Host Separation Syndrome and Chemical Dependency of Ectosymbiotic Decapods Associated with Echinoderms

Alexia Lourtie



A dissertation submitted to the University of Mons and the Catholic University of Louvain in fulfillment of the requirements for the degree of Doctor of Philosophy in Biological Science.

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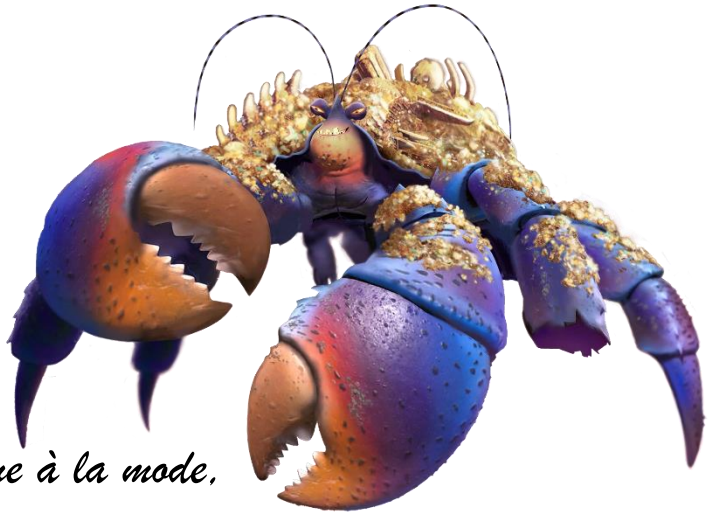
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« Un demi-dieu, même à la mode,

Ne vaut pas mieux qu'un décapode » *Tamatoa (Vaiana), 2016*

A ma famille,

chiens compris

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Thesis summary

Symbiotic relationships, defined as close and long-lasting associations between at least two different species, are ubiquitous throughout the biosphere. One key factor that sustains symbioses across generations is the establishment of a host-specific chemical environment allowing, amongst others, the survival of the symbiont and its ability to select a suitable host. These chemical signals, true metabolites from the host, are also known as kairomones as they provide benefits for the symbiont. In echinoderms, the nature of the kairomones attracting symbionts is only known for associations involving sea cucumbers and sea urchins hosts. Moreover, when separated from their hosts, the environment (both chemical and nutritional) of the symbionts becomes unsuitable leading to a "host separation syndrome". This syndrome, only described in a sea urchin-shrimps association, is characterized by an alteration of the behavior and the state of health, potentially leading to the death of symbionts.

The general aim of this thesis is to contribute to the knowledge of these kairomones playing a crucial role in symbiotic associations, notably those associating decapods with echinoderms which are frequent symbioses in the marine environment. First, the chemical characterization of the kairomonal chemical signature allowing host recognition was investigated in crinoid and asteroid hosts. Then, the host separation on various symbiotic decapods was monitored to characterize the syndrome in several species and to understand its impact at the behavioral, anatomical and physiological levels.

The **introduction** of this thesis begins with a review of the diversity of macro-ectosymbiotic decapods associated with echinoderms. This includes an exhaustive list of 220 decapod species associated with all five classes of echinoderms, and an analysis of all these associations, described since 1849. The review also investigated association characteristics, such as the benefits for symbionts and their dependency on hosts, as well as co-evolution involving morphological, physiological, and behavioral adaptations. The introduction is followed by four chapters that provide new insights on the host separation syndrome and the host chemical dependency of decapod symbionts.

Chapter I characterizes the chemical environment produced by crinoids and explores host recognition behavior in the symbiotic pistol shrimp, *Synalpheus stimpsonii*. The chemical attractiveness of two crinoid hosts and one non-host species, as well as commercial

anthraquinones, were tested in an olfactometer. Mass spectrometry revealed, from the chemical extraction of the crinoid *Phanogenia distincta*, the presence of three different anthraquinones (rhodoptilometrin, comantherin, and a new crinoid anthraquinone) that trigger host recognition.

Chapter II investigates host recognition in the adult and larvae of the shrimp *Zenopontonia soror*, an obligate symbiont of sea stars. Semiochemicals influencing host selection were revealed using chemical extractions, behavioral experiments in olfactometers, and mass spectrometry analyses. Our results demonstrate that asterosaponins are species-specific and play a role in host recognition, including symbiont larval recruitment. While adult shrimps were attracted only by their original host species *Culcita novaeguineae*, larvae were attracted by different asteroid species. This study provides, for the first time, the chemical identification of an olfactory cue used by larvae of symbiotic organisms to locate their hosts for recruitment.

Chapter III explored the host separation syndrome using a transcriptomic approach. Paired-end Illumina HiSeq technology has been used to analyze transcriptomes from *Arete indicus* and *Tulеariocaris holthuisi* confronted with three different conditions used previously to investigate host separation syndrome. The three conditions analyzed were: individuals associated with their host, individuals separated from their host and individuals separated from their host but conditioned by naphthoquinones that is a kairomone involved in the host recognition by these symbionts. A total of 217,832 assembled unigenes were obtained, with an N50 of 2,061 bp. Our results show a proportion of 16.5% of differentially expressed genes in isolated *T. holthuisi* shrimp compared to the control treatment and a proportion of 8.5% of DEGs in isolated *T. holthuisi* shrimps in presence of spinochromes compared to the control. For *A. indicus*, there were fewer variations in DEG proportions among the same comparisons. Yet, analyses highlight a complex transcriptomic effect on both symbiotic species, with notably a differential expression of heat shock protein in isolated *T. holthuisi*.

Chapter IV focuses on the mimetic pigmentation of four symbiotic models: *Z. soror – C. novaeguineae, S. stimpsonii – P. distincta, T. holthuisi – E. mathaei* and *A. indicus – E. mathaei*. The results suggested a strong host dependency of all four symbionts, but only three of them exhibited a host chemical dependency. The host isolation impacted the pigmentation of the symbionts, with a nuanced and dynamic pigmentation variation depending on the species. The chemical extraction highlights similar carotenoid pigments in *Z. soror* and *C. novaeguineae*. At the opposite, the crinoid *P. distincta* did not possess the carotenoids found in its associated

shrimp. Histological sections show the presence of chromatophores in all the species and when shrimps discolored, their chromatophores agglomerated.

The final discussion aims to incorporate the results obtained in the different thesis chapters to provide a broader understanding and potential future research objectives that may be carried out to better understand marine symbiotic associations and their integration in an ecosystem-level. It sheds light on the symbiont response to specific chemicals produced by the host and to the host separation. This research will contribute significantly to future studies on co-evolution, host recognition, and chemical ecology within symbiotic systems.



Résumé de thèse

Les relations symbiotiques, définies comme des associations intimes et durables entre au moins deux espèces différentes, sont omniprésentes dans la biosphère. L'un des facteurs clés permettant aux symbioses de perdurer au fil des générations est la mise en place d'un environnement chimique spécifique à l'hôte permettant, entre autres, la survie du symbiote et sa capacité à sélectionner un hôte approprié. Ces signaux chimiques, véritables métabolites de l'hôte, sont également connus sous le nom de kairomones, car ils apportent des avantages au symbiote. Chez les échinodermes, la nature des kairomones attirant les symbiotes n'est connue que pour deux associations impliquant des hôtes concombres de mer et oursins. De plus, lorsqu'ils sont séparés de leurs hôtes, l'environnement (potentiellement à la fois chimique et nutritionnel) des symbiotes devient inadapté, ce qui entraîne un "syndrome de séparation de l'hôte". Ce syndrome, uniquement décrit dans une association oursin-crevette, est caractérisé par une altération du comportement et de l'état de santé, pouvant conduire à la mort des symbiotes.

L'objectif général de cette thèse est de contribuer à la connaissance de ces kairomones jouant un rôle crucial dans les associations symbiotiques, notamment celles associant les décapodes aux échinodermes, constituant des symbioses fréquentes en milieu marin. Tout d'abord, la caractérisation de la signature chimique des kairomones permettant la reconnaissance de l'hôte a été étudiée chez des hôtes crinoïdes et astéries. Ensuite, la séparation de l'hôte pour différents décapodes symbiotiques a été suivie afin de caractériser ce syndrome chez plusieurs espèces et de comprendre son impact au niveau comportemental, anatomique et physiologique.

L'**introduction** de cette thèse commence par une revue de la diversité des décapodes macro-ectosymbiotiques associés aux échinodermes. Ceci inclut une liste exhaustive de 220 espèces de décapodes associés aux cinq classes d'échinodermes, et une analyse de toutes ces associations, décrites depuis 1849. L'étude porte également sur les caractéristiques des associations, telles que les avantages reçus par les symbiotes et leur dépendance vis-à-vis des hôtes, ainsi que sur la coévolution impliquant des adaptations morphologiques, physiologiques et comportementales. L'introduction est suivie de quatre chapitres qui fournissent de nouvelles informations sur le syndrome de séparation de l'hôte et la dépendance chimique des symbiotes décapodes vis-à-vis de leur hôte.

Le **chapitre I** caractérise l'environnement chimique produit par les crinoïdes et explore le comportement de reconnaissance de l'hôte chez la crevette pistolet symbiotique *Synalpheus stimpsonii*. L'attractivité chimique de deux crinoïdes hôtes et d'une espèce non-hôte, ainsi que d'anthraquinones commerciales, a été testée dans un olfactomètre. Les analyses en spectrométrie de masse ont révélé, à partir de l'extraction chimique du crinoïde *Phanogenia distincta*, la présence de trois anthraquinones différentes (rhodoptilométrine, comanthérine et une nouvelle anthraquinone de crinoïde) qui déclenchent la reconnaissance de l'hôte.

Le **chapitre II** étudie la reconnaissance de l'hôte chez les adultes et les larves de la crevette *Zenopontonia soror*, un symbiote obligatoire des étoiles de mer. Les substances sémiochimiques influençant la sélection de l'hôte ont été révélées à l'aide d'extractions chimiques, d'expériences comportementales dans des olfactomètres et d'analyses par spectrométrie de masse. Nos résultats démontrent que les astérosaponines sont spécifiques aux espèces et jouent un rôle dans la reconnaissance de l'hôte, y compris dans le recrutement des larves du symbiote. Alors que les crevettes adultes n'étaient attirées que par leur espèce hôte d'origine, *Culcita novaeguineae*, les larves étaient attirées par différentes espèces d'astéries. Cette étude fournit, pour la première fois, l'identification chimique d'un signal olfactif utilisé par les larves d'organismes symbiotiques pour localiser leurs hôtes en vue du recrutement.

Le **chapitre III** a exploré le syndrome de séparation de l’hôte par une approche transcriptomique. La technologie « paired-end Illumina HiSeq » a été utilisée pour analyser les transcriptomes d'*Arete indicus* et de *Tulеariocaris holthuisi* confrontés à trois conditions différentes utilisées précédemment pour étudier le syndrome de séparation de l’hôte. Les trois conditions analysées étaient les suivantes : individus associés à leur hôte, individus séparés de leur hôte et individus séparés de leur hôte mais conditionnés par des naphtoquinones, une kairomone impliquée dans la reconnaissance de l'hôte par ces symbiotes. Un total de 217 832 unigènes assemblés a été obtenu, avec un N50 de 2,061 pb. Nos résultats montrent une proportion de 16,5% de gènes différentiellement exprimés chez les crevettes *T. holthuisi* isolées par rapport au traitement témoin et une proportion de 8,5% de DEGs chez les crevettes *T. holthuisi* isolées en présence de spinochromes par rapport au contrôle. Pour *A. indicus*, il y a moins de variations dans les proportions de DEGs entre les mêmes comparaisons. Cependant, les analyses mettent en évidence un effet transcriptomique complexe sur les deux espèces symbiotiques, avec notamment une expression différentielle des protéines de choc thermique chez les crevettes *T. holthuisi* isolées.

Le **chapitre IV** se concentre sur la pigmentation mimétique de quatre modèles symbiotiques : *Z. soror - C. novaeguineae*, *S. stimpsonii - P. distincta*, *T. holthuisi - E. mathaei* et *A. indicus - E. mathaei*. Les résultats suggèrent une forte dépendance à l'hôte pour les quatre symbiotes, mais seulement trois d'entre eux présentent une dépendance chimique à l'hôte. L'isolement de l'hôte a eu un impact sur la pigmentation des symbiotes, avec une variation nuancée et différentes dynamiques de la pigmentation en fonction de l'espèce. L'extraction chimique met en évidence des pigments caroténoïdes similaires chez *Z. soror* et *C. novaeguineae*. A l'inverse, le crinoïde *P. distincta* ne possède pas les caroténoïdes retrouvés chez les crevettes qui lui sont associées. Les coupes histologiques montrent la présence de chromatophores chez toutes les espèces et lorsque les crevettes se décolorent, leurs chromatophores s'agglomèrent.

La **discussion finale** vise à intégrer les résultats obtenus dans les différents chapitres de la thèse afin de fournir une compréhension plus large et des objectifs de recherche futurs potentiels qui pourraient être réalisés pour mieux comprendre les associations symbiotiques marines et leur intégration au niveau de l'écosystème. Il met en lumière la réponse du symbiote à des produits chimiques spécifiques produits par l'hôte et à la séparation de l'hôte. Cette recherche contribuera de manière significative aux études futures sur la coévolution hôte-symbiote, la reconnaissance de l'hôte et l'écologie chimique au sein des systèmes symbiotiques.

Foreword

At the boundary between ecology and chemistry, this thesis explores the pivotal role played by metabolites (ecomones) that mediate and perpetuate symbiotic associations. The decision to delve into chemical ecology involving echinoderms hosts and crustaceans symbionts, corresponds to the continuity of two previous PhD theses that were undertaken in the Biology of Marine Organisms and Biomimetics unit of the University of Mons (Caulier 2016; Brasseur 2018). This PhD project has been funded by a FRIA grant (Fund for Research training in Industry and Agriculture) from the Fund for Scientific Research (F.R.S.–FNRS) of Belgium obtained in 2019. The initial research plan has been considerably impacted by the COVID-19 (Coronavirus disease 2019) pandemic that limited our possibilities to travel to scientific stations abroad, particularly to the biological station of Belaza (Madagascar) where we planned to go initially. However, the resilience allowed by scientific collaboration allowed this project to evolve and adapt by finding new partners and new biological symbiotic models. This preamble aims to explain the thematic and the structure of this PhD thesis.

(1) Contextualizing the research landscape

Within the intricate thematic of biological interaction, the exchange of information through chemical signaling stands as the most ancient and ubiquitous mode of communication (Kittredge et al. 1971). This phenomenon, known as chemical communication, extends its influence across all levels of the biosphere, from the cellular level to entire ecosystems (Krieger and Breer 1999). Furthermore, the emergence of complex organisms can be linked to the development of molecular signaling between cells, tissues, and organs (Hartwell et al. 1999). These communication pathways within an organism are facilitated by the production of molecules, called primary and secondary metabolites, which enable the entire metabolic processes (Elliot and Elliot 1997).

The chemical ecology focus on the same metabolites when they are released into the external environment (Eisner and Meinwald 1995). In this concept, they are called ecomones or semiochemicals. They can be crucial to structure the ecosystems as they will facilitate a myriad of interactions (Eisner and Meinwald 1995). Ecomones secreted by one organism navigate the external environment, potentially intercepted and chemodetected by the sensory system of another organism (Wyatt 2014). Cues interpretation carried by these chemical stimuli, produced by the emitting organisms, induces a behavioral and/or physiological response in the receiving organism (Wyatt 2014). In marine environment, each organism navigates through a complex

solution of chemical signals produced by the entirety of biotic and abiotic entities within its ecosystem (Brönmark and Hansson 2012). During this thesis, we decided to focus on one of the less investigated chemical interaction between organisms : symbiotic associations. The chosen biological model encompasses echinoderm hosts and crustacean symbionts.

(2) Echinoderms – crustaceans interests

Thanks to the rich symbiotic community they usually host, echinoderms are extensively investigated in marine chemical ecology (Williamson et al. 2012; Caulier et al. 2013b; De Bruyn et al. 2016; Brasseur et al. 2018; Antokhina and Britayev 2020a; Claereboudt et al. 2023). This phylum is characterized by class-specific semiochemicals that may act as specific chemical signatures (Kornprobst 2005). Quinone pigments, rare in the animal kingdom (a few insects and sponges), are very common in crinoids and sea urchins (Thomson 1991; El-Najjar et al. 2011; Hou et al. 2018). Saponins, almost absent from the animal kingdom, are massively present in sea stars and sea cucumbers (Netala et al. 2009; Caulier et al. 2011; Savarino et al. 2021). As for brittle stars, although they contain a few quinone pigments, they are clearly distinguished from the other four classes by the exclusive presence of 3α-hydroxysterols (Kornprobst 2005). Thus, the study of echinoderm metabolites suggests indisputable analogies between crinoids and echinoids, on the one hand, and asteroids and holothuroidss on the other. We considered sea stars, sea urchins and crinoids because of their availability on the field and the high symbiotic prevalence in these classes (**Introduction**). We decided to not focus on holothuroidss as they were already studied in host recognition and host separation (Ayotte and DeMaintenon 2005; Caulier et al. 2013b). And finally, we did not investigate brittle stars as we did not find any species involved in symbiotic association during the scientific field missions realized during this PhD thesis.

Crustaceans are common symbionts found on/in echinoderms (Ng and Jeng 1999) and they constitute suitable biosensors to study the ecological role of chemical sensing. Indeed, despite their visual acuity (Glantz et al. 2014), crustaceans are built as chemical receivers having numerous chemical receptors distributed on various body parts (Hay 2011). They use chemical sensing to find mates or food, and their high metabolic rates, limited mating periods, and short life spans have imposed the need to rapidly respond to chemical cues and signals (Hay 2011). Additionally, their rapid maturation, small size, and ease of maintenance in the lab conditions make them excellent assay organisms. Not only they are exceptionally suited to investigate the behavioral response to various chemical signals (*e.g.* in olfactometers), but they also may be

used to measure the costs and benefits of this in terms of survivorship, growth, and fecundity (Cruz-Rivera and Hay 2003). Previous works have already identified the chemical nature of cues used to find and assess mates, signal dominance, recognize known conspecifics, find favored foods and appropriate habitats, and assess threats such as the presence of predators (Breithaupt and Thiel 2010). However, only two studies, performed by colleagues from my host lab have highlighted the chemical nature of the kairomones involved in host selection for symbiotic crustaceans (Caulier et al. 2013b; Brasseur et al. 2018).

In order to introduce this thesis, a comprehensive inventory describing the diversity of macro-ectosymbiotic decapod crustaceans associated with echinoderms is provided. This review also investigates the types of association, the benefits for the symbionts and their dependency on the host, and their co-evolution involving morphological, physiological, developmental, and behavioral adaptations.

General introduction



Exploring the Ecological and Evolutionary Significance of Decapod-Echinoderm Symbioses: a review

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Abstract

The marine biosphere is structured by symbiotic relationships, with hosts and symbionts playing crucial roles in the dynamics and stability of these diverse and complex ecosystems. Crustaceans and echinoderms are particularly interesting to investigate as both taxa display broad morphological plasticity due to diverse ecological niches, offering scope to explore co-adaptation pathways. The objective of this review is to provide the state of the art on the diversity of macro-ectosymbiotic decapod crustaceans associated with echinoderms. This review is both a comprehensive inventory, and an investigation of the types of associations, the benefits for the symbionts and their dependency on the host, and their co-evolution involving morphological, physiological, developmental, and behavioral adaptations. The inventory reveals that 221 species of decapods (representing 1.3 % of Decapoda) have been reported as symbionts with at least 228 species of echinoderms, mainly reported in tropical areas and shallow waters. Crinoidea is the class involving the highest number of decapod symbionts (87) despite being less diverse in terms of species. Crinoids have greater body complexity than other echinoderms and offer their decapod symbionts a range of ecological niches. Echinoderms are, unsurprisingly, populated by symbionts from the most species-diversified infra-orders of decapods, carideans and brachyurans (n=103 and 81 species, respectively). The relationships show different levels of symbiotic specificities, from species-specific to inter-phyla generalists. Phylogeny of symbiotic decapods reveals independent pathways leading to the extant decapod-echinoderm symbioses. This review summarizes symbiont benefits including mechanical and chemical defenses against predators, diverse food intake strategies or a potential impact of host chemicals on the symbiont metabolism, as well as co-adaptation leading to decapod-echinoderm symbioses. Concurrently, various morphological, behavioral and ontogenic adaptations have been highlighted for the symbionts, like the transformation of essential organs such as pereiopods, mouthparts or sensory systems. Specific behavioral adaptations, such as symbiont locomotion, host selection, and host switch were also described. Finally, the adaptations of the biological cycle of symbionts and their reproduction are discussed.

Keywords Ectosymbiotic association; Crustaceans; Phenotypic adaptations; Host separation syndrome; Host recognition; Co-evolution.

I. Symbiosis: definitions and concepts

While the term "parasite" already existed in the ancient Greek language (παράσιτος), the terms "commensalism", "mutualism" and "symbiosis" are more recent and owe their origin to 19th century biologists (Martin and Schwab 2012). The term "*symbiosis*", in particular, appeared following the experiments and reflections of German lichenologists (de Bary 1879; Martin and Schwab 2012). Indeed, the discovery of the algae-fungi duality, which is at the origin of lichens, prompted these scientists to find a new term, other than parasite, to qualify these relationships between phylogenetically distant species. In 1877, Albert Bernhard Frank coined the word "*Symbiotismus*" by indicating "*We must group together all cases where two different species live on top of each other or in each other under an overall concept which does not consider the role that the two species play but is based on simple coexistence and for which the term symbiotismus is to be recommended* "(Frank 1877). Frank conducted detailed studies on different lichens and hypothesized that there might be cases in which the algal component had become so adapted to fungi that it could no longer live independently (Frank 1877). He then studied various other types of associations between microbes and plants and collected them in his work "*Lehrbuch der Botanik*" (Frank 1892) which was frequently cited by 19th century botanists who were interested in symbioses. Although Frank's studies are well known and he has become one of the leading proponents of the idea that many associations involving microorganisms cannot be called parasitism, the origin of the term "*symbiosis*" was not assigned to him (Martin and Schwab 2012). The creation of the term "*symbiosis*" is attributed to Henrich Anton de Bary, which he used for the first time in a speech entitled "*The Phenomena of Symbiosis*" delivered at a general meeting of the Association of German Naturalists and Physicians in Cassel in 1878 (Oulhen et al. 2016). He defined it as "*the life together of different organisms*". Although he never referred to Frank in this speech, de Bary, referred readers to Frank's 1877 article in which he first used the term "*Symbiotismus*" (de Bary 1879). In the symbiosis concept, de Bary included various types of complex associations, extending along a continuum from parasitic to mutualistic relationships. The term "*mutualism*" and "*commensalism*" had been introduced a few years earlier by the Belgian zoologist Pierre-Joseph van Beneden in a lecture at the Royal Academy of Belgium entitled "*A word on the social life of lower animals*" (van Beneden 1873). In his popular book "*Animal Parasites and Messmates*" (van Beneden 1878), van Beneden opposed the view, common at his time, that all intimate relationships involving "lower animals" living in or on "higher animals" were parasites. Instead, he argued that the types of social relationships in animal societies were as varied as those found

in human societies and should be classified in terms of "parasitism", "commensalism", and "mutualism".

Today, symbiotic associations are still divided into three main categories: parasitism, commensalism, and mutualism (Paracer and Ahmadjian 2000; Eeckhaut 2003; Parmentier and Michel 2013). The effects of the association on the symbiont being always beneficial, these categories are only characterized by the effects on the host. Mutualism is the category where both species benefit from the interaction (Eeckhaut 2003; Morita and Schmidt 2018), commensalism implies no effect on the host fitness (Paracer and Ahmadjian 2000; Eeckhaut 2003; Parmentier and Michel 2013) and parasitism is when the symbiont benefits at the expense of the host, with a significant effect on its health and survival (*i.e.* biological fitness) (Jangoux 1987; Paracer and Ahmadjian 2000; Eeckhaut 2003; Parmentier and Michel 2013). However, these discrete classifications are unrealistic as the boundaries between these categories are often blurry, and many symbiotic relationships can be better understood as a continuum, with varying degrees of mutualism, commensalism, and parasitism (Johnstone and Bshary 2002; Trilles and Hipeau-Jacquotte 2012; Parmentier and Michel 2013; Canestrari et al. 2014). The concept of a symbiotic continuum highlights the dynamic and evolving nature of symbiotic relationships, with various biotic and abiotic factors potentially inducing shifts between these categories, and underscores the importance of understanding these interactions in the context of their ecological and evolutionary history (Parmentier and Michel 2013). For example, factors that may influence the nature of symbiotic relationships include the quantity of symbionts present on a particular individual host, the stage of development of the symbiont, and the abundance of food resources available in the environment (Eeckhaut and Jangoux 1997; Johnstone and Bshary 2002; Caulier et al. 2012; Canestrari et al. 2014; González et al. 2021).

Symbiotic associates can be appropriate models to investigate co-evolution and sometimes co-speciation as they induce intrinsic adaptations that are advantageous for the symbiont fitness, favoring host dependency (Gherardi 1991; Lanterbecq et al. 2010; Horká et al. 2016). This is exemplified by host switching, which can play a significant role in the adaptation and the emergence of new species of symbiotic associates (Millanes et al. 2014; Horká et al. 2016; Li et al. 2017). Symbiotic associations are often conserved across host phylogeny, meaning that closely related symbiont species tend to associate with similar symbiotic partners. Therefore, host switches between phylogenetically distant hosts are atypical (Gómez et al. 2010). Indeed, a series of behavioral, morphological and physiological adaptations allowing a specific

symbiont to efficiently live in symbiosis with a single or a few closely related hosts (Horká et al. 2016; Brasseur et al. 2018) constrains the establishment of a new relationship with a phylogenetically distant potential host (Gómez et al. 2010; Horká 2017). However, symbiotic lineages do occasionally switch to new taxa and even, sometimes, distantly related host species (Gómez et al. 2010).

Marine ecosystems are notably structured by symbiotic relationships as hosts and symbionts play crucial roles in the stability of these complex systems (Lopanik 2014). Symbiotic relationships usually confer adaptive advantages to partners, that may allow them to cope with changing environmental conditions, face ongoing anthropogenic impacts, or provide environmental resilience (Duffy and Stachowicz 2006; Buhl-Mortensen et al. 2010; Ziegler et al. 2018). Symbioses may also improve nutrient cycling and waste recycling, which would help maintain nutrient balance in the ecosystem and would support the growth/productivity of various organisms (Wang and Douglas 1998; Zhang et al. 2019). Moreover, associating with one or several symbiotic partner(s) potentially creates new ecological niches for symbionts, that may be inaccessible otherwise (Buhl-Mortensen et al. 2010; Chapman et al. 2018).

Echinoderms constitute a major deuterostomian phylum present in all marine ecosystems around the globe (Belaústegui et al. 2017; DeSalle 2021). Being composed of five classes, they can host an important symbiotic biodiversity and can be considered as true micro-ecosystems (Jangoux 1987; Antokhina and Britayev 2012; Virgili et al. 2020). Symbiotic associations between crustaceans and echinoderms are particularly interesting to investigate as both taxa display a large morphology plasticity and diverse ecological niches, offering a large scope to explore co-adaptation pathways (Dworschak et al. 2009; Horká 2017; Sotelo-Casas et al. 2018; DeSalle 2021; Chow et al. 2021; Hamel et al. 2022)

II. Introduction to echinoderm-associated decapods and objectives of the review

Decapod crustaceans are found globally in nearly all marine and freshwater environments. (Briones-Fourzán and Hendrickx 2022). The evolution of decapod crustaceans spans more than 455 million years and there are approximately 17,500 extant species with another 3,000 species in the fossil record (Wolfe et al. 2019). The characteristics of decapods greatly vary across species and display numerous specific morphological, physiological and behavioral adaptations (Horká 2017). The majority of the species belonging to the order Decapoda are considered as free living but many are also adapted to symbiotic associations involving a large variety of hosts such as sponges, cnidarians, mollusks, ascidians and echinoderms (Dworschak et al. 2009; Horká 2017; Chow et al. 2021).

Echinoderms, with more than 7,000 species described (DeSalle 2021), show diverse body plans and take part in essential ecological services that help in maintaining the delicate balance of a healthy marine environment. The five classes of echinoderms include (*i*) the Holothuroidea (sea cucumbers); (*ii*) the Crinoidea (feather stars); (*iii*) the Asteroidea (sea stars); (*iv*) the Echinoidea (sea urchins) and (*v*) the Ophiuroidea (brittle stars) (Bruce 1975b; Horká 2017). Several echinoids, asteroids, ophiuroids, holothuroids and crinoids are known to be low or important "bioturbators" as they move and stir the sediment while feeding, burrowing, or crawling on/in the seabed (Li et al. 2013; Belaústegui et al. 2017; Hamel et al. 2022). This process enhances nutrient cycling, oxygenation, and the distribution of organic matter in the sediment, which is essential for the health of benthic ecosystems (Mermillod-Blondin and Rosenberg 2006; Meysman et al. 2006). Other species can filter feed, be scavengers or necrophagous, therefore returning essential nutrients to the water column and sediment and supporting the growth of primary producers, finally sustaining the entire food web (McClintock 1994). Some can influence the structure of marine habitats by consuming algae or other benthic organisms, creating available free spaces of substrate allowing a possible turnover of benthic biodiversity (Sotelo-Casas et al. 2018).

Therefore, both echinoderms and crustaceans are known to promote the biodiversity by maintaining, in collaboration with diverse organisms, the balance of marine environment and regulate the population of benthic groups such as corals, mollusks, algae, and polychaetes (Glynn and Enochs 2011; Horká 2017; Sotelo-Casas et al. 2018; Briones-Fourzán and

Hendrickx 2022). They can also be both predators and preys in marine food webs (Juanes 1992; Campbell et al. 2001). As predators, they help control populations of other invertebrates and maintain ecological balance (Anger et al. 1977; Juanes 1992; McClintock 1994). On the other hand, they serve as a food source for various marine organisms, including vertebrates (*e.g.* fish and seabirds), other decapods and echinoderms (Lawrence and Vasquez 1996; Maynou and Cartes 2000; Campbell et al. 2001). Their ecological services have far-reaching impacts on the biodiversity and functioning of coastal and deep-sea environments.

Echinoderm-associated decapods are mostly represented by ectosymbionts and the most popular families associated with this taxon are the Palaemonidae, Alpheidae, Pinnotheridae and Porcellanidae (Baeza and Thiel 2007; Horká 2017). Echinoderms hosting decapods can be found in each of the five classes (**Figure 1**) (Bruce 1975b; Horká 2017).

Despite the evolutionary success of the associations between echinoderms and crustacean decapods, no review has ever summarized the species involved and highlighted characteristics of those symbiotic associations. The objective of this review is to provide the state of the art on the diversity of the macro-ectosymbiotic decapods associated with echinoderms. This review also investigates association characteristics such as the benefit for the symbiont and its dependency to the host, and the co-evolution of host/symbiont involving morphological, physiological, developmental and behavioral adaptations.



Figure 1: Eectosymbiotic decapods associated with the five echinoderms classes **(A)** *Dissodactylus primitivus* on the sea urchin *Meoma ventricosa*. **(B)** Sea star shrimp *Zenopontonia soror* on the sea star *Culcita novaeguineae*, **(C)** Snapping shrimp *Synalpheus stimpsonii* on the crinoid *Phanogenia distincta*, **(D)** Sea cucumber Arlequin crab *Lissocarcinus orbicularis* on the holothuroid *Bohadschia argus*. **(E)** Alpheid shrimp *Athanas ornithorhynchus* on the ophiuroid *Macrophiothrix longipeda*. Illustration 1E from Marin et al. (2005). Scale bars = 0.5cm in A, B; 0.8cm in C ; 1cm in D and 0.25cm in E.

III. Inventory and characterization of decapod-echinoderm symbioses

Table S1 lists all the ectosymbiotic decapods associated to echinoderms to the species level. In total, this inventory compiles 324 references from the scientific literature, published between 1849 and 2023. It also mentions the type of symbiotic association when detailed in the original reference. For practical reasons (*i.e.* length-limit of the current review), **Table 1**, to the genus level, has been generated as a summary of **Table S1**.

Table 1: Summary of the inventory S1 displaying echinoderms-associated decapods, ordered by their phylogenetic classification to the genus level. When a decapod genus has been described as a symbiont of different echinoderms classes, numbers mentioned in brackets correspond to the number of symbiont species reported for each host class. If the sum of those numbers is higher than the number of species, it means that one species can be found on two different host classes. Unknown = species described as associated to an undetermined echinoderm. Corresponding detailed **Table S1** can be found in supplementary material.

Decapod taxonomy	Total Direct-children	Echinoderms symbionts	Echinoderm Class involved
Order Decapoda	±17 500 species	221 species	All classes
Suborder Pleocyemata	11 infraorders	5 infraorders	
Infraorder Achelata	2 families	1 family	
Family Palinuridae	12 genera	1 genus	
Genus Panulirus	22 species	1 species	Echinoidea
Infraorder Anomura	6 superfamilies	2 superfamilies	
Superfamily Chirostyloidea	4 families	2 families	
Family Chirostylidae	6 genera	2 genera	
Genus Uroptychodes	14 species	2 species	Crinoidea
Genus Uroptychus	286 species	3 species	Crinoidea
Family Sternostylidae	1 genus	1 genus	
Genus Sternostylus	14 species	1 species	Asteroidea
Superfamily Galatheoidea	4 families	4 families	
Family Galatheidae	12 genera	2 genera	
Genus Allogalathea	4 species	3 species	Crinoidea
Genus Galathea	177 species	6 species	Crinoidea

Family Munididae	34 genera	1 genus	
Genus Munida	7 species	1 species	Crinoidea
Family Munidopsidae	4 genera	1 genus	
Genus Munidopsis	275 species	3 species	Holothuroidea (1), Crinoidea (1), Echinoidea (1)
Family Porcellanidae	29 genera	7 genera	
Genus Allopetrolisthes	3 species	1 species	Asteroidea
Genus Clastotoechus	6 species	2 species	Echinoidea
Genus Liopetrolisthes	2 species	2 species	Echinoidea
Genus Lissoporcellana	10 species	1 species	Crinoidea
Genus Pachycheles	48 species	1 species	Asteroidea
Genus Petrolisthes	113 species	4 species	Echinoidea
Genus Pisidia	14 species	2 species	Crinoidea
Superfamily Paguroidea	7 families	1 family	
Family Diogenidae	22 genera	1 genus	
Genus Calcinus	47 species	1 species	Echinoidea
Infraorder Brachyura	35 superfamilies	7 superfamilies	
Superfamily Grapsoidea	9 families	1 family	
Family Percnidae	1 genus	1 genus	
Genus Percnon	7 species	1 species	Echinoidea
Superfamily Hymenosomatoidea	1 family	1 family	
Family Hymenosomatidae	26 genera	1 genus	
Genus Neohymenicus	2 species	1 species	Ophiuroidea
Superfamily Majoidea	7 families	4 families	
Family Epialtidae	96 genera	1 genus	
Genus Menaethius	2 species	1 species	Holothuroidea
Family Inachidae	34 genera	1 genus	
Genus Achaeus	39 species	1 species	Holothuroidea
Family Inachoididae	13 genera	1 genus	

Genus Stenorhynchus	2 species	2 species	Crinoidea (1), Echinoidea (2)
Family Mithracidae	14 genera	1 genus	
Genus Mithraculus	8 species	1 species	Echinoidea
Superfamily Nematocarcinoidea	4 families	1 family	
Family Rhynchocinetidae	2 genera	1 genus	
Genus Rhynchocinetes	15 species	1 species	Echinoidea
Superfamily Pilumnoidea	3 families	1 family	
Family Pilumnidae	72 genera	13 genera	
Genus Cebudonus	1 species	1 species	Echinoidea
Genus Ceratocarcinus	3 species	3 species	Crinoidea
Genus Echinoecus	3 species	3 species	Echinoidea
Genus Eumedonus	5 species	5 species	Echinoidea
Genus Gonatonotus	3 species	3 species	Holothuroidea (1), Asteroidea (1), Echinoidea (3)
Genus Hapalonotus	1 species	1 species	Holothuroidea
Genus Harrovia	7 species	7 species	Crinoidea
Genus Permanotus	1 species	1 species	Crinoidea
Genus Rhabdonotus	3 species	3 species	Crinoidea
Genus Tauropus	1 species	1 species	Echinoidea
Genus Tiaramedon	1 species	1 species	Crinoidea
Genus Zebrida	3 species	3 species	Echinoidea
Genus Zebridonus	1 species	1 species	Echinoidea
Superfamily Pinnotheroidea	2 families	1 family	
Family Pinnotheridae	62 genera	12 genera	
Genus Alain	2 species	2 species	Holothuroidea
Genus Austrotheres	2 species	1 species	Holothuroidea
Genus Buergeres	4 species	4 species	Holothuroidea
Genus Calyptraeotheres	5 species	1 species	Asteroidea

Genus Clypeasterophilus	4 species	4 species	Echinoidea
Genus Dissodactylus	15 species	8 species	Echinoidea
Genus Glassella	12 species	1 species	Holothuroidea
Genus Holotheres	5 species	5 species	Holothuroidea
Genus Holothuriophilus	2 species	2 species	Holothuroidea
Genus Opisthopus	1 species	1 species	Holothuroidea
Genus Pinnaxodes	7 species	4 species	Holothuroidea (2), Echinoidea (2)
Genus Pinnixa	32 species	3 species	Holothuroidea
Genus Trichobezoares	2 species	2 species	Holothuroidea
Superfamily Portunoidea	7 families	1 family	
Family Portunidae	42 genera	1 genus	
Genus Lissocarcinus	9 species	2 species	Holothuroidea
Infraorder Caridea	14 Superfamilies	2 superfamilies	
Superfamily Alpheoidea	8 families	3 families	
Family Alpheidae	52 genera	5 genera	
Genus Alpheus	326 species	3 species	Crinoidea (1), Echinoidea (1), Ophiuroidea (1)
Genus Arete	4 species	4 species	Echinoidea
Genus Athanas	41 species	4 species	Crinoidea (3), Echinoidea (1), Ophiuroidea (1)
Genus Betaeus	16 species	1 species	Echinoidea
Genus Synalpheus	168 species	8 species	Crinoidea
Family Hippolytidae	16 genera	2 genera	
Genus Hippolyte	40 species	2 species	Crinoidea

Genus Latreutes	19 species	1 species	Echinoidea
Family Thoridae	8 genera	1 genus	
Genus Thor	17 species	2 species	Crinoidea
Superfamily Palaemonoidea	2 families	1 family	
Family Palaemonidae	149 genera	32 genera	
Genus Actinimenes	4 species	1 species	Echinoidea
Genus Allopontonia	2 species	2 species	Echinoidea
Genus Araiopontonia	1 species	1 species	Crinoidea
Genus Bathymenes	17 species	1 species	Echinoidea
Genus Brucecaris	1 species	1 species	Crinoidea
Genus Conchodytes	11 species	1 species	Holothuroidea
Genus Crinotonia	2 species	2 species	Crinoidea
Genus Cristimenes	4 species	4 species	Crinoidea (2), Echinoidea (2)
Genus Cuapetes	30 species	1 species	Crinoidea
Genus Diapontonia	1 species	1 species	Echinoidea
Genus Echinopericlimenes	4 species	4 species	Echinoidea
Genus Gnathophylloides	2 species	2 species	Echinoidea
Genus Gnathophyllum	10 species	3 species	Holothuroidea (1), Asteroidea (1), Echinoidea (2)
Genus Hymenocera	1 species	1 species	Asteroidea
Genus Laomenes	12 species	10 species	Crinoidea
Genus Levicaris	1 species	1 species	Echinoidea
Genus Lipkebe	1 species	1 species	Crinoidea
Genus Lipkemenes	1 species	1 species	Echinoidea (1), Ophiuroidea (1)
Genus Margitonia	1 species	1 species	Echinoidea
Genus Palaemonella	23 species	1 species	Crinoidea
Genus Periclimenaeus	87 species	1 species	Crinoidea
Genus Periclimenella	3 species	1 species	Crinoidea

Genus Periclimenes	117 species	18 species	Holothuroidea (2), Crinoidea (10), Echinoidea (5), Ophiuroidea (2)
Genus Phyllognathia	2 species	1 species	Asteroidea (1), Echinoidea (1)
Genus Pontoniopsis	1 species	1 species	Crinoidea
Genus Pseudocoutierea	8 species	1 species	Echinoidea
Genus Pycnocaris	1 species	1 species	Unknown
Genus Sandimenes	2 species	2 species	Echinoidea (1), Ophiuroidea (1), Unknown (1)
Genus Stegopontonia	1 species	1 species	Echinoidea
Genus Tuleariocaris	4 species	4 species	Echinoidea
Genus Unguicaris	4 species	4 species	Crinoidea
Genus Zenopontonia	3 species	3 species	Holothuroidea (1), Asteroidea (3)
Infraorder Stenopodidea	3 families	2 families	
Family Spongicolidae	7 genera	1 genus	
Genus Microprosthema	16 species	1 species	Crinoidea
Family Stenopodidae	4 genera	2 genera	
Genus Odontozona	23 species	1 species	Crinoidea
Genus Stenopus	11 species	1 species	Echinoidea

As it is fairly common when compiling a scientific review, it is important to mention that all the studies combined in **Table 1** and **S1** do not usually integrate the same research effort/ methodologies, some studies being specifically focused on symbiotic associations (*e.g.* Gherardi 1991; De Bruyn et al. 2010; Brasseur et al. 2018) while others only provide a general inventory of crustaceans in a specific location (*e.g.* Castro 1971b; Bruce 1982, 1984). In many cases, the scientific literature does not specify the nature of the symbiosis (*i.e.* commensalism, parasitism, mutualism) developed by the decapod colonized echinoderms. On the 221 symbiotic decapods listed in **Table S1**, the relationships of 108 species were not assigned to a specific

symbiotic category (**Table S1**). For 48 species (referred to as "accurate" in **Table S1 and Figure 2**), the relationship has been described in at least two studies, but the authors chose to refer to the decapods as symbionts without specific classification. 35 species (referred to as "suggested as" in **Table S1** and **Figure 2**), are suggested as "symbionts" of echinoderms because they were either rarely collected with echinoderm, or most frequently associated with other, non-echinoderm hosts, or even found mostly free-living. Twenty-five species of decapods were described as symbionts of echinoderms, but only one or a maximum of two papers describe the association (referred as "poorly studied" in **Table S1** and **Figure 2**). Finally, 113 species were assigned to a symbiotic category, with 86 species described as commensals, 16 as parasites, 1 as mutualist and 9 as "straddling between parasitism and commensalism" (**Figure 2)**.

In a parasitic relationship, a specific decapod species will negatively impact its host, and therefore may leave obvious negative evidence of its effect, tissue damage, altered behavior, or compromised fitness (Jangoux 1987). For example, crabs *Hapalonotus pinnotheroides* live in membranous cysts within the respiratory tree or close to the host cloacum (VandenSpiegel et al. 1992; Chia et al. 1999) or *Zebrida adamsii* feeds on external tissue of echinoids (appendages, skin and peripheral dermis), producing naked test areas (Suzuki and Takeda 1974; Saravanan et al. 2015). The only species classified as mutualist is *Harrovia albolineata*, as it was suggested that these crabs may use long and relatively powerful chelipeds to defend the crinoid host against potential predators (Castro 2015). The last category, commensalism, can be the most challenging to determine as it involves a relationship without any apparent harm or benefit for the host and without any morphological or behavioral impact on it. Therefore, researchers must rely on careful observations and data analysis, as performed by Gherardi (1991) on the commensal *A. indicus* found on the sea urchin *E. mathaei*, to suggest this category. However, apart from a few exceptions (*e.g. A. indicus*, *D. primitivus*, *E. pentagonus*, etc.), literature often provides a limited description of the association, without precise eco-ethological data. Consequently, the relationships are often suggested to be commensalism without a real understanding of the entire life cycle and integrative cost/benefit studies (and then no analysis of the impact of the association on host fitness) have rarely been conducted. It is therefore important to consider these data with the appropriate and critical scientific mind.

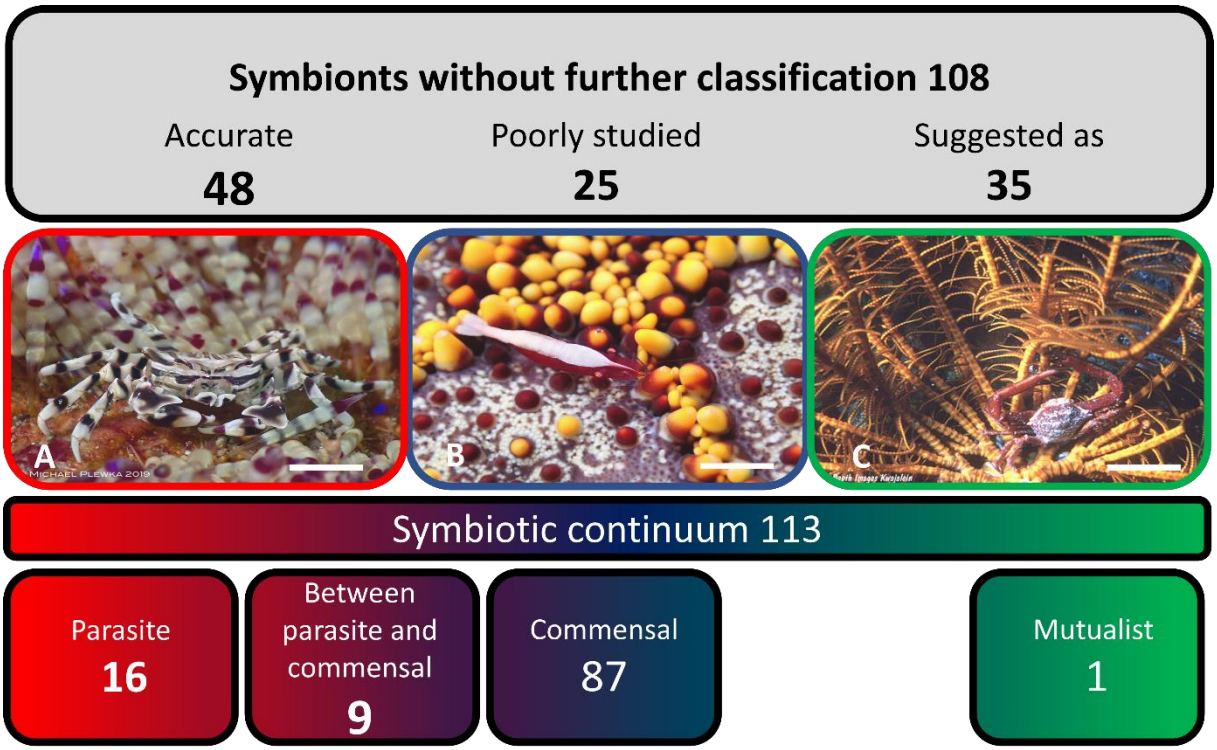


Figure 2: Illustration of the symbiotic categories mentioned in **Table S1**, with an example of association for each category. (A) Parasitic *Zebrida adamsii* on its host *Asthenosoma varium* (from @Michael Plewka 201 (B) Commensal *Zenopontonia soror* on its host *Culcita novaeguineae*, and (C) mutualist *Harrovia albolineata* on its crinoid host (from @in-Depth Images Kwajalein 2018). Without further classification = symbionts that were not classified as parasite, commensal or mutualist. Accurate = investigated in more than 2 studies; Poorly studied = investigated in less than 2 studies; Suggested as = only suggested as symbiont because of their rare occurrence, their collection with dense deep echinoderms populations or because they are usually associated with non-echinoderms hosts. Scale bars = 0.5cm in A; 0.7cm in B and 1cm in C.

To date, more than 7,000 echinoderm species have been described (DeSalle 2021) and at least 228 species were listed as decapod-associated (3.3%). On the other hand, nearly 1.3% of the 17,500 decapod species (n = 221 species) have been described in association with echinoderms (**Tables 1** and **Supplementary Table 1**). In echinoderms, crinoids and echinoids are the most frequent classes reported to be involved in decapod associations, with 87 and 80 decapod species found on these classes, respectively **(Figure 3)**. Holothuroids are the third echinoderms class that host the most species of decapods, with 33 species **(Figure 3)**. Asteroids and ophiuroids harbor fewer decapod species, with only 7 and 3 respectively **(Figure 3)**.

Paradoxically, these differences cannot be explained by the species diversity that is encountered in each of these classes (DeSalle 2021). The brittle stars and the sea stars are among the most represented classes in terms of number of species, with some 2,100 and almost 1,900 species, respectively. After, the most diversified is the sea cucumbers with 1,700 species followed by the sea urchins and their 850 species (Miller et al. 2017; DeSalle 2021). The crinoids represent the smallest class of echinoderms with fewer than 700 species. The decreasing symbiotic

prevalence for each class (*i.e.* hosts species/ total species for each class), is 9.8% (crinoids), 9.3% (sea urchins), 2.2% (sea cucumbers), 1.7% (sea stars) and 0.3% (brittle stars). These differences between the echinoderm classes may be better explained by a combination of various criteria such as body dimensions and complexity; the body wall texture their efficiency to deter predators, and/or the food availability for their symbionts (Baeza 2007; Caulier et al. 2012; Kasumyan et al. 2020). Indeed, echinoids and crinoids, the classes displaying the highest decapod colonization, also display the most complex external body shape. Echinoids usually have spines, some of which are venomous, along with pedicellariae while crinoids have numerous arms with pinnules and cirri (Ruppert et al. 2004). These classes harbor therefore ideal hiding spots for ectocommensal fauna seeking refuge from potential threats. Interestingly, the majority of holothuroids, while hosting a significant number of decapod species, usually display a smooth exterior devoid of external anatomical structures for symbionts (Ruppert et al. 2004). A few species, however, exhibit more complex external anatomy (*e.g. Thelenota ananas)* and can be covered in large, soft, pointed and sometimes branched papillae (Pinn et al. 2014). Numerous symbionts can enter in the holothuroid cloacal or buccal cavities to seek refuge (Hultgren et al. 2022). Holothuroids are also known to produce noxious saponins that deter many predators (Van Dyck et al. 2009; Caulier et al. 2013b; Kamyab et al. 2020). Some asteroids can also display a complex body wall with protuberances and pedicellariae, possessing however significatively less anatomical structures for symbionts to hide from predators than previous classes (Ruppert et al. 2004). As holothuroids, they produce efficient chemical defenses to deter predators (Demeyer et al. 2014; Kamyab et al. 2020; Stonik et al. 2020). Ophiuroids, on the other hand, are the least colonized echinoderms by decapods. These creatures usually show a small and simple body surface/volume, relying on mostly autotomy or fleeing mechanisms for defense against predators (Ruppert et al. 2004), being probably less advantageous as a host species. An extensive section discussing the advantages offered by all five classes of echinoderm hosts to their decapod associates can be found below (see section VIII).

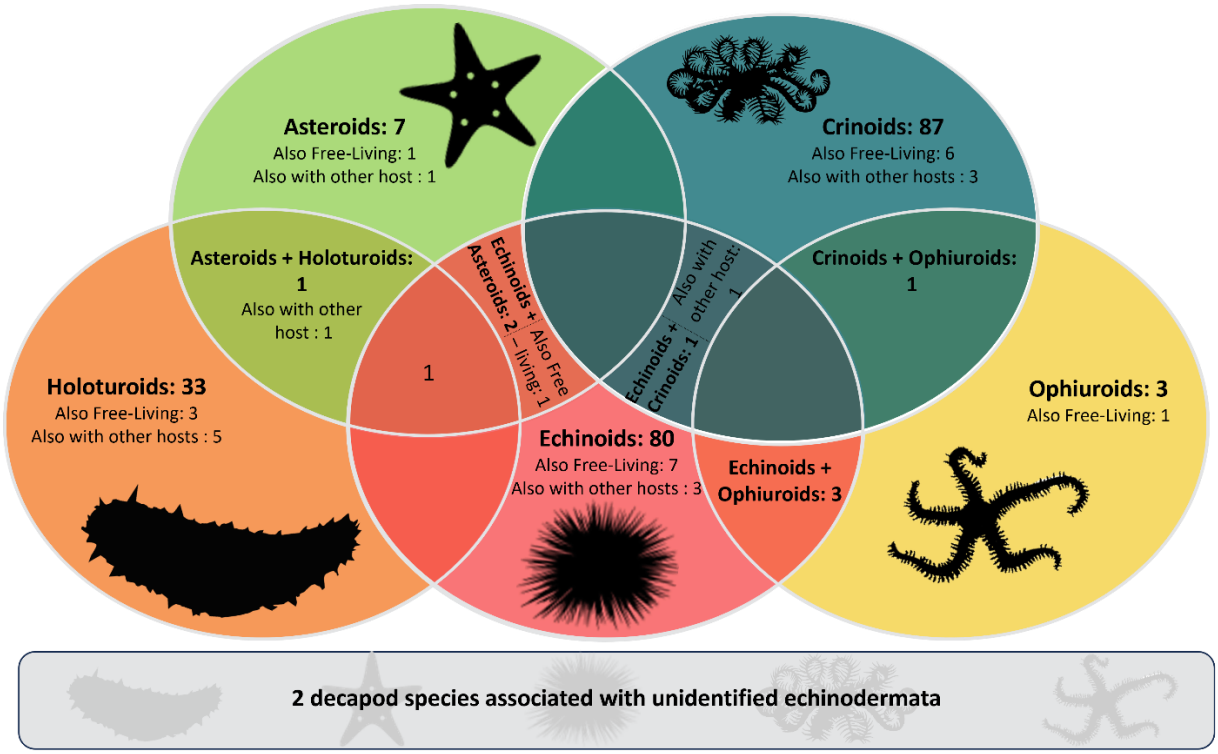


Figure 3: Venn diagram showing the number of decapod species associated with the 5 different echinoderms classes. green = sea stars; blue = feather stars; orange = sea cucumbers; red = sea urchins and yellow = brittle stars. Overlapping areas indicate decapods associated with multiple echinoderm classes. Total decapod species associated with each class of echinoderms is indicated including the number of decapods also reported as free-living or associated with non-echinoderm hosts.

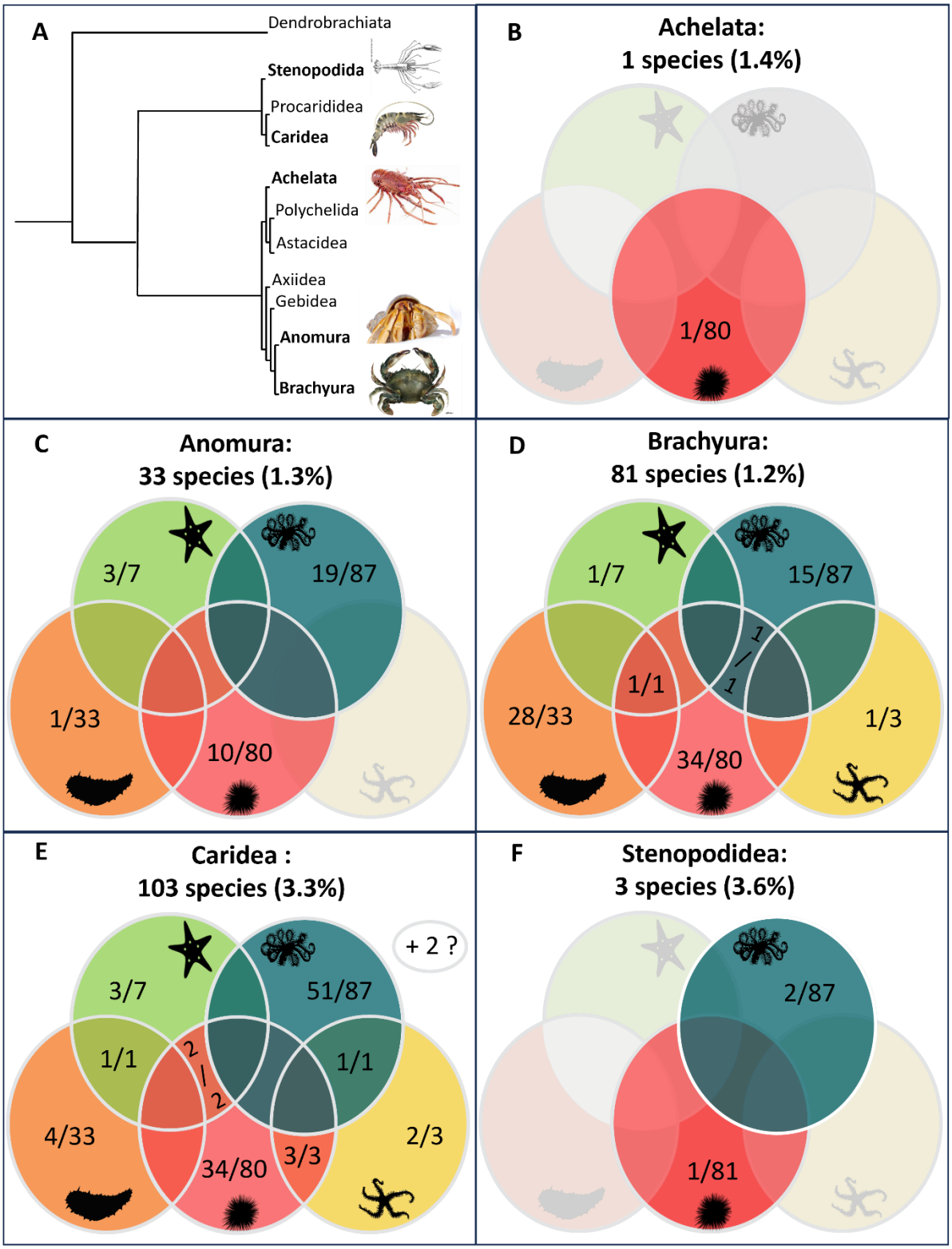


Figure 4: (A) Cladogram of decapods simplified from (Wolfe et al. 2019), with the five infraorders including echinoderms associated highlighted in **bold**. (B-F) Venn diagrams of the number of decapod species associated with the 5 different echinoderms class. (B) Infraorder Achelata, (C) Infraorder Anomura, (D) Infraorder Brachyura, (E) Infraorder Caridea, (F) Infraorder Stenopodidea. Number represented in each (inter-)circle corresponds to the fraction of the number of symbiotic decapods of a specific infra-order associated to a specific echinoderm class on the total number of decapods associated with the corresponding echinoderms class. Colored disks: green = sea star; blue = crinoids; orange = sea cucumber; red = sea urchins and yellow = ophiuroids. Overlapping areas indicate decapods associated with multiple echinoderm classes.

In total, 221 species of decapods are associated with echinoderms, spanning across 93 genera, 24 families and 5 infraorders. These 5 infraorders are Achelata (*i.e.* spiny lobsters; 72 extant species; Bracken-Grissom et al. 2014), Anomura (*i.e.* squat lobsters, porcelain crabs and hermit crabs; 2,500 extant species; Bracken-Grissom et al. 2013), Brachyura (*i.e.* crabs; 6,800 extant species; Ng et al. 2008), Caridea (*i.e.* shrimps; 3,100 extant species; Martin et al. 2016) and Stenopodidea (*i.e.* coral shrimps; 83 extant species; Chen et al. 2016) (**Table 1, Figure 4).** On the five infraorders, species within Caridea are the most frequently reported symbionts of echinoderms (n = 103; 3.3% of infraorder species, followed by Brachyura (n = 81; 1.2%), and Anomura (n = 33; 1.3%) (**Figure 4**). In contrast, Achelata and Stenopodidea show lower numbers, each accounting for 1 (1.4%) and 3 species (3.6%) associated with echinoderms, respectively (**Figure 4**). The variation in the number of decapods adopting a symbiotic lifestyle with echinoderms can be partly attributed to the inherent diversity within each infraorder. In addition, it is well-documented that Anomura, Brachyura and Caridea gather numerous symbiotic representatives (Horká 2017; de Gier and Becker 2020). While we focus only on echinoderm hosts, decapod symbionts are also found hosted by cnidaria and mollusca among other marine or freshwater organisms (Zitzler and Cai 2006; Chow et al. 2021; Lu et al. 2022a). And, conversely, Decapoda are not the only organisms capable of colonizing echinoderms.

The single species of the infraorder Achelata is associated with sea urchins, accounting for 1.25% of the symbiont diversity within Echinoidea. Among the 33 echinoderm-associated species of Anomura, 19 are found on crinoids (57.6% of total Anomura), 10 on sea urchins (30.3%), 3 on sea stars (9.1%), and 1 on holothuroids (3.0%) **(Figure 4)**. These percentages respectively represent 21.8%, 12.5%, 42.8%, and 3% of the symbiont diversity for these four echinoderm classes.

The 81 echinoderm-associated brachyuran species are found associated with the five echinoderm classes. Specifically, 34 species are found on sea urchins (42.0% of total Brachyura), 28 on holothuroids (34.6%), 15 on crinoids (18.5%), 1 on sea stars (1.2%), and 1 on ophiuroids (1.2%). Additionally, two species are found on more than one echinoderm class: (i) *Gonatonotus nasutus* is associated with three classes, primarily with echinoids and occasionally with holothuroids and asteroids (Chia et al. 1999; Castro 2015; Endo and Naruse 2016; Limviriyakul et al. 2016; Ng and Lin 2021); (ii) *Stenorhynchus seticornis* has been described in various symbiotic associations, including echinoids and a possible association with crinoids (Hayes et al. 1998b; Thiel et al. 2003a; Hayes 2007; Saravanan et al. 2015; Ghory et

al. 2018), thus appearing at the boundary between crinoids and echinoids **(Figure 4)**. Brachyura represents 42.5% of the symbiont diversity associated with sea urchins, 75.8% associated with holothuroids, 17.2% associated with crinoids, 14.3% associated with sea stars, and 33.3% associated with ophiuroids.

Caridea comprises 103 species of echinoderms-associated decapods, with 49.5% (51 species) associated with crinoids, 33% (34 species) with sea urchins, 3.8% (4 species) with holothuroids, 2.9% (3 species) with sea stars, and 1.9% (2 species) with ophiuroids. Additionally, there are 3 species described in ambiguous and questionable associations with both sea urchins and ophiuroids [*Athanas ornithorhynchus* (Fabricius and Dale 1993; Marin et al. 2005; Deheyn et al. 2006; Anker and Marin 2007), *Periclimenes parvus* (Gan et al. 2015), and *Sandimenes hirsutus* (Bruce 1971a; Gan et al. 2015; Horká et al. 2016; Horká 2017; Chow et al. 2021; Tseng et al. 2022)], 2 species in association with both sea stars and sea urchins [*Gnathophyllum americanum* (Bruce 1976b; Takeda et al. 1976; Titgen 1989; Araújo 2002; Gan et al. 2015; Tavares et al. 2017; Neves 2020) and *Phyllognathia ceratophthalma* (Gan et al. 2015)], one species in association with both sea stars and holothuroids [*Zenopontonia rex* (Fransen and Hoeksema 2014; Horká 2017; Hamel et al. 2022)], as well as one in association with both crinoids and ophiuroids [*Athanas ornithorhynchus* (Fabricius and Dale 1993; Marin et al. 2005; Deheyn et al. 2006; Anker and Marin 2007)]. In terms of symbiontic diversity per echinoderm classes, Caridea represents 58.6% of decapods associated with crinoids, 42.5% of symbionts associated with sea urchins, 12.1% of those associated with holothuroids, 42.8% of decapods associated with sea stars, and 66.6% of ophiuroid-associated decapods (**Figure 4**).
The last infraorder, the Stenopodidae, are represented by 3 species associated with 2 echinoderm classes. Two of these species are linked with crinoids (66.6% of total Stenopodidea), and one is associated with sea urchins (33.3%). They account for 2.3% and 1.2% of symbiont diversity with crinoids and sea urchins, respectively.

As a result, crinoids primarily host carideans (58.6%), sea urchins are predominantly inhabited by a combination of brachyurans and carideaans (42.5% each), holothuroids are predominantly associated with brachyurans (84%), sea stars are mostly linked with a combination of anomurans and carideans (42.8% each), and ophiuroids are primarily colonized by carideans (66.6%).

IV. Symbiotic specificity

Host specificity is another fundamental concept used in the study of symbiotic relationships. It refers to the range and diversity of host species that a symbiont is able to associate with and it quantifies the selectivity of a symbiont in choosing its host (Becker and Türkay 2017; Wells and Clark 2019). High host specificity usually indicates a strong dependence on a particular host, while low host specificity suggests a more generalist relationship across various host species (Scott 1973; Van As and Van As 2019). Therefore, host specificity is not an all-or-nothing concept; rather, it exists along a continuous spectrum. All 221 symbiotic decapods are listed with their potential hosts in **table S1**, which may assess their host specificity. To avoid inaccurate classification, we chose to provide examples for each degree of host specificity, ranging from high host-specificity to low host-specificity (*i.e.* high opportunism) (**Figure 5**). We focus here below on extensively studied species where host specificity was reliably estimated.

Species-Specific. These symbionts are highly specialized and found exclusively with one species of echinoderm. Here are some examples of decapods listed as associated with only one species of echinoderm. *Arete acanthocarpus* is only found with the sea urchin *Echinometra mathaei* (Miya and Miyake 1968; Suzuki 1970; Ayyagari and Kondamudi 2014; Ghory and Kazmi 2021). The genus *Laomenes* includes 10 species associated with only crinoids and several with only one host species [*e.g. Laomenes clarki* is associated with *Zygometra punctata* (Marin et al. 2012; Gan et al. 2015; Virgili et al. 2020), *L. cornutus* is only found on *Comaster schlegelii* (Potts 1915; Huang et al. 2005a; Deheyn et al. 2006; Marin and Chan 2006; Marin et al. 2012) and *L. pardus* is hosted by *Comanthus briareus* (Mekhova and Britayev 2013; Kou et al. 2015; Virgili et al. 2020)]. In ophiuroids, *Periclimenes perryae* is only found on *Astrophyton muricatum* (**Figure 5A;** Chace 1969, 1972; Criales 1984; Dobson et al. 2014; Horká et al. 2016). Considering species-specific symbiotic decapods associated with holothuroids, the genus Buergeres comprises four examples. *Buergeres choprai* is only found on *Actinopyga echinites* (Ahyong and Ng 2020; de Gier and Becker 2020), *B. deccanensis* lives with *Holothuria scabra* (Castro 2015; Hamel et al. 2019, 2022; Hultgren et al. 2022), *B. holothuriae* is associated with *Stichopus horrens* (Semper 1880; Ng and Manning 2003; Castro 2015; de Gier and Becker 2020) and *B. ortmanni* is a symbiont of *Holothuria fuscocinerea* (Ng and Manning 2003; Ahyong and Ng 2007, 2020; Castro 2015; de Gier and Becker 2020). There is also the Holotheres genus presenting 4 out of 5 species that are highly specific to holothuroids species (Ahyong 2010; Hamel et al. 2019; de Gier and Becker 2020).

Genus-Specific. These symbionts only associate with a few or all members of a particular host genus. For example, *Crinotonia anastasiae* is considered as a specialized decapod found only on the crinoids *Phanogenia gracilis* and *P. multibrachiata* (Marin 2006, 2008, 2009; Marin and Chan 2006; Gan et al. 2015; Virgili et al. 2020; Chow et al. 2021); *Clypeasterophilus ususfructus* is associated with the echinoids *Clypeaster europacificus* and *C. speciosus* (Griffith 1987; Campos and Griffith 1990; Campos et al. 1992, 2009; de Gier and Becker 2020); *Holotheres semperi* is found on both *Holothuria fusocinerea* and *H. scabra* (Lanchester 1900; Ng and Manning 2003; Ahyong and Ng 2007; Castro 2015; Hamel et al. 2019, 2022; de Gier and Becker 2020); *Holothuriophilus trapeziformis* is listed with *Holothuria inornate*, *H. kefersteinii* and *H. lubrica* (**Figure 5B**; Ahyong and Ng 2007; Campos et al. 2012; Castro 2015; de Gier and Becker 2020; Cortés-Carrasco et al. 2022).

Family-Specific. These symbionts are associated with different host species within the same family. For example, several species belonging to the snapping shrimp genus *Synalpheus* can be classified in this category as *S. carinatus* that is associated with the crinoids *Comanthus parvicirrus*, *Comaster schlegelii*, *Comatula purpurea*, *Phanogenia gracilis* and *P. multibrachiata*, all part of the family Comatulidae (Potts 1915; Sastry 1981; Bruce 1982; Zmarzly 1984; Banner and Banner 1985; Fabricius and Dale 1993; Prakash and Marimuthu 2020; Virgili et al. 2020). Another example is *Gnathophylloides mineri* found on the echinoids of the Toxopneustidae family, including *Lytechinus variegatus*, *Pseudoboletia indiana*, *Tripneustes depressus*, *T. gratilla* and *T. ventricosus* (**Figure 5B**; Patton et al. 1985; Okuno and Tanaka 2001; Thiel et al. 2003; Williamson et al. 2012; De Grave et al. 2021; Salas-Moya et al. 2021).

Class-Specific. These decapods are associated with different families of a particular echinoderm class. A good example to illustrate this category is the sea star shrimp *Zenopontonia soror*, known to be associated with a large spectrum of species, all belonging to the Asteroidea. It can be found on 27 species included on 8 different families: *Acanthasteridae*, *Asteriidae*, *Asteropseidae*, *Echinasteridae*, *Mithrodiidae*, *Ophidiasteridae*, *Oreasteridae* and *Pterasteridae* (**Figure 5D**; Horká 2017; Chow et al. 2021; De Grave et al. 2021; Salas-Moya et al. 2021; Tseng et al. 2022).

Phylum-Specific. These symbionts are primarily associated with several host organisms, belonging to different classes of echinoderms. However, none of the symbiotic decapods of the 221 listed can be classified as echinoderm-specific (intensively studied, associated with several classes of echinoderms and without other hosts that do not belong to echinoderms).

Inter-phyla generalists. Some symbionts are found on a large variety of hosts, including echinoderms, but also other marine organisms as cnidarians or molluscans. In this category, we can cite *Opisthopus transversus* (Rathbun 1894; Jones and Mahadevan 1965; Jangoux 1987; Ng and Manning 2003; Castro 2015; Ahyong 2018; de Gier and Becker 2020), *Stenorhynchus seticornis* (Hayes et al. 1998a, a; Thiel et al. 2003b; Hayes 2007; Saravanan et al. 2015; Ghory et al. 2018) and *Zenopontonia rex* (**Figure 5E**; Fransen and Goud 1999; Antokhina and Britayev 2012; Antokhina et al. 2012; Gan et al. 2015; Horká et al. 2016; Chow et al. 2021; Hamel et al. 2022)

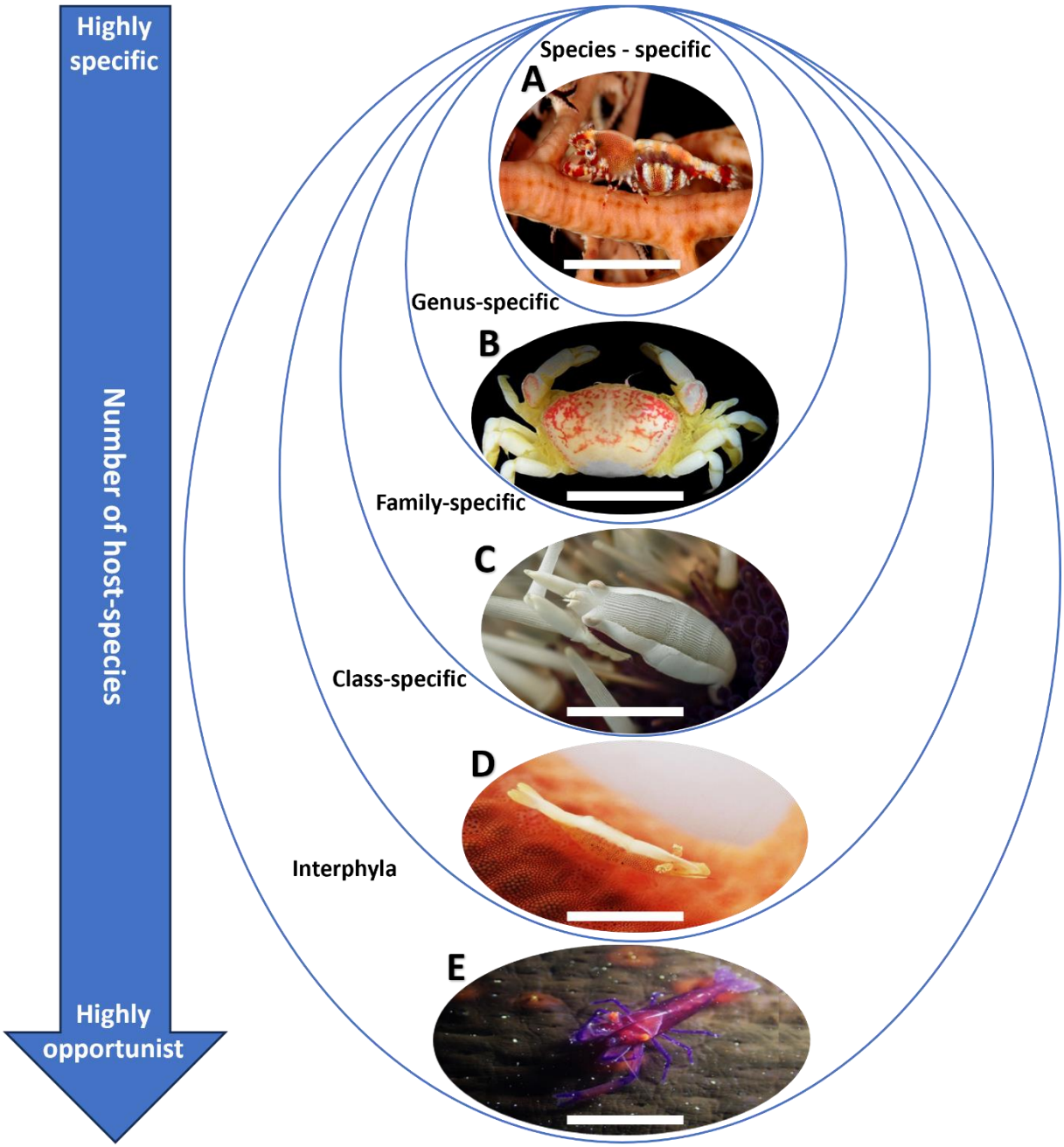


Figure 5: Representation of the host specificity level, with an example for every category. (A) the species-specific *Periclimenes perryae* on the giant basket star *Astrophyton muricatum* (from @flickr 2012) (B) the genus-specific *Holothuriophilus trapeziformis* found with several species of the genus *Holothuria* (from Cortés-Carrasco et al. 2022) (C) the family-specific *Gnathophylloides mineri* on an unidentified sea-urchins host (from @Carl-Johan Nilsson Romblon) (D) the class-specific asteroid shrimp *Zenopontonia soror* on a sea star *Linckia multiflora*. (E) the highly opportunist *Zenopontonia rex* on a holothuroid *Bohadschia argus*. Scale bars = 0.8cm in A and D; 1cm in B and C; 1.3cm in E.

The degree of host specificity has significant ecological implications. Highly host-specific decapods may have evolved specialized adaptations to live exclusively with their associated host, whereas less specific ones may have more general adaptations that allow them to switch hosts more readily (Becker and Türkay 2017; Wells and Clark 2019). However, by focusing solely on the degree of specificity, researchers might miss valuable ecological insights. Decapods in class-specific associations may have evolved unique mechanisms to coexist with their echinoderm hosts. For example, *Lissocarcinus orbicularis* can be found on various species of holothuroids and therefore are class-specific (Ayotte and DeMaintenon 2005; Pinn et al. 2014; Caulier et al. 2014). However, they are obligate symbionts (*i.e.* they cannot be found free living) that display several morphological and behavioral adaptations (see below for more information). It is therefore important to make the distinction between host specificity and other fundamental concepts, such as specialization and obligatory aspects. Another bias, especially in the least studied organisms, is the accuracy of available data in literature. Various symbionts associated with echinoderms appear as highly specific, sometimes pairing with only one host species, but that may not reflect ecological reality and may be limited by the overall study effort. For example, more extensive studies should be performed on *Synalpheus tijou* (Banner and Banner 1982, 1985), *S. townsendi* (Criales 1984), *Hippolyte catagrapha* (Hempson and Griffiths 2008) or *Margitonia insolitus* (Bruce 1975a; Horká 2017) to properly assess their specificity. The same can be noted about species that appear less specific than they may really be. For example, *Arete indicus* are highly specific with *Echinometra mathaei*, but there are two questionable reports of this species reporting it to be associated with a *Diadema* sp. and *Heliocidaris crassispina* (see **Table S1**). This decapod, which seems to be a genuine species-specific symbiont, may be misidentified as class-specific instead. For some species, geographic factors, environmental conditions, and genetic diversity among the hosts can all contribute to local specificity. *Tuleariocaris holthuisi* can be used to illustrate this statement; it is a specific species to the echinoids class, being primarily found on *E. mathaei,* but it can also associate with *Astropyga radiata, A. pulvinata, Diadema mexicanum, Echinothrix diadema* and *Stomopneustes variolaris*, as it has been shown that they only associate with several of these secondary host species during specific time of the year (see **Table S1**). The specificity of this species would therefore depend on the local availability of the host and the period of the recording.

V. Biogeography of decapods associated with echinoderms

To investigate the geographical distribution of decapod-echinoderm symbiotic associations, we gathered data from the World Register of Marine Species (WoRMS Editorial Board 2023; Documented distribution section). Among the 221 species of symbionts listed in **Table S1**, we excluded four species: one fossil species and three unidentified ones (identified to the genus, but not to the species). Out of the remaining 217 species, 54 lacked geographical data in the WoRMS database (**Table S2**). **Figure 6A** displays the number of symbiotic associations between echinoderms and arthropods in a climatic zone or between different climatic zones, defined by latitudes limits. An alternative analysis is proposed in **Figure 6B** used the Food and Agriculture Organization (FAO) map of the major fishing areas of the world (FAO 2023). This map, based on ecological, oceanographic, and geopolitical factors, categorizes global oceans into distinct fishing areas with specific numerical designations (FAO 2023). It is widely used for statistical analyses of marine species abundance, particularly commercially targeted ones. We adopted this representation to assess the worldwide distribution of decapod species associated with echinoderms (**Figure 6B**). For these two maps, symbioses numbers are not cumulative as some associations have been reported in different areas in the world.

Among the 163 species of decapods associated with echinoderms with geographical information (**Table S2**), distinct ecological patterns emerged, notably within the main climatic zones (**Figure 6A**). The tropical region, limited to the area between the Tropics of Cancer and Capricorn, is the area where the most associations between decapods and echinoderms were reported, with 147 species of echinoderm-associated decapods found at least within this area (**Figure 6A**). Within the Tropical Zone exclusively, 77 species of decapods were documented (**Figure 6A**). Subtropical regions also hosted numerous species, with 82 decapods species recorded (**Figure 6A**), including 9 in North-Subtropical and 2 in South-Subtropical regions exclusively (**Figure 6A**). Temperate zones accounted for 14 species, including 4 species exclusively inhabiting these areas (3 in North-temperate and 1 in South-temperate zones) (**Figure 6A**). Notably, no decapod-echinoderm symbiotic associations were reported in the Polar zone (**Figure 6A**). Consistently, no species were reported in areas corresponding to the Arctic (18), Atlantic Antarctic (48), Southern Indian Ocean (58), or Pacific Antarctic Sea (88). Additionally, no species were recorded in the Northeast Pacific Ocean (67) Six areas hosted less than 10 species each (**Figure 6B**, Blue). In ascending order, these are the Southeast Pacific

(87), with only one species, followed by the Northwest and Northeast Atlantic (21 and 27), each containing two species of echinoderm-associated decapods, then the Mediterranean and Black Sea region (37) that hosted 4 species, the Eastern Central Pacific (77) included 7 species, and finally the Southern Atlantic (47) had 8 species reported. Four areas contained between eleven and twenty species each (**Figure 6B**, Green). These are the Eastern Central Atlantic (34), the Southwest Atlantic (41), the Northwest Pacific (61), and the Southwest Pacific (81), housing 11, 12, 17, and 19 species, respectively. The Western Central Atlantic (31), Western Indian Ocean (51), and Western Indian Ocean (57) were found to host a higher number of species, with 40, 50, and 59 decapods listed, respectively (**Figure 6B**, Orange). The area with the highest number of listed species was the Western Central Pacific (71), boasting 92 species (**Figure 6B**, Red).

This geographical analysis underscores the significance of climatic zones and specific oceanic regions in influencing the distribution of decapod-echinoderm symbiotic associations, shedding light on the ecological patterns of these intriguing relationships (**Figure 6**). One of the most prominent findings is the dominance of tropical regions in hosting decapod-echinoderm symbiotic associations. This phenomenon resonates with the well-established patterns of heightened biodiversity and productivity typically observed in tropical marine ecosystems (Lœuff and von Cosel 1998; Cheung et al. 2009; Bowen et al. 2013). The warm, nutrient-rich waters of these regions likely create highly favorable conditions for biodiversity, including both echinoderms and decapods (Fabricius and Dale 1993; Cannicci et al. 2008). Therefore, that may contribute significantly to the abundance of these kinds of symbiotic associations. In opposition, temperate zones show fewer decapod-echinoderm associations. This can be attributed to the lower species diversity and less stable conditions in these areas compared to the tropics (Lœuff and von Cosel 1998; Cheung et al. 2009; Bowen et al. 2013). Additionally, the absence of these associations in polar regions is expected due to harsh conditions. The Western Central Pacific area, that strongly stands out when compared to others, may be partially explained by the presence of two biodiversity hotspots: Coral sea (Australia) and French Polynesia Islands (Poupin 1998; Randall et al. 1998; Bowen et al. 2013).

However, it is essential to consider the potential influence of research intensity in these geographical distributions (Gray 2001). We hypothesize that areas subjected to more extensive research efforts yield higher counts of the documented decapod-echinoderm associations. It is crucial to acknowledge that limited research has been conducted in polar regions and deep sea areas, especially in comparison to tropical regions, which may contribute to the dearth of

reported associations (Gray 2001). Due to this bias, the range distribution of symbiotic associations has not been considered in this analysis.

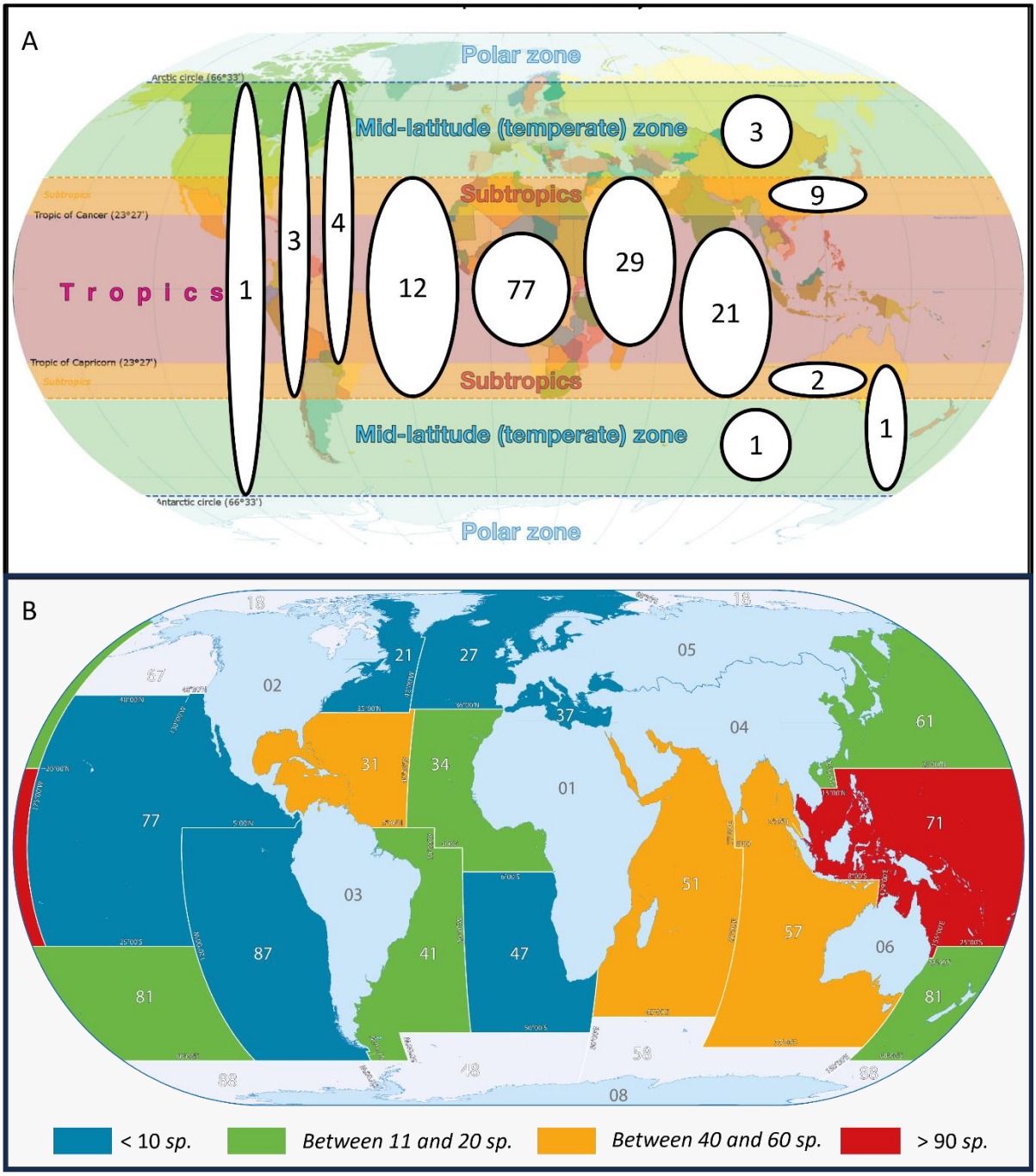


Figure 6: Decapods-echinoderms symbioses world distribution. (A) Correspondence between the number of symbioses reported in main climatic zones, each white oval corresponding to the number of associations reported in each corresponding (trans)zone. (B) Number of associations reported in each marine region, as defined by the Food and Agriculture Organization (FAO): Arctic Sea (18); Northwest Atlantic (21); Northeast Atlantic (27), Western-Central Atlantic (31) Eastern Central Atlantic (34), Mediterranean And Black Sea (37), Southwest Atlantic (41), Southeast Atlantic (47), Atlantic Antarctic (48), Western Indian Ocean (51), Eastern Indian Ocean (57), Antarctic and Southern Indian Ocean (58), Northwest Pacific (61), Northeast Pacific (67), Western Central Pacific (71), Eastern Central Pacific (77), Southwest Pacific (81), Southeast Pacific (87) and Pacific Antarctic (88). Data in (A) and (B) were listed from the World Register of Marine Species (WORMS; Documented distribution section).

VI. Decapod-echinoderm symbiosis at a population level

At the individual level, a host may represent a micro-habitat (sometimes even considered as a micro-ecosystem) where a multitude of organisms coexist and interact with each other (Baeza and Thiel 2003). Within this context, multiple decapods may inhabit a single echinoderm individual and engage in interactions with various other symbiotic organisms, either from the same species (*i.e.* population) or different species (*i.e.* community) leading to potential competition for resources. Although limited research has been conducted on this topic, multiple micro-populations of symbiotic organisms coexist on echinoderm hosts. The most studied have been those on crinoids; there have been nine studies focused on crinoid associated fauna (**Table 2**).

Table 2: Studies focusing on crinoids and their complete symbiotic fauna

NUMBER OF SYMBIOTIC SPECIES	LOCATION	REFERENCE
18	Marshall Islands	Zmarzly 1984
5	Hong Kong, China	Morton and Mladenov 1992
46	Great Barrier Reef, Australia	Fabricius and Dale 1993
11	Taiwan	Huang et al. 2005a
70	Nhatrang Bay, Vietnam	Britayev and Mekhova 2011
14	Maldive Islands	Tchesunov 1989
47	Papua New Guinea	Deheyn et al. 2006
39	Bangka Islands, Indonesia	Virgili et al. 2020
20-25	Great Reef of Toliara, Madagascar	Terrana et al. Submitted

In most cases, the taxa that colonized crinoids most frequently are Polychaeta, Myzostomida, Gasteropoda, Pisces, Ophiuroidea and Crustacea (Zmarzly 1984; Fabricius and Dale 1993; Huang et al. 2005a; Deheyn et al. 2006; Britayev and Mekhova 2011; Virgili et al. 2020; Terrana et al. Submitted). Amongst crustaceans, shrimps, galatheans and crabs regularly colonized crinoids (Zmarzly 1984; Morton and Mladenov 1992; Fabricius and Dale 1993; Huang et al. 2005a; Deheyn et al. 2006; Britayev and Mekhova 2011; Virgili et al. 2020; Terrana et al. Submitted). In particular, *Cristimenes commensalis*, *Allogalathea elegans* and *Synalpheus stimpsonii* were extremely abundant in various locations and on various hosts (**Figure 7**). In the 9 analyses made on populations of crinoid symbionts, the number of species

(all symbionts considered) varies from 5 (Hong-Kong) to 70 (Vietnam). In the four analyses where the symbiont species where >35 (Indonesia, Papua New Guinea, Vietnam and Australia), the two most abundant taxa (in terms of individuals and species) were myzostomids and crustaceans. There was no evidence of negative interactions between symbionts. Moreover, the size of the crinoid correlated with the number of shrimp individuals (Deheyn et al. 2006; Britayev and Mekhova 2011; Virgili et al. 2020). The host-specificity of myzostomids was greater compared to shrimps or polychaetes, indicating intriguing patterns of specialization within these symbiotic associations (Deheyn et al. 2006; Virgili et al. 2020). Usually, decapod symbionts were found individually or by pair. Some species, as *Brucecaris tenuis*, can be found in higher numbers (30 per host) with a large proportion of them being juveniles (Fabricius and Dale 1993). Some species can co-occur with other species in a some host individual, as in the case of *B. tenuis* that often co-occurred with *C. commensalis* (Fabricius and Dale 1993). When there is co-occurrence of two or more species, they may not be found in the same area of the host, living on different micro-ecological niches offered by the crinoid morphology (**Figure 7)**. For example, *B. tenuis* and *C. commensalis* shared the distal parts of the host arms, synalpheids were most often observed at the level of the calyx, or between cirri and galatheids occurred mainly on the centro-dorsal arm (Fabricius and Dale 1993; Huang et al. 2005a; Virgili et al. 2020; Terrana et al. Submitted).

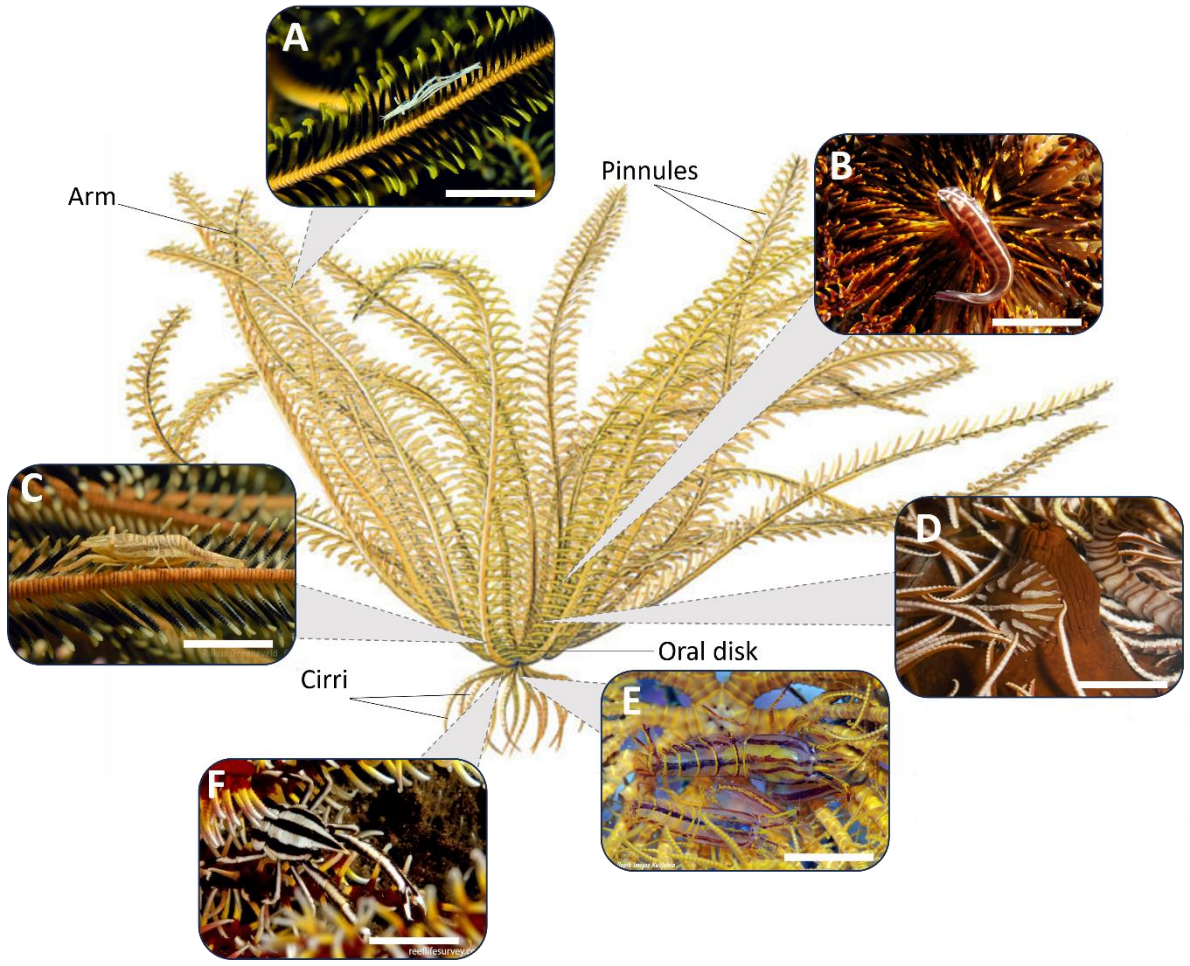


Figure 7: Schematic illustration of the localization and potential co-occurrence of symbionts on a crinoid host, based on (Fabricius and Dale 1993; Huang et al. 2005a; Virgili et al. 2020; Terrana et al. Submitted). (A) *Brucecaris tenuis* (from @Trent Burkholder 2008) (B) *Discotrema crinophila* (From Bray 2018) (C) *Cristimenes commensalis* (@Rokus Groeneveld 2008) (D) *Myzostoma insigne* (From Virgili et al. 2020) (E) *Synalpheus stimpsonii* (from @in-Depth Images Kwajalein 2018) (F) *Allogalathea elegans* (from @reeflifesurvey.com). Scale bars represent = 0.6cm in A; 1.6cm in B; 1cm in C and E; 0.5cm in D; 0.7cm in F.

VII. Decapod-echinoderm interactions: evolutionary history

The evolutionary history of symbionts and their hosts can be approached by the study of fossils which, when they exist, provide with a rough indication of the time when the symbiosis originated (Dworschak et al. 2009; Lanterbecq et al. 2009; Ahyong et al. 2011). Also, phylogenetic inference allows hypotheses on the co-phylogenesis of the two associated taxa and, therefore, potential co-speciation and host switch events (Horká et al. 2016; Wolfe et al. 2019; Chow et al. 2021). Indeed, the evolutionary pathways of symbiotic interactions between hosts and symbionts usually begins with a loosely (*i.e.* generalist) associated relationship involving diverse hosts and then progresses towards a more dependent and specialized association (Scott 1973; Van As and Van As 2019). While this straightforward orthogenetic concept may apply to certain coevolutionary systems (Thompson 1994; Palopoli et al. 2017), it

may be more complex in some evolutionary pathways where host switching plays a crucial role (Horká et al. 2016; Davis et al. 2018; Chow et al. 2021). Co-adaptations have been recognized as important drivers of speciation in co-evolutionary studies, although concrete evidence of co-speciation remains relatively rare, especially between two metazoan lineages (Eeckhaut and Lanterbecq 2005; Hoberg and Brooks 2008; Weising et al. 2010; de Vienne et al. 2013). Studies have shown that host associations are generally conserved across phylogeny, with infrequent switches occurring between distantly related hosts (Hoberg and Brooks 2008; Gómez et al. 2010). This conservation of host association seems logical since symbionts with morphological, behavioral, and physiological specializations are well suited to exploit a specific host but may face challenges when attempting to exploit new, phylogenetically distant hosts (Horká et al. 2016; Horká 2017). Despite the general trend of host conservatism, there are many cases showing that symbiotic lineages switch to new hosts, even being distantly related species (Goto et al. 2012). While host switching has been extensively studied in terrestrial ecosystems, particularly in herbivorous insects and parasites, it remains largely understudied in marine environments (Boyko and Williams 2009; Lanterbecq et al. 2010; Goto et al. 2012).

To our knowledge, there is only one fossil indicating the association between decapods and echinoderms in the past. This may seem normal if we consider the type of symbiosis characterizing today's decapods: they are mainly ectosymbionts and leave no trace on the skeleton of echinoderms. This is not the case for the myzostomids for example, which include parasites deforming the skeleton of crinoids: this symbiosis would certainly have appeared before 300 million years ago (Eeckhaut and Lanterbecq 2005; Lanterbecq et al. 2009; Bleidorn et al. 2014). Regarding the fossils of free-living decapods, terrestrial crabs have been discovered in amber 100 million years ago (Luque et al. 2021). The fossil records of Galatheoidea indicate a Jurassic origin of squat lobsters and their expansion in the late Cretaceous (Ahyong et al. 2011). Fossil records, patterns of distribution and phylogeny of Palaeomonidae suggest a minimum age for this taxon from late Jurassic to early Cretaceous (Pereira 1991). In the context of echinoderm-decapod associations, the unique fossil involves a porcellanid crab species described as *Petrolisthes myakkensis*, which was found in association with abundant and still extant asteroids, *Heliaster microbrachius* (Bishop and Portell 1989). This association dates back to the late Pliocene and was conserved in calcareous quartz sandstone within the Pinecrest Beds of the Tamiami Formation, near El Jobean in Charlotte County, Florida, USA. The crab-asteroid association is preserved with minor amounts of shell fragments and the crab hollow

specimen was preserved as a whole, articulated, fragile specimen, preserved with complex sea stars (**Figure 8**). The fossil local fauna is prominently characterized by the presence of these asteroids and crabs, although barnacles specimens are also present (Bishop and Portell 1989). The fossil specimens morphology suggests a lack of mobility capacity of the crabs, and the close proximity of these crabs to the abundant sea stars (along with the known commensal behaviors of other extant symbiotic porcellanid crab species and their associations with echinoderms) lead to the suggestion that *Petrolisthes myakkensis* and *Heliaster microbrachius* had an ectocommensal relationship (Bishop and Portell 1989).

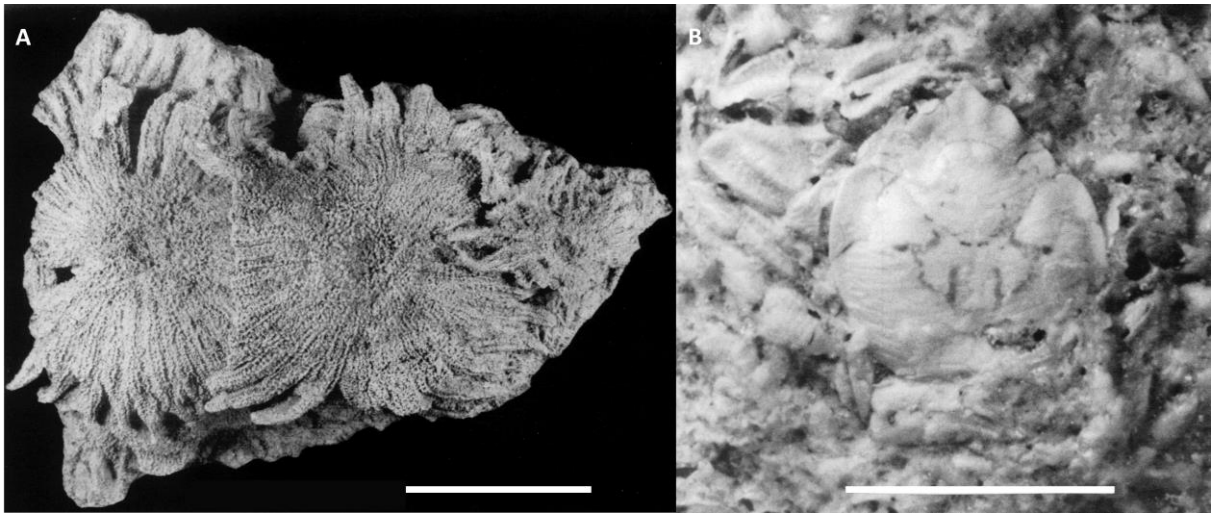


Figure 8: Crab-Sea star Fossil Association. (A) Slab of sandstone preserving over 12 individual stacked specimens of *Heliaster microbrachius*. The two most visible specimens are photographed showing the abactinal (aboral) side. Numerous other specimens in various orientations can be seen below the two major overlapping specimens. Scale = 10 cm. (B) Dorsal aspect of holotype of *Petrolisthes myakkensis*, showing left cheliped and proximal portions of two walking legs (UF 8678). Scale bars = 5 mm. Both illustrations are from Bishop and Portell (1989).

Recent studies have shed light on the divergent evolutionary pathways and host shifts of symbiotic palaemonid shrimps, specifically within the subfamily Pontoniinae (Gan et al. 2015; Kou et al. 2015; Horká et al. 2016; Chow et al. 2021). Phylogenetic reconstruction, character mapping and ancestral state analyses can be powerful tools used in evolutionary biology to understand the divergent evolutionary pathways and "host shift" events in symbiotic relationships, especially when studying host spectrum (the range of hosts a symbiont can infect or associate with) (Miles and Dunham 1993; Bleidorn et al. 2014; Chow et al. 2021). Practically, once the symbiont phylogeny is constructed, it is possible to map the known host associations onto the tree (character-mapping analyses) and by examining the tree, it is possible to identify clades (groups of closely related species) that have switched hosts during their evolutionary history (Bollback 2006; Maddison and Maddison 2011). Ancestral state reconstruction aims to infer the most likely states, here host associations, of ancestral nodes

(common ancestors) in the symbiont phylogeny. It can help to estimate, in a host switch event, the ancestral host taxa on which decapods were associate before the event, and the derivate host taxa, on which symbionts were living after the event (Maddison and Maddison 2011; Horká et al. 2016; Chow et al. 2021).

Kou et al. (2015) conducted a preliminary analysis, focusing on 26 species of Pontoniinae from 23 genera, representing a small fraction of the total diversity within the taxon. Another study by Gan et al. (2015) explored a dataset of 101 species of Palaemonidae from 42 genera of Indo-West Pacific area, employing mitochondrial genes (16S rRNA and 12S rRNA) and nuclear protein-coding genes (histone 3 and sodium-potassium ATPase a-subunit). Building upon these works, Horka et al. (2016) conducted a more extensive analysis based on a first dataset of 87 species from 43 genera in a four-marker analysis (based on sequences of two nuclear genes, 18S rRNA and histone 3, and of two mitochondrial genes, cytochrome c oxidase subunit I and 16S rRNA), and a second dataset of 107 species from 48 genera in a three-marker analysis (Histone 3, 16S rRNA and cytochrome c oxidase subunit I sequence). In a comprehensive study by Chow et al. (2021), the phylogeny of palaemonid shrimps, including both symbiotic and free-living taxa, was reconstructed using multiple mitochondrial and nuclear markers. Their analysis incorporated 166 species from 75 genera, representing approximately 50% of the symbiotic palaemonid diversity.

The above-mentioned studies revealed multiple occurrences of progression into symbiosis within the evolutionary history of palaemonid shrimps (Horká et al. 2016; Chow et al. 2021) (**Figure 9**). Specifically, they identified at least two different occurrences of host switching, leading to echinoderm association (**Figure 9,** Clade III A and Clade IIIB-H) (Chow et al. 2021). The diverse symbioses observed in Clade III B to H, which includes all echinoderm-associated species except *P. pottsi*, seems to have originated from a cnidarian associate (ancestral state) and subsequently colonized echinoderms at least seven times independently (**Figure 9**; Horká et al. 2016; Chow et al. 2021). Transition rates among host affiliations indicated directional switches from cnidarians and mollusks to echinoderms (Horká et al. 2016; Chow et al. 2021). When echinoderm associates were classified at the class level, multiple host switch events were highlighted for crinoid, echinoid, holothuroid, and ophiuroid associates, with 6, 5, 2 and 2 switches respectively (Chow et al. 2021). The analysis of Chow et al. (2021) suggests that the transition from cnidarians and mollusks to echinoderms was typically accompanied by an increase in specificity.

The evolution of the host spectrum in Palaemonidae is essentially not a single unified process, it is a convergent evolution leading to similar ways of life, to the multiple origins of symbioses along independent evolutionary pathways. Host switching is probably playing a significant role in shaping the dynamic host spectrum throughout the phylogeny of Palaemonidae, and potentially disrupts and redirects the ongoing adaptive evolution and results in an interspersed distribution of species with different host affiliations.

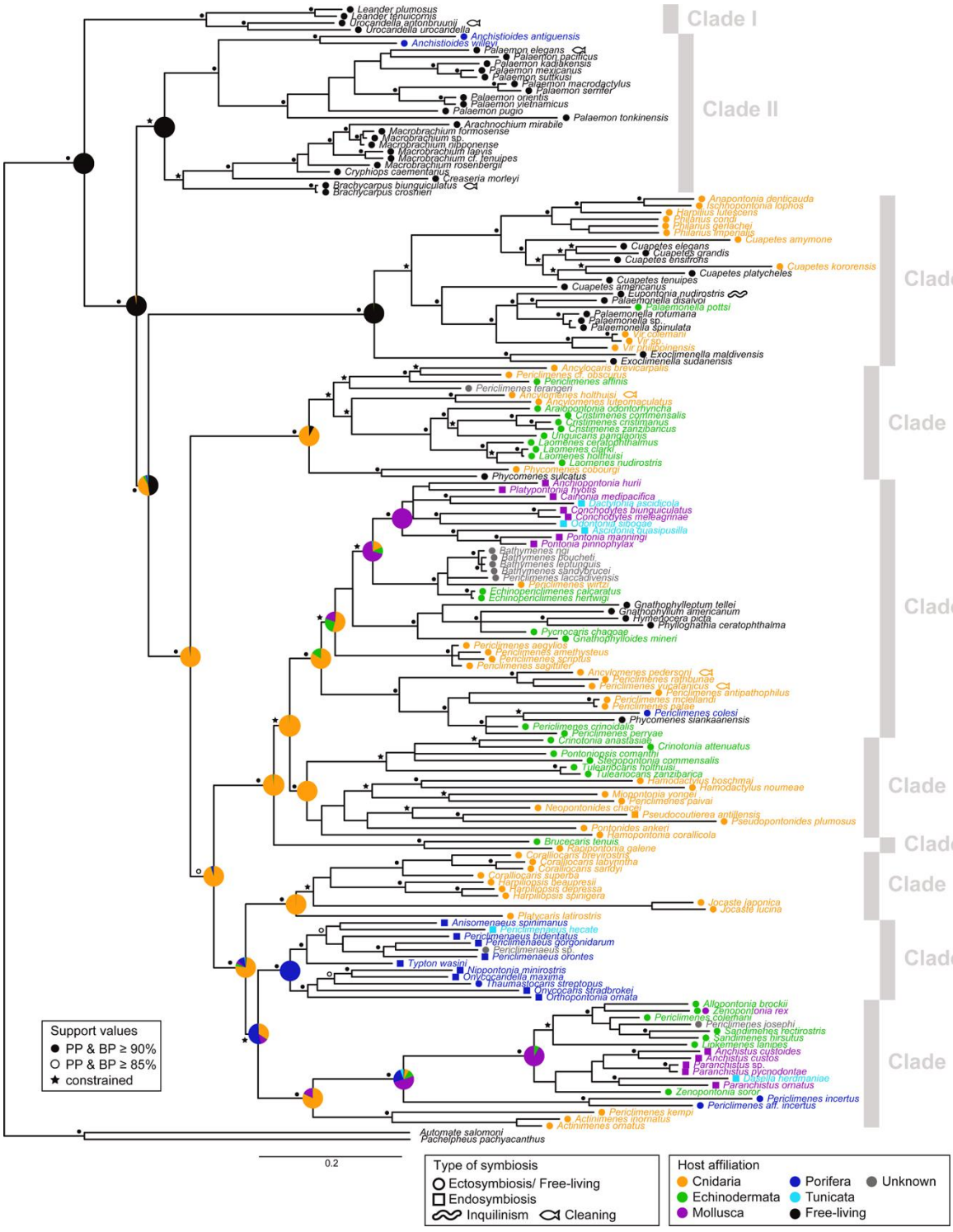


Figure 9: Final phylogenetic tree of Palaemonidae resolved by Bayesian Inference with a major host indication (from Chow et al. 2021). Support values are denoted by closed circles, open circles or stars, except for poorly resolved nodes. Major host affiliations and types of symbiosis are indicated by different colors and shapes at branch tips, respectively. Posterior probabilities of ancestral states of host affiliation are indicated in the form of pie charts.

VIII. Benefits acquired by decapods by associating with echinoderms

Like most biological concepts, symbiotic relationships are diverse and complex, involving a multitude of benefits and costs for symbionts and/or hosts (Holbrook and Schmitt 2005). The benefits acquired by the decapod symbionts associated with echinoderms take four main forms (**Table S1)**: (i) a protection of adults against predators (ii) a protection for their eggs and juveniles (iii) feeding strategies and (iv) host-secreted chemical assimilation.

(1) Protection against predators

(a) Mechanical defense

One of the most studied symbiont benefits when hosted by echinoderms is the mechanical protection from predators provided by the host (Kalinin et al. 1996a, b). Several species, belonging for example to the genera *Arete*, *Echinopericlimenes*, *Tuleariocaris*, *Dissodactylus* or *Echinoecus* seek refuge on the spines of their sea urchin hosts, which lowers their predation rate (Sastry 1981; Horká et al. 2016; Brasseur et al. 2018; Chow et al. 2021). Echinoids and asteroids also possess minute, pincer-like structures on their surface called pedicellariae (Dubois and Ameye 2001; Coppard et al. 2012). These organs consist of a "head" composed of two to five articulated "jaws" known as valves, which may be associated with venomous glands, and attached to a stalk called the pedicel, which allows the structure to move and pivot (Campbell 1983; Dubois and Ameye 2001; Coppard et al. 2012). Pedicellariae have different functions depending on the species of echinoderm, such as sensory purposes, cleaning functions, or protection against parasites and predators (Campbell 1983; Ghyoot et al. 1987; Coppard et al. 2012). The flower sea urchin (*Toxopneustes* spp.) possesses unique large globiferous pedicellariae, which contain bioactive substances that cause deleterious effects (Edo et al. 2012). When threatened, the sea urchin can use its pedicellariae to create a protective barrier, making it more difficult for predators to attack (Ghyoot et al. 1987; Coppard et al. 2012). This mechanical barrier does not only benefit the sea urchin but may also benefit symbionts present on the sea urchin by providing an additional layer of protection against predators.

Another strategy is to hide from predators by entering the internal cavity of the host. Indeed, portunid crabs, like *Lissocarcinus orbicularis*, or sea urchin crab, as *Echinoecus pentagonus*, can also benefit from the protective shelter provided by their respective holothuroids and

echinoids host (Castro 1978; Jangoux 1987; Caulier et al. 2012; Pinn et al. 2014). These crabs can seek refuge inside their hosts by entering their cloaca or mouth, where they are mechanically shielded from predators thanks to the tough and/or spiny outer body wall of their host (Bryan et al. 1997; Chia et al. 1999; Caulier et al. 2012; Meher and Thiruchitrambalam 2019).

Some species of holothuroids that exclusively belong to the Holothuriidae family possesses specialized structures called Cuvierian tubules (Hamel and Mercier 2000; Flammang et al. 2002). They are elongated, thread-like structures that can vary in both morphology and composition depending on the species (Flammang et al. 2002; Becker and Flammang 2010). They can be classified in two main types: the lobulated and smooth (Flammang et al. 2002; Becker and Flammang 2010). When sea cucumbers are threatened or disturbed, they can rapidly evert and expel the tubules through their cloaca (Flammang et al. 2002; Becker and Flammang 2010). The smooth tubules possess a unique combination of adhesiveness (*i.e.* glue) and tensile strength, allowing them to efficiently entangle and immobilize most predators (Flammang et al. 2002; Becker and Flammang 2010). This enables the sea cucumber and its symbionts to escape from danger and avoid being consumed (Caulier et al. 2013b). Being non-sticky in the interior of the cloaca (*i.e.* they only become sticky when expelled out of the anus in surrounding water), these defensive organs do not negatively impact the survival of the symbionts.

(b) Chemical defense

In addition to mechanical defenses, all five classes of echinoderms are well known to secrete chemical molecules to deter their predators (Rideout et al. 1979; Bryan et al. 1997; D'Auria et al. 2002; Caulier et al. 2011). The most deleterious of these molecules are saponins produced by holothuroids and asteroids (Bryan et al. 1997; Van Dyck et al. 2009; Caulier et al. 2011, 2013a; Kamyab et al. 2020). Their chemical structure provides them with amphiphilic properties and deleterious membranolytic effects associated with several biological activities (*i.e.* ichthyotoxic, cytotoxic, hemolytic, fungicide, etc.) (Caulier et al. 2011; Savarino et al. 2021). Holothuroids and asteroids can produce toxic saponins in their body wall or viscera, including the Cuvierian tubules (Van Dyck et al. 2009; Caulier et al. 2013a). Sea cucumber saponins are described as triterpene glycosides while sea stars saponins (*i.e.* asterosaponins) are classified as steroidic glycosides, both being composed of a steroid oligosaccharide chain made up to 6 sugar units (the polar moiety) and an aglycone composed of different aromatic cycles

(Bryan et al. 1997; Tang et al. 2005; Van Dyck et al. 2009; Netala et al. 2009; Caulier et al. 2011, 2013a; Ngoan et al. 2015; Stonik et al. 2020; Savarino et al. 2021; Ha et al. 2021).

Echinoids and crinoids use quinones (another class of organic compounds) for their chemical defense (Rideout et al. 1979; Kornprobst 2005; Kamyab et al. 2017). These compounds are effective in deterring predators, including fish and free-living crustaceans, and can also act as feeding deterrents by reducing the palatability of the echinoderms (Rideout et al. 1979; Bryan et al. 1997; Kasumyan et al. 2020). It is important to note that quinones have a variety of biological functions in addition to their defensive role, such as providing pigmentation, antioxidant properties, and antimicrobial/fungal effects (Anderson et al. 1969; Brasseur et al. 2017). Due to their nature, quinones are an important aspect of the chemical defense system of echinoderms, allowing them to protect themselves and their adapted symbionts from potential predators and harmful microorganisms (Kasumyan et al. 2020). The quinones exclusively produced by echinoids are called spinochromes or echinochromes (Anderson et al. 1969; Hou et al. 2018; Shikov et al. 2018). Their structures are characterized by a bicyclic aromatic structure consisting of a quinone or quinone-imine ring and a benzene ring (El-Najjar et al. 2011; Brasseur et al. 2017, 2018). Anthraquinones, which are specific to crinoids, are characterized by a three-ring aromatic structure composed of two benzene rings connected by a quinone ring (El-Najjar et al. 2011; Caulier et al. 2022). Both spinochromes and anthraquinones can display various functional groups such as ethyl, acetyl, methoxy or amino groups on their aromatic structures (D'Auria et al. 2002; El-Najjar et al. 2011; Shikov et al. 2018). Ophiuroids may be considered as chemically marginal in the phylum of echinoderms as they share some chemical characteristics with both crinoids and echinoids, and some with asteroids and holothuroids (Kornprobst 2005; Claereboudt et al. 2019). However, they also display numerous fatty acids and sterols, including a majority of 3α-hydroxysterols (Bryan et al. 1997; D'Auria et al. 2002; Kornprobst 2005; Claereboudt et al. 2019; Zhukova 2023). These compounds may deter predators by affecting their feeding behavior or disrupting their cellular processes and their ability to absorb food (Bryan et al. 1997; Zhukova 2023).

(2) Protection for eggs and juveniles

The defense mechanisms previously described can be exploited by the echinoderm host and adult decapod symbionts to improve their fitness, but they also may provide a nursery platform for the symbiont to protect or lay their eggs. This reproductive benefit has been well documented in other symbiotic associations, with one of the most iconic examples being the mutualistic relationship between the anemonefish *Amphiprion* spp. (clownfish) and anemones (Mitchell 2003; Holbrook and Schmitt 2005). Similarly, in echinoderm-decapod symbioses, these adaptations may provide a safe and stable environment for the offspring to develop, increasing their chances of survival and success during various stages of their life cycle, including embryo development, larvae and juveniles (Gherardi 1991).

In addition, many decapods are known to perform significant parental care (Ramirez Llodra 2002). Several species have evolved mechanisms to protect and nurture their offspring (Ramirez Llodra 2002). This includes decapods that carry fertilized eggs attached to their abdomens which take care of them until the larvae hatch, or species that keep the eggs or newly hatched larvae in a specialized chamber, often located under their carapace or within their body (brooding species). To maximize their survivability, some females may also defend the eggs and offspring against potential predators by using their claws or other defensive mechanisms, and symbiosis may be one of them. This hypothesis is highly likely, as the presence of breeding females and juvenile decapods on echinoderms has been mentioned in numerous inventory papers (Bruce 1971b; Baba 2004; Marin 2006). However, there has been limited research on the detailed description of larval/juvenile stages and none on the complete life cycle of decapods associated with echinoderms. Some studies, such as Wear (1976) and Lim and Ng (1988), have described the first zoeal larval stage of symbiotic shrimps, but comprehensive surveys encompassing all larval stages are lacking.

(3) Feeding strategies

Decapod symbionts, and probably their juveniles, may benefit from increased food availability through their association with echinoderms (Castro 1971a; Caulier et al. 2014; Horká 2017). However, to date, only a few studies have focused on their feeding habits. The researchers used various techniques to estimate their diets, as feces or gut contents (Castro 1971a; Telford 1982; Caulier et al. 2014), stable isotopes (Caulier et al. 2014; Terrana et al. Submitted), DNA barcoding and metabarcoding (Caulier et al. 2014), microscopy observation on mouthparts (Telford 1982; VandenSpiegel et al. 1992), in situ observation (Sastry 1981; VandenSpiegel et al. 1998; Britayev and Zamishliak 2012) and feeding experiments (Castro

1971a). Usually, echinoderm associated symbionts can be classified in five feeding guilds detailed here below.

(i) Mucus and host-generated waste feeding: decapods associated with echinoderms are known to actively feed on the mucus, fecal pellets and coelomocytes aggregates produced by their echinoderms hosts without an impact on the host (Castro 1971a; Sastry 1981; Britayev and Zamishliak 2012).

(ii) Feeding on host tissues: several studies tend to classify symbionts feeding from their host tissues as parasitic, but multiple factors should be considered when assessing the impact on the host fitness. The feeding behavior studied on four species of *Dissodactylus* crabs, considered as echinoid parasites, reveals that they obtain up to 80% of their diet by consuming the tissues of their respective hosts, partially by removing healthy spines and consuming mucus and tissues from the created wound (Telford 1982). Similarly, *Zebrida adamsii* consumes the integument, and peripheral dermal tissues of echinoids, resulting in the formation of noticeable exposed areas on the test and therefore negatively impacts its host (Jangoux 1987). Another example is *Lissocarcinus orbicularis* that can partially feed on the host, as suggested by their gut content containing ossicles originating from the papillae, podia and cloacal integument (Caulier et al. 2014). The symbiont impact on the host will therefore depend on the species, size, age and sex of symbiotic crabs as well as the abundance of other food resources (Telford 1978; Jangoux 1987; Caulier et al. 2014). The adult female of *Echinoecus pentagonus* can also feed from epithelial tissues and tube feet constantly renewing, the damages caused by the symbionts being in equilibrium with the regenerating capacities of the host (Castro 1971a; Horká 2017).

(iii) Predation on other symbionts: decapods may feed on other epibionts attached to the same host. By removing potential parasites, the symbiont not only gains a stable food source but also helps to reduce the burden on the echinoderm host. While these feeding methods were not studied extensively in decapod–echinoderm associations, it was described for other echinoderm symbionts, such as turbellarian *Meara stichopi* (Westblad 1949) and it is likely that it also happens in decapod-echinoderm associations. In captivity, *Brucecaris tenuis* was observed feeding on copepods attached to the arm of their crinoid host (Marin and Chan 2006).

(iv) Kleptoparasitism: refers to symbionts feeding from the same food source its host. Stealing food from the host is common in the marine environment and symbioses involving decapods (Iyengar 2002; Masuda et al. 2008; Gonçalves et al. 2020). Kleptoparasitic organisms tend to

be the first in line at the water current, or near the host's mouth, digestive and ambulatory systems, where they can easily intercept the food particles collected by the host before consumption (Iyengar 2002). It is particularly likely for decapod species associated with echinoderms to live close to the ambulacral system and digestive apparatus of their host, where food particles are easily accessible. While this facultative or obligate kleptoparasitic behavior may not have a significant impact on the host fitness, it may deprive the host of some food intakes (Dominey and Snyder 1988; Iyengar 2002). An example for this category is detailed in Terrana et al. (submitted) in which they performed stable isotopic analyses on crinoids ectosymbiont (submitted). The squat lobster, *Allogalathea elegans*, and the crab *Ceratocarcinus spinosus*, presented similar values of carbon (δ^{15}C) and nitrogen (δ^{13} N) stable isotope ratios with their hosts, with an overlap between the trophic niches of these species and the crinoids, suggesting similar food sources between hosts and symbionts. Both crustaceans were found on cirri or along the oral side of the arm base near the ambulacral grooves of the crinoids, suggesting a high probability of kleptoparasitism behavior.

(v) External food sources: Some symbionts can sustain their usual diet while being protected by their host, without kleptoparasitism. For example, the crab *Hapalonotus reticulatus*, living in the holothuroids respiratory tree, is known to be a suspension feeder (VandenSpiegel et al. 1992). Another study involving the shrimp *Synalpheus stimpsoni* and the polychaete worm *Paradyte crinoidicola* revealed both symbionts, found on crinoids, display a stable isotopic δ^{13}C composition different from their host, suggesting they feed on different food sources. A previous study of Vanden Spiegel et al. (1998) confirms this hypothesis showing that these snapping shrimps were grazing on macroalgae around the calyx.

Because of the different strategies of obtaining food through symbiosis, the majority of symbionts, especially the obligate one, may be reliant on their hosts for food. Previous studies have shown that when symbionts are isolated from their host, they may lose their food source, which may quickly lead to their death (Gherardi 1991).

(4) Host-secreted chemical assimilation

A lesser-known benefit was suggested by Brasseur et al. in 2018. They suggest that some metabolites secreted by the urchin host *Echinometra mathaei* may be essential for the survivability of the shrimp *Tulearіocaris holthuisi*. The study indicates that the chemical environment, including a specific cocktail of spinochromes (Brasseur et al 2017), has a crucial role in the physiological processes involved in the survival of *T. holthuisi*. Indeed, when isolated

from their host in two types of water (seawater versus seawater containing a spinochrome chemical extraction), the survival rate of isolated *T. holthuisi* was significantly lower than with the spinochrome treatment (**Figure 10**; Brasseur et al. 2018).

These observations led to the proposition of a new term: the host separation syndrome, which potentially encompasses anatomical, physiological, and behavioral alterations observed in symbiotic organisms upon separation from their hosts. This syndrome can also be defined as an altered health status of obligate symbionts when isolated from their hosts, potentially leading to their death. An unusual phenotypic manifestation occurred during the host separation events: host discoloration/depigmentation. This depigmentation may result from the absence of specific chemical cues or compounds produced by the host that influence the decapod pigmentation (Brasseur et al. 2018). Host separation syndrome indicates strong dependence and co-evolutionary relationship between some decapods and echinoderms. It implies that the symbiotic association is not merely a casual or opportunistic relationship but rather an intricate and specialized partnership. The exact mechanisms underlying the host separation syndrome, including the specific compounds or signals involved, are still not fully understood, and require further investigation. Understanding this phenomenon and its implications is crucial for the comprehension of the dynamics of symbiotic relationships, as well as the ecological and evolutionary consequences for both the decapod and the echinoderm species involved. It emphasizes the significance of maintaining the symbiotic association for the survival and overall fitness of the symbiont and is particularly crucial as numerous species are currently under threat from the 6^{th} mass extinction (Naggs 2017).

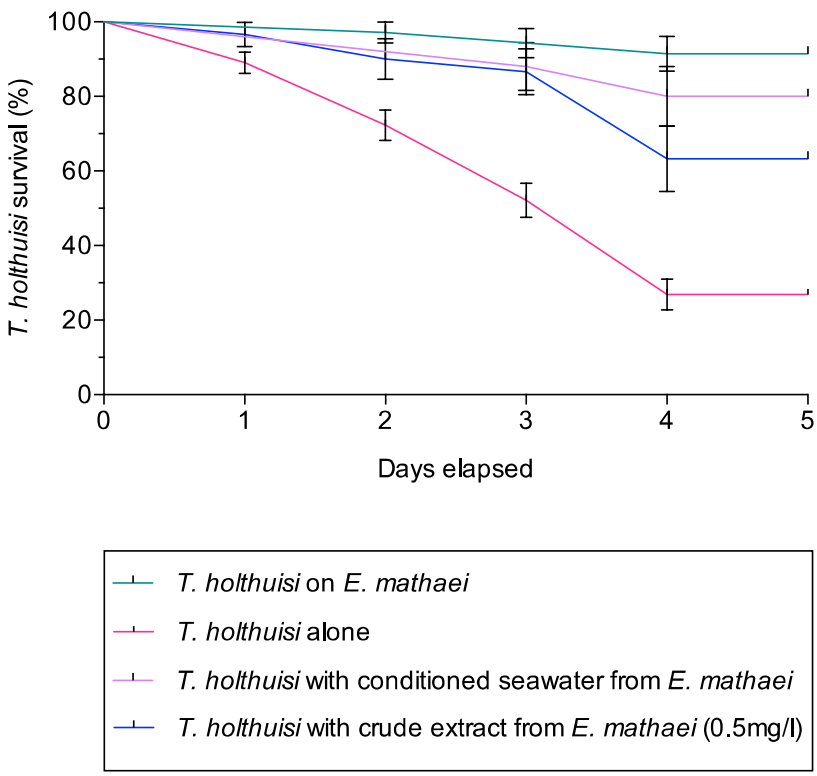


Figure 10: Survival curves of *T. holthuisi* according to different conditions. The data is expressed as the mean of living shrimps (standard error = standard deviation) (from Brasseur et al. 2018).

IX. Adaptations to symbiotic life

Each symbiotic species may exhibit various adaptations, but the extent varies depending on the specific ecological demands and evolutionary history of the symbiotic relationship (Horká 2017; de Gier and Becker 2020; Chow et al. 2021). Some species have a host-adapted morphology but, on the other hand, not all host-specific symbionts exhibit extensive morphological adaptations. Some species may have fewer observable morphological adaptations and/or may rely more on behavioral or physiological strategies rather than anatomical modifications (Gherardi 1991; Caulier et al. 2012). Therefore, host-specificity does not seem to be directly linked to morphological adaptation.

(1) Morphological adaptations

Usually, decapods that live in symbiosis with echinoderms display a specialized body shape, cheliped morphology, coloration pattern, or mouthpart appendages (Bauer 2005; Horká 2017). Few ecological studies focus on the morphological adaptations of echinoderm symbiotic decapods (Hultgren et al. 2022). However, the taxonomic literature on decapods investigates morphological traits, essentially to describe new species or differentiate two close species (Davenport 2015; de Gier and Becker 2020; Chow et al. 2021; Hultgren et al. 2022). These traits may highlight adaptations that can be attributed to symbiotic life.

(a) Carapace shape

Some symbiotic decapods display a specialized body shape that enables them to move easily on the echinoderm surface (Zmarzly 1992; de Gier and Becker 2020; Hultgren et al. 2022). Some species harbor a dorso-ventral flattened body that allows them to move on the body wall of holothuroids or between the spines of echinoids (Baeza and Thiel 2000; Ayotte and DeMaintenon 2005; Caulier et al. 2012; Campos 2016). Ectosymbiotic crab species associated with holothuroids may sometimes be found inside the cloaca of their host (*e.g.* to avoid predators during daytime) and exhibit a smaller carapace area compared to species associated with echinoids that favors their mobility as ectosymbionts (Hultgren et al. 2022). Thanks to their specialized body shape, various crab genera as *Lissocarcinus, Dissodactylus* and *Clypeasterophilus* species can hide themselves by taking up the soft tissues of their holothuroids host, creating a slight depression in the soft body wall by benefiting from the mutable connective tissues of their host that can change shape easily (**Figure 11 A-C**; De Bruyn 2010; Caulier et al. 2012). Dissodactylidae crabs (also known as pea crabs) known to live inside internal cavities or tissues of echinoderms (*e.g.*, genera *Alain*, *Buergeres*, *Holotheres*,

Pinnaxodes) display a small round to subangular firm carapace (**Figure 11 D-F**; De Bruyn 2010; de Gier and Becker 2020). Some sea-urchin shrimp symbionts, such as *Stegopontonia*, *Tulеariocaris* and *Inachus*, have an elongated body shape that mimics the host spine perfectly, with long bowed pereiopods that can wrap around the host spines to secure their position (**Figure 11 G-H;** Horká 2017; Brasseur et al. 2018). These body shapes also help them to blend in with their surroundings (*i.e.* camouflage or biomimetics) and avoid predator visual detection (Castro 1974; Brasseur et al. 2018).

In addition, most decapod species are glabrous or only have a sparsely setose epidermis, in combination with some setae for feeding (de Gier and Becker 2020). The holothuroid-associated genera *Alain*, *Holotheres*, *Holothuriophilus*, and *Trichobezoares*, possess very setosed carapace or carapace margins, which may be an adaptation for holothuroid infestation allowing them to perform chemical mimicry as a defense mechanism (Ng and Manning 2003; Campos et al. 2012; Ahyong 2018; Ng 2018; de Gier and Becker 2020). Indeed, it is possible that the short setae on the crab may collect mucus, effectively concealing the crab's smell signature and reducing its palatability when it ventures away from its host (Luckenbach and Orth 1990). The crab *Dissodactylus primitivus* also shows numerous setae on its carapace, potentially to provide an appropriate culture medium for a biofilm that the crab would feed on (Jossart et al. 2020).

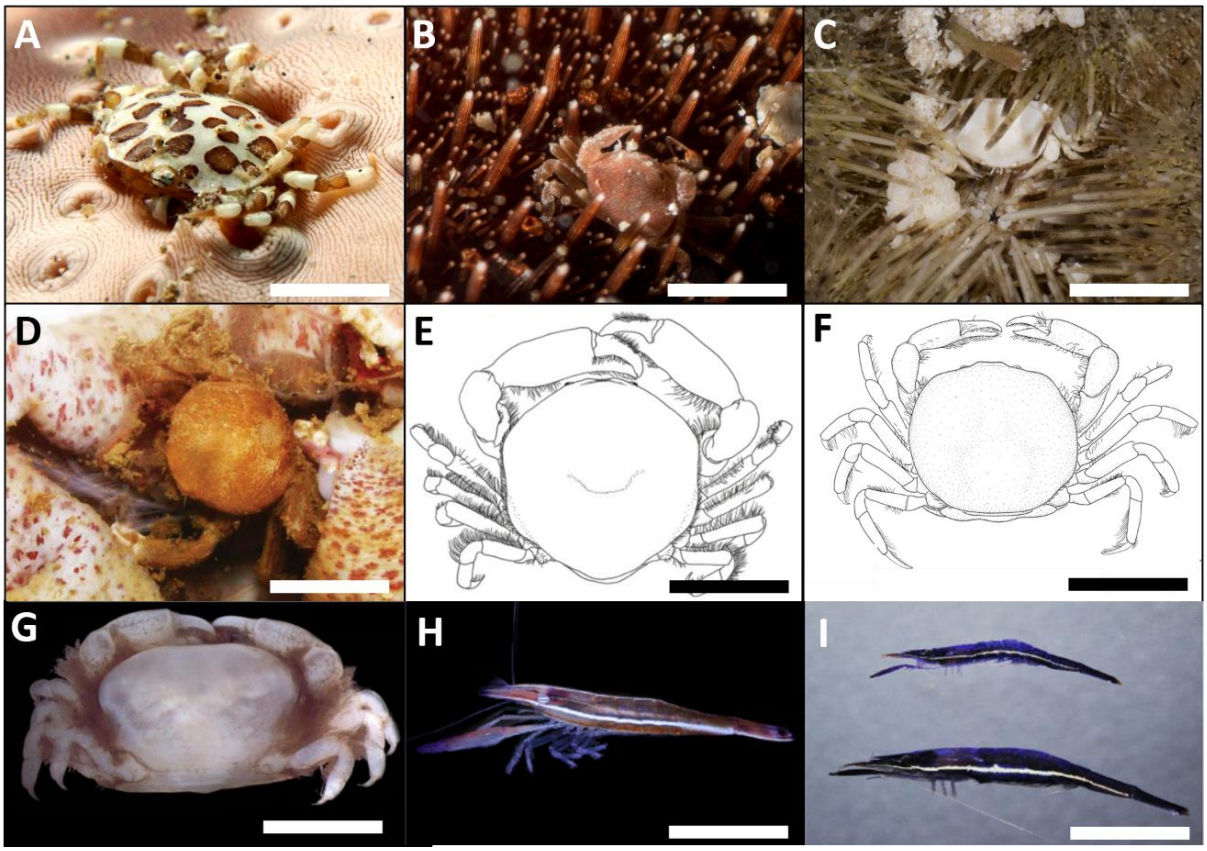


Figure 11: Example of echinoderms associated decapods harboring a particular body shape. (A) *Lissocarcinus orbicularis* (from Florent Charpin 2023), (B) *Dissodactylus mellitae* (from Randall Spangler 2017), (C) *Clypeasterophilus stebbingi* (from Museum of Comparative Zoology, Harvard University), (D) *Alain raymondi* (From Ahyong and Ng 2008), (E) *Holotheres danielae* (From de Gier et Becker 2020), (F) *Buergeres choprai* (From Ahyong and Ng 2020), (G*) Holothuriophilus trapeziformis* (From de Gier and Becker 2020), (H) *Stegopontonia commensalis* (From Limviriyakul et al 2022) and (I) *Tulеariocaris zanzibaricus* (From Limviriyakul et al 2022). Scale bars = 0.6cm in A; 0.3cm in B, C and I; 0.5cm in D; 0.8cm in E; 0.2cm in F and G; 0.4cm in H

(b) Cryptic pigmentation and ornamentation

Another adaptation of some symbiotic decapods is their ability to visually mimic the color and texture of their echinoderm hosts, making them less visible to predators (Caro 2018). Camouflage by mimicry is an effective defense strategy increasing the symbiont chances of survival (Guinot and Wicksten 2015). Decapods have two major mimicry strategies. Firstly, they can be partly or totally translucent, becoming close to invisible. This strategy is used, completely or partially, by numerous decapods, especially shrimp species and juveniles decapods as observed in genera as *Allopontonia*, *Periclimenes*, *Synalpheus* and *Zenopontonia* for example (**Figure 12 A-C**; Britayev et al. 2013; Yogesh Kumar et al. 2015; Antokhina and Britayev 2020; Lourtie et al. 2023). In the second strategy, decapods will mimic their host by matching their coloration (de Gier and Becker 2020). This strategy can be easily observed in some symbiotic genera, including *Clypeasterophilus*, *Dissodactylus*, *Lissocarcinus*, *Synalpheus* and *Zenopontonia* (**Figure 12 D-O**; De Bruyn et al. 2016; Antokhina and Britayev

2020; de Gier and Becker 2020; Caulier et al. 2022). The two first genera use the white color to mimic shell fragments, or coral rubble that their hosts hold using their tube feet (*i.e.* covering behavior). The two others harbor vivid colors that are used to match the host coloration (Brasseur et al. 2018; Caulier et al. 2022). They can show uniform coloration or display different colored stripes or spots called ornaments, which add textures to their camouflage (Gherardi 1991; Caro 2018; de Gier and Becker 2020). For example, *Arete indicus*, all *Stegopontonia* and some *Periclimenes* are extremely cryptic, with their longitudinal bands that mimic the urchin spines or crinoids pinules **(Figure 12 J-K and M-N**; Bruce 1982; Gherardi 1991; Bauer 2023). In crabs also, there are a lot of ornaments, as observed on Clypeasterophilus or Zebrida species, that have a black-and-white colored pattern on their ambulatory legs that mimic their host (**Figure 12 L and O**; de Gier and Becker 2020). More elaborate coloration observed in adult decapods may actually rely less on crypsis to survive and ornamentation could be more adapted for mate recognition or intrasexual selection (Caro 2018). As for non-symbiotic decapods, the coloration is obtained through a combination of pigments, including carotenoids that is associated with orange-red coloration, contained in specific cells called chromatophores (Caro 2018). The *de novo* synthesis of carotenoids is impossible in crustaceans (Maoka 2020). Therefore, decapods usually obtain their pigments with the food and/or a partial metabolization of the pigments of their preys (Maoka 2020). To perfectly mimic the color of their host, some symbiotic decapods feed on the hosts and reuse their pigments for their own coloration and other essential function as their pigments may have an additional essential metabolic impact for the symbiont (Brasseur et al. 2018). Pigments may also be obtained thanks to microbial symbiosis or horizontal gene transfer, as proven for others organisms (Nugraheni et al. 2010; Sloan and Moran 2012; Orlandi et al. 2022; David et al. 2023). But, to date, these phenomena were not observed in decapods. Some species also display various structural ornamentation on their carapaces, including tubercules, lamellae and upturned margins (Ng and Manning 2003; Campos et al. 2012; Ng 2018). Currently, the function of these structures remains unknown, but may be the result of adaptive evolution, and could be linked to host specificity (de Gier and Becker 2020).



Figure 12: Example of echinoderms associated decapods harboring cryptic coloration (A) *Allopontonia brockii* (from Eric Keung Photography 2014), (B) *Zenopontonia soror*. On this picture, arrows show translucent juveniles. (C) *Synalpheus carinatus* (From Virgili et al. 2020), (D-F) Different color morphotypes of *Zenopontonia soror*, matching the background host color. (G-I) Different color morphotypes of *Lissocarcinus orbicularis*, matching the background host color (G from Patrick Ess 2019; H from Michael Eisenbart 2022; I from Brian Mayes 2008). (J) *Periclimenes affinis* (From Virgili et al. 2020), (K) *Arete indicus* (from Patrick Eschweiler 2022), (L) *Zebrida adamsii* (from Jim Anderson 2017), (M) *Periclimenes colemani* (from Zubi 2008), (N) *Laomenes cornatus* (from Zubi 2007) and (O) *Ceratocarcinus longimanus* (From Virgili et al. 2020). Scalebar = 0.5 cm in A, M and N; 1 cm in B, C, F, G and I; 0.8 cm in D and E; 0.6 cm in H and K; 0.2cm in J; 1.2cm in L and 0.4cm in O.

(c) Chelipeds

Some symbiotic decapods have modified chelipeds and ambulatory appendices (*i.e.* pereiopods) that are adapted to grip onto the spines of echinoids or the soft tissues of other echinoderms (Telford 1982). *Dissodactylus* and *Lissocarcinus* have robust, broad, curved claws with cutting edges of various morphologies, that may be linked to their spine grip ability (**Figure 13 A**; Bell 1984; Bruyn 2010). They are specifically adapted to clip their host's porous spines yet cause a minimum of physical damage (Telford 1982). Other species, as *Clypeasterophilus rugatus*, have long slender claws with a pointed tip that can grasp onto the host tube feet and may be linked to the podia feeding habits (Telford 1982). These types of elongated chelipeds are also widely observed in Palaemonidae shrimp, and may also serve to grip the host podia (**Figure 13 B**; Horká 2017). Some *Alpheus* species, such as the snapping shrimps *Synalpheus*, possess a dimorphism between their two chelipeds with one claw that is significantly bigger and elongated, while the other claw is smaller and more typical in shape (**Figure 13 C**; Banner and Banner 1975; VandenSpiegel et al. 1998; Wang and Zhongli 2015; Caulier et al. 2022). Their function in predation, competition and host guarding are well known, as they can produce a cavitation bubble allowing them to strike at a distance (Ritzmann 1974; VandenSpiegel et al. 1998; Schmitz and Herberholz 1998; Qian et al. 2018; Kasumyan et al. 2020). Chelipeds can also impact the host negatively. In the case of *Buergeres deccanensis,* it is known to pierce the body wall of its host with its chelae while inhabiting the respiratory system (Jones and Mahadevan 1965).

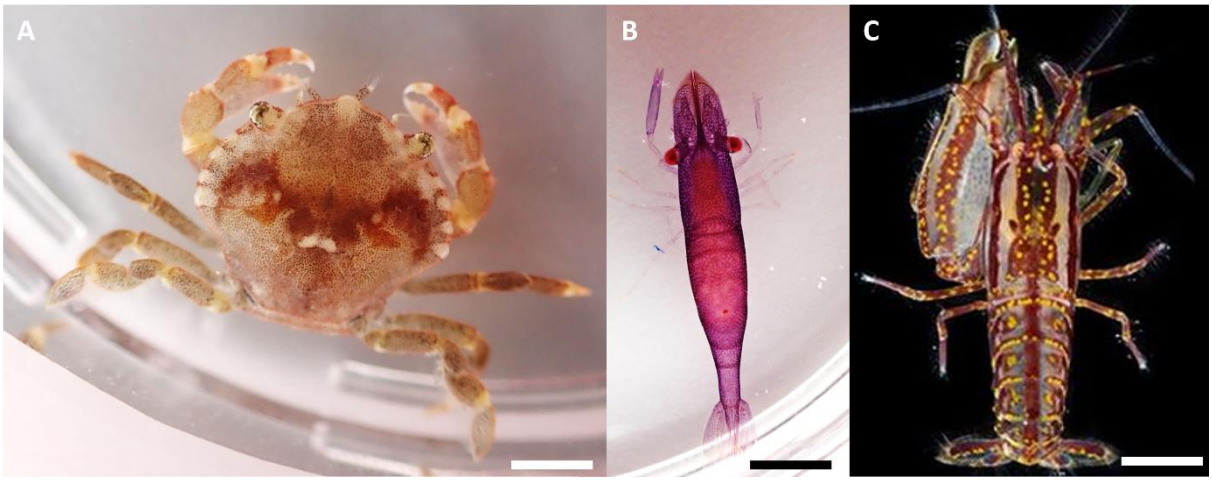


Figure 13: Chelipeds of decapods associated to echinoderms (A) *Lissocarcinus orbicularis*, with is typical robust, broad claws with a curved shape and a large range of morphologies, (B) *Zenopontonia soror* harboring a long slender claw with a pointed tip and (C) *Synalpheus stimpsonii* with its enormous and oclaw typical of this genus (from Arthur Anker). Scalebar = 3cm in A; 0.4cm in B and 0.75cm in C.

(d) Ambulatory legs (pereiopods)

In addition, the pereiopods, also called ambulatory legs, can also display adaptations to the symbiotic lifestyle. Most variations in the ambulatory legs can be found in the most distal segment, the dactylus. *Dissodactylus* and *Clypeasterophilus* have bifurcated dactyli in their first three pairs of ambulatory legs, which are thought to aid in moving between the spines of their sea urchin hosts (Rathbun 1918; Bell 1984; Campos and Griffith 1990). Morphological adaptations in the dactyli of other species can also be linked to their host choice and position on the host (Hamel et al. 1999). For example, the holothuroid-associated *Holotheres halingi* possesses falciform sharp dactyli in all ambulatory legs, used to cling to the respiratory tree of the host (Hamel et al. 1999). This pereiopodial modification, in addition to an enlarged pleon, seems to make it impossible for this species to walk (Hamel et al. 1999). Other adaptations are present in holothuroid commensal crustaceans, such as *Lissocarcinus orbicularis*, that possess hook-shaped pereiopods with a rounded tip, morphologically adapted to fix and move on the host integument without causing injuries to it (Mariappan et al. 2000; Caulier et al. 2012). In addition, the greatly specialized echinoid-associated genera *Stegopontonia* and *Tuleariocaris* have unequal second pereiopods and short ambulatory legs ending with a hooked dactyli used to remain on the host spines (Hipeau-Jacquotte 1965; Castro 1971b, 1974; Horká 2017). The second pereiopods that are unequally developed (and show great differentiation in the size and shape) are also observed in highly specialized crinoid-associated as *Crinotonia* or *Pontoniopsis* (Fishelson 1974; Bruce 1982; Marin 2006). But most echinoderm-associated shrimps (such as *Allopontonia alastairi* or *Palaemonella pottsi*, observed in association with sea urchins and crinoids, respectively) have relatively long ambulatory legs bearing simple dactyli (Bruce 1974, 1982). An intriguing study has provided insights into the morphological adaptations of ambulatory dactyli in relation to the phylogeny of palaemonid shrimps (**Figure 14**; Chow et al. 2021). The findings suggest that the evolution of ambulatory dactyli is influenced by both inheritance by descent and convergent evolution. Notably, it appears that they are evolving toward more specialized ambulatory dactyli, particularly in symbiotic species, including those with echinoderms (depicted in **Figure 14** in green). Interestingly, these specialized ambulatory dactyli are often observed in species with similar host affiliations, whether they are closely related (as seen in type III and VIII) or not (as observed in type VI and VII). In cases where these adaptations occur in unrelated species, it may signify instances of evolutionary convergence.

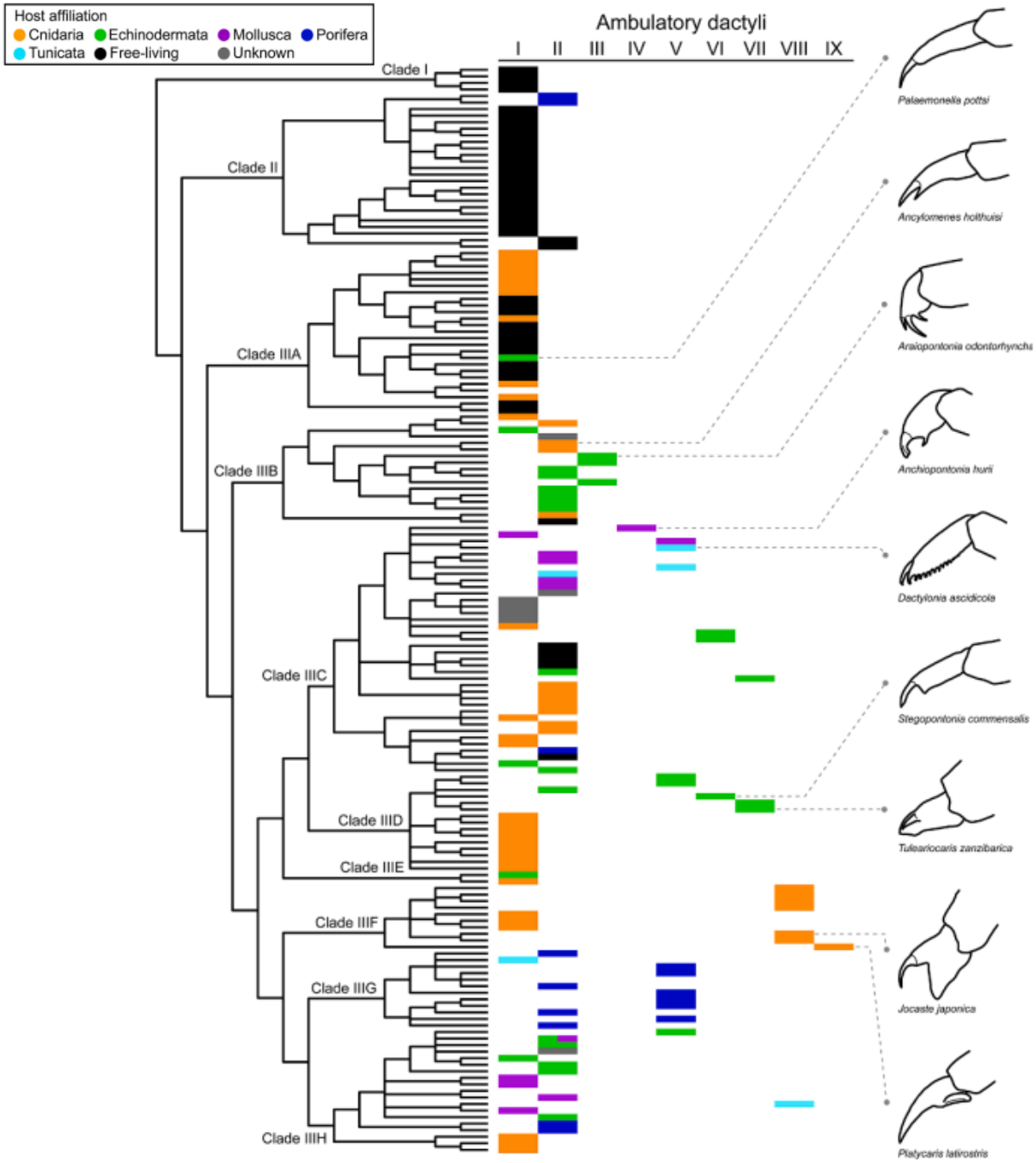


Figure 14: Character mapping of classified types of ambulatory dactyli on palaemonid phylogeny. Major host affiliations are indicated by different colors with yellow = Cnidaria, Green = Echinodermata, Purple = Mollusca, Blue = Porifera; Turquoise = Tunicata, Black = Free-living and Gray = Unknown. From Chow et al, 2021.

(e) Frontal appendages

Echinoderm associated decapods, like many symbiotic organisms, may display various adaptations of their frontal appendages. Frontal appendages play a role in sensory perception of the exterior environment and therefore responsible for sensory interactions with the host. Firstly, there are appendages used for host recognition (see below: behavioral adaptation)

(Caulier et al. 2013b, 2022; Jossart et al. 2020). They consist of the eyes and antennulae, respectively involved in visual and chemical communication at a distance (Reeves and Brooks 2001; Huang et al. 2005b; Williamson et al. 2012). Decapods display broad variation in the position and size of their eyes, therefore they are commonly used for identification in morphological taxonomy, and in some species, this characteristic may be linked to their specific host range (de Gier and Becker 2020). In addition, previous research suggested that the eye size can reflect the feeding behavior of decapods. Small eyed species are usually filter, detritus, or grazer feeding but also may appear as symbionts that consume the host (Hiller-Adams and Case 1985; Dobson et al. 2014). Free-living organisms also seem to possess bigger eyes than symbionts, and ectosymbionts may have bigger eyes than endosymbionts (Dobson et al. 2014). Decapods species, as Alpheidae, Palaemonidae, Pinnotheridae and Portunidae, include model organisms used to study host recognition in marine organisms (Dimock and Davenport 1971; Ache and Davenport 1972; Souza et al. 2019; Jossart et al. 2020; Antokhina and Britayev 2020). They all possess antennulae, the primary distance chemoreceptor organs, and some species possess elongated setae near the eyes, which also have a chemoreceptive function (Davie et al. 2015).

(f) Mouthparts

A few research studies highlight different types of modified mouth parts in echinoderm-associated decapods, but these may be more linked to the variety of diets allowed by the association rather than true adaptations to symbiotic life itself. Holothuroid-associated crab genera *Pinnaxodes, Holotheres* and *Holothuriophilus* possess palps (*i.e.* distal part of the mandible) covered with long setae used to capture planktonic food resulting in an adapted filter feeding diet (Wells and Wells 1961; Ng and Manning 2003).This specific morphology was also observed in other filter feeder decapods associated with bivalves or ascidians (Christensen and McDermott 1958; Ocampo et al. 2012; Becker and Türkay 2017). Species belonging to the genera *Dissodactylus* and *Clypeasterophilus*, feed on tube feet, spines and tissues of their spatangoid echinoid hosts, and display a small dactylus on the third maxillipeds that may be linked to their diets (de Gier and Becker 2020). The mandibles and chelae of *Zenopontonia soror* indicate that this shrimp may feed on mucus or mucus-entrapped particles (Tseng et al. 2022). *Gnathophylloides mineri* display toothed setae on the second maxillipeds that may help to collect the epithelium covering the sea urchin test and spines (Patton et al. 1985). Chow et al. (2020) show that the mandible remained relatively unchanged in Palaemonidae, with most

species displaying a conserved simple type I configuration, which is considered to be the ancestral state (**Figure 15**). However, as observed in symbiotic amphipods that have specific mouthparts based on their feeding habits (Vader 1984), echinoderm associated decapods, which have a wide-ranging diet, thanks to their association, may have similar eco-functional mandible adaptations and therefore have other specialized mouthparts.

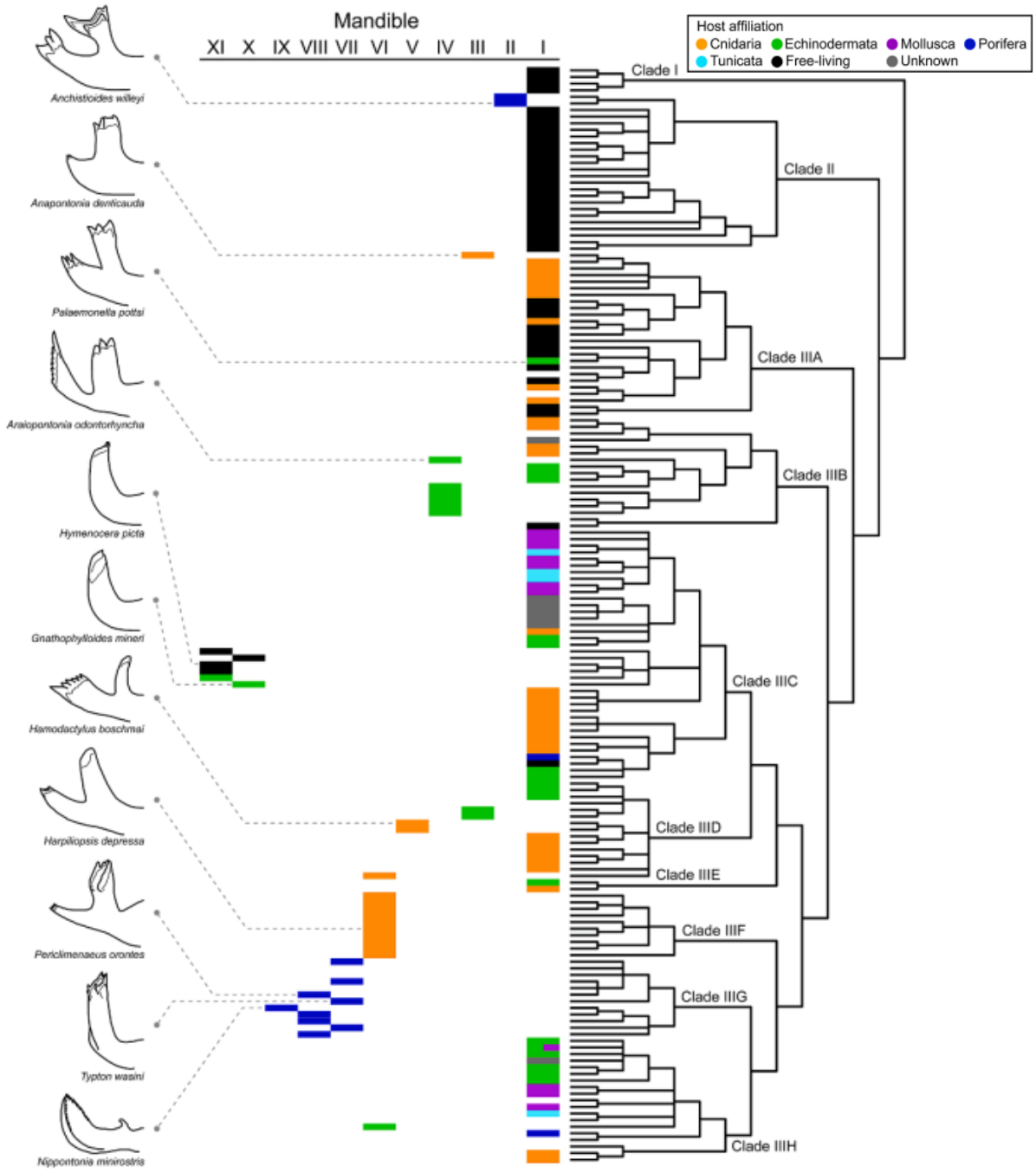


Figure 15: Character mapping of classified types of mandibles on palaemonid phylogeny. Major host affiliations are indicated by different colors with yellow = Cnidaria, Green = Echinodermata, Purple = Mollusca, Blue = Porifera; Turquoise = Tunicata, Black = Free-living and Gray = Unknown. From Chow et al, 2021.

(2) Behavioral adaptations

(a) Host selection

To allow the establishment and the perennity of symbiotic associations, host recognition by symbiotic adults and larvae is essential. Recognition is made possible by a multimodal communication including various sensory (*e.g.* visual, acoustic and chemical) pathways. However, to date, only vision and olfaction are known to be involved in decapod-echinoderm associations (Breithaupt and Thiel 2010; Antokhina and Britayev 2020). These sensory modalities work together to provide decapods with a comprehensive understanding of their environment and facilitate successful interactions with their hosts.

Chemical sensing is considered as the most ancient and ubiquitous mode of communication throughout the biosphere, especially in aquatic environments (Hay 1996). Chemical communication involves the production and secretion of secondary metabolites (*i.e.* the ecomones or semiochemicals) that will be transported in the environment and will induce a behavioral or physiological change in the organism that will perceive it (Caulier et al. 2013b; Wyatt 2014). These molecules can be subcategorized depending on the impact they will elicit on the producing or receiving organism. Considering symbiotic associations, ecomones involved in a parasitic or commensal association are called kairomones, while they are named synomones in a mutualistic relationship (Caulier et al. 2013b). Despite their abundance in marine environments, the exact chemical nature of the ecomones involved in symbioses is under-studied. Until now, only two kairomones and one synomone involved in marine symbioses have been clearly described (Murata et al. 1986; Caulier et al. 2013b; Brasseur et al. 2018). They include: (*i)* spinochromes that are produced by the sea urchin host *E. mathaei* that attract the symbiotic shrimps *T. holthuisi* and *A. indicus* (Brasseur et al. 2018), (*ii*) saponins produced by different sea cucumber species that attract the symbiotic crab *L. orbicularis* (Caulier et al. 2013b) and (*iii*) the synomone amphikuamin involved into the mutualistic relationship between sea anemones and clown fishes (Murata et al. 1986) (**Figure 16**).

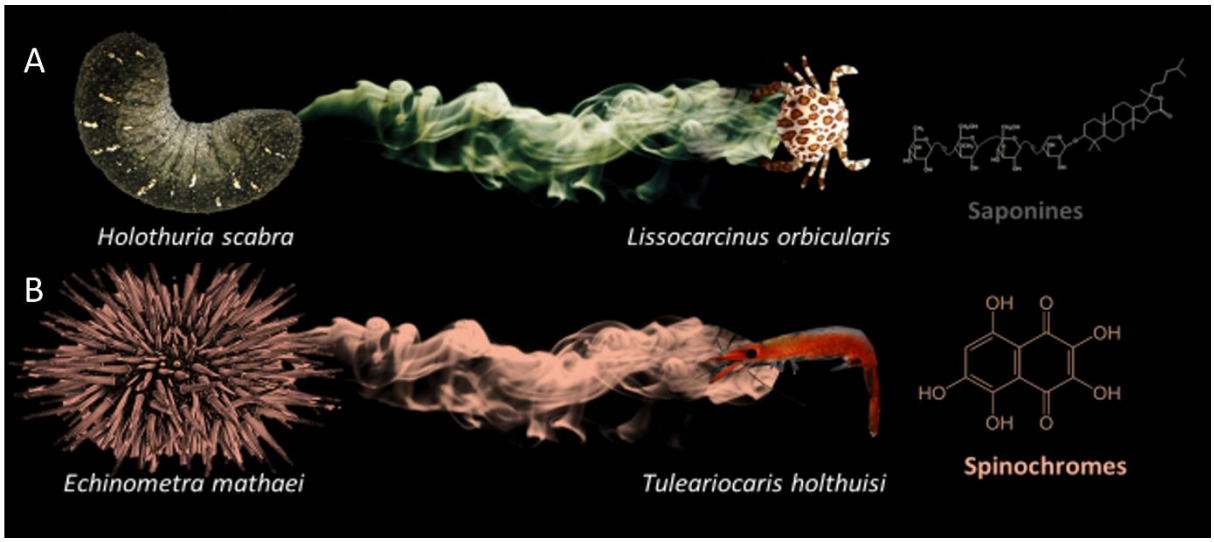


Figure 16: Semiochemicals involved in echinoderms - decapods association.

L. orbicularis is chemically attracted by several suitable host species, and may even be able to diagnose the state of health of its hosts (Caulier et al. 2013b, Unpublished Result). Other studies, on *Dissodactylus crinitichelis* and *D. primitivus* suggest that chemical stimuli associated with perivisceral fluid or host stress are potentially a repellent for symbionts (Souza et al. 2019, Caulier et al. 2023). Adult decapods will display typical behaviors when stimulated by chemical cues (Dimock and Davenport 1971; Caulier et al. 2013b; Souza et al. 2019; Antokhina and Britayev 2020). Decapods have sensory structures on their antennules, antennae and mouthparts, which are involved in detecting and interpreting chemical signals in their environment. Antennular grooming and flicking behaviors are observed during the chemical host recognition process. During antennular grooming behaviors, shrimps use their mouthpart, especially the third maxillipeds, to actively clean and remove epizoites, molecules, and particles that may have settled on their sensory structures (Bauer 1977, 1981; Cericola and Daniel 2010). This grooming behavior is important because it helps to maintain clean chemosensory organs, and therefore their sensitivity and functionality (Bauer 1977, 1981). Antennular flicking behavior involves periodic rapid movements of the antennae (Schmitt and Ache 1979; Solari et al. 2017). This behavior helps to enhance the contact between the sensory structures and the surrounding water or substrate, increasing the chances of encountering and capturing chemical cues released by the host (Schmitt and Ache 1979). The flicking movements can also aid in dispersing and spreading the captured chemical signals across the sensory surfaces, optimizing the reception and processing of the information by the chemoreceptors (Schmitt and Ache 1979).

In addition to chemical sensing, decapods have well-developed visual systems that enable them to perceive and respond to visual stimuli in their environment (Huang et al. 2005b; Williamson et al. 2012; Jossart et al. 2020). One of the key visual cues used in host recognition is the shape (like the general body shape or distinctive body part) and physical characteristics (like patterns, texture and coloration) of the echinoderm (Huang et al. 2005b; Williamson et al. 2012; Jossart et al. 2020). Decapods may therefore have evolved visual adaptations that allow them to differentiate between various host species based on their distinctive body shapes, patterns, and colors (Dobson et al. 2014; de Gier and Becker 2020).

It is important to note that while visual recognition or chemical sensing plays a crucial role in host identification, other sensory modalities, such as acoustic and tactile cues, may also be important in the overall process of host recognition and selection. These sensory modalities work together to provide decapods with a comprehensive understanding of their environment and facilitate successful interactions with their specific host organisms.

(b) Host switch

Host recognition is particularly useful during another important behavior in symbiotic association: host switching. It refers to situations where a decapod species changes its association from one host organism to another (Baeza and Thiel 2007; Caulier et al. 2012; Horká et al. 2016). The concept of host switch is widely used but depending on the kind of research (eco-ethological study or phylogenetic analyses), this concept will involve important differences.

In eco-ethological studies, host switches refer to switches performed by a symbiont individual when they move between one host to another during its lifetime. Numerous decapods are known to switch between different host individuals during their life cycle (Caulier et al. 2012; Antokhina and Britayev 2020). This kind of switch is frequently observed during reproduction, as in some species males and/or females have to switch from one host to another to reproduce (*i.e.* sexual partner recognition), increasing their reproductive fitness (Knolton 1980; Baeza and Thiel 2007; Asakura 2009; Caulier et al. 2012). It can also be observed when multiple decapod individuals or species compete for the same host resources: host switching may occur as a mean to reduce competition and find less crowded or unoccupied hosts (Martinelli Filho et al. 2014; Souza et al. 2019).

In phylogenetic analyses, host switch events refer to the occurrence of a derived state created when a symbiont lineage, which was originally associated with one host lineage, successfully

establishes and maintains associations with a new host lineage. (**See before section VI**; *e.g.* Boyko and Williams 2009; Lanterbecq et al. 2010; Horká et al. 2016; Chow et al. 2021). Usually, these kind of host switches between phylogenetical distant hosts are atypical because of the numerous adaptations that symbionts have (Gómez et al. 2010; Horká et al. 2016). However, symbiotic lineages do occasionally switch to new, and even sometimes, distantly related host species (Gómez et al. 2010; Horká et al. 2016).

(c) Coordination of host/symbiont movements and swimming adaptations

Decapods engaged in symbiotic relationships with echinoderms exhibit various specialized morphologies and behaviors, which contribute to the coordination of the host/symbiont movement and swimming adaptations. They may move in close proximity to their host, following its movements (Baeza and Thiel 2007; Dumont et al. 2010; Baeza et al. 2016; Parmentier et al. 2021; Hayes et al. 2022). When threatened, they can also quickly hide or flee from potential predators, by crawling close to the echinoderm body or its immediate surroundings (Bruce 1975b; Olliff 2013; Horká 2017; Kasumyan et al. 2020). Species of the genus *Tulеariocaris* and *Stegopontonia* exhibit a specific locomotion behavior when threatened (**Figure 17 C**; Bruce 1975; Horká 2017). They swim around spines with the head-down similarly to the fish *Aeoliscus strigatus* (Bruce 1975b; Horká 2017). Symbionts that can enter the mouth and cloaca of their host, as the *Lissocarcinus* species, flee inside their host when disturbed. Some symbionts remain motionless to avoid detection by predators (Caulier et al. 2012). They may remain still and blend in with the host body, leveraging camouflage and mimicry to appear inconspicuous (Fishelson 1974; Olliff 2013). *Arete indicus* or *Gnathophylloides mineri* are good examples of this behavior as they are known to stay still

between the spines of their host, always in a head pointed outwards position (**Figure 17 A-B**; Patton et al. 1985; Olliff 2013)

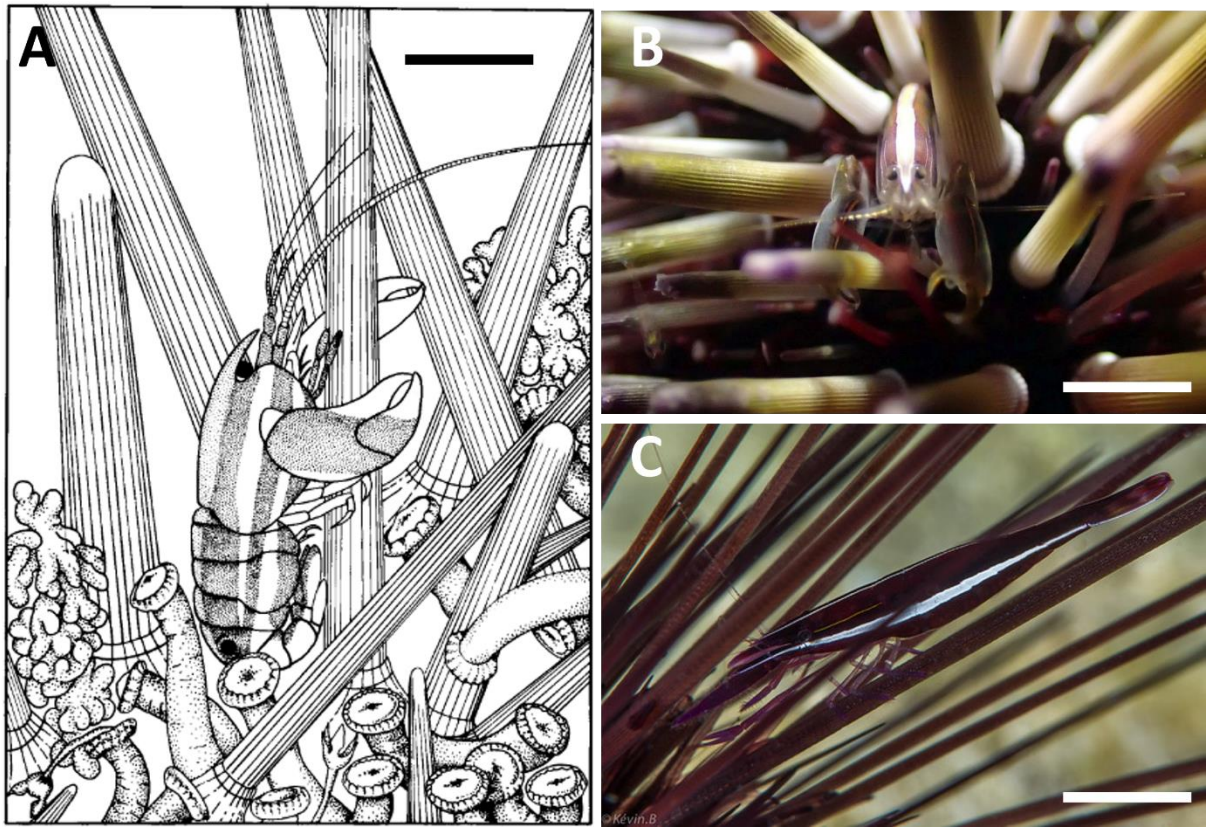


Figure 17: Ectocommensal species showing specific behaviors. (A-B) *Arete indicus* on its sea urchin *Echinometra mathaei*, with its typical head pointed outwards position (A from Gherardi 1991; B from Eschweiler 2022). (C) *Stegopontonia commensalis* in head-down position (from Bourdon 2014). Scale bars = 0.1cm in A: 0.5cm in B and 0.3cm in C.

(d) Host guarding: competitive and protective symbionts

Host guarding refers to a behavior commonly observed in decapods, and it can be classified into two main categories: protective host guarding and competitive host guarding. In protective host guarding, the symbionts exhibit interspecific antagonist behaviors to protect their echinoderm host from predators (Huang et al. 2005b; Baeza and Thiel 2007; Britayev and Mekhova 2011; McCammon and Brooks 2014). These decapods may actively defend their host by warding off potential threats and predators that may harm or consume the host organism. They often exhibit aggressive behaviors, such as aggressive displays, claw waving, or even physical attacks, to deter predators from approaching the host and potentially, to limit the settlement of parasites or other potential symbionts (Thiel et al. 2003a; Huang et al. 2005b). For example, the crinoid associates, Synalpheus species produce strong noises with chelipeds when a predator approaches (snapping behavior) (Schmitz and Herberholz 1998; Glynn and Enochs 2011). *Harrovia albolineata* was suggested as protective mutualist as the crab seems to

use its long and relatively powerful chelipeds to defend the crinoid against potential predators (Castro 2015).

On the other hand, in competitive host guarding, the symbionts display strong territorial behaviors (Wilson 2000; Baeza 2007; Caulier et al. 2012; Martinelli Filho et al. 2014). They defend their territory (*i.e.* symbiotic ecological niche) directly on or around the hosts, primarily to secure resources as food, shelter, or mating partners, and prevent other individuals, including potential competitors from accessing those resources (Baeza and Thiel 2007; Caulier et al. 2012; Martinelli Filho et al. 2014). Competitive symbionts engage in aggressive interactions with intruders that may be from the same species, chasing away competitors, or even engaging in physical combat (De Bruyn et al. 2016). They show strong intraspecies competition behavior towards other adults of the same sex and fight each other until there remains usually one adult, one heterosexual couple or a heterosexual pair with juveniles (Caulier et al. 2012). Some species of pinnotherid crabs show these intraspecific patterns that are stronger in females (Hultgren et al. 2022).

Depending notably on the degree of host guarding behavior, organisms can be found as (*i*) solitary individuals, such as *B. deccanensis, H. longipes* or *Z. adamsii* (Jones and Mahadevan 1965; Yanagisawa and Hamaishi 1986; Eeckhaut et al. 2000; Hui 2012), (*ii*) in heterosexual pairs, such as *H. semperi, S. stimpsonii* or *T. spinosum* (Fujita and Shokita 2003; Huang et al. 2005b; Castro 2015; Hamel et al. 2019; Caulier et al. 2022) or (*iii*) dense unstructured aggregations of individuals, such as *Z. soror* (Antokhina and Britayev 2020). By securing exclusive access to resources, these decapods increase their own fitness and reproductive success (Baeza and Thiel 2007). Both host guarding behaviors are similar, sometimes interconnected, and may be easily mixed up. The key difference between protective and competitive symbionts lies in their primary focus. Protective symbionts prioritize the defense of their host and themselves, ensuring their safety and survival. On the other hand, competitive symbionts are primarily concerned with securing and defending their own resources and territory, often engaging in aggressive interactions with other individuals.

(3) Physiological adaptations

Decapods associated with echinoderms may have undergone various physiological adaptations to thrive in their symbiotic relationships and cope with the challenges of their specific symbiotic habitats (Gherardi 1991; Brasseur et al. 2018). However, up to now, little is known about these possible adaptations and additional studies on this subject are crucial to highlight or better understand these characteristics. At least, we can mention two different categories that are associated to chemical environment produced by echinoderm hosts.

(a) The use of host metabolites by symbionts for its own metabolism

It is well known in endoparasitic species that the conditions offered by the micro-ecosystem provided by the host is essential for their development and their survival (Fisher et al. 2017; Schmidt et al. 2022). But this type of dependency is much less studied in ectosymbionts and only one study suggests the dependency of certain decapods on specific metabolites from echinoderm hosts, the study of Brasseur et al. (2018). This work sheds light on a chemical dependency observed in the shrimp species *T. holthuisi* towards the spinochrome of its sea urchin host (see also above). The researchers put forward three hypotheses to explain the increased mortality of *T. holthuisi* in the absence of spinochromes: (i) spinochromes may serve as a primary source of food for the shrimp, (ii) the antibacterial and antioxidant properties of spinochromes may play a crucial role in protecting the shrimp from harmful events and (iii) spinochromes may promote essential physiological pathways for the shrimp metabolism.

Understanding the mechanisms of chemical dependencies in symbiotic relationships can provide valuable insights into the co-evolutionary processes between symbionts and their hosts (Brasseur et al. 2018). Further research in this area is needed to unravel the intricacies of these physiological adaptations and their implications for the overall functioning of symbiotic ecosystems.

(b) Resistance to noxious molecules produced by the host

To date, there is no study exploring the potential adaptations of decapods to the noxious molecules of echinoderm hosts. It can, however, be reasonably suggested that symbiotic decapods must show some adaptations to live in these habitats that sometimes contain cocktails of toxic molecules, especially when living on sea stars or sea cucumbers that both secrete saponins. Other non-decapod symbionts associated with echinoderms exhibit distinct structures and behaviors that enable them to resist and coexist in enduring partnerships. For instance,

carapid fish whose most live inside the respiratory trees of holothuroids, are not impacted by the normally ichthyotoxic saponins produced by the internal organs and Cuvierian tubules of holothuroids (Eeckhaut et al. 2015). While the gill structure of carapids is similar to that of free-living fishes, it does not account for their resistance to saponins. Their ability to withstand these toxins is linked to the quantity and composition of the mucus they secrete (Eeckhaut et al. 2015). Further research into the mechanisms underlying symbiotic decapod resistance to noxious molecules would provide valuable insights into decapod-echinoderm co-adaptations.

X. Life cycle and co-evolution

(a) Reproduction and life cycle

Most decapod species, including symbiotic ones, are gonochoric (*i.e.* one separate sex during the entire life cycle), but decapods also comprise many species that can change sex or that are simultaneous hermaphrodites (Bauer 1976; Bell and Stancyk 1983; Correa and Thiel 2003; Baeza and Thiel 2007; Sagi 2008; Asakura 2009; Dabbagh et al. 2019). Their reproduction typically involves internal or external fertilization and various mating behaviors (Bauer 1976; Bell and Stancyk 1983; Correa and Thiel 2003; Baeza and Thiel 2007; Sagi 2008; Asakura 2009; Dabbagh et al. 2019). The life cycle involves multiple developmental stages, including larval, juvenile and adult phases (Herring 1974; Anger et al. 1994; Ramirez Llodra 2002; Sagi 2008). After fertilization, eggs are usually incubated by females on their abdomen, thanks to their pleopods, until they hatch into free-swimming larvae, which undergo a series of developmental stages (Bauer 1976; Anger et al. 1994; Correa and Thiel 2003; Asakura 2009). Regarding symbiotic decapods associated with echinoderms, pelagic larvae are supposed to disperse in the water column until they detect a host, moving towards it (*i.e.* host recruitment), realizing larval metamorphosis. Upon finding a suitable echinoderm host, the larvae would settle and undergo metamorphosis into juveniles (Antokhina and Sorokin 2010; Lourtie et al. 2023).

At the adult stage, specific reproductive behaviors and strategies develop depending on the decapod species and the nature of the symbiotic association (Baeza and Thiel 2007; Asakura 2009; Dabbagh et al. 2019). Baeza and Thiel (2007) proposed a conceptual model based on the mating system of symbiotic crustaceans, including decapods associated with echinoderms (**Figure 18**). In this model, it is assumed that males and females within symbiotic crustaceans possess distinct optimal mating strategies, a concept already accepted in many animals: males

will tend to maximize their reproductive success by reproducing with as many females as possible, while females aim to select a high-quality male (Darwin 1872; Bateman 1948; Trivers 1972; Emlen and Oring 1977; Hunt et al. 2004). They strive to achieve optimal mating through host guarding and host switching (Knolton 1980; Christy 1987; Horká et al. 2016; Baeza et al. 2016). These behavioral options of symbiotic individuals are limited by predation risk, as well as abundance, relative size morphological complexity of the available host (Baeza and Thiel 2007). The model predicts the existence of five mating systems among symbiotic crustaceans (**Figure 18**):

(i) Monogamy: when predation risk is high, and hosts are unabundant; the host displays a small body size with simple morphologically features

(ii) Host-defense polygyny: when predation risk is high, and hosts are unabundant; the host displays an intermediate body size with simple morphologically features

(iii) Pure-search polygynandry: when symbiont females are mobile, predation risk is low, and hosts are abundant; the host have large body size with complex morphologically features

(iv) Pure-search polygynandry: when female symbionts are sedentary, predation risk ranges from moderate to high and hosts are moderately abundant and have extremely small body size

(v) Female-centered polygyny, which is predicted when predation risk is moderate and hosts moderately abundant but with intermediate body size

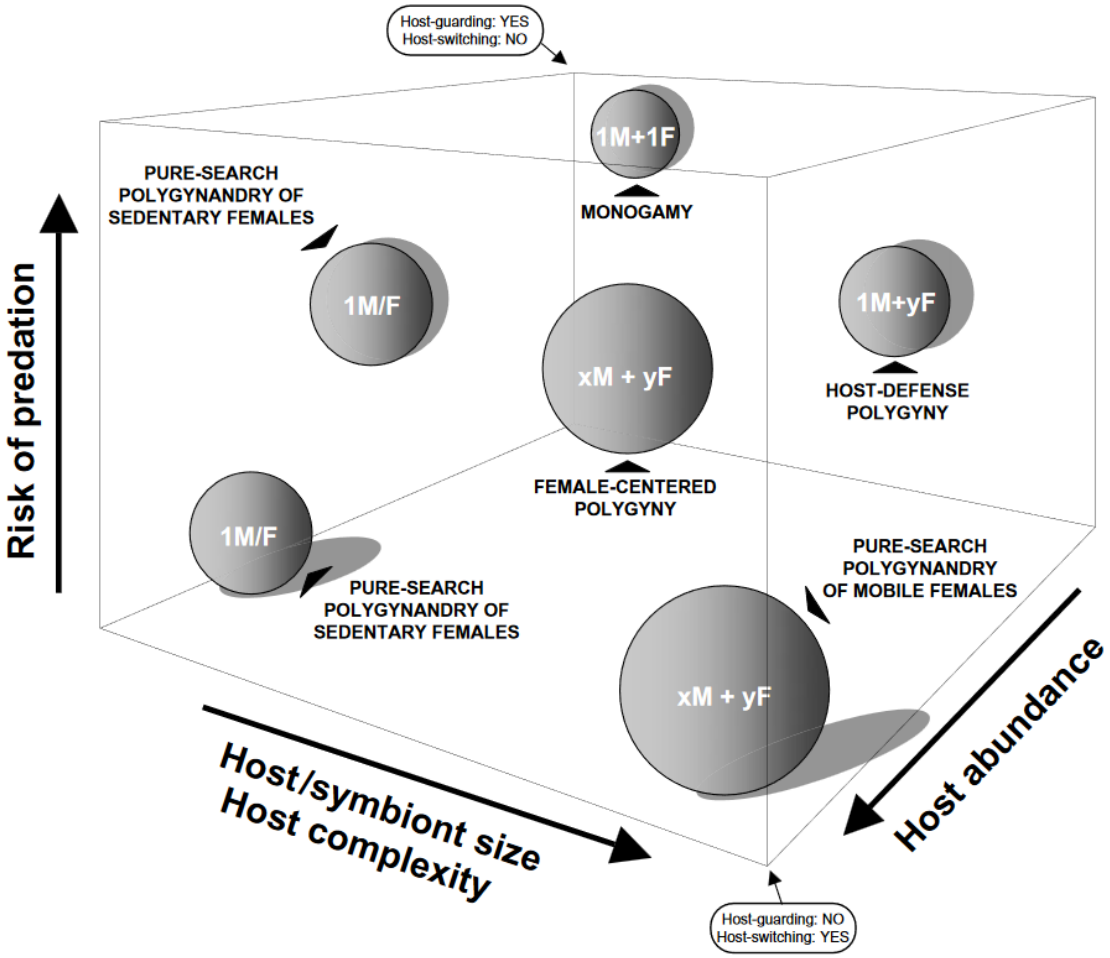


Figure 18: Mating systems predicted for symbiotic crustaceans according to a specific set of environmental conditions (Baeza and Thiel 2007). The interaction among predation risk, host relative size, and abundance is modelised as a three-dimensional space in which different mating systems occur. x and y, number of males (M) and females (F), respectively, found in a host under the different mating systems.

Goals of the thesis



Goals of the thesis

In marine ecosystems, particularly in tropical environments, 221 symbiotic associations between echinoderms and crustacean decapods have been reported. Such relationships can be defined as an intimate and long-lasting association between one echinoderm host and at least one symbiotic decapod, where the decapod notably relies on the host for protection and access to resources. Despite the fundamental role of these symbioses, little is known about the "host separation syndrome", a phenomenon observed when the obligate symbiont is separated from its host and suffers from detrimental effects, potentially leading to its death.

The primary objective of this PhD thesis is to investigate the parameters influencing the "host separation syndrome" phenomenon in four obligate symbiotic associations involving four shrimp species hosted by three classes of echinoderm hosts: asteroids, crinoids and echinoids. This research aims to shed light notably on the chemical dependencies shown by the symbionts in these associations. This research will encompass a multidisciplinary approach to better understand this phenomenon, involving sample collections, behavioral experiments, characterization of the chemical environment produced by the hosts (*i.e.* ecomones secreted by the hosts), as well as investigation on the physiological or transcriptomic responses of symbionts when they are totally isolated from their hosts or in water containing the ecomones of the host (*i.e.* host conditioned water). Scientific activities occurred during different scientific field missions in IHSM (Toliara - Madagascar), Criobe (Moorea - French Polynesia), Marine Station of Concarneau (Concarneau - France), UMONS (Mons – Belgium), UCL (Louvain-la-Neuve – Belgium) and ULiège (Liège – Belgium).
An extensive literature review of symbiotic relations between echinoderms (hosts) and decapods (symbionts), presented in the introduction, is followed by four chapters that provide new insights on the host separation syndrome and the host chemical dependency of decapod symbionts.

Chapter I highlights the chemical environment (ecomones) produced by crinoids and explores host recognition behavior in the symbiotic pistol shrimps, *Synalpheus stimpsonii*. The chemical attractiveness of two crinoid hosts and one non-host species, as well as commercial anthraquinone, were tested in olfactometry and characterized in mass spectrometry.

Chapter II, similarly to **chapter I**, investigates host recognition abilities in the adults and larvae of another symbiotic decapod, *Zenopontonia soror*, an obligate symbiont of sea stars.

We examine the ecomones that influence host selection using chemical extractions, behavioral experiments in olfactometers, and mass spectrometry analyses

Chapter III explores the host separation syndrome effects on two obligate symbionts associated with the echinoid *Echinometra mathaei*. Paired-end Illumina HiSeq technology has been used to analyze transcriptomes from the *Arete indicus* and *Tulеariocaris holthuisi* confronted with three different conditions used previously to investigate host separation syndrome. The three conditions analyzed were: (1) individuals associated with their host (control), (2) individuals separated from their host in seawater and (3) individuals separated from their host but in seawater conditioned by naphthoquinone, an ecomone involved in the host recognition occurring in these two shrimps.

Chapter IV focuses on the survivability of four symbiotic pairs presenting various characteristics and different host - specificities: *Z. soror – C. novaeguineae, S. stimpsonii – P. distincta, T. holthuisi* and *A. indicus – E. mathaei*. It also investigates the mimetic pigmentation, a physiological parameter that can be influenced by host separation and the presence/absence of chemical environment.

The four chapters describing results is followed by a **general discussion** that aims to incorporate the results obtained in the different thesis chapters to provide a broader understanding and potential future research objectives that may be carried out to better understand marine symbiotic associations and their integration at an ecosystem-level.

Finally, this thesis aims to provide new insights into symbiotic associations between echinoderm hosts and their decapod symbionts. It sheds notable light on the symbiont response to specific chemicals produced by the hosts and how they are impacted by host isolation. This research will therefore contribute significantly to future studies on co-evolution, host recognition, and chemical ecology within marine symbiotic systems. Obtained results provide crucial clues to understand how this host separation may be triggered in natural environments when a host disappears, due to predation, biotope disturbances, or because of the global biodiversity loss that actually negatively impacts the marine biosphere.



Selection of the biological models



Selection of the biological models

Four biological models were chosen to investigate the effect of metabolites on symbiotic associates. They were selected for their unique attributes and were chosen for direct comparisons, underlining the complexity inherent to the host separation syndrome (see the final discussion).

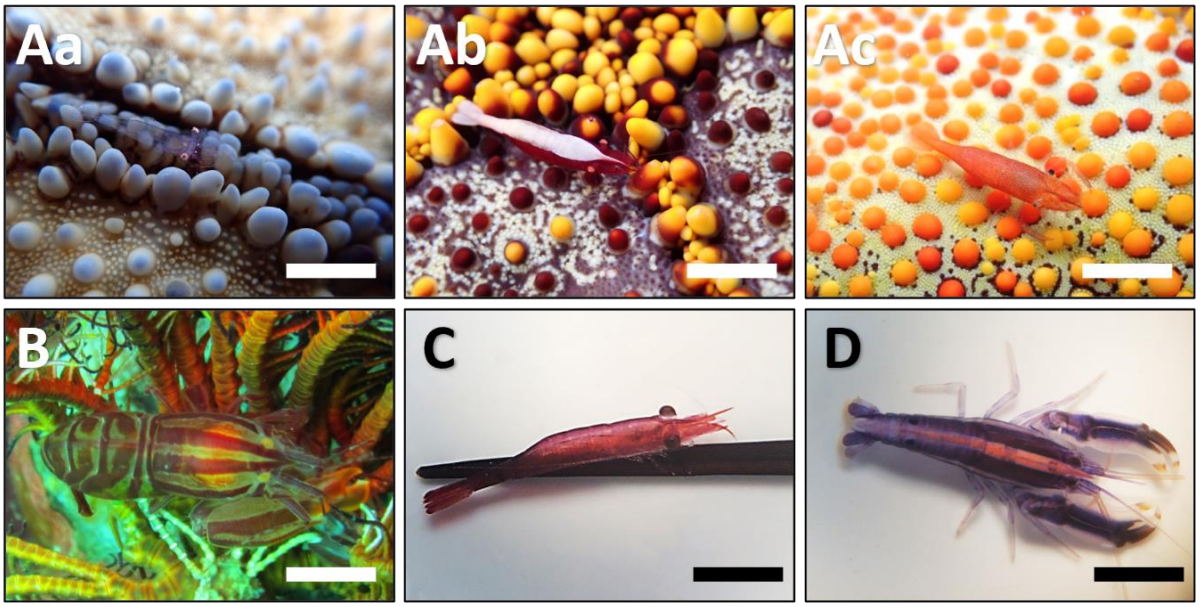


Figure 1: Investigated symbiotic models. (Aa-b) *Zenopontonia soror*, (B) *Synalpheus stimpsonii*, (C) *Tulеariocaris holthuisi* and (D) *Arete indicus*. Scalebar = 0.5 cm in Aa: 0.6 cm in Ab-c; 0.85 cm in B and 0.2 cm in C-D.

The four investigated symbiotic associations comprised 4 shrimps species belonging to the Caridae infraorder, two of them being in the Alpheidae family and the two others from the Palaemonidae family. They are (i)) the sea star shrimp *Zenopontonia soror* (Nobili, 1904) (**Figure 1A**), which notably coexists with the cushion sea star *Culcita novaeguineae* Müller & Troschel, 1842, (ii) the snapping shrimp *Synalpheus stimpsonii* (De Man, 1888) (**Figure 1B**), commonly found on the crinoid *Phanogenia distincta* (Carpenter, 1888), (iii) *Tulеariocaris holthuisi* Hipeau-Jacquotte, 1965 (**Figure 1C**), and (iv), *Arete indicus* Coutière, 1903 (**Figure 1D**), both residing on the sea urchin *Echinometra mathaei* (Blainville, 1825). These marine symbiotic relationships are predominantly observed in diverse tropical marine environments, ranging from shallow tropical lagoons, reef flats or reef channels. As highlighted in the introduction of this thesis, most symbiotic associations between decapods and echinoderms occur in tropical environments like Madagascar or French Polynesia where four scientific field

missions were conducted during this PhD thesis. This may be explained by a higher predation rate of decapods in a generally oligotrophic water (Dorn et al. 2006), promoting decapods to associate with a host to search for protection, but also by higher research efforts than in colder or deeper ecosystems (Gray 2001).

A. indicus represents the most specific association within our study. Usually, literature suggests that it exclusively forms symbiosis with *E. mathaei* (Gherardi 1991). This species measures approximately 1 to 1.2 cm, and its coloration closely matches that of its host, exhibiting a distinct purple hue with a few minutes vertical bands and two dark spots on the dorsal part of the abdomen (Gherardi 1991; pers. obs.). *A. indicus* is frequently observed in solitary or paired arrangements at the base of the sea urchin spines, showing its cephalon toward the exterior of the host, with its body and chelipeds oriented parallelly to the host spine (Gherardi 1991; pers. obs.). In comparison, the second symbiont, *T. holthuisi*, while being primarily observed on *E. mathaei* in Toliara (*i.e.* the town being at the origin of its genus name), occasionally or seasonally forms associations with at least six other sea urchins species (Bruce 1982; Khan et al. 2004; Marin and Anker 2009). Therefore, depending on the source, it has been described as species-specific (Brasseur et al. 2018) or family-specific symbiont (Chow et al. 2021). This shrimp also presents a body shape measuring approximately 1 to 1.2 cm, but with a slender and more elongated body than *A. indicus*, perfectly mimicking the spine of its sea urchin host (Brasseur et al. 2018; pers. obs.). This shrimp species ranks as the smallest among those investigated. Its coloration closely resembles its host, with a slight pinkish hue. *T. holthuisi* is commonly found individually or in pairs, on the spines head down toward the test of the sea urchin and often coexisting with *A. indicus* (Gherardi 1991; Brasseur et al. 2018). No competition behaviors have been observed between the two species that may exploit different ecological niches within their host (Gherardi 1991; pers. obs.). Indeed, *A. indicus* is usually found on the test of *E. mathaei* while *T. holthuisi* is mostly observed living and mimicking the spines of their hosts (Gherardi 1991; pers. obs.).

S. stimpsonii falls into the class-specific symbiont category, only associating with crinoids and settling interactions with up to 10 host species (see **Introduction**). This species stands as the largest among those studied in this project, with adults' individuals typically measuring around 2 cm or more (pers. obs.). All populations exhibit a consistent external appearance characterized by a robust body adorned with longitudinal and transversal-colored bands, ranging from translucent to vivid orange bands, delimiting the body metamers (pers. obs.). *S. stimpsonii* is

often found in heterosexual pairs, typically in proximity to the cirri or at the base of the host calyx (VandenSpiegel et al. 1998; Terrana et al. Submitted; pers. obs.).

Z. soror stands out as one of the most studied symbiotic shrimps associated with echinoderms. It has been described in association with a wide array of sea star hosts (currently encompassing 27 described host species), classifying it as a class-specific symbiont (see **Introduction**). These shrimps exhibit an elongated and relatively wide body, with slender claws and three distinct morphotypes: translucent, striped coloration (*i.e.* with a white dorsal band), and fully colored (Antokhina and Britayev 2020; Fernández-Lereé et al. 2023; pers. obs.). These morphotypes display variations in pigmentation, often harboring translucent or mimetic colors with vivid tints such as red, purple, yellow, or orange (Antokhina and Britayev 2020; Fernández-Lereé et al. 2023; pers. obs.). This species presents an intermediate size compared to the other investigated species with adults typically reaching a size of 1.5 to 2 cm (pers. obs.). Notably, *Z. soror* is often found in small to large aggregations, with groups reaching up to 14 individuals observed in our survey in Moorea (Antokhina and Britayev 2020; pers. obs.). Their frequent proximity to the ambulacral groove or the sea star mouth, where they can even enter, challenges prior assumptions that classified them exclusively as ectosymbionts (pers. obs.).

Chapter I



Crinoid anthraquinones as kairomones allowing host selection for the symbiotic snapping shrimp *Synalpheus stimpsonii*

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Abstract

Quinones are one of the major pigment groups that provide such bright colors to feather stars (Echinodermata, Crinoidea). These secondary metabolites also act as defensive molecules rendering crinoids unpalatable and repellent to other organisms. However, feather stars are usually associated with numerous symbiotic organisms, amongst which the ectocommensal snapping shrimp *Synalpheus stimpsonii*. We investigated the chemical stimulus allowing host selection in *S. stimpsonii* through the combination of behavioral tests, chemical extractions, and mass spectrometry analyses. The individuals of *S. stimpsonii* used in the experiments were sampled around the Great Reef of Toliara (Madagascar) where they are found in association with two crinoid species: *Comanthus wahlbergii* and *Phanogenia distincta*. The chemical attractiveness of the two crinoid hosts and a non-host species, *Cenometra bella*, was tested in an olfactometer. The three crinoids produced attractive kairomones allowing the snapping shrimp to recognize them. Mass spectrometry analyses on purified extracts of *P. distincta* revealed the presence of three different anthraquinones (rhodoptilometrin, comantherin, and a new crinoid anthraquinone). Compared to the existing literature, this anthraquinonic cocktail is specific to *P. distincta*. When these extracts were injected in the olfactometer, they triggered similar attracting behavior suggesting that crinoid anthraquinones are kairomones allowing host selection for *S. stimpsonii*. This hypothesis is also supported by the fact that shrimps were chemically attracted by pure commercial anthraquinones. In addition to their traditional defensive role (allomones), anthraquinones would therefore also function as kairomones, maintaining the symbiosis between *S. stimpsonii* and its crinoid hosts.

Keywords: Marine chemical ecology; Echinoderm; Crustacean; Ectocommensal; Y-tube; Olfactometer.

I. Introduction

Quinones represent a major family of polyketides widely distributed in the biosphere. More than 1,200 quinones have been described so far. They are composed of diones that form diverse aromatic structures differing by the number of aromatic cycles and their associated radicals (El-Najjar *et al.* 2011). Quinones are essential to many biological processes: some derivatives of quinones serve as electron acceptors in electron transport chains playing a role in photosynthesis and aerobic respiration (Elling *et al.* 2016; Lubitz 2003; Nohl *et al.* 1986); they play a role in the cellular membrane stabilization (Rao *et al.* 2008) and in the formation of vitamins and antioxidants (Beattie *et al.* 2007; Gong *et al.* 2008). Quinone-related compounds are of high interest for human health. For example, they constitute a component of nearly every modern drug to cure cancer (Asche 2005; Lu *et al.* 2013). Quinones are found in a wide variety of plants, bacteria, and fungi (El-Najjar *et al.* 2011; Sunassee and Davies-Coleman 2012). Except for the ubiquitous ubiquinones, these molecules are rare in the animal kingdom and only found in small concentrations in marine sponges, cnidarians, mollusks, arthropods, tunicates, and echinoderms (Gordaliza 2010; Singh *et al.* 1967; Sunassee and Davies-Coleman 2012; Thomson 2012). Concerning echinoderms, naphthoquinones are produced exclusively by echinoids while anthraquinones are specifics to crinoids (*i.e.* feather stars) (Kornprobst 2005; Shikov *et al.* 2018).

In crinoids, neither the biogenesis nor the function of quinones are well documented (Feng *et al.* 2017). Previous literature has highlighted the presence of anthraquinones in different organs, from the skeleton to the viscera of feather stars (Bartolini *et al.* 1973; Ghaly *et al.* 2009; Kornprobst 2005; Thomson 2012). Also, numerous studies underline their importance as natural pigments, partially causing the colorful appearance of most species (Baker and Sutherland 1968; Feng *et al.* 2017; Kent *et al.* 1970; Powell and Sutherland 1967; Rideout and Sutherland 1981, 1985; Singh *et al.* 1967; Smith and Sutherland 1971; Sutherland and Wells 1967). Quinonic pigments were even detected in the macrofossils crinoids *Liliocrinus* sp. dated from the Trias-Jurassic period (Wolkenstein *et al.* 2006). It has also been shown that polyketides such as anthraquinonic pigments, especially when sulfated, have a feeding-deterrent activity on fishes and could play the role of chemical defense to deter predators and parasites (Kasumyan *et al.* 2020; Rideout *et al.* 1979; Takahashi *et al.* 2002). Several studies also detail the chemical defense mechanism provided by the secretion of quinones in insects (Berenbaum 1995; Kittredge *et al.* 1974; Pasteels *et al.* 1988). Quinones cytotoxicity is mainly attributed to their

ease of reduction and therefore to their ability to act as oxidizing or dehydrogenating agents (El-Najjar *et al.* 2011).

Although they produce repellent chemicals (Tinkova *et al.* 2014), comatulids usually host various symbiotic metazoans like crustaceans, fishes, gastropods, myzostomids, nematodes, ophiuroids, and polychaetes (Britayev and Mekhova 2011; Deheyn *et al.* 2006; Lanterbecq *et al.* 2010; Zmarzly 1984). Amongst them, the snapping shrimp *Synalpheus stimpsonii* (Alpheidae) is an obligatory ectocommensal regularly found alone or in a heterosexual pair under the calix of their crinoid host (**Figure1a**) where they are protected from predators (VandenSpiegel *et al.* 1998). Shrimps sometimes leave their host to reproduce with a mate located on another host. This host switching behavior has been well explored in ectosymbiotic crustaceans (Baeza and Thiel 2007; De Bruyn *et al.* 2016) and highlights the existence of a specific sensory system allowing the symbiont to select its host. Our team already performed host selection experiments testing the visual and chemical recognition of crinoids by the snapping shrimp *S. stimpsonii* in Hansa Bay, Papua New Guinea (VandenSpiegel *et al.* 1998). The results suggested that the shrimp uses both visual and chemical information to recognize its host at a distance. Chemical communication was much more accurate than the visual one. However, to date, no study has ever identified the kairomone (*i.e.* chemical signal emitted by hosts when they elicit a commensal or a parasitic symbiosis) released by crinoids that influence the shrimp behavior.

In a previous study, we highlighted that the repellent, holothuroid-specific saponins were the attractive kairomones allowing host selection by the holothuroid associate Harlequin crab *Lissocarcinus orbicularis* (Caulier *et al.* 2013). Recently, we also demonstrated that spinochromes (naphthoquinones) produced by the sea urchins *Echinometra mathaei* are recognized by its shrimp symbiont *Tuleariocaris holthuisi* (Brasseur *et al.* 2018b). We hypothesize that many other symbiotic organisms have diverted a natural chemical defense to their advantage: these defensive molecules would be used by symbionts to identify their hosts and to protect themselves against their predators. The present study aims to investigate whether crinoid anthraquinones could be the kairomones attracting the snapping shrimp *S. stimpsonii*. For this purpose, we carried out olfactometry behavioral experiments, anthraquinone chemical extractions, and mass spectrometry analyses.

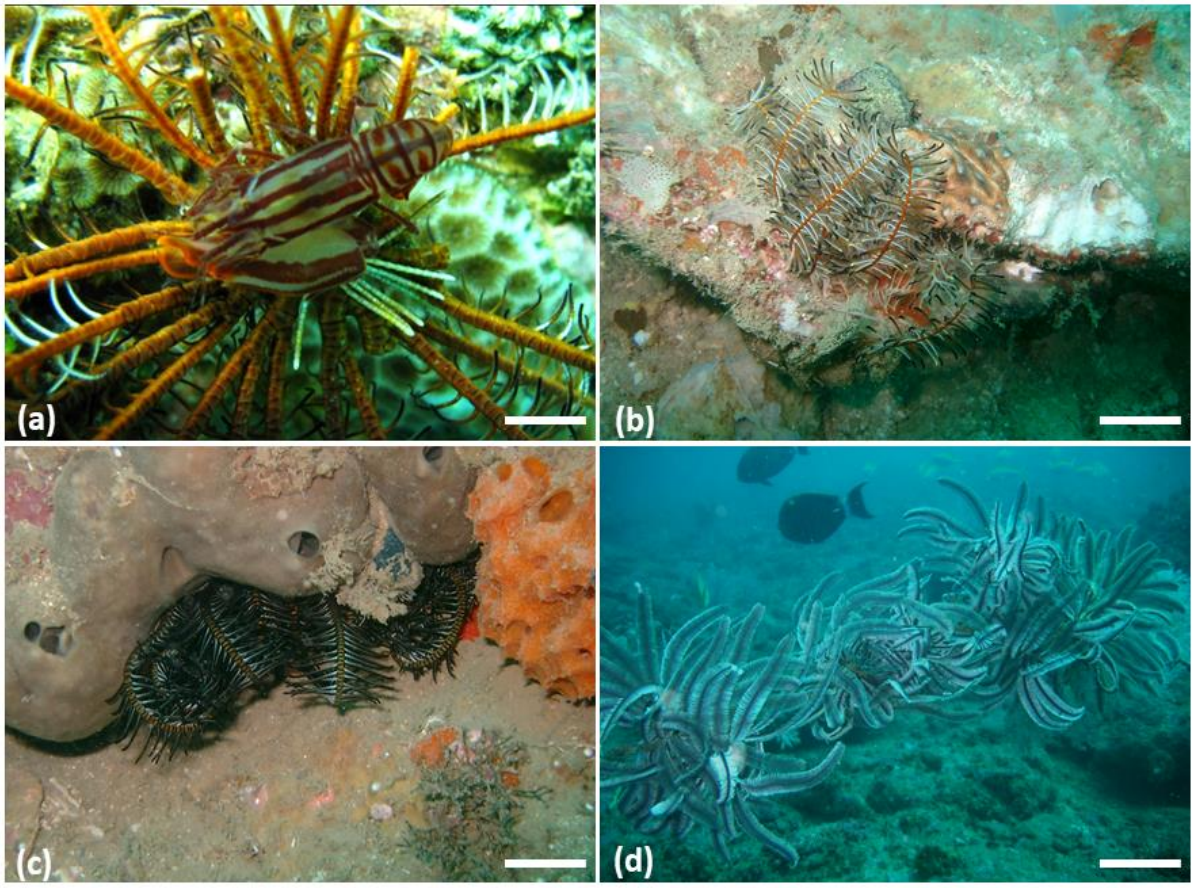


Figure 1: a The snapping shrimp *Synalpheus stimpsonii* under the calyx of a crinoid host *Phanogenia distincta*. b The crinoid *Phanogenia distincta* partially hidden in rock anfractuosity. c The crinoid *Comanthus wahlbergii* partially hidden in rock anfractuosity. d The crinoids *Cenometra bella* attached to a whip black coral. The scale bar represents 6 mm in (a); 26 mm in (b); 28 mm in (c) and 53 mm in (d)

II. Methods and materials

Samplings, chemical extractions, and behavioral experiments were conducted during five scientific field missions in the Fishery and Marine Science Institute of the University of Toliara, South-West of Madagascar (January-February 2011 and 2012; November-December 2013, 2014, and 2019). Chemical purification and mass spectrometry analyses were performed at the University of Mons (Belgium).

(1) Sampling Procedure

Crinoids and their symbiotic shrimps were hand-collected by scuba diving between 18 and 32 m depth on the northern external slope of the Great Reef of Toliara (23° 20' 59" S; 43°36' 45" E). Three crinoid species were commonly found in this area: *Phanogenia distincta* (Carpenter 1888) (**Figure 1b**); *Comanthus wahlbergii* (Müller 1843) (**Figure 1c**) and *Cenometra bella* (Hartlaub 1890) (**Figure 1d**) (Marshall and Rowe 1981). We noticed a

modification in comatulid distribution in the sampling area from one year to another, with a rarefaction of *C. bella* and *C. wahlbergii* along with an increase of the *P. distincta* population. Anthraquinonic extractions (see here below) were then only performed on *P. distincta*. Each sampled crinoid was carefully stored in hermetic plastic bags to prevent the loss of symbiotic shrimps. Once in the laboratory, the crinoid and symbiont species were identified and the number of *Synalpheus stimpsonii* (de Man 1888) recorded. Hosts and symbionts were then kept separately in two semi-open water aquaria for at least 24 h before the behavioral experiments. The seawater used in this study was 1 µm filtered natural seawater, 27 °C and 35 ‰ salinity, pumped directly from the sea. Animals used in our experiments were maintained and treated in compliance with the guidelines specified by the Belgian Ministry of Trade and Agriculture.

(2) Behavioral Experiments

Because host fidelity implies host choice preferences by olfactory imprinting (Hasler *et al.* 1978; VandenSpiegel *et al.* 1998), we only used shrimps collected on *P. distincta* for the behavioral experiments. The chemical communication between symbiotic shrimps and comatulids was tested in a Y-tube olfactometer system (Davenport 1950), following the methodology used in (Brasseur *et al.* 2018b; Caulier *et al.* 2013). The system consisted of a 20 cm long unpaired branch and two 10 cm paired branches that were each connected to a 5-L aquarium. The tube diameter was 3 cm. One of the aquaria (aquarium A) contained test seawater and the other (aquarium B) was filled with control seawater (**Figure S1**). Test water consisted of seawater conditioned either with living crinoids (*i.e.* conditioned with 3 individuals in a 5-L aquarium for 20 minutes) or with purified anthraquinones. Water flowed from these two aquaria through the olfactometer and was evacuated at the base of the unpaired branch of the Y-tube where the shrimp was introduced at the beginning of each test. Flow laminarity inside the olfactometer was controlled using fluorescein salt before the experiments and the water flux was regulated to a speed of 2-3 cm s^{-1}.

An attractive chemical communication was recorded when a significant proportion of shrimps moved up the flow and orientated towards the olfactory stimulus source. During a typical trial, one snapping shrimp *S. stimpsonii* was introduced at the base of the unpaired branch of the Y tube. If it was not stimulated, the shrimp remained at the base of the branch without moving and the run was aborted and considered as null after 10 minutes. If the shrimp was stimulated, it moved into the unpaired branch up to the junction of the two-paired branches where it usually stopped moving, tested the water fluxes, and entered one of the paired branches. Two types of

behaviors were therefore recorded in the Y tube: the motion behavior, when shrimps moved for at least 10 cm into the unpaired branch towards the stimulation source, and the orientation behavior, when shrimps entered one of the two paired branches and reached the corresponding aquarium (Brasseur *et al.* 2018b; Caulier *et al.* 2013). Videos SI1 and SI2 illustrate the motion and the orientation behaviors of the symbiont *S. stimpsonii* in the Y-tube during the end of a test. Those videos were recorded for illustration only as typical tests were not filmed to avoid any bias (*i.e.* the shrimp could visually detect the camera or the motion of the scientist filming).

At least twenty different shrimps were tested for each experiment and aquaria A and B were exchanged every five runs. Each shrimp individual was only tested once to avoid any potential habituation effect. The entire device was washed after each trial (*i.e.* after each shrimp). To test the significance of the motion behavior, the number of times shrimps started to move under a chemical stimulation (*i.e.* chemotaxis) was compared by a Pearson's chi-squared test with Yates continuity correction ($\chi^2 = 0.05$, df = 1) to the number of times shrimps were moving when control seawater filled both aquaria. The orientation behavior was assessed using a binomial test by comparing the percentage of shrimps that entered aquarium A to a random distribution (Probability of success = 0.5). All statistical tests were performed with the R software (R project, x64 3.2.3).

Shrimp behaviors were first recorded when only seawater filled the olfactometer (negative control). Then, chemically mediated host selection was tested with water conditioned by each species of crinoid. Finally, anthraquinones extracted from the host *P. distincta* and pure commercial anthraquinones (Alfa Aesar; CAS 84-65-1) were tested for their potential attractiveness to *S. stimpsonii*. The chemical structure of this commercial compound can be found in supplementary material (**Figure S2**). All extracts were diluted to 0.05 g L^{-1} in artificial seawater (made by dissolving 26 g of NaCl, 6 g of $MgCl_2$, 0.7 g of KCl, 1.47 g of $CaCl_2$ and 0.2 g of $NaHCO_3$ in 1 L ultrapure MilliQ water) and introduced into one of the two aquaria of the olfactometer at a flow rate of 10 ml min^{-1} using a peristaltic pump (Amersham P-1). The natural concentration of anthraquinones released in surrounding water by crinoids is still unknown. Therefore, different anthraquinones concentration used in this study were tested while setting up the protocol of this experiment and the most efficient dose to induce the positive chemotaxis of the symbiotic shrimp *S. stimpsonii* was 0.05 g L^{-1} (data not shown due to low replication). A second negative control was realized with seawater injected by the peristaltic pump.

(3) Anthraquinones Purification

Twenty-six entire *P. distincta* were used for anthraquinone extraction. Each crinoid was briefly rinsed with distilled water in order to remove the salt excess on their integument and was then crushed with a mortar and macerated in 50 ml of acetone for 6 h. The homogenate was then filtrated under vacuum with a Buchner flask and the extract was evaporated at low pressure at 60 °C (Zhu *et al.* 2008) using a rotary evaporator (Laborota 4001 efficient, Heidolph) or an oven. The dried crude extract was dissolved in 50 ml milliQ water and was then partitioned against diethyl ether (v/v; 1:1). The aqueous phase was recovered and dried. Anthraquinone concentrations were then calculated by dividing the dry weight of the anthraquinone extracts by the wet weight of crinoids.

Twenty extracts from twenty different individuals *P. distincta* were combined, dissolved in artificial seawater and tested in the Y-tube olfactometer. Combining the extracts resulted in a homogenous chemical stimulus to be tested in the olfactometer to potentially attract the shrimps. The remaining six extracts were kept separately for mass spectrometry analyses: the aqueous phases were recovered, evaporated to less than 2 ml and centrifuged at 10,000 g for 10 min to remove potential solid residues. Finally, each supernatant was evaporated to dryness using a Speed Vac (RC 10.22, VWR international) and was re-dissolved in 80 % methanol.

(4) *Mass Spectrometry Analyses*

For the LC-MS analyses, a Waters Alliance 2695 liquid chromatography device (HPLC) was used. The system comprises a quaternary pump, a vacuum degasser, and an autosampler. The chromatography was carried out on a reversed-phase column (Kinetex® 5 µm Biphenyl 100 Å, 50 x 4.6 mm, Phenomenex) at 30 °C and with a sample volume injected of 10 µl and a constant flow (1.25 ml min^{-1}) of a gradient of eluent A (water, 0.1 % formic acid) and eluent B (acetonitrile) (Table SI1).

Mass spectra of the purified extracts were first obtained on a Waters Quattro Ultima in the negative ionization mode by scanning between m/z 100 and 1,500 using Electrospray ionization (ESI) source. The ESI conditions were: capillary voltage 3.1 kV; cone voltage 40 V; source temperature 120 °C; desolvation temperature 300 °C. Dry nitrogen was used as the ESI gas with a flow rate of 70 L h^{-1} for the gas cone and 600 L h^{-1} for the desolvation gas. Accurate mass measurements were then performed on a Waters Q-Tof Premier using ESI source, in the negative ionization mode by scanning between m/z 50 and 600 with scan durations of 1s and an interscan time of 0.1 s. The ESI conditions were: capillary voltage 3.1 kV; cone voltage 40 V; source temperature 120 °C; desolvation temperature 300 °C. Dry nitrogen was used as the

ESI gas with a flow rate of 50 L h^{-1} for the gas cone and 600 L h^{-1} for the desolvation gas. The mass spectrometer was equipped with a lockspray source to obtain high mass accuracy of anthraquinones. Sodium iodide ($[I\text{-}Na]^-$= 126.9045), was used as a lock mass, being continuously sprayed into a second ESI source and sampled every 10 s.

Mass spectrum analyses were performed on MassLynx 4.1. mass spectrometry software (Waters). Chemical structures were determined from accurate mass databases and from existing literature. Compositions were validated only under a mass error of 10 ppm. Anthraquinone structures were finally drawn using BKChem software.

III. Results

(1) Occurrence of Shrimps on Crinoids

Table 1 describes the number of collected individuals for each species of crinoids and the occurrence of the snapping shrimps *Synalpheus stimpsonii*. In total, 245 crinoids from three species, *Cenometra bella*, *Comanthus wahlbergii*, and *Phanogenia distincta* were collected. They were associated with 247 *S. stimpsonii*. Only the two species *C. wahlbergii* and *P. distincta* hosted this shrimp species with the prevalence of 84 and 68 %, respectively. No snapping shrimp was found on *C. bella*. Shrimps displayed mimetic colors corresponding to their hosts; individuals collected on *C. wahlbergii* were darker than the individuals collected on *P. distincta*. Sometimes, after several days in an aquarium without hosts, the symbiotic shrimps lost their colors, becoming transparent. Comatulids never hosted more than two snapping shrimps but other symbionts such as myzostomids, eulimids, clingfishes *Discotrema crinophilum*, squat lobsters *Allogalathea elegans* and shrimps *Laoemenes* sp. and *Periclimenes* sp. were regularly found on the same host. Most organisms were finally released in different diving sites near the coral reef after behavioral experiments.

Table 1 Occurrence of *Synalpheus stimpsonii* on three comatulid species in Toliara Bay, Madagascar.

Crinoids			**Shrimps *S. stimpsonii***		
Species	Observed specimens	Colonized specimens	Heterosexual pairs	Single	Total
Cenometra bella	38	0	/	/	0
Comanthus wahlbergii	36	30	24	6	54
Phanogenia distincta	171	116	77	39	193
Total	**245**	**146**	**101**	**45**	**247**

(2) Host Selection Behavioral Experiments

Most *S. stimpsonii* did not move in the olfactometer only filled with seawater ($P > 0.05$) (**Figure 2.1**). Therefore, there is no attractive chemical or rheological stimulus in regular seawater. On the contrary, water conditioned by *C. wahlbergii* or *P. distincta* triggered specific crustacean chemodetection behaviors (*i.e.* flicking of the antennae and the antennules) that subsequently initiated movements of the shrimps towards their host by chemotaxis. Shrimps significantly ($P < 0.01$) performed the "motion behavior" (*i.e.* they moved up-flow in the unpaired branch of the olfactometer) and 80 to 90 % of them significantly ($P < 0.01$) performed the "orientation behavior" (*i.e.* they chose the branch corresponding to their host conditioned water) (**Figure 2.2-3; Video SI1**). The mean time (± SD) required to enter the conditioned aquarium was 1.8 (± 1.1) min. Similar results were obtained with the non-host species *C. bella* (**Figure 2.4**) with a mean time of 2.3 (± 0.8) min. When given the choice between *P. distincta* and *C. bella*, 18 shrimps out of 20 preferred the water conditioned by their host (**Figure 2.5**) in 2.1 (± 0.4) min. Finally, when confronted with odors issued from the two host species *C. wahlbergii* and *P. distincta*, shrimps were attracted by the conditioned water ($P < 0.01$) (no symbiont remained at the origin of the olfactometer) but they did not orientate significantly towards one aquarium ($P > 0.05$) (**Figure 2.6**). In this last test, the mean time of an assay was 3.9 (± 1.6) min.

	Trial	Shrimp choice (%)	Motion	Orientation
1	(A) Seawater vs (B) Seawater	(5) (85) (10)	/	1
2	(A) *C. whalbergii* vs (B) Seawater	(90) (0) (10)	**$3.1\ 10^{-7}$** ***	**$4\ 10^{-4}$** ***
3	(A) *P. distincta* vs (B) Seawater	(80) (0) (20)	**$6.2\ 10^{-7}$** ***	**$4.1\ 10^{-3}$** **
4	(A) *C. bella* vs (B) Seawater	(85) (0) (15)	**$3.1\ 10^{-7}$** ***	**$2.6\ 10^{-3}$** **
5	(A) *P. distincta* vs (B) *C. bella*	(90) (0) (10)	**$3.1\ 10^{-7}$** ***	**$4\ 10^{-4}$** ***
6	(A) *C. whalbergii* vs (B) *P. distincta*	(60) (0) (40)	**$3.1\ 10^{-7}$** ***	0.5

Shrimps that orientated adequately towards the aquarium A
Shrimps that remained stationary in the impaired branch after 10 minutes
Shrimps that orientated adequately towards the aquarium B

Figure 2: Results of the behavioral experiments conducted with a Y-tube olfactometer on the symbiotic shrimp Synalpheus stimpsonii with crinoid conditioned water. The horizontal bar charts represent the percentage of shrimps (value in brackets) that remained stationary in the impaired branch after 10 min (white bars), that orientated adequately towards the aquarium A (black bars) or orientated towards the control aquarium B (striped bars). The P value of the statistic results for motion (Pearson's chi-squared test with Yates continuity correction, $\chi 2 = 0.05$, df = 1) and orientation (binomial test, 0.5 proportion) behaviors can be found in the corresponding columns. Significant results are in bold and characterized by asterisks (*for $p < 0.05$; ** for $p < 0.01$ or *** for $p < 0.001$). N = 20 replicates for each test except for test n° 3 that was realized on 25 replicates. "vs" stands for versus.

(3) Anthraquinones Mass Spectrometry Analyses

Mass spectrometry analyses and accurate mass measurements on the final extracts revealed a cocktail of three anthraquinones (**Table 2**). Two of them had already been described in crinoids (Bartolini *et al.* 1973):) 1,3,8-trihydroxy-6-[(1S)-1-hydroxypropyl] anthracene-9,10-dione (**Figure 3a**) - also known as rhodoptilometrin -, and 4-hydroxy-5,6 dimethoxy-2-

methylbenzo[g]chromen-8-one (**Figure 3b**) - also known as comantherin. The third one is a new anthraquinone congener bearing one or a few alkyl sidechains with a total of 6 carbons. The distribution of the relative intensity of the three ion peaks is well conserved across all replicates (n=6) with rhodoptilometrin as the major ion peak (around 50 times more intense than the other anthraquinone ion peaks) (**Figure 4**). Other ion peaks were observed in the spectra but their composition, derived from their accurate masses, suggests they are not anthraquinone derivatives. Even if the anthraquinonic extracts are not 100% pure, we provide a first attempt to quantify the concentration of these molecules in crinoids. Concentration was calculated as the ratio of the extract dry weight (11.36 (± 3.33) mg) to the entire crinoid wet weight (4.95 (± 1.34) g). The mean concentration of the extract was therefore 3.63 (± 1.2) mg g^{-1}.

Table 2 Anthraquinones extracted from the crinoid *Phanogenia distincta*. Corresponding chemical structures for (a) and (b) can be found in **Figure 3**. Chemical names are provided following the International Union of Pure and Applied Chemistry (IUPAC) nomenclature standard.

Anthraquinone identification	Retention time (min)	Elemental Composition (Neutral)	Theoretical mass $[M-H]^-$ (m/z)	Measured mass $[M-H]^-$ (m/z)	Error (ppm)
(a)1,3,8-trihydroxy-6-[(1S)-1-hydroxypropyl] anthracene-9,10-dione also known as rhodoptilometrin	2.7 – 3.5	$C_{17}H_{14}O_6$	313.0712	313.0700	-3.8
(b)4-hydroxy-5,6 dimethoxy-2 methylbenzo[g]chromen-8-one also known as comantherin	8.3 – 9.0	$C_{16}H_{14}O_5$	285.0763	285.0742	-7.4
(c) Unknown 1	6.0 – 6.7	$C_{20}H_{20}O_2$	291.1385	291.1366	-6.5

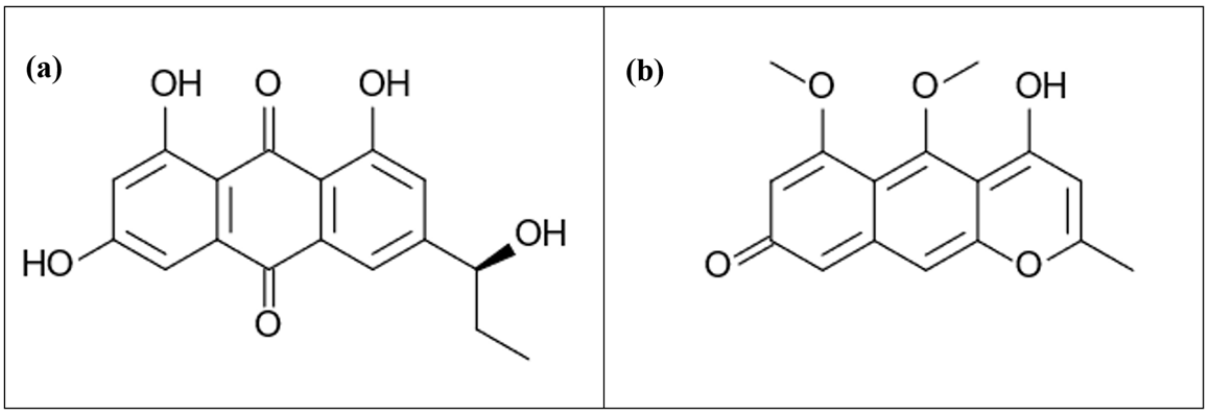


Figure 3: Presumptive molecular structures of the anthraquinones detected in the crinoid *Phanogenia distincta.* a 1,3,8-Trihydroxy- 6-[(1S)-1-hydroxypropyl] anthracene-9,10-dione or rhodoptilometrin and b 4-hydroxy-5,6 dimethoxy-2-methylbenzo[g]chromen-8-one or comantherin. The chemical structure of the new anthraquinone c is not shown as it cannot be confirmed only by mass spectrometry

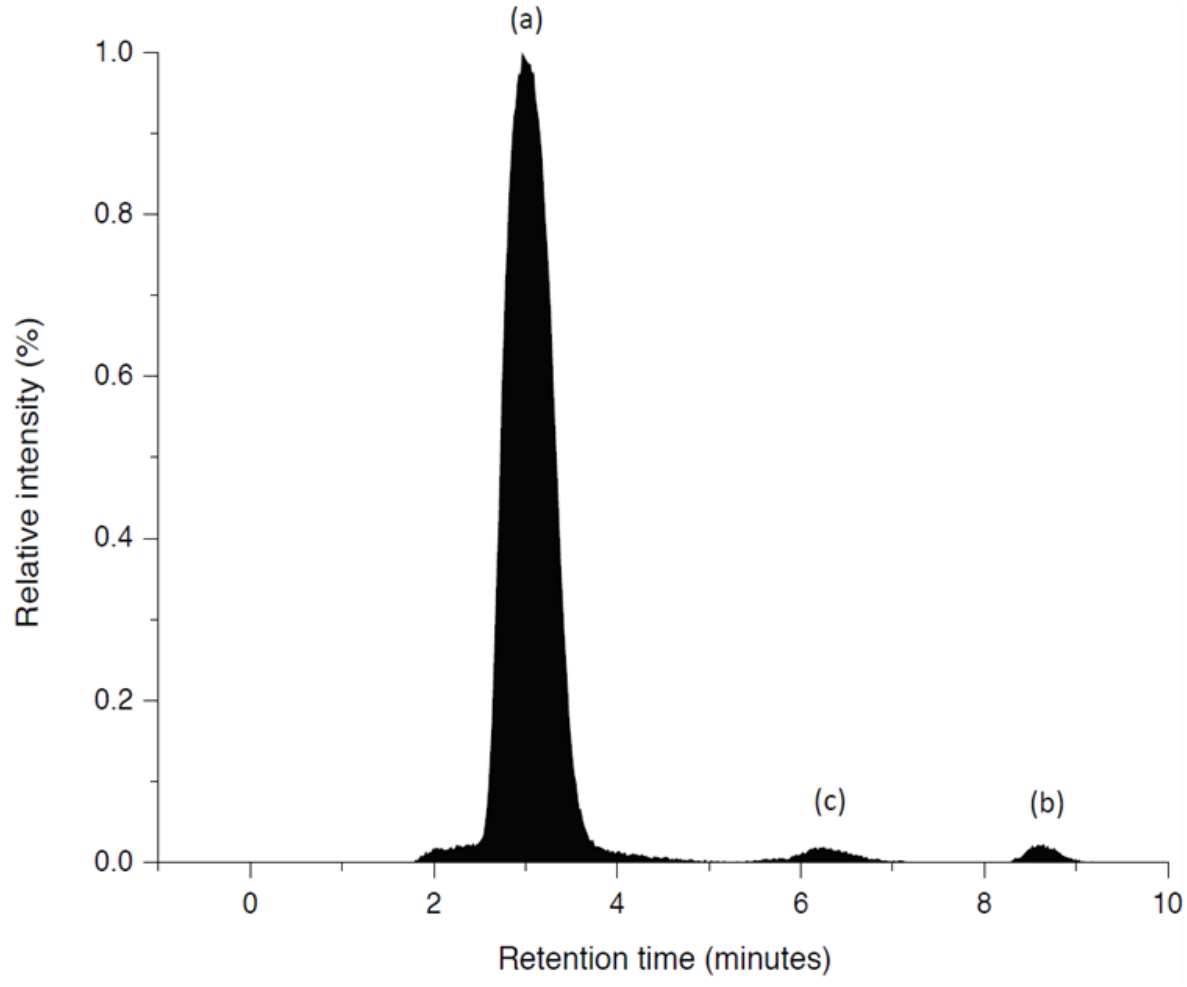


Figure 4: LC–MS consensus chromatogram of Phanogenia distinct anthraquinone extracts from all individuals. a 1,3,8-Trihydroxy-6-[(1S)-1-hydroxypropyl] anthracene-9,10-dione or rhodoptilometrin; b 4-hydroxy-5,6 dimethoxy-2-methylbenzo[g]chromen-8-one or comantherin; c unknown 1. Corresponding MS results can be seen in **Table 2** and corresponding chemical structures for (a) and (b) in **Figure 3**.

(4) Snapping Shrimp Chemotaxis Towards Anthraquinone-Conditioned Water

The use of the peristaltic pump in the olfactometer had no impact on the behavior of *S. stimpsonii* (P > 0.05) (negative control; **Figure 5.1**). Purified anthraquinone extracts from *P. distincta* triggered the same behavior pattern as seawater conditioned by live comatulids. Several shrimp individuals were even found closely in contact with the distal tube of the peristaltic pump. Seventeen individuals out of 20 were chemically attracted by the purified anthraquinones (**Figure 5.2; Video S2**). Commercial anthraquinones were also attractive to the symbiotic shrimps (**Figure 5.3**). The mean time shrimps required to enter the conditioned aquarium was 2.3 (± 0.8) min with *P. distincta* extracts and 3.5 (± 1.7) min for synthetic anthraquinones.

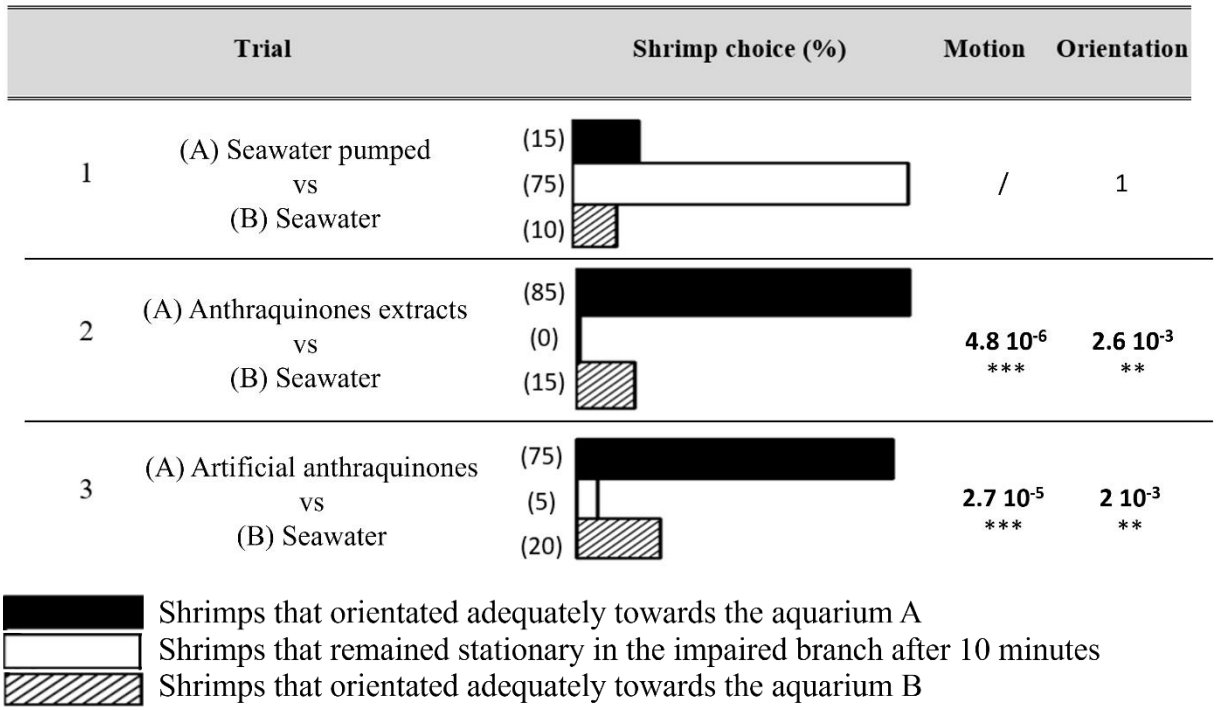


Figure 5: Results of the behavioral experiments conducted with a Y-tube olfactometer on the symbiotic shrimp *Synalpheus stimpsonii* with water conditioned by anthraquinones extracted from *Phanogenia distincta* or by commercial anthraquinones. The horizontal bar charts represent the percentage of shrimps (values in brackets) that remained stationary in the impaired branch after 10 min (white bars), orientated adequately towards aquarium A (black bars) or orientated towards the control aquarium B (striped bars). The P value of the statistic results for motion (Pearson's chi-squared test with Yates continuity correction, $\chi 2 = 0.05$, df = 1) and orientation (binomial test, 0.5 proportion) behaviors can be found in the corresponding columns. Significant results are in bold and characterized by asterisks (*for $p < 0.05$; ** for $p < 0.01$ or *** for $p < 0.001$). N = 20 replicates for each test. "vs" stands for versus

IV. Discussion

In addition to the present work, two other studies, those of VandenSpiegel *et al.* (1998) and Eeckhaut *et al.* (2001) focused on the chemical attractiveness of crinoids on symbionts. The Papuan *Synalpheus stimpsonii* studied by VandenSpiegel *et al.* (1998) were observed on 4 crinoid species out of the 25 recorded in the research area (Hansa Bay) (Deheyn *et al.* 2006). The crab *Harrovia longipes*, also studied in Papua New Guinea by Eeckhaut *et al.* (2001), was observed on 9 crinoid species out of 25 (Deheyn *et al.* 2006). In the two cases, olfactory experiments highlighted some general rules about the chemoattraction exerted by crinoids on crustacean symbionts: (*i*) symbionts are chemically attracted by their crinoid hosts, (*ii*) they also are chemically attracted by non-host crinoids and (*iii*) they can chemically distinguish host crinoids from non-host crinoids. Eeckhaut *et al.* (2001) also demonstrated that the crabs *H. longipes* are not attracted by other echinoderms (holothuroids and echinoids). From an ecological point of view, the ability to detect the proper host is an advantage that ensures the obligatory and specific symbiont to live at the safest place: they can hide on crinoids and be protected by their repellent semiochemicals. In nature, the crinoid semiochemicals allow shrimps to discriminate hosts from non-hosts with a significant preference for the hosts although the specificity of the observed associations is due to multiple factors. In Madagascar, the crinoid specificity shown by *S. stimpsonii*, recorded on the crinoids *Phanogenia distincta* and *Comanthus wahlbergii* but not on *Cenometra bella*, can be explained by ecological factors. The way of life of the first two species is very different from the one of the last species: *C. bella* lives mainly tied on the top of black coral branches, always exposing their whole body to water current, whereas the two hosts are usually hidden in crevices of the coral reef (Tinkova *et al.* 2014). The shrimps settling on *C. bella* would be much less protected than those on the hosts and would potentially be eliminated through predation.

The anthraquinonic content of crinoids has been reported for several species (Bartolini *et al.* 1973; Feng *et al.* 2017; Kemami Wangun *et al.* 2010; Kent *et al.* 1970; Lee and Kim 1995; Rideout *et al.* 1979; Thomson 2012; Tseng *et al.* 2019; Wolkenstein *et al.* 2018; Wolkenstein *et al.* 2009). As in other studies, only a few different anthraquinones molecular structures were detected in *P. distincta*. Indeed, in general, only two or three congeners have been found in each species investigated, and even though Bartolini *et al.* (1973) found five different pigments in *Comanthus bennetti*, four were very close and only differed in their degree of oxidation. However, one anthraquinonic pigment, rhodoptilometrin, was found across all species studied

until now. This particular molecule could play an important metabolic/ecological role or could have a major function in the biosynthesis of other anthraquinones.

As an obligatory symbiont, the snapping shrimp could have adapted its chemosensory system to detect preferentially anthraquinones specific to crinoids. However, since they are also attracted by synthetic anthraquinones, a broad range of anthraquinones may attract *S. stimpsonii*. It can also discriminate the chemical signals between a host and non-host feather star what could be explained by different anthraquinonic cocktails or the presence of other semiochemicals than anthraquinones acting as attracting kairomones. In vivo, host selection by snapping shrimps is not only performed by chemical detection of anthraquinones but also by visual stimuli perception (Huang *et al.* 2005; VandenSpiegel *et al.* 1998). Therefore, visual communication probably also plays a role to avoid non-hidden or colorful feather stars like *C. bella* (Huang *et al.* 2005; VandenSpiegel *et al.* 1998).

Mimetic colors of the shrimps could be caused by the accumulation of anthraquinonic pigments in the symbiont tissues (see Brasseur *et al.* 2018b). Comparison of our results with those of the literature shows that *P. distincta* has its own cocktail of three different anthraquinone pigments, giving it a proper chemical signature. Two of these anthraquinones are well known in the literature (Feng *et al.* 2017; Kent *et al.* 1970; Rideout *et al.* 1979) and the third one is a potentially new anthraquinone. Although mass spectrometry can only provide accurate mass information and so potential composition, we can estimate that this new molecule is organized as an anthraquinone derivative with one or a few alkyl sidechains (with 6 carbons in total). Future works may concentrate on the anthraquinone extraction method detailed in this study in order to obtain enough pure dry extract to conduct NMR-spectroscopy analyses that could reveal the precise chemical structure of this new anthraquinone and its name. The relative intensity of ion peaks showed a large overabundance of rhodoptilometrin compared to the other anthraquinones. This result can be directly related to the molecule abundance in the crinoid extract but maybe also to its higher ionization potential. Only additional analyses with internal referents could allow quantifying the relative proportion of each congener in the anthraquinone cocktail. The natural concentration of anthraquinones contained in the tissues of crinoids is not currently known. A similar question concerns the amount of kairomones released in surrounding water by crinoids.

So far, only three host chemical factors involved in marine symbiotic associations have been identified in comparison to the thousands of molecules highlighted in plant-insect interactions,

which underlines the significant lack of knowledge existing in marine chemical ecology. The first group of molecules is the amphikuemins (Murata *et al.* 1986) which acts in the symbiosis between sea anemones and anemonefishes (Konno *et al.* 1990). The second involves the saponins (Caulier *et al.* 2013) which mediates partner-recognition between the Harlequin crabs *Lissocarcinus orbicularis* and their holothuroid hosts. The kairomonal function of holothuroid saponins has also been confirmed in another symbiotic interaction, these molecules being chemoattractant for the symbiotic pearlfish *Encheliophis vermicularis* (Miyazaki *et al.* 2014). The third group of molecules is spinochromes (naphthoquinones) (Brasseur *et al.* 2017) which are produced by the sea urchins *Echinometra mathaei* and recognized by its shrimp symbiont *Tulеariocaris holtuisi* (Brasseur *et al.* 2018b). We here demonstrate that crinoid anthraquinones are attractive to a symbiotic shrimp. In addition to their traditional defensive role (repellent allomones), quinones therefore would also function as attracting kairomones, maintaining the symbiosis between *S. stimpsonii* and its crinoid hosts.

Chapter II



Species-specific metabolites mediate host selection and larval recruitment of the symbiotic sea star shrimp

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Abstract

In marine environments, host selection, defining how symbiotic organisms recognize and interact with their hosts, is often mediated by olfactory communication. Although adult symbionts may select their hosts detecting chemosensory cues, no information is available concerning the recruitment of symbiotic larvae which is a crucial step to sustain symbioses over generations. This study investigates the olfactory recognition of sea star hosts by adult *Zenopontonia soror* shrimps and the recruitment of their larvae. We examine the semiochemicals that influence host selection using chemical extractions, behavioral experiments in olfactometers, and mass spectrometry analyses. After describing the symbiotic population and the embryonic development of shrimps, our results demonstrate that asterosaponins, which are traditionally considered as chemical defences in sea stars, are species-specific and play a role in attracting the symbiotic shrimps. Adult shrimps were found to be attracted only by their original host species *Culcita novaeguineae*, while larvae were attracted by different species of sea stars. This study provides the first chemical identification of an olfactory cue used by larvae of symbiotic organisms to locate their host for recruitment. These findings highlight the importance of chemical communication in the mediation of symbiotic associations, which has broader significant implications for understanding the ecological dynamics of marine ecosystems.

Keywords
Marine Chemical Ecology; Chemical communication; Asterosaponins; Kairomones; Decapoda; Echinodermata.

I. Introduction

Symbiotic relationships, defined as close and long-lasting associations between two different species, are ubiquitous throughout the biosphere (Paracer and Ahmadjian 2000; Parmentier and Michel 2013). The impact of a symbiont on the host fitness varies depending on factors such as the type of symbiosis, the developmental stage of the symbiont, or external stressors like food scarcity or environmental changes (Johnstone and Bshary 2002; Trilles and Hipeau-Jacquotte 2012; González et al. 2021). However, symbionts always drift off a fitness advantage from the symbiotic relationship, often resulting in a strong host dependence (Paracer and Ahmadjian 2000; Lanterbecq et al. 2010; Horká et al. 2016). Co-evolution is frequent in symbiotic associations and leads to intrinsic physiological, morphological, and behavioral adaptations (Gherardi 1991; Eeckhaut and Jangoux 1997; Lanterbecq et al. 2010; Horká et al. 2016). One key factor that sustains symbiotic relationships across generations is the establishment of a specific recognition, allowing the symbiont to selectively associate with its host (Derby and Sorensen 2008).

Host recognition is achieved through multimodal communication driven by various stimuli such as visual, acoustic, and chemical signals (Ache and Davenport 1972; Antokhina and Britayev 2020). Chemical sensing is considered as the most common form of communication in marine environments (Eeckhaut et al. 2000; Fourgon et al. 2007; Caulier et al. 2013b). Chemical signals are specific metabolites referred to as ecomones or semiochemicals (Eeckhaut et al. 2000; Fourgon et al. 2007; Caulier et al. 2013b). In interspecific relationships, three categories of ecomones can be distinguished: allomones, kairomones, and synomones. These molecules provide benefits for either the producing organism, the receiving organism, or both, respectively(Dicke and Sabelis 1988). As the symbiont always receives a benefit from the symbiosis, the molecules involved in host recognition are either kairomones in parasitic or commensal associations or synomones in mutual relationships (Caulier et al. 2013b).

Although chemical signalling plays a crucial role in the establishment of symbiotic relationships (Gage 1966; Dimock and Davenport 1971; Eeckhaut et al. 2000; Zimmer and Butman 2000; Fourgon et al. 2007), the exact chemical nature of these signals remains largely unidentified in marine organisms. Currently, only four different host recognition semiochemicals have been identified so far in marine environments (Murata et al. 1986; Caulier et al. 2013b, 2022; Brasseur et al. 2018). These structurally and functionally diverse molecules include the amphiphilic steroid saponins involved in the association between sea cucumbers and Harlequin

crabs (Caulier et al. 2013b) and the hydrophobic amphikuemin acting as a synomone allowing the sea anemone – clown fish symbiosis (Murata et al. 1986). Also, two hydrophobic kairomones were recently discovered, the spinochromes produced by sea urchins and recognized by symbiotic shrimps (Brasseur et al. 2018) and anthraquinones that enable recognition between crinoids and pistol shrimps (**Chapter I**). These cocktails of molecules are chemical signatures that are specific to the hosts, allowing a precise host selection by the symbionts. In comparison with the large number of kairomones that have been identified in terrestrial environment (Metcalf and Metcalf 1992) and given the widespread occurrence of symbioses in marine environments, a multitude of other host recognition kairomones certainly await discovery.

Considering that most marine symbiotic invertebrates have an indirect life cycle (Vance 1973), generating one or several pelagic larval stage(s), the question on how these symbiont larvae can recognize their benthic host to ensure a successful recruitment and settlement remains unanswered. While previous studies have suggested that chemical sensing plays a role in larval settlement (Marsden 1987; Hadfield and Paul 2001; Cowan et al. 2016; Lecchini et al. 2017; Doll et al. 2023), this has yet to be demonstrated in the context of marine symbiotic associations. This study aims to address this gap by examining the chemical signals detected by adults and larvae of the symbiotic sea star shrimps *Zenopontonia* (ex. *Periclimenes*) *soror* (**Figure 1C-E**) living on the cushion sea star *Culcita novaeguineae* (**Figure 1G**). *Z. soror* is an obligatory symbiont of tropical sea stars and is known to be associated with 23 species of asteroids (Antokhina and Britayev 2020). Asteroid specific steroidal glycosides, also known as asterosaponins (Demeyer et al. 2014), primarily described as metabolites having a role in chemical defence (Demeyer et al. 2014), were here investigated as kairomone candidates. This hypothesis is founded on recent studies proving that echinoderm-specific defensive semiochemicals could also act as pheromones (Claereboudt et al. 2023) but also as kairomones in symbioses (Caulier et al. 2013b; Brasseur et al. 2018). In the present study, these molecules were tested for the host recognition of adult shrimps, and, for the first time, for the host recognition of their larvae. The scientific strategy involved (i) behavioral tests in two different olfactometer models, (ii) saponin extractions from the original host *C. novaeguineae*, potential hosts (*Acanthaster planci*; *Linckia laevigata*) (Bruce 1976a; 英男 and 喬司 1988; Guenther et al. 2007; Olliff 2013; Schelvis 2017; Antokhina and Britayev 2020), and non-host species (*Holothuria scabra*; *Asterias rubens*; *Echinaster sepositus*), and (iii) investigation of the diversity of asterosaponins by mass spectrometry analyses.

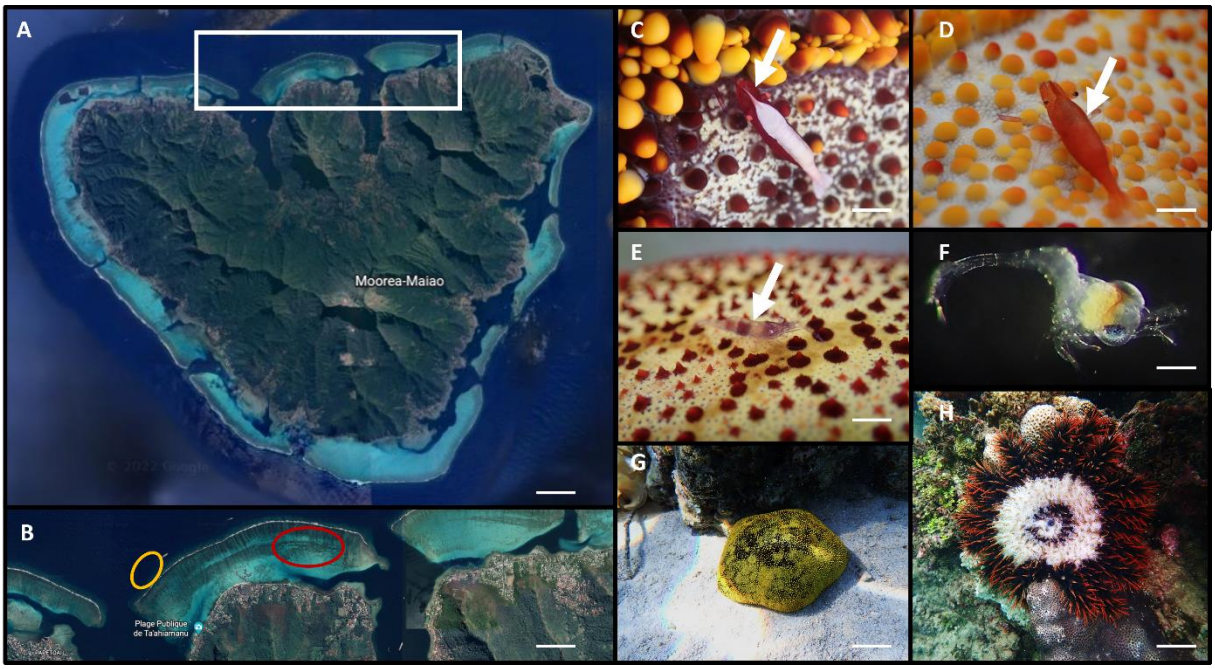


Figure 1: Description of the model organisms investigated and localization: (A) Map of Mo'orea Island highlighting the collection area (white rectangle); (B) Zoom of map A showing the precise collection site of *Culcita novaeguineae* in red, and the collection area of *Acanthaster planci* in orange. (C – E) Different morphotypes of the symbiotic shrimps *Zenopontonia soror*, with a white arrow highlighting the symbionts. (F) Zoeal larval stage of *Z. soror*. (G) The host *C. novaeguineae* in its environment and (H) *A. planci* on a coral colony. A and B were modified from ©Google Earth, version 9.181.0.1. Scale bars represent 0.2 km in A; 1.6 km in B; 0.5 cm in C; 0.6 cm in D; 0.5 cm in E; 100 µm in F; 10 cm in G and H.

II. Method

Two scientific expeditions were conducted during July and August 2021 and 2022 at the Centre for Island Research and Environmental Observatory (CRIOBE) located in Mo'orea, French Polynesia. Sample collection, larval rearing, behavioral experiments, and chemical extractions were performed on site. Chemical purification and mass spectrometry analyses were performed at the University of Mons (Belgium).

(1) Sampling, organism maintenance, larval rearing

Zenopontonia soror (Nobili, 1904) and its most frequent host in the area, *Culcita novaeguineae* Müller & Troschel, 1842, were hand-collected using free diving techniques in depths ranging from 1 to 5 metres on the northern coast of Mo'orea, located between the Opunohu and Cook Bays (17° 28′ 50" S; 149° 50′ 00" W; **Figure 1A-B**). The collected sea stars were carefully stored in 3L-hermetic plastic bags filled with seawater to prevent the loss of symbiotic shrimps. Upon arrival in the laboratory, the symbionts were inventoried, their length carefully measured using graph paper, their number recorded and their morphotype determined (**Figure 1C-E**). Each shrimp individual investigated in this study originated from a cushion sea

star. Hosts and symbionts were then kept together in a 300 L open-water tank with a sandy substrate for a minimum of 24 hours until further experimentation. During that time, the symbionts could switch from one host individual to another. Prior to the behavioral experiments, the sea stars and shrimps were separated and isolated individually in 30 L tanks for a minimum of three hours. All behavioral experiments were performed using 1 µm-filtered seawater, with a temperature of 28-29°C and a salinity of 35‰, pumped directly from the Opunohu Bay facing the marine station. For asterosaponin extractions (see here below), five *C. novaeguineae* and one crown-of-thorns sea star *Acanthaster planci* (Linnaeus, 1758) were harvested. The latter was hand-collected by scuba diving at 20 metres depth, on the external reef outside the Opunohu Bay (17° 28′ 51” S; 149° 51′ 21” W; **Figure 1A-B**).

To obtain *Z. soror* larvae (**Figure 1F**), 15 gravid shrimps were kept in low-volume individual containers under a constant light and moderate elevated temperature (29°C) for 6 hours at different periods of the day. The eggs of five females hatched around 10 PM and the larvae were used directly for behavioral experiments. During these treatments, eggs were regularly collected on the bottom of the container and characterized under binoculars to evaluate the embryonic development of the egg. For each developmental stage investigated, n=6 propagules and n=10 zoeal larvae were considered for the description.

After experimentation, all cushion sea stars and their associated shrimps were returned to their original location in the lagoon, with the exception of those preserved for saponin extractions. Animals used for experiments were maintained and treated in compliance with the guidelines specified by the French Polynesia’s Environment Department (DIREN) and by the Belgian Ministry of Trade and Agriculture.

(2) Chemical characterization

The behavioral experiments on adult shrimps and their larvae (see here below) required the chemical extraction of asterosaponins from three hosts and three non-host species. The non-host species, *Echinaster sepositus, Asterias rubens,* and *Holothuria scabra* were not collected in Mo’orea but in the Mediterranean Sea (Algiers bay; Pointe Pescade; Algeria) and North Atlantic Ocean (Audresselles; Opal Coast; France) for the first two sea stars, and the Mozambican channel (Great Reef of Toliara; Madagascar) for the third one, a sea cucumber. They have not been previously recorded as associates of *Z. soror* which is exclusively associated with tropical sea stars (Bruce 1976a; 英男 and 喬司 1988; Guenther et al. 2007; Olliff 2013; Schelvis 2017; Antokhina and Britayev 2020). The contents of saponins in these

species have previously been thoroughly characterized in our laboratory(Demeyer et al. 2014; Dahmoune et al. 2021; Savarino et al. 2022) and preserved chemical extracts from these studies were used for olfactometer experiments. The extraction and mass spectrometry analyses of asterosaponins from the potential host species, *C. novaeguineae*, *A. planci*, collected in Mo'orea, and *Linckia laevigata*, from the Great Reef of Toliara (Madagascar) were conducted using the following standardized methodology.

(3) Saponin extractions

The body walls of each species were dissected and lyophilized with an Alpha 1-2 freeze dryer for 48 hours to preserve the chemical nature of the metabolites. The dried samples were then finely ground using a mechanical crusher IKA A11 and homogenized in 100% methanol technical grade solution for 12 hours. The methanolic extract was recovered through centrifugation (10 min; 4500 rpm) and diluted with Milli-Q water to reach a 70:30 MeOH:H_2O ratio. It was partitioned successively through three liquid-liquid extractions using 100 % *n*-hexane C_6H_{14} technical grade (*v*:*v*), 100% chloroform $CHCl_3$ technical grade (*v*:*v*) and finally 100% dichloromethane CH_2Cl_2 technical grade (*v*:*v*). The final hydromethanolic solution was evaporated using a rotary evaporotator (Laborata 4001 efficient, Heidolph) at low pressure in a double boiler at 60/65 °C. The dry residue was redissolved in Milli-Q water and was then purified using the last liquid/liquid extraction with isobutanol HPLC grade (*v*:*v*). The solution was washed three times with Milli-Q water to remove inorganic salts and recover the purified saponins, and this butanolic fraction, containing the dissolved saponins, was re-dried using the rotary evaporator with the same parameters described earlier. The dry extracts were weighed and stored in the dark at -20°C until use in olfactometry experiments or mass spectrometry analyses.

(4) Mass Spectrometry analyses

The purified asterosaponin extracts underwent two types of spectrometric analyses(Savarino et al. 2021): (i) identification and quantification of the saponins present in the extracts and (ii) exact mass analysis. All the mass spectra were obtained using a mass spectrometer (Waters SYNAPT G2-S*i*) coupled with a liquid chromatography device (Waters Acquity H-class) and analyzed with MassLynx 4.1 software (Waters).

Liquid chromatography was performed with 5 µl of the sample on an ACQUITY UPLC BEH C18 reverse phase UPLC column, 130 Å, 1.7 µm, 2.1 mm X 50 mm at 40°C with a gradient of eluent A, consisting of a 60/40 mixture of a solution of milli-Q water and formic acid HPLC

grade (HCOOH, 0.1%) and eluant B composed of acetonitrile HPLC grade at a constant rate of 250 µl/min as explained in Brasseur et al. 2018. Considering that the vast majority of currently described asterosaponins in sea stars have a sulphate moiety(Demeyer et al. 2014; Kicha et al. 2014; Stonik et al. 2020; Ha et al. 2021), mass spectrometry analyses were performed via electrospray negative (-) or positive (+) ionization of the molecules (ESI). Note that, in the present study, we did not intend to structurally characterize all the saponin molecules which usually requires other techniques such as proton high magnetic resonance. We thus only reported the saponin elemental compositions with regards to the LC elution times, see **Table 1**.

The following parameters were used for the ESI: a capillary voltage of 2.5 kV (-) or 3,1 kV (+), a cone voltage of 40 V, a source offset of 80 V, a source temperature of 100 °C and a desolvation temperature of 300 °C. The ESI gas used was dry nitrogen supplied at a flow rate of 60 L/h for the gas cone and 500 L/h for the desolvation gas. The quadrupole was set to pass ions from *m/z* 50 to 2000, all the ions were transmitted to the pusher region of the time-of-flight analyser with an integration time of 1 second. A quantitative comparison was made for each congener by comparing the area under their LC spectrum peaks to an internal reference, hederacoside C, which also allowed determining the sample purity. To obtain the exact masses of saponin ions, the analyses were carried out in positive mode via electrospray ionization (ESI) of the molecules with the Hederacoside C ions ($[M-H]^-$ - *m/z* 1219.6, $[M+H]^+$ - *m/z* 1221.6) as the internal standard (lock mass).

The relative quantification of natural extracts was achieved by adding a known quantity (0.1 $mg \cdot mL^{-1}$) of commercially available hederacoside C (Sigma-Aldrich – Product n° 97151 – M-ClarityTM Program MQ100), as an internal standard in all solutions of saponin extract at a given concentration, typically 0.1 $mg \cdot mL^{-1}$. The spiked solution was analyzed using LC-MS (injection of 5 µL) using the experimental conditions described here above. For each saponin molecule, including hederacoside C, the corresponding LC-MS ion signals - including all the isotopic compositions - were integrated using the integration algorithm available under MassLynxTM 4.1 Software. The global ion counts were then used to estimate the relative concentration (mol-%) of each saponin congener within the saponin cocktail. The %-weights in extract correspond to the mass percentages of saponin congeners relative to hederacoside C within the saponin extracts. Note that the sum of the %-weight does not reach 100 % allowing to estimate the saponin content (purity) within the extract.

(5) Behavioral experiments

The chemical communication behavior between the sea star shrimps and the different host and non-host species was studied using two types of olfactometers (**Figure 2**) including an innovative Y-tube device made-to-measure larval chemotaxis under low flow condition (**Figure 2 B** and **E**). The experiments were conducted in two stages: (i) investigation of the chemical communication between adult and larvae of *Z. soror* and their host species *C. novaeguineae*, and (ii) identification of the potential kairomonal role of saponins extracted from various species. One of the aquaria (A) of the olfactometer contained conditioned seawater with either living sea stars or purified saponins, while the other (B) was filled with control seawater. Test water consisted of seawater conditioned either with living sea stars (*i.e.* conditioned with 2 individuals in a 10 L-aquarium for 2 hours) or with purified asterosaponins from *C. novaeguineae* or *A. planci* diluted with a concentration of 0.01 mgL^{-1} (determined empirically in preliminary assays). Flow laminarity, flow speed inside the olfactometers was controlled using fluorescein salt before the experiments (**Figure 2 D** and **E**). A minimum of 20 replicates using 20 adult shrimps (using the three different morphotypes) were performed for each experiment, and the aquaria were exchanged every 10 runs (to consider any variations that could exist on either side of the olfactometers). Once tested, each adult shrimp individual was left on their host for 10 days between experiments. A minimum of twenty larvae were also used in one experiment (20 assays) and each larva was tested only once directly after hatching. The entire olfactometer was washed after each trial. A negative control, with both aquaria filled with seawater, was performed on experiments using adults and larvae. Experiments were performed in a dark room with artificial low-intensity LED lights.

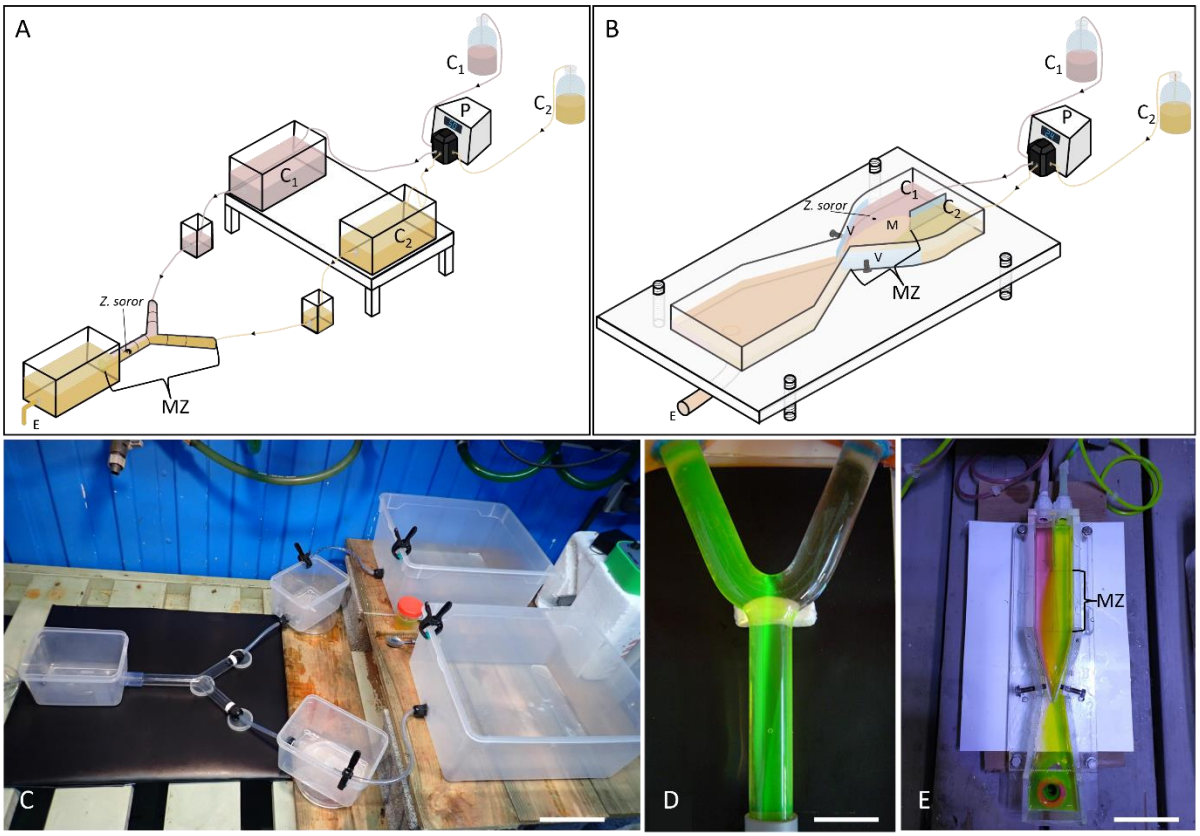


Figure 2: Experimental devices used in behavioral experimentations : (A) olfactometer used for adults *Zenopontonia soror*. (B) low-flow olfactometer used for *Z. soror* larvae. C1 = chemical cue 1; C2 = chemical cue 2; M = Mix of both cues; MZ = monitoring zone; P= peristaltic pump; V = Valve composing the valve regulation system; E = Exit. (C) real experimental device used for adult Z. soror. (D) Zoom of the Y-tube (corresponding to the monitoring zone) used for adult *Z. soror*, with the visualization of the flow laminarity using fluorescein and (E) real low-flow olfactometer used for *Z. soror* larvae, with the visualization of the flow laminarity using food colorants. MZ = monitoring zone. Scale bars represent 13.3cm in C; 3.3cm in D; 5cm in E.

(6) Olfactometer experiments on Z. soror adults

The chemical attraction of *Z. soror* adults towards *C. novaeguineae* was evaluated using a Y-tube olfactometer system (**Figure 2 A, C** and **D**) similar to the one described in several previous studies(Caulier et al. 2013b, 2022; Brasseur et al. 2018). The system consisted of a 20 cm long unpaired branch connected to two 10 cm paired branches that were each connected to a 10 L-aquarium. The glass tube diameter was 3 cm. Water flowed from these two aquaria through the olfactometer and was evacuated at the base of the unpaired branch of the Y-tube where the shrimp was introduced at the beginning of each test and the water flow was regulated to a speed of 2–3 cm s^{-1}. An attractive chemical communication was recorded when a significant proportion of shrimps moved up the flow and orientated towards the olfactory stimulus source. During a typical trial, one *Z. soror* was introduced at the base of the unpaired branch of the Y tube. If it was not stimulated, the shrimp remained at the base of the branch without moving and the run was aborted and considered as null after 5 min (Null). If the shrimp

was stimulated, it moved into the unpaired branch up to the junction of the two-paired branches where it usually stopped moving, tested the water fluxes, and entered one of the paired branches. Two types of behaviors were therefore recorded in the Y tube: the motion behavior, when shrimps moved for at least 10 cm into the unpaired branch towards the stimulation source, and the orientation behavior, when shrimps entered one of the two paired branches and reached the corresponding aquarium [13,15,16]. **Videos SI1** and **SI2** illustrate the motion and the orientation behaviors of the shrimp *Z. soror* in Y-tubes. **Video SI1** represents a typical *Z. soror* that behaves in response to a positive chemical stimulus (positive motion and orientation behavior) and **video SI2** corresponds to a shrimp that is not attracted by any chemical cue and remains still in the device (null result). Those videos were recorded for illustration only as typical tests were not filmed to avoid any bias and were performed in low light condition.

(7) Olfactometer experiments on Z. soror larvae

The chemical attraction of *Z. soror* larvae towards *C. novaeguineae* was evaluated in a new innovative low-flow olfactometer device **Figure 2 B** and **E**) designed to measure chemotaxis in a low current allowing the larvae to swim in the system. It consisted of a 25 cm long plexiglass compartment, with the upper extremity connected to a dual flow peristaltic Cole-Parmer MasterFlex® pump bringing cues from two 10 L-aquaria. Water flowed from the upper extremities through the olfactometer and was evacuated at the base of the bottom extremity. The two flows were kept separated by a separation thin wall on the first 3 cm of the device, to stabilize and discriminate the two laminar flows. Downstream in the system, the flows mixed creating a chemical gradient (**Figure 2 B** and **E**). The monitoring zone (*i.e.* where the larvae were introduced, and their behaviors were recorded) was comprised between the boundary where cues mix together and the valve regulation system limiting the flow speed. Flow laminarity inside the olfactometer was controlled using two different food coloring before experiments (**Figure 2 E**) and the water current was regulated with a pump to 24 mL min^{-1}. An attractive chemical communication was recorded when a significant proportion of larvae swam actively and orientated towards the olfactory stimulus source. If it was not stimulated, the larvae remained immobile and were passively transported (*i.e.* drifting) through the monitoring area without a clear orientation in less than 30 seconds. In that case, the trial was considered as Null. During a typical trial, if larvae were stimulated, they moved actively and could choose a side of the device while actively progressing against the low current. Two types of behaviors were therefore recorded in larvae olfactometers: the motion behavior corresponding to the observation of swimming movements of the larvae in the monitoring zone and the orientation

behavior that was measured with the position record occurring every 5 seconds (left, middle or right in the monitoring zone) of the device. The drifting time the larvae that stayed inside the monitoring zone were also recorded, with a maximum of 200 seconds that corresponded to the end of the experimentation.

(8) Statistical analyses

The motion behavior of adult shrimps and larvae was evaluated by comparing the number of times they started to move under cue stimulation (chemotaxis) to the number of times they moved in response to control seawater (negative test), using a Pearson's chi-squared test with Yates continuity correction ($\chi 2 = 0.05$, df = 1). The orientation behavior of adult shrimps and larvae was determined by comparing the percentage of individuals that oriented towards the side with a chemical to a random distribution (binomial test, probability of success = 0.5). The duration that larvae remained in the low-flow device was assessed using an analysis of variance (ANOVA) considering a significant drifting time difference between tests at the α level of 5%. Multiple comparisons of means (Tukey HSD) were performed to compare the results. All statistical tests were performed with the R software (R project, .64 3.2.3; R Core Team). For every test, results were considered significant when the P-value is egal or inferior to 0.01.

III. Results

(1) Description of the symbiotic associates

A total of 71 *C. novaeguineae* sea stars, with a diameter varying from 12 to 19 cm (mean ± SD of 14.5 ± 1.3 cm) were collected in the inter-bays of the lagoon of Mo'orea. They were usually hidden under coral patch reefs or rocks during the day and more visible and active during the night in natural and artificial conditions (*e.g.* climbing the tanks vertical walls each night from the sunset to the sunrise). Sampled cushion stars hosted 152 symbiotic shrimps *Z. soror*. The symbiotic occurrence of the association was 69.2%, most shrimps being found externally on the aboral side of their host with 1 to 14 individual(s) per host (**Figure 3A**). The mean of the symbiotic load was 2.1 ± 2.6 (mean ± SD) shrimps per sea star. Interestingly, one shrimp individual was observed alive inside the mouth of its host (**Figure 3B**). Three color morphotypes were observed for the symbiotic shrimps: 14.5% (n=22) were colored with a dorsal white band (**Figure 3C**), 13.2 % (n=20) were fully colored (**Figure 3D**) and 72.4 % (n=110) were transparent (**Figure 3E**). Another variability amongst the symbionts concerned

their cheliped dimorphism, with 29% (n=44) of the shrimps presenting an enlarged left claw, 35% (n=52) having an enlarged right claw and 36 % (n=54) showing no difference between left and right claws. The length of the shrimps ranged from 0.4 to 1.5 cm (mean ± SD of 0.9 ± 0.2 cm) long and 18% (n=27) were gravid females (**Figure 3CX**). Gravid females were the biggest individuals measuring 1.2 ± 0.1 cm long. We also noticed that the occurrence of the symbiosis could dramatically vary inside the lagoon between two micro-populations of cushion sea stars that were separated by a navigation channel of 22 m depth in which the flow was stronger, probably limiting the dispersion of the pelagic larvae that could not reach the isolated *C. novaeguineae* population. Indeed, no symbionts were found in this isolated population that was not considered in our symbiotic population description. In addition, no symbiotic shrimp was found on the 11 *A. planci* observed during the diving collections on the outer reef.

(2) Description of the developmental cycle

The primary goal of monitoring the shrimp eggs being to obtain larvae that could be tested in a low-flow olfactometer, their developmental stage was regularly monitored, and several observations could be made on the development of the embryos. Various developmental stages (from unfertilized oocytes to well-developed embryos) were present at the same time in the population, but for a given female, all eggs presented a homogenous developmental stage A general growth of the egg was observed between the first and the last stage of embryo development, with the most immature eggs (Stage I) measuring 460 µm ± 28 µm, to the final stage VIII, that exhibited a 639 ± 19 µm diameter (**Figure 3C**). In the stages I and II (length = 518 ± 33 µm), only yolk was observed in the eggs through transparency. While the stage I was characterized by numerous "micro-droplets" (probably corresponding to blastomeres) that appeared homogeneous, the next stage comprised bigger "micro-droplets" gathering in one side of the egg (probably corresponding to the growth and migration of the blastomeres toward one pole of the embryo). During stages III (length = 525 ± 36 µm), IV (length = 544 ± 37 µm), and V (length = 555 ± 34µm), the embryos were visible in the eggs, while a reduction in yolk could be observed. At stage III, an embryo emerged at one pole of the egg. During stage IV, this embryo occupied a larger volume. In stage V, the anterior-posterior axis of the organism could be discerned, as well as the beginning of the segmentation of the locomotor appendages. From stage VI (length = 571 ± 12µm), the two eyes began to form and presumably contained melanin pigment, developed through stage VII (length = 595 ± 29 µm) and ultimately led to the formation of ommatidia during stage VIII (length = 639 ± 19 µm). Following this stage, the embryo continued to develop, ultimately resulting in the emergence of a zoeal larva. This first

stage of zoeal larvae exhibited well-developed appendages, including legs, telson, antennae, and prominent eyes. Measuring 825 ± 35 µm in length, the basal region of the head presented an orange-yellow substance that seemed similar to the yolk. Following larvae developmental stages (*e.g.* post hatched stages), were not observed in this study.

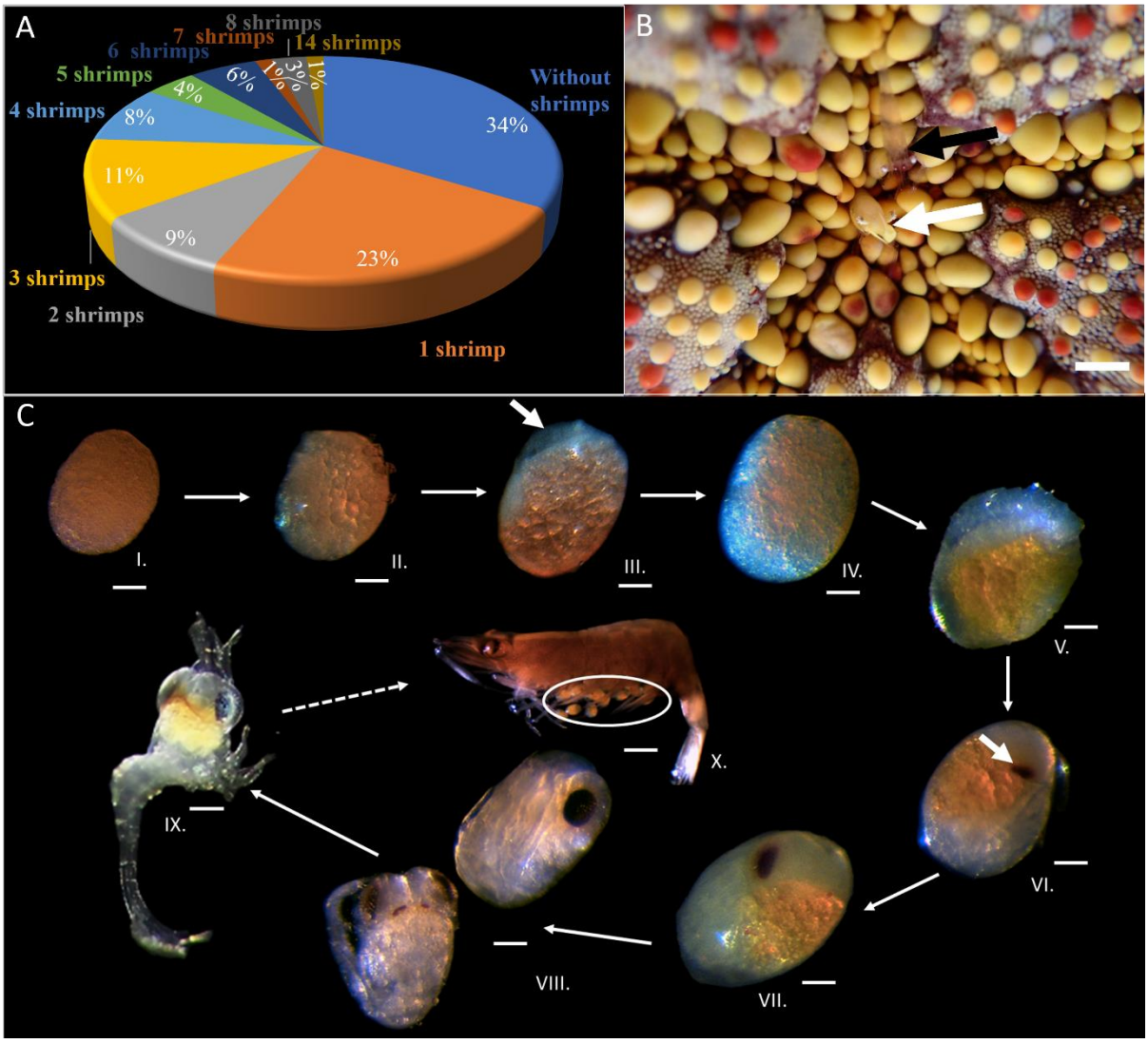


Figure 3: Description of the symbiotic population and the embryonic development of *Zenopontonia soror*: **(**A) Occurrence (%) and symbiotic load of sea star shrimps per host individual. (B) Two symbiotic *Z. soror* in association with their host *Culcita novaeguineae*: one transparent shrimp close to the mouth (black arrow) and one colored morphotype was observed inside the mouth (white arrow). C = Developmental cycle of the shrimp *Z. soror*. I. Pigmented egg that represents either an unfertilized egg or one of the first development stages. II. Pigmented egg with a large quantity of yolk and larger blastomeres. III. Pigmented egg with a beginning of embryo development (white arrow). IV. Egg with a slight growth of the embryo. V. Pigmented egg with embryo development presenting a discernible antero-posterior axis. VI. Pigmented egg presenting a black vesicle corresponding to the development of an eye (white arrow) and decrease in the quantity of yolk. VII. Advanced developed embryo showing a development of the eye, segmented body and a reduction of the pigmented yolk. VIII. Developed embryo with very little yolk content and developed body and complex eyes displaying ommatidia. IX. First zoeal larval stage. X. Adult gravid *Z. soror* with eggs (white circle). Scalebar represents 0.5cm in B; 140µm for C I to -C IX and 700µm in C X.

(3) Characterization of saponins

Globally, 51 different saponin molecules were detected in the three potential host species of sea stars. Each investigated sea star species presents a specific cocktail of saponins (**Table 1**; **Figure 4**). The saponin congeners were distinguished based on their elemental compositions and LC elution times that are specifically associated with their aglycon/sugar moieties and the presence/absence of a sulphate group (**Table 1**). The number of detected saponins varied according to the species, with *C. novaeguineae*, *A. planci* and *L. laevigata* presenting 13, 18 and 20 saponin congeners, respectively. Each cocktail was composed of 3 or 4 major congeners that, taken together, represented approximately 75%, 69% and 64% (in relative abundances, mol-%) of the total saponin contents in the natural extract for *C. novaeguineae*, *A. planci* and *L. laevigata*, respectively (**Table 1**, in bold; **Figure 4**). Most of the saponins (n = 37) were species-specific but some saponins (n=7) were common to both *C. novaeguineae* and *A. planci* (same molecular formula and retention time). Based on mass spectrometry analyses, we estimated the saponin contents within the natural extracts to be between 92% and 95% (in %-weight).

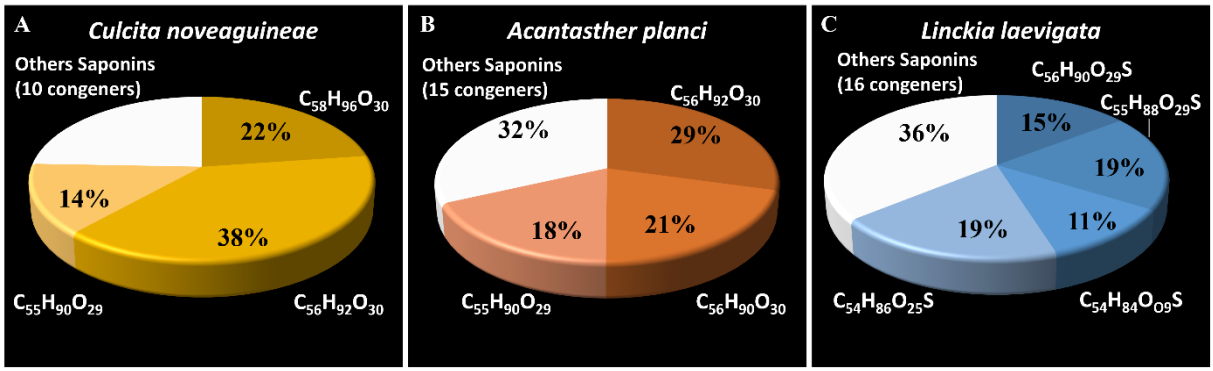


Figure 4: Relative abundances (mol-%) of the asterosaponins of the three potential host sea stars: (A) *Culcita novaeguineae*, (B) *Acanthaster planci* and (C) *Linckia laevigata*.

Table 1: Asterosaponins extracted from the three potential sea star hosts, *i.e. Culcita novaeguineae*, *Acanthaster planci* and *Linckia laevigata*. Saponin contents (%-weight) were determined relative to the internal standard, hederacoside C (Sigma-Aldrich, Belgium). Major congeners are in bold. Common congeners are highlighted with an asterisk*.

***C. novaeguineae* - Natural extract - LC-MS(-) – Saponin content (%-weight) : 95.7%**						
Saponin	Composition	m/z [M-H]-	Δ (ppm)	Retention time (min)	Relative proportion (%)	%-weight in extract (%)
1	$C_{64}H_{106}O_{34}$	1417.6486	0.1	11.13	2.75	2.63
2*	$C_{63}H_{104}O_{34}$	1403.6331	0.0	8.23	1.41	1.35
3*	$C_{62}H_{102}O_{34}$	1389.6173	0.1	8.35	5.56	5.32
4	$C_{59}H_{98}O_{30}$	1285.6135	4.0	10.30	2.91	2.78
5*	**$C_{58}H_{96}O_{30}$**	**1271.5898**	**0.8**	**8.74**	**22.44**	**23.30**
6				11.19	1.91	
7*	$C_{57}H_{94}O_{30}$	1257.5754	0.2	9.10	1.44	3.83
8				9.24	2.57	
9	**$C_{56}H_{92}O_{30}$**	**1243.5591**	**0.3**	**8.42**	**38.39**	**36.74**
10*	$C_{56}H_{92}O_{29}$	1227.5629	1.4	9.55	3.30	5.94
11*				10.35	2.91	
12*	**$C_{55}H_{90}O_{29}$**	**1213.5499**	**0.7**	**8.72**	**13.65**	**13.05**
13	$C_{42}H_{62}O_{15}$	805.3999	1.5	8.94	0.80	0.77
***A. planci* - Natural extract - LC-MS(-) – Saponin content (%-weight) : 92.4%**						
14*	$C_{63}H_{104}O_{34}$	1403.6331	0.0	8.23	2.15	2.70
15				9.74	0.78	
16*	$C_{62}H_{102}O_{34}$	1389.6173	0.1	8.35	1.14	1.05
17	$C_{58}H_{96}O_{31}$	1287.6042	4.0	9.37	0.40	1.81
18				10.30	1.56	
19*	$C_{58}H_{96}O_{30}$	1271.5898	0.8	8.74	1.26	1.16
20*	$C_{57}H_{94}O_{30}$	1257.5790	3.0	9.10	4.00	4.09
21				9.87	0.44	
22	**$C_{56}H_{92}O_{30}$**	**1243.5599**	**0.3**	**8.84**	**29.30**	**27.07**
23	**$C_{56}H_{90}O_{30}$**	**1241.5413**	**2.0**	**8.83**	**1.06**	**21.70**
24				**9.99**	**20.80**	
25				10.80	1.64	

26*	$C_{56}H_{92}O_{29}$	1227.5629	1.4	9.55	5.14	15.34
27*				10.35	2.43	
28				10.57	9.04	
29*	**$C_{55}H_{90}O_{29}$**	**1213.5499**	**0.7**	**8.72**	**18.08**	**16.70**
30	$C_{54}H_{82}O_{24}$	1113.5160	3.9	10.42	0.30	0.27
31	$C_{42}H_{62}O_{15}$	805.3999	1.5	9.15	0.50	0.46
***L. laevigata* - Natural extract - LC-MS(-) – Saponin content (%-weight) : 94.1%**						
32	$C_{61}H_{98}O_{33}S$	1391.5740	1.4	7.56	3.93	3.70
33	$C_{61}H_{98}O_{32}S$	1375.5801	0.7	7.95	1.28	1.20
34	$C_{60}H_{94}O_{32}S$	1359.5469	2.2	8.91	0.92	0.86
35	$C_{60}H_{94}O_{31}S$	1343.5530	1.5	8.04	0.62	0.59
36	$C_{60}H_{96}O_{31}$	1313.6005	1.5	7.52	1.26	1.18
37	$C_{60}H_{96}O_{30}$	1297.6066	2.3	8.05	2.38	2.24
38	$C_{59}H_{94}O_{30}$	1283.5860	1.6	7.63	2.73	2.57
39	**$C_{56}H_{90}O_{29}S$**	**1259.5330**	**0.8**	**8.03**	**14.66**	**13.79**
40	**$C_{55}H_{88}O_{29}S$**	**1245.5214**	**2.4**	**7.61**	**19.08**	**17.96**
41	$C_{55}H_{86}O_{29}S$	1243.4978	4.0	8.62	5.33	5.02
42	$C_{56}H_{88}O_{28}S$	1241.5245	0.8	7.81	4.10	3.86
43	**$C_{54}H_{84}O_{29}S$**	**1229.4852**	**1.6**	**7.98**	**11.33**	**10.67**
44	$C_{55}H_{82}O_{28}S$	1223.4727	3.3	7.59	1.57	1.48
45	$C_{54}H_{84}O_{28}S$	1213.4933	0.8	8.28	3.73	3.51
46	$C_{56}H_{90}O_{25}S$	1195.5524	1.7	8.98	1.04	0.98
47	**$C_{54}H_{86}O_{25}S$**	**1167.5232**	**0.0**	**11.23**	**19.13**	**18.00**
48	$C_{54}H_{84}O_{25}S$	1165.5056	1.7	10.6	1.61	1.52
49	$C_{54}H_{84}O_{24}S$	1149.5097	2.6	11.23	0.93	0.87
50	$C_{56}H_{86}O_{23}$	1127.5603	0.9	11.23	1.72	1.62
51	$C_{55}H_{84}O_{23}$	1113.5447	0.9	8.03	2.65	2.50

(4) behavioral olfactometry analyses on adult Z. soror

During the negative control with both aquaria of the olfactometer exclusively filled with non-conditioned seawater, 90% (n=18/20) of shrimps remained in the entry of the olfactometer and did not show any positive chemotaxis (**Table 2; Video SI2**). Therefore, *Z. soror* individuals did not elicit attractive rheological or chemically-mediated behavior when testing non-conditioned seawater flows. When an aquarium was conditioned with the initial host, *C. novaeguineae* or its extracted asterosaponins, the shrimps usually displayed grooming and flicking behaviors with their A1 and A2 antennae and pereiopods, that are associated to chemoreception patterns(Caulier et al. 2013b). Chemoreception led to the successful detection and orientation of more than 82% (n=33/40) of the shrimps (**Video SI1**), which confirms that *Z. soror* can detect semiochemicals produced by their initial host but also that asterosaponins play the role of kairomones allowing host selection.

Asterosaponins extracted from the potential host crown of thorns sea star *A. planci* and the azure sea star *L. laevigata*, elicited chemosensation behaviors but were not attractive to the *Z. soror* with 65% (n=13/20) and 75% (n=15/20) of the tested shrimps remaining at the origin of the Y-tube olfactometer and displaying no positive chemotaxis. Finally, saponins extracted from the two non-host sea star species (*A. rubens* and *E. sepositus*) and the sea cucumber *H. scabra* revealed similar results as the negative control and were thus considered non-attractive for *Z. soror*.

Table 2: Results of behavioral experiments using the Y-tube olfactometer on adult *Zenopontonia soror*. Eight different tests were performed on a minimum of 20 different adult shrimps. Results show the number of trials, spread out among the shrimps that remained stationary in the beginning of the impaired branch (Null), those that orientated adequately towards the cues source (A) and those of orientated towards the control aquarium (B). The P-values of the chi^2 test and the binomial test are respectively found in motion and orientation behavior columns. Significant results (P-value, 0.01) are underlined and **bolded**.

Aquarium A VS Aquarium B	Results				Statistics	
	Trials	**Null**	**A**	**B**	**Motion behavior**	**Orientation behavior**
Negative control						
Seawater (A) VS seawater (B)	20	18	1	1	/	**1**
Water conditioned by the initial host						
***Culcita novaeguineae* (A) VS seawater (B)**	40	4	**33**	3	**$1.8\ 10^{-10}$**	**$2.3\ 10^{-7}$**
Water conditioned by asterosaponins extracted from potential host asteroid species						
***C. novaeguineae* extract (A) VS seawater (B)**	40	4	**33**	3	**$1.8\ 10^{-10}$**	**$2.3\ 10^{-7}$**
***Acanthaster planci* extract (A) VS seawater (B)**	20	13	6	1	0.1	**0.1**
***Linckia laevigata* extract (A) VS seawater (B)**	20	15	5	0	0.4	**$6\ 10^{-2}$**
Water conditioned by saponins from non-host echinoderm species						
***Asterias rubens* extract (A) VS seawater (B)**	20	18	1	1	1	**1**
***Echinaster sepositus* extract (A) VS seawater (B)**	20	18	1	1	1	**1**
***Holothuria scabra* extract (A) VS seawater (B)**	**20**	**18**	**1**	**1**	**1**	**1**

(5) behavioral low-flow olfactometry analyses on larvae Z. soror

The low-flow Y-tube device is adapted to test larval chemotaxis (**Figure 2**). Results are summarized in **Table 3.** When performing the negative control (*i.e.* both aquaria filled with non-conditioned seawater), the larvae remained quite inactive and were transported passively into the Y-tube with the current generated by the peristaltic pump. The mean (± SD) drifting time of the larvae in the system was 64.3 ± 49.4 s. No detection/orientation behavior was

observed (12 Null on 20 larvae). Indeed, larvae almost never moved actively in the device and when they did drift off to the right or the left part with an equivalent percentage (50%). When one of the aquaria was filled with water conditioned by the host species, larvae were more active and swam periodically and remained for a significatively longer time in the monitoring zone (ANOVA, $F(4, 175) = 7.1$, p-value = $2.7\ 10^{-5}$). The larvae could strongly orientate themselves toward the host side of the Y-tube (33 larvae oriented adequately on 40). Those results confirm the validity of the low-flow olfactometer device that can be used to identify larvae chemotaxis. Interestingly, the zoeal larvae displayed similar orientation results with water conditioned with the saponins extracted from the two potential host species *C. novaeguineae* and *A. planci*. However, when we performed a test to simultaneously compare the cues originating from the two potential host species, the larvae oriented themselves significatively towards *C. novaeguineae*, indicating a preference for this cue compared to the one of *A. planci*.

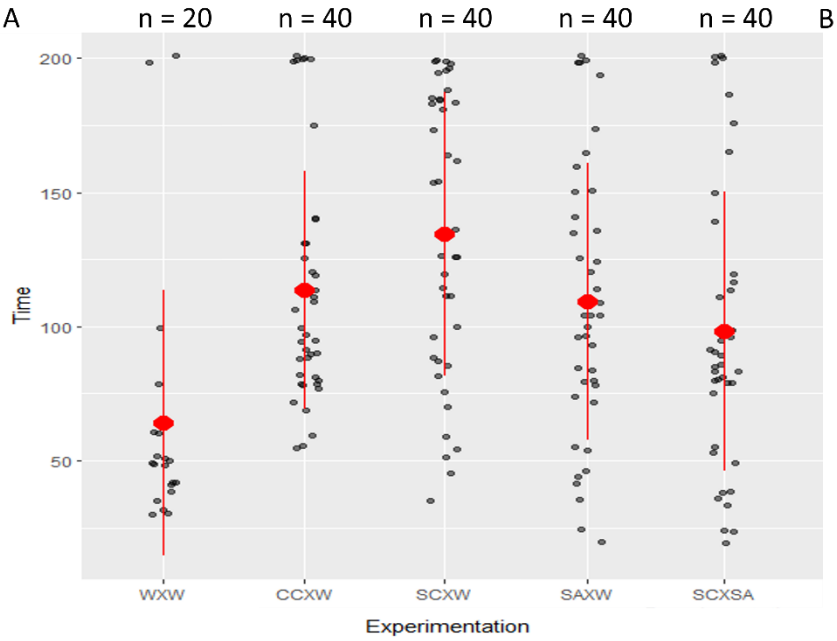
Table 3: Results of behavioral experiments using the olfactometer on larvae of *Zenopontonia soror*. Five different tests were performed on a minimum of 20 different larvae. Three individuals of *Culcita novaeguineae* were used to prepare conditioned water and, for each saponins extract, at least 3 individuals for each species were used. Results show the number of trials, spread out among the shrimps that did not converge significatively toward the device (Null), those that orientated adequately toward the cues from aquarium A (A) and those that orientated toward the cues coming from the aquarium B (B). The P-value of the chi^2 test and the binomial test are respectively found in motion and orientation behavior columns. Significant results (P-value, 0.01) are underlined and in **bold**.

Aquarium A VS Aquarium B	Results				Statistics	
	Trials	**Null**	**A**	**B**	**Motion behavior**	**Orientation behavior**
Negative control						
Seawater (A) VS seawater (B)	20	12	4	4	/	**1**
Water conditioned by the initial host						
***Culcita novaeguineae* (A) VS seawater (B)**	40	4	**33**	3	**1.3 10-^{4}**	**2.3 10^{-7}**
Water conditioned by asterosaponins extracted from potential host asteroids						
***C. novaeguineae* extract (A) VS seawater (B)**	40	5	**33**	2	**3.9 10^{-4}**	**3.7 10^{-8}**
***Acanthaster planci* extract (A) VS seawater (B)**	40	6	**32**	2	**1 10^{-3}**	**6.4 10^{-8}**
Comparison of two waters conditioned by asteroids saponins extracts						
***C. novaeguineae* extract (A) VS *A. planci* extract (B)**	**40**	**8**	**29**	**3**	**4.5 10^{-3}**	**2.6 10^{-5}**

The homoscedasticity (Bartlett Test, $K^2 = 1.5$, df = 4, p-value = 0.8) and the distribution of the residuals were verified using a quantile-quantile plot (**Figure SI-1**) and showed that they did not differ significantly from a normal distribution. Multiple comparisons of means (Tukey HSD) demonstrated (**Figure 5**) that larvae had a shorter drifting time in the device in the negative control test (seawater VS seawater) than when they are confronted with the cues of one of their hosts [(Linear hypotheses = *C. novaeguineae* conditioned water VS seawater - seawater VS seawater = 0, t = -0.4, p-value = 4 10^{-3}); (Linear hypotheses = *C. novaeguineae* extract VS seawater - seawater VS seawater = 0, t = -5.1, p-value = 1 10^{-3}); (Linear hypotheses = *A. planci* extract VS seawater- seawater VS seawater = 0, t = -3.3, p-value = 1 10^{-2})]. When both host extracts were injected together, larvae stayed slightly more time in the device in comparison to the negative controls, but the mean times are not significantly different (linear hypotheses = *C. novaeguineae* extract VS *A. planci* extract - seawater VS seawater = 0, t = -

2.5, p-value = 0.1). However, a significant mean time difference is observed between the test where both extracts are tested together and the test with only the asterosaponins of *C. novaeguineae* are injected, with the larvae staying more time in the second one (linear hypotheses = *C. novaeguineae* extract VS seawater - *C. novaeguineae* extract VS *A. planci* extract = 0, t = 3.2, p-value = 1 10^{-2}).

A



B

Linear Hypotheses	P-value
WXW - CCXW = 0	**4.1 10^{-3}**
WXW - SCXW = 0	**1 10^{-3}**
WXW - SAXW = 0	**1.1 10^{-2}**
WXW - SCXSA = 0	0.1
SCXW - SCXSA = 0	**1.2 10^{-2}**
SCXSA - CCXW = 0	0.7
SCXW - CCXW = 0	0.3
SCXSA - SAXW = 0	0.9
SCXW - SAXW = 0	0.2

Figure 5: Drifting time of *Zenopontonia soror* larvae test under different chemical cues: (A) *Z. soror* larvae drifting time (s) inside the olfactometer device during the different experimentations. For each experiment, the red spot corresponds to the meantime values and the red line represents the standard deviation. The grey dots represent the measures of the larvae drifting time, with small random horizontal shifts to visually separate them. The number n above each box represents the number of larvae observed in each experimentation. All experiments are abbreviated as the following (i) WXW: seawater VS seawater (negative control); (ii) CCXW: *C. novaeguineae* conditioned water VS seawater; (iii) SCXW: Saponins from *C. novaeguineae* VS seawater; (iv) SAXW: Saponins from *A. planci* VS seawater. (B) Results of the multiple comparisons of mean (Tukey) test. Significant results (P-value, 0.01) are underlined and bolded.

IV. Discussion

(1) Notes on the symbiosis between Z. soror and C. novaeguineae in Mo'orea

Z. soror is an obligate associate that inhabits at least 23 different asteroid host species, making it a generalist symbiont at the species level and a specific symbiont at the Asteroidea class. In the northern Mo'orea lagoon, more than two third of *C. novaeguineae* hosted sea star shrimp individual(s), with a maximum of 14 shrimps coexisting on a same host. To date, the highest occurrence ever recorded reached up to 53 individuals of *Z. soror* on one *C. novaeguineae* (英男 and 喬司 1988; Antokhina and Britayev 2020). Even if previous studies reported the association between *Z. soror* and *C. novaeguineae* in Tuamotu islands and Society islands (Poupin 1998; Li 2008) and even describe partially the host preference and food habit of the symbiont(Olliff 2013; Schelvis 2017), this study is the first to provide an exhaustive description of the symbiotic population in French Polynesia. The crown-of-thorns *A. planci* monitored in this study did not host any symbiont but were only found on the external reef and not in the lagoon of the North of Mo'orea at the time of the field missions (**Figure 1B)**. This corallivorous species may be abundant in the waters of Mo'orea including in the lagoons of French Polynesia in general (Krupa and Reeves 2004; Clark and Weitzman 2008; Kayal and Kayal 2017). *L. laevigata* and *L. multiflora* may also be natural hosts in this area (Adjeroud and Salvat 1996; Schelvis 2017) but were not observed. These four species are known to coexist in some other geographic areas, such as, for example, the Nhatrang Bay in Vietnam where the symbiotic shrimp was also reported (Antokhina and Britayev 2012). Additionally, we report, for the first time, that *Z. soror*, referred exclusively as an ectosymbiont (Bruce 1976a; 英男 and 喬司 1988; Guenther et al. 2007; Olliff 2013; Schelvis 2017; Antokhina and Britayev 2020), may enter the buccal cavity of its host (**Figure 3B)** and could be, under certain conditions (*e.g.* predation stress), a temporary endosymbiont. As no study has ever investigated specifically the buccal cavity of *C. novaeguineae* to monitor the presence of symbionts, the occurrence and symbiotic load of *Z. soror* described in literature (Antokhina and Britayev 2012; Tseng et al. 2022) may be biased as we know that some symbiotic crustaceans associated with echinoderms frequently exhibit an endosymbiotic lifestyle (*e.g.* the Harlequin crab *L. orbicularis* (Caulier et al. 2012)).

We partially described the embryonic development of *Z. soror* providing further insights into the life cycle of this species. Similar to non-symbiotic palaemonid shrimps, the development of *Z. soror* is complex and involves multiple stages, from fertilization to hatching(Little 1968;

Nazari et al. 2003; Wowor et al. 2009; Cuvin-Aralar 2014); during which the embryos undergo a series of morphological changes. Each stage is characterized by specific timing and the modification of the size, shape, and functionality of the developing organs and structures (Nazari et al. 2003; Cuvin-Aralar 2014). The embryonic description complement precedent study describing the larva of *Z. soror* (Wear 1976). The post hatched zoeal stages have never been observed before. However, in Palaemonidae, the free-swimming larvae may go through 9 to 13 zoeal stages before reaching the juvenile stage (Wowor et al. 2009). Traditionally studied for Commercial Palaemonid species (Nazari et al. 2003; Wowor et al. 2009; Cuvin-Aralar 2014), understanding the embryonic development of symbiotic organisms is also important for the management and conservation of symbiotic marine biodiversity.

(2) Host selection of the sea star shrimp through chemical sensing

behavioral experiments in olfactometers with adults *Z. soror* confirm that host selection of the symbiont is mediated though chemical sensing, as already shown in recent literature (Olliff 2013; Antokhina and Britayev 2020). Antokhina and Britayev (2020) showed that *Z. soror* recognizes its original host species through chemical cues, but not the other host species present in the environment (Antokhina and Britayev 2020). Our innovative low-flow Y-tube olfactometer results prove that sea star chemical cues have an attractive effect on the larvae, eliciting a positive chemotaxis and enabling pelagic larvae to selectively recruit on their benthic hosts before metamorphosis. This ensures the sustainability of the symbiotic association across generations (**Figure 6**) and could also explain why molecular genetic study have found that different *Z. soror* populations associated with different asteroid species are genetically homogeneous (Antokhina and Sorokin 2010). *Z. soror* exhibits a remarkable ability to form associations with diverse sea star hosts (英男 and 喬司 1988; Correa and Thiel 2003; Guenther et al. 2007; Li 2008; Olliff 2013; Caulier et al. 2013b), thereby accessing a wide range of habitats and promoting gene flow among populations. Essentially, the *Z. soror* larvae can find suitable habitats in tropics and drift with the water current if any of the host species is present.

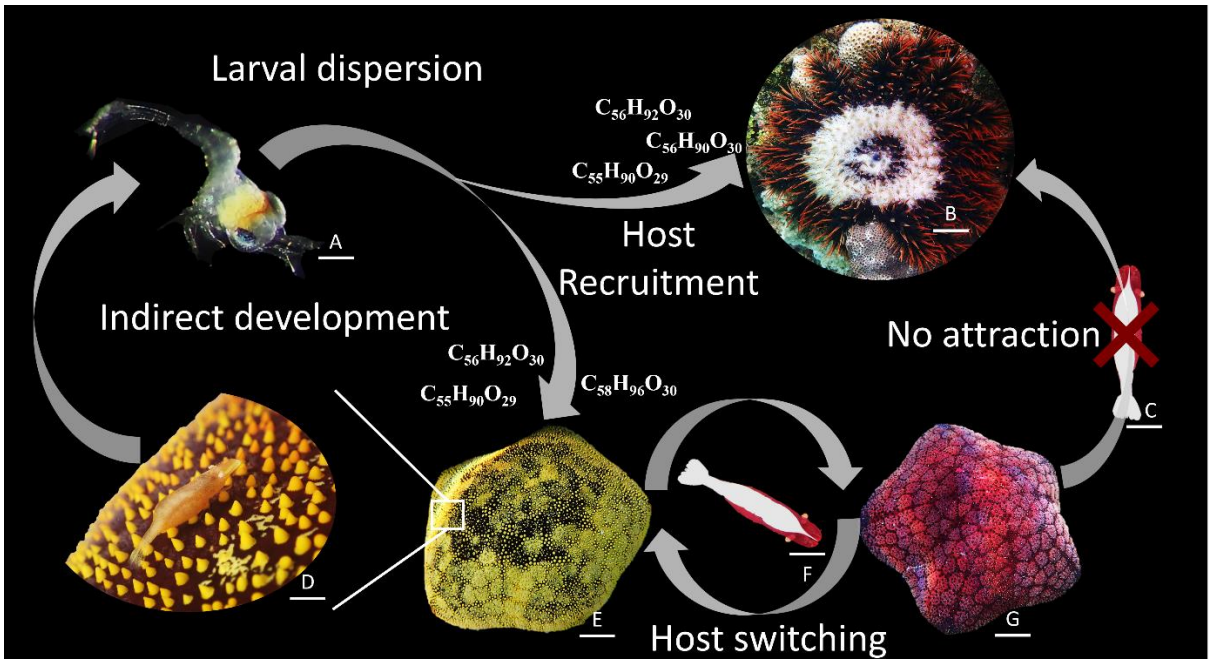


Figure 6: Summary diagram of the mechanisms involved in host recognition, host switch and larval settlements in the symbiotic association between *C. novaeguineae* and *Z. soror*. Scale bars represent 110µm in A; 3,6 cm in B; 250µm in C, D and F; 2 cm in E and G.

For the first time, the study reveals that these cocktails of asterosaponins act as chemical cues (*i.e.* kairomones) allowing host selection by both adults and larvae of *Z. soror*. Asterosaponins from *Acanthaster planci* have previously been suggested to serve as a pheromonal recruitment factor allowing (i) its larvae to perform their metamorphosis in areas where other conspecifics are present and (ii) the aggregation of adult crown of thorns sea stars (Motti et al. 2018). In the present study, we compared the asterosaponin contents from different host and non-host species (Demeyer et al. 2014; Dahmoune et al. 2021; Savarino et al. 2022) (**Table 1**) and found that each sea star species produces a unique mixture of asterosaponins. This specificity explains the differences exhibited by *Z. soror* adults and larvae when exposed to water conditioned by saponins extracted from different potential host species. Indeed, the *Z. soror* adults were only attracted by their original host species *C. novaeguineae* saponins, on which they were exclusively collected. They were not attracted by the saponins extracted from 5 other echinoderms, two potential host species where *Z. soror* adults have already been recorded in literature (*A. planci* and *L. laevigata*) and 3 non-host species (*Asterias rubens, Echinaster sepositus* and *Holothuria scabra*). It is not surprising that *Z. soror* adults are not attracted by non-host species, but the fact that saponins from *A. planci* do not elicit a positive chemotaxis on the adults but do attract their larvae can be explained by a phenomenon known as symbiotic host imprinting. Host imprinting explains how a symbiont may co-adapt its chemoreception capacities to recognize the specific host species on which it is found (Immelmann 1975;

Antokhina and Britayev 2020). It is well established that host imprinting is a common phenomenon among decapods, for host recognition, habitat preference or food habit for example (Immelmann 1975; VandenSpiegel et al. 1998; Antokhina and Britayev 2020; **Chapter I)**. At the opposite, Caulier et al. (2013) showed that adults of tropical Harlequin crabs *L. orbicularis* positively recognize the saponins from *Holothuria forskali* (a temperate sea cucumber where they never occur) suggesting that imprinting is not a general rule for all decapods. As soon as they are capable of swimming efficiently, *Z. soror* larvae are certainly transported by natural seawater currents to different places in the benthos. If, by chance, they contact or are close to a potential host, our experiments suggest that their swimming activity will allow them to stay in contact with that host. Probably taking advantage of the surface of this host, they could stay there long enough to metamorphose. Indeed, we demonstrated that they could detect the presence of different species of potential hosts in their environment and to orient themselves towards them. Then, adults *Z. soror* will be imprinted by the original host species, specializing its chemotaxis capacities and being no more attracted by asterosaponins produced by other potential hosts present in the environment.

Our results confirm previous findings that each species of holothuroids and asteroids produces a unique mixture of different saponin congeners, resulting in a distinct cocktail of molecules that can be considered as a chemical signature (Demeyer et al. 2014; Kicha et al. 2014; Ha et al. 2021; Dahmoune et al. 2021; Savarino et al. 2022). These molecules can have a role in both pheromonal communication (Claereboudt et al. 2023) and host recognition phenomenon (Caulier et al. 2013b). Some asteroid species exclusively produce sulphated saponins while others only produce non-sulphated saponins, and some produce both (Ngoan et al. 2015; Stonik et al. 2020; Ha et al. 2021). Moreover, saponins are known to play a crucial role in chemical defence in marine environments and have strong ichthyotoxic properties that effectively deter potential predators (Kasumyan et al. 2020). Therefore, symbiotic fauna must evolve mechanisms to counteract the harmful effects of asterosaponins. Furthermore, they may also leverage their host chemical defence mechanisms to protect themselves from their own predators. In the present study, we found that *C. novaeguineae* and *A. planci* only produce non-sulphated saponins, while *L. laevigata* produces both sulphated and non-sulphated asterosaponins. Interestingly, previous studies have shown differences in saponin congener profiles within populations of the same species, which may be explained by the geographical distance between the populations. For example, *C. novaeguineae* has been previously studied in South China (Tang et al. 2005; Ma et al. 2009; Lu et al. 2018, 2020, 2022b), Vietnam (Ngoan

et al. 2015), Japan (Iorizzi et al. 1991) and Seychelles (Levina et al. 2010). Similar analyses have also been conducted for *A. planci* in Japan (Kitagawa and Kobayashi 1977) and Vietnam (Kicha et al. 2014; Ha et al. 2021). Therefore, it would be interesting to compare the potential host attractiveness of asteroids from different parts of the world to their larvae and adult symbionts in future studies. The differences in saponin mixtures among different populations of the same species may also impact intraspecific recognition and aggregation, underlining a potential emergence of a speciation event.

V. Conclusion

In this study, we investigated the recruitment of symbiont larvae through chemical sensing. We have shown that the host chemical recognition is not limited to adult symbionts, but also occurs in symbiont larvae. Adult shrimps become imprinted by their initial host species and are no longer attracted to asterosaponins produced by other potential hosts. These results shed light on the mystery of the symbiont larval recruitment and provide valuable information on how these symbionts complete their life cycle.

Chapter III



Exploring symbiont gene expression in two echinoid-associated shrimp species under host separation

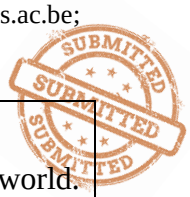
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Abstract

Symbiotic relationships are omnipresent in nature and particularly diverse in the marine world. One example occurs in the Western Indian Ocean, where the tropical sea urchin *Echinometra mathaei* associates with two obligate ectosymbiotic shrimp species, *Tulearіocaris holthuisi* and *Arete indicus*. Previous studies have underscored the host-dependent nature of these shrimps, particularly the severe host separation syndrome exhibited by *T. holthuisi*: isolated individuals manifest distinct signs of stress, including a loss of coloration and a decline in overall health leading to rapid mortality. Specific host pigments, known as spinochromes, have been identified as essential for the survival of *T. holthuisi* in isolation. Considering these observations, our study envisages a transcriptomic approach to assess the stress induced by host separation on these two symbiotic shrimps. Using paired-end Illumina HiSeq technology, we analysed transcriptomes of both shrimp species under three conditions: (i) symbionts residing on their host, the sea urchin *E. mathaei* (control condition = CC), (ii) symbionts isolated from their host (isolated condition = IC), and (iii) symbionts isolated from their host but placed in seawater containing spinochromes produced by the sea urchin (isolated + spinochromes conditions = IC + S). Sequencing revealed a total of 217,832 assembled unigenes for both species, with an N50 value of 2,061 bp. Our findings reveal that isolated *T. holthuisi* shrimp exhibit a proportion of 16.5% of differentially expressed genes (DEGs) compared to the control treatment (IC/CC), whereas *T. holthuisi* in the presence of spinochromes showed a proportion of 8.5% of DEGs compared to the control (IC+S/CC), demonstrating a notable reduction in DEGs when spinochromes are present. Further analyses of stress-related genes show that *T. holthuisi* expressed some stress-related genes when isolated in comparison to the control (IC/CC). Notably, heat shock proteins (HSPs), recognized for their role in stress responses, were significantly up-regulated in *T. holthuisi* under isolation conditions, especially in the absence of spinochromes, compared to the control condition. In contrast, *A. indicus* displayed differential expression of diverse genes, suggesting micro-regulation as an adaptive mechanism to cope with isolation stress, at least for a limited duration. In conclusion, this study is the first to use Next Generation Sequencing to explore the transcriptomic responses of two obligate symbiotic shrimp species associated with sea urchin host, shedding some light on the molecular impact of the host-separation syndrome and the crucial role of chemical dependencies in these intricate ecological relationships.

Keywords: Symbiosis, Crustacea, Echinoderm, Stress response, Transcriptome, RNA-seq

I. Introduction

Most marine organisms are associated with at least one or several individual(s) belonging to another species (Castro 1988). Symbioses are considered as intimate and durable associations between two heterospecific organisms, commonly named the host (*i.e.*, the biggest partner) and the symbiont (*i.e.*, the smallest one) (Paracer and Ahmadjian 2000; Parmentier and Michel 2013; Brasseur et al. 2018). Symbioses are generally classified into three categories - parasitism, commensalism and mutualism - which are distinguished by the effects of the symbionts on their hosts (negative, neutral and positive effects on the host, respectively) (Paracer and Ahmadjian 2000; Parmentier and Michel 2013). A symbiotic association can however be switched from one category to another depending on the timing of the symbiont's life cycle (*e.g.*, most myzostomids switch from a parasitic to a commensal style during their life) or the environmental conditions (*e.g.*, bacteria in their digestive tracts may switch from a mutualistic to a parasitic lifestyle) (Parmentier and Michel 2013). Symbionts may present various degrees of host-specificity, which can vary significantly from living with a single host species (host-specific symbiont) to multiple host species belonging to varied taxa (opportunistic symbiont) (Eeckhaut and Jangoux 1993, 1995, 1997; Paracer and Ahmadjian 2000). Because symbiotic species have lived together intimately for a significant period (*i.e.*, usually over numerous generations), hosts and symbionts have coevolved together. This co-evolution involves adaptations (e.g., morphological, metabolic) in symbionts, hosts and/or both. These adaptations lead to evolutionary advantages for the symbiont and/or the host and ensure, in the case of symbionts, to live in appropriate habitats over time (Eeckhaut and Jangoux 1993, 1995; VandenSpiegel et al. 1998; Lanterbecq et al. 2009; Rouse et al. 2013; Terrana et al. 2016). Some symbionts may be highly dependent on their host (VandenSpiegel et al. 1998; Terrana et al. 2016; Brasseur et al. 2018) and, very often, their life cycle cannot be completed without the symbiotic association (Olsen 1986; Baker 2003). Those symbionts are qualified as "obligatory" (Eeckhaut 2003; Bauer 2023).

While presenting a large set of mechanical and chemical defences against biotic and abiotic threats (Brasseur et al. 2017, 2018; **Chapter I**), numerous echinoderms are "infested" by a wide range of symbionts (Lanterbecq et al. 2010; Horká et al. 2016; Chow et al. 2021). As an example, the sea urchin *Echinometra mathaei* (Blainville, 1825) can be colonised by two symbiotic shrimps, *Arete indicus* Coutière, 1903 (Alpheidae) and *Tuleariocaris holthuisi* Hipeau-Jacquotte, 1965 (Palaemonidae) (Hipeau-Jacquotte 1965; Gherardi 1991; Marin and

Anker 2009; De Grave and Fransen 2011; Brasseur et al. 2018) (**Figure 1**). The mimetic pigmentation of both shrimps relies on spinochromes which are specific pigment metabolites belonging to the naphthoquinone category and are specifically produced by echinoids (Brasseur et al. 2017). Indeed, mass spectrometry analyses revealed different cocktails of molecules containing at least nine different spinochromes in *E. mathaei*, five in *T. holthuisi* and three in *A. indicus* (Brasseur et al. 2017). Gut content and stable isotope analyses ($\delta^{15}C$, $\delta^{13}N$) demonstrated that the host integument constitutes the exclusive food of *T. holtuisi* but not for *A. indicus*, which also feed on other organic matters (Brasseur et al. 2018). Both shrimps are attracted by their echinoid host by chemical sensing, spinochromes being attractive stimulus allowing host selection. When *T. holthuisi* shrimps are isolated from their hosts, and more precisely from the chemical environment produced by them, they experience a "host separation syndrome" that results in a fast alteration of their health status associated with a visible depigmentation, loss of mobility and high mortality rate, most individuals dying within four days following the host separation. In comparison, the survival rate of *A. indicus* was less impacted by the host separation, and no significative differences were observed, neither in pigmentation nor in mobility for the same period (Brasseur et al. 2018). Interestingly, the host-separation syndrome is attenuated when isolated *T. holthuisi* individuals were placed in a solution of spinochromes which significantly increased the shrimp survival rate after 4 days of experiment. These experiments suggest that echinoid host spinochromes influence to some extent the physiology of the host-chemically-dependent shrimp *T. holthuisi*.

To investigate this hypothesis, we here explore the gene expression of *T. holthuisi* and *A. indicus* maintained in various conditions, with or without a host and with or without echinoid spinochromes through comparative transcriptomic analyses.

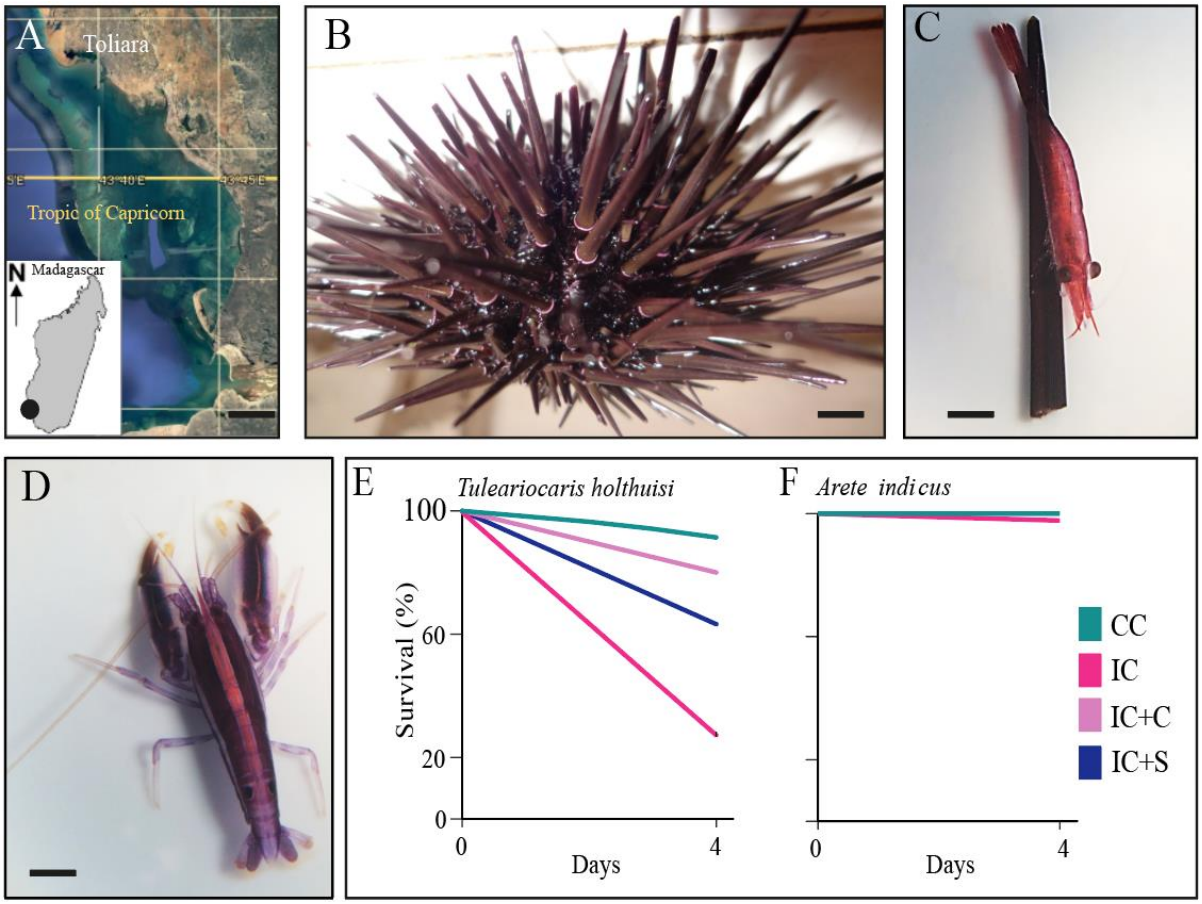


Figure 1. Biology of the symbiosis between *Tuleariocaris holthuisi*, *Arete indicus*, and *Echinometra mathaei* in the South-West of Madagascar. (A) South-West of Madagascar and Great Reef of Toliara, (B) *E. mathaei* sea urchin host, (C) *T. holthuisi* on a host spine, (D) isolated *A. indicus* (E-F) Survival rate of *T. holthuisi* and *A. indicus* shrimps under different conditions: control condition (CC, shrimps are associated with their host); isolated condition (IC, shrimps are isolated from their host); isolated shrimps in host-conditioned water (IC+C); isolated shrimps with spinochrome conditioned seawater (IC+S). (E-F) Modified from Brasseur et al. 2018. Scale bars represent 7 km in A, 1 cm in B and 0.2 cm in C and D.

II. Material and method

(1) *Sample collection*

The sea urchin *Echinometra mathaei* (Blainville, 1825) and its symbionts, *Tuleariocaris holthuisi* Hipeau-Jacquotte, 1965 and *Arete indicus* Coutière, 1903, were hand collected during low tide on the flat of the Great Reef of Toliara, Madagascar (23°23′34″S, 43°38′47″E) in November 2015 and November 2016. *E. mathaei* (n = 50) were sampled with *T. holthuisi* (n = 18) and *A. indicus* (n = 18) for the experiments. The organisms were maintained together (= Control Condition) in tanks (directly supplied with seawater from the sea) in the Halieutic Institute and Marine Sciences (IHSM) of the University of Toliara (Madagascar) for a minimum of 24 hours before any experimentation occurred.

(2) In vivo *host separation experiments*

Based on Brasseur et al. (2018), the following experimental design was used: (*i*) five *T. holthuisi* individuals were placed on one *E. mathaei* during 5 days (control condition, CC), (*ii*) five *T. holthuisi* individuals were placed in a second aquarium without their host (host isolation condition, IC), (*iii*) five *T. holthuisi* individuals were incubated in seawater with 0.5 mg/L of *E. mathaei* spinochromes crude extract from tests and spines (isolation condition + spinochromes, IC+S) (**Figure 2**). The methodology associated with the spinochromes crude extraction is explained in details by Brasseur et al. (2018). For the control CC and the treatment IC, the seawater was replaced every day with fresh 1 µm-filtered seawater. For the treatment IC+S, the seawater was replaced every day with fresh 1 µm-filtered seawater containing 0.5 mg/L of spinochrome extract. The same experimental procedure was used for the species *A. indicus*. All experiments were performed in tanks of 14 cm x 18 cm x 15 cm containing 1 L of 1 µm-filtered seawater at 28 °C and 35 PSU of salinity mimicking the experiments of Brasseur et al. (2018), the different treatments were stopped after 4 days (96 hours). Sexing the selected individuals was not possible for both symbiotic shrimps.

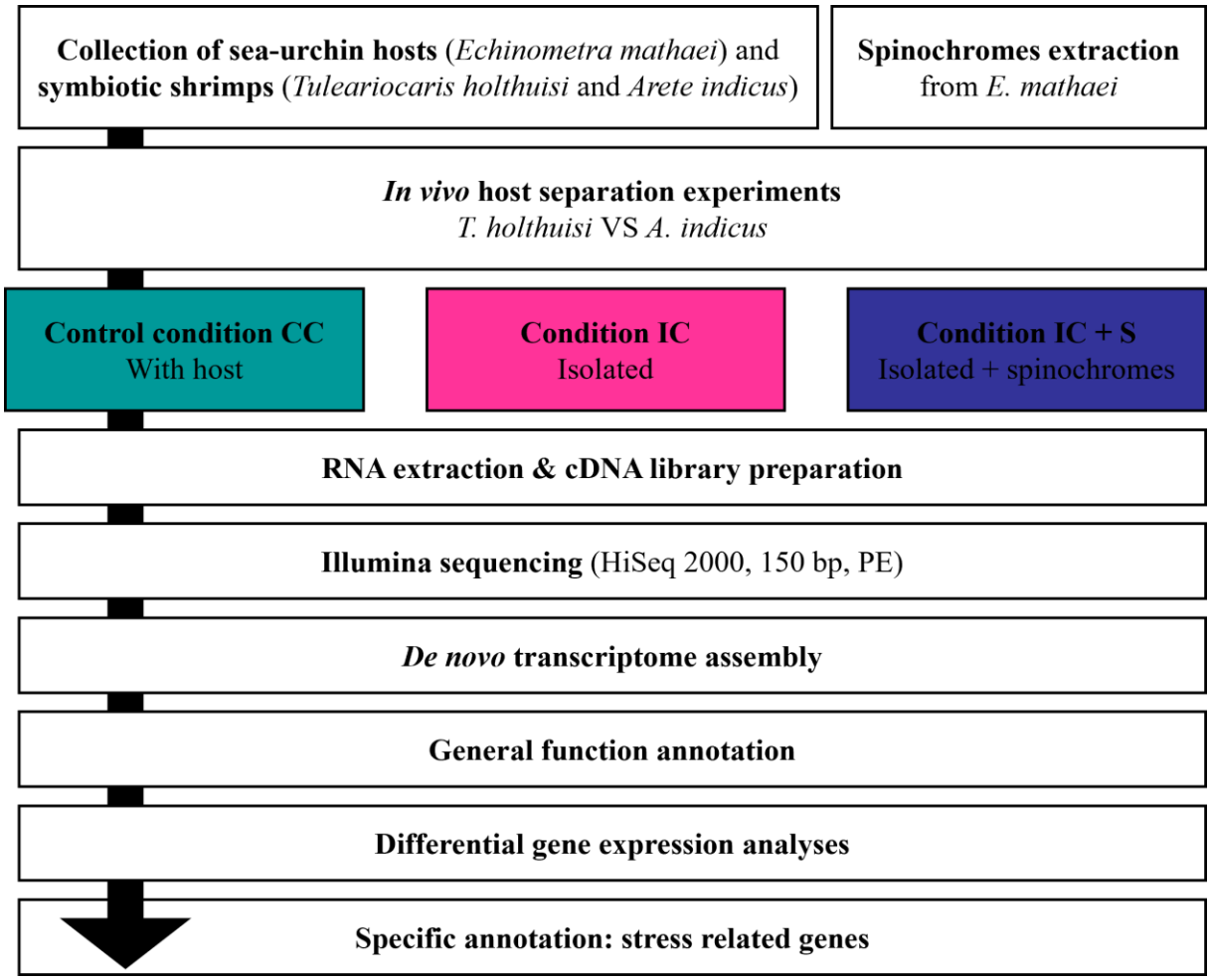


Figure 2. Study methodological pipeline to investigate the differential gene expression associated with the host separation syndrome in *Tuleariocaris holthuisi* and *Arete indicus*, both symbionts of *Echinometra mathaei*. . Bp = base pairs; PE = Paired-End sequencing.

(3) RNA extraction, cDNA library preparation and sequencing

For each species and each treatment, three individuals were randomly selected for the RNA extractions and the following transcriptomic analyses (a total of 18 RNA extractions). Entire shrimp individuals were stabilized in RNA later (Life Technologies) for one night at 4°C following the manufacturer's instructions. Samples were then stored at 4°C for a few days according to our field imperatives, until the return of the field mission. Samples were finally stored, in our laboratory in Belgium, at -80°C until RNA extraction. For RNA extractions, frozen samples were processed using the RNeasy Mini Kit (QIAGEN) following the manufacturer's instructions. The quality of the RNA extracts was checked by gel electrophoresis on a 1.2 M TAE agarose gel, and by size-exclusion chromatography with an Agilent Technologies 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA).

After treatment of the total RNA with DNase I, magnetic beads with Oligo(dT) were used to isolate mRNAs. The purified mRNAs were then mixed with fragmentation buffer and

fragmented into 100-400 bp pieces using divalent cations at 94°C for 5 minutes. Using these fragments as a template, random hexamer primers (Illumina) were used to synthesise the first-strand cDNA, followed by the synthesis of the second-strand cDNA using RNase H and DNA polymerase I. End reparation and single nucleotide A (adenine) addition were performed on the synthesised cDNAs. Next, Illumina paired-end adapters were ligated to the ends of these 3' adenylated short cDNA fragments. To select the proper templates for downstream enrichment, the products of the ligation reaction were purified on a 2% agarose gel. The cDNA fragments of about 200 bp were excised from the gel. Fifteen rounds of PCR amplification were carried out to enrich the purified cDNA template using PCR primer PE 1.0 and 2.0 (Illumina Inc., San Diego, CA, USA) with Phusion DNA polymerase. Finally, the cDNA library was constructed with 200 bp insertion fragments. After validation on the Agilent Technologies 2100 Bioanalyzer, the library was sequenced using Illumina HiSeq 2000 (Illumina Inc., San Diego, CA, USA), and the workflow was as follows: template hybridisation, isothermal amplification, linearisation, blocking, sequencing primer hybridisation, and first read sequencing. After completion of the first read, the templates are regenerated *in situ* to enable a second read from the opposite end of the fragments. Once the original templates are cleaved and removed, the reverse strands undergo sequencing by synthesis. High-throughput sequencing was conducted using the Illumina HiSeq 2000 platform to generate 150 bp paired-end reads. cDNA library preparation and sequencing were performed at Beijing Genomics Institute (BGI, Hong Kong) according to the manufacturer's instructions (Illumina, San Diego, CA). After sequencing, raw image data were transformed by base calling into sequence data, which were called raw reads and stored in the fastq format. Transcriptome quantity and quality control of reads were checked using the FastQC software (Andrews 2010).

(4) De novo *transcriptome assembly*

De novo transcriptome assembly was performed separately for *A. indicus* and *T. holthuisi* samples (**Figure 2**). Before the transcriptome assembly, the raw sequences were filtered to remove the low-quality reads. The filtration steps were as follows: 1) removal of reads containing only the adaptor sequence; 2) removal of reads containing over 5% of unknown nucleotides ''N"; and 3) removal of low-quality reads (those comprising more than 20% of bases with a quality value lower than 10). These filtration steps are routinely employed (Delroisse et al. 2015, 2018). The remaining clean reads were used for further analysis.

Transcriptome *de novo* assembly was carried out with short paired-end reads using the Trinity software (Grabherr et al. 2011) (version v2.0.6, parameters: min_contig_length 150, CPU 8, min_kmer_cov 3, min_glue 3, bfly_opts '-V 5, edge-thr=0.1, stderr'). After Trinity assembly, the TGI Clustering Tool (TGICL, version: v2.0.6, parameters: -l 40 -c 10 -v 25 -O '-repeaT. stringency 0.95 -minmatch 35 -minscore 35'), followed by Phrap assembler (http://www.phrap.org) were used for obtaining distinct sequences. These sequences are defined as unigenes (*i.e.*, unique predicted transcripts generated during the process of transcriptome assembly) in the following manuscript. Unigenes can either form clusters in which the similarity among overlapping sequences is superior to 94%, or singletons that are unique unigenes. As the length of sequences assembled is a criterion for assembly success, we calculated the size distribution of both contigs and unigenes.

BLASTx alignments (E-value threshold < $1e^{-5}$) between unigenes and public databases including non-redundant proteins database (Nr, NCBI), non-reductant nucleotides (Nt, NCBI), SwissProt, Kyoto Encyclopedia of Genes and Genomes (KEGG), InterPro protein families and domains (InterPro), Genes Ontology (GO) and Clusters of Orthologous Genes (COG), were performed, and the best-aligning results were used to identify the sequence direction of unigenes. When results from different databases were conflicting, the priority order Nr, Swiss-Prot, KEGG, and COG was followed to decide on sequence direction for unigenes. When a unigene was unaligned in all the above databases, the ESTScan software (v3.0.2) was used to decide on its sequence direction. ESTScan produces a nucleotide sequence (5'–3') direction and the amino sequence of the predicted coding region. The BUSCO software (Benchmarking Universal Single-Copy Orthologs, v4) was also used to evaluate the completeness of the assembled unigenes using the "Eukaryota odb" dataset (Simão et al. 2015).

To evaluate unigene expression levels, all usable reads were mapped back to the unigenes using SOAP aligner with the default settings (Li et al. 2008). For both transcriptomes, unigene expression was evaluated using the FPKM (Fragments per kilobase of transcript, per million fragments sequenced) method. The FPKM value is calculated following the specific formula $FPKM = \frac{10^6 C}{NL/10^3}$ where C is the number of fragments shown as uniquely aligned to the concerned unigene, N is the total number of fragments that uniquely align any unigene, and L is the base number in the coding DNA sequence of the concerned unigene. The FPKM method integrates the influence of different gene lengths and sequencing levels on the calculation of gene expression.

(5) Functional gene annotations

Following the pipeline described in **Figure 2**, all unigenes were used for homology searches against the various protein databases cited above. Best annotation results were selected to annotate the unigenes. Unigene sequences were also compared to nucleotide databases nt (non-redundant NCBI nucleotide database, E-value < $1e^{-05}$, BLASTn).

To estimate the number of assembled transcripts that appear to be nearly full-length, the unigenes were aligned against all known proteins from the nr database and the numbers of unique top matching proteins (E-value < $1e^{-05}$) that align across more than a certain percentage of the reference protein length were counted.

To further annotate the unigenes, the Blast2GO program was used with nr annotation to get Gene Ontology annotation according to molecular function, biological process, and cellular component ontologies (http://www.geneontology.org/).

The unigene sequences were also compared to the COG database to predict and classify possible functions (Tatusov et al. 2000). Finally, pathway assignments were performed according to the KEGG pathway database (Kanehisa et al. 2017). Potential molecular pathways involving predicting unigenes can be highlighted from KEGG pathway annotations.

(6) Global differential gene expression analyses

NOIseq was used to detect differentially expressed genes (Parameters: Fold Change >= 2.00 and Probability >= 0.8) (Tarazona et al. 2011). Hierarchical clustering for DEGs was performed, after a $\log_{10}$ normalisation, using the R function *PlotHeatMap* implemented in the web tool Metaboanalyst (Chong and Xia 2018; Pang et al. 2022). The following R script was used: mSet, "heatmap_0_", "norm", "row", "euclidean", "ward.D","bwm", 8, "overview", T, T, NULL, T, F, T, T, T). PCA analyses were performed using the web tool MetaboAnalyst and using the following R script mSet<-Normalization(mSet, "NULL", "LogNorm", "NULL", ratio=FALSE, ratioNum=20 ; mSet<-PlotPCAScree(mSet, "pca_scree_0_", 5); mSet<-PlotPCA2DScore(mSet, "pca_score2d_0_", 1,2,0.95,0,0); mSet<-PlotPCALoading(mSet, "pca_loading_0_", 1,2); mSet<-PlotPCABiplot(mSet, "pca_biplot_0_", 1,2).

To highlight the most representative differential gene expression (DEG), we created an additional filtered dataset of 100 DEGs (for all pairwise comparisons and both species) which present the smallest intra-condition mean deviation (i.e., minimal standard deviation between replicates of the same condition (IC, IC+S or CC)). The selection was performed using the web

tool MetaboAnalyst. For visualization purposes, heatmaps of the 100 DEGs presenting to smallest intra-condition mean deviation were performed.

(7) BLAST/ reciprocal BLAST analyses to highlight stress-related genes

Genes that are specifically expressed under stress conditions have been mined from the literature with a particular emphasis on crustacean-specific studies. Gene sequences were then obtained from open-access NCBI online databases (http://www.ncbi.nlm.nih.gov) and used for a 'tBLASTn/reciprocal BLASTx' approach. The reference sequences are listed in the **Table S1** (Chen et al. 2013; Kim et al. 2017; Armstrong et al. 2019). Homologous sequences (*i.e.*, best candidate potentially corresponding to the closest orthologous) to reference stress-related genes were found in the transcriptomes of *A. indicus* and *T. holtuisi*. The expression profiles of the genes of interest were characterised based on our differential gene expression analysis.

III. Results

(1) Illumina sequencing and read assembly

Eighteen cDNA libraries were constructed to perform Illumina HiSeq sequencing for both shrimp species. A total of 742 million raw reads were generated. After removing the adapter sequences, the low-quality sequences and the ambiguous nucleotides, we obtained a total of 642 million clean reads, representing 96 Gigabases. The GC content for the clean reads was 39% (**Table 1**). The mean value of Q20 was 98.5% and the one for Q30 percentage was 95.5%. After the assemblies, 217,832 unigenes were obtained, with a total length, an average length and a N50 of 225.166.173 bp, 1033 bp and 2,061 bp respectively (**Table S2** and **S3**).

Table 1. Description of the output sequenced data for *A. indicus* and *T. holthuisi* transcriptomes. i. Conditions were "CC: Control condition (with host)"; "IC: Isolation condition"; "IC+S: Isolation condition + spinochromes". R = Replicate, M=Million, Gb = Giga bases.

Sample	Total raw reads (M)	Total clean reads (M)	Total clean bases (Gb)	Clean read Q20 (%)	Clean read ratio (%)
A. indicus CC, R1	42.34	36.59	5.49	98.47	86.42
A. indicus CC, R2	42.34	36.05	5.41	98.54	85.16
A. indicus CC, R3	41.67	35.60	5.34	98.53	85.43
A. indicus IC, R1	40.71	36.52	5.48	98.50	89.71
A. indicus IC, R2	43.96	36.06	5.41	98.50	82.02
A. indicus IC, R3	42.34	36.98	5.55	98.45	87.35
A. indicus IC+S, R1	40.71	35.81	5.37	98.55	87.96
A. indicus IC+S, R2	38.75	31.85	4.78	98.55	82.20
A. indicus IC+S, R3	41.93	34.59	5.19	98.57	82.51
T. holtuisi CC, R1	39.97	34.53	5.18	98.54	86.38
T. holtuisi CC, R2	39.08	35.67	5.35	98.48	91.28
T. holtuisi CC, R3	39.08	35.68	5.35	98.54	91.31
T. holtuisi IC, R1	40.71	36.12	5.42	98.53	88.72
T. holtuisi IC, R2	42.34	35.87	5.38	98.36	84.72
T. holtuisi IC, R3	39.08	35.89	5.38	98.36	91.85
T. holtuisi IC+S, R1	43.96	36.44	5.47	98.52	82.89
T. holtuisi IC+S, R2	42.34	36.51	5.48	98.62	86.24
T. holtuisi IC+S, R3	40.71	35.84	5.38	98.55	88.05

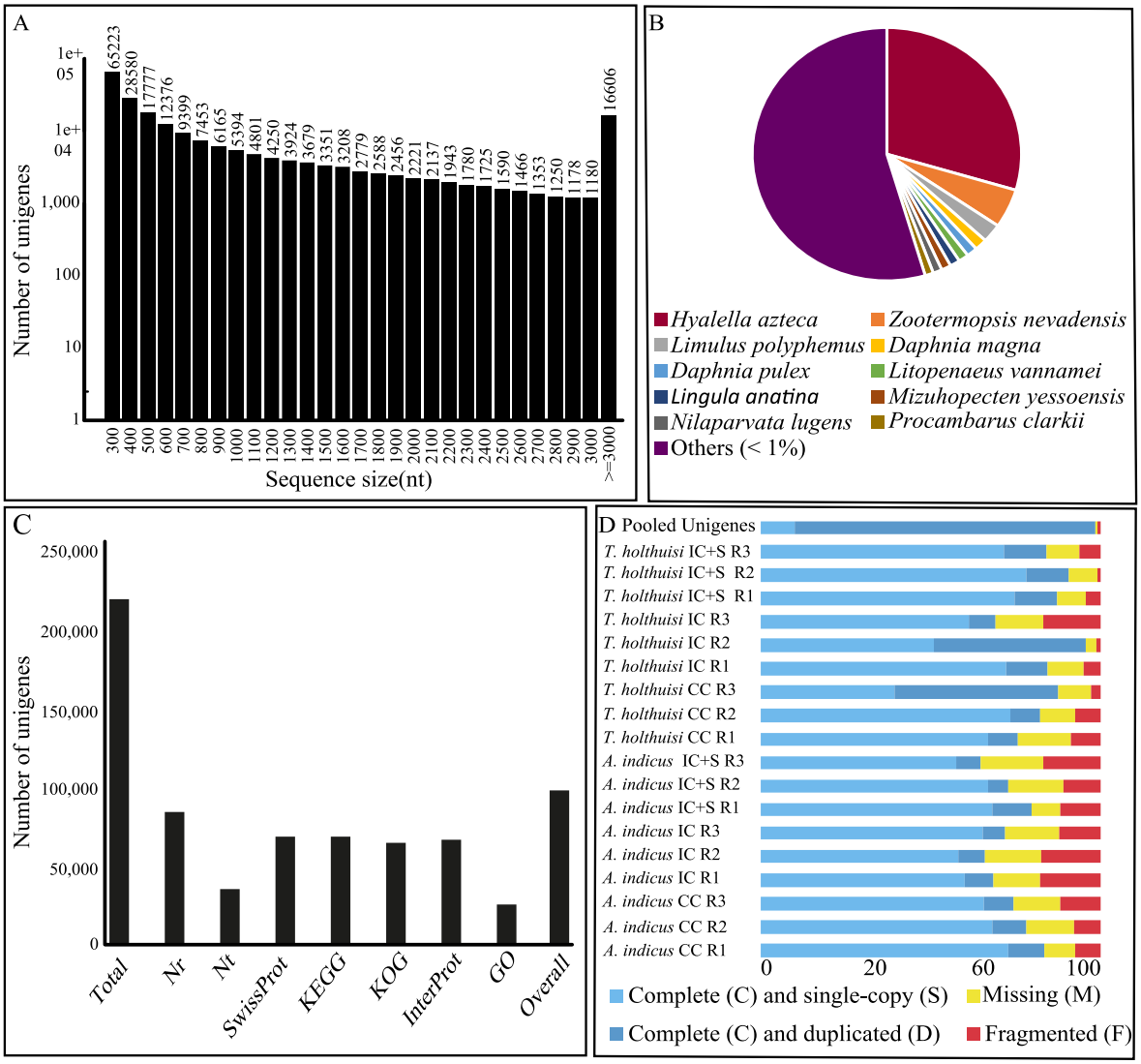


Figure 3. (A) Length distribution of all assembled unigenes (pooled for *Tulerіocaris holthuisi* and *Arete indicus*, (B) species annotation of all assembled unigenes, (C) global annotation of all assembled unigenes, (D) Benchmarking Universal Single-Copy Orthologs (BUSCO) analyses of all isolated transcriptome unigenes (Eukaryota database). Databases include *non-redundant protein* (Nr, NCBI), *non-redundant nucleotide* (Nt, NCBI), SwissProt, *Kyoto Encyclopedia of Genes and Genomes* (KEGG), *InterPro protein families and domains* (InterPro), *Gene Ontology* (GO) and *Clusters of Orthologous Genes* (COG).

(2) Transcriptome annotation

The length distribution of unigenes provided important information about the size and complexity of the transcriptome and can be used to determine the completeness of the assembly **(Figure 3A)**. Around 36% of unigenes presented lengths between 500 and 1500 base pairs. A significant proportion of unigenes were shorter (43%) or longer (20.9%) than this range. Taxonomic distribution analyses revealed that the main represented species within the unigene annotation is the highly studied amphipod *Hyalella azteca* (29.38%), followed by the insect model species *Zootermopsis nevadensis* (4.94%) (**Figure 3B**). A global annotation of assembled unigenes, which refers to the process of assigning predicted functional information

to the sequences generated from transcriptome assembly was performed (**Figure 3C**). Out of the 217,832 annotated genes, the majority found matches in various databases: 38.32% in NCBI's NR database, 31.29% in KEGG pathway database, and 30.74% in the SwissProt database, among others. The completeness of the transcriptome was evaluated by BUSCO (Benchmarking Universal Single-Copy Orthologs) analyses (**Figure 3D**). The pooled unigenes of *T. holtuisi* and *A. indicus* transcriptomes show 98.5% of complete (C) BUSCO (9.8% of single and 88.6% of duplicated copies), indicating a high level of completeness of the transcriptomes. For descriptive purposes, the 10 most expressed unigenes within the different samples and treatments were compiled in **Table S4**, with their predicted annotations.

(3) Differentially expressed unigenes under host separation

Differentially expressed genes (DEGs) between the control condition (CC; *i.e.*, shrimp on its host) and the treatments were analysed for both species (**Figure 4**). The list of all DEGs, with their corresponding annotation, are shown in **Table S5**.

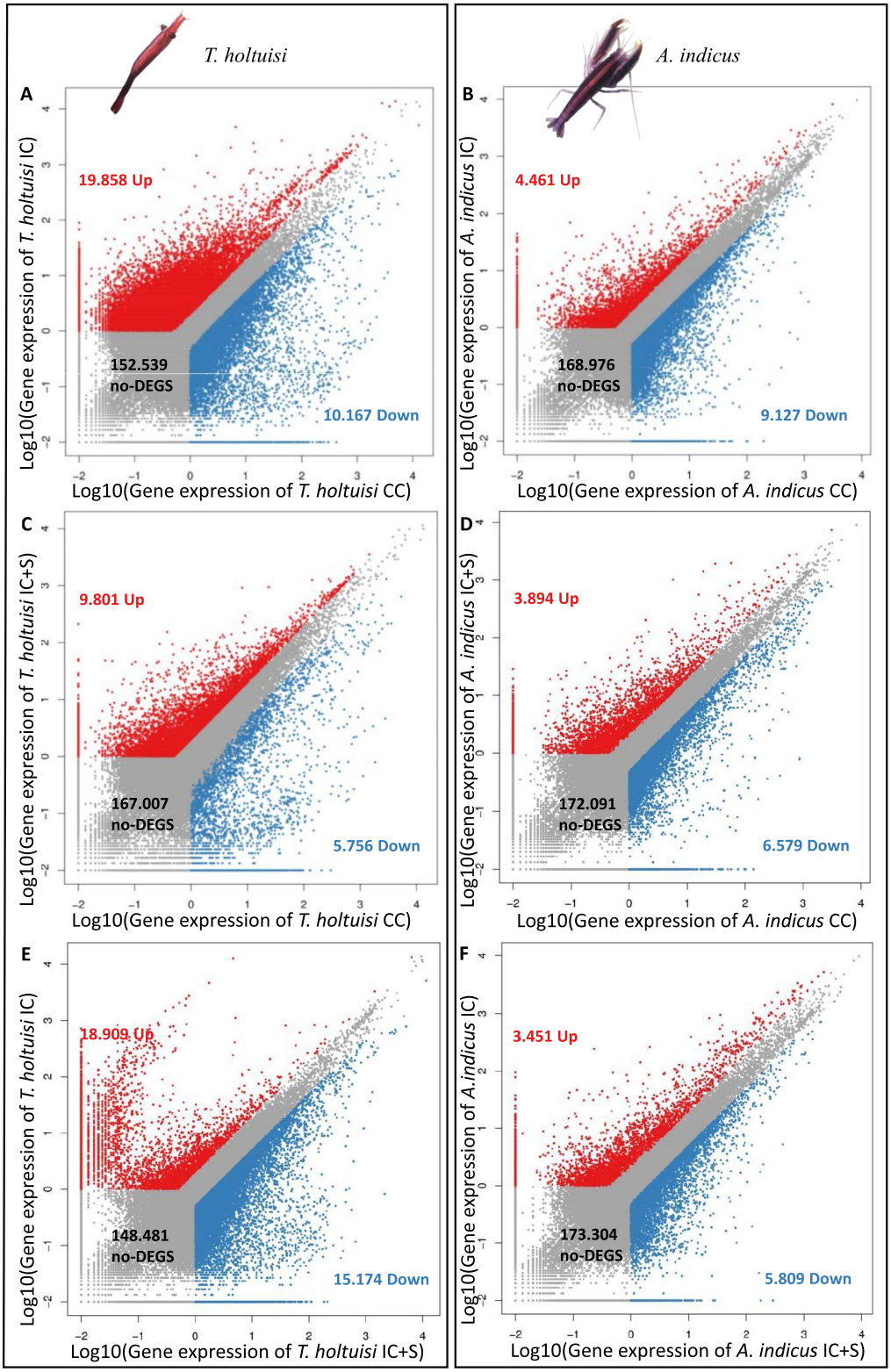


Figure 4. Differential unigene expression related to *Tulearіocaris holthuisi* (A; C; E); related to *Arete indicus* (B; D; F). Conditions were "CC: Control condition (with host)"; "IC: Isolation condition"; "IC+S: Isolation condition + spinochromes". For every comparison (A and D = IC/CC; B and E= IC+S/CC and C and F =IC/IC+S), if fold change > 1 = gene(s) is upregulated the first condition compared to the second condition and appears in red, fold change < -1 = gene(s) is downregulated the first condition compared to the second condition and appear in blue, and if fold change is comprised between -1 and +1 = gene(s) is no differentially expressed in the corresponding comparison and appear in grey. The graph was generated using $\log_{10}$ (FPKM+ 0.01) values.

Based on Brasseur et al. (2018) and according to their host-separation experimental framework, we suggested the hypothesis that the shrimp *A. indicus* exhibits reduced chemical host dependency. The shrimp's short-term survival rates were not reduced when *A. indicus* individuals were isolated from their hosts in comparison to the shrimps that remained on the sea urchin. Under this premise, we anticipated that species observing minimal number of differential gene expression in the following comparisons: (i) between shrimps in contact with their hosts (control; CC) and isolated shrimps (IC), (ii) between the control group (CC) and isolated shrimps treated with spinochromes (IC + S), (iii) between isolated shrimps (IC) and isolated shrimps with spinochromes (IC + S). Our findings reveal that the proportion of DEGs in *A. indicus* accounts for 7.4%, 5.7%, and 5% of the total predicted unigenes for each comparison (CC vs IC, CC vs IC+S, IC vs IC+S), respectively.

On the other hand, the shrimp species *T. holthuisi* develops a pronounced host separation syndrome (Brasseur et al. 2018). The shrimp survival was maximised when they were on their hosts (CC) or when they were isolated from their hosts with spinochromes (IC+S) (Brasseur et al. 2018). The shrimp survival was maximized when they were on their hosts (CC) or when they were isolated from their hosts with spinochromes (IC+S) (Brasseur et al. 2018). We then hypothesised notable variations in differential gene expression in the following comparisons: (i) between shrimps in contact with their hosts (the control; CC) and isolated shrimps (IC) and (ii) between isolated shrimps (IC) and isolated shrimps with spinochromes (IC + S). Conversely, we expected less variation when comparing control individuals (CC) with isolated shrimps with spinochromes (IC+S). For *T. holthuisi*, the proportions of DEGs are 16.5%, 18.6%, and 8.5% of the total unigenes, respectively, when comparing CC with IC, IC with IC+S, and CC with IC+S (**Figure 4**). Furthermore, we observed a 50% decrease in DEG numbers in the presence of spinochromes (IC+S) compared to the control condition (CC).

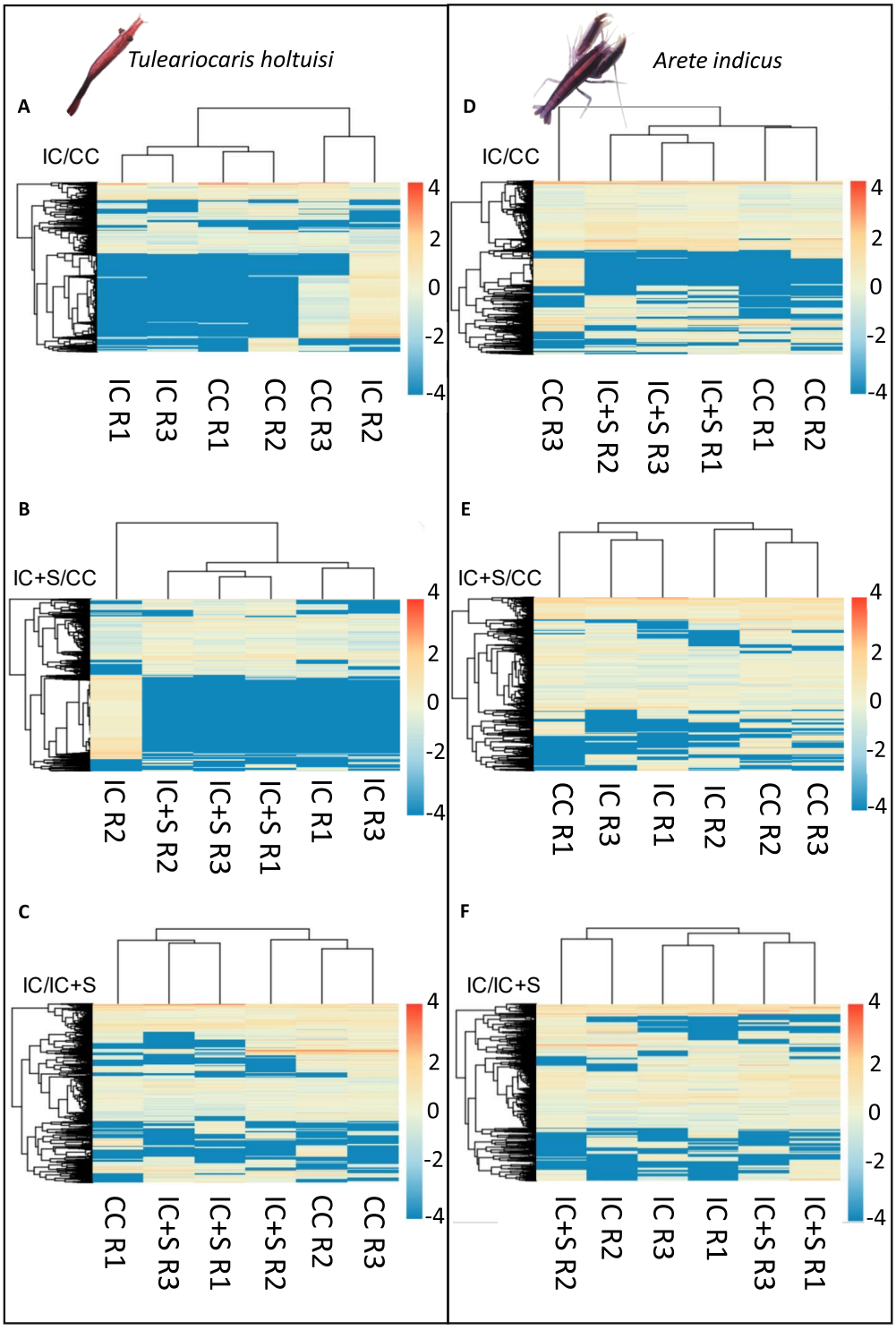


Figure 5. Clustering heatmap of the fold change of all statistically significant DEGs in relation to the host separation and the chemicals dependency for *Tulearіocaris holthuisi* (A, B and C) and *Arete indicus* (D, E and F). The hierarchical clustering analysis is made according to the expression profile. Each row represents a DEG, and each column represents a sample. The color displays the fold change. For every comparison (A and D = IC/CC; B and E= IC+S/CC and C and F =IC/IC+S), if fold change is > 1, the unigene is up-regulated in the first condition compared to the second condition and appears in red, if fold change is < -1, the unigene is down-regulated in the first condition compared to the second condition and appears in blue. If the fold change is comprised between -1 and +1, the unigene is not differentially expressed in the corresponding comparison and appears in beige. The darker in color, the greater the fold change. Both species were exposed to three conditions: "CC: Control condition (with host)"; "IC: Isolation condition"; "IC+S: Isolation condition + spinochromes". The differentially expressed genes were determined in 3 independent replicates (shown as columns of the heatmaps).

We conducted a differential gene expression analysis at the individual scale (i.e., biological replicate) and performed clustering of individuals based on their similarity of gene expression profiles (**Figure 5**). We postulated here that in *A. indicus*, a clustering by condition would not be observed. On the contrary, we anticipated encountering distinct clusters for isolated individuals (IC) and the two other conditions for *T. holthuisi* (CC, IC + S) since shrimp survival was maximised in these latter two conditions. However, the results of the analysis for *T. holthuisi* (**Figure 5 A-C**) did not reveal a condition-based clustering and our data showed a wide intra-condition variability (**Figure 5 A-C**). *For A. indicus*, we also did not observe a well-defined clustering, which may be more consistent with our expectation (**Figure 5 E-G**).

For all condition comparisons, the 40 most significant differentially expressed unigenes (20 up-regulated and 20 down-regulated) are detailed in **Table S6** and **Figure S1**. In *T. holthuisi*, a comparison between control (CC) and isolated treatments (IC) revealed a significant up-regulation of genes related to the production of cuticle and chitin proteins in isolated individuals. This up-regulation concerned 16 unigenes (e.g., *cuticle protein 19-like, cuticle protein CB6, early cuticle protein 3, chitin-binding protein, calcification associated soluble matrix protein 2*). However, this up-regulation was mainly observed in the second replicate of *T. holthuisi*'s IC. The other unigenes differentially expressed in this comparison were associated with diverse functions, without highlighting a grouping in their functions. Moreover, 12 unigenes were not annotated in this comparison, with one up-regulated and 11 down-regulated in isolated individuals compared to the control condition. For this comparison, no clear replicate clustering per condition was revealed by clustering heatmap and PCA analyses (**Figure S1**).

For *A. indicus*, the comparison between the control (CC) and the host isolation condition (IC) revealed differentially expressed unigenes with various functions. For the IC condition, we observed three up-regulated genes coding for structural proteins (*glycine-rich cell wall structural protein 1.8-like, cell wall protein AWA1-like, and vegetative cell wall protein gp1-like*) and one coding for a predicted histone activity regulator (*YEATS domain-containing protein 2-like*). Down-regulated unigenes included, as selected examples, a cuticle-associated gene (*chitin-binding protein*), and a ligase (*ubiquitin-protein ligase*). Three unigenes known to be expressed in stress conditions (*mitochondrial heme-binding protein* that facilitates necrosis and cell death, two *cathepsin L* that are lysosomal cysteine proteases) (Chen et al. 2013; Kim et al. 2017; Armstrong et al. 2019) were also up-regulated in isolated individuals. In this comparison, twenty sequences were not annotated (7 up-regulated/ 13 down-regulated in IC).

For this species, the results of the heatmap and the PCA analyses revealed a more significant clustering, especially in comparison between isolated shrimps with spinochromes (IC+S) (**Figure S1 and Figure S2**).

Based on our results and considering the strong inter-replicate/condition variability, we completed our DEG analyses by performing an additional filtering step on the DEGs. We therefore selected the 100 DEGs for all pairwise comparisons and for both species (**Figure S2 and Figure 6**), which present the smallest mean deviation within the same condition. Our hypothesis is that these filtered DEGs will better represent the impact of host isolation than the whole DEG dataset.

For both species, the three experimental replicates clustered well by treatment in every comparison. For *T. holthuisi*, the heatmap of the top 100 differentially expressed unigenes in the CC-IC comparison presents a clear expression pattern with two main DEG clusters (**Figure 6A**). In both clusters, most unigenes seemed to be up-regulated in the IC+S condition. In the first cluster (45 DEGs), the DEGs were up-regulated in IC and down-regulated in CC, and in the second cluster (55 DEGS), the DEGs were up-regulated in CC and down-regulated in IC+S. The majority of DEGs were not annotated in the GO database (79/100, *E* value $>10^{-5}$). The GO annotated of the remaining DEGs are diverse and distributed across the three main ontologies (*biological process, molecular function and cellular component*). There are 8 GO affiliated to the *biological process*, including for example the *lipid storage* (GO:0019915) in cluster 1 and the *chitin process* (GO:0006030) found in cluster 2. The cellular component ontology included 8 ontologies, like the *integral component of membrane* (GO:0016021) that contained 7 different DEGs, including 4 in cluster 1 and 3 in cluster 2. The *molecular function* included most of the GO terms detected, with 24 different ontologies like the *metal ion binding* (GO:0046872) that contained 3 different DEGs found in both clusters (1 and 2 respectively), the *extracellular matrix structural constituent* (GO:0005201) characterized by one DEG sequence found in cluster 2, or the *GTP binding* (GO:0005525) that includes 1 DEG of cluster 1.

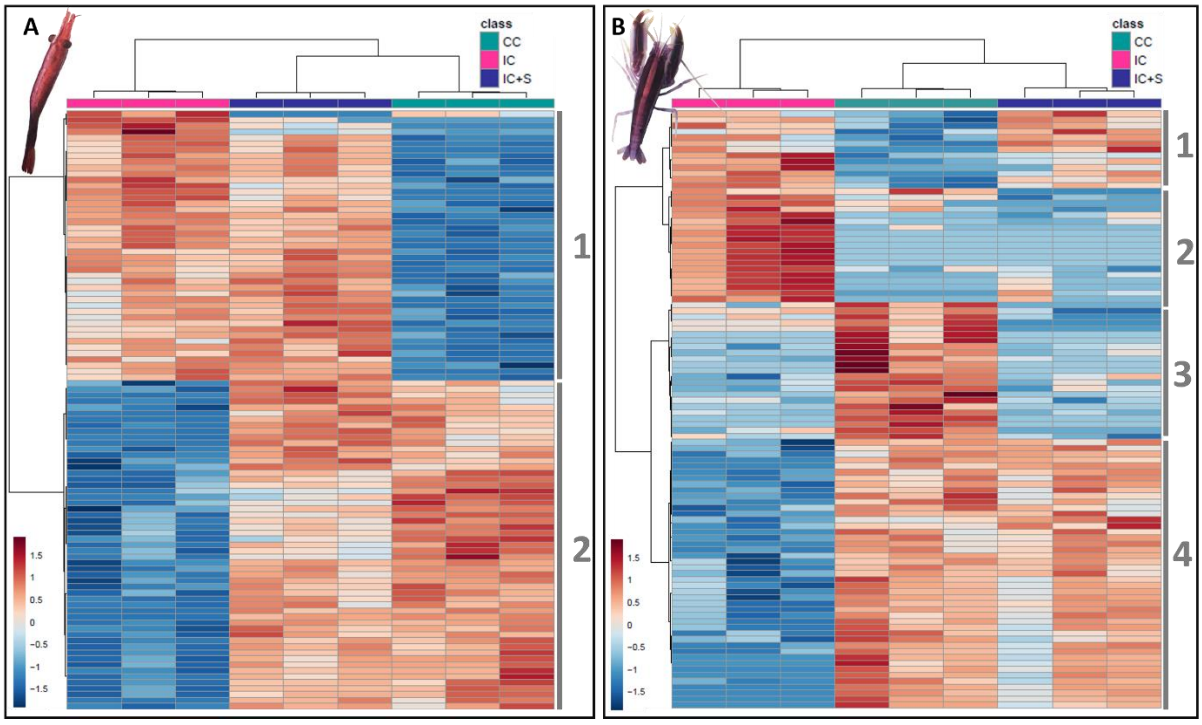


Figure 6. Cluster heatmap of the top 100 differentially expressed unigenes expression in the context of the host separation and chemicals dependency for *Tulearіocaris holthuisi* (A) and *Arete indicus* (B). Both species were exposed to three conditions: "CC: Control condition (with host)"; "IC: Isolation condition"; "IC+S: Isolation condition + spinochromes". The differentially expressed genes were determined in all independent biological replicates (shown as columns of the heatmaps). The heatmaps are based on all differentially expressed genes for every comparison (shown as a row of the heatmaps). The similarity of the gene expression patterns is indicated by Pearson's distance dendrogram at the top and left side. The colors of the heatmap indicate the unigene expression level. For every comparison (A and D = IC/CC; B and E= IC+S/CC and C and F =IC/IC+S), if the fold change is > 1, the unigene is up-regulated in the first condition compared to the second condition and appears in red. If the fold change is < -1, the unigene is down-regulated in the first condition compared to the second condition and appears in blue. If the fold change is comprised between -1 and +, the unigene is not differentially expressed in the corresponding comparison and appears in grey. The heatmap was generated using $\log_{10}$ (FPKM+ 0.001) values.

For *A. indicus*, four main clusters of DEGs were highlighted: (i) cluster 1 (13 DEGs) brings together DEGs up-regulated in IC and IC+S but down-regulated in CC, (ii) the second cluster included 19 DEGs up-regulated in IC but down-regulated in both CC and IC+S, (*iii*) cluster 3 gathered 23 DEGs up-regulated in CC and down-regulated in both IC and IC+S and (*iv*) the last cluster grouped 45 DEGs up-regulated in both CC and IC+S but down-regulated in IC. The majority of DEGs were not annotated in the GO database (86/100, E value $>10^{-5}$). As for *T. holthuisi*, represented GO ontologies were diverse and distributed across the three main ontologies (*biological process, molecular function and cellular component*). Twelve different GO ontologies were highlighted for the *biological process* such as *oxidation-reduction process* (GO:0055114) which contained 1 DEG found in cluster 1 or the *chitin metabolic process* (GO:0006030) which annotated one DEG in cluster 2. There were 9 ontologies affiliated to the *cellular component category*, including, for example, the *integral component of membrane* (GO:0016021) that caracterised 3 DEGs found each in clusters 1, 3 and 4, or the *extracellular*

region (GO:0005576), which were highlighted for 2 DEGs found in clusters 1 and 2, for example. The *molecular function* included 22 different detected ontologies such as the *glucose transmembrane transporter activity* (GO:0005355) found one time in cluster 4 or the *actin-binding* (GO:0003779) that caracterised 1 DEG of cluster 4.

(4) Expression of stress-related genes in the host separation condition

To examine the potential differential expression of stress-related genes, genes of interest were selected based on previously published literature (Chen et al. 2013; Kim et al. 2017; Armstrong et al. 2019) (**Table S1**) and were obtained from NCBI. Using a BLAST approach, the corresponding closest orthologous genes were identified in both species, and their expression levels were visualised (**Table S7** and **Figure S3**). The selected genes of interest included cytochrome P450 genes, heat shock protein genes, structural protein genes, genes linked to apoptosis and cell cycle, cellular defence effector genes, immune regulator genes, protease and protease inhibitor genes, genes involved in signal transduction, and the crustacean hyperglycemic hormone protein gene.

The analysis showed that *T. holtuisi* had more differential expression than *A. indicus*, with 27 DEGs in control individuals (CC) compared to isolated individuals (IC), 30 for the control individuals (CC) compared to isolated individuals with spinochromes (IC+S), and 21 for the isolated individuals (IC) compared to isolated individuals with spinochromes (IC+S) (**Table S7** and **Figure S3)**. For *A. indicus*, fewer unigenes were differentially expressed, with 20 DEGs in the CC/IC comparison, 11 DEGs in both the CC/IC+S and IC/IC+S comparisons (**Table S7** and **Figure S3)**.

When each unigene was considered separately, differentially expressed categories of genes between treatments did not reveal marked differences except for *heat shock proteins* (HSP, see here below). The cytochrome P450, antioxidant response, apoptosis, immune regulator cell cycle, and genes involved in signal transduction, categories showed heterogeneity of expression, particularly in *T. holtuisi*, and several corresponding unigenes were not differentially expressed. The unigenes associated with the categories *structural protein*, *cellular defense effector* and *protease and protease inhibitor* showed only a few sequences with differential expression in pairwise comparison. The crustacean hyperglycemic hormone protein, LITAF, and the signal transduction categories showed no strong expression in any treatment for both species.

Out of the nine targeted HSP unigenes, in *A. indicus*, the control individuals (CC) compared to isolated individuals (IC) highlighted three differentially expressed unigenes, and the two other comparisons showed one differentially expressed gene each (**Table S7**; **Figure 7)**. For *T. holtuisi*, out of the nine selected sequences, six were mostly expressed in the IC and three in IC+S. (**Table S7**; **Figure 7**).

The HSP heatmap (**Figure 7**) showed that the control shrimps were well-grouped together because most of the nine targeted HSP unigenes were down-regulated: this is obvious for the control individuals 1 and 2 and less clear for the individual 3 where only 4 unigenes were down-regulated. The isolated individuals and those isolated with spinochromes were mainly clustered together (5 out of 6 individuals) except the isolated individual 2. The last had 5 down-regulated HSP unigenes but all the others had from 4 to 7 up-regulated HSP unigenes. Between the 5 individuals that showed an up-regulation of the HSP unigenes, there was however no clear distinction between those shrimps treated with spinochromes and those that were not. We observed that HSP21 and 67B1-like genes were highly expressed in isolated individuals with spinochromes (IC+S). Differential expression was also observed between IC and CC of the HSP21 gene, with the smallest up-regulation in IC. The HSP68-like, 68-like isoform X3, and HSP90 alpha genes were up-regulated in IC and IC+S compared to the control condition, without showing a strong difference between the two treatments. The HSP75 and 90 beta genes were highly expressed in IC compared to both IC+S and the control condition. A small down-regulation was also observed in IC+S compared with the control condition.

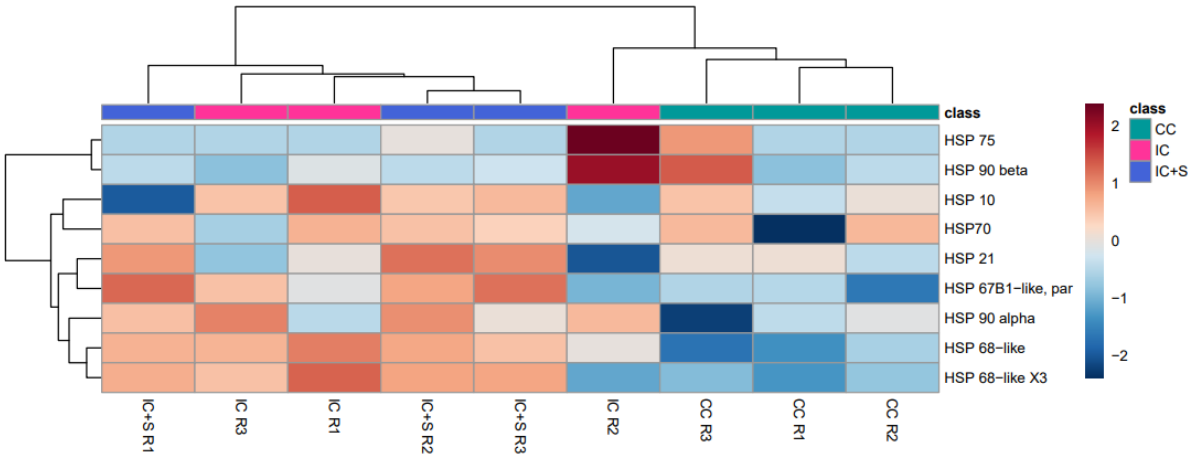


Figure 7. Heatmaps of Heat Shock Protein (HSP) in *Tuleariocaris holtuisi* subjected to host separation syndrome. Every unigenes ID is shown on the right side of the heapmap. There are three conditions: "CC: Control condition (with the host)"; "IC: Isolation condition"; "IC+S: Isolation condition + spinochromes". The differentially expressed genes were determined in 3 independent replicates (shown as columns of the heatmaps). The heatmaps are based on HSP unigene expression (shown as a row of the heatmaps). The similarity of the gene expression patterns is indicated by Pearson's distance dendrogram at the top and left side. The colors of the heatmap indicate the relative gene regulation level above (red) or below (blue) the average for each row. The heatmap was generated using $\log_{10}$ (FPKM+ 0.001) values.

IV. Discussion

Symbiotic relationships between decapods and echinoderms are common in marine ecosystems, with many associations being obligatory for the symbionts to complete their life cycle (Castro 1988; Gherardi 1991; De Grave and Fransen 2011; Horká 2017). *T. holthuisi* is mainly found on *E. mathaei*, its primary host, especially on the Great Reef of Toliara area (Hipeau-Jacquotte 1965; Brasseur et al. 2018), but it can also be found on other secondary sea urchin hosts such as *Diadema mexicanum*, *Astropyga radiata*, or *Stomopneustes variolaris* (Castro 1971b; Bruce 1974; Wicksten and Hernández 2000; Marin and Anker 2009; Salas-Moya et al. 2021). The second obligate symbiont, *A. indicus*, appears to be strictly species-specific with *E. mathaei* (Gherardi 1991; Dabbagh et al. 2019; Limviriyakul et al. 2020; Ghory and Kazmi 2021), but questionable occurrences of this species have been described on *Diadema* sp. and *Heliocidaris crassispina* (Miya and Miyake 1968; Ganapati and Sastry 1972; Al-Kandari et al. 2020). Their hosts provide them with various benefits, such as a safe shelter from predators and food platforms, which may make them host-dependent (Gherardi 1991; Brasseur et al. 2018). *T. holthuisi* feeds directly on its host, while *A. indicus* prefers seaweeds and it can sweep up the crumbs from the sea urchin meal (Gherardi 1991; Brasseur et al. 2018). They both present specific adaptations to efficiently live in symbiosis with their host, such as a specific motion behavior or a pigmentation that mimics the color of their host (Gherardi 1991; Horká et al. 2016; Brasseur et al. 2017; Horká 2017; Salas-Moya et al. 2021). *A. indicus* is always found grasping onto a sea urchin spine with the head pointed outwards while *T. holthuisi* shows a similar behavior with the head directed in the opposite direction (Gherardi 1991; Horká 2017). *T. holthuisi* is particularly efficient in mimicry of the host spine, as its body shape and color are almost identical to the host spines (Hipeau-Jacquotte 1965; Brasseur et al. 2017; Horká 2017). Moreover, Brasseur et al. (2018) demonstrated that the survival of *T. holthuisi* separated from its host could be extended if it was placed in the presence of the chemical environment produced by its host, the spinochromes (Brasseur et al. 2018). Although both *A. indicus* and *T. holthuisi* are obligate and highly host-dependent symbionts, the latter has a stronger chemical dependence on its host.

Primarily aiming to investigate the effects of host separation on both symbionts through a transcriptomic approach, this study also wants to examine the chemical dependency of *T. holthuisi* and the effect of spinochromes which seem to attenuate the separation stress in this species (Brasseur et al. 2018). For that purpose, we compared the gene expression of both

species in three conditions: unstressed symbionts living on their hosts, isolated stressed individuals, and isolated individuals in seawater presenting the chemical environment of the host, notably the spinochrome pigments. Due to the small size of the echinoid-associated shrimp specimens, it was impossible to determine the sex of these individuals accurately. This limitation is a common constraint encountered in studies involving small, cryptic species inhabiting complex ecosystems (Ghiselli et al. 2012). The inability to distinguish between male and female individuals may have implications for understanding the transcriptomic responses observed in this study. Sex-specific differences in gene expression can be crucial in shaping the symbiotic relationships between hosts and symbionts. Therefore, the lack of sex information in our dataset should be considered when interpreting the results and their implications is the host-separation context.

We observed significant differences in the number of differentially expressed genes (DEGs). For *A. indicus*, the three comparisons showed a similar number of DEGs (CC/IC = 7.4%; CC/IC+S = 5.7% and IC/IC+S = 5%). For *T. holthuisi*, the total predicted unigenes showed similar differential expression levels (CC/IC = 16.5% and IC/IC+S = 18.6%) when comparing isolated individuals with the other conditions (CC/IC+S). The presence of spinochromes, however, is associated with a 50% decrease in DEGs in the comparison between control individuals and those isolated in spinochrome-containing environment. This finding suggests that spinochromes have a significant effect at the molecular level, bringing the health status of isolated shrimp closer to that of individuals living on hosts. However, the number of DEGs remains high in other comparisons (*T. holthuisi*: CC/IC+S; *A. indicus*: CC/IC, CC/IC+S and IC/IC+S) which also suggest a transcriptomic regulation in conditions that appear less impactful (IC+S in both species and IC for *A. indicus*) in the survival experiments performed by Brasseur et al. (2018).

Having observed certain differences in the number of DEGs according to the categories of individuals, we expected to obtain well-defined clusters depending on the condition involved, which was not often the case. We first focused on the 40 most significant expressed unigenes (20 up-regulated and 20 down-regulated). In *T. holthuisi*, no clear grouping was found in any of the three conditions which indicates a high interindividual variability. Variations in the age, sex and proximity to the moulting time could have greatly influenced the clustering of individual gene expression profiles. In filtered DEGs, *T. holthuisi* presents two clear clusters of DEGs, while *A. indicus* present 5 clusters of differentially expressed genes in the IC condition

compared to the CC condition. This heterogeneity of differential expression of genes in this comparison may highlight a more complex process of regulation to cope with host separation for *A. indicus* and may explain why this species does not seem to be affected by host separation. While *T. holthuisi* individuals are affected by the host separation and do not survive long, *A. indicus* may present alternative expressions of genes to cope longer with this stress. For at least a short period, this species will not appear to be impacted in their survivability by the host separation.

We then determine a list of genes expressed during stress conditions in crustaceans based on the scientific literature to investigate their expression in our study (Chen et al. 2013; Kim et al. 2017; Armstrong et al. 2019). By comparing conditions (CC, IC and IC + S), we observed that *T. holtuisi* had more differential expression, in all pairwise comparisons, than *A. indicus*. This suggests that, in all cases of isolation, with or without spinochromes, the stress genes are more differentially expressed in the species *T. holthuisi*. We finally focused on heat shock proteins (HSPs) which are highly conserved and ubiquitous proteins primarily functioning as molecular chaperones (Liu et al. 2018). These proteins facilitate the folding, refolding, and unfolding of partially denatured proteins, enabling protein movement across cellular compartments and membranes, as well as degradation of unstable proteins and dissolution of protein complexes and aggregates (Lindquist 1986; Feder and Hofmann 1999; King and MacRae 2015; Zhang et al. 2017; Kim et al. 2017; Liu et al. 2018) . Although initially discovered as proteins that respond to high thermal stress, HSPs are now known to perform a variety of other functions, and their up-regulation, especially under environmental and non-environmental stress conditions, has been described in various organisms, including crustaceans (Lindquist 1986; McLennan and Miller 1990; Portune et al. 2010; King and MacRae 2015; Liu et al. 2018). HSPs participate in various stress responses, such as oxidative stress, osmotic shock, starvation, toxic chemicals, bacterial infections, and immune reactions (Beck et al. 2000; Han et al. 2012; Yang et al. 2013; Liu et al. 2015, 2018; Ikwegbue et al. 2017; Ghosh et al. 2018; Meng et al. 2019; Ren et al. 2020). HSPs are generally categorised into different families based on their molecular weights (Beck et al. 2000). In the current study, *T. holtuisi* showed a general up-regulation of HSPs in both isolation treatments compared to the control condition, particularly in the complete host isolation condition. Also, several stress-related genes that have been previously identified in crustaceans, such as the crustacean hyperglycemic hormone and antioxidant-response proteins were not differentially expressed in all pairwise comparisons for both species in these analyses. Since our stress-related gene searches only focus on the recovery of the closest homolog in both

species, the selected sequences do not correspond systematically with the most expressed unigenes coding for protein homologues, which can cause a bias in our analyses as it is always the case in non-model studies.

During this study, numerous DEGs highlighted appear not to be annotated using the classical public databases. This may be explained by the fact that the investigated species are poorly studied non-model organisms. Stress-related genes of interest may not have been captured by the analyses, as the experiments were performed on whole-body tissue of the symbionts. The gene expression of crustacean stress-response has been more frequently evaluated on targeted tissue, such as gills (Li et al. 2014; De Vos et al. 2019; Tang et al. 2020), muscles (Li et al. 2014), or the hepatopancreas (Meng et al. 2019), but the stress was very different including salinity (Li et al. 2014; De Vos et al. 2019), temperature (McLennan and Miller 1990), and various chemical exposures (Li et al. 2014; Meng et al. 2019; Ren et al. 2020). This bias was, however, unavoidable even though it was considered at the start of the study: the symbiotic organisms studied were very small and a dissection to sample an organ on which we would have had to assess the effect of stress was impossible, especially since the stress studied in the present paper (host separation) has never been investigated to the best of our knowledge.

In the case of environmental stress, the energy balance of an organism can be significantly disrupted, as additional energy is required to maintain metabolic integrity and recover from stress-induced damages (Straalen and Hoffmann 2000; Sokolova et al. 2012). Previous studies have identified two main survival strategies in response to stress: the "limiting process" and the "compensatory process" (Péqueux 1995; Rainbow and Black 2001; Sokolova et al. 2012; Lv et al. 2013). The limiting process occurs in low-level stress environments and relies on cellular, physiological, and behavioral mechanisms to minimize and cope with the negative impact of stress before it causes significant damage to the organisms (Rainbow and Black 2001; Sokolova et al. 2012). Although more energy-intensive, the compensatory process offsets the impact of stress, such as the production of reactive oxygen species that may damage DNA, which can lead to the death of the organism or a reduction in fitness-related functions (Rainbow and Black 2001; Sokolova et al. 2012). In our study, the transcriptomic approach yielded mixed results, that indicate that both species feel host isolation stress but with different degrees of coping. *A. indicus* can cope with this kind of stress, at least for a few times (more than 4 days), without a decrease in its survival rate (Brasseur et al. 2017). However, various genes with essential functions appeared differentially expressed which indicated potential micro-adaptation of the

different individuals to the isolation stress. In addition, *A. indicus* is a bigger symbiont than *T. holthuisi*, and this size can play a key role in its survivability as the biggest species is usually more able to cope with stressful situation (Linden et al. 2009; McCormick et al. 2018). For *T. holthuisi*, the species exhibiting a clear host-separation syndrome and a strong host chemical dependence (Brasseur et al. 2018), results indicated a strong transcriptomic response. In both conditions, shrimps may be stressed but the addition of spinochromes may enable the shrimp to cope more efficiently with the isolation situation. The up-regulation of HSPs may have played a key role in the compensatory process used by the symbiont to defend itself against the stress of isolation until the stress became too severe, eventually resulting in organism death.

V. Conclusion

In non-model species, the emergence of Next Generation Sequencing (NGS) technologies has enabled the generation of molecular data, allowing for the detection of new genes and the analysis of their expression. In this study, we reported the transcriptome sequencing and analysis of two symbiotic shrimps associated with the sea urchin *E. mathaei* in the southwest of Madagascar. Our work presents the first report on the use of NGS techniques for studying the host-separation syndrome involving a new kind of host reliance: the chemical dependency.

We generated a transcriptome dataset for two symbiont species, *A. indicus* and *T. holtuisi*, by obtaining more than 96.41 clean Giga bases. For *T. holthuisi*, the results highlight a strong transcriptomic response, especially in isolation without the addition of the chemical environment produced by the host. Based on a comparison of gene expression, genes coding for heat shock proteins were found to be differentially expressed in response to the chemical host-isolation experiment. The addition of spinochromes would enable this shrimp to better cope with the stressful situation. In addition, *A. indicus appears* to be also stressed by the host isolation but can potentially cope with this stress thanks to transcriptomic adaptation. Our results contribute to the understanding of the host-separation syndrome and the symbiont's dependence on semiochemicals produced by their host.



Chapter IV



Exploring the mimetic pigmentation of symbiotic shrimps associated with echinoderms

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Abstract
In marine ecosystems, many organisms undergo an intimate and long-lasting association with at least one other species, various benthic hosts being considered as micro-ecosystems hosting a rich symbiotic community. Among the adaptive strategies developed to lower their predation rate, symbionts may display a color resembling that of their hosts - known as mimetic pigmentation. Although frequently described, this phenomenon has scarcely been characterized. The objective of this research is to investigate the pigmentation dynamics within four symbiotic pairs associating crustaceans to echinoderms: (i) the shrimp *Zenopontonia soror* cohabiting with the sea star *Culcita novaeguineae*, (ii) the snapping shrimp *Synalpheus stimpsonii* hosted by the crinoid *Phanogenia distincta*, and (iii) the shrimps *Tuleariocaris holthuisi*, and (iv) *Arete indicus*, both associated with the sea urchin *Echinometra mathaei*. The scientific approach involved: (i) monitoring symbiont survivability across diverse conditions, (ii) employing an innovative standardized photography technique to measure color variation, (iii) locating pigment-associated cells in symbionts tissues through histological sections, and (iv) characterizing the chemical composition of the pigments using HPLC chromatography. The results suggest a strong host dependency for all four symbionts, three of them also exhibiting a reliance to their host metabolites. Host-symbiont separation also impacts the pigmentation of the symbionts, leading to a dynamic color variation depending on the species. Histological sections highlight the presence of chromatophores in all the species, with *A. indicus* harboring two specific layers of chromatophores containing different pigments. Finally, pigment chemical analyses reveal divergence according to the symbiotic couples, with carotenoids being responsible for the pigmentation of both associates *Z. soror* and *C. novaeguineae*, while being only present in the symbiont *S. stimpsonii* but being scarce in the host crinoid *P. distincta*. These discoveries shed light on the mechanisms underlying the mimetic coloration of symbiotic organisms, expanding our knowledge on the ecology of symbionts and their interaction with their hosts.

Keywords : Crustacean; Ectosymbiont; Coloration; Mimicry; Chromatophore

I. Introduction

The biosphere relies on an intricate interactome binding biological entities together in dynamic ecosystems (Levin 1998). It includes symbiotic associations, which are defined as intimate and lasting interactions between interspecific organisms, called host and symbionts (Paracer and Ahmadjian 2000; Eeckhaut 2003). Symbionts always benefit from an advantage arising from these relationships, often resulting in a particularly strong host-dependency (Caulier et al. 2014; Morita and Schmidt 2018; Brasseur et al. 2018; Lourtie et al. 2023). A key factor for increasing the survivability of many symbionts is their ability to adopt a mimetic coloration, replicating the texture and color of their host body wall (Maciá and Robinson 2009; Caro 2018). Such mimicry makes them almost invisible and consequently decreases their predation rate for predators relying on visual cues (Maciá and Robinson 2009; Virgili et al. 2020; Bauer 2023). This mimetic coloration has been observed in many symbionts belonging to different taxa, including symbiotic decapods (Gherardi 1991; Horká 2017; Brasseur et al. 2018; Virgili et al. 2020; Bauer 2023). These crustaceans are known to display a large phenotypic plasticity, with two different strategies leading to mimicry. Firstly, a partial or complete translucence of their body allows some decapods to be nearly imperceptible (Britayev et al. 2013; Yogesh Kumar et al. 2015; Fernández-Lereé et al. 2023). The second strategy involves coloration mimicry, where symbiotic decapods fit to the appearance of their hosts, frequently adopting specific colors containing shades of multiple colors (de Gier and Becker 2020; Fernández-Lereé et al. 2023). For example, the white coloration may be used by the symbiont to simulate shell fragments of their host, while the vivid colors are more adapted to match some host integument coloration (Gherardi 1991; Caro 2018; Virgili et al. 2020; Caulier et al. 2022; Fernández-Lereé et al. 2023). These adaptive coloration strategies may take the form of uniform shades, textured patterns, or ornamentation, such as distinct colored stripes or spots, which contribute to their effective mimicry (Gherardi 1991; Virgili et al. 2020; de Gier and Becker 2020).

Despite the numerous examples of specific symbiotic coloration and the essential character of this mimetic function on symbiont survival, there is scarce research aimed at understanding its underlying mechanism. Only a few studies were realized in applied research aiming to provide a vivid pigmentation to commercialized shrimps in seafood or ornamental shrimp industry to sell the final product at a higher price (Wade et al. 2017a, b; Lu et al. 2022). It has been shown previously that the non-symbiotic crustacean coloration is mainly mediated by carotenoid

pigments such as astaxanthin (named after its first characterization in the European crayfish, *Astacus astacus*). Carotenoids can only be synthesized by photosynthetic organisms (Cazzonelli 2011) and *de novo* synthesis of these chemicals is unfeasible in the vast majority of animals, these essential pigments being thus probably obtained by partial metabolization of their diet or by the help of mutualistic bacteria (Cobbs et al. 2013; Maoka 2020; Lu et al. 2022). In order to achieve an efficient mimicry of their host coloration, some decapod species may acquire pigments by fully or partially feeding on their hosts tissues (Zmarzly 1992; Brasseur et al. 2018). Consequently, when kept separated from their hosts, their pigmentation may vary with different time dynamics, from a few hours to several days or months (Ayotte and DeMaintenon 2005; Brasseur et al. 2018). Moreover, crustaceans coloration can vary depending on abiotic parameters such as the background color or ambient luminosity, and the time period (*i.e.* nychthemeral rhythm) (Joss 1973; Ayotte and DeMaintenon 2005; Tlusty et al. 2009).

In addition to coloration, carotenoids are also involved in other essential metabolic functions as they are antioxidants, precursors of vitamin A and retinol, photo-protectors, enhancers of immunity and they may also play a key function in mating strategy (Higuera-Ciapara et al. 2006; Maoka 2020; David et al. 2023). Pigments are stored in specialized cells known as chromatophores, present in the integument and containing various pigments such as melanin or pterins for examples (Lu et al. 2022; Fernández-Lereé et al. 2023). Some chromatophores, called erythrophores, are characterized by reddish colors and a distinctive dendritic form (Lu et al. 2022; Fernández-Lereé et al. 2023). In general, their pigmentation is due to the presence of carotenoids or their derivatives (Matsumoto 1965; Matsumoto and Obika 1968). Chromatophores may play a role in pigmentation changes in organisms (Fingerman 1985; Fernández-Lereé et al. 2023) through two distinct pathways: (i) a fast pathway involving muscle contractions (*e.g.* in cephalopods; Mäthger et al. 2008), and (ii) a slower pathway involving the endocrine system (*e.g.* in chameleons; Duarte et al. 2017). Considering the endocrine pathway, specific hormones called chromatophorotropins, such as PDH (Pigment Dispersion Hormone) or PCH (Pigment Concentration Hormone), produce opposite effects on chromatophore diameter, impacting ultimately the organism coloration (Josefsson 1975; Lu et al. 2022).

Performing host separation experiments on symbiotic shrimps, Brasseur *et al.* (2018) highlighted the chemical dependency of the shrimp *Tulearіocaris holthuisi* to its sea urchin host, *Echinometra mathaei*. This research suggests the essential role of spinochromes, specifically produced by the echinoid class (Hatate et al. 2002; Brasseur et al. 2017; Hou et al. 2018), in (1) the host recognition/selection and (2) the short-term survival of *T. holthuisi*.

Indeed, this study reveals a higher survival rate among isolated *T. holthuisi* individuals when incubated in spinochrome extracts or sea urchin-conditioned water (Brasseur et al. 2018) in comparison with shrimps isolated in normal seawater. This phenomenon was described as a host separation syndrome, defined as a degradation of the health status of a specific symbiotic organism when isolated from its host, leading potentially to the symbiont death. The authors suggested that the pigmentation may be influenced by this dependency, as the isolated shrimps seemed to show less mimetic pigmentation than the healthy ones that remained on their host. In opposition to *T. holthuisi*, the coloration and short-term survival of a second host-specific shrimp, *Arete indicus,* were not affected by the host isolation (Gherardi 1991; Brasseur et al. 2018).

The goal of the present study is to investigate the host separation syndrome, the chemical dependency and the mimetic coloration of a larger panel of symbiotic shrimps associated with echinoderms. The mimetic coloration of shrimps is investigated using a pluri-disciplinary approach, combining (1) the monitoring of the color changes during the time of separation from the host with a photographic standardized method, (2) the identification and quantification of the host and symbiont pigments through chemical extraction and chromatography analyses and (3) the localization of colored cells and their changes in the body wall using histological techniques. Four tropical species of shrimp living on echinoderms are investigated, all harboring typical mimetic coloration (**Figure 1**). The first is the shrimp *Zenopontonia soror* associated with the cushion sea star *Culcita novaeguineae* (**Figure 1Aa-d**). This species is known to have three morphotypes: translucent, fully colored and stripe-colored (Antokhina and Sorokin 2010; Antokhina and Britayev 2020a; Lourtie et al. 2023; Fernández-Lereé et al. 2023). The second association involves the yellow-orange snapping shrimp *Synalpheus stimpsonii* living on the crinoid *Phanogenia distincta* **(Figure 1Ba-b;** VandenSpiegel et al. 1998; Caulier et al. 2022). The third and fourth shrimps are those found on the sea urchin *Echinometra mathaei*: *Tuleariocaris holthuisi* and *Aretes indicus* (**Figure 1Ca-c**). This last symbiotic couple was already investigated by Brasseur et al. (2018) but was investigated during a shorter time period than the present study. Both symbionts present mimetic pigmentation: *A. indicus* shows a purple coloration with two whiter stripes on the body sides and *T. holthuisi* is characterized by a fully pinkish-purple color (Gherardi 1991; Marin and Anker 2009; Brasseur et al. 2018; Dabbagh et al. 2019; Ghory and Kazmi 2021). Both species are known to share common spinochrome pigments with their host, obtained either partially or completely by feeding to their host tegument to gather specific spinochrome pigments (Brasseur et al. 2018).

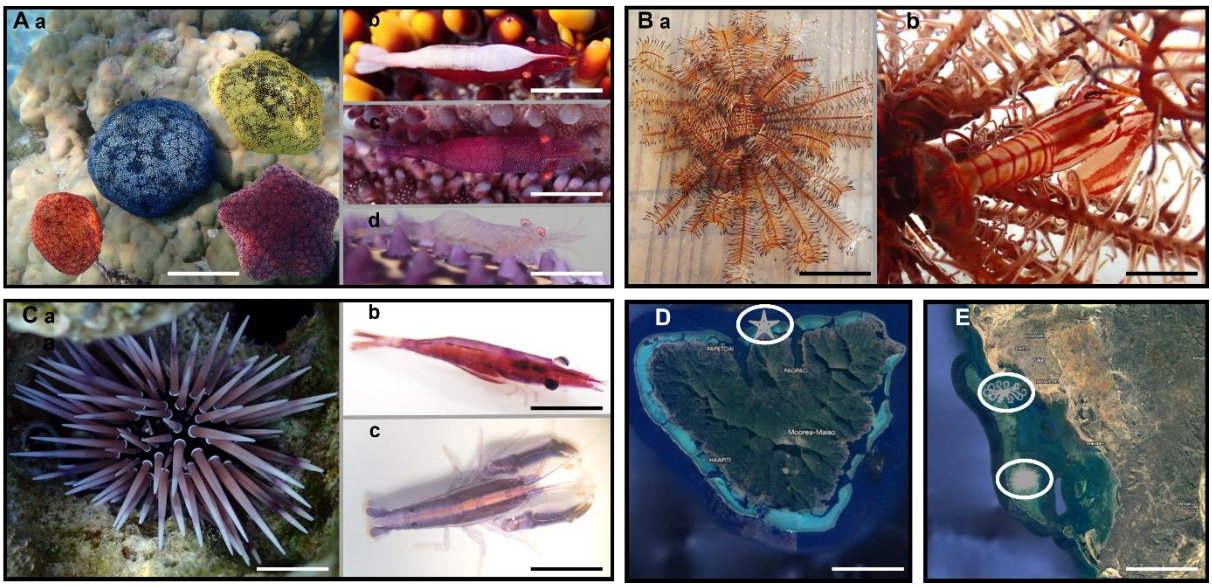


Figure 1: Symbiotic model organisms investigated for host separation experiments and their respective field locations. (A) the association with *Culcita novaeguineae* and *Zenopontonia soror*. (Aa) different color morphotypes of the sea star host, (Ab) stripe-colored shrimp morphotype, (Ac) full-colored shrimp morphotype and (Ad) translucent shrimp morphotype. (B) *Phanogenia distincta* associated with *Synalpheus stimpsonii*. (Ba) the crinoid host, (Bb) the symbiotic shrimp. (Cc) the sea urchin host, *Echinometra mathaei*, that can host two different shrimps named (Cb) *Tuleariocaris holthuisi* and (Cc) *Arete indicus*. (D) Moorea island, French Polynesia, where the asteroids were collected (white circle). (E) the Great Reef of Toliara, Madagascar, where the crinoids and echinoids were collected (white circles). (D,E) Modified from ©Google Earth, version 9.181.0.1. Scalebar = 8 cm in Aa; 6 mm in Ab-c and Cc; 4 mm in Ad and Cb; 6.2 cm in Ba; 9 mm in Bb; 2 cm in Ca; 4.8 km in D; 7.2km in E.

II. Material and Methods

Two scientific field trips took place in July - August 2021 and 2022 at the Center for Island Research and Environmental Observatory (CRIOBE) located in Moorea, French Polynesia. Similarly, two field missions were conducted in April and May 2022 and 2023, at the Halieutic Institute and Marine Sciences (IHSM, University of Toliara) and the Marine Station of Belaza, Madagascar. Organisms collection, sample preparation and photographic monitoring were performed on site. Carotenoid extractions and subsequent analyses were conducted at the Marine Station of Concarneau, France. Finally, the assessment of pigmentation variations and the chromatophore analysis in optical microscopy were carried out at the University of Mons, Belgium.

The study of the host separation syndrome, including survival and color change monitoring, and histological analyses was performed on the four symbiont species: *Z. soror*, *S. stimpsonii*, *A. indicus* and *T. holthuisi*. The HPLC chemical identification of the pigments was only realized on the two symbiotic couples *Z. soror – C. novaeguineae* and *S. stimpsonii – P. distincta* due to sample availability.

(1) Sampling and organisms maintenance

Culcita novaeguineae Müller & Troschel, 1842 hosting *Zenopontonia soror* (Nobili, 1904) were free-diving hand-collected in depths ranging from 1 to 5 meters on the northern coast of Moorea near the Opunohu Bay, French Polynesia (**Figure 1D**; 17°28’50’’S; 149°50’00’’W). *Phanogenia distincta* (Carpenter, 1888) hosting *Synalpheus stimpsonii* (De Man, 1888) were collected by scuba diving on the north reef pass of the Great Reef of Toliara in Madagascar (**Figure 1E**; 23°23'14"S; 43°39'04 "E), at depths ranging from 20 to 28 meters. *Echinometra mathaei* (Blainville, 1825) hosting *Tuleariocaris holthuisi* Hipeau-Jacquotte, 1965 and *Arete indicus* Coutière, 1903 were hand-collected at low tide in depths ranging from 0 to 1 meter on the flat intertidal zone of the Great Reef of Toliara in Madagascar (**Figure 1E**; 23°22’48’’S 43°38’13’’E). All the collected hosts were stored for a short term (max. 2h) in a 3l hermetic plastic bag filled with seawater to preserve all the symbionts during the collection and transport period. Upon reaching the laboratory, an inventory and characterization of the organisms were conducted including the determination of the morphotype and the number of symbionts per host. When possible, the sex of the organisms was determined based on morphological sexual dimorphism or based on eggs presence. For each shrimp, a picture was taken and considered as the initial standard picture at T_0. Subsequently, the specimens were

maintained in 6L open water aquaria supplied with 1µm-filtered seawater, sourced directly from the sea. The tanks were aerated with an air bubbler and carefully maintained at a constant temperature of 28-29°C, a salinity of 35 PSU and a pH of 8.

(2) Host separation experiments

To assess the influence of the host and its olfactory environment on the symbiont fitness and coloration, three distinct treatments were established for the four decapod symbiotic species. Those experiments started directly after the inventories, except if the considered symbiont die during the first 12 hours (if it was the case, it was not considerate for further analyses). Five treatments were realized for a duration ranging from 10 to 60 days according to the survival of symbionts:

(1) **Control treatment** where symbionts remained associated with their hosts. We used 22 *Z. soror*, 16 *S. stimpsonii*, 10 *A. indicus* and 10 *T. holthuisi* (**Figure 2A**). All organisms were maintained on their host in the 6L aquaria, respecting the natural symbiotic load found in the field. Therefore, we preserved 2 hosts per aquarium, and a maximum of two symbionts per hosts for *Arete indicus* (Gherardi 1991; Dabbagh et al. 2019), *Tulеariocaris holthuisi* (Marin and Anker 2009; Brasseur et al. 2018) and *S. stimpsonii* (VandenSpiegel et al. 1998; Caulier et al. 2022). For *Z. soror*, as this species can live on large aggregations (Antokhina and Britayev 2020b; Fernández-Lereé et al. 2023), we decide to preserved them on 2 hosts per aquarium, with a larger number of symbionts composed of 4 adults (colored morphotype) and 4 juveniles (translucent morphotype).

(2) **Isolated treatment** where symbionts were isolated from their hosts in seawater. Twenty-two *Z. soror*, 11 *S. stimpsonii*, 10 *A. indicus* and 10 *T. holthuisi* were used (**Figure 2B).** Symbionts were each isolated in a vial container of 30 mL sealed with a mosquito net to allow oxygen and metabolites diffusion and placed in a 6L aquarium containing only filtered seawater.

(3) **Host-conditioned treatment** where symbionts were isolated from their hosts in conditioned seawater. Twenty-two *Z. soror*, 11 *S. stimpsonii*, 10 *A. indicus* and 10 *T. holthuisi* were used (**Figure 2C)**. In this treatment, the symbionts were isolated in the same vials used in the previous condition but the water in the tank was conditioned by two host individuals that were also preserved in the 6L tank. This configuration allowed the exchange of metabolites without the need for direct contact between symbionts and host.

Additionally, two supplementary conditions were introduced for *S. stimpsonii* to further investigate the influence of food and artificial kairomones on symbiont survival.

(4) **Kairomone-conditioned treatment** where symbionts were isolated from their hosts in kairomone-conditioned seawater (**Figure 2D)**. Sixteen *S. stimpsonii* were isolated in the same device than (2 and 3), but using seawater conditioned by a 0.01 g/l of commercial anthraquinone (CAS number: 84-65-1; $C_{14}H_8O_2$; Anthraquinone; 98%, Thermo Scientific Chemicals) in seawater. This specific molecule was proven to act as an attractant from the shrimp in a behavioral olfactometer, thus considered to play a kairomonal role allowing host selection (Caulier et al. 2022). After every monitoring, the water contained in these aquaria were completely renewed and 0.06g (0.01g/L, for an aquarium of 6L) of anthraquinones were add and homogenized manually.

(5) **Food treatment** where symbionts were isolated from their hosts and daily fed (**Figure 2E)**. Six *S. stimpsonii* were isolated in the same vials used in the previous condition, placed in 6L tank filled with seawater. As this species is known to feed during the night on external resources by grazing or filtering (VandenSpiegel et al. 1998; Terrana et al. Submitted), we decided to feed the shrimps at 10pm every day with a mixture of 10ml of a *Chaetoceros gracilis* and 15ml of an *Isochrysis galbana* liquid culture at a concentration of 20,000 cells/ml.

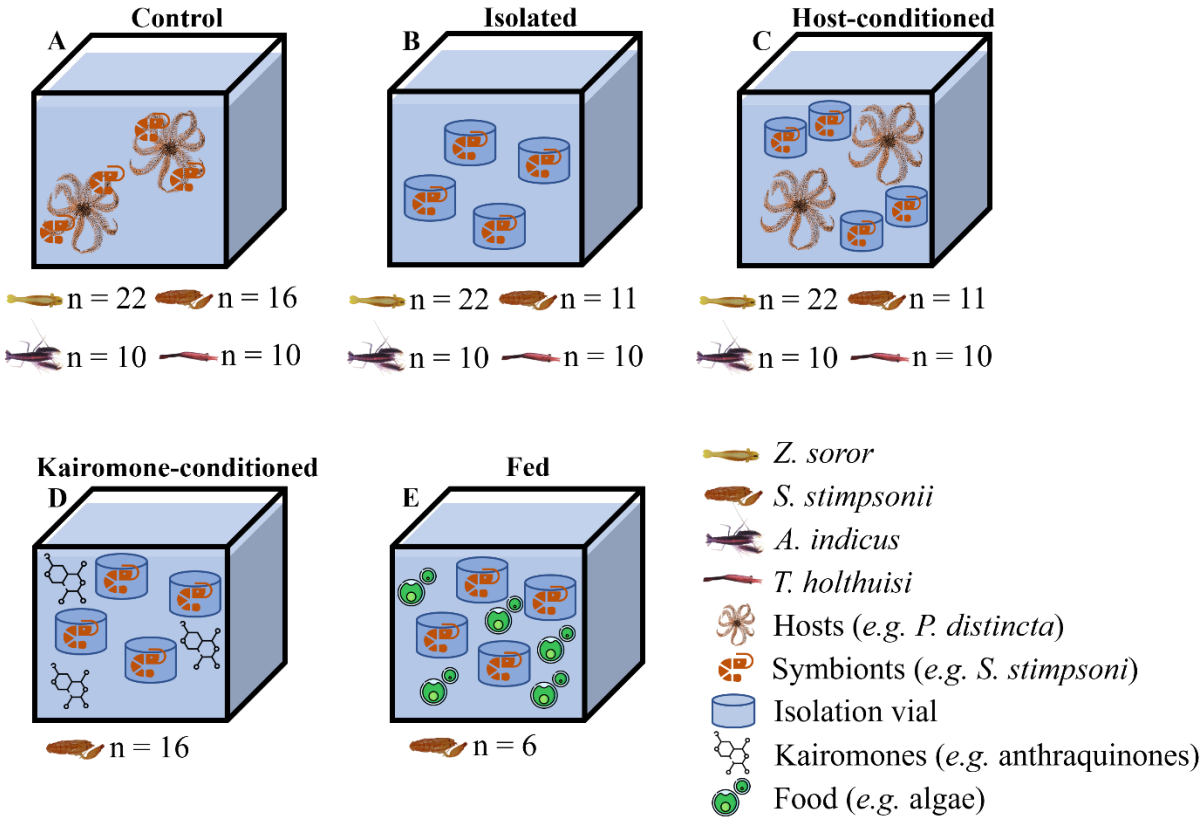


Figure 2: Scheme displaying the different experimental conditions applied to the 4 symbiotic couples investigated in this study and the number of replicates used for each species and treatment. (A) Control treatment with symbionts on their hosts (B) Symbiont isolation treatment in pure seawater (C) Symbiont isolation treatment in host-conditioned treatment (D) Symbiont isolation treatment in kairomone (anthraquinone)-conditioned treatment (E) Symbiont fed ad libitum by cultured microalgae. The proportions of the organisms are not to scale.

For each species and treatment, survival checks were conducted four times daily at 6 a.m., 12 a.m., 6 p.m., and 12 p.m. Detection of dead individuals was based on three criteria: immobility (*e.g.* shrimp not moving even when stimulated with light and antennae A1 and A2 not moving), unusual body position (*e.g.* shrimp on the back) and, in some cases, an unusual pigmentation (*e.g.*, observed in *T. holthuisi*). Dead organisms were fixed for future analyses like histological investigations.

(3) Monitoring of symbionts pigmentation

(a) Long-term monitoring

All symbiotic shrimps were regularly photographed in standardized conditions from t_0 to monitor the pigmentation variation through the time of separation from their host and to allow comparison between treatments and species. Photos were taken four times a day (6 a.m., 12 a.m., 6 p.m., and 12 p.m.) over a period from 6 to 18 days, depending on the studied species, following the survival monitoring (see here above). The experiment was carried out until the death of individuals or, for *T. holthuisi* and *Z. soror* for up to 10 days, *A. indicus* for up to 18 days and *S. stimpsonii* for up to 20 days. Symbionts were isolated while alive in 6ml vials submerged with seawater from their respective aquariums (see here above), within a light-sealed photographic chamber using a ColorChecker (ColorChecker® mscc model) **(Figure 3b)**. The photography process was conducted under the same LED lighting quality/intensity (1300 lumen, 5500k, ®HAVOX). The distance between the individuals and the camera was kept at 25 cm. Photos were taken with an Olympus TG-6 camera in RAW format, using the BKT (Bracketed Focus Technique), which means that several photos were taken simultaneously at different focus distances. For this experiment, according to the number of individual available for each symbiont species, a total of five individuals of *Z. soror*, seven individuals of *S. stimpsonii*, ten individuals of *A. indicus* and ten individuals of *T. holthuisi* were used. Each photo contained a maximum of six shrimps together with a label inscription and a ColorChecker (ColorChecker® mscc model) for color calibration. The ColorChecker is a flat, rectangular chart with a grid of colored patches, each of which representing a specific color with known and standardized values; it was used for color calibration to ensure accurate and consistent color reproduction in images analyses. This allows data to be standardized by creating a photographic profile (**Figure 3**). The latter enables the creation of a colorimetric profile, as well as brightness adjustment. This makes it possible to standardize photos and eliminate biases linked to the brightness of the environment.

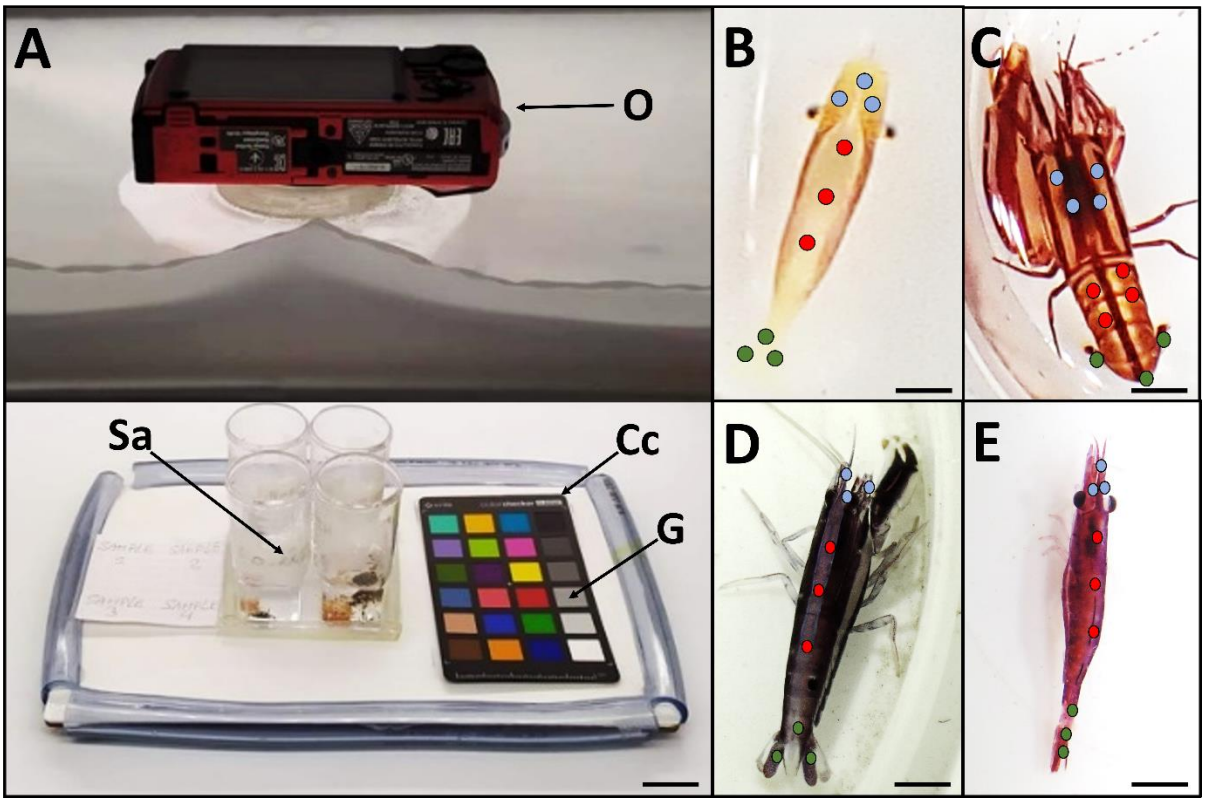


Figure 3: (A) Color monitoring experimental device using a colorchecker in a lightroom. (B) Representation of the points sampled on *Z. soror* (C) Representation of the points sampled on *S. stimpsonii* (D) Representation of the points sampled on *A. indicus* (E) Representation of the points sampled on *T. holthuisi.* (B, C, D and E) Pictures taken in the represented device in (A), with the blue dots indicating points sampled on the cephalon, red dots indicating points sampled on the thorax or abdomen, green dots represent points sampled on the telson/uropods. Cc: ColorChekcker; G: neutral Gray (R : 128, G:128, B: 128); O: camera Olympus TG-6; Sa: Samples. Scalebar = 5cm in A, 2mm in B, 3mm in C, D and E 2mm.

In a native file colored digital picture, each pixel can be represented by a vector of three numbers (each ranging from 0 to 255) for the three primary color channels: red, green, and blue (RGB). The color analysis involved the extraction of these RGB values from three distinct spots scattered on three different body regions of the symbiotic organisms represented by the blue (anterior part of the body), red (mid part of the body) and green (posterior part of the body) spots on **Figure 3** (corresponding respectively to the anterior extremity of the cephalothorax, to the posterior extremity the cephalothorax plus the abdomen, and to the telson/uropods, **Figure 3**). Subsequently, the lights and colors of the images were standardized (RGB values of the neutral gray aligned to fit the RGB value of 128:128:128) and RGB values of the nine dots of each shrimp were measured using Adobe Photoshop software (Adobe Photoshop 2022 V25.5.2). For each triple RGB values obtained for the different regions of the shrimps, the median value was taken and transformed into one average grayscale value using the formula Luminance = 0.2126 × Red + 0.7152 × Green + 0.0722 × Blue (Gonzalez et al. 2016) (**Figure 4**). This formula is used to calculate relative luminance, *i.e.* the variation in gray in relation to the RGB (Red, Green, Blue) values obtained. It is derived from a two-dimensional Fourier transformation (Sazzad et al. 2013) .

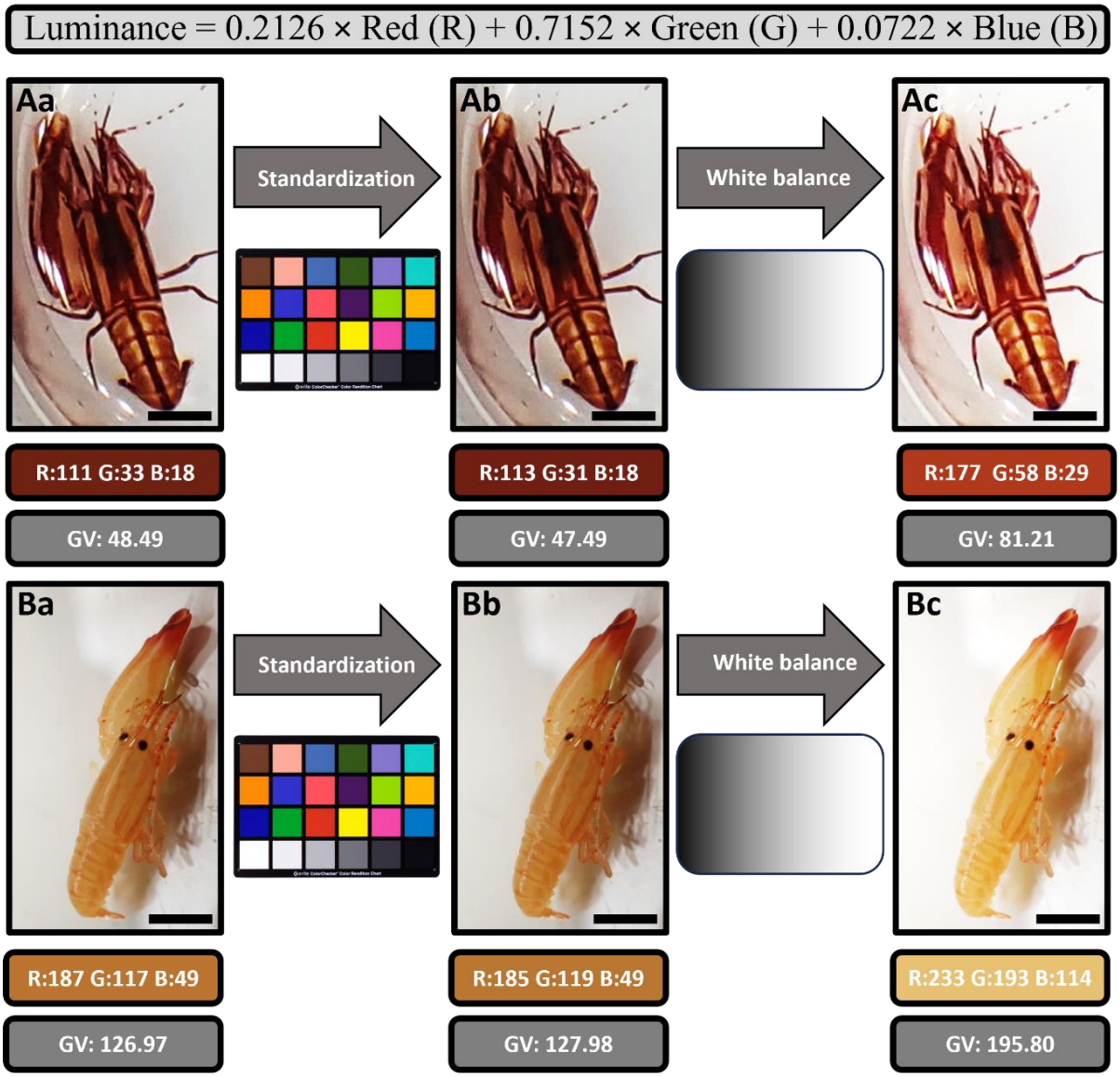


Figure 4: Illustration of the image processing standardization on the pictures realized during the color monitoring experiment taking a darker (Aa-Ac) and a lighter (Ba-Bc) individuals of *S. stimpsonii* as examples. RGB represents the Red-Green-Blue values sampled using Adobe Photoshop ®. Gray value (GV) is calculated from the formula : (0.2126 × Red + 0.7152 × Green + 0.0722 × Blue). (A) *S. stimpsonii* presenting a vivid pigmentation (B) another individual of *S. stimpsonii* presenting a lighter coloration. Aa and Ba : Raw picture of the symbiont taken by the camera without photo standardization; Ab and Bb : picture Aa and Ba standardized using the colorimetric profile using ColorChecker; Ac and Bc: picture Ab and Bb standardized using the white balance. Scalebar = 0.5cm.

(b) Short-term monitoring

To investigate short-term color changes that could occur between the first hour of host isolation (*i.e.* between the two first monitoring times), short monitoring has been realized on *Z. soror*. Symbionts were photographed in 6ml vials full of seawater (28-29°C, a salinity of 35 PSU and pH of 8) renewed every hour to limit variation of temperature or oxygenation. The organisms were kept during all the short-monitoring within the light-sealed photographic chamber at constant LED lighting intensity (1300 lumen) and the distance between the individuals and the camera was kept at 25 cm (**Figure 3a**). Photos were taken with an Olympus TG-6 camera in RAW format, using the time lapse programs, which means that photos were taken every 5 minutes of the 8 hours experimentation. Color variation was analyzed on four individuals of each morphotype of *Z. soror* (*i.e.* 4 translucent, 4 stripe-colored and 4 fully-colored) thanks to the addition of the ColorChecker (ColorChecker® mscc model) (**Figure 3**). Once the photographs were taken, they were analyzed using the same methodology as explained above in the "long-monitoring" section.

(4) Histological analyses

Samples for histology were fixed in formol 4% (diluted in artificial seawater) in the field. As carotenoids are fat-soluble, it is not possible to use a conventional histology protocol involving solvents baths to characterize them (Krinsky 1994). Therefore, they were frozen-sectioned using a cryostat at the University of Mons. For that purpose, they were placed in plastic boxes specific to the cryostat and then embedded with OCT medium (Optimal Cutting Temperature) (Roth®). The OCT medium becomes solid at -10°C and is used as a coating medium for the cryostat. The OCT blocks were put in the freezer at -20°C overnight. The blocks were then sectioned, at a thickness of 8 µm, using a cryostat maintained at -20°C (Leica ®). The longitudinal sections of the shrimps were collected on SuperFrostPlus® glass slides, mounted with Vectashield® Antifade Mounting Media, and directly observed under a Zeiss Axioscope A1 microscope connected to a Zeiss AxioCam 305 color camera. Pictures were observed with the ZEN Blue Software (ZEN 3.2, ZEN lite). Considering the size of the individual, this method allows a cross-section of the entire organism.

(5) Biochemical analyses

Carotenoid pigment extractions and analyses were only conducted for the two symbiotic couples *Z. soror* - *C. novaeguineae*, and *S. stimpsonii* - *P. distincta.* The entire bodies of the symbiont organisms were preserved for extraction. Concerning the hosts, small parts of their body wall were sampled for extractions without the need to sacrifice the organisms that were later released on their collection spots. A dissection was performed to obtain 40-100 mg of the external body wall of *C. novaeguineae* and 5-15 mg of the arms of *P. distincta.* In order to preserve the maximum quality/quantity of carotenoids, all samples were directly preserved in a freezer (-20°C) and lyophilized for 48 hours using a Martin Christ alpha 2-4 freeze dryer. Samples were preserved in the dark until extraction to avoid pigment deterioration.

At the Marine Station of Concarneau, dried samples were placed in 1.5 mL of 95% methanol (buffered with 2% w:v ammonium acetate) along with a stainless-steel bead in a 2 mL plastic tube. The samples were then ground using a MM 400 Retsch bead mill at 30 Hz for 10 minutes to allow complete and homogenous pigment transfer from the biological tissues to the solvent. The resulting extracts were filtered using 0.2 µm PTFE syringe filters and analyzed using an Agilent 1260 Infinity HPLC system. The HPLC system comprised a quaternary pump (VL 400 bar), a UV-VIS photodiode array detector (DAD 1260 VL, 250-900 nm), and a 100 µl automatic sample injector maintained at 4°C in the dark. Chromatographic separation was achieved using a reverse-phase C18 column (Supelcosil, 25 cm long, 4.6 mm inner diameter). The mobile phases consisted of A: 0.5 M ammonium acetate in methanol and water (85:15, v:v), B: acetonitrile and water (90:10, v:v), and C: 100% ethyl acetate. Peak identification and calibration in the HPLC chromatograms were performed using 8-apobeta-carotene (Roth ®) as a reference compound (David et al. 2023). For carotenoid quantification, the area under the curve formed by the spectrum is calculated for each peak. The quantity of pigments is calculated thanks to the surface present under the curve of the standard whose mass is known. By dividing these values by the mass of the sample used, a value of mg of pigment/g of sample is obtained (David et al, 2013). The number of individuals replicate used for the experimentation were : n=3 for translucent *Z.* soror, n= 6 for colored *Z. soror*, n = 6 for *C. novaeguineae*, n = 9 for *P. distincta* and n = 7 for *S. stimpsonii*

(6) Statistical analyses

For the survivability experiment, A Mantel-cox statistical test (p-value < 0,05) was used to compare the potential impact of the different host separation treatments on the survival of symbionts, as performed by Brasseur et al. (2018). Survival statistical analyses and graphs were performed using GraphPad Prism (V10.0.0).

Considering Gray values (GV) variations, linear regressions were performed for each individual for all three body parts (**Figure S1)** and for the abdomen part only (**Figure 6**). The choice to simplify the analysis to the abdomen was chosen for its higher representativeness of the color changes observed on the organism (**Figure S1)**. Indeed, the abdomen was less impacted by carapace ornamentation and color pollution caused by an underlying organ. When a linear regression shows an increasing slope, that indicates a decrease in color intensity (*i.e.* discoloration), when the linear regression goes down (negative slope), that indicates an increase in color intensity (*i.e.* coloration/ recoloration) and when a slope is flat, that indicates no change in color intensity (*i.e.* stable coloration). When a line stops, it means the corresponding individual was found dead, stopping the monitoring of this individual (**Figure S1**, **Figure 6**). Linear regression coefficients and the GV difference between the initial and final GV are then calculated for the abdominal region of each individuals (**Table 3**). A negative coefficient and GV difference indicate recoloration of the individual, while a positive coefficient and GV difference indicate discoloration of the individual (**Table 3**).

The treatment of the carotenoids extraction data was made using RStudio software (v2022.12.0).

III. Results

(1) Symbiotic metrics

A total of 74 sea stars *C. novaeguineae* were collected and revealed hosting 119 shrimps *Z. soror* that were mostly found on the oral face of the host, around the mouth or near one of their ambulacral grooves. The symbiotic prevalence was 60.2% with 1 to 14 shrimps being observed on a single host individual, with a majority being alone (29.4%) or by pairs (25.5%). The three morphotypes (translucent, stripe-colored and fully-colored) were part of this symbiotic population (**Figure 1 Ab-d**). The majority of the shrimps were translucent (72.2%; **Figure 1 Ad**) while the stripe-colored (**Figure 1 Ab**) and full-colored morphotypes (**Figure 1 Ac**) were similarly abundant, representing respectively 14.3% and 13.6% of the population. The stripe-colored morphotype individuals were characterized by a dorsal band that always showed a white coloration while the other part of their anatomy harbored vivid colors, similarly to the fully-colored morphotypes (**Figure 1 Ab-c**).

The three other species investigated in this study presented a unique morphotype with a single, unique pigmentation. A total of 148 crinoids *P. distincta* were collected hosting 134 snapping shrimps *S. stimpsonii*, with a symbiotic prevalence of 50.3%. These shrimps were always found single (22.2% of single females and 13.8% of single males) or per heterosexual pair (63.8%) on the cirri, calyx or on one of the arms of their host. The body of all individuals presented an orange coloration with dark orange to red horizontal and vertical stripes on their body (**Figure 1 Bb**). The exact appearance of these stripes could vary among individuals, being wider and more colored when the general coloration of the shrimp was brighter, with sometimes stripes overlapping each other making their distinction difficult. Usually, on the cephalothorax, four longitudinal stripes were observed, and on the abdomen, there was a unique longitudinal stripe centered on the dorsal side of the organisms (**Figure 1 Bb**). Transversal stripes, when visible, might not be as distinct as the longitudinal ones, and could vary in their number and appearance, especially in small individuals. They were found only on the abdomen and were positioned in a perpendicular direction to the longitudinal stripes (**Figure 1 Bb**). These transversal stripes seemed to be located on the delimitation between two abdominal metameres (**Figure 1 Bb**).

On the 106 sea urchins *E. mathaei*, 44 shrimps *A. indicus* and 18 *T. holthuisi* were found, showing a symbiotic prevalence of 68.3% and 28.6%, respectively. *A. indicus* was always found close to the sea urchin test, at the base of the spine, with is body and chelipeds parallel to a spine and the head toward the exterior of its host. *T. holthuisi* was always located on a spine,

with its elongated body parallel to the spine, head toward the test of its host. These two species could be found together on the same host (68.9%), both species being found as a single individual (9%) or by pair on the host (91%). All *A. indicus* presented a similar coloration, characterized by a highly mimetic purple color with a small lighter mid-dorsal band that stretched to the inner edge of the eyestalk and extended from the rostrum to the fifth abdominal segment (**Figure 1 Cc**). Along each lateral side of the body, there was a minute blue stripe running the entire shrimp length, ultimately converging at the posterior edge of the abdomen where the orange band fades away (**Figure 1 Cc**). On the fourth abdominal segment, two prominent dark blue spots could be observed symmetrically placed at each side of the mid-dorsal band (**Figure 1 Cc**). For *T. holthuisi*, all individuals arbored a specific elongated body shape, highly similar to a host spine, making them difficult to find on the host. Their coloration consisted of a purple-pink uniform color, with a minute mid-dorsal stripe that had a darker coloration and two-minute lateral white stripes running from the cephalothorax to the end of the abdomen (**Figure 1 Cb**).

(2) Survivability and chemical dependency

Figure 5, Tables 1 and **2** display the different survival curves and statistical parameters resulting from the host separation experiment for the four symbionts investigated. Concerning *Z. soror*, individuals separated from their host showed a rapid mortality for both isolated and host-conditioned treatments (**Figure 5 A; Tables 1** and **2**). Indeed, after 10 days, 90% of the individuals in the control were still alive (20/22 symbionts) while only 22.7% (5/22 symbionts) in the host-conditioned treatment and 18.1% (4/22 symbionts) in the isolated treatment. The Mantel-Cox test indicated that the survival curve distribution of the controls was significantly different from the distributions of the two other treatments (both p-value $< 10^{-4}$) but there was no significant difference between the survival curve distributions of symbionts maintained isolated or in water conditioned by the host (p-value = 0.5).

Considering the three other host species, the survival rates are positively impacted by the chemical environment produced by the hosts (**Table 2**). Indeed, after 60 days of experiment using *S. stimpsonii*, we observed a survival rate of 100% (16/16 symbionts) in the control, 45.4% (5/11 symbionts) in conditioned sea water and only 9.1% (1/11 symbionts) in the isolated treatment (**Figure 5 B**). The Mantel-Cox test revealed a significant difference between the three different treatments, each corresponding p-value being under 0.05. In pairwise comparisons, the control condition is significatively different from the isolated treatment (p-value $<10^{-4}$) and

from host-conditioned treatment (p-value = 0.6 10^{-4}). The isolated and host-conditioned symbionts survival curves were also significatively different (p-value = 4.1 10^{-2}). In addition, shrimps in the anthraquinone-conditioned sea water presented a survival of 56.25% (9/16 symbionts) while daily fed symbionts survival was of 60.0% (3/5 symbionts) (**Figure 5 B**). These two additional conditions were both significatively different in comparison to the control (p-value = 5 10^{-4} and 7.5 10^{-3} respectively) but not significatively different of each other (p-value = 0.7) or from the host-conditioned treatments (p-value = 0.9 and 0.7, respectively).

Concerning *T. holthuisi*, the isolated symbionts survived up to a maximum of 8 days (0/10 shrimps) (**Figure 5 C**). After 10 days, only 20% (2/10 symbionts) of host-conditioned individuals remained alive while 100% (10/10 symbionts) of the control symbionts remained (**Figure 5 C**). The Mantel-Cox test showed significant differences between the control and the isolated symbionts (p-value $<10^{-4}$), between the control and the host-conditioned treatment (p-value $<10^{-4}$) and between the isolated and host-conditioned symbionts (p-value = 2.8 10^{-2}). Concerning *A. indicus*, after 48 days of experimentation, all isolated symbionts did not survive (0/10 symbionts), while 64.8% (6/9 symbionts) of conditioned shrimps and 100% (10/10 symbionts) of the control remained after 60 days (**Figure 5 D**). Statistically, the Mantel-cox test also shows a significant difference with a p-value of $< 10^{-4}$ in control versus isolated treatments, a p-value of 4.6 10^{-2} in the comparison between control and host-conditioned symbionts and a p-value of 1.2 10^{-2} in the remaining comparison.

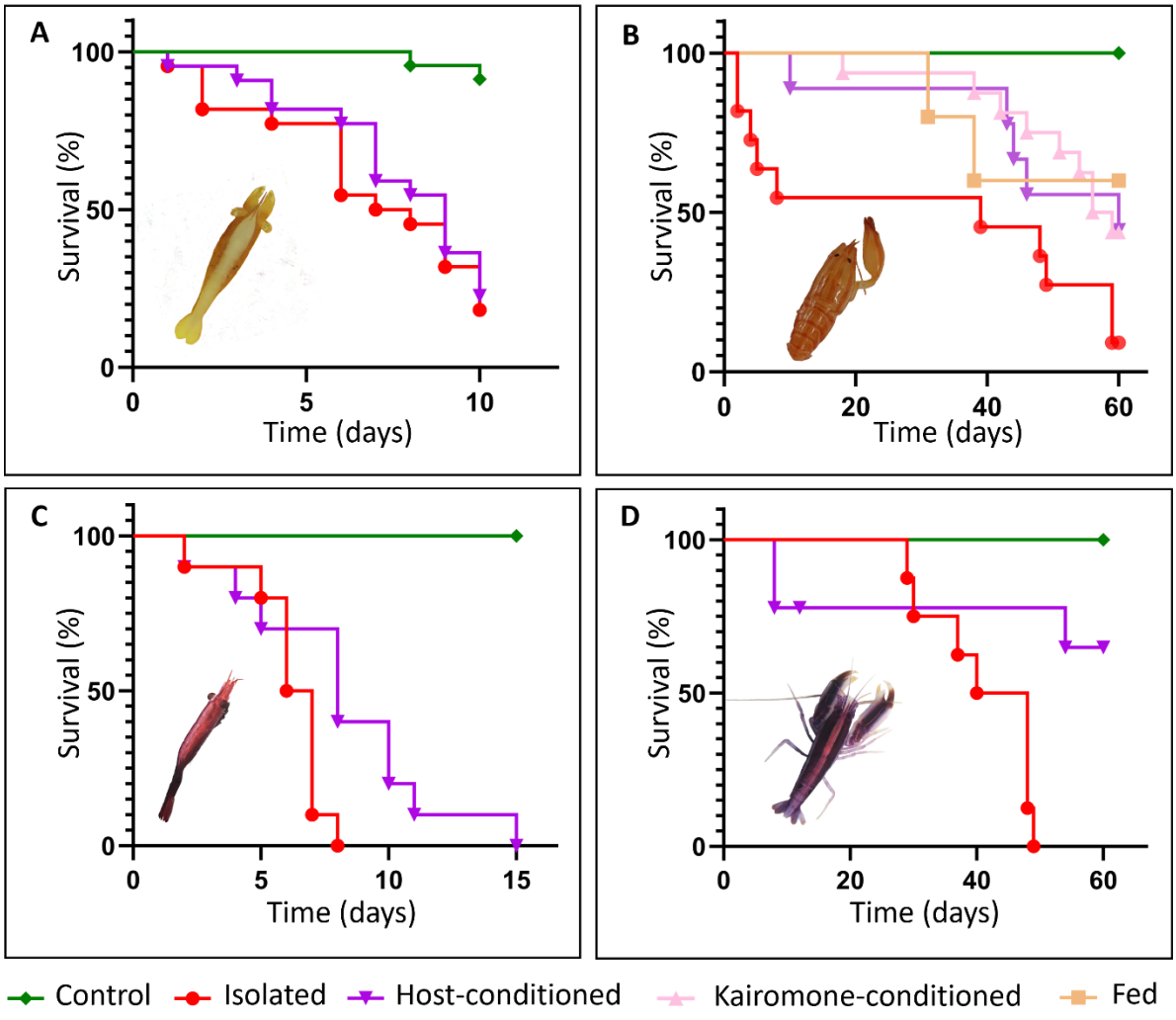


Figure 5: Survival curves of the four species of shrimps associated with echinoderms. (A) *Zenopontonia soror*, (B) *Synalpheus stimpsonii*, (C) *Tuleariocaris holthuisi* and (D) *Arete indicus*. For all species, three conditions are represented with (i) the control shrimps in green, (ii) the isolated treatment in red and (iii) the host-conditioned in purple. For *S. stimpsonii*, two supplementary treatments were applied: (iv) kairomone-conditioned and (v) Fed with algae. The x-axis represents the number of days of the experimentation and the y-axis shows the percentage of survivability of the species. Number of replicas for each treatment can be seen in **Figure 2** or in the Material and methods section.

Table 1: Median number of days of survival after a host separation experiment using four symbiotic shrimp species associated with echinoderms. Replicates associated to the number of species tested per condition can be obtained in **Figure 2**. N/A = Not Applicable (as not tested).

	Z. soror	***S. stimpsonii***	***T. holthuisi***	***A. indicus***
Control	Undefined (90% of survival at the end of the test, 10 days)	Undefined (100% of survival at the end of the test, 60 days)	Undefined (100% of survival at the end of the test, 10 days)	Undefined (100% of survival at the end of the test, 60 days)
Isolated	7.5	39	6.5 days	44
Host-conditioned	9	60	8 days	Undefined (64.8% of survival after 60 days)
Kairomone-conditioned	N/A	57.5	N/A	N/A
Food treatment	N/A	Undefined (60% of survival after 60 days)	N/A	N/A

Table 2: Statistical analyses comparing the different host separation treatments displaying P-values obtained using Mentel-Cox statistic tests (see material and methods for more information). P-value < 0.05 are highlighted in bold. N/A = Not Applicable.

	Z. soror	***S. stimpsonii***	***T. holthuisi***	***A. indicus***
Control VS Isolated	**$<10^{-4}$**	**$<10^{-4}$**	**$<10^{-4}$**	**$<10^{-4}$**
Control VS Host-conditioned	**$<10^{-4}$**	**$6\ 10^{-4}$**	**$<10^{-4}$**	**$4.6\ 10^{-2}$**
Isolated VS Host-conditioned	0.5	**$4.1\ 10^{-2}$**	**$2.8\ 10^{-2}$**	**$1.2\ 10^{-2}$**
Control VS Fed	NA	**$7.5\ 10^{-3}$**	NA	NA
Control VS Kairomones-conditioned	NA	**$5\ 10^{-4}$**	NA	NA
Host-conditioned vs Fed	NA	0.8	NA	NA
Host-conditioned VS kairomones-conditioned	NA	0.9	NA	NA
Fed VS Kairomone-conditioned	NA	0.7	NA	NA

(3) Symbiont pigmentation variation associated with host separation

(a) Long-term monitoring

During long-term isolation, observations revealed diverse discoloration and coloration events, depending on the treatment (isolated or host-conditioned) or the species investigated. When individuals were maintained in complete isolation, mainly discoloration was monitored for all four species studied, with some exceptions being observed at the individual level. For *S. stimpsonii*, *A. indicus* and *T. holthuisi*, discoloration was less pronounced when individuals were exposed to kairomones produced by their respective hosts but there was no difference in color variation between these treatments for the asteroid shrimp *Z. soror*. For *S. stimpsonii*, a nychthemeral cycle was highlighted, resulting in a more pronounced discoloration usually observed during the night, and recoloration starting from the sunrise until the sunset. In addition, when using artificial lights (*i.e.* during the midnight monitoring), some individuals could recolor rapidly, varying from transparent to redder individuals in a few seconds to a few minutes. It must be pointed out that some individuals of *T. holthuisi* and *Z. soror* especially, seemed to show a specific recoloration pattern before dying prematurely.

Gray values (GV) variations of the abdomen are illustrated in **Figure 6** (only the abdomen) and **Figure S1** (all body parts considered) for isolated and host conditioned symbionts. Considering the five isolated *Z. soror* individuals (**Figure 6Aa**), three showed an increase in GV of their abdomen, suggesting a tendency towards discoloration; one individual shows a relatively stable GV, indicating little color change; and one individual showed a decrease in GV over its abdomen, suggesting potential recoloration (**Figure 6 Aa**)**.** It is important to note that this last individual died prematurely compared to the other replicates. Similarly, *Z. soror* individuals conditioned by the host environment (**Figure 6 Ab**) showed comparable patterns of GV variation**.** Four of these individuals showed a tendency towards discoloration, while another appeared to undergo partial recoloration before prematurely dying**.** In summary, a trend towards increased mean GV, indicative of discoloration, was observed in the abdomen, whether the individual was isolated or conditioned by the host's olfactory environment (**Figure 6 Aa-b**)**.**

For the isolated *S. stimpsonii* (**Figure 6 Ba)**, a trend toward discoloration of the abdomen was observed. Indeed, on seven individuals examined, six showed a marked increase in gray values (GV), while one individual exhibited stagnation in GV. For shrimp conditioned in host-specific seawater **(Figure 6 Bb)**, GV revealed greater diversity in coloration patterns among specimens**.** Indeed, two individuals showed a tendency to discoloration, three appeared to have brighter

coloration, and one individual remained relatively stable**.** In conclusion, in isolated *S. stimpsonii*, a general discoloration can be suggested. In contrast, when conditioned by the host's olfactory environment, isolated individuals appear to show variable responses in their coloration.

Considering *A. indicus*, important variations are observed depending on the treatment, may the shrimps be isolated or in conditioned seawater (**Figure 6 C**). The individual number 4 died prematurely (yellow slope) and showed a substantial increase in GV, indicating significant discoloration, while the other eight individuals also showed an increase in GV associated with discoloration **(Figure 6 Ca).** In the host-conditioned shrimps **(Figure 6 Cb)**, the linear regression of GV appeared to be more stable, with a smaller linear coefficient**.** Five individuals showed an increase in GV, suggesting a tendency towards coloration**.** Four individuals remained more stable in their GV values, reflecting more consistent coloration, while one individual showed a decrease in GV, suggesting potential discoloration**.** In summary, despite a general increase in GV in both conditions, this trend appears to be more pronounced in totally isolated individuals, indicating more marked discoloration in this condition **(Figure 6 Cb)**.

In isolated *T. holthuisi* (**Figure 6 Da**), isolated individual 3 is not shown as it died rapidly. The other individuals show a general increase in GV. For host-conditioned shrimps (**Figure 6 Db**), the graph reveals nuanced results as seven individuals show an intensification of coloration, while three others show a discoloration**.** Therefore, there is a notable difference in the trend of GV variations between the two conditions.

Table 3 shows the differences observed in grey values (GV) between the initial and final points, together with the linear regression coefficients obtained from the graphs in **Figure 6**. For *Z. soror,* an increase in GV is observed both in total isolation and in host-conditioned environments, with no difference visualized between the 2 conditions. For *S. stimpsonii,* this numerical increase corresponds to the graphical observations. Similar trends are observed for *A. indicus* and *T. holthuisi*.

Table 3: Table showing the differences in gray variations (GV) of the long term analysis of the abdomen color variations in four ectosymbiotic decapod species associated with echinoderms in isolated and host-conditioned treatments. The initial and final GV and the linear regression coefficients obtained of each individual for the 4 species studied. Differences of GV are in RGB units, linear regression coefficients have not units. δ = differences; GV = Gray variation; LR = Linear regression; I =Isolated and HC = Host-conditioned

	Z. soror				
ID	**1**	**2**	**3**	**4**	**5**
δ GV (I)	-4.6	-13.6	6.3	45.6	88
δ GV (HC)	-8.5	-5	25	41.6	-1.2
LR coefficient (I)	-25.2	35	-12.3	49.6	51.2
LR coefficient (HC)	-28.2	38.5	39	22.9	-2.6

	S. stimpsonii						
ID	**1**	**2**	**3**	**4**	**5**	**6**	**7**
δ GV (I)	24.2	-25.2	67.4	30.4	37.7	49.4	84.8
δ GV (HC)	71.3	-0.3	-0.5	22.2	-2.3	19.6	NA
LR coefficient (I)	1.6	4.5	-9.6	12.2	-33.5	16,5	2.1
LR coefficient (HC)	9.2	2.8	-0.8	11.9	7.3	4	NA

	A. indicus									
ID	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
δ GV (I)	63.4	59.5	-5.1	NA	-26.9	-33	36.2	19.8	13	23.7
δ GV (HC)	4.05	27.7	8.7	40.8	18.7	52.2	29.7	32.8	29.8	46.3
LR coefficient (I)	5.9	8.6	-20.6	NA	-41.3	-66.7	-2.1	-13.7	-12.6	23.6
LR coefficient (HC)	-19.4	21.7	-30.7	15.1	4	2.8	-1.4	9	0.3	33.5

	T. holthuisi									
ID	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
δ GV (I)	61.2	39.4	NA	25.6	7.7	-22.6	-14.8	-47	0.3	-2.5
δ GV (HC)	26.5	-28.9	11.8	-4.5	-32	-37.2	4.1	25.3	16.8	-23
LR coefficient (I)	30	29	NA	-26.4	-28.4	-65	-46.9	-53.5	13.8	-37.7
LR coefficient (HC)	-65.9	-67.4	-67.8	-4.45	-50.3	-37.2	-43.2	-22.3	11.3	-6

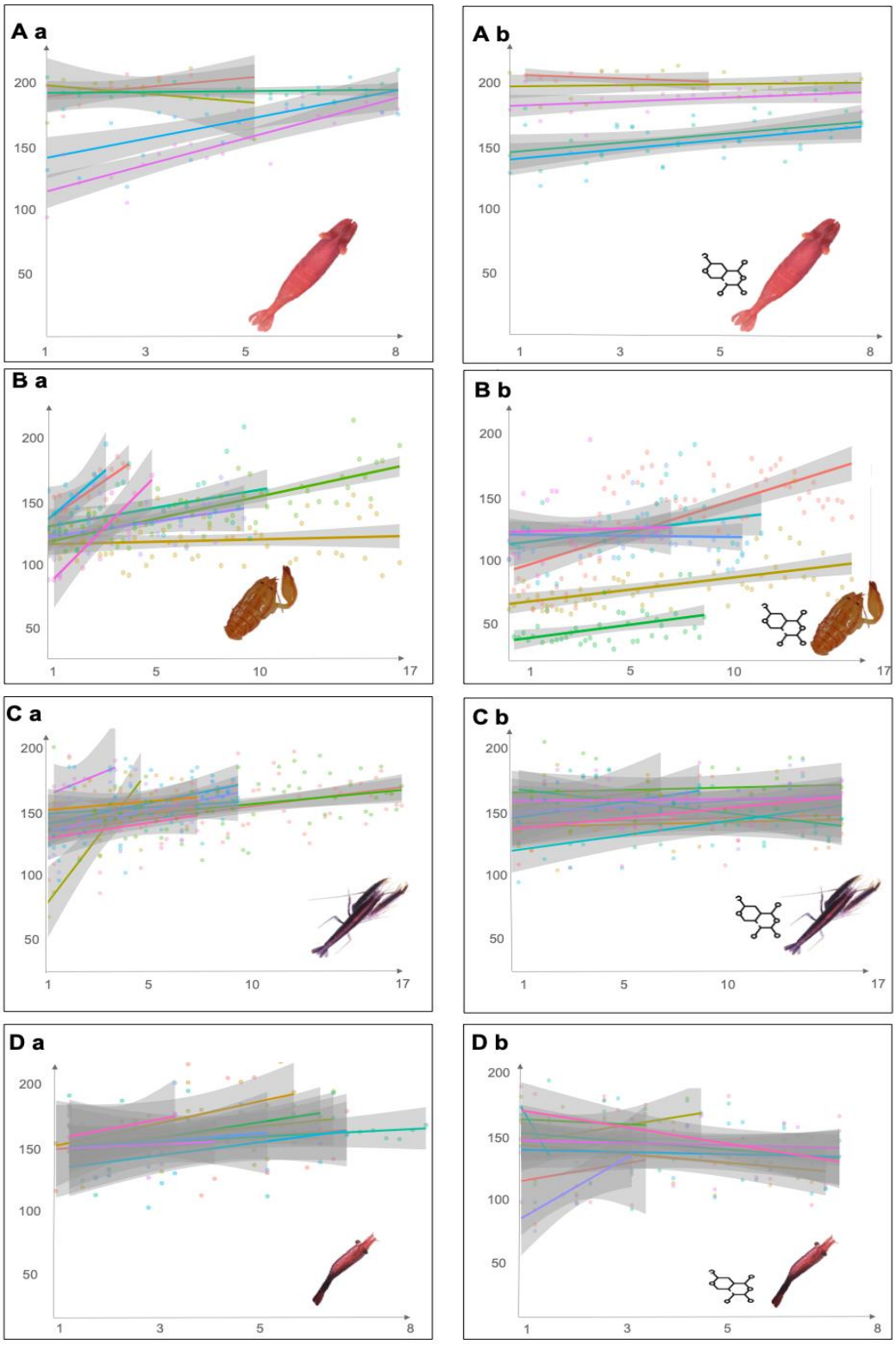


Figure 6: Long term analysis of the abdomen color variations in four species of ectosymbiotic decapods associated with echinoderms in isolated (Aa, Ba, Ca, Da) and host-conditioned (Ab, Bb, Cb, Db) treatments. Each graph represents the gray-scale values of the transformed RGB values obtained from photos taken at the time of the experiments on (A) *Z. soror*, (B) *S. stimpsonii*, (C) *T. holthuisi*, and (D) *S. stimpsonii*. Each colored line represents the variation in gray values of an individual. Lines that extend to the end of the graph represent individuals that survived throughout the experiment, while lines that do not reach the end of the graph indicate that these individuals did not survive to the end of the experiment. These trajectories were generated from the linear regression of grey variation values, represented by the points on the graph. The x-axis represents time with day, the y-axis represents grey values. Each line represents an individual. Short-term monitoring

(b) Short-term monitoring

As a reminder, short-term color monitoring was only realized on the three morphotypes of *Z. soror* for 8 hours. For the three morphotypes and all body part investigated, including the abdomen, color intensity seems to decrease when individuals were isolated from their hosts, as all the GV linear regression tent to increase (**Figure 7A-C, Figure S2, Table 4**). Therefore, in this short-term monitoring, results indicating that all *Z. soror*, independently of their morphotypes or the body part investigate, has a discoloration when separated from the hosts.

Table 4: Table showing the differences in gray variations (GV) of the short term analysis of the abdomen color variations in the three morphotypes of *Zenopontonia soror* associated to *Culcita novaeguineae* in isolated conditions. Table represents the initial and final GV and the linear regression coefficients obtained of each individual. Differences of GV are in RGB units, linear regression coefficients have not units.

	Full colored morphotype			
ID	**1**	**2**	**3**	**4**
δ GV	26.4	33.8	52.4	19.3
LR coefficient	4.7	4.2	3.3	1
	Strip-colored morphotype			
ID	**1**	**2**	**3**	**4**
δ GV	29.1	32.3	28.3	66.2
LR coefficient	2.4	2.2	2.1	4.5
	Strip-colored morphotype			
ID	**1**	**2**	**3**	**4**
δ GV	28.6	37.8	26.5	50.2
LR coefficient	2.5	4.5	2.4	5.6

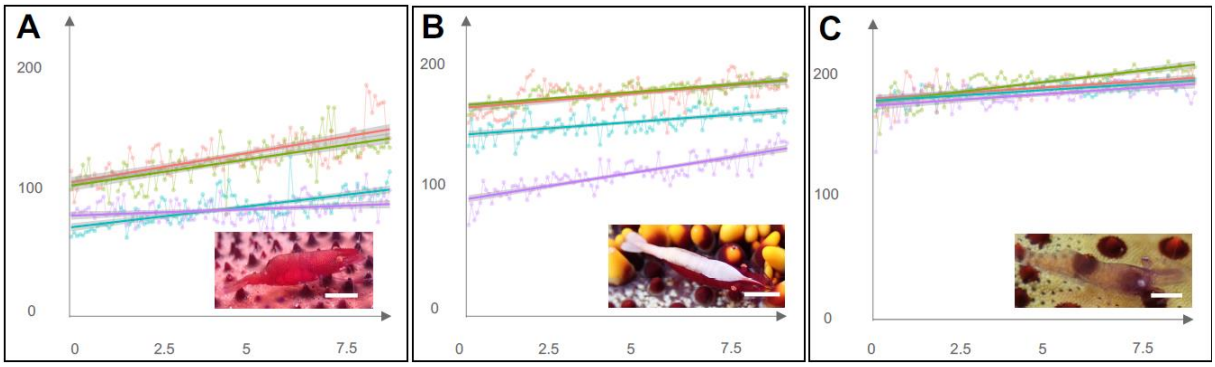


Figure 7: Short term analysis of the abdomen color variations in three morphotypes of the decapod shrimp *Zenopontonia soror* in isolated conditions. Color variation during a short time under constant light (1300 Lumen) (A) Graph showing the variation in RGB values of 4 *Z. soror* individuals of full-colored morphotype separated from their hosts. The x-axis represents time in hours, the y-axis represents RGB values transformed into gray. (B) Graph showing the variation in RGB values of 4 *Z. soror* individuals of stripe-colored separated from their hosts. The x-axis represents time in hours, the y-axis represents RGB values transformed into gray. (C) Graph showing the variation in RGB values of 4 *Z. soror* individuals of translucent morphotype separated from their hosts. The x-axis represents time in hours, the y-axis represents RGB values transformed into gray.

(4) Chromatophore analysis with histological sections

Under light microscopy, histological sections revealed the presence of chromatophores located within the dermal layer of the shrimp, under their transparent cuticle. Chromatophores were identified in all species with significant variation in size and pigment covers. They ranged from single chromatophore measuring 20 ± 8.5 µm (in *Z. soror*; **Figure 8 Aa**) to a maximum of 36 ± 13 µm (in *S. stimpsonii*; **Figure 8 Bd**). Multiple chromatophores generally packed together making cell boundaries difficult to identify because of their proximity (in colored *S. stimpsonii* for example; **Figure 8 Bb**). Their shape can vary from amoeboid, characterized by radial arrangement with elongated and pigmented arms extending outward from a central cell body (*e.g.* **Figure 8 Aa or Cc**) to spherical presenting a condensed uniform round shape that have less cell process (*e.g.* **Figure 8 Bd or Cd**). The chromatophore displayed varying degrees of pigmentation, with some appearing densely colored (*e.g.* **Figure 8 Bb**), while others were relatively pale (*e.g.* **Figure 8 Cb**). Usually, the color of the pigment in these chromatophores arbored different tints of orange/red. This coloration was observed in all four species. However, one species (*A. indicus*) also presented a second type of chromatophores containing pigments with purple coloration.

In the case of *Z. soror*, two morphotypes were investigated: colored (*e.g.* **Figure 8 Ae**) and translucent (*e.g.* **Figure 8 Af**). In colored morphotypes, numerous spherical and amoeboid chromatophores were visible with a pigmentation covering of 50.7% (± 37.9%) of the total

dermis surface (**Figure 8 Aa-b**). When translucent, no chromatophore were observed in the entire histological section (pigmentation covering = 0%) (**Figure 8 Ac-d**).

For *S. stimpsonii*, individuals with a vivid coloration (*e.g.* **Figure 8 Be**) or discolored because of the nychthemeral cycle of this species (*e.g.* **Figure 8 Bf**) were investigated. The dermal surface covered by chromatophores was 75.2% (±26.6%) in the colored individuals and 35.4% (±19.2%) for the discolored ones (**Figure 8 Bc-d**). For this species also, spherical and amoeboid chromatophores of various sizes were apparent throughout the dermal and muscular layers **(Figure 8 Ba-b)**. During discoloration, a noticeable aggregation of chromatophores became apparent (**Figure 8 Bc-d**).

For the third species *T. holthuisi*, histological sections were performed on colored individuals (*e.g.* **Figure 8 Ce**), as well as host-isolated individuals that have discolored (*e.g.* **Figure 8 Cf**). The pigmentation covering in colored individuals represent 54.4% (±13.4%) of the total dermal surface. In comparison, in discolored organisms, they only had 16.5% (±10.5%) of their dermal surface covered by pigmentation (**Figure 8 Cc-d**). In colored *T. holthuisi*, chromatophores do not exhibit a unique and distinct shape, harboring different degrees of amoeboid and spherical chromatophores, usually densely concentrated together in unstructured aggregates. Conversely, in discolored individuals, chromatophores coalesce into well-defined, intensely orange spherical formations **(Figure 8 Ca-b).**

On the latest species, *A. indicus*, no observation was made on “discolored” morphotype as this species did not show any transparent morphotype and only colored individuals were investigated (*e.g.* **Figure 8 Dc**). Histological sections highlight two distinct pigment layers in the dermis: with an inner purple layer and an outer red layer (**Figure 8 Da-b**). The purple layer represented 65.8% (±19.6%) of the total surface of the dermis and the red layer covered a surface of 59.7% (±37%).

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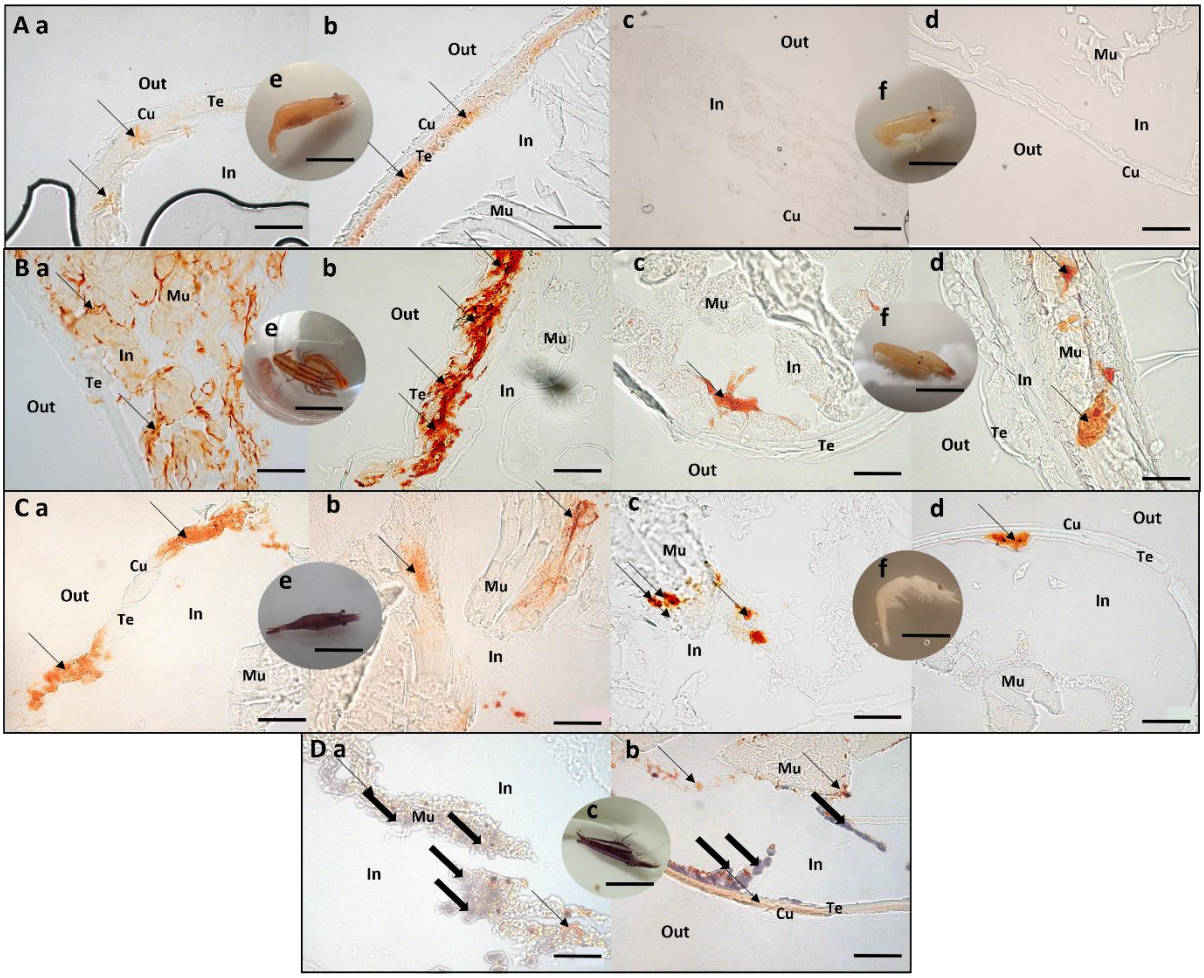


Figure 8 Histological sections reveal chromatophores in four symbiotic shrimp species: (A) *Z. soror*. (Aa-b) histological sections of a fully colored *Z. soror* individual. (Ac-d) histological sections of a translucent *Z. soror* individual (Ad-e) colored adult *Z. soror*. (Af) represents a translucent adult *Z. soror*. (B) *S. stimpsonii*. (Ba-b) histological sections of a colored *S. stimpsonii* individual. (Bc-d) histological sections of a discolored *S. stimpsonii* individual. (Be) represents a colored adult *S. stimpsonii*. (Bf) represents a discolored adult *S. stimpsonii*. (C) *T. holthuisi*. (Ca-b) histological sections of a colored T. holthuisi individual. (Cc-d) histological sections of a discolored *T. holthuisi* individual. (Ce) colored adult *T. holthuisi*. (Cf) discolored adult *T. holthuisi*. (D) A. indicus. (Da-b) histological sections of a colored *A. indicus* individual. (Dc) colored adult *A. indicus*. Thin arrows indicate erythrophores, while the thick arrow indicates cells containing spinochromes. Out: Outside the individual In: Inside the individual Te: tegument Mu: muscles Cu: Cuticle. Scale bar = 20 µm in Aa-d; 200 µm in Ac; 1 cm in Af; 20 µm in Ba-d; 1 cm in Be-f; 20 µm in Ca-d; 1 cm in Ce-f; 20 µm in Da-d; 1 cm in Dc.

(5) Pigments characterization by HPLC

As pigments of red/orange colors were observed in histological section, we suspected they may be composed of carotenoids, a major pigment found in shrimps and usually obtained through their diets. To investigate this, HPLC characterization were performed on two of the four symbiotic couples: *Z. soror* - *C. novaeguineae* and *S. stimpsonii* - *P. distincta*. Depending on the species, several pigments were separated and highlighted using the HPLC system (**Figure 9; table S1 and S2**). The sea star *C. novaeguineae* presents the most complex carotenoid assemblage, with 11 to 15 different peaks corresponding to two isomers of astaxanthin, lutein, zeaxanthin, canthaxanthin and their derivative (**Figure 9A**). The associated

shrimps *Z. soror* present fewer pigments, with a carotenoid assemblage of both isomers of astaxanthin and a few traces of zeaxanthin and beta-carotene in colored morphotype (**Figure 9B**). In addition, no pigments were detected in the translucent morphotypes of this species. The second host, *P. distincta* has the less complex assemblage, with only a few traces of carotenoids detected (**Figure 9C**). This only peak, visible on **Figure 9C,** was identified as fucoxanthin, a typical constituent of microalgae (Kim et al. 2012), and therefore was considerate as a contaminant. However, its symbiotic shrimps, *S. stimpsonii*, contains several pigments dominated by zeaxanthin with astaxanthin (both isomers), beta-carotene (both isomers), and traces of canthaxanthin, lutein and other pigment derivatives (**Figure 9D**). As our system was not optimal for the quantification of this pigment (long-tailed peak of the standard and graphical overlay of the different peak), we decided to report the sum of all pigment covering (**Table S2**). *S. stimpsonii* are organisms presenting the biggest proportions of pigments per sample mass (mean: 97.7 ± SD: 23.4 mg per sample g), followed by *C. novaeguineae* (mean: 64.6 ± SD: 9.7 mg per sample g). *Z. soror* present significatively fewer pigments, with a mean of 7.1 (± SD: 2.2) mg per sample gramme. For *P. distincta*, as no carotenoids (except contaminant) were surely detected, we did not calculate this value as it was not relevant.

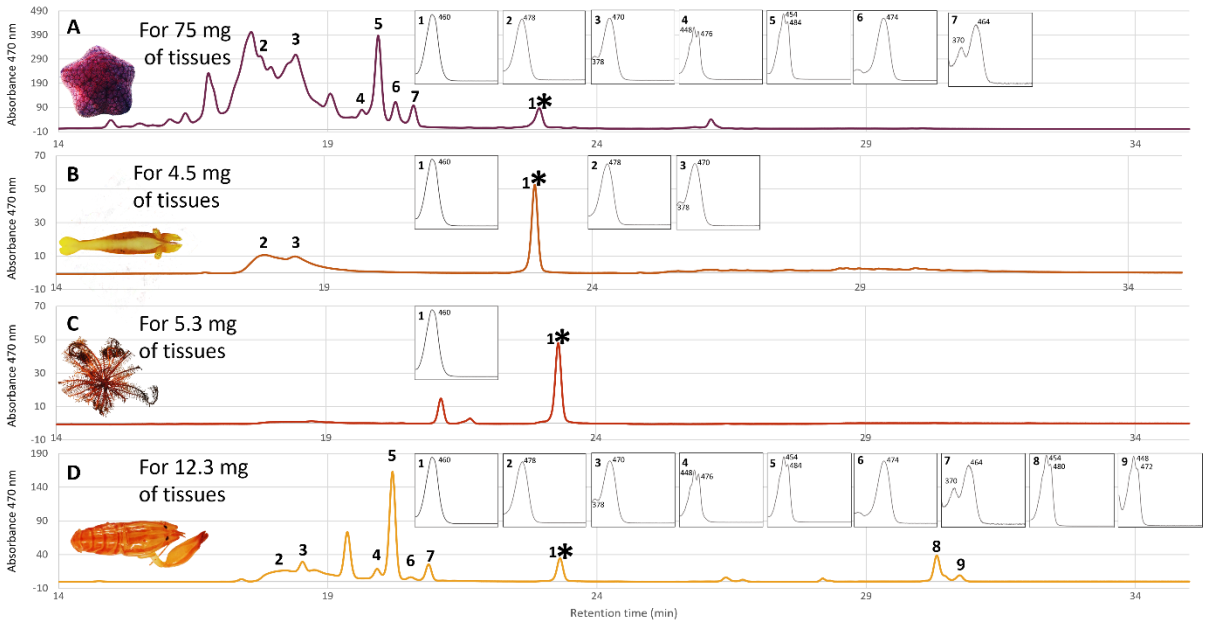


Figure 9 : High-performance liquid chromatography (HPLC) carotenoid profile with y-axis as absorbance at 470 nm and x-axis as retention time. (A) *Culcita novaeguineae*, (B) *Zenopontonia soror*, (C) *Phanogenia distincta* and (D) *Synalpheus stimpsonii*. Only peaks identified with experimental standards were highlighted. Peak 1* highlight the peak obtain with 20µg of internal standard (8-apobeta-carotene (Roth ®)). Peak 2+3 = Astaxanthin; 4 = Lutein; 5 = Zeaxanthin; 6+7 = canthaxanthin; 8+9 = Beta-carotene.

IV. Discussion

The shrimps *Z. soror*, *S. stimpsonii*, *T. holthuisi* and *A. indicus* are four obligatory ectocommensals associated with 3 different classes of echinoderms. These symbionts share a similar way of life, and all present a mimetic pigmentation as well as a specific morphology resulting from co-adaptation with their host. As an example, more than harboring similar colors than their host, *T. holthuisi* also shows an elongated morphology that allows it to be confused with an urchin spine (Marin and Anker 2009; Horká 2017). The level of morphological adaptation is usually related to the level of specificity/dependency of the association. *Z. soror* is the most generalist symbiont being found on at least 23 asteroid species (Antokhina and Britayev 2020a; De Grave et al. 2021; Lourtie et al. 2023; Fernández-Lereé et al. 2023). *S. stimpsonii* is also a generalist that can be found on at least 10 crinoid species (VandenSpiegel et al. 1998; Virgili et al. 2020; Caulier et al. 2022). However, these two species remain specifically associated to the class level, being only associated with asteroids and crinoid hosts, respectively. In contrary, both *A. indicus* and *T. holthuisi* are highly specific symbionts, primarily or exclusively found on *E. mathaei* (Gherardi 1991; Brasseur et al. 2018; Dabbagh et al. 2019; Al-Kandari et al. 2020; Ghory and Kazmi 2021; Hayes et al. 2022).

The results obtained in this study support the concept of host separation syndrome introduced by Brasseur et al. (2018). Indeed, the obligate ectosymbionts investigated here reveal a decline in their health when isolated from their hosts, leading ultimately to the organism's death, particularly in the absence of the chemical environment produced by the host. However, our multi-species data offer a more nuanced understanding of the dynamics of host separation and olfactory dependence, with a particular emphasis on coloration variation. All four decapod species are impacted by host separation, but notable differences exist. Firstly, *Z. soror* does not appear to be highly dependent on the chemical signals produced by its host starfish, unlike the other species (*i.e.* no significant differences were measured between the isolated and conditioning treatments). Also, *T. holthuisi* and *Z. soror* react more quickly to separation from their host than *A. indicus* and *S. stimpsonii*, probably because of their diets that depend on their hosts and because of their smaller body size than the latter species. This divergence also raises questions about the link existing between survivability and loss of coloration. *T. holthuisi*, *A. indicus* and *S. stimpsonii* show distinct coloration variation between the two isolated treatments. When isolated in seawater, discoloration is observed, but when isolated in the presence of the host-produced chemical environment, their coloration remains more stable. This suggests that

host-derived chemicals, including sea urchin spinochromes (Brasseur et al. 2018) or crinoid anthraquinones (Caulier et al. 2022), may contribute to maintaining coloration stability. Both of these molecules were proven to be specific quinones used notably as pigments in these respective classes of echinoderms (Hatate et al. 2002; Zhu et al. 2008; Brasseur et al. 2017; Hou et al. 2018; Mishchenko et al. 2020). Interestingly, for *Z. soror*, survival data show host dependence, but this does not appear to rely on host semiochemicals. However, its separation from the host leads to a discoloration, even when the shrimps are in the presence of semiochemicals produced by the sea star. One of these semiochemicals is asterosaponins which are defensive molecules against predators but also attractive kairomones for *Z. soror* (Lourtie et al. 2023). Contrarily to the attractive kairomones of crinoids, the anthraquinones (see Lourtie et al. 2023), and the attractive kairomones of sea urchins (Brasseur et al. 2018), the spinochromes, asterosaponins are not pigments and was never associate to other function than chemical defenses (Rideout et al. 1979; Netala et al. 2009; Brasseur et al. 2018; Caulier et al. 2022).

Considering that essential pigments for symbionts are carotenoids and quinones, future works should focus on the potential trophic link existing between symbionts and their hosts (Latscha 1989; Maoka 2011; Wade et al. 2017b). Whereas *T. holthuisi* feeds exclusively on host tissues, *A. indicus* feeds only partially on them and may rely on other food sources to obtain its spinochromes (Brasseur et al. 2018). But we suspect that, at least a part of their pigmentation may be attributed to additional carotenoids. Similar hypothesis may be true for *S. stimpsonii*, which feeds on particles available in the water column or by grazing close to the cirri of their host, rather than on host tissue (VandenSpiegel et al. 1998; Terrana et al. Submitted). In particular, our HPLC results suggest that this shrimp contains a high proportion of carotenoids, with diverse pigments such as zeaxanthin, astaxanthin or beta-carotene, whereas its crinoid host has no or only a very low proportion of carotenoids. Previous studies suggest that the symbiont mimetic coloration, and the persistence of this coloration in the presence of host kairomones, may be facilitated by the transfer of anthraquinone pigments produced by the host (Caulier et al. 2022). Concerning *Z. soror*, some results suggest that it may be a kleptoparasite, feeding both on the same food source as its host and even potentially on the host itself (unpublished data). Notably, they are usually found close to the ambulacral system and the mouth of the host (Lourtie et al. 2023). Since it shares common pigments with its host, such as astaxanthin or beta-carotene, it is possible that it obtains these carotenoids either by feeding at the same source as its host, or by directly consuming its host.

Also, the possibility of coloration being influenced by the color of the environment cannot be ignored. Previous research shows that the holothuroid associated crab *Lissocarcinus orbicularis* were able to adapt its pigmentation and shell ornamentation to match the colors of its host or the color of the bottom of the aquarium (Ayotte and DeMaintenon 2005; Fernández-Lereé et al. 2023). When isolated in uniform environments, such as aquariums without a host, symbionts may undergo discoloration to reduce visibility to potential predators (Gherardi 1991; Ayotte and DeMaintenon 2005). In contrast, in conditioned treatments, the shrimps are isolated in the same aquarium as their host where they can see them or be close to them. Therefore, they may remain more colored in response to the presence of the host.

Intraspecific characteristics may also play a role, particularly in *A. indicus* and *S. stimpsonii*. *A. indicus* exhibits a unique two-layered colored tissue, potentially containing carotenoids (red layer) in the first layer and spinochromes (purple layer) in the second one. Previous study highlights the presence of spinochromes in this species by chemical extraction (Caulier et al. 2022). During isolation, carotenoids may be metabolized to ensure survival while maintaining the purple layer's coloration remained, inducing only small coloration change. For *S. stimpsonii*, which exhibits a nychthemeral coloration/discoloration cycle, the color variations can be significantly influenced by the light in monitoring and may be more related to the quantity of light present during the experimentation (Joss 1973; Tlusty et al. 2009).

According to literature, carotenoids are usually suggested as the dominant pigments in shrimp species (Latscha 1989; Higuera-Ceiapara et al. 2006; Maoka 2011; Wade et al. 2017a). Our results indicated that the pigments are found in mainly one, or two, layers of pigmented tissue located just beneath the transparent cuticle. Histological sections reveal erythrophores containing red-orange pigment dendritic cells, probably containing carotenoids, in the first layer found in all species, and a second layer with a purple coloration that we attributed to spinochromes only in *A. indicus*. The erythrophores are present in large quantities in colored individuals of all species but appear less abundant in *Z. soror* translucent morphotype and in a condensed form in discolored *S. stimpsonii*. For *Z. soror* translucent morphotype, the HPLC analyses highlighted that the lack of coloration is linked to the absence of carotenoids. However, for *S. stimpsonii*, carotenoids are still present in the organisms, but their chromatophores seem to have the ability to change their size/diameter by condensing or decondensing, influencing their coloration. This dynamic system may be regulated by different mechanisms (i) hormonal regulation, (ii) neuronal regulation or (iii) direct impact on the metabolism of carotenoids. In

shrimps, mechanisms involving PCH (Pigment Concentrate Hormone) or PDH (Pigment Dispersion Hormone) were already observed (Fingerman, 1969). These mechanisms may be involved in reversal color variation as a fast coloration/discoloration event or nychthemeral cycle. However, the discoloration observed in response to host separation may be primarily due to a lack of pigments obtained through feeding or through the host's chemical environment. As carotenoids are involved in various functions of metabolism (Matsuno, 2001; Vershinin, 1999; Wade et al., 2017), symbionts may reuse them to more fundamental functions leading to an overall depigmentation of the organisms. But as often in complex and dynamic environmental systems, it is evident that the observed color variations in these symbionts are influenced by a combination of factors, including perhaps hormonal regulation, neuronal regulation, and direct impacts on pigment metabolism. Further research is needed to unravel the precise mechanisms underlying these coloration variations and their ecological significance.

V. Conclusion

Our study revealed a host dependence for all four species and a dependence to host semiochemicals in *A. indicus*, *T. holthuisi* and *S. stimpsonii*. We observed discoloration in all four species studied in the case of separation from the host. Complete isolation of these symbionts revealed a noticeable discoloration, which seems to be limited in the presence of the chemical environment produced by their host. This discoloration was attributed to the presence of carotenoids, or other pigments such as spinochromes, stored in pigment cells. These dendritic pigment cells were found just beneath the transparent cuticle and were red-orange pigmented. This study highlights several potential drivers of coloration variation. The presence of host-derived chemicals (carotenoids, spinochromes and/or anthraquinones), dietary choices, environmental coloration adaptation, and intraspecific characteristics all seems to play roles in this mimetic color variation.

General discussion



New insights into the host separation syndrome

The primary objective of this research project was to investigate the essential role of chemical ecology in initiating and perpetuating symbiotic relationships within the marine tropical ecosystems. More specifically, one of our working hypotheses was centered on the fact that kairomones secreted by marine invertebrate hosts could play a vital role in the survival/fitness of diverse obligatory ectosymbionts. Based on previous experimental works and on the observations that some symbionts died quickly when separated from their hosts, we suggested that these chemical cues not only enable these organisms to specifically recognize their hosts but may also directly influence their metabolic processes. In other words, without an optimal olfactory environment, these symbionts might face rapid mortality due to a phenomenon called «host separation syndrome» which was defined as a deterioration of the health status of an obligate symbiont when isolated from its host. To explore this hypothesis, we selected four distinct shrimp species, each associated with an echinoderm host and characterized by diverse ecological traits.

This final discussion aims to incorporate the results obtained in the different thesis chapters to provide a broader understanding and potential future research objectives that may be carried out to better understand marine symbiotic associations and their integration at an ecosystem-level. While the introduction of this thesis was centered on symbiotic associations, the beginning of this general discussion aims to provide a foreword on chemical ecology to better understand the contribution of this PhD thesis to this scientific field.

I. Chemical communication in marine ecosystem

Chemical communication is an ubiquitous and crucial process to the survival and regulation of interactions between living organisms within ecosystems (Kittredge et al. 1971; Brönmark and Hansson 2012). This mode of communication is made possible by the production of chemical signals, involving mainly secondary metabolites (Elliot and Elliot 1997). These molecules were originally defined as “waste products” from the primary metabolism and considered as non-essential to the survival of the producing organisms. However, it has become clear that these natural products play fundamental ecological roles, impact organisms survival, and do not always result for catabolism (Kornprobst 2005). They are secreted by every species in the biosphere, from microorganisms to vertebrates, including poriferans, bryozoans,

annelids, mollusks, arthropods, echinoderms and ascidians for example (Krieger and Breer 1999; Hay 2009).The physico-chemical characteristics of the external fluid in which organisms live play a crucial role in shaping the dynamics and success of ecological interactions, influencing the characteristics of the chemical messengers (Hay 1996). In terrestrial environments, the array of chemical compounds is constrained by the aerial medium, permitting only the transport of volatile compounds (Brönmark and Hansson 2012). Consequently, the majority of terrestrial semiochemicals consist of low molecular weight molecules (Brönmark and Hansson 2012). This limitation, however, does not apply to aquatic environments, where an extensive range of molecular structures, both hydrophilic and hydrophobic, are used to communicate (Zimmer and Butman 2000; Hay 2011; Brönmark and Hansson 2012). This impressive diversity makes marine ecosystems the greatest resource of natural products that could, in the future, constitute bioactive molecules that may be useful in the creation of new medicines (Malve 2016).

The chemical cues transported in this environment are referred to as semiochemicals or ecomones when they have a behavioral or physiological impact on another organism. These compounds fall into two main categories: pheromones and allelomones (**Figure 1**) (Ezzat et al. 2020). Pheromones play a fundamental role in intraspecific communication (Wyatt 2014). Concurrently, allelomones are involved in interspecific interactions and can be classified into three subcategories: (i) allomones, which are beneficial for the emitter; (ii) kairomones, beneficial to the receiver; and (iii) synomones, beneficial to both the emitter and receiver (Wyatt 2014). Some of the different ecological functions allowed by these molecules are shown in **Figure 1**. The complexity of semiochemical classification lies in the fact that one chemical stimulus secreted by a one producer can play multiple diverse roles depending on different receivers of the signal. For example, a chemical substance may act as a pheromone within one species while being perceived by another species as a kairomone or an allomone. As an example, some saponins produced by sea stars and sea cucumbers were identified as pheromones allowing intraspecific recognition and promoting gregarious behavior (Motti et al. 2018; Claereboudt et al. 2023), allomones allowing chemical defenses (Rideout et al. 1979) and kairomones allowing the symbionts to precisely recognize their hosts (Caulier et al. 2013; Brasseur et al. 2018; **Chapter I and II**).

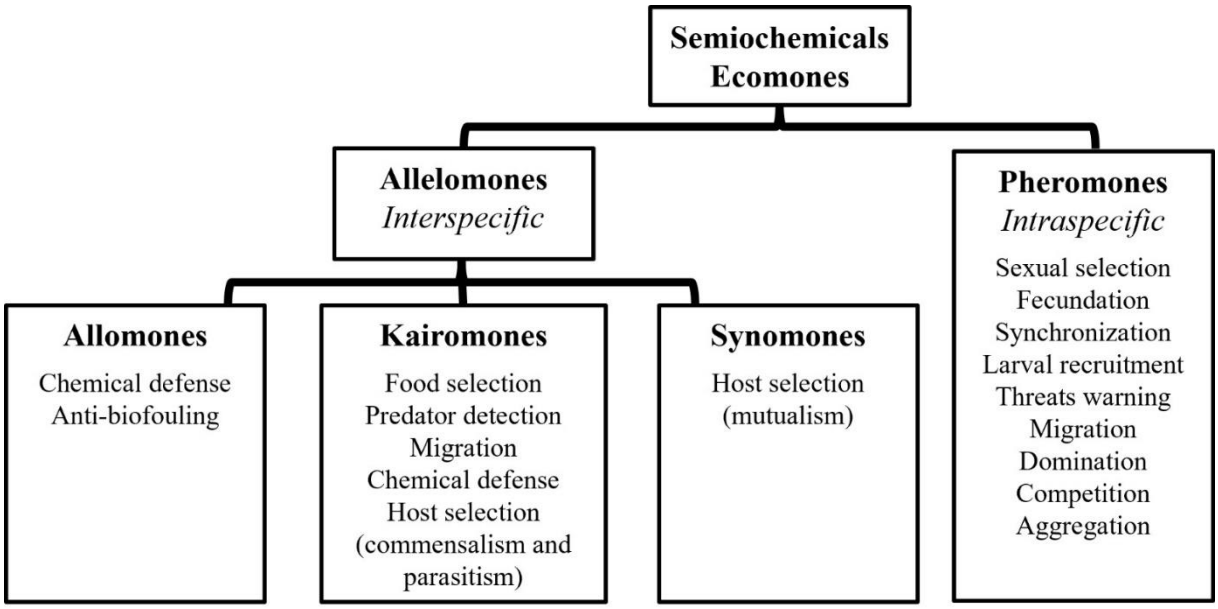


Figure 1: Classification of the different types of ecomones and non-exhaustive list of the ecological functions allowed by each category.

(1) Intraspecific chemical communication

Pheromones play a pivotal role in facilitating a myriad of intraspecific biological interactions. They have been extensively studied in the context of sexual communication, including the recognition and identification of sexually mature partners, the synchronization of external fecundation, and larval recruitment (Hay 2009; Wyatt 2014). For instance, female crustaceans exhibit the synthesis of pheromones in their urine, chemically attracting males just before molting. In 2000, Asai et al. synthesized a ceramide (*i.e.* a molecule composed of linear sphingosines and branched fatty acids), replicating the effects of female Horsehair crab *Erimacrus isenbeckii* pheromones. The sexual functions of pheromones extend to other examples, such as barnacles; sessile adults produce a recruitment pheromone that forms dense populations by attracting larvae through a chemical cocktail composed of the cuticular glycoprotein arthropodin and other protein molecules, thereby inducing metamorphosis (Clare and Matsumura 2000). Pheromones being species-specific, they can also be used as taxonomic proxies, allowing chemotaxonomy (Singh 2016).

Beyond sexual interactions, pheromonal communication also serves non-sexual functions. In the marine environment, certain intertidal organisms, like chitons and limpets, demonstrate a "homing" behavior, consistently returning to the same location after foraging for food (Cook 1969; Chelazzi 1990). These mollusks synthesize specific pheromones in the left mucus along their path, recognizing them through chemosensing to navigate the reverse route (Cook 1969;

Chelazzi 1990). Similarly, some pelagic organisms, such as salmonids, can detect chemical signals produced by sympatric populations to facilitate their migration (Nordeng 1977).

(2) Interspecific chemical communication

Allelomones are intensively studied in prey-predator interactions where some cocktails of molecules were characterized and chemically identified. Generalist consumers generally respond to primary metabolites (sugars, proteins, amino acids, metabolic waste products, ...) as they indicate the presence of nutritive resources in general (Rittschof and Cohen 2004; Hay 2011). For example, numerous laboratory experiments demonstrate that crustaceans are attracted to amino acids or peptides (Rittschof and Cohen 2004). Specialist consumers, on the other hand, will be sensitive to specific metabolites produced only by their nutritional sources (Rittschof and Cohen 2004). There are many cases where crabs, amphipods or isopods are attracted by specific secondary metabolites only produced by certain preys, which are repellent to most other organisms (Rittschof and Cohen 2004). An example of this is the crab *Caphyra rotundifrons*, which feeds solely on the algae *Chlorodesmis fastigiate* (Hay et al. 1989). The latter has the particularity to produce a cytotoxic metabolite called chlorodesmine to which the predator is resistant (Hay et al. 1989). At the opposite, a prey may also detect the smell of a predator to reduce its predation rate. In 1970, Mackie demonstrated that the escape behaviors observed on mollusks were induced by the chemoreception of steroidal saponins, molecules that are specific to asteroids such as the predatory sea star *Marthasterias glacialis*. In crustaceans, gammarids *Gammarus pulex* have been shown to avoid a predator, the trout *Salmo trutta*, by olfaction rather than vision (Dahl et al. 1998).

However, chemical communication is not restrained to prey-predator interactions. It is also extremely important in symbiotic associations. Indeed, it may be involved in the establishment and sustainability of these relationships mainly through two processes: the specific host recognition (Dimock and Davenport 1971; Murata et al. 1986; Souza et al. 2019; Antokhina and Britayev 2020) and the essential reuse of host metabolites by the symbiont (chemical dependency).

II. The host recognition/selection process

Similarly to prey-predator interactions, symbionts should detect class-specific, family-specific or species-specific chemical signals to allow an efficient host selection (depending on the host specificity) (Bartolini et al. 1973; Netala et al. 2009; Caulier et al. 2013b; Hou et al. 2018; Stonik et al. 2020). The first chemical component that was identified as a host selection synomone was the amphikuemin, involved in the recognition of anemones by clown fishes (ref). Subsequent investigations have focused on echinoderms (Caulier et al. 2013; Brasseur et al. 2018; **Chapter I and II**). At the University of Mons, our research team has identified four distinctive cocktails of kairomones composed of host-derived chemical metabolites facilitating the host recognition process:

(i) **Triterpenic saponins (Figure 2A).** Produced by various sea cucumber species, these compounds serve as attractants for the symbiotic crab *L. orbicularis* (Caulier et al. 2013b). The symbiotic crabs are therefore able to recognize and move toward several host species. In addition, it is suggested that they are also able to determine the health status of potential hosts, are they can recognize diseased sea cucumbers thanks to the modified semiochemicals they secrete (Caulier et al., *in prep.*).

(ii) **Spinochromes (Figure 2B).** Produced by sea urchin hosts and studied in the host *E. mathaei*. The kairomonal cocktail attracts both symbiotic shrimps, *Tulеariocaris holthuisi* and *Arete indicus* (Brasseur et al. 2018).

(iii) **Anthraquinones (Figure 2C).** Those molecules are involved in the relationship between the crinoid *P. distincta* and the shrimp *S. stimpsonii* (**Chapter I**).

(iv) **Asterosaponins (Figure 2D).** Produced by sea-stars, these compounds serve as attractants for both adult shrimps and zoeal larvae of *Z. soror* (**Chapter II**). This research not only shows that adults *Z. soror* are attracted by asterosaponins produced by their natural hosts, but also reveals a remarkable aspect related to their larvae: *Z. soror* larvae possess the ability to recognize different potential hosts present in their environment, expanding our understanding of the chemical cues involved in larval symbiotic recognition allowing potential recruitment (**Chapter II**).

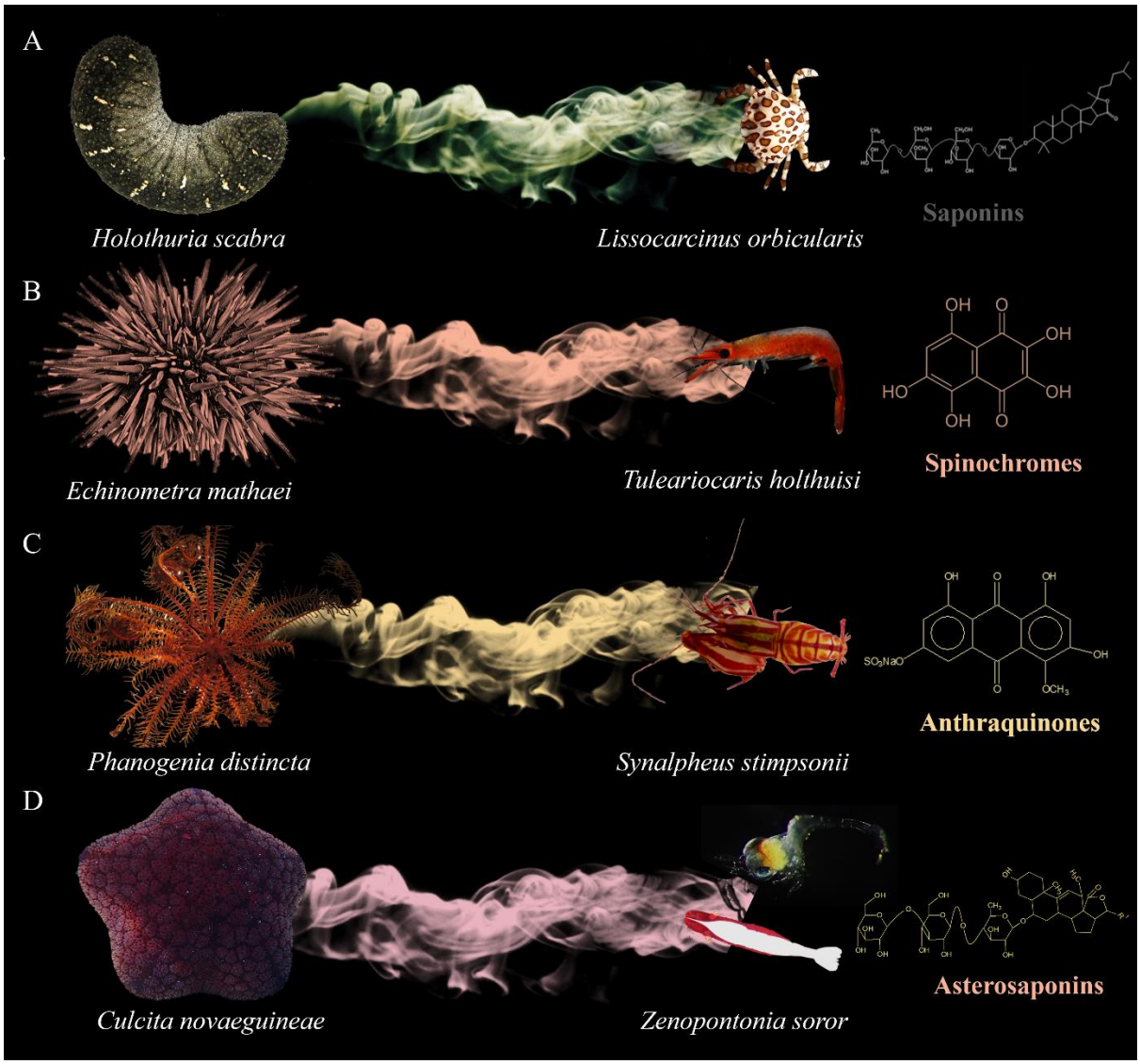


Figure 2: Characterization of kairomones involved in chemical communication and host selection in 4 symbiotic pairs between decapods and echinoderms. (A) Saponins involved in the recognition between different holothuroid species and the Harlequin crab *Lissocarcinus orbicularis* . (B) Sea urchin spinochromes acting as kairomones for the shrimp *Tulearіocaris holthuisi*. (C) Crinoid anthraquinones attracting the pistol shrimp *Synalpheus stimpsonii*. (D) Cushion sea star *Culcita novaeguineae* attracting the adult and larval shrimp *Zenopontonia soror*. The proportions of the organisms are not to scale.

Perspectives:

Actually, with the exception of two studies focusing on the host selection of symbiotic fishes (Murata et al. 1986; Miyazaki et al. 2014), our research team is the only laboratory working on the identification of host selection kairomones/synomones in the marine environment. To complete our investigations on all echinoderm classes, the ophiuroids still need to be investigated. This echinoderm class is known to host the smallest variety of decapod symbionts (**Introduction**). A good candidate could be the shrimp *Athanas ornithorhynchus* that is reported as an obligate symbiont of *Macrophiothrix longipeda* (Fabricius and Dale 1993; Marin et al. 2005; Deheyn et al. 2006; Anker and Marin 2007). This large ophiuroid is abundantly found in depths usually ranging from 0 to approximatively 70m in the Indian and Pacific Oceans (Pers. obs.; WoRMS Editorial Board 2023), making it possible to collect easily and in important quantities to investigate chemical communication and dependency. Another interesting association to investigate is the "babysitting" symbiosis between *Ophiomastix venosa* and *Ophiocoma scolopendrina* (Fourgon et al. 2007) that is already known for their host selection: symbiotic juveniles of *O. venosa* specifically recognizing *O. scolopendrina* adults in olfactometry behavioral experiments. However, the nature of the involved chemicals still remains to be discovered. Also, testing other echinoderms symbionts should be realized to confirm the paradigm shown in this work.

In addition, it may be interesting to investigate other symbiotic associations involving other hosts than echinoderms. Scleractinian corals, among others, may be good candidates as they display essential roles in coral reef ecosystems and host a large variety of ectosymbionts (Plaisance et al. 2011) but still remain largely under-investigated.

Finally, one of the major inputs of this thesis is made in **Chapter II**. For the first time, the identification of the kairomones involved in larval host recognition was proven and characterized. Considering that most invertebrates display one or several pelagic larval stage(s) (Herring 1974; Anger et al. 1994; Ramirez Llodra 2002; Sagi 2008; Gonzalez-Canales et al. 2018), lots of different symbionts may be investigated, starting with the models that were already investigated by our team for host selections of the adults (Caulier et al. 2013; Brasseur et al. 2018; **Chapter I**).

III. The host separation syndrome and chemical dependency

As symbiotic associations have a positive impact on the fitness of the symbiont, it may seem obvious that when a symbiont, especially if highly specialist, is separated from its host, it often dies, sometimes rapidly. It is particularly the case for many parasites that cannot complete their life cycle without their hosts (Dobson 1988). However, until recently, the impact of the separation between the host and the symbiont had never been investigated for ectocommensals that are yet numerous in marine ecosystem. Brasseur et al. (2018) introduced a novel term: the 'host separation syndrome.' It manifests as a compromised health status among obligate symbionts when isolated from their hosts, potentially leading to their demise. This phenomenon may result from the absence of specific chemical cues or compounds produced by the host. This hypothesis was supported by the fact that one unusual phenotypic manifestation had been observed during host separation events: symbiont depigmentation (Brasseur et al. 2018).

The study of Brasseur et al. (2018) primarily focused on short-term isolation and only considered chemical dependency without considering other potential detrimental factors that could impact the survival of the symbiont. To better understand the concept of host separation, we conducted longer experiments and integrated a multi-stressor approach to explain the potential emergence of the host separation syndrome. We suggest it is crucial to include multiple parameters of stress and long-term experiments for several reasons:

(i) It is a more accurate representation of real-world conditions (*i.e.* ecologically relevant) (Heugens et al. 2001). When separated from their hosts, symbionts will experience a panel of benefit lost, as the chemicals and food provisions, or the shelter offered by the host defense (*e.g.* morphological or chemical defenses), especially if they are dependent of their hosts.

(ii) Different stressors can interact in unexpected ways, leading to amplified or diminished effects. This interaction can be synergistic (where the combined effect is greater than the sum of individual effects) or antagonistic (where the combined effect is lower than the sum of individual effects). For example, corals are known to be exposed to a panel of stress factors (UV light, temperature and pollution), some being categorized as synergetic factors (sedimentation and temperature for example) (Maina et al. 2011).

(iii) Organisms may have mechanisms (*e.g.*, physiological responses) to cope with stress in low exposure or short term, (Godefroid 2023, **Chapter III**). However, prolonged

exposure to multiple stressors can challenge an organism resilience and adaptation abilities, eventually leading to a decline in their health (Matheny et al. 1986; Heugens et al. 2001; Guh et al. 2021).

Our results confirm that *T. holthuisi* is the most impacted by the host separation syndrome (**Figure 3D and H**). As suggested by Brasseur et al. (2018), they display a chemical dependency toward their host (**Figure 3B**). However, this fast mortality may also be explained by the food dependency, which is high for this species (**Figure 3A**). The smallest size of that species suggests that it does not have high internal nutritional reserves to cope with the multi-factorial stress (**Figure 3C**).

The second species that is the most impacted by the host separation is *Z. soror* (**Figure 3E and H**). While this species does not seem to display a chemical dependency toward its host, they only rely on the host to obtain their food (**Figure 3B**). This species may therefore present a food-dependency toward its host (**Figure 3A**).

The third species to be impacted by the host separation is *A. indicus* (**Figure 3F and H**). In this species, our results show that individuals are chemically dependent of their host, but their food sources are varied (**Figure 3B**). This species may therefore be more impacted by the chemical privation than by the food privation (**Figure 3A**).

The last species, *S. stimpsonii*, is the biggest species, and therefore individuals might have the biggest internal reserves (**Figure 3G-H**). They are not food-dependent toward their hosts (**Figure 3A**), but they display a chemical-dependency toward them (**Figure 3B**).

Altogether, our results suggest that *T. holthuisi* is the most impacted by the host separation syndrome due to both starvation and loss of the beneficial chemical environment. We can suggest a synergy between the host's kairomone lack and food loss coupled with its small size. *Z. soror* seems to have a food-dependency, making it the second most affected. *A. indicus* shows chemical dependency but less reliance on its host for food. These differences between *Z. soror* and *A. indicus* suggest that food-dependency has a greater impact on symbionts than the chemical-dependency for these models. Lastly *S. stimpsonii*, the largest species, appears to have the most resilience due to its size and chemical dependency but no food-dependency. The differences between *A. indicus* and *S. stimpsonii* may be primarily explained by size differences (**Figure 3**).

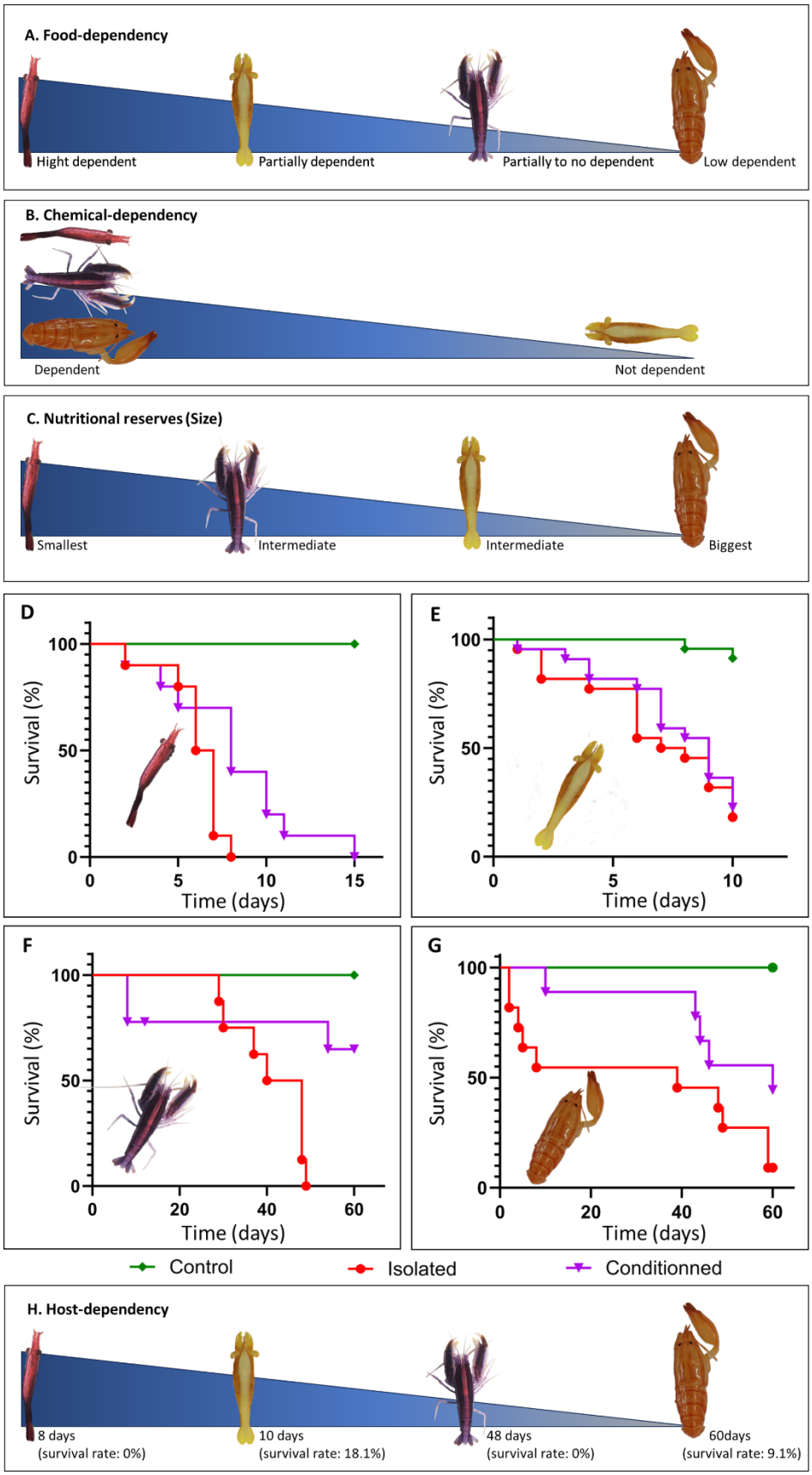


Figure 3: Conclusion schematic representation summarizing the different parameters measured on the four different symbiotic shrimps associated with echinoderms during this PhD thesis. **(A)** Food dependency (**B**) Chemical dependency (**C**) Nutritive reserves of the symbionts (**D-G**) Survival curves measured during host separation syndrome isolation tests on the four shrimps. (**H**) Host dependency for the symbionts.

We therefore currently hypothesize that the impact on the symbiont upon host isolation depends on three main factors: (i) the nature of the chemical environment of the symbiont, (ii) the diet preferences of the symbiont and (iii) the size of the symbiont and the quantity of nutritional reserves.

(1) Nature of chemical environment (purely noxious or partially beneficial)

In the context of symbiotic relationships, a host can be considered as a micro-ecosystem displaying its own specific chemical environment. Echinoderm hosts, in particular, are renowned for their specific chemicals to each class: triterpenoid saponins in holothuroids (Kamyab et al. 2017; **Figure 4A**); steroidic saponins (*i.e.* asterosaponins) for asteroids (Motti et al. 2018; **Chapter II**; **Figure 4B**); anthraquinones for crinoids (Bartolini et al. 1973; **Chapter I** ; **Figure 4C**) and naphtoquinones (*i.e.* spinochromes) for echinoids (Shikov et al. 2018; **Figure 4D**). Even if those molecules are frequently produced in other biological kingdoms (Thomson 1991; Vincken et al. 2007; Savarino et al. 2021), they are quite rare in the animal kingdom and can be considered as chemical signatures for each echinoderm class (Kornprobst 2005). Producing such specific chemicals display a metabolic cost for the host (Mebs 2001; Enzor et al. 2011) and those molecules usually display multitasking ecological roles, from aggregating pheromones (Claereboudt et al. 2023) to symbionts kairomones (Caulier et al. 2013; **Chapter I and II**) and chemical deterrent allomones (Van Dyck et al. 2011).

Indeed, saponins and quinones are known for their status as noxious molecules (Rideout et al. 1979; McClintock and Vernon 1990) that are associated with a need of metabolic adaptation for echinoderms to cope with those biotoxic properties. Considering saponins that interact with biological membranes and are cytotoxic, sea stars and sea cucumbers have adapted their sterol composition by displaying a significantly higher proportion of delta 7 ,9, 11 sterols to make their cell membranes saponin-resistant (Claereboudt et al. 2018).

But how can symbionts adapt to these defense allomones? First of all, it seems that decapods, by their peculiar anatomy/physiology, are less sensitive to detrimental chemicals than other organisms like fishes for example. Taking sea cucumbers as an example, while saponins are reported as ichthyotoxic, damaging the gills of most fishes (with the exception of the endosymbiont carapids; Eeckhaut et al. 2015), the highest predator of the aqua cultivated sea cucumbers *Holothuria scabra* is the predatory crab *Thalamita crenata* (Lavitra et al. 2009; Eeckhaut et al. 2020). Also, it seems that symbionts are adapted to the natural concentration produced by their host in open-sea environment, but they may be impacted by higher

concentrations. This was frequently observed during this thesis, as the seawater containing the hosts could be tinted in orange/red for crinoids and pink/purple for echinoids, or be foamy for sea cucumbers and sea stars, especially when they were stressed (*e.g.* with the experimenter manipulations). We suspect that those observations are the consequence of the secretion of anthraquinones/spinochromes and saponins that are produced in higher quantities by the stressed hosts, being responsible of this pigmentation/foam. Besides, it was shown that the concentration and quality of the cocktail of saponins could change when the sea cucumber were stressed (Van Dyck et al. 2011; Bahrami et al. 2018). Intriguingly, during such events, all symbionts present within this environment succumbed rapidly (pers. obs). This observation prompts the hypothesis that under normal conditions, symbionts associated with sea urchins, crinoids, sea stars and sea cucumbers remain unharmed by the noxious allomones that also repel their own predators (*i.e.* as they benefit from the chemical defenses of their host). However, they may be negatively impacted and die if the host is stressed, especially in a closed environment, triggering a concentration rise, that actually should only happens in *in vitro* conditions (*i.e.* in tanks) or in closed pools formatting at low tides (Kooistra et al. 1989).

Considering the potential effects of those host's ecomones on symbiont metabolism, no beneficial effect has ever been reported for holothuroid and asteroid saponins, they would therefore have no beneficial effect on the symbionts except the environmental role of chemical defense (Vincken et al. 2007; Netala et al. 2009; Demeyer et al. 2014). Conversely, quinones are associated with a broad spectrum of advantageous properties, in addition to their chemical defenses effects (El-Najjar et al. 2011; Lu et al. 2013). More generally speaking, quinones are known to play a key role in numerous biological processes (Thomson 1991; El-Najjar et al. 2011; Lu et al. 2013; Brasseur et al. 2017, 2018). Certain quinone derivatives act as electron acceptors in electron transport chains, contributing to processes like photosynthesis and aerobic respiration (Nohl et al. 1986; Elling et al. 2016). Additionally, they play a role in the cellular membrane stabilization (Nageswara Rao et al. 2008). They were also proven to have a role in the formation of vitamins and antioxidants (Beattie et al. 2007; Gong et al. 2008) or in antimicrobial activities (Stekhova et al. 1988).

The chemical environment produced by the host may impact the symbiont chemical-dependency (**Figure 3B)**. In this thesis, *Z. soror* does not appear to be highly dependent on the chemical signals produced by sea stars (*i.e.* no difference between the isolated and conditioning treatments) as asterosaponins are exclusively described as noxious molecules and as our results suggest non-chemical dependency of this species. For the three other species, there is a significative increasing of the survival when individuals are isolated with the semiochemicals produced by the hosts (Brasseur et al. 2018; **Chapter IV**). This therefore suggests a chemical dependency of *S. stimpsonii*, *A. indicus* and *T. holthuisi* toward their respective hosts and both spinochromes and anthraquinones may be beneficial to a certain extent for the symbionts. It is important to note that, while our study focused on targeted specific molecules (asterosaponins, anthraquinones and spinochromes), the entire chemical environment may surely be more complex. It may also include supplementary molecules with beneficial impact (*e.g.* carotenoids), that were not targeted in this study.

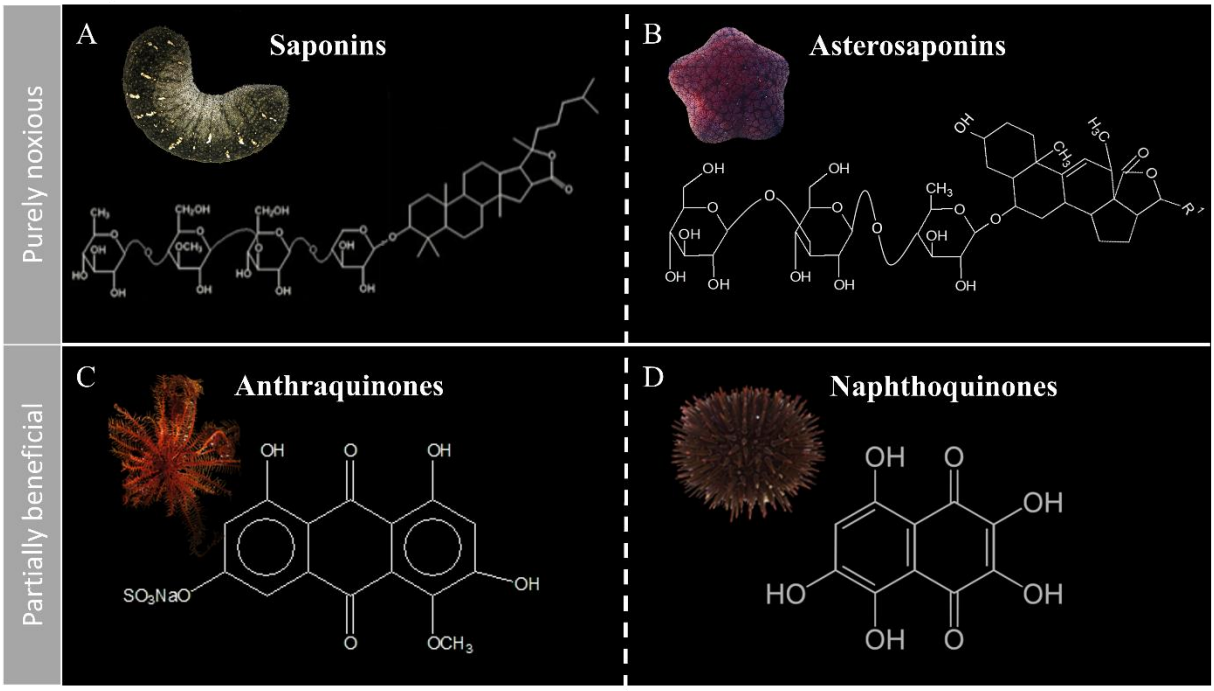


Figure 4: Characterization of kairomones involved in the chemical communication and host selection in 4 symbiotic pairs decapods-echinoderms associations. (A) Triterpenic saponins involved in the recognition between different holothuroid species and the Harlequin crab *Lissocarcinus orbicularis*. (B) Steroidic asterosaponins involved in the recognition between the asteroid species *Culcita novaeguineae* and the shrimp *Zenopontonia soror* (C) Crinoid anthraquinones attractive to the pistol shrimp *Synalpheus stimpsonii*. (D) Sea urchin spinochromes acting as kairomones for the shrimp *Tuleariocaris holthuisi*. The proportions of the organisms are not to scale.

Perspectives:

To better understand the chemical environment produced by a host, it seems interesting to investigate two major thematics. First, it is essential to investigate the potential modification of the chemical environment produced by the host when they are unwell, stressed or sick. The concentration (quantity), as well as the nature (quality) of the cocktail of molecules may vary and become more or less noxious, having a potential detrimental impact on the symbionts. To do this, analytic analyses (*e.g.* mass spectrometry, nuclear magnetic resonance spectroscopy,…) should be performed on various organisms having different health status (healthy, sick, stressed or dead for example). It may also be interesting to develop a methodology to investigate directly the molecules contained in host-conditioned seawater. Currently, we extracted the molecules directly from whole-organisms or various host tissues as teguments, viscera and Cuvierian tubules (Kornprobst 2005; Van Dyck et al. 2010; Demeyer et al. 2014; Brasseur et al. 2017; Bahrami et al. 2018). However, it is not sure that the metabolites secreted in the outside environement will match with the molecules found inside the host organisms tissues. Currently, only one research investigate directly the saponins that are present in the sea cucumber *Holothuria forskali* conditioned seawater (Van Dyck et al. 2011).

Another crucial dimension of the chemical environment that warrants exploration: the impact of a modified chemical environment generated by stressed organisms. We conducted preliminary investigations into this aspect using the associated fauna of the coral species *Pocillopora acuta* (Flandroit et al. 2022; Ver Hoeye et al. 2023). Our research employed a methodology similar to the host-separation investigations carried out on echinoderms (Brasseur et al. 2018, **Chapter IV**). During the course of our investigations, we encountered instances where some coral colonies became unhealthy and began to bleach (Flandroit et al. 2022; Ver Hoeye et al. 2023). Subsequently, we observed that both the host-conditioned symbionts and the control group exhibited a higher mortality rate compared to their isolated counterparts (Flandroit et al. 2022; Ver Hoeye et al. 2023). These initial findings suggest that an unhealthy chemical environment may have a detrimental impact on the symbiotic fauna, potentially leading to their mortality. Given the global prevalence of coral bleaching events (Panithanarak 2015; Heron et al. 2017; Hédouin et al. 2020), it becomes increasingly pertinent to gain a deeper understanding of the effects of such events on the chemical environment of coral ecosystems and the potential consequences they may have on the associated fauna.

(2) <u>The diet preferences of the symbionts (exclusively, partially or not feeding on the host)</u>

Decapod symbionts usually seem to benefit from increased food availability through their association (Castro 1971a; Caulier et al. 2014; Horká 2017). As a reminder, symbionts can be classified in five feeding guilds : (i) mucus and host-generated waste feeding (*e.g.* Castro 1971; Britayev and Zamishliak 2012), (ii) feeding on host tissues (*e.g.* Jangoux 1987; Caulier et al. 2014), (iii) predation on other symbionts (*e.g.* Westblad 1949; Marin and Chan 2006), (iv) kleptoparasitism (*e.g.* Mussoi et al. 2023; Terrana et al. Submitted) and (v) external food sources (*e.g.* VandenSpiegel et al. 1998).

The four symbionts here investigated present different feeding strategies and therefore exhibit diverse host nutritional dependencies as illustrated in **Figure 5**.

Considering *T. holthuisi*, stable isotopes analysis based on $\delta^{13}C$ and $\delta^{15}N$ ratios suggests that sea urchins serve as the primary food source for this symbiont (Brasseur et al. 2018). The $\delta^{13}C$ ratio of *T. holthuisi* closely resembles that of its host, but with a higher $\delta^{15}N/^{14}N$ ratio. In the case of symbiontic organisms, this difference often signifies exclusive feeding from their hosts (guild ii). This implies a strong nutritional dependency for this species (**Figure 5A**).

Z. soror has a mixed feeding strategy (**Figure 5B**). Stable isotopic $\delta^{13}C$ analysis (Mussoi et al. 2023, *In Prep*) reveals similar ^{13}C and $\delta^{15}N$ values between the host and the symbionts, with a slight augmentation in $\delta^{15}N$ for the symbionts in comparison to its host and a minor overlap between the isotopic niches of both organisms. This suggests that *Z. soror* may engage in opportunistic kleptoparasitism mixed with an external intake of food and, maybe, small proportions of host tissues consumption (Mussoi et al. 2023, *In Prep*). This hypothesis gains strength when considering their location on their hosts, often near the ambulacral groove and the host mouth or evaginated cardiac stomach during the night when the sea stars feed (**Chapter II**). Further evidence is found in the gut content analysis which indicates the presence of small coral fragments, the primary food source of their hosts (Mussoi et al. 2023, *In Prep*). However, the analysis also highlighted sea star ossicles in their guts, demonstrating partial consumption of their hosts, as already detected in the gut content of the symbiotic crabs *Lissocarcinus orbicularis* consuming tissues from their holothuroid hosts (Caulier et al. 2014). Additional food sources, such as algae or planktonic particles, were also observed. When kept on their hosts, numerous feeding behaviors were observed, especially kleptoparasitic behaviors where

shrimps stole food particles directly from the ambulacral groove of their host (**Figure 3Aa**). As a summary, *Z. soror* have an opportunistic feeding approach, combining primarily kleptoparasitism (guild iv), limited consumption of host tissues (guild ii), and other potential food sources (guild v) (**Figure 5B**). However, as both kleptoparasitism and host tissue consumption strictly rely on the host presence, this symbiont may partially rely on its hosts to obtain its food (**Figure 5B**).

For the third species, *A. indicus*, stable isotope analyses did not reveal host-dependent feeding patterns (Brasseur et al. 2018). The considerable standard deviation observed in $\delta^{13}C$ values suggests a diverse array of food sources (Brasseur et al. 2018). Additionally, minimal overlap with *T. holthuisi* isotopic niche indicates a distinct dietary preference (guild v). While *A. indicus* may feed on *E. mathaei* (guild ii), potentially for the ingestion of naphthoquinones (Brasseur et al. 2018), this is not their exclusive diet as Gherardi et al. (1999) did not find any evidence of host tissues ingestions in the gut contents which suggests that they will "sweep up the crumbs" from their host meals, which suggest a kleptoparasitism activity (guild iv). But it is likely that various other food items, such as benthic organisms, organic matter and various seaweeds, are the major part of their nutritional intake (guild v) (Gherardi 1991). Therefore, *A. indicus* do not rely strongly on their host for their food, being more partially or non-food-dependent toward its host (**Figure 5C**).

Studies have revealed that the last symbiont, *S. stimpsonii*, exhibits a stable isotopic $\delta^{13}C$ composition that is distinct from that of its host (Terrana et al. Submitted). This difference suggests that this symbiont feeds on different food sources than its host and can therefore be classified in the guild v (**Figure 5D**). Previous research by VandenSpiegel et al. (1998) supports this hypothesis, demonstrating that these snapping shrimps graze on macroalgae located around the calyx of their host crinoids. Also, when isolated from their host, the addition of micro-algae significantly increases their survival rate (**Chapter IV**). Therefore, they do not directly rely on their host for feeding and only present small nutritional dependency toward their host (**Figure 5D**).

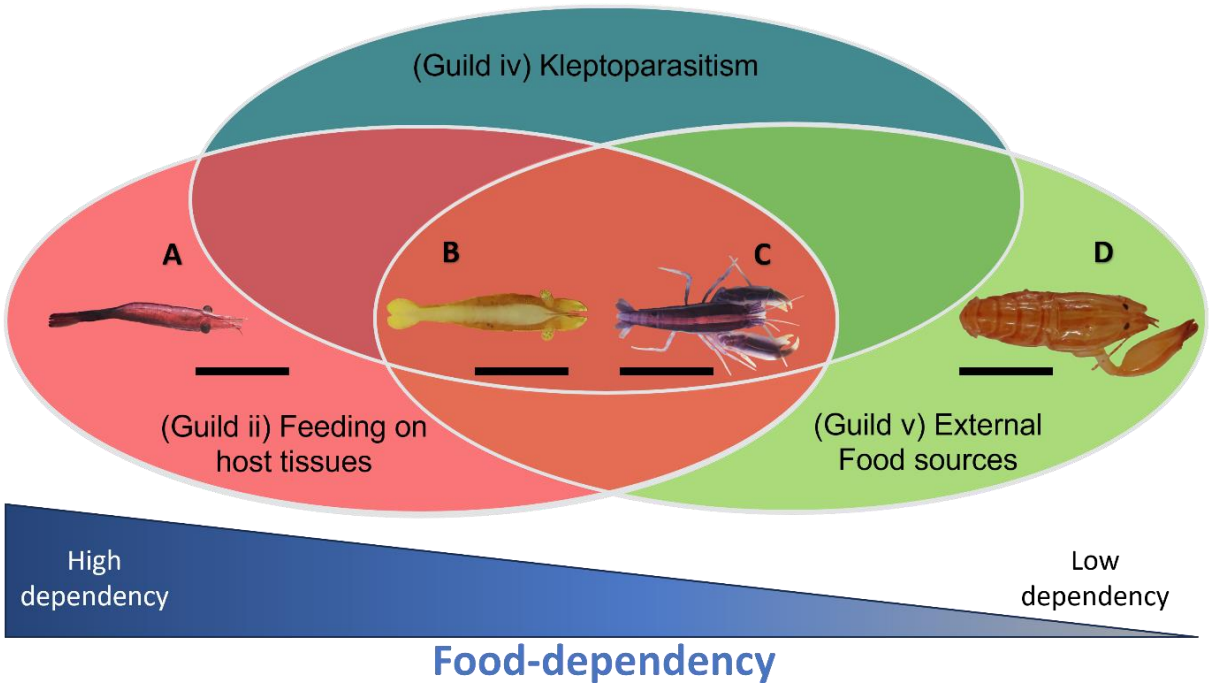


Figure 5: Venn diagram showing the feeding guilds of the four symbiotic shrimp species investigated in this project. Pink = Guild ii: Feeding on host tissues; blue = guild iv: kleptoparasitism and green = guild v: feeders on external food resources. Overlapping areas indicate decapods associated with multiple feeding guilds. (A) *Tulearіocaris holthuisi*, (B) *Zenopontonia soror*, (C) *Arete indicus*, and (D) *Synalpheus stimpsonii*. Scalebar = 0.55cm in A; 0.5cm in B; 0.6 in C and 0.65 in D.

In summary, these four symbiotic species exhibit a diverse array of nutritional strategies. While some predominantly rely on host-derived resources, others display opportunistic feeding behaviors, often supplementing their diets with external food sources. *T. holthuisi* may stand out as the most host-dependent, primarily feeding on host tissues. *Z. soror* appears to have an intermediate nutritional dependency on its hosts, displaying kleptoparasitic tendencies while also incorporating host tissues into its diet. In contrast, *A. indicus* and *S. stimpsonii* may be the less influenced by the presence of their hosts when it comes to feeding. In the absence of predators and with access to external food sources, they demonstrate the ability to efficiently sustain themselves (pers. Obs).

Perspectives:

The nutritional aspects of echinoderms-associated decapods are currently understudied. Some studies have investigated the modified mouthparts of these decapods, with the majority of these adaptations attributed to the diversity of diets facilitated by these associations. For instance, the genera *Pinnaxodes*, *Holotheres*, and *Holothuriophilus* possess palps covered with long setae, enabling them to capture planktonic food effectively, indicating a filter-feeding diet (Wells and Wells 1961; Ng and Manning 2003). In *Dissodactylus* and *Clypeasterophilus,* adaptations such as the presence of a small dactylus on the third maxillipeds indicates that their diet is composed primarily of sea urchins tissues (de Gier et al. 2022). Similarly, *Gnathophylloides mineri* displays toothed setae on the second maxillipeds, possibly to collect the epithelium covering sea urchin tests (Patton et al. 1985). Considering *Z. soror*, the morphology of its mandibles and chelae suggests a feeding strategy involving the host mucus or mucus-entrapped particles (Tseng et al. 2022).

As we move forward in understanding the nutrition of symbionts associated with echinoderms, it becomes apparent that there is still much to uncover regarding the adaptations of mouthparts and their correlation with diet preferences. Future research in this area could shed light on the eco-functional mandible adaptations in symbiotic decapods. Coupled with stable isotopes and guts content analyses, it will enable to better understand the nutritional aspect of these symbioses.

(3) The size and body shape of the symbionts (small, medium or big symbiont)

In general, larger organisms tend to exhibit greater resilience to stress compared to their smaller counterparts (Fernandes and McMeans 2019). This can be attributed to the greater internal resources available in the bodies of larger organisms, as smaller ones may have comparatively fewer resources to cope with stressful conditions (Shuter and Post 1990; Fernandes and McMeans 2019). Additionally, smaller organisms typically have higher metabolic rates relatively to their body mass when compared to larger organisms (Shuter and Post 1990; Fernandes and McMeans 2019). Consequently, they tend to deplete their resources at a faster rate than larger organisms. Considering our four species, the smallest is *T. holthuisi*, followed by *A. indicus*, which has a similar size but a more robust body than *T. holthuisi*. Follows *Z. soror* that is slightly bigger and *S. stimpsonii which* is the biggest species here considered (**Figure 3C**).

IV. Proxy to estimate the host separation induced stress

(1) Host specificity vs host dependency

Brasseur et al. (2018) suggest that host dependency was correlated to host specificity. However, our results suggest the opposite. For example, the most specific symbionts *A. indicus*, is less impacted than *Z. soror*, which is a class-specific symbiont and the most opportunist species of our investigation (**Introduction; Figure 3 and 6**). We therefore propose that host-specificity alone may not be a sufficient criterion to estimate the impact of host separation among symbiotic organisms. Instead, we suggest that host imprinting phenomenon may be a crucial aspect for understanding the consequences of host separation.

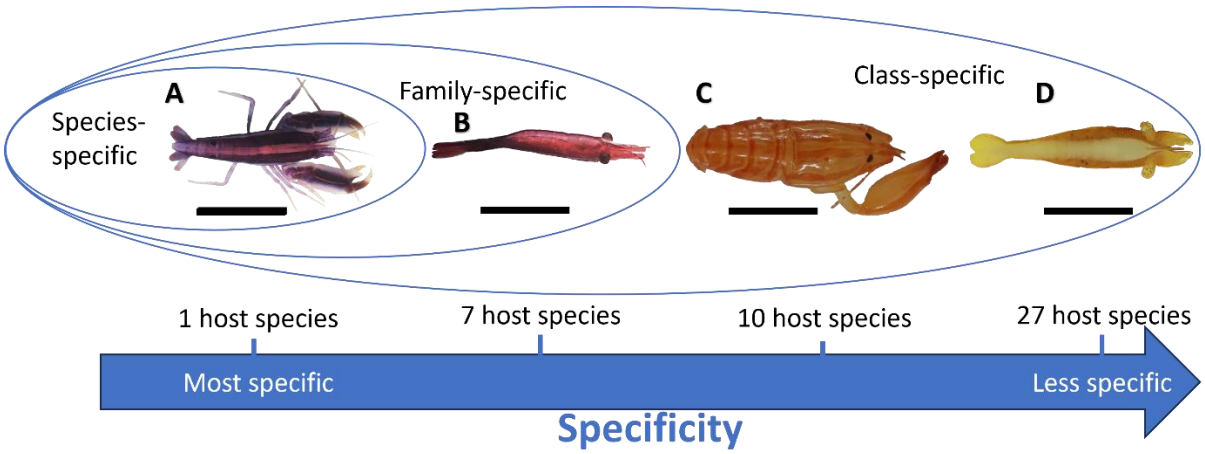


Figure 6: Representation of the host specificity level on the four species investigated in the thesis. (A) the species-specific *Arete indicus* (B) the family-specific *Tulеariocaris holthuisi* (C) the class-specific *Synalpheus stimpsonii*, and (D) the class-specific *Zenopontonia soror*. Scalebar = 0.55cm in A; 0.5cm in B; 0.6 in C and 0.65 in D.

In behavioral biology, the imprinting concept involves the acquisition of specific behaviors or preferences based on the exposure to certain stimuli (*e.g.* food , specific chemical; etc.), which can have a lasting impact on an individual behavior and interaction with its environment (Immelmann 1975). Host imprinting is often irreversible (Immelmann 1975) and can significantly influence an organism survival. In symbiotic species, imprinting may play a key role in host preferences, and impact both their feeding and chemical response behavior (Immelmann 1975; Schelvis 2017; Antokhina and Britayev 2020b; **Chapter II**).

To illustrate this statement, our results on *Z. soror* suggest that they are imprinted by their host semiochemicals during their host recognition behavior (**Chapter II**): the adult *Z. soror* individuals displayed a clear preference for the asterosaponins produced by their native host species, *C. novaeguineae,* as they were only attracted by them. In contrast, they did not exhibit attraction towards the two other potential host species, *A. planci* and *L. laevigata*, previously documented as hosts for adult *Z. soror* in the scientific literature, as well as by three non-host species, namely *A. rubens*, *E. sepositus,* and *H. scabra*. However, *Z. soror* larvae were both attracted by *C. novaeguineae* and *A. planci,* which suggest an imprinting behavior only impacting the adult symbiont but not the larvae.

In addition, symbiotic host imprinting is a well-documented phenomenon observed in various decapod species (Immelmann 1975; Schelvis 2017; Antokhina and Britayev 2020a). This adaptation phenomenon is not limited to *Z. soror* but has been widely observed in decapods, impacting various aspects such as host recognition, habitat selection, and dietary preferences in these organisms (Immelmann 1975; Schelvis 2017; Antokhina and Britayev 2020a). After host imprinting, a symbiont individual may be more specific than expected.

(2) A transcriptomic approach of the host separation syndrome

In non-model species, New Generation Sequencing (NGS) technologies has revolutionized molecular data generation, allowing for the discovery of novel genes and comprehensive expression analyses. **Chapter III**, describing the transcriptomic sequencing and analysis of *T. holthuisi* and *A. indicus*, is the first to employ NGS techniques to investigate the host-separation syndrome and the transcriptomic response induced by the chemical environment loss.

Our study yielded extensive transcriptome datasets for both symbiont species, amassing over 96.41 Giga clean bases of data. The results for *T. holthuisi* displayed an important

transcriptomic response, especially when isolated from its host without exposure to the host-produced chemical environment. Notably, the gene expression analysis revealed differentially expressed genes coding for heat shock proteins in response to the host-isolation experiment. The introduction of spinochromes seemed to enhance this shrimp ability to adapt to the stressful conditions. In parallel, *A. indicus* also exhibited signs of stress upon host isolation, which was not suggested by the results of Brasseur et al. (2018). The results suggested that this species appeared to employ transcriptomic adaptation mechanisms to cope with this host-separation stress, which was confirmed by **Chapter IV**.

A transcriptomic approach alone may not be useful, especially to study non-model organisms that are under environmental stresses like host-separation. But coupled with a pluri-disciplinary approach involving behavioral experiments and *in vivo* stress assessment, it can help to provide new hypotheses and shed light on the mechanisms set up by organisms to cope with stresses.

(3) Discoloration induced by host separation

In a prior study, Brasseur et al. (2018) described depigmentation on *T. holtuisi* when individuals were separated from echinoids. Strictly monitoring symbionts colors, our findings reveal that it is not a general rule as it depends on the investigated species and individual (**Chapter IV**). Notably, when examining *T. holthuisi* in isolation from its host, we observed a consistent trend of uniform discoloration until the last hour before its death, where a specific recoloration appears (**Figure 3Fc**). Conversely, host-conditioned shrimps tended to keep their coloration or even showing brighter colors (**Chapter IV**).

However, considering other species, our investigations on *Z. soror* yield some interspecific variations. Out of the five individuals studied, four exhibited discolorations when separated from their host (either completely or when placed in host-conditioned seawater) (**Chapter IV**). In this species, the chemical environment alone did not seem sufficient to influence pigmentation significantly (**Chapter IV**). Consequently, it may appear that this species relies primarily on its food sources to obtain pigments, as it lacks efficient mechanisms to reuse potential pigments from the environment.

For the last two species (*A. indicus* and *S. stimpsonii*), which exhibit a slower host separation syndrome, we observed substantial interspecific and interindividual variations in terms of discoloration (**Chapter IV**). For both species, some individuals showed stable or even

increased coloration in both isolated and host-conditioned treatments. In these cases, it is challenging to discern any consistent patterns related to coloration, making it an unreliable proxy for assessing potential host separation stress.

Additionally, while certain species have specific characteristics that may influence their coloration (*e.g.*, the nychthemeral cycle in *S. stimpsonii* or the presence of multiple chromatophore layers in *A. indicus*), more general factors are known to also influence the coloration of all the species maintained in aquariums (Joss 1973; Ayotte 2005; Tlusty et al. 2009). Environmental factors like light conditions and the background color of the environment (*e.g.* host or aquarium, for example) may play a significant role in influencing of these commercial crustacean coloration (Joss 1973; Ayotte 2005; Tlusty et al. 2009). In this context, we hypothesized that the transparent plastic vials in which symbionts were kept within the aquarium allowed them to maintain visual contact with their hosts. Consequently, some symbionts might have continued to exhibit their typical mimetic coloration in response to the presence of their hosts.

V. Mimetic coloration and its variation:

The relationship between coloration and diet has been confirmed in numerous symbiotic associations (Gherardi 1991; Horká 2017; Brasseur et al. 2018; Virgili et al. 2020; Bauer 2023). In the context of this study, it is strongly suggested that shrimps are incapable of producing *de novo* carotenoids, or other less common pigments such as spinochromes or anthraquinones (Cazzonelli 2011; Caro 2018). Therefore, they obtain such essential pigments from their diets (Gherardi 1991; Horká 2017; Brasseur et al. 2018; Virgili et al. 2020; Bauer 2023). *T. holthuisi*, which solely feeds on its host, likely acquires all its pigments from this source (Brasseur et al. 2018, **Chapter IV**). *A. indicus* may obtain a portion of its pigments from its host but primarily may also rely on other dietary sources for additional pigments (Gherardi 1991; Brasseur et al. 2018, **Chapter IV**). In contrast, *S. stimpsonii*, which feeds on algae rather than host tissue (VandenSpiegel et al. 1998; Terrana et al. submitted), exhibits a rich array of carotenoids, including zeaxanthin, astaxanthin, and beta-carotene (**Chapter IV**). Its crinoid host has no carotenoid pigments, which reinforces the hypothesis that *S. stimpsonii* obtains its carotenoids from other feeding resources (**Chapter IV**). Considering *Z. soror* (an opportunistic feeder including kleptoparasitic habits, small consumption of host tissues and consumptions of external food sources), pigment acquisition is likely derived from its various feeding sources (Mussoi et al. 2023). It may obtain astaxanthin, trace of zeaxanthin and beta-carotene directly

from its host food (**Chapter IV**). Additionally, the shrimp may consume a small proportion of its host, which contains a high concentration of diverse pigments as astaxanthin, canthaxanthin or zeaxanthin (**Chapter IV**). This mix of dietary sources enables *Z. soror* to efficiently mimic the pigmentation of its host.

Therefore, all four symbiotic decapod shrimp species exhibit a remarkable ability to adapt their coloration to their hosts, demonstrating a type of environmental coloration adaptation (**Figure 7**).This phenomenon is particularly evident in *Z. soror*, which displays a large range of mimetic colorations composed of three distinct morphotypes: translucent, stripe-colored, and full-colored individuals (respectively **Figure 7A, B, C**), each appearing in various intensities and shade of colors (Antokhina and Britayev 2020; Fernández-Lereé et al. 2023; **Chapter IV**). A significant proportion of the population is composed of translucent juveniles (**Chapter IV**), allowing them to look barely visible on their host (**Figure 7Aa-c**). The colored morphotypes (striped and full-colored; **Figure 7Ba-c and 7Ca-c**) are arbored by adult shrimps, and characterized by vivid pigmentations that match the color of the host faithfully (Fernández-Lereé et al. 2023). The unique differences between these two morphotype is the presence of a vertical mid-dorsal white band in the striped morphotype (**Chapter IV**). In addition, this species undergoes remarkable coloration changes when transitioning between hosts of the same species, but with different color morphotypes (Mussoi et al. 2023). This work is one of the first to highlight that the transparent morphotypes are actually juvenile individuals acquiring their colors when getting bigger/older (Mussoi et al. 2023). This hypothesis has recently been confirmed by Fernández-Lereé et al. (2023) who also suggested that juveniles of *Z. soror* undergo rapid coloration changes during their development stages thanks to an enhancement of pigments density in dermal chromatophores. Therefore, the color intensity increased along ontogeny development from translucent (juveniles) to vivid coloration (adults) (Fernández-Lereé et al. 2023). This hypothesis is strengthened by the **Figure 7Ac** showing a transparent shrimp displaying an emerging white stripe.

Another noteworthy example is *S. stimpsonii* (**Figure 7Da-c**). This species harbors a vibrant coloration, featuring an orange hue adorned with dark orange to red horizontal and vertical stripes (**Figure 7Da-c**). The exact manifestation of these stripes can exhibit significant diversity among individual shrimp as variations in stripe width, intensity, and coloration are commonly observed (**Chapter IV**). When the coloration is particularly vivid and the stripes are widespread, they may merge, making it challenging to discern individual stripes (**Chapter IV**).

Typically, on the cephalothorax of *S. stimpsonii*, four vertical stripes can be observed (**Figure 7Da-c**). Moving to the abdomen, a single vertical stripe is centered on the dorsal side of the organism (**Figure 7Da-c**). In contrast, the horizontal stripes, when present, may not exhibit the same level of distinctness. Their presence can vary both in terms of the number and appearance, particularly among smaller individuals (**Chapter IV**). These horizontal stripes are exclusively found on the abdomen and are oriented perpendicularly to the vertical stripes (**Figure 7Da-c**). Interestingly, it appears that these horizontal stripes are positioned at the boundaries between the abdominal metameres (**Chapter IV**). We observed that this species, which responds dynamically to changes in environmental light conditions, displays a nychthemeral cycle and displays pronounced coloration responses to alterations in light availability (**Chapter IV**).

T. holthuisi and *A. indicus* stand out as extremely mimetic, making them difficult to discern with the naked eye when they are on their host (Gherardi 1991; Brasseur et al. 2018; **Chapter IV**)**.** All individuals of *A. indicus* display a consistent and highly mimetic purple coloration, characterized by a predominant purple hue, with a narrow, lighter mid-dorsal band extending from the rostrum to the fifth abdominal segment and stretching outward to the inner edge of the eyestalk (**Figure Ea-c**). Along the lateral sides of the body, a minute blue stripe runs the entire length, eventually converging at the rear where it fades into an orange band (**Figure 7Ea-c**). On the fourth abdominal segment, two prominent dark spots adorn the dorsal surface, encircled by blue semi-circles, symmetrically positioned on either side of the mid-dorsal band (**Figure 7Ea-c**). *T. holthuisi* exhibits a specific body shape that closely mimics the spines of its host, with an overall uniform purple-pink coloration, further enhancing their camouflage (Brasseur et al. 2018; **Chapter 4; Figure 7Fa-b**). They possess a subtle mid-dorsal band with a slightly darker hue, extending from the cephalothorax to the end of the abdomen. Additionally, two small lateral white stripes run along the entire length of their bodies, beginning at the cephalothorax and lasting at the end of the abdomen (**Figure 7Fa-b**).

The mechanisms underlying pigmentation variation in these shrimp species are multifaceted (**Chapter 4**). Histological sections of all shrimp species reveal erythrophores containing red-orange pigments, indicative of carotenoids (Fernández-Lereé et al. 2023; **Chapter 4**). Typically, these pigments are primarily located in a single layer of pigmented tissue found in the derma, but *A. indicus* also displays a second layer with a purple coloration, that may be attributed to spinochromes (**Chapter 4**) as highlighted by Brasseur et al. in 2018. In the case of *Z. soror* translucent morphotype, the absence of carotenoids is attributed to its lack of coloration

(**Chapter 4**). Conversely, discolored *S. stimpsonii* seems to keep their carotenoids but can dynamically adjust the size of their chromatophores, thereby influencing their coloration (**Chapter 4**). This adaptive mechanism in *S. stimpsonii* may allow them to undergo rapid coloration/discoloration events or to follow nychthemeral cycles.

Although poorly studied before this project, especially in marine environments, the rapid dynamics of pigmentation changes have been investigated in cephalopods (Mäthger et al. 2008). In order to adapt to their environment, these organisms are capable of rapidly changing color through muscular regulation (Mäthger et al. 2008; Lu et al. 2022a). Slower pathway, where there is passage in the endocrine system, were investigate in some reptiles (Duarte et al. 2017). Similar regulations mechanisms may appears in decapods, but were never prove up to now.

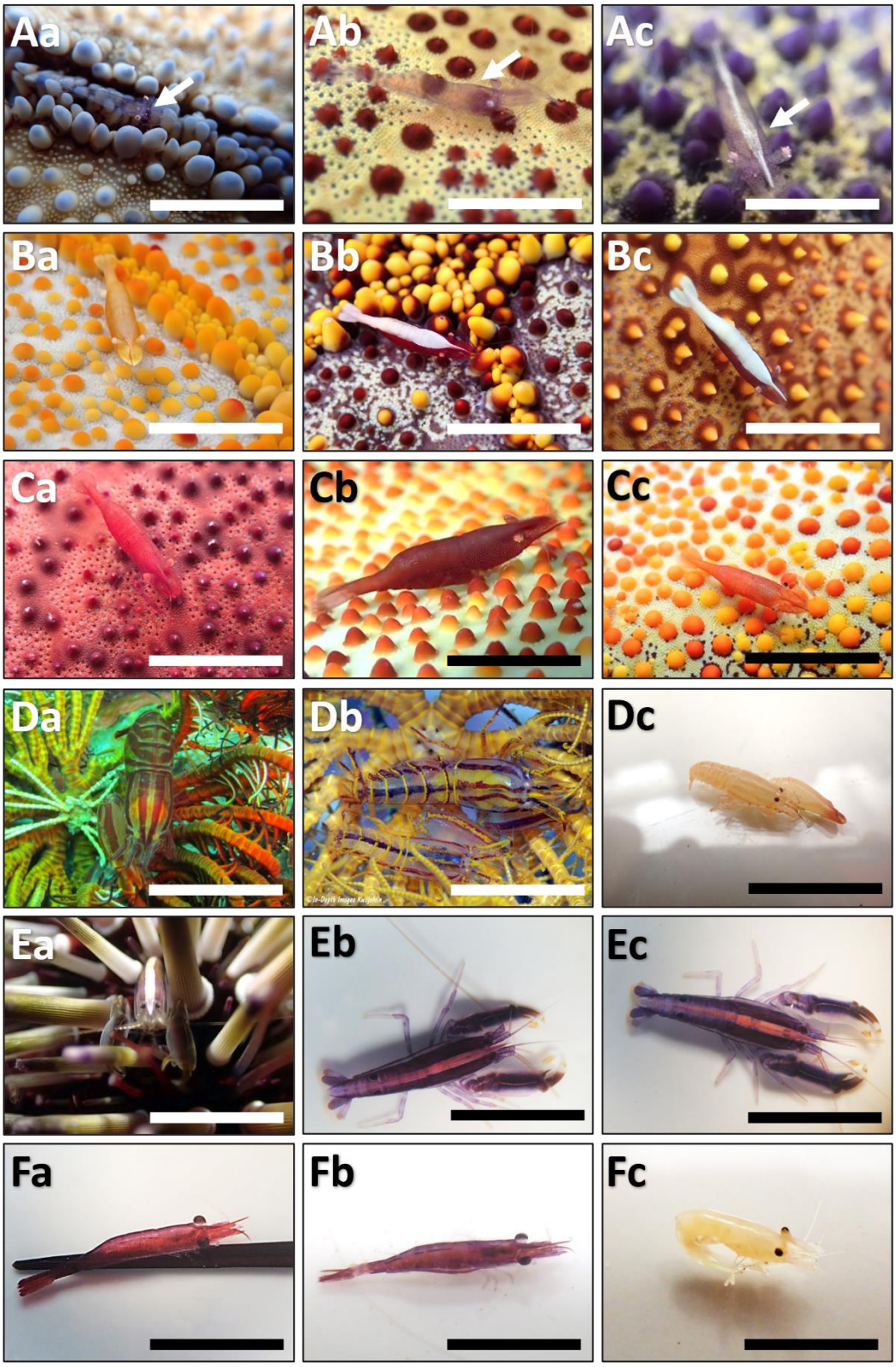


Figure 7: Mimetic coloration of the four investigated species. (A-C) *Zenopontonia soror* on its sea urchin host *Culcita novaeguineae* (Aa-c) translucent morphotype, (Ba-c) striped colored morphotype and (Ca-c) full colored morphotype. (D) *Synalpheus stimpsonii* (Da-b) on its crinoid host *P. distincta* (Db from @in-Depth Images Kwajalein 2018) and (Dc) an isolated discolored individual. (E) *Arete indicus* (Ea) on its sea urchin host *Echinometra mathaei* (from Patrick Eschweiler 2022) and (Eb-c) isolated from its host. (F) *Tulеariocaris holthuisi* (Fa) on a spine of its sea urchin host, *E. mathaei* (Fb) isolated but still displaying a normal pigmentation and (Fc) isolated and discolored. Scalebar = 1cm in Aa; 0.5 cm in Ab; 0.6 cm in Ac; 1.5 in Ba-b; 1.3 cm in Bc; 1.1 cm in Ca; 0.8 cm in Cb; 1.6 cm in Cc; 1.9 cm in Da; 1.5 in Db; 2.5 cm in Dc; 0.5 cm in Ea-c; 0.9 cm in Fa-c.

Perspectives:

Within tropical marine environments, numerous colorful benthic invertebrates host a community of symbiotic organisms (Levin 1998). In most cases, these symbionts mimic their hosts coloration and potentially exhibiting variations in hues over time (Caro 2018; Virgili et al. 2020; de Gier and Becker 2020). However, the composition of the pigments involved in this coloration and the dynamics of chromatic variation remain poorly investigated. Therefore, it woud be interesting to investigate the coloration of other symbionts [*i.e.* decapods (crabs and shrimps in particular), polychaetes, myzostomids or fishes], that display mimetic pigment profiles. As carotenoids are one of the most abundant pigments found in metazoans (Vershinin 1999), it seems interesting to primarily focus on them. However, other pigments may be as crucial as carotenoid in some association. For example, in echinoderms association involving a sea urchin host, Brasseur et al. (2018) demonstrate the presence of spinochromes within its symbiotic shrimps. Potentially, the same mechanism may happen in symbioses involving crinoids as hosts, as they are known to produce another specific pigments called anthraquinones (Bartolini et al. 1973; Wolkenstein et al. 2009; **Chapter I**). The further research would have to use a multidisciplinary approach combining:

- in vivo behavioral experiments such as photographic monitoring in different treatments (with or without additional pigments, with different color backgrounds, etc.)
- Microscopy observations to characterize the chromatophores and the pigments inside. For that, an interesting approach would be to combine optic microscopy and Transmission Electron Microscopy (TEM).
- Characterization of the pigments quality/quantity by High-Performance Liquid Chromatography (HPLC) coupled with (Tandem) Mass Spectrometry (MS and MS/MS) and Maldi-imaging to identify precisely the chemical nature and the location of the pigments.
- Histological sections of organism across their ontology (from eggs to adults).

VI. Host separation syndrome in natural environment

In symbiotic relationships, organisms derive specific advantages from their associations, enhancing their survival and/or reproduction. Symbionts may typically benefit through the protective shield offered by the host, facilitated feeding strategies, and the assimilation of host-secreted chemicals into their metabolisms, thereby improving their fitness (Parmentier and Michel 2013). As pressure of predation is high in marine environment, usually symbionts will benefit from the symbioses by being more or less protected (Dahl et al. 1998). The adaptation of symbionts to blend with their hosts, seen across various phyla, further supports the notion that symbiotic associations confer protection against predators (Virgili et al. 2020; Mussoi et al. *In Prep*; **Chapter IV**). When isolated in natural environments (and not on control aquaria), symbionts are likely to become easy targets for predators, impacting their fitness. Although not addressed in this PhD thesis, it seems logical that isolation may significantly decrease symbiont fitness in natural settings. Another common advantage of symbiotic associations is that they often provide symbionts with a reliable and potentially increased source of food. The enhanced food availability resulting from the symbiotic association contributes to the overall fitness of the symbiont. This assessment highlights one of the fundamental reasons why symbiosis is prevalent in the natural world, as it provides a mechanism for organisms to enhance their access to essential resources for survival and growth. However, as previously discussed, symbionts may present various degrees of food dependency toward their hosts.

This thesis is one to the first project that explores how metabolites, constituting the hosts chemical environment, enhance the survival rates of isolated marine symbionts. This underlines a potential recycling of host-produced chemicals within the symbiont metabolism, which had never been investigated on an ectosymbiont in previous literature, except for Brasseur et al. (2018). Comparatively, endosymbiotic mutualists obtaining metabolites from their hosts offer a relevant analogy. In marine environments, the iconic symbiosis between zooxanthellae and coral polyps exemplifies a chemical dependency, improving symbiont fitness (Michalek-Wagner 1999). Coral hosts supply essential inorganic nutrients for photosynthesis, such as nitrogen and phosphorus (Dimond and Carrington 2008; Allemand and Furla 2018). Additionally, zooxanthellae assimilate metabolic waste products from the coral, including carbon dioxide generated during cellular respiration. This relationship provides protection against predators and a controlled homeostatic medium that is close to the light emitted by the sun, a crucial advantage for a unicellular photosynthetic alga (Rowan 1998).

We therefore propose a final hypothesis that the degree of dependency a symbiont has on the various benefits provided by its host correlates with its susceptibility to rapid demise when isolated in natural environments. Potential causes include predation, loss of food sources, and deprivation of host-derived metabolites, that may form synergy of dependencies.

VII. Symbiotic associations and host separation syndrome in a changing environment

In addition to the proposed scientific perspectives at the end of each of the sections of this general discussion, we also need to consider that global biosphere is threatened by changes in biotic and abiotic parameters, having a significative detrimental impact on global biodiversity and biomass (*i.e.* sometimes considered as the 6th mass extinction, (Naggs 2017), including marine ecosystems (Worm et al. 2006; Solan et al. 2012; Jacob et al. 2018). With the exception of the impact of those changes on the endosymbiosis between scleractinian corals and zooxanthellae algae that are under an important monitoring in recent years (*e.g.* McNeil et al. 2004; Anthony et al. 2011; Cantin and Lough 2014), very few studies focused on the epibionts of those corals for example (Johnson et al. 2021). It is urgent to focus on the cryptic symbiotic biodiversity, usually underestimated because understudied. Results provided by this thesis already highlight the impact of host separation syndrome on the survival of the decapod epibionts, but also show how chemical ecology is crucial to understand the symbiotic biosphere. Future research may investigate how chemical communication will be impacted in the future.

Physico-chemical parameters, including acidification (Gobler and Baumann 2016), rising temperatures (Cheng et al. 2019) and chemical pollution (Lecchini et al. 2017) might indeed display an impact on the ecomones and therefore on larval recruitment, host switch, but also on the chemical environment of the host itself which may impact the efficiency of their chemical defenses for example. As a semiochemical is specifically recognized by a receptor in the chemosensitive system of the receiving organism, any change in the structure of the chemical stimulus will undoubtedly lead to a change in the conformation of the ligand, which will no longer be able to interact with its receptor (Brönmark and Hansson 2012). A few examples have been characterized in the literature. For example, Roggatz et al. (2016)'s study demonstrates the impact of ocean acidification on the conformational change of a peptide produced by *Carcinus maenas* females and acting as a pheromone to activate egg aeration behavior at pH 8.1 (Roggatz et al. 2016). Under more acidic pH conditions (7.7), protonation changes the conformation of the molecule, which no longer enables activation of egg aeration behavior by

the female (Roggatz et al. 2016). Anthraquinones, on the other hand, can be formed in a polluted environment by the photo-oxidation of plastic derivatives such as polycyclic aromatic hydrocarbons (Mallakin et al. 1999). In 2019, a review summarizes the impact of climate warming on sexual chemical signaling in terrestrial environment (Groot and Zizzari 2019).

Generally speaking, host separation may occur naturally in various environmental scenarios. Currently, numerous organisms, including potential hosts, are threatened by the actual 6th mass extinction. They therefore may be prompted to sudden disappearance and their symbiont may be impacted by the host separation syndrome, being left unable to adapt or even find another host. In consequence, this host separation syndrome may lead a chain of biodiversity loss in the obligate associated symbionts. This project may therefore shed light on one of the most important challenges for biodiversity.

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Glossary



Glossary

- **Allomones:** Chemical substances emitted by an organism that influence the behavior or physiology of another organism to the benefit of the emitter.
- **Chemical cue:** A chemical substance that conveys information and elicits a response in another organism.
- **Chemical dependency:** The reliance on specific chemical signals for essential biological functions or behaviors.
- **Chemoattraction:** The movement of an organism or cell toward a higher concentration of a particular chemical substance.
- **Chemoreceptor:** A sensory receptor that responds to chemical stimuli, often involved in detecting changes in the chemical environment.
- **Chemosensor:** A sensory organ or cell that detects and responds to chemical stimuli.
- **Chemotaxis:** The directed movement of an organism or cell in response to chemical gradients in the environment.
- **Co-adaptations:** Reciprocal evolutionary changes in interacting species that enhance the fitness of both.
- **Co-evolution:** The process in which two or more species reciprocally influence each other's evolutionary trajectories.
- **Commensalism:** A type of symbiotic relationship where one organism finds benefits (the symbiont), and the other is neither harmed nor benefited (the host).
- **Co-phylogeneses:** The co-evolutionary process where the evolutionary history of one species is intertwined with that of another.
- **Co-speciation:** The simultaneous speciation of two or more interacting species due to mutual influence on each other's evolution.
- **Host dependency:** The reliance of a symbiotic organism on a host for essential resources.
    - **Species-specific:** Pertaining to characteristics or interactions that are specific to a particular taxonomic species.
    - **Genus-specific:** Pertaining to characteristics or interactions that are specific to a particular taxonomic genus.
    - **Family-specific:** Pertaining to characteristics or interactions that are specific to a particular taxonomic family.

- **Class-specific:** Pertaining to characteristics or interactions that are specific to a particular taxonomic class.
- **Phylum-specific:** Pertaining to characteristics or interactions that are specific to a particular taxonomic phylum.
- **Intra-phyla generalist:** A generalist organism that can interact with a variety of host species within the same phylum.

- **Ecomones:** Chemical substances emitted by an organism that affect the behavior or physiology of other organisms in the same ecological community.
- **Ectosymbiot:** An organism that lives on the external surface of another organism in a symbiotic relationship.
- **Endosymbiont:** An organism that lives inside the tissue of another organism in a symbiotic relationship.
- **Emitter Organism:** The organism that releases or produces chemical substances, signals, or cues into the environment. These emitted substances, such as pheromones or allelochemicals, serve various functions, including communication, defense, or attraction.
- **Facultative symbiont:** A symbiotic organism that can live independently of its host under certain conditions.
- **Fitness:** The ability of an organism to survive and reproduce in its environment, contributing to the persistence of its genetic traits.
- **Generalist:** An organism that can thrive in a variety of environmental conditions or exploit a wide range of resources.
- **Habituation effect:** A decrease in response to a stimulus after repeated or prolonged exposure.
- **Host:** An organism that harbors/host another organism.
- **Host imprinting:** The process by which an organism learns and recognizes specific criteria (such as host, food, odors,…).
- **Host selection:** The choice made by a symbiotic organism in selecting its host.
- **Host separation syndrome:** Behavioral or physiological changes in a dependent symbiont upon separation from its host, that can lead ultimately to the organism death .
- **Host specificity:** The degree to which a symbiotic organism is restricted to a particular host species.
- **Kairomones:** Chemical substances emitted by one species that benefit another species.

- **Kleptoparasite:** An organism that steals food or resources from another organism.
- **Metabolites:** Small molecules produced by metabolic processes in an organism.
- **Mimetic pigmentation:** The resemblance of an organism's coloration to its surroundings or host for protective or predatory purposes.
- **Multimodal communication:** Communication that involves multiple sensory modalities, such as visual, auditory, and chemical signals.
- **Mutualism:** A symbiotic relationship where both participating species benefit.
- **Obligatory symbiont:** A symbiotic organism that cannot survive independently of its host.
- **Olfactometers:** Devices used to measure or control the delivery of odor stimuli.
- **Olfactometry:** The study or measurement of odor.
- **Opportunistic symbiont:** A symbiotic organism that can live independently but takes advantage of opportunities to form symbiotic relationships.
- **Parasite:** An organism that lives on or in another organism (the host) and benefits at the host's expense.
- **Parasitism:** A symbiotic relationship where one organism (the symbiont) benefits at the expense of another (the host).
- **Pheromone:** A chemical substance produced by an organism to elicit a specific behavioral response in another organism of the same species.
- **Primary metabolite:** Metabolites essential for the basic physiological functions of an organism.
- **Receiver Organism:** The organism that detects and responds to the chemical signals, cues, or stimuli produced by another organism. This response may involve behavioral changes, physiological reactions, or other adaptations influenced by the chemical information received from the emitter organism.
- **Secondary metabolite:** Metabolites not directly involved in the basic life processes but often play a role in ecological interactions.
- **Semiochemicals:** Chemical substances that convey information between organisms, including pheromones and allelochemicals.
- **Specific symbiont:** A symbiotic organism with a narrow host range.
- **Symbionts:** Organisms that live with, is hosted by another organism, in a symbiotic relationship.

- **Symbiosis:** A close and long-term biological interaction between at least two different species.
- **Symbiotic:** Pertaining to a symbiotic relationship, where two organisms live in close association.
- **Synomones:** Chemical substances produced by one organism that benefit another organism.



Appendix 1



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Placoid scales in bioluminescent sharks: Scaling their evolution using morphology and elemental composition

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Elasmobranchs are characterised by the presence of placoid scales on their skin. These scales, structurally homologous to gnathostome teeth, are thought to have various ecological functions related to drag reduction, predator defense or abrasion reduction. Some scales, particularly those present in the ventral area, are also thought to be functionally involved in the transmission of bioluminescent light in deep-sea environments. In the deep parts of the oceans, elasmobranchs are mainly represented by squaliform sharks. This study compares ventral placoid scale morphology and elemental composition of more than thirty deep-sea squaliform species. Scanning Electron Microscopy and Energy Dispersive X-ray spectrometry, associated with morphometric and elemental composition measurements were used to characterise differences among species. A maximum likelihood molecular phylogeny was computed for 43 shark species incuding all known families of Squaliformes. Character mapping was based on this phylogeny to estimate ancestral character states among the squaliform lineages. Our results highlight a conserved and stereotypical elemental composition of the external layer among the examined species. Phosphorus-calcium proportion ratios (Ca/P) slightly vary from 1.8-1.9, and fluorine is typically found in the placoid scale. By contrast, there is striking variation in shape in ventral placoid scales among the investigated families. Character-mapping reconstructions indicated that the shield-shaped placoid scale morphotype is likely to be ancestral among squaliform taxa. The skin surface occupied by scales appears to be reduced in luminous clades which reflects a relationship between scale coverage and the ability to emit light. In luminous species, the placoid scale morphotypes are restricted to pavement, bristle- and spine-shaped except for the only luminescent somniosid, *Zameus squamulosus*, and the dalatiid *Mollisquama mississippiensis*. These results, deriving from an unprecedented sampling, show extensive morphological diversity in placoid scale shape but little variation in elemental composition among Squaliformes.

KEYWORDS

dermal denticles, deep-sea sharks, morpho-mapping, scanning electron microscopy, Energy-dispersive X-ray spectrometer (EDX), Dalatiidae, Etmopteridae, Somniosidae

Introduction

Although deep-sea research is steadily increasing, the mesopelagic and bathyal zones, which extend from 200 to 4,000 meters in depth, remain poorly known and studied (Howell et al., 2021). Abiotic conditions in these environments are extreme, with (*i*) pressures reaching several hundred atmospheres, (*ii*) a constant low temperature, (*iii*) a low concentration of dissolved oxygen, and (*iv*) an absence of sunlight at depths below 1,000 meters (MacDonald, 1975). Among the diversity of fishes in the deep-sea, various chondrichthyans [i.e., rays (batomorphs), sharks (selachians), and chimaeras] are found (Herring and Morin, 1978; Gage and Tyler, 1992; Herring, 2001). Selachians comprise two major super-orders: (*i*) the galeomorphs (Galeomorphii) and (*ii*) the squalomorphs (Squalomorphii) (Naylor et al., 2012; Straube et al., 2015). Within the squalomorphs, the order Squaliformes contains 31 genera and 187 species (Pollerspöck and Straube, 2021). The Squaliform order includes numerous deep-sea sharks and is composed of six families (*i*) Squalidae, (*ii*) Centrophoridae, (*iii*) Dalatiidae, (*iv*) Somniosidae, (*v*) Oxynotidae and (*vi*) Etmopteridae (Figure 1A). Molecular phylogenetic studies show Oxynotidae nested within Somniosidae (Straube et al., 2010; Naylor et al., 2012; Straube et al., 2015).

Three Squaliforme families contain bioluminescent species, i.e., species able to produce light: the Etmopteridae (Straube et al., 2015; Duchatelet et al., 2020b; Duchatelet et al., 2021a), the Dalatiidae (Claes et al., 2012; Straube et al., 2015; Delroisse et al., 2021; Mallefet et al., 2021; Duchatelet et al., 2021a) and the Somniosidae (Straube et al., 2015; Mallefet et al., 2021). In bioluminescent sharks, the photophores are present within the integument and are located in between the placoid scales (Ohshima, 1911; Hubbs et al., 1967; Claes and Mallefet, 2009; Renwart et al., 2014; Renwart et al., 2015; Mallefet et al., 2021; Duchatelet et al., 2021a). Interestingly, bioluminescent sharks probably have the highest number of photophores within bioluminescent animals (Hubbs et al., 1967; Herring and Morin, 1978). Reif (1985a) suggested an evolutionary trade-off in space occupancy between photophores and placoid scales (i.e., the dermal denticle covering the skin defined as one of the main synapomorphies characterising chondrichthyans) (Applegate, 1967; Deynat, 2014; Lund and Grogan, 2015; Rodríguez-Cabello et al., 2016). In mesopelagic luminous sharks, placoid scale coverage was reduced allowing the coexistence of epidermal photophores and placoid scales in luminescent

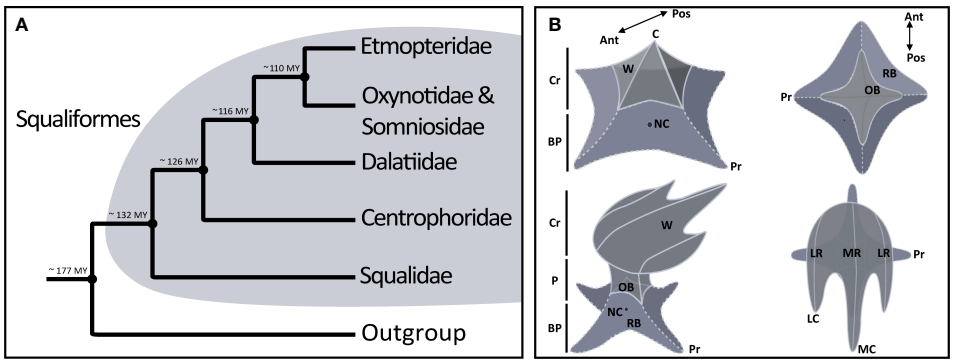


FIGURE 1
Generalised structure of placoid scales with annotated structures in sharks including Squaliformes. **(A)** Phylogeny of Squaliformes based on Straube et al., 2015, with the estimated divergence times of the families. The divergence shown are corresponding to mean ages. **(B)** Schematic representations of a dermal denticle illustrating the morphological terminology: Cr, crown; P, peduncle; R, ridge, LR, lateral ridge; MR, median ridge); OB, observed base; RB, real base; NC, neck-canal; BP, basal plate; C, cusp (LC, lateral cusp and MC, median cusp); Pr, process; W, wing. There is a 2D top view and a 2.5 D lateral view with the anteroposterior orientation (Ant.= Anterior and Pos.= Posterior).

sharks (Reif, 1985b; Ferrón and Botella, 2017). Claes and Mallefet (2009) highlighted different types of placoid scales in luminescent sharks (e.g., pavement-shaped and bristle-shaped). By comparison, non-bioluminescent sharks usually have shield- or leaf-shaped denticles (Compagno, 2001). Recently, two new types of placoid scales were described for the luminescent ventral area of *Zameus squamulosus* and the rostral area of *Dalatias licha* (Duchatelet et al., 2021a; Duchatelet et al., 2021b; Mallefet et al., 2021). Surprisingly (i.e., it appears counterintuitive to the trade-off hypothesis of Reif, 1985a), both species have overlapping shield-shaped placoid scales with light organs embedded in the skin underneath (Straube et al., 2015; Duchatelet et al., 2021a; Duchatelet et al., 2021b; Mallefet et al., 2021). The emitted light is able to pass through the placoid scale (Straube et al., 2015) and the scale might act as a bioluminescence enhancer (Duchatelet et al., 2021a; Duchatelet et al., 2021b). This last hypothetical function could be added to the traditionally described ecological functions of placoid scales which are (i) enhancement of hydrodynamic properties, (ii) defense against parasites, (iii) anti-fouling, (iv) protection against predators, and (v) protection against substrate abrasion (Reif, 1985a; Han et al., 2008; Han and Wang, 2011; Oeffner and Lauder, 2012; Wen et al., 2014; Ferrón et al., 2018; Munther et al., 2018; Feld et al., 2019; Lloyd et al., 2021).

Placoid scales are homologous to gnathostome teeth (Kawasaki et al., 1980; Reif, 1985b; Reif, 1985a; Enax et al., 2012; Enault et al., 2015) and their general morphology and morphogenesis are very similar, as well as their chemical composition (Kawasaki et al., 1980; Reif, 1985a). Chemically, placoid scales are mainly composed of hydroxyapatite crystals interspersed with a dense network of collagen and other proteins such as phospho- and glycoproteins forming a compact structure (Kawasaki et al., 1980; Kawaguchi, 1985; Samuel et al., 1987; Kemp, 1989). Structurally, placoid scales comprise two distinct layers: the dentin and the enameloid (Reif, 1985a; Rodríguez-Cabello et al., 2020). The chemical formula for the enameloid mineral part is Ca5 [PO4]3 [OH] (Frank, 1961; Lussi et al., 2015). The Fluorine element can bind to this molecule to form crystals of calcium fluorohydroxyapatite (Ca5 [PO4]3 F) making the structure less prone to demineralisation (Lussi et al., 2015). However, scarce information is available on the elemental composition of placoid scales and no studies have been carried out on deep-sea sharks. Placoid scales exhibit several typical morphological features: a scale base that is not visible externally as it is covered by the tegument, a visible base corresponding to the junction between the placoid scale and the tegument, a peduncle, a crown, wings, ridges, and cusps (Figure 1B; Ebert, 2015; Rodríguez-Cabello et al., 2020). The morphology and arrangement of placoid scales can be species-specific and is often used in species identification (Reif, 1985b; Raschi and Tabit, 1992; Deynat, 2000; Gravendeel et al., 2002; Claes and Mallefet, 2009; Dillon et al., 2017; Ferrón and Botella, 2017; Ankhelyi et al., 2018; Duchatelet et al., 2020a). Intraspecific variation of placoid scale morphology is observed at different body regions (Duchatelet et al., 2020a; Feichtinger et al., 2021; Gabler-Smith et al., 2021; Naylor et al., 2021) but, within a dedicated body region, the intraspecific variation is low (authors' pers. obs.). Differences - in both morphology and squamation pattern - are often present between two different skin areas (e.g., ventral, dorsal) of an individual (Reif, 1985b; Raschi and Tabit, 1992; Deynat, 2000; Gravendeel et al., 2002; Claes and Mallefet, 2009; Dillon et al., 2017; Ferrón and Botella, 2017; Ankhelyi et al., 2018; Feld et al., 2019; Duchatelet et al., 2020a; Lloyd et al., 2021).

The present work examines the general morphology and distribution pattern as well as the elemental composition of placoid scales from different deep-sea inhabiting Squaliformes. Our study focuses on the ventral skin area that is serving the main function in bioluminescent shark species for countershading (i.e., silhouette cloaking) (Claes et al., 2010; Claes et al., 2014; Duchatelet et al., 2021a).

Materials and methods

Sample collection

A total of 33 different shark species were sampled *via* field collecting or in museum collections (Supplementary Table S1). Due to the rarity of some species, only one adult individual was sampled for each species. *Etmopterus spinax* specimens were harvested by longlines in January 2017 during a campaign in the Raunefjord (60°15'54'N; 05°07'46'E; Norway) at 250 m depth. *Etmopterus molleri*, *Heptranchias perlo*, *Squalus mitsukurii*, and *Centrophorus moluccensis* specimens were sampled with an electric fishing rod in November 2017 in the East China Sea near the coast of Sesoko Island in Okinawa (26°28'94'N; 127° 41'20'E; Japan) 480 to 510 m depth. *Pristiophorus japonicus*, *Squalus japonicus*, *Squalus brevirostris*, *Etmopterus pusillus*, *Etmopterus brachyurus*, *Etmopterus bigelowi*, *Etmopterus polli*, and *Centroscymnus owstonii* specimens were obtained from the collection of the Churaumi Aquarium of Okinawa. *Etmopterus lucifer*, *Squalus acanthias*, *Etmopterus granulosus*, *Centrophorus squamosus*, *Deania calcea*, *Dalatias licha*, *Scymnodon macracanthus*, and *Oxynotus bruniensis* were collected during the "Chatham survey 2018" campaign (Hoki Survey, Chatham Rise Survey - February 2018 - R/V Tangaroa) off New Zealand. *Zameus squamulosus* specimens were obtained from a collection at the Te Papa Museum in New Zealand. *Squaliolus aliae*, *Trigonognathus kabeyai*, and *Etmopterus splendidus* specimens were collected from industrial fishing vessels operating from the port of Donggang, Taiwan. The first two species were fished with open water nets at depths between 50 and 400 m. *E. splendidus* was obtained by bottom trawling at depths of 300 – 600 m. The holotype (TU 203676; 142.0 mm in total length and 14.6 g in total weight) of *M. mississippiensis* (which represents the only

known specimen of this species) was collected in the central Gulf of Mexico on 4 February 2010 by the NOAA Ship PISCES (survey 7620101001, station 053). The remaining species (*Etmopterus robinsi*, *E. sheikoi*, *Aculeola nigra*, *Isistius brasiliensis*, *Squaliolus laticaudus*, *Centroscyllium nigrum* and *Centroselachus crepidater*) were provided by the Bavarian State Collection of Zoology (Munich, Germany), the Museum für Naturkunde Berlin (Germany) and the National History Museum Paris (France). Living individuals were euthanised according to the ethical regulations of the host country. A ventral skin patch of about 1 cm^2 was dissected from the ventral area located between the two pectoral fins and stored in 90% ethanol until use.

Scanning electron microscopy (SEM) and microanalyses

For scanning electron microscope observations, ventral skin patches were fixed in Bouin's fluid (saturated aqueous picric acid solution - formalin - glacial acetic acid, 15:5:1, Rai et al., 2016) at room temperature for 24 h, then dehydrated in sequential concentrations of ethanol and dried by the critical-point method using CO_2 as the transition fluid (Renwart et al., 2015; Delroisse et al., 2017, for technical details). Samples were mounted on aluminium stubs, coated with a mix of gold/palladium (40/60) in a sputter coater, and observed with a JEOL JSM 6100 scanning electron microscope. To perform the observation, the sample was placed inside the SEM observation chamber and the working distance (WD) was set up to 10 mm. The voltage was set to 10 kV with average beam width to photographic optimisation. Under these conditions and with a fixed 100 X magnification, three micrographs were made for each skin sample (i.e., technical replicate illustrating three different regions of the sample). Subsequently, three scales were selected inside a random area of the ventral skin sample according to three selection criteria: (*i*) entire and in good condition (*ii*) visible as a whole and (*iii*) oriented to avoid shading effects due to electrons.

Each picture was used for obtaining four datapoints: (*i*) the maximal distance between the base tips, (*ii*) the maximal distance between the epidermis and the higher points on the scale (*iii*) the number of scales under a 100 X magnification and (*iv*) the percentage of epidermis covered by placoid scale under a 100 X magnification (i.e., scale coverage). Measurements were performed using the Fiji software (Schindelin et al., 2012).

A determination key was created, based on these characteristics, to classify the different placoid scale types (Supplementary Figure S1). To clarify the terminology used in the study, we created a generalised scheme representing the dermal denticle using the program Adobe Illustrator (Figure 1B). Three main morphotypes are recognised. First, pavement-shaped scales (M1) are the only ones to have a scale's height lower than the scale base's length. The other two morphotypes are differentiated by the presence or absence of a peduncle. The scales without peduncles are classified as bristle/spine-shaped (M2), which are sub-divided into either ("Bristle-shaped", M2a) or ("Spine-shaped", M2b) based on their relative heights. The pedunculated scales are grouped as shield-shaped scales (M3). Depending on the characteristics of the ridges, several sub-categories are defined for type M3. Some shield-shaped scales do not have ridges or show only small ridges, visible on the peduncle (M3a). The others have ridges visible in SEM, which can be divergent (M3b) or running parallel (M3c) (Supplementary Figure S1). SEM data was obtained for all 33 shark species listed in the "sample collection" section. In addition, literature data was used to characterise *Somniosus microcephalus* (Somniosidae) (i.e., 34 investigated species in total) (Castro, 2010).

The elemental composition was analysed using Oxford X-MaxN Energy-dispersive X-ray spectrometer (EDS-EDX) equipped with an 80 mm2 silicon drift detector (and coupled with the SEM). Acquisition conditions on the SEM were 15 kV, 10 mm working distance and 10 s live time acquisition at approximately 30–40% dead time. The spectra were acquired with an AZtec (Oxford Instrument) EDS data processing software. Two kinds of elemental analyses were performed. First, maps of the general composition are produced for each main element (C, O, F, Na, Mg, Al, P, S, K, Ca). All maps were processed using the AZtec true map function. Second, the elemental composition of three points on the scale and one off-scale point are analysed to determine the elements typically found inside and outside the scale. For each species, skin samples from single individuals were investigated and several technical replicates were performed (i.e., three different points on a scale, for three different scales). Because of the importance of the ratio Ca/P in dental analyses, which are similar in elemental composition to dermal denticles, we included them in this study. An Etmopteridae tooth (*E. molleri*) was used as an external reference for the elemental composition analyses. Descriptive statistics were performed, and the element proportions are expressed as the median/mean values in weight per cent (Wt%) associated with a standard deviation (SD) (median/mean ± SD). To illustrate the variation of the element composition (F, P, Ca, C, O, Na, Mg, Al, S, K, M, N) across the investigated species/families, we performed a principal component analysis (PCA) based on a variance-covariance matrix using PAST 4.02 (Hammer et al., 2001). Descriptive statistics were conducted in the R software package (R Core Team, 2021) using the "ggplot2" Package (Wickham, 2011) and using Prism 9 (GraphPad Software).

Analyses of character evolution

Initially, a species phylogeny including suitable outgroups based on available DNA sequences of the mitochondrial NADH-ubiquinone oxidoreductase chain 2 (NADH2) was estimated. Used samples are listed in Supplementary Table S2 and were downloaded from Genbank (https://www.ncbi.nlm.nih.gov/genbank) and additional sequences were provided by the Chondrichthyan Tree of Life Project (https:///sharksrays.org). Sequences were subsequently aligned in Geneious® 7.1.9 using the MUSCLE sequence aligner (Katoh et al., 2002; Katoh et al., 2005). A maximum likelihood phylogeny was computed using RAxML vers. 8.2.12 (Stamatakis, 2014). We used the general time reversible substitution model with a GAMMA distribution of rate heterogeneity. The phylogeny based on nuclear genes in Straube et al. (2015) was used as backbone tree under RAxML's -g option where the maximum likelihood tree is constrained to the provided backbone topology. Bootstrapping was carried out with 500 replicates and mapped onto the maximum likelihood tree (Supplementary Figure S2).

In a next step, the maximum likelihood tree was used for the ancestral character state reconstruction for placoid scale morphotypes. Beforehand, the phylogenetic tree (including 43 species) was pruned to match our species sampling for the ancestral character state reconstructions (32 species, Supplementary Table S2). Ancestral character states were performed in Mesquite v.3.40 (Maddison and Maddison, 2011) using a parsimony model with characters treated as unordered. We based ancestral state reconstructions on the topology of our newly generated phylogeny. Morpho-mapping analyses were performed to estimate placoid scale evolution across the 32 shark species. Three variables were analysed (*i*) the denticle morphotype, (*ii*) the coverage percentage of the epidermis with placoid scales (denticle coverage), and (*iii*) the ability to emit light, i.e., the presence of photophores (see morphological data and corresponding character states matrix in Supplementary Tables S3, S4). The morphotype character comprises the aforementioned three main character states: (*i*) pavement-shaped scales (M1), (*ii*) bristle/spine-shaped scales (M2), which is represented in two subcategories (M2a: bristle-shaped; M2b: spine-shaped) and *(iii*) shield shaped (M3), subdivided in three categories (M3a: without ridges; M3b: with diverging ridges; M3c: with parallel ridges). The placoid scale coverage, corresponding to the percentage of epidermis overlapped by the placoid scales, was categorised into four-character states: individuals (*i*) with less than 10% denticle coverage; (*ii*) with a denticle coverage between 10% and 30%; (*iii*) with a denticle coverage of 31% to 90% and (*iv*) with scales covering almost all their epidermis (>90%). In a comparative approach, our sampling includes 34 species (i.e., 33 were sampled), including 20 luminescent species. Two sampled species (*E. robinsi, S. mitsukurii*) were not included in the ancestral character state reconstructions because the NADH Dehydrogenase Subunit 2 sequence data was lacking, and we were not able to clarify their precise phylogenetic position.

Results

Phylogeny of Squaliformes

The NADH2 sequence alignment comprises 1044 nucleotide sites. The alignment has 600 distinct patterns and a proportion of gaps and undetermined characters of 0.21%. The tree topology resulting from the maximum likelihood analysis recovers clades as in Straube et al. (2015) due to the backbone tree used for guiding the phylogenetic inference. In summary, Squaliformes are monophyletic, Squalidae are sister to all remaining families where *Squalus* and *Cirrhigaleus* form sister clades. Centrophoridae comprise sister clades *Deania* and *Centrophorus*. The family splits sequentially after Squalidae and is sister to a clade comprising Dalatiidae, Somniosidae (including Oxynotidae) and Etmopteridae. Dalatiidae split in two clades, one comprising *Isistius*, *Dalatias*, and *Mollisquama*, the other *Squaliolus* and *Euprotomicrus*. Somniosidae are sister to Etmopteridae and comprises two major clades representing the *Somniosus* lineage as well as all other somniosid genera also including Oxynotidae. Etmopteridae are split in four clades corresponding to the previously described intrageneric *Etmopterus* clades, i.e., the *E. lucifer* clade, the *E. spinax* clade, the *E. gracilispinis*, and the *E. pusillus* clades (Supplementary Figure S2).

External morphology of ventral placoid scales in 34 Squaliformes species

Our analyses, focusing on the ventral placoid scales only, highlight morphological variability among the 34 Squaliformes species (Figure 2, Supplementary Figures S1, S3).

Different morphotypes are found in the investigated Etmopteridae species. *E. bigelowi*, *E. pusillus*, *E. sheikoi* and *T. kabeyai* have pavement-shaped scales (M1) with anaverage denticle height and length of 25 µm and 225 µm, respectively. Other Etmopteridae species have bristle/spine-shaped scales (M2) with an average denticle height and length of 275 µm and 100 µm, respectively, subdivided into two subcategories (M2a and M2b). *E. robinsi*, *E. spinax* and *E. brachyurus* have longer scales, assigned to the bristle-shaped morphotype (M2a). *A. nigra*, *C. nigrum*, *E. granulosus*, *E. molleri*, *E. polli* and *E. splendidus* represent the spine-shaped scales (M2b). All Etmopteridae have a low denticle coverage (*i.e.*, percentage of epidermis overlapped by the placoid scales), which cover less than 30% of the epidermis.

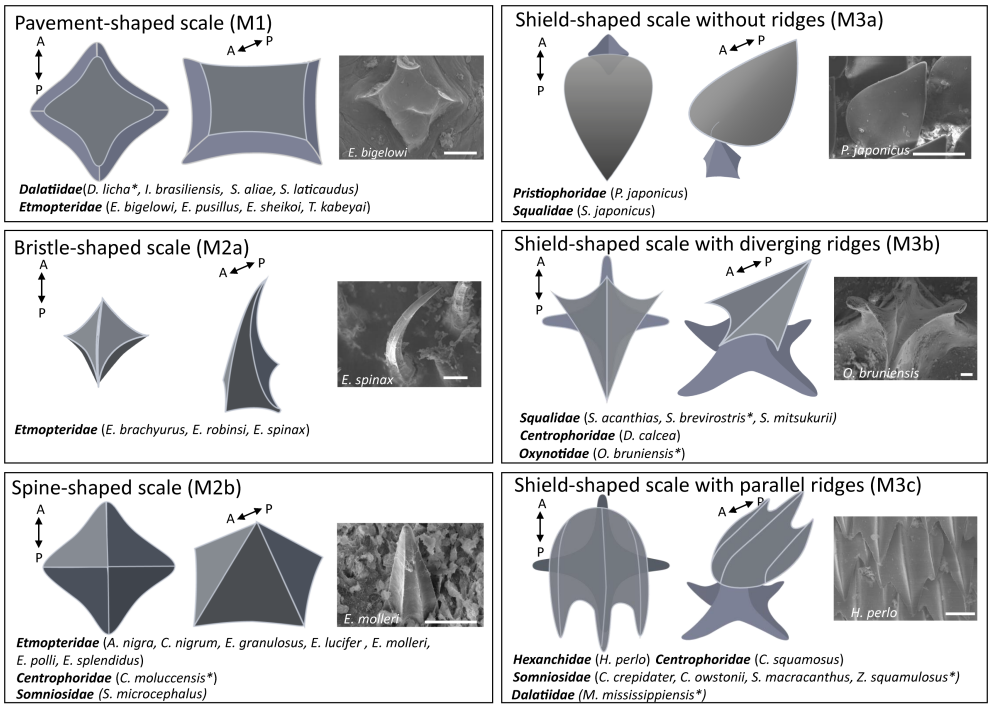


FIGURE 2
Assignation of the ventral placoid scale morphotypes observed in the 34 investigated species. For each morphotype, two schematic representations of the denticle are shown, the left picture represents a 2D top view and the middle picture represents a 2.5 D lateral view with the anteroposterior information (A= Anterior and P= Posterior). The right picture is a typical denticle observed under the scanning electron microscope (SEM). Scale bars: 100 µm. The * corresponds to the species exhibiting atypical scales within the different morphotypes.

Four species of Dalatiidae (*D. licha*, *I. brasiliensis*, *S. aliae* and *S. laticaudus)* are characterised by pavement-shaped scales (M1) and low denticle coverage (i.e., less than 40% coverage). *I. brasiliensis*, *S. aliae* and *S. laticaudus* have morphologically similar denticles with an average denticle height and length of 25 µm and 250 µm, respectively. However, *D. licha* presents scales formed by large pavement-shaped denticles with a central ridge and bilateral symmetry with an average of 55 µm and 850 µm of denticle height and length (Supplementary Figure S3).

In Centrophoridae, *D. calcea, C. squamosus* have shield-shaped scales (M3). *C. moluccensis*, shows scales as assigned to the spine-shaped morphotype (M2b), which is characterised by very similar height and length (300 µm on average). *P. japonicus* and *S. japonicus* have shield-shaped scale, without ridges (M3a).

The Squalidae members *S. mitsukurii*, *S. brevirostris* and *S. acanthias*, the centrophorids *D. calcea* and oxynotid *O. bruniensis* have shield-shaped scales with diverging ridges (M3b). *Oxynotus bruniensis* has a structurally typical M3b morphotype but they present extreme dimensions (height: about 2 cm; base length: about 1 cm). The squalid *S. brevirostris* has atypical M3b scales with rounded extremities and a very small peduncle (Supplementary Figure S3). The last morphotype group the species with shield-shaped scales with parallel ridges (M3c) with the dalatiid *M. mississippiensis*, the somniosids *Z. squamulosus*, *C. crepidater*, *C. owstonii* and *S. macracanthus*, as well as the centrophorid *C. squamosus* and the hexanchid *H. perlo* (Supplementary Figure S3). *M. mississippiensis* and *Z. squamulosus* were classified in the M3c category because their ridges present a parallelism, but they also appear more complex than the other shield-shaped scales with parallel ridges observed. Secondary ridges are present and form a honeycomb structure (Supplementary Figures S3, S5). The grouping of the two species in this category indicates a morphological similarity but does not mean that the two scale structures are easily comparable or structurally homologous.

Morphological evolution of ventral placoid scale in Squaliformes

Morpho-mapping analyses were performed to estimate placoid scale evolution across the investigated shark species. Two characteristics were analysed (*i*) the denticle morphotype (Figure 3) and (*ii*) the percentage coverage of epidermis by placoid scales (denticle coverage). This last character has been investigated in relation to the bioluminescence abilities of the sharks (Figure 4).

Etmopteridae have different ventral scale morphotypes (i.e., pavement-shaped, spine/bristle-shaped types). The ancestral character state analysis indicates a spine-shaped morphotype (M2b) in the common ancestor of Etmopteridae (Figure 3) as most likely ancestral character state. Pavement-shaped (M1) and bristle-shaped (M2a) denticles would instead correspond to derived characters within the family. The majority of Dalatiidae (*D. licha*, *I. brasiliensis*, *S. aliae* and *S. laticaudus*), with an exception in *M. mississippiensis*, have pavement-shaped denticles (M1) as some Etmopteridae (*E. bigelowi*, *E. pusillus*, *E. sheikoi*, and *T. kabeyai*). The ancestral character state analysis is unconclusive for Dalatiidae. It indicates a pavement-shaped morphotype (M1) or a shield-shaped morphotype with parallel ridges (M3c) as most likely ancestral character state in Dalatiidae.

Most investigated species of Somniosidae have shield-shaped scales with parallel ridges (M3c) except for *Somniosus microcephalus* which has spine-shaped scales (M2b). The prickly dogfish, *O. bruniensis* shows a large shield-shaped morphotype with diverging ridges (M3b). Similar to Dalatiidae, the ancestral character state analysis is unconclusive for the clade Oxynotidae and Somniosidae and indicates a spine-shaped morphotype (M2b) or a shield-shaped morphotype with parallel ridges (M3c) as most likely ancestral character state.

All other families (Squalidae, Centrophoridae, and the outgroup species) show more variability in the ridge's disposition on the shield-shaped denticles. Our parsimony analyses identified the shield-shaped scale (M3) as ancestral, potentially with parallel ridges (M3c).

The Etmopteridae have the lowest denticle coverage with less than 30% of coverage for all species. The majority of Dalatiidae (i.e., *D. licha*, *I. brasiliensis*, *S. aliae*, *S. laticaudus*) have a higher denticle coverage than the Etmopteridae, ranging from 20% and 50%. A similar coverage is shown in species of Squalidae (*S. acanthias*) and Centrophoridae (*D. calcea*, *C. mollucensis)* analysed herein. All other species investigated in the present study have placoid scales covering 90% or more of their ventral epidermis. Parsimony analyses estimated the ancestral denticle coverage of the common ancestor of Squaliformes to cover the largest part of the ventral epidermis, i.e., 90% or more.

Based on the literature (Haddock et al., 2010; Straube et al., 2015; Duchatelet et al., 2021a; Duchatelet et al., 2021b), we mapped the presence of bioluminescence as character of Etmopteridae and Dalatiidae families, as well as the only known bioluminescent somniosid *Z. squamulosus*, to link it to the denticle coverage (Figure 4). A significant correlation between the scale coverage and the bioluminescence is observed (point-biserial correlation of 0.72): bioluminescent species being generally characterised by a lower scale coverage. In comparison, the scale coverage doesn't appear to be correlated with scale length or height (Pearson correlation of 0.36 and 0.16, respectively). Bioluminescence doesn't appear to be correlated with scale length or height (point-biserial correlation of 0.38 and 0.52), either.

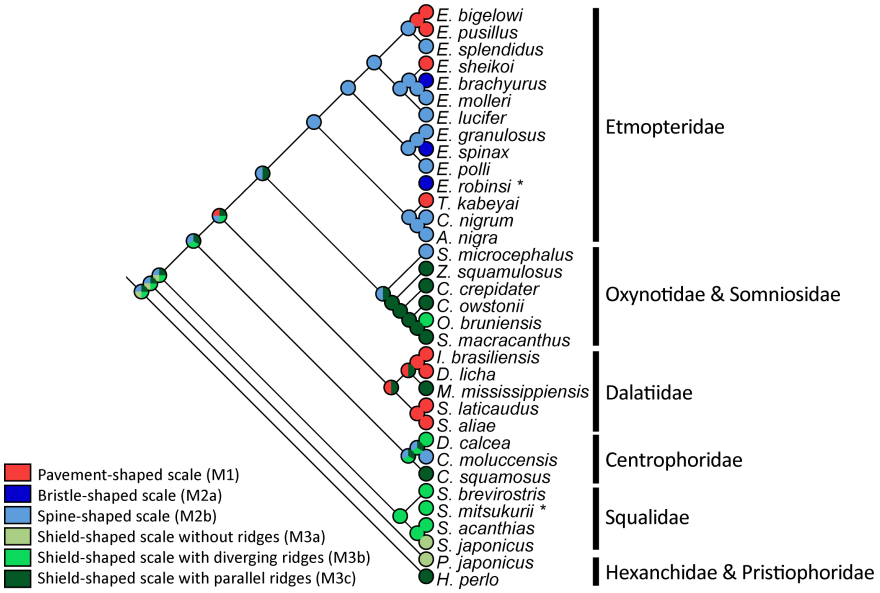


FIGURE 3
Estimation morphotype evolution of ventral placoid scale of the 34 studied squalomorph shark species. The phylogenetic representation is based on the phylogenetic tree shown in Supplementary Figure 2. Asterisks correspond to shark species for which NADH2 sequences are not available. Their position was manually assigned based on their corresponding genus.

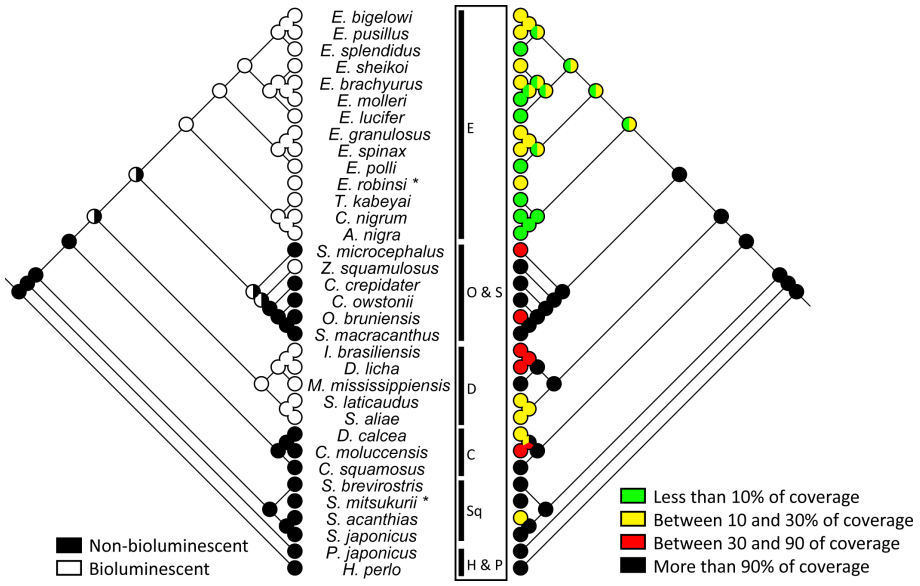


FIGURE 4
Comparison between percentage of ventral epidermis covered by placoid scales and the presence of bioluminescence abilities across the 34 studied squalomorph shark species. The phylogenetic representation is based on the phylogenetic tree shown in Supplementary Figure 2. Asterisks correspond to shark species for which NADH2 sequences are not available. Their position was manually assigned based on their corresponding genus. C, Centrophidae; D, Dalatiidae; E, Etmopteridae; H, Hexanchidae; O, Oxynotidae; P, Pristiophoridae; S, Somniosidae; Sq, Squalidae.

Elemental compositions of placoid scales in 32 Squalomorph species

The elemental composition of ventral placoid scales and their surrounding epidermis was analysed using Energy-dispersive X-ray Spectroscopy (EDX) coupled with Scanning Electron Microscopy (SEM). EDX analyses gave a rough overview of the main elements observed in the external layer of the placoid scales: carbon (C), oxygen (O), calcium (Ca), phosphorus (P), and to a lesser extent fluorine (F) (Figure 5A; Supplementary Table S5). Micrographies of elemental composition were generated to identify the elements typically found in the scales (Figure 5B for *E. molleri*/morphotype M2b; Supplementary Figure S4 for other representative species). On the epidermis, C and O elements were detected in higher proportions relative to the other elements. These elements show important inter-specific and intra-individual variations (20.5/23.5 ± 12% for C, 45/43 ± 7% for O) (Figure 5, Supplementary Table S5). The three other main elements also vary, but to a lesser extent. The proportion of Ca element ranges between 26.8/24.55 ± 4.25% in the Etmopterid *E. brachyurus* and 11.85/12 ± 1.9% in the Centrophorid *C. squamosus*. The P element proportion ranges between 13.9/12.3 ± 2.7% in the Etmopterid *T. kabeyai* and 7.5/7.2 ± 1.2% in the Centrophorid *D. calcea*, while it is rather stable in all other taxa. A ratio of the percentage of Ca to P was calculated, and these elements appear to be correlated (Figures 5E, F; Supplementary Table S5). The Ca/P ratio variation between the different species is small and about 1.8-1.9. The proportion of F varies in a narrower range of values and ranges between 1.4/1.7 ± 0.8% in the Somniosid *Z. squamulosus* and 5.4/5.4 ± 0.7% in the Pristiophorid *P. japonicus*. It was not possible to investigate the elemental composition of the scale of the species *M. mississippiensis*.

Discussion

Evolution of placoid scale in squaliform deep-sea sharks

Shark squamation is adapted to different lifestyles and ecological niches (Reif, 1985a; Reif, 1985b; Raschi and Tabit, 1992; Dean and Bhushan, 2010; Chernova and Vorob'eva, 2012; Lang et al., 2012; Fletcher et al., 2014; Ferrón and Botella, 2017). The morphological plasticity of placoid scales is potentially linked to adaptations to ecological conditions (Reif, 1985b; Reif, 1985a; Raschi and Tabit, 1992; Dean and Bhushan, 2010; Chernova and Vorob'eva, 2012; Ferrón and Botella, 2017). In this study, the phylogenetic distribution of dermal denticle morphotypes (and coverage) was investigated for the first time in squaliform deep-sea sharks. Character mapping was used to test different hypotheses and estimate ancestral character states.

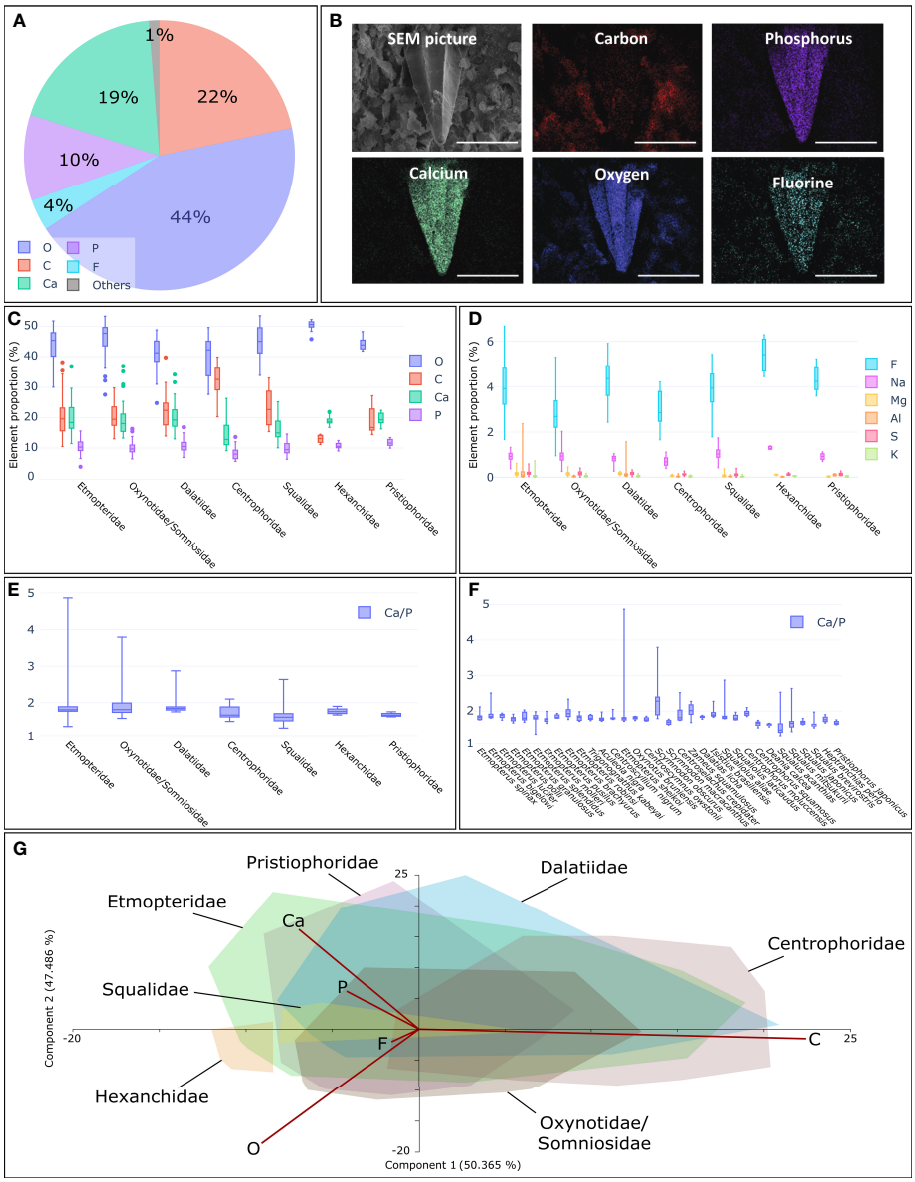


FIGURE 5
Elemental compositions of ventral placoid scales using EDX-SEM technic. **(A)** Average elemental compositions of the placoid scales of *E. molleri*. C, carbon; O, oxygen; F, fluorine, Na, sodium; Mg, magnesium; Al, aluminium; P, phosphorus; S, sulphur; K, potassium; Ca, calcium. **(B)** SEM picture of a placoid scale of *E. molleri* (morphotype M2b) without any EDX analyses and the localisation of the most abundant elements detected by Energy Dispersive X-ray spectrometry (EDX). For every picture, scale= 200µm. **(C)** Elemental proportion (%) of the four main elements (O, C, Ca and P) found on placoid scale clustered by families. **(D)** Elemental proportion (%) of the six other main elements (F, Na, Mg, Al, S and K) found on placoid scale clustered by families **(E)** Ca/P ratio clustered by families For C to E, families are *Etmopteridae*, *Oxynotidae/Somniosidae*, *Dalatiidae*, *Centrophoridae*, *Squalidae*, *Hexanchidae* and *Pristiophoridae*. **(F)** Ca/P ratio obtain for the 32 species (except *M. mississippiensis*) targeted by this study. **(G)** PCA illustrating the elemental proportion for all investigated shark families. Only the convex hulls representing the families are shown. Two principal factors extracted from the PCA, PC1 and PC2, accounted for 97.9% of the cumulative variance.

Our parsimony analyses identified the shield-shaped scale (M3) as the most probable ancestral state of ventral placoid scale morphotype among squaliform sharks. Historically, shield-shaped denticles have been assumed to be linked to drag reduction and hydrodynamics functions (Reif, 1985a; Raschi and Tabit, 1992; Ferrón and Botella, 2017; Feld et al., 2019; Lloyd et al., 2021). During the entire diversification of the Squaliformes, the evolutionary emergence of divergent ridges (M3b) is estimated to have occurred potentially three times independently. The evolutionary emergence of shield-shaped

scales without ridges (M3a) may have occurred twice. Other ventral scale morphotypes are estimated as derived character states, some of which appear to be convergent. The appearance of spine-shaped scales (M2b) seems to coincide with the radiation of the Etmopteridae. The bristle-shaped derived state (M2a) may have appeared at least two times in Etmopteridae. Pavement-shaped scales (M1) potentially evolved four times (once in the Dalatiidae and three times in the Etmopteridae).

The Squaliformes were estimated to have diverged about 177 Ma ago diversifying during the transition from the Lower to the Upper Cretaceous. (Straube et al., 2015). According to the character-mapping analyses, the common ancestor of all 32 species analysed herein had shield-shaped scales, potentially with parallel ridges (M3c). Derived characters may have evolved multiple times independently. The ancestral morphotypes of placoid scales at the base of each squaliform family may be different. In the Dalatiidae, which is estimated to have split from the Etmopteridae/Somniosidae-Oxynotidae clade 116 Ma ago (Straube et al., 2015), the morphotype could be either shield-shaped with parallel ridges (M3c) or pavement-shaped (M1). This latter character state is the most frequent type in extant species of this family (except for *M. mississippiensis*). Within Etmopteridae, which split from the Somniosidae/ Oxynotidae clade about 110 Ma ago (Straube et al., 2015), different morphotypes are observed. Most species have spine-shaped scales (M2b), which is assumed to be the ancestral scale type of this family. Bristle-shaped (M2a) and pavement-shaped scales (M1) are estimated to be derived character states within this family.

Species belonging to the bioluminescent Etmopteridae and the majority of Dalatiidae have ventral dermal denticles potentially adapted to facilitate functional ventral bioluminescence. The ventral placoid scale morphology of Etmopteridae can vary depending on the species between spine-shaped morphotype (M2b), pavement-shaped denticles (M1) and the bristle-shaped morphotype (M2a). The majority of Dalatiidae (*D. licha*, *I. brasiliensis*, *S. aliae* and *S. laticaudus*) have pavement-shaped denticles (M1) which could be analogous to the pavement-shaped scales of some Etmopteridae (*E. bigelowi*, *E. pusillus*, *E. sheikoi* and *T. kabeyai*). However, all these scale types have something in common: their distribution results in a small percentage of denticle coverage ventrally allowing coexistence with bioluminescent organs located in the skin. A clear space trade-off between ventral skin photophores and denticles is observed in Etmopteridae. A higher level of denticle coverage is observed in Dalatiidae and the single known bioluminescent Somniosid for which the bioluminescence function is supposed to be limited to countershading. In these two families, the denticle coverage can reach up to 90% (defined as the ancestral character state in Squaliforms) (Figure 3).

Our results support a scenario where a first diversification of non-luminescent squaliforms is characterised by the ancestral M3c denticle morphotype and later evolution of derived morphotypes facilitating bioluminescence, which is estimated to have emerged in sharks during the diversification of Dalatiidae, Somniosidae/ Oxynotidae and Etmopteridae during the transition from the Lower to the Upper Cretaceous (Straube et al. 2015). Although bioluminescence seems to perfectly correlate with a low denticle coverage (mainly in Etmopteridae and most Dalatiidae), two bioluminescent sharks are characterised by a high ventral denticle coverage: *Z. squamulosus* (Somniosidae) and *M. mississippiensis* (Dalatiidae). Both species show a third, entirely different type of shield-shaped denticle with additional honeycomb structures (See for *M. mississippiensis*, Supplementary Figures S3, S5). In *Z. squamulosus*, the denticle appears translucent to luminescence (Duchatelet et al., 2021b). This alternative scale organisation and morphology may represent a by-passing of the trade-off etmopterids and other dalatiid species encounter, where denticles are assumed to compete in space with photophores. In *M. mississippiensis*, ventral photophore aggregations are mentioned for the only specimen observed to date (Grace et al., 2019). Also, numerous dark spots (assumed to be photophores) were mentioned around denticle pedicles and found on the body (Grace et al., 2015; Grace et al., 2019). However, conclusive histology was not possible due to the freezing and thawing of tissue (Julien Claes, pers. comm.). *M. mississippiensis* also has translucent denticles but the light transmissivity has not yet been investigated. A dark area is present in the central part of the scale and is corresponding to the pedicle that anchors into the dermis (Supplementary Figure S5). Further analyses are needed to discuss the potential functional link between these placoid scales and the bioluminescence. It is important to state that the correlation we highlighted between the presence of bioluminescence and the low denticle coverage is not necessarily corresponding to a causative relationship. Alternative hypotheses related to other ecological features (e.g., paucity of food, resource allocations) of these organisms might be related to the specific structures of the dermal denticles in bioluminescent species.

All non-bioluminescent species present shield-shaped denticles, except for *C. moluccensis* (spine-shaped scales). The majority of Somniosidae analysed herein with the exception of *S. microcephalus* (M2a type scales), is equipped with shield-shaped scales with parallel ridges (M3c), a character that also appears as the ancestral state in this shark family. The Oxynotid *O. bruniensis* shows a large shield-shaped morphotype with diverging ridges (M3b). All other families (Squalidae, Centrophoridae and the outgroup species) show more variability on the ridges disposition on the shield-shaped denticles. Shield-shaped denticles can therefore be an advantage for deep-sea sharks, for example serving hydrodynamic functions, which may explain why this placoid scale type is widespread within Squalomorph sharks.

Based on the ventral coverage estimations, the evolution of less denticle coverage allowing for coexistence of denticles and

photophores, could have evolved in Etmopteridae and Dalatiidae independently. The Etmopteridae are estimated to have undergone adaptive radiations in a period of intense modifications in the marine environment, including the deep-sea (Fordyce, 1977; Straube et al., 2010; Claes et al., 2015; Straube et al., 2015). The diversification of *Etmopterus*, the genus showing most complex bioluminescence functions today, was likely rapid and may have been additionally fuelled by the establishment of the circum-antarctic current at the beginning of the Oligocene (Kennett et al., 1974; Fordyce, 1977; Lawver and Gahagan, 2003; Straube et al., 2010; Straube et al., 2015). As such, the pavement-shaped scales occurring in Dalatiidae and some species of Etmopteridae could have been convergently evolved, however bristle and spine-shaped scale type (M2a and b) are specific to the Etmopteridae.

Elemental composition of ventral placoid scales in deep-sea sharks

Various authors hypothesised that the elemental composition of placoid scales may vary due to biological functions, as it is the case for tetth (Murray, 1936; Hennemann, 2001; Riede, 2004). Our EDX analyses highlighted five main elements: C, O, Ca, P and F. C element is found mostly on the epidermis, while Ca, P and F are found almost exclusively on the surface of the placoid scales. Considering these element proportions is interesting because resources in F, Ca or P within mesopelagic and bathyal environments are limited (Gage and Tyler, 1992; Herring, 2001; Ifremer, 2015). Oxygen (O) is present on all samples. This general elemental distribution was conserved in all samples. The O and C element proportions are sometimes high in a single placoid scale. Previous studies have shown that fast swimming sharks produce a nano-layer of mucus that covers their placoid scales to improve their hydrodynamics (Dean and Bhushan, 2010; Lang et al., 2012; Bixler and Bhushan, 2013; Luo, 2015). If such a nano-layer is present in the species studied herein as well, it could influence our elemental composition analyses despite performing various rinsing steps during sample preparation.

While the elemental proportions could vary within the same placoid scale or among species (particularly for Ca and P elements), the Ca/P ratio is homogeneous within placoid scales (ranging from 1.42 to 2.28 for median values per species, from 1.60 to 1.80, median values per family) (Figure 5) and similar to the Ca/P ratio of the *E. molleri* tooth (median value of 1.46, data not shown). They are also similar to that of human dental enamel (Frank, 1961; Lussi et al., 2015). The latter ranges between 1.67 and 2.0 (Murray, 1936; Frank, 1961; Sánchez-Quevedo et al., 1998; Arnold and Gaengler, 2007; Lussi et al., 2015).

The F signal varies in the same value ranges for the different species and all their percentages had low values (global average value of 3.77 for all investigated species, in comparison the *E. molleri* tooth F proportion was 3.11 on average). The relative amount of fluorine in the upper layer of the placoid scales would therefore be relatively constant and conserved during evolution. The presence of this element results in more resistant and less soluble denticles in the surrounding environment (Lussi et al., 2015). The placoid scales are sometimes described as "non-growing structures" because their span of growth is limited and so they are only replaced when they are too damaged or when they fall out (Reif, 1985a; Reif, 1985b; Kemp, 1989; Helfman et al., 2009; Rodríguez-Cabello et al., 2020). Premature destruction or surface damage of these structures would not allow them to perform their functions and renewal should therefore be faster.

Conclusion

Our study, based on an unprecedented sampling of squalomorph sharks, sheds light on the evolution of the extant morphological diversity and relative homogeneity of the elemental composition of ventral placoid scales in Squaliformes for the first time.

Our sampling highlights the presence of a typical ventral squamation type in most bioluminescent species, with comprising pavement-shaped, spine-shaped, or bristle-shaped placoid scales scarcely located on the epidermis. However, two bioluminescent species, *Z. squamulosus* and *M. mississippiensis*, have a different kind of squamation, with shield-shaped placoid scales almost fully covering the epidermis. For *Z. squamulosus*, a previous study shows that this scale type is translucent and allows the emitted light to pass through the scale (Duchatelet et al., 2021b). Our results suggest that this morphology serves for additional functionality hitherto not associated with the functions of placoid scales.

Our analyses estimate the ancestral ventral morphotypes of Squaliform placoid scales to be a shield-shaped scale (as observed in most extant sharks). Other morphotypes are derived from this ancestral state. Squamation that would allow coexistence with photophores possibly evolved three times independently in luminescent families Etmopteridae, Dalatiidae and Somniosidae. Ancestral denticles of Etmopteridae are estimated to have a spine-shaped morphotype, a state that is highly preserved in many extant species. Conversely, the pavement-shaped morphotype is widely present in Dalatiidae, but also present in some Etmopteridae. However, the Dalatiidae *M. mississippiensis* and the only Somniosidae known to be bioluminescent, *Z. squamulosus*, show a third ventral scale morphotype, with shield-shaped denticles with honeycomb structures.

All dermal denticles appear to have a very similar chemical composition of their external layer. The elements mostly detected on placoid scales are oxygen, carbon, phosphorus,

calcium, and fluorine. The chemical composition seems to be conserved in Squaliformes belonging to mesopelagic and bathyal systems, regardless of the morphological characteristics of the scales or other ecological or biological characteristics of the different species of sharks.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

Ethical review and approval were not required for this study. The shark specimens were either captured as bycatch by local fishermen or from Museum collections.

Author contributions

AL, LD, and JD performed, analysed and interpreted the SEM pictures. AL and JD performed morpho-mapping, and statistical analyses. AL, JD, and LD performed the first draft manuscript. LD and JD collected and gathered tissue samples. NS provided samples and performed phylogenetic analyses. AL, JD, and NP prepared the figures. NP created schematics of the Figures 1A, 2. JD and LD supervised the work. MG, and GN provided molecular data for the phylogenetic analyses and contributed to the manuscript revision. JD coordinated the preparation of the publication with all co-authors. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2022.908237/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Determination key for the observed ventral morphotypes. * Most of the scales are overlapping, which prevents direct observation of the peduncles.

SUPPLEMENTARY FIGURE 2
Maximum likelihood phylogeny of squalomorph sharks based on mitochondrial NADH-ubiquinone oxidoreductase chain 2 (NADH2) gene under a GTR + Gamma model using RAxML (Stamatakis, 2014). Analysed specimens are listed in Supplementary Table 2. Bootstrap

values (500 replicates) are indicated at each node. The phylogeny based on nuclear genes in Straube et al. (2015) was used as backbone tree under RAxML's -g option where the maximum likelihood tree is constrained to the provided backbone topology.

SUPPLEMENTARY FIGURE 3
Ventral placoid scale micrographs of the different studied species at constant magnification (X100). For all micrographs, scale = 400 µm. The color code indicates the families to which the individuals belong. Red: Centrophoridae; Blue: Dalatiidae; Green: Etmopteridae; Yellow: Hexanchidae and Pristiophoridae; Purple: Oxynotidae and Somniosidae; Orange: Squalidae. For *C. squamosus* and *S. macrocanthus*, a lower magnification image (X90 and X65) was added in the upper left corner, because the entire scale was not visible at X100 magnification. For *C. nigrum*, a higher magnification photograph (X700) was also added.

SUPPLEMENTARY FIGURE 4
SEM picture of ventral placoid scales corresponding to the different morphotypes and Energy Dispersive X-ray spectrometry (EDX) maps highlighting the most abundant elements. **(A)** Placoid scale of *E. bigelowi* (pavement-shaped; M1). **(B)** Placoid scale of *E. spinax* (bristle-shaped; M2a). **(C)** Placoid scale of *P. japonicus* (shield-shaped without ridges; M3a). **(D)** Placoid scale of *O. bruniensis* (shield-shaped with diverging ridge; M3b). **(E)** Placoid scale of *H. perlo* (shield-shaped with parallel ridge; M3c).

SUPPLEMENTARY FIGURE 5
Ventral placoid scale of *M. mississippiensis* illuminated from below. **(A)** Scale present on the tegument, **(B)** an isolated placoid scale. Scales = 150µm.

SUPPLEMENTARY TABLE 1
Specimen collection data. The table includes the taxonomic information (family, species), the sampling site, the collection ID, the collection depth, the sampling method, and the collection date.

SUPPLEMENTARY TABLE 2
List of the species investigated for the Squalomorph phylogeny reconstruction including GenBank accession numbers for the NADH Dehydrogenase Subunit 2 sequences and the corresponding tissue sample numbers.

SUPPLEMENTARY TABLE 3
Raw data for the morphological analyses (scale height, scale length, number of visible scales under a magnification of X100, scale coverage).

SUPPLEMENTARY TABLE 4
Character state matrix used during the Mesquite analyses for the 33 studied shark species. Morphotype: pavement-shaped (M1), bristle-shaped (M2a), spine-shaped (M2b), shield-shaped without ridge (M3a), shield-shaped with diverging ridges (M3b), shield-shaped with parallel ridges (M3c); surface coverage: 1 – less than 10% of coverage, 2 – between 10 and 30% of coverage, 3 – between 30 and 90% of coverage, 4 – more than 90% of coverage.

SUPPLEMENTARY TABLE 5
Raw data obtained using EDX elemental proportion analyses for C= carbon, O= oxygen, F= fluorine, Na= sodium, Mg= magnesium, Al= aluminium, P= phosphorus, S= sulphur, K= potassium, Ca= calcium. For all species, three measurements represent the elemental composition analysed on the placoid scale, and one measurement was performed outside the scale (on the tegument).

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Supplementary Materials : Introduction



Table S1 *Summary of the symbiotic decapod crustaceans known to symbiotically interact with echinoderm hosts. The species were ordered following their taxonomic classification : family, genus and species. The species name is given following WoRMS (WoRMS Editorial Board 2023) and, aspiring to be the most complete inventory and to be the most useful to scientists working in this field, all known synonyms were also investigated and mentioned in the table S1. Because the information level is rarely equivalent when comparing different studies, some adjectives were added to qualify the suitability of the information :*

Symbiont		**Host Species**	**Relationship**	**References**
Species	**Synonym**			
Crustacea, Malacostraca, Decapoda				
Symbiont Family: Alpheidae Symbiont Genus: Alpheus				
Alpheus cristulifrons Rathbun, 1900		*Crinoid* *Nemaster grandis* (questionable) Free-living On reef edge intertidal level with rocks, coral and sponges, sand beach and rock outcrops	Free-living, maybe occasionally facultative ectosymbiont of echinoderms	(Criales 1984; Hendrickx and Hermoso-Salazar 2005; Anker et al. 2008)
Alpheus serenei Tiwari, 1964		Ophiuroid *Macrophiothrix longipeda* Free-living In coral block	Free-living and occasionally facultative ectosymbiont of ophiuroid	(Banner and Banner 1982; Bruce 1986a; Anker and De Grave 2016)
Alpheus splendidus Coutière, 1897	*A.pomatoceros*	Echinoid Unspecified (questionable) Free-living In muddy sandflats, in burrows and under large rocks and rubble.	Free-living, maybe occasionally facultative ectosymbiont of echinoderms	(Anker and De Grave 2016; Kazmi 2016; Ghory and Kazmi 2021)

Table S1

Symbiont Genus: Arete				
Arete acanthocarpus (Miya & Miyake, 1968)	*Athanas acanthocarpus*	Echinoid *Echinometra mathaei*	Commensal	(Miya and Miyake 1968; Suzuki 1970; Ayyagari and Kondamudi 2014; Ghory and Kazmi 2021)
Arete amboinensis de Man, 1910		Echinoid *Heterocentrotus sp.*	Symbiont (poorly studied)	(Banner and Banner 1960)
Arete dorsalis Stimpson, 1860	*Athanas dorsalis; A. mascarenicus*	Echinoid *Centrostephanus rodgersii; C. tenuispinus* *Echinothrix calamaris; E. diadema* *Heliocidaris erythrogramma; H. tuberculata* *Stomopneustes variolaris* *Tripneustes gratilla*	Obligate commensal	(Hipeau-Jacquotte 1965; Miya and Miyake 1968; Suzuki 1970; Ganapati and Sastry 1972; Ache 1974; Sastry 1977, 1981; Miya 1984; Gherardi 1991; James 1995; Coopard and Campbell 2004; Ayyagari and Kondamudi 2014)

Table S1

Arete indicus Coutière, 1903	*Arete dorsalis; A. intermedius; A. iphianassa; Athanas indicus; A. kominatoensis*	Echinoid *Echinometra mathaei* (primary host) *Diadema sp.* (questionable) *Heliocidaris crassispina* (questionnable)	Obligate commensal	(Coutière 1903, 1904, 1905; Banner and Banner 1960; Hipeau-Jacquotte 1965; Miya and Miyake 1968; Suzuki 1970; Ganapati and Sastry 1972; Bruce 1976b; Pomeranz and Tsurnamal 1976; Sastry 1977, 1981; James 1983; Gherardi 1991; Thiel et al. 2003; Ayyagari and Kondamudi 2014; Saravanan et al. 2015; Ghory et al. 2018; Brasseur et al. 2018; Dabbagh et al. 2019; Limviriyakul et al. 2020; Al-Kandari et al. 2020)
Symbiont Genus: Athanas				
Athanas anatidactylus Anker & Marin, 2007		Crinoid *Comanthus parvicirrus*	Suggested as free-living and occasionally facultative ectosymbiont symbiont	(Anker and Marin 2007; Mekhova and Britayev 2013)
Athanas areteformis Coutière, 1903	*Athanas crosslandi; A. dubius; A.erythraeus; A. naifaroensis*	Echinoid *Echinometra mathaei* *Toxopneustes gratilla, T. pileolus*	Symbiont	(Venmathi Maran et al. 2017; Ghory and Kazmi 2021)
Athanas ornithorhynchus Banner & Banner, 1973		Crinoid *Himerometra sp.* (maybe mis-associated) Ophiuroid *Macrophiothrix longipeda*	Symbiont Suggested as obligate	(Fabricius and Dale 1993; Marin et al. 2005; Deheyn et al. 2006; Anker and Marin 2007)

Table S1

Athanas parvus De Man, 1910	*Athanas sibogae*	Crinoid One unidentified species Free-living In dead coral heads, pools under rocks, and sandy or muddy beaches.	Free-living and occasionally facultative ectosymbiont	(Yang and Kim 2003; Al-Kandari et al. 2020; Virgili et al. 2020)
Symbiont Genus: Betaeus				
Betaeus macginitieae J.F.L. Hart, 1964		Echinoid *Mesocentrotus franciscanus* (primary host) *Strongylocentrotus purpuratus* (secondary host)	Symbiont	(Holmes 1900; Hart 1964; Gray et al. 1968; Ache and Davenport 1972; Ache 1974; Brasseur et al. 2018)
Symbiont Genus: Synalpheus				
Synalpheus carinatus (de Man, 1888)		Crinoid *Comanthus parvicirrus* *Comaster schlegelii* *Comatula purpurea* *Phanogenia gracilis; P. multibrachiata*	Symbiont	(Potts 1915; Banner and Banner 1975; Sastry 1981; Bruce 1982; Zmarzly 1984; Banner and Banner 1985; Fabricius and Dale 1993; Huang et al. 2005a; Prakash and Marimuthu 2020; Virgili et al. 2020)
Synalpheus comatularum (Haswell, 1882)		Crinoid *Clarkcomanthus albinotus* *Comanthus parvicirrus* *Comaster schlegelii* *Phanogenia distincta; P. gracilis* *Stephanometra indica*	Symbiont	(Potts 1915; Banner and Banner 1975; Bruce 1982; VandenSpiegel et al. 1998; Deheyn et al. 2006; Prakash and Marimuthu 2020)

Table S1

Synalpheus demani Borradaile, 1900	*Alpheus triunguiculatus; Synalpheus brockii*	Crinoid *Capillaster multiradiatus* *Cenometra bella* *Clarkcomanthus littoralis* *Comanthus parvicirrus; C. purpurea* *Comaster schlegelii* *Himerometra robustipinna*	Symbiont	(Banner and Banner 1975, 1985; Bruce 1982; Zmarzly 1984; Fabricius and Dale 1993; VandenSpiegel et al. 1998; Fujita and Baba 1999; Huang et al. 2005a, b; Deheyn et al. 2006; Wicksten and McClure 2007; Anker and De Grave 2016; Britayev et al. 2016; Virgili et al. 2020)
Synalpheus odontophorus de Man, 1909		Crinoid (questionable) Unidentified	Free living, maybe misidentified in the past	(de Man 1909; Banner and Banner 1975; Bruce 1982)

Table S1

Synalpheus stimpsoni (de Man, 1888)	*Alpheinus tridens; Alpheus amboinae; A. stimpsonii; Synalpheus brucei; S. consobrinus; S. striatus*	Crinoid *Anneissia japonica* *Clarkcomanthus alternans* *Comanthus parvicirrus* *Comaster gracilis; C. multifidus; C. schlegelii; C. nobilis* *Comatula purpurea* *Phanogenia gracilis; P. multibrachiata*	(Protective) Commensal	(Coutière and Chace 1899; Potts 1915; Banner and Banner 1975, 1985; Sastry 1981; Bruce 1982; Zmarzly 1984; Ng and Lim 1990; Gherardi 1991; Fabricius and Dale 1993; VandenSpiegel et al. 1998; Fujita and Baba 1999; Deheyn et al. 2006; Wicksten and McClure 2007; Mekhova and Britayev 2013; Anker and De Grave 2016; Brasseur et al. 2018; Prakash and Marimuthu 2020; Kasumyan et al. 2020; Virgili et al. 2020; Caulier et al. 2022)
Synalpheus tijou Banner & Banner, 1982		Crinoid *Comanthus suavia*	Symbiont (poorly studied)	(Banner and Banner 1982, 1985)
Synalpheus townsendi Coutière, 1909		Crinoid *Davidaster rubiginosus*	Symbiont (poorly studied)	(Criales 1984)
Synalpheus tropidodactylus Banner & Banner, 1975		Crinoid *Comanthus briareus* *Comaster nobilis; C. schlegelii*	Symbiont	(Bruce 1982; Fabricius and Dale 1993; Mekhova and Britayev 2013)

Table S1

Symbiont Family: *Diogenidae* Symbiont Genus: *Calcinus*				
Calcinus californiensis Bouvier, 1898		Echinoid *Diadema mexicanum* Free-living	Free-living and occasionally facultative ectosymbiont	(Hayes et al. 2022)
Symbiont Family: Chirostylidae Symbiont Genus: Uroptychodes				
Uroptychodes barunae Baba, 2004		Crinoid "collected with dense Crinoid"	Suggest as symbiont	(Baba 2004; Baba et al. 2009)
Uroptychodes grandirostris (Yokoya, 1933)	*Uroptychus grandirostris*	Crinoid "collected with dense Crinoid"	Suggest as symbiont	(Baba 2004; Baba et al. 2009)
Symbiont Genus: Uroptychus				
Uroptychus babai Ahyong & Poore, 2004		Crinoid *Glyptometra inaequalis*	Symbiont (poorly studied)	(Ahyong and Poore 2004; Baba et al. 2009; Baeza 2011)
Uroptychus capillatus Benedict, 1902		Crinoid *Crinometra brevipinna*	Symbiont (poorly studied)	(Rice and Miller 1991; Baeza 2011)
Uroptychus rugosus (A. Milne-Edwards, 1880)	*Diptychus rugosus*	Crinoid Probably *Stylometra spinifera*	Symbiont (poorly studied)	(Rice and Miller 1991; Baeza 2011)

Table S1

Symbiont Family: Epialtidae Symbiont Genus: Menaethius				
Menaethius monoceros (Latreille, 1825)	*Inachus arabicus* *Menaethiops monoceros* *Menaethius angustus;* *M. areolatus ; M. dentatus ; M. depressus ; M. inornatus ; M. porcellus: M. rugosus;* *M. subserratus: M. tuberculatus* *Pisa monoceros*	Holothuroid Living in the cloaca of an Holothurian Free-living in rocky substrates encrusted among algal growths and within fronds of the sea grasses at sandy areas	Free-living or facultative commensal or recently establish commensal	(Chopra 1931; Jones and Mahadevan 1965; El-Sayed 1997)
Symbiont Family: Galatheidae Symbiont Genus: Allogalathea				
Allogalathea babai Cabezas, Macpherson & Machordom, 2011		Crinoid *Anneissia bennetti*	Symbiont	(Cabezas et al. 2011; Baeza 2011; Virgili et al. 2020)

Table S1

Allogalathea elegans (Adams & White, 1848)	*Galathea deflexifrons; G. elegans; G. grandirostris; G. longirostris*	Crinoid *Amphimetra ensifera; A. tessellata* *Anneissia bennetti;A. japonica; A. pinguis* *Capillaster multiradiatus* *Cenometra bella* *Clarkcomanthus alternans* *Colobometra perspinosa* *Comanthus gisleni; C. parvicirrus; C. wahlbergii* *Comaster nobilis; C. schlegeli* *Comatella nigra; C. stelligera* *Dichrometra palmata; D. flagellate; D. regalis* *Heterometra savignii* *Himerometra robustipinna* *Lamprometra palmata* *Oxymetra finschii* *Stephanometra indica; S. tenuipinna* *Tropiometra carinata; T. macrodiscus* *Zygometra comata; Z. elegans*	Commensal	(Potts 1915; Barnard 1950; Serène et al. 1957; Jones and Sankarankutty 1960; Baba 1969, 1979; Lewinsohn 1969; Haig 1973; Fishelson 1974; Zmarzly 1984; Morton and Mladenov 1992; Fabricius and Dale 1993; Huang et al. 2005a; Deheyn et al. 2006; Baba and Fujita 2008; Baba et al. 2009; Cabezas et al. 2011; Baeza 2011; Dgebuadze et al. 2012; Mekhova and Britayev 2013; Mekhova et al. 2015; Limviriyakul et al. 2016; Britayev et al. 2016; Prakash and Marimuthu 2020; Kasumyan et al. 2020; Virgili et al. 2020)
Allogalathea inermis Cabezas, Macpherson & Machordom, 2011		Crinoid *Anneissia bennetti* *Himerometra robustipinna*	Symbiont	(Cabezas et al. 2011; Baeza 2011; Britayev et al. 2016; Virgili et al. 2020)

Table S1

Symbiont Genus: Galathea				
Galathea amboinensis de Man, 1888	*Galathea minuta*	Crinoid *Dichrometra sp.* *Capillaster multiradiatus* *Comanthus gisleni; C. parvicirrus* *Comaster nobilis; C. schlegelii* *Comatella nigra* *Phanogenia gracilis* *Stephanometra indica* *Tropiometra macrodiscus*	Commensal	(Potts 1915; Baba 1979; Fujita and Baba 1999; Baba and Fujita 2008; Saito and Fujita 2009; Baeza 2011; Mekhova and Britayev 2013)
Galathea continua Baba & Fujita, 2008		Crinoid *Capillaster multiradiatus* *Clarkcomanthus littoralis* *Comatella nigra; C. stelligera,* *Comanthus gisleni*	Symbiont	(Fujita and Baba 1999; Baba and Fujita 2008; Baeza 2011; Virgili et al. 2020)
Galathea genkai Miyake & Baba, 1964		Crinoid *Decametra chadwicki* *Heterometra savignii* *Oligometra serripinna*	Symbiont (poorly study)	(Fishelson 1974; Baba et al. 2009; Baeza 2011)
Galathea inflata Potts, 1915		Crinoid *Anneissia bennetti* *Comanthus parvicirrus* *Comaster nobilis; C. schlegelii* *Phanogenia gracilis*	Commensal	(Potts 1915; Baba 1979; Fujita and Baba 1999; Deheyn et al. 2006; Baba and Fujita 2008; Baba et al. 2009; Baeza 2011; Virgili et al. 2020)
Galathea leptocheir Baba & Fujita, 2008		Crinoid *Phanogenia gracilis*	Symbiont	(Baba and Fujita 2008; Saito and Fujita 2009; Baeza 2011)

Table S1

Galathea orientalis Stimpson, 1858		Crinoid *Anneissia japonica* Free-living Sand, sandy mud, coral head, rocky shore, between boulders, and on basal part of seaweed on rocks	Free-living, occasionally ectosymbiont	(Baba et al. 2009)
Symbiont Family: Hippolytidae Symbiont Genus: Hippolyte				
Hippolyte catagrapha d'Udekem d'Acoz, 2007		Crinoid *Tropiometra carinata*	Obligate symbionts	(Hempson and Griffiths 2008)
Hippolyte prideauxiana Leach, 1817	*Bellidia huntii* *Hippolyte hunti; H. moorii*	Crinoid *Antedon bifida*	Symbiont (poorly study)	(Barel and Kramers 1977)
Symbiont Genus: Latreutes				
Latreutes parvulus (Stimpson, 1871)	*Concordia gibberosus* *Rhynchocyclus parvulus*	Echinoid *Astropyga magnifica*	Symbiont (poorly study)	(Criales 1984)
Symbiont Family: Hymenosomatidae Symbiont Genus: Neohymenicus				
Neohymenicus orientalis (Sakai, 1932)	*Halicarcinus orientalis*	Ophiuroids *Breviturma brevipes; B. dentata*	Symbiont (poorly study)	(Takeda et al. 1976; Sloan et al. 1979)

Table S1

Symbiont Family: Inachidae Symbiont Genus: Achaeus				
Achaeus brevirostris (Haswell, 1879)	*Achaeus affinis* *Stenorhynchus brevirostris*	Holothuroid Living in the cloaca of an Holothurian Free-living In muddy habitat, found in sublittoral to shallow offshore	Free-living and occasional facultative symbiont	(Chopra 1931; Jones and Mahadevan 1965)
Symbiont Family: Inachoididae Symbiont Genus: Stenorhynchus				
Stenorhynchus debilis (Smith, 1871)	*Leptopodia debilis; L. sagittaria*	Echinoid *Diadema mexicanum*	Symbiont (poorly study)	(Salas-Moya et al. 2021)
Stenorhynchus seticornis (Herbst, 1788)	*Cancer sagittarius; C. seticornis* *Leptopodia lineata; L. ornata; L. sagittaria*	Crinoid Unspecified (questionable) Echinoid *Diadema antillarum* *Eucidaris tribuloides* Other Sponges; Stony corals; Soft corals; Gorgonians; Anemones	Generalist symbiont	(Hayes et al. 1998; Thiel et al. 2003; Hayes 2007; Saravanan et al. 2015; Ghory et al. 2018)
Symbiont Family: Mithracidae Symbiont Genus: Mithraculus				
Mithraculus sculptus (Lamarck, 1818)	*Maia sculptus* *Mithraculus coronatus* *Mithrax minutus; M. sculptus*	Echinoid *Diadema antillarum* *Tripneustes ventricosus*	Symbiont (poorly study)	(Hayes 2007)

Table S1

Symbiont Family: Munididae Symbiont Genus: Munida				
Munida intermedia A. Milne-Edwards & Bouvier, 1899	*Munida bamffia; M. sarsi*	Free-living In muddy bottoms Crinoid *Antedon mediterranea*	Free-living (adult) and symbiont (juvenile) (poorly studied)	(De Domenico et al. 2010; Baeza 2011)
Symbiont Family: Munidopsidae Symbiont Genus: Munidopsis				
Munidopsis abdominalis (A. Milne-Edwards, 1880)	*Elasmonotus abdominalis*	Echinoid *Cidaris blakei; C. rugosa*	Symbiont (poorly study)	(Rice and Miller 1991; Baeza 2011)
Munidopsis alaminos Pequegnat & Pequegnat, 1970		Holothuroid *Mesothuria gargantua*	Symbiont (poorly study)	(Rice and Miller 1991; Baeza 2011)
Munidopsis spinifer (A. Milne-Edwards, 1880)	*Galathodes spinifer*	Crinoid *Crinometra brevipinna*	Symbiont (poorly study)	(Rice and Miller 1991; Baeza 2011)
Symbiont Family: Palaemonidae Symbiont Genus: Actinimenes				
Actinimenes inornatus (Kemp, 1922)	*Periclimenes (Ancylocaris) inornatus* *Periclimenes paraornatus*	Echinoid Unspecified (questionable) *Other* Cnidaria (primary host)	Commensal	(Gan et al. 2015; de Gier et al. 2022)

Table S1

Symbiont Genus: Allopontonia				
Allopontonia alastairi Bruce, 2010		Echinoid *Salmacis belli*	Commensal	(Bruce 2010; Horká 2017)
Allopontonia brockii (de Man, 1888)	*Allopontonia iaini* *Anchistia brockii* *Periclimenes signatus; P. priodactylus*	Echinoid *Asthenosoma sp.* *Salmaciella dussumieri; S. erythracis* *Salmacis bicolor; S. sphaeroides*	Commensal	(Bruce 1972a, a, 1987, 1992; Wicksten and Hernández 2000; Britayev et al. 2013; Chow et al. 2021)
Symbiont Genus: Araiopontonia (monotypic)				
Araiopontonia odontorhyncha Fujino & Miyake, 1970		Crinoid *Anneissia bennetti* *Comaster schlegelii*	Commensal	(Bruce 1982; Zmarzly 1984; Huang et al. 2005a; Marin and Chan 2006; Marin 2009; Mekhova and Britayev 2013; Gan et al. 2015; Horká et al. 2016; Park et al. 2019; Chow et al. 2021)
Symbiont Genus: Bathymenes				
Bathymenes aleator (Bruce, 1991)	*Periclimenes aleator*	Echinoid *Aspidodiadema* sp.	Commensal	(Bruce 1991; Li 2008; Kou et al. 2016; Horká 2017)

Table S1

Symbiont Genus: Brucecaris (monotypic)				
Brucecaris tenuis (Bruce, 1969)	*Periclimenes tenuis*	Crinoid *Amphimetra sp.* *Anneissia bennetti; A. pinguis* *Cenometra bella* *Comaster schlegelii* *Decametra chadwicki* *Dichrometra palmata* *Heterometra africana; H. savignii* *Himerometra robustipinna* *Oxymetra sp.* *Tropiometra carinata* *Zygometra comata*	Commensal	(Bruce 1969, 1974a; Fishelson 1974; Bruce 1982; Zmarzly 1984; Fabricius and Dale 1993; Huang et al. 2005a; Deheyn et al. 2006; Marin and Chan 2006; Marin 2009; Mekhova and Britayev 2013; Mekhova et al. 2015; Horká et al. 2016; Virgili et al. 2020; Chow et al. 2021)
Symbiont Genus: Conchodytes				
Conchodytes tridacnae Peters, 1852		Holothuroid Living in a Holothurian (unusual host) Other Bivalva (primary host)	Commensal	(Chopra 1931)
Symbiont Genus: Crinotonia				
Crinotonia anastasiae Marin, 2006		Crinoid *Phanogenia gracilis; P.multibrachiata*	Obligate and highly specialized commensal	(Marin 2006, 2008, 2009; Marin and Chan 2006; Gan et al. 2015; Virgili et al. 2020; Chow et al. 2021)

Table S1

Crinotonia attenuatus (Bruce, 1971)	*Periclimenes attenuatus*	Crinoid *Comaster multifidus* *Phanogenia gracilis*	Obligate and highly specialized commensal	(Bruce 1971b, 1982, 1992; Fabricius and Dale 1993; Li 2004; Deheyn et al. 2006; Marin 2006; Marin and Chan 2006; Marin 2008; Saito and Fujita 2009; Marin 2009; Gan et al. 2015; Virgili et al. 2020; Chow et al. 2021)
Symbiont Genus: Cristimenes				
Cristimenes brucei Park, De Grave & Kim, 2019		Crinoid *Anneissia japonica; A. solaster* *Catoptometra rubroflava*	Commensal	(Thiel et al. 2003)

Table S1

Cristimenes commensalis (Borradaile, 1915)	*Periclimenes commensalis*	Crinoid *Amphimetra ensifera* *Anneissia bennetti; A. japonica; A. pinguis* *Capillaster multiradiatus* *Cenometra bella* *Clarkcomanthus alternans* *Colobometra perspinosa* *Comanthus gisleni; C. parvicirrus; C. wahlbergii* *Comaster multifidus; C. nobilis; C.schlegelii* *Comatella nigra; C. stelligera* *Dichrometra flagellata* *Heterometra africana* *Himerometra robustipinna* *Phanogenia distinta* *Stephanometra tenuipinna* *Tropiometra afra; T. macrodiscus* *Zygometra microdiscus*	Obligate commensal	(Potts 1915; Bruce 1971c, 1974a, 1980b; Zmarzly 1984; Morton and Mladenov 1992; Fabricius and Dale 1993; Fujita and Baba 1999; Huang et al. 2005a; Deheyn et al. 2006; Marin and Chan 2006; Dgebuadze et al. 2012; Mekhova and Britayev 2013; Gan et al. 2015; Mekhova et al. 2015; Anker and De Grave 2016; Horká et al. 2016; Britayev et al. 2016; van der Meij et al. 2018; Park et al. 2019; Virgili et al. 2020; Chow et al. 2021)
Cristimenes cristimanus (Bruce, 1965)	*Periclimenes cristimanus*	Echinoid *Diadema setosum (Primary host)* *Echinothrix calamaris (occasionnaly)*	Obligate commensal	(Bruce 1965, 1971a; Gan et al. 2015; Anker and De Grave 2016; Horká et al. 2016; Park et al. 2019; Chow et al. 2021)
Cristimenes zanzibaricus (Bruce, 1967)	*Periclimenes zanzibaricus*	Echinoid *Astropyga* sp. *Centrostephanus tenuispinus* *Diadema savignyi; D. setosum* *Echinothrix calamaris*	Commensal	(Bruce 1965, 1971a, 1973b, 1974a, 1975; Horká et al. 2016; Park et al. 2019; Limviriyakul et al. 2020; Chow et al. 2021)

Table S1

Symbiont Genus: Cuapetes				
Cuapetes americanus (Kingsley, 1878)	*Anchistia americana* *Kemponia americana* *Periclimenes bermudensis; P. rhizophorae; P. americanus*	Crinoid *Nemaster grandis* (occasionnal) Free-living In sandy and rocky bottoms, macroalgae, seagrass beds, and mangrove roots	Free-living and facultative commensal	(Criales 1984; Negri et al. 2017)
Symbiont Genus: Diapontonia (monotypic)				
Diapontonia maranulus Bruce, 1986		Echinoid *Paleopneustes tholoformis*	Commensal	(Bruce 1986b; Horká et al. 2016)
Symbiont Genus: Echinopericlimenes				
Echinopericlimenes aurorae Marin & Chan, 2014		Echinoid Unknown; suggest in association with a deep-water sea urchin	Suggest as symbiont	(Marin and Chan 2014)
Echinopericlimenes calcaratus (Chace & Bruce, 1993)	*Periclimenes calcaratus*	Echinoid Unknow, suggest in association with a deep-water sea urchin of the family Echinothuriidae	Suggest as symbiont	(Chace and Bruce 1993; Marin and Chan 2014; Horká 2017; Chow et al. 2021)
Echinopericlimenes dentidactylus (Bruce, 1984)		Echinoid Unknown, suggest in association with a deep-water sea urchin	Suggest as symbiont	(Marin and Chan 2014; Horká et al. 2016; Horká 2017)
Echinopericlimenes hertwigi (Balss, 1913)	*Periclimenes (Ancylocaris) gracilirostris* *Periclimenes hertwigi*	Echinoid *Araeosoma owstonii; A. thetidis* *Asthenosoma* sp. *Phormosoma* sp.	Commensal	(Balss 1914; Bruce 1965, 1971a, 1975, 1991; Marin and Chan 2014; Gan et al. 2015; Horká et al. 2016; Horká 2017; Dabbagh et al. 2019; Chow et al. 2021)

Table S1

Symbiont Genus: Gnathophylloides				
Gnathophylloides mineri Schmitt, 1933		Echinoid *Lytechinus variegatus* *Pseudoboletia indiana* *Tripneustes depressus; T. gratilla; T. ventricosus*	Obligate commensal	(Lewis 1956, 1958; Chace 1969; Castro 1971a, b; Chace 1972; Bruce 1974b; Abele 1975; Bruce 1976b; Criales 1984; Patton et al. 1985; Bruce 1988; Titgen 1989; Gherardi 1991; VandenSpiegel et al. 1998; Wicksten and Hernández 2000; Okuno and Tanaka 2001; Thiel et al. 2003; Wirtz et al. 2009; Williamson et al. 2012; Maciá and Robinson 2012; Saravanan et al. 2015; Salas-Moya et al. 2021; De Grave et al. 2021)
Gnathophylloides robustus Bruce, 1973		Echinoid *Centrostephanus tenuispinus*	Commensal	(Bruce 1973a)

Table S1

Symbiont Genus: Gnathophyllum				
Gnathophyllum americanum Guérin-Méneville, 1855	*Gnathophyllum fasciolatum; G. minuscularium; G. pallidum; G. triden; G. zebra*	Asteroid: *Echinometra lucunter* (occasionally) Echinoid *Lytechinus variegatus* (occasionally) Free-living Report as free living under rocks or within rock cracks in tide pools covered with algae	Free living and occasionally facultative associated with echinoderms	(Bruce 1976b; Takeda et al. 1976; Titgen 1989; Araújo 2002; Gan et al. 2015; Tavares et al. 2017; Neves 2020)
Gnathophyllum precipuum Titgen, 1989		Holothuroid *Stichopus horrens* Free-living In reef crevice	Fee living and occasionally facultative associated with echinoderms or a predator of them	(Titgen 1989; De Grave and Fransen 2011)
Gnathophyllum taylori Ahyong, 2003		Echinoid *Centrostephanus rodgersii* Free-living Under boulders or amongst marine algae	Free living and occasionally facultative associated with echinoid	(Ahyong 2003)
Symbiont Genus: Hymenocera (monotypic)				
Hymenocera picta Dana, 1852	*Hymenocera elegans; H. latreillii*	Asteroid Various undertermined asteroids *Acanthaster planci* Free-living In the lagoons within the fringing reef	Fee living and occasionally facultative associated with echinoderms or a predator of them	(Rainbow 1974; Titgen 1989; Wicksten and Hernández 2000; Gan et al. 2015)

Table S1

Symbiont Genus: Laomenes				
Laomenes amboinensis (de Man, 1888)	*Anchistia amboinensis* *Periclimenes amboinensis*	Crinoid *Anneissia bennetti* *Capillaster multiradiatus* *Comanthus briareus; C. zy; C. wahlbergii* *Comaster nobilis; C. schlegelii* *Himerometra robustipinna*	Commensal	(Bruce 1982, 1992, 2007; Zmarzly 1984; Fabricius and Dale 1993; Fujita and Baba 1999; Huang et al. 2005a; Deheyn et al. 2006; Marin and Chan 2006; Marin 2009; Mekhova and Britayev 2013; Horká et al. 2016; Park et al. 2019; Virgili et al. 2020)
Laomenes ceratophthalmus (Borradaile, 1915)	*Periclimenes ceratophthalmus*	Crinoid *Lamprometra palmata* *Dichrometra flagellate; D. palmata* *Himerometra robustipinna* *Stephanometra indica*	Commensal	(Potts 1915; Bruce 1974a, 1982, 2007; Chace and Bruce 1993; Fujita and Baba 1999; Deheyn et al. 2006; Marin and Chan 2006; Marin 2009; Mekhova and Britayev 2013; Gan et al. 2015; Horká et al. 2016; Limviriyakul et al. 2020; Virgili et al. 2020; Chow et al. 2021)
Laomenes clarki Marin, 2009		Crinoid *Zygometra punctata*	Commensal	(Marin 2009; Marin et al. 2012; Gan et al. 2015; Virgili et al. 2020; Chow et al. 2021)
Laomenes cornutus (Borradaile, 1915)	*Periclimenes cornutus*	Crinoid *Comaster schlegelii*	Commensal	(Potts 1915; Bruce 1982, 2007; Huang et al. 2005a; Deheyn et al. 2006; Marin and Chan 2006; Marin 2009; Marin et al. 2012)

Table S1

Laomenes holthuisi Marin & Okuno, 2010		Crinoid *Comaster nobilis*	Commensal	(Marin and Okuno 2010; Marin et al. 2012; Gan et al. 2015; Chow et al. 2021)
Laomenes jackhintoni (Bruce, 2006)	*Periclimenes jackhintoni*	Crinoid One species of unidentified crinoid	Commensal	(Bruce 2006, 2007; Marin 2009)
Laomenes nudirostris (Bruce, 1968)	*Parapontonia nudirostris*	Crinoid *Amphimetra tessellata* *Himerometra robustipinna* *Pontiometra andersoni* *Stephanometra tenuipinna* *Tropiometra afra*	Commensal	(Bruce 1971c, 1982, 1992, 2007; Fabricius and Dale 1993; Fujita and Baba 1999; Marin and Chan 2006; Marin 2009; Gan et al. 2015; Mekhova et al. 2015; Virgili et al. 2020; Chow et al. 2021)
Laomenes pardus Marin, 2009		Crinoid *Comanthus briareus*	Commensal	(Marin 2009; Mekhova and Britayev 2013; Gan et al. 2015; Kou et al. 2015; Virgili et al. 2020)
Laomenes pestrushka Marin, Chan & Okuno, 2012		Crinoid Maybe *Capillaster multiradiatus*	Commensal	(Marin et al. 2012; Gan et al. 2015)
Laomenes tigris Marin, 2009		Crinoid *Stephanometra* sp.	Commensal	(Marin 2009; Gan et al. 2015)

Table S1

Symbiont Genus: Levicaris				
*Levicaris mammillata (*Edmondson, 1931)	*Coralliocaris mammilatus*	Echinoid *Heterocentrotus mamillatus*	Obligate commensal	(Castro 1971b; Fujino and Takeda 1977; Patton et al. 1985; Titgen 1989)
Symbiont Genus: Lipkebe (monotypic)				
Lipkebe holthuisi Chace, 1969		Crinoid *Comactinia meridionalis* *Nemaster grandis*	(Protective) Commensal	(Chace 1969; Criales 1984)
Symbiont Genus: Lipkemenes (monotypic)				
Lipkemenes lanipes (Kemp, 1922)	*Periclimenes lanipes*	Echinoid *Unspecified* (questionable) Ophiuroids *Astroboa nuda* *Astroglymma sculptum* *Euryale purpurea*	Commensal	(Bruce 1974a, 1982, 1992; Marin et al. 2005; Gan et al. 2015; Kou et al. 2015; Horká et al. 2016; Horká 2017; Chow et al. 2021)
Symbiont Genus: Margitonia (monotypic)				
Margitonia insolitus (Bruce, 1974)	*Periclimenes insolitus*	Echinoid *Pseudoboletia indiana*	Commensal	(Bruce 1975; Horká 2017)

Table S1

Symbiont Genus: Palaemonella				
Palaemonella pottsi (Borradaile, 1915)	*Periclimenes pottsi*	Crinoid *Anneissia bennetti* *Clarkcomanthus alternans* *Comanthina schlegeli* *Comanthus briareus; C. parvicirrus* *Comaster gracilis; C. nobilis* *Himerometra robustipinna* *Phanogenia gracilis*	Obligate commensal	(Potts 1915; Bruce 1982; Zmarzly 1984; Ng and Lim 1990; Chace and Bruce 1993; Fabricius and Dale 1993; Fujita and Baba 1999; Li and Lui 2003; Deheyn et al. 2006; Marin and Chan 2006; Saito and Fujita 2009; Marin 2009; Mekhova and Britayev 2013; Gan et al. 2015; Mekhova et al. 2015; Horká et al. 2016; Park et al. 2019; Prakash and Marimuthu 2020; Limviriyakul et al. 2020; Virgili et al. 2020; Chow et al. 2021)
Symbiont Genus: Periclimenaeus				
Periclimenaeus djiboutensis Bruce, 1970	*Periclimenes djiboutensis (lapsus)*	Crinoid *Dichrometra palmata* *Heterometra savignii.*	Commensal	(Bruce 1970; Fishelson 1974)
Symbiont Genus : Periclimenella				
Periclimenella spinifera (de Man, 1902)	*Periclimenes petitthouarsi; P. spiniferus*	Crinoid Unspecified (questionable or accidental) Other Corals (primary host)	Commensal	(Sastry 1981)

Table S1

Symbiont Genus: Periclimenes				
Periclimenes affinis (Zehntner, 1894)	*Palaemonella affinis* *Periclimenes brocketti*	Crinoid *Comatula cratera* *Comanthus sp.* *Comaster nobilis; C. schlegelii*	Commensal	(Potts 1915; Bruce 1980a, 1982; Fabricius and Dale 1993; Fujita and Baba 1999; Marin and Chan 2006; Mekhova and Britayev 2013; Gan et al. 2015; Virgili et al. 2020; Chow et al. 2021)
Periclimenes albolineatus Bruce & Coombes, 1997		Crinoid Unspecified	Suggest as symbiont	(Fabricius and Dale 1993; Bruce and Coombes 1997)
Periclimenes bowmani Chace, 1972		Crinoid either *Davidaster rubiginosus* or *Tropiometra carinata*	Commensal	(Chace 1972; Criales 1984)
Periclimenes carinidactylus Bruce, 1969		Crinoid Unspecified	Commensal	(Bruce 1969, 1982; Marin and Chan 2006)
Periclimenes colemani Bruce, 1975		Echinoid *Asthenosoma intermedium*	Commensal	(Bruce 1975; Horká et al. 2016; Chow et al. 2021; de Gier et al. 2022)
Periclimenes compressus Borradaile, 1915		Crinoid Unspecified	Commensal	(Marin and Chan 2006)
Periclimenes crinoidalis Chace, 1969		Crinoid *Nemaster grandis*	Commensal	(Chace 1969; Criales 1984; Horká et al. 2016; Chow et al. 2021)
Periclimenes hongkongensis Bruce, 1969		Holothuroid Unspecified	Suggest as symbiont	(Bruce 1969; Horká 2017)

Periclimenes ingressicolumbi Berggren & Svane, 1989		Echinoid *Paleopneustes tholoformis*	Commensal	(Berggren and Svane 1989; Bruce 1992)
Periclimenes maldivensis Bruce, 1969		Echinoid Unspecified	Commensal	(Bruce 1969, 1971a; Dabbagh et al. 2019)
Periclimenes meyeri Chace, 1969		Crinoid *potentially Nemaster sp.*	Commensal	(Chace 1969, 1972; Criales 1984)
Periclimenes milleri Bruce, 1986		Echinoid *Heterobrissus sp.*	Commensal	(Bruce 1986c, 1992)
Periclimenes novaffinis Bruce & Coombes, 1997		Crinoid *Zygometra sp.*	Commensal	(Marin 2006; Marin and Chan 2006)
Periclimenes pectiniferus Holthuis, 1952		Holothuroid Unspecified	Commensal	(Horká 2017)
Periclimenes pectinipes Bruce, 1991		Crinoid Unspecified	Commensal	(Marin and Chan 2006)
Periclimenes parvus Borradaile, 1898		Echinoid Unspecified Ophiuroids Unspecified	Commensal	(Gan et al. 2015)
Periclimenes perryae Chace, 1942		Ophiuroids *Astrophyton muricatum*	Commensal	(Chace 1969, 1972; Criales 1984; Marin et al. 2005; Dobson et al. 2014; Horká et al. 2016; Chow et al. 2021)
Periclimenes ruber Bruce, 1982		Crinoid *Zygometra microdiscus*	Commensal	(Bruce 1982; Marin and Chan 2006)

Table S1

Symbiont Genus: Phyllognathia				
Phyllognathia ceratophthalma (Balss, 1913)		Asteroid Unspecified Echinoid Unspecified	Commensal	(Gan et al. 2015)
Symbiont Genus : Pontoniopsis (monotypic)				
Pontoniopsis comanthi Borradaile, 1915		Crinoid *Anneissia bennetti; A. pinguis* *Cenometra bella* *Colobometra perspinosa* *Comanthus gisleni; C. parvicirrus* *Comaster nobilis* *Comatula pectinate; C. purpurea* *Comatella stelligera* *Clarkcomanthus alternans* *Dichrometra palmata; D. flagellata; D. regalis* *Heterometra savignii* *Himerometra robustipinna* *Phanogenia distinta* *Stephanometra indica; S. tenuipinna* *Tropiometra carinata* *Zygometra comata*	Obligate and highly crinoid-specialized commensal	(Fishelson 1974; Bruce 1982; Zmarzly 1984; Fabricius and Dale 1993; Fujita and Baba 1999; Deheyn et al. 2006; Marin and Chan 2006; Marin 2009; Dgebuadze et al. 2012; Mekhova and Britayev 2013; Gan et al. 2015; Mekhova et al. 2015; Anker and De Grave 2016; Horká et al. 2016; Britayev et al. 2016; van der Meij et al. 2018; Prakash and Marimuthu 2020; Chow et al. 2021)
Symbiont Genus: Pseudocoutierea				
Pseudocoutierea elegans Holthuis, 1951		Echinoid *Centrocidaris doederleini* Others: *Octocorallia, Hydrozoa*	Commensal	(Salas-Moya et al. 2021)

Table S1

Symbiont Genus : Pycnocaris (monotypic)				
Pycnocaris chagoae Bruce, 1972		Echinodermata Suggest as a echinodermata symbiont	Suggest of commensal	(Bruce 1972b; Chow et al. 2021)
Symbiont Genus: Sandimenes				
Sandimenes hirsutus (Bruce, 1971)	*Periclimenes hirsutus*	Echinoid *Astropyga radiata* Ophiuroids Unspecified (questionnable)	Commensal	(Bruce 1971a; Li 2009; Gan et al. 2015; Chow et al. 2021)
Sandimenes rectirostris (Bruce, 1981)	*Periclimenes rectirostris*	Echinodermata (unspecified)		(Chow et al. 2021)
Symbiont Genus: Stegopontonia (monotypic)				
Stegopontonia commensalis Nobili, 1906		Echinoid *Diadema paucispinum* *Echinothrix calamaris; E. diadema*	Commensal	(Castro 1971b; Gan et al. 2015; Horká et al. 2016; Horká 2017; Limviriyakul et al. 2020; Chow et al. 2021)
Symbiont Genus : Tulеariocaris				
Tulеariocaris sarec Berggren, 1994		Echinoid *Echinometra mathaei*	Commensal	(Berggren 1994; Marin 2006)
Tulеariocaris holthuisi Hipeau-Jacquotte, 1965	/	Echinoid *Echinometra mathaei* (primary host) *Astropyga radiata; A. pulvinata* (secondary seasonal host) *Diadema mexicanum* (secondary seasonal host) *Echinothrix diadema* *Stomopneustes variolaris*	Commensal	(Hipeau-Jacquotte 1965; Castro 1971b; Bruce 1974a; Berggren 1994; Wicksten and Hernández 2000; Gan et al. 2015; Horká et al. 2016; Brasseur et al. 2018; Chow et al. 2021; Ghory and Kazmi 2021; Hayes et al. 2022)

Table S1

Tulearіocaris neglecta Chace, 1969		Echinoid *Diadema antillarum (primary host)* *Astropyga magnifica (occasionnaly)*	Commensal	(Chace 1969; Castro 1974; Criales 1984; Berggren 1994; Hayes et al. 1998; Thiel et al. 2003; Saravanan et al. 2015; Tavares et al. 2017)
Tulearіocaris zanzibarica Bruce, 1967		Echinoid *Astropyga radiata* *Diadema savignyi; D. setosum* *Echinothrix calamaris; E. diadema*	Commensal	(Bruce 1974a; Castro 1974; Gherardi 1991; Berggren 1994; Thiel et al. 2003; Gan et al. 2015; Saravanan et al. 2015; Limviriyakul et al. 2020; Chow et al. 2021)
Symbiont Genus : Unguicaris				
Unguicaris alegrias (Bruce, 1986)	*Periclimenes alegrias*	Crinoid *Comaster multifidus* *Dichrometra palmata* *Stephanometra indica*	Commensal	(Bruce 1986d; Marin 2009)
Unguicaris novaecaledoniae (Bruce, 1968)	*Periclimenes novaecaledoniae*	Crinoid *Dichrometra palmata* *Tropiometra afra*	Commensal	(Bruce 1980a, 1982; Marin and Chan 2006; Marin 2009; Horká et al. 2016; Virgili et al. 2020)
Unguicaris panglaonis Marin & Chan, 2006		Crinoid *Dichrometra palmata* *Stephanometra indica*	Commensal	(Marin and Chan 2006; Marin 2009; Gan et al. 2015; Kou et al. 2015; Chow et al. 2021)
Unguicaris pilipes (Bruce & Zmarzly, 1983)	*Periclimenes pilipes*	Crinoid *Comanthina schlegeli*	Commensal	(Zmarzly 1984; Fujita and Baba 1999; Marin and Chan 2006; Marin 2009; Gan et al. 2015; Park et al. 2019)

Table S1

Symbiont Genus : Zenopontonia				
Zenopontonia noverca (Kemp, 1922)	*Periclimenes noverca*	Asteroid *Pentaceraster mammillatus; P. tuberculatus* *Poraster superbus* *Protoreaster lincki*	Commensal	(Bruce 1974a, 1976a; Horká 2017)
Zenopontonia rex (Kemp, 1922)	*Periclimenes imperator; P. rex*	Holothuroid *Actinopyga bannwarthi* *Bohadschia argus* *Euapta godeffroyi* *Holothuria fuscopunctata; H. scabra* *Opheodesoma spectabilis* *Stichopus chloronotus; S. horrens* *Synapta maculata* *Thelenota ananas: T. anax* Asteroid *Euretaster insignis* *Gomophia egyptiaca* Other Gasteropoda	Generalist commensal	(Bruce 1974a, 1976b; Fransen and Goud 1999; Antokhina and Britayev 2012; Antokhina et al. 2012; Fransen and Hoeksema 2014; Gan et al. 2015; Horká et al. 2016; Horká 2017; Chow et al. 2021; Hamel et al. 2022)

Table S1

Zenopontonia soror (Nobili, 1904)	*Periclimenes bicolor* *Periclimenes frater* *Periclimenes parasiticus* *Periclimenes soror*	Asteroid *Acanthaster planci; A. brevispinus* *Asteropsis carinifera* *Astrometis sertulifera* *Choriaster granulatus* *Culcita noveaguineae; C. schmideliana* *Echinaster luzonicus; E. purpureus* *Euretaster insignis* *Halityle regularis* *Linckia laevigata; L. multifora* *Mithrodia clavigera; M. bradleyi* *Nardoa frianti* *Nidorellia armata* *Pentaceraster cumingi; P. horridus; P. mammillatus; P. regulus; P. tuberculatus* *Pharia pyramidata* *Phataria unifascialis* *Plectaster decanus* *Protoreaster lincki; P. nodosus*	Obligate commensal Class-specific but genus/ species-generalist	(Castro 1971b; Bruce 1974a, 1976b, a, 1982; Takeda et al. 1976; Wear 1976; Wicksten and Hendrycks 1986; 英男 and 喬司 1988; Poupin 1998; Guenther et al. 2007; Crandall et al. 2008; Li 2008; Antokhina and Sorokin 2010; Antokhina and Britayev 2012, 2020; Antokhina et al. 2012; Olliff 2013; Gan et al. 2015; Kou et al. 2015; Horká et al. 2016; Schelvis 2017; Horká 2017; Salas-Moya et al. 2021; Chow et al. 2021; De Grave et al. 2021; Tseng et al. 2022; Lourtie et al. 2023)
Symbiont Family: Palinuridae Symbiont Genus: Panulirus				
Panulirus argus (Latreille, 1804)	*Palinurus americanus; P. argus; P. ricordi; P. argus westoniie*	Echinoid *Diadema antillarum*	Commensal	(Davis 1971; Hayes et al. 1998; Hayes 2007; Ghory et al. 2018)

Table S1

Symbiont Family: Percnidae Symbiont Genus: Percnon				
Percnon gibbesi (H. Milne Edwards, 1853)	*Acanthopus gibbesi* *Lonchophorus anceps* *Plagusia delaunayi* *Zoea boscii*	Echinoid *Diadema antillarum*	Commensal	(Hayes et al. 1998, 2022; Thiel et al. 2003; Hayes 2007; Cannicci et al. 2008; Saravanan et al. 2015; Ghory et al. 2018)
Symbiont Family: Pilumnidae Symbiont Genus: Cebudonus (monotypic)				
Cebudonus poppeorum Ng, 2014		Echinoid Unspecified	Suggest as obligate symbionts of Echinoid	(Ng 2014; Castro 2015)
Symbiont Genus: Ceratocarcinus				
Ceratocarcinus frontodentata (Chen & Dai in Shen, Dai & Chen, 1982)	*Harrovia frontodentata*	Crinoid Unknown, possibly comatulid Crinoid	Suggest as obligate symbionts of Crinoid	(Chen and Dai 1982; Castro 2015)
Ceratocarcinus longimanus White, 1847	*Ceratocarcinus dilatatus ; C. intermedius; C. speciosus*	Crinoid *Anneissia bennetti* *Capillaster multiradiatus* *Clarkcomanthus* sp. *Comanthus gisleni; C. parvicirrus* *Comaster schlegelii* *Comatella nigra; C. stelligera* *Comatula purpurea* *Himerometra robustipinna* *Zygometra* sp.	Obligate symbiont	(Serène et al. 1957; Števčić et al. 1988; Fabricius and Dale 1993; Castro et al. 1995; Chia and Ng 1998; Deheyn et al. 2006; Castro 2015; Nigam et al. 2020; Virgili et al. 2020)
Ceratocarcinus trilobatus (T. Sakai, 1938)	*Harrovia trilobata*	Crinoid *Comanthus* sp.	Obligate symbiont	(Lanchester 1900; Sakai 1938; Serène et al. 1957; Chia and Ng 1998; Castro 2015)

Table S1

Symbiont Genus : Echinoecus				
Echinoecus pentagonus (A. Milne-Edwards, 1879)	*Echinoecus convictor; E. klunzingeri; E. pentagonus ; E. petiti ; E. rathbunae Eumedon convictor; E. pentagonus; E. petiti Liomedon pentagonus*	Echinoid *Diadema savignyi; D. setosum Echinothrix calamaris; E. diadema Heliocidaris crassispina Heterocentrotus mamillatus Pseudocentrotus depressus Phyllacanthus dubius*	Parasite for males and small immature females Commensal for large mature females Obligate symbiont	(Bouvier and Seurat 1905; Mortensen 1935; Miyake 1939; Serène et al. 1957; Sakai 1965; Castro 1971a, 1978, 2015; Suzuki and Takeda 1974; Sastry 1977, 1981; Jangoux 1987; Gherardi 1991; VandenSpiegel et al. 1998; Ng and Jeng 1999; Coopard and Campbell 2004; Limviriyakul et al. 2016; Nigam et al. 2020)
Echinoecus nipponicus Miyake, 1939	*Echinoecus petiti nipponicus*	Echinoid *Heliocidaris crassispina*	Obligate symbiont	(Miyake 1939; Ng and Jeng 1999; Castro 2015)
Echinoecus sculptus (Ward, 1935)	*Proechinoecus sculptus*	Echinoid *Colobocentrotus (Podophora) atratus*	Obligate symbiont	(Ward 1934; Serène et al. 1957; Serène and Romimohtarto 1963; Ng and Jeng 1999; Castro 2015)
Symbiont Genus : Eumedonus				
Eumedonus brevirhynchus Chia & Ng, 1999		Echinoid Suggest as obligate symbionts of Echinoid	Obligate symbiont	(Chia et al. 1999; Castro 2015)
Eumedonus intermedius Chia & Ng, 1999		Echinoid Suggest as obligate symbionts of Echinoid	Obligate symbiont	(Chia et al. 1999; Castro 2015)
Eumedonus niger H. Milne Edwards, 1834		Echinoid *Goniocidaris (Petalocidaris) biserialis*	Obligate symbiont	(Chia et al. 1999; Castro 2015; Ng and Lin 2021)

Table S1

Eumedonus vicinus Rathbun, 1918		Echinoid *Colobocentrotus (Podophora) atratus*	Obligate symbiont	(Chia et al. 1999; Castro 2015)
Eumedonus zebra Alcock, 1895		Echinoid Unspecified	Suggest as obligate symbionts of Echinoid	(Alcock 1895; Chia et al. 1999; Castro 2015; Viswanathan et al. 2020)
Symbiont Genus: Gonatonotus				
Gonatonotus granulosus (MacGilchrist, 1905)	*Eumedonus granulosus*	*Echinoid* Unspecified	Suggest as obligate symbionts of Echinoid	(Chia et al. 1999; Castro 2015)
Gonatonotus nasutus Chia & Ng, 1999		Echinoid (primary host) *Echinothrix calamaris* *Echinometra mathaei* *Eucidaris metularia* *Phyllacanthus imperialis ; P. parvispinus* *Plococidaris verticillate* *Prionocidaris baculosa* *Toxopneustes pileolus* Holothuroid (occasional host) *Holothuria (Halodeima) atra* *Thelenota anax* Asteroid (occasional host) *Choriaster granulatus*	Obligate symbiont	(Chia et al. 1999; Castro 2015; Endo and Naruse 2016; Limviriyakul et al. 2016; Ng and Lin 2021)
Gonatonotus pentagonus White, 1847		Echinoid Unspecified	Suggest as obligate symbionts of Echinoid	(Chia et al. 1999; Castro 2015)

Table S1

Symbiont Genus: Hapalonotus (monotypic)				
Hapalonotus pinnotheroides (Guérin-Méneville, 1838)	*Cyclocarcinus pinnotheroides* *Hapalonotus reticulatus* *Malacosoma reticulatus*	Holothuroid *Holothuria scabra*	Obligate parasite	(VandenSpiegel et al. 1992; Chia et al. 1999; Hamel et al. 1999, 2022; Castro 2015)
Symbiont Genus: Harrovia				
Harrovia albolineata Adams & White, 1849	Sometimes misidentified as *Harrovia elegans*	Crinoid *Comatula purpurea* *Comaster gracilis; C. multifidus* *Lamprometra spp.*	Obligate (protective) mutualist	(Lanchester 1900; Serène et al. 1957; Garth 1964; Sakai 1976; James 1983; Lim and Ng 1988; Castro 1989, 2015; Ng and Lim 1990; Chia et al. 1993; Fabricius and Dale 1993; Chia and Ng 1998; VandenSpiegel et al. 1998; Ng and Jeng 1999; Kazmi 2016; Virgili et al. 2020)
Harrovia cognata Chia & Ng, 1998		Crinoid *Comatulid Crinoid*	Obligate symbiont	(Chia and Ng 1998; Castro 2015)
Harrovia elegans de Man, 1887		Crinoid *Comanthus japonicus; C. parvicirrus; C. wahlbergii* *Lamprometra* sp.	Obligate symbiont	(Sakai 1938, 1976; Serène et al. 1957; Castro 1989, 2015; Chia and Ng 1998; Ng and Jeng 1999; Nigam et al. 2020)
Harrovia japonica Balss, 1921		Crinoid *Comatulid Crinoid*	Obligate symbiont	(Lee and Ko 2009; Castro 2015)

Table S1

Harrovia longipes Lanchester, 1900	*Harrovia albolineata longipes* *Harrovia plana*	Crinoid *Comaster multifidus* *Comissia* sp. *Dichrometra palmata* *Phanogenia gracilis; P. multibrachiata; P. typica*	Obligate commensal	(Hartlaub 1891; Lanchester 1900; Serène et al. 1957; Castro 1989, 2015; Chia et al. 1993; Chia and Ng 1998; Fujita and Baba 1999; Ng and Jeng 1999; Fujita and Shokita 2003; Deheyn et al. 2006; Hui 2012)
Harrovia ngi Chen & Xu, 1992		Crinoid *Comatulid Crinoid*	Obligate symbiont	(Castro 2015)
Harrovia tuberculate Haswell, 1880		*Crinoid* *Actinometra multiradiata*	Obligate symbiont	(Lanchester 1900; Serène et al. 1957; Ng and Jeng 1999; Castro 2015)
Symbiont Genus: Permanotus (monotypic)				
Permanotus purpureus (Gordon, 1934)	*Harrovia bituberculata; H. purpurea*	Crinoid *Comanthus benetti* *Comaster schlegelii* *Phanogenia gracilis* *Himerometroidea* sp.	Obligate commensal	(Gordon 1934; Holthuis 1953; Castro 1989, 2015; Chia and Ng 1998; Fujita and Baba 1999; Ng and Jeng 1999; Huang et al. 2005a; Deheyn et al. 2006; Prakash and Marimuthu 2020; Mariyambi et al. 2020; Virgili et al. 2020)
Symbiont Genus: Rhabdonotus				
Rhabdonotus pictus A. Milne-Edwards, 1879		Crinoid *Cyllometra manca*	Obligate symbiont	(Serène and Romimohtarto 1963; Stevcic 1991; Chia and Ng 1995; Castro 2015)
Rhabdonotus pilipes Chia & Ng, 1995		Crinoid *Heterometra crenulata*	Obligate symbiont	(Chia and Ng 1995; Castro 2015)

Table S1

Rhabdonotus xynon Chia & Ng, 1995		Crinoid *Comatulid Crinoid*	Obligate symbiont	(Chia and Ng 1995; Castro 2015)
Symbiont Genus: Tauropus (monotypic)				
Tauropus egeriae (Gordon, 1947)	*Harrovia egeriae*	Echinoid Unspecified	Suggest as obligate symbionts of Echinoid	(Gordon 1947; Castro 2015)
Symbiont Genus: Tiaramedon (monotypic)				
Tiaramedon spinosum (Miers, 1879)	*Ceratocarcinus spinosus*	Crinoid *Clarkcomanthus littoralis* *Comanthus gisleni; C. parvicirrus; C. suavia* *Comatella nigra* *Decametra chadwicki* *Dichrometra palmata* *Heterometra savignii.* *Stephanometra indica*	Obligate commensal	(Sakai 1938; Serène et al. 1957; Fishelson 1974; Števčić et al. 1988; Chia and Ng 1998; Ng and Jeng 1999; Nigam et al. 2020; Mariyambi et al. 2020)
Symbiont Genus: Zebrida				
Zebrida adamsii White, 1847	*Acanthonyx zebrida* *Zebrida paucidentata*	Echinoid *Acanthocidaris* sp. *Asthenosoma ijimai* *Diadema setosum* *Heliocidaris crassispina* *Pseudocentrotus depressus* *Salmacis bicolor; S. sphaeroides; S. virgulata* *Toxopneustes elegans; T. pileolus* *Tripneustes gratilla*	Obligate parasite	(Serène et al. 1957; Serène and Romimohtarto 1963; Suzuki and Takeda 1974; Takeda et al. 1976; Yanagisawa and Hamaishi 1986; Jangoux 1987; Ng and Chia 1999; Thiel et al. 2003; Britayev et al. 2013; Saravanan et al. 2015)
Zebrida brevicarinata Ng & Chia, 1999		Echinoid Unspecified	Suggest as obligate symbionts of Echinoid	(Ng and Chia 1999; Castro 2015)
Zebrida longispina Haswell, 1880		Echinoid Unspecified	Suggest as obligate symbionts of Echinoid	(Ng and Chia 1999; Castro 2015)

Table S1

Symbiont Genus: Zebridonus (monotypic)				
Zebridonus mirabilis Chia, Ng & Castro, 1995		Echinoid Unspecified	Suggest as obligate symbionts of Echinoid	(Castro 2015)
Symbiont Family: Pinnotheridae Symbiont Genus: Alain				
Alain crosnieri R.B. Manning, 1998		Holothuroid *Molpadia sp.*	Symbiont	(Manning 1998; Ng and Manning 2003; Castro 2015; de Gier and Becker 2020)
Alain raymondi Ahyong & P.K.L. Ng, 2008		Holothuroid Unidentified deep-water holothurian	Commensal	(Ahyong and Ng 2008; Castro 2015; de Gier and Becker 2020; Hultgren et al. 2022)
Symbiont Genus: Austrotheres				
Austrotheres holothuriensis (Baker, 1907)	*Ostracotheres holothuriensis* *Pinnotheres holothuriensis*	Other (Primary host) Ascidia (*Herdmania grandis* and *H. momus*) Holothuroid (questionable) Unidentified	Suggest as facultative holothurian associate (questionable)	(Baker 1907; Ng and Manning 2003; Ahyong 2018; de Gier and Becker 2020)
Symbiont Genus: Buergeres				
Buergeres choprai Ahyong & PKL Ng, 2020		Holothuroid *Actinopyga echinites*	Symbiont	(Ahyong and Ng 2020; de Gier and Becker 2020)

Table S1

Buergeres deccanensis (Chopra, 1931)	*Pinnotheres deccanensis; P. decanus*	Holothuroid *Holothuria scabra*	Female are obligate parasite Male are probably free-living	(Chopra 1931; Jones and Mahadevan 1965; Jangoux 1987; James 1995; Ng and Manning 2003; Eeckhaut et al. 2003; Pinn et al. 2014; Castro 2015; Hamel et al. 2019, 2022; de Gier and Becker 2020; Hultgren et al. 2022)
Buergeres holothuriae (Semper, 1880)	*Pinnotheres holothuriae*	Holothuroid *Stichopus horrens*	Symbiont	(Semper 1880; Ng and Manning 2003; Castro 2015; de Gier and Becker 2020)
Buergeres ortmanni (Bürger, 1895)	*Buergeres tenuipes Pinnotheres ortmanni; P. tenuipes*	Holothuroid *Holothuria (Stauropora) fursocinerea*	Symbiont	(Ng and Manning 2003; Ahyong and Ng 2007, 2020; Castro 2015; de Gier and Becker 2020)
Symbiont Genus: Calyptraeotheres				
Calyptraeotheres sp.		Asteroid *Asteropsis carinifera*	Symbiont (poorly studied)	(Salas-Moya et al. 2021)
Symbiont Genus: Clypeasterophilus				
Clypeasterophilus juvenilis (Bouvier, 1917)	*Dissodactylus alcocki; D. juvenilis*	Echinoid *Clypeaster subdepressus* *Meoma ventricosa*	Symbiont	(Bouvier 1917; Rathbun 1918; Campos and Griffith 1990; Castro 2015; de Gier and Becker 2020; Hultgren et al. 2022)

Table S1

Clypeasterophilus rugatus (Bouvier, 1917)	*Dissodactylus calmani; D. rugatus*	Echinoid *Clypeaster rosaceus* *Encope michelini*	Parasite	(Bouvier 1917; Telford 1982; Jangoux 1987; Campos and Griffith 1990; Reeves and Brooks 2001; Castro 2015; de Gier and Becker 2020; Hultgren et al. 2022)
Clypeasterophilus stebbingi (Rathbun, 1918)	*Dissodactylus stebbingi*	Echinoid *Clypeaster subdepressus*	Species-specific (protective) parasite or commensal	(Rathbun 1918; Campos and Griffith 1990; Martinelli Filho et al. 2014; Castro 2015; de Gier and Becker 2020; Hultgren et al. 2022)
Clypeasterophilus ususfructus (H. Griffith, 1987)	*Dissodactylus ususfructus*	Echinoid *Clypeaster europacificus; C. speciosus*	Symbiont	(Griffith 1987; Campos and Griffith 1990; Campos et al. 1992, 2009; de Gier and Becker 2020)
Symbiont Genus: Dissodactylus				
Dissodactylus crinitichelis Moreira, 1901	*Dissodactylus encopei*	Echinoid *Clypeaster rosaceus; C. subdepressus* *Encope emarginata; E. michelini* *Leodia sexiesperforata* *Mellita quinquiesperforata* *Meoma ventricosa*	Parasite or commensal	(Moreira 1901; Telford 1978, 1982; Bell 1984; Jangoux 1987; Bishop and Portell 1989; Reeves and Brooks 2001; Thiel et al. 2003; Wirtz et al. 2009; Lima et al. 2014; Martinelli Filho et al. 2014; Saravanan et al. 2015; de Gier and Becker 2020)

Table S1

Dissodactylus glasselli Rioja, 1944		Echinoid *Clypeaster europacificus* *Encope micropora; E. californica* *Lanthonia grantii; L. longifissa* *Mellita kanako*	Parasite	(Jangoux, 1987; de Gier and Becker, 2020)
Dissodactylus latus H. Griffith, 1987		Echinoid *Clypeaster subdepressus* *Encope michelini* *Leodia sexiesperforata*	Symbiont	(Griffith 1987; Lima et al. 2014; de Gier and Becker 2020; Hultgren et al. 2022)
Dissodactylus lockingtoni Glassell, 1935	*Dissodactylus smithi*	Echinoid *Encope californica; E. grandis; E. micropora* *Lanthonia grantii; L. longifissa* *Leodia sexiesperforata* *Mellita kanako*	Parasite or commensal	(Telford 1978; Jangoux 1987; Griffith 1987; Campos et al. 2009; Pohle 2011; Campos 2017; de Gier and Becker 2020)
Dissodactylus mellitae (Rathbun, 1900)		Echinoid *Mellita quinquiesperforata (primary host)* *Clypeaster subdepressus* *Echinarachnius parma* *Encope michelini* *Leodia sexiesperforata* *Mellita isometra; M. tenuis*	Parasite or commensal	(Gray et al. 1968; Ache and Davenport 1972; Bell and Stancyk 1983; Bell 1984; Jangoux 1987; Bishop and Portell 1989; Gherardi 1991; VandenSpiegel et al. 1998; Thiel et al. 2003; Lima et al. 2014; Saravanan et al. 2015; Brasseur et al. 2018; de Gier and Becker 2020; Hultgren et al. 2022)

Table S1

Dissodactylus nitidus Smith, 1870	*Dissodactylus meyerabichi*	Echinoid *Encope grandis; E. micropora; E. californica* *Mellitella stokesii* ~~*Lanthonia longifissa*~~ (removed)	Parasite	(Glassell 1936; Jangoux 1987; Campos et al. 2009; Pohle 2011; Campos 2017; de Gier and Becker 2020; Salas-Moya et al. 2021; Hultgren et al. 2022)
Dissodactylus primitivus Bouvier, 1917	*Dissodactylus borradailei*	Echinoid *Meoma ventricosa* *Plagiobrissus grandis*	Parasite	(Bouvier 1917; Telford 1982; Jangoux 1987; Thiel et al. 2003; De Bruyn et al. 2009, 2010, 2011; Jossart et al. 2013, 2017, 2020; Lima et al. 2014; Saravanan et al. 2015; Bruyn et al. 2016; de Gier and Becker 2020; Caulier et al. 2023)
Dissodactylus xantusi Glassell, 1936		Echinoid *Encope grandis; E. micropora* *Lanthonia longifissa* *Mellitella stokesii*	Parasite or commensal	(Glassell 1936; Telford 1978; Jangoux 1987; Campos et al. 2009; Pohle 2011; de Gier and Becker 2020)
Symbiont Genus: Glassella				
Glassella leptosynaptae (Wass, 1968)	*Pinnixa leptosynaptae*	Holothuroid *Leptosynapta crassipatina*	Commensal	(Wass and University 1968; de Gier and Becker 2020)
Symbiont Genus: Holotheres				
Holotheres danielae Ahyong, 2010		Holothuroid *Acaudina molpadioides*	Symbiont	(Ahyong 2010; Castro 2015; de Gier and Becker 2020)
Holotheres flavus (Nauck, 1880)	*Pinnotheres flavus*	Holothuroid Unidentified holothuroid	Obligate symbiont	(Ng and Manning 2003; Castro 2015; de Gier and Becker 2020)

Table S1

Holotheres halingi (Hamel, P.K.L. Ng & Mercier, 1999)	*Pinnotheres halingi*	Holothuroid *Holothuria scabra*	Obligate parasite	(Hamel et al. 1999, 2019, 2022; Eeckhaut 2003; Ng and Manning 2003; Castro 2015; de Gier and Becker 2020)
Holotheres semperi (Bürger, 1895)	*Pinnotheres semperi*	Holothuroid *Holothuria scabra* *Holothuria (Stauropora) fursocinerea*	Obligate symbiont	(Lanchester 1900; Ng and Manning 2003; Ahyong and Ng 2007; Castro 2015; Hamel et al. 2019, 2022; de Gier and Becker 2020)
Holotheres setnai (Chopra, 1931)	*Pinnotheres setnai*	Holothuroid *Actinopyga mauritiana*	Parasite	(Chopra 1931; James 1983; Jangoux 1987; Ng and Manning 2003; Castro 2015; de Gier and Becker 2020)
Symbiont Genus: Holothuriophilus				
Holothuriophilus pacificus (Poeppig, 1836)	*Leucosia pacifica* *Pinnaxodes meinerti* *Pinnoteres Silvestrii*	Holothuroid *Athyonidium chilensis*	Symbiont	(Ng and Manning 2003; Castro 2015; Campos 2017; de Gier and Becker 2020; Hultgren et al. 2022; Cortés-Carrasco et al. 2022)
Holothuriophilus trapeziformis Nauck, 1880		Holothuroid *Holothuria (Halodeima) inornate; H. kefersteinii* *Holothuria (Selenkothuria) lubrica*	Parasite	(Ahyong and Ng 2007; Campos et al. 2012; Castro 2015; de Gier and Becker 2020; Cortés-Carrasco et al. 2022)

Table S1

Symbiont Genus: Opisthopus (monotypic)				
Opisthopus transversus Rathbun, 1894	*Pinnotheres nudus*	Holothuroid (occasional) *Apostichopus californicus; A. parvimensis* *Caudina sp.* Other *Polyplacophora; Gastropoda; Bivalvia; Polychaeta*	Occasional facultative parasite on holothurians	(Rathbun 1894; Jones and Mahadevan 1965; Jangoux 1987; Ng and Manning 2003; Castro 2015; Ahyong 2018; de Gier and Becker 2020)
Symbiont Genus: Pinnaxodes				
Pinnaxodes bipunctatus (Nicolet in Gay, 1849)	*Pinnotheres bipunctatum*	Echinoid Unknown, suggest as sea-urchins associate	Suggest as symbiont	(Gay and Johnston 1849; Campos 2017; de Gier and Becker 2020)
Pinnaxodes chilensis (H. Milne Edwards, 1837)	*Pinnaxodes hirtipes* *Pinnotheres chilensis* Often identified *Fabia chilensis*	Echinoid *Caenocentrotus gibbosus* *Loxechinus albus* *Tetrapygus niger*	Parasite or commensal	(Jangoux 1987; Lardies and Castilla 2001; Rojas and Bunster 2011; Campos 2017; Gonzalez-Canales et al. 2018; de Gier and Becker 2020; Hultgren et al. 2022)
Pinnaxodes floridensis H.W. Wells & M.J. Wells, 1961		Holothuroid *Holothuria princeps*	Commensal	(Wells and Wells 1961; de Gier and Becker 2020)
Pinnaxodes major Ortmann, 1894		Holothuroid *Holothuria hilla* Others Bivalvia	Symbiont	(Ng and Manning 2003; Jiang and Liu 2011; de Gier and Becker 2020)

Table S1

Symbiont Genus: Pinnixa				
Pinnixa barnharti Rathbun, 1918		Holothuroid *Caudina arenícola* Others Polychaeta *Urechis unicinctus*	Obligate Parasite	(Rathbun 1918; Jones and Mahadevan 1965; Jangoux 1987; Zmarzly 1992; Campos and Campos 2012; Marin 2016)
Pinnixa huffmani Glassell, 1935		Holothuroid *Thyone* sp.	Symbiont (poorly studied)	(Griffith 1987)
Pinnixa tumida Stimpson, 1858	*Pinnixia tumida*	Holothuroid *Caudina arenicola* *Paracaudina chilensis*	Parasite	(Jones and Mahadevan 1965; Jangoux 1987; Takeda et al. 1997; Marin 2018)
Symbiont Genus: Trichobezoares				
Trichobezoares pilumnoides (Nobili, 1906)	*Pinnotheres pilumnoides*	Holothuroid *Holothuria scabra*	Symbiont	(Nobili 1906; Ng 2018; de Gier and Becker 2020)
Trichobezoares villosissimus (Doflein, 1904)	*Holotheres villosissimus* *Pinnotheres villosissimus*	Holothuroid *Actinopyga mauritiana; A. lecanora*	Parasite	(Chopra 1931; Jangoux 1987; VandenSpiegel and Jangoux 1989; Ng and Manning 2003; Castro 2015; Ng 2018; de Gier and Becker 2020)
Symbiont Family: Porcellanidae Symbiont Genus: Allopetrolisthes				
Allopetrolisthes spinifrons (H. Milne Edwards, 1837)	*Porcellana spinifrons*	Others Sea anemone (Primary host); Gasteropod Asteroid: *Heliaster helianthus* *Meyenaster gelatinosus*	Commensal Occasional young specimens on asteroids	(Milne-Edwards 1837; Baeza et al. 2001, 2002; Baeza and Stotz 2001; Hiller et al. 2010)

Table S1

Symbiont Genus: Clastotoechus				
Clastotoechus gorgonensis Werding & Haig, 1983		Echinoid *Echinometra vanbrunti*	Commensal	(Werding and Haig 1982; Schoppe and Werding 1997; Harvey 1999; Lazarus et al. 2004; Vallejo and Monroy 2012)
Clastotoechus vanderhorsti (Schmitt, 1924)	*Madarateuchus vanderhorsti* *Petrolisthes vanderhorsti*	Echinoid *Echinometra lucunter*	Commensal	(Werding and Haig 1982; Schoppe and Werding 1997; Harvey 1999; Thiel et al. 2003; Vallejo and Monroy 2012; Saravanan et al. 2015)
Symbiont Genus: Liopetrolisthes				
Liopetrolisthes mitra (Dana, 1852)	*Porcellana mitra; P. pulchelulla; P. spinosa*	Echinoid *Tetrapygus niger*	Symbiont	(Weber and Galelguillos 1991; Baeza and Thiel 2000; Thiel and Baeza 2001; Thiel et al. 2003; Baeza 2007; Saravanan et al. 2015)
Liopetrolisthes patagonicus (Cunningham, 1871)	*Porcellana patagonica*	Echinoid *Loxechinus albus*	Symbiont (poorly studied)	(Weber and Galelguillos 1991)
Symbiont Genus: Lissoporcellana				
Lissoporcellana sp. Haig, 1978		Crinoid *Himerometra robustipinna*	Symbiont (poorly studied)	(Britayev et al. 2016)

Table S1

Symbiont Genus: Pachycheles				
Pachycheles biocellatus (Lockington, 1878)	*Petrolisthes (Pisosoma) biocellatus* *Petrolisthes gibbosicarpus* *Pisosoma aphrodita*	Asteroid *Asteropsis carinifera*	Symbiont (poorly studied)	(Salas-Moya et al. 2021)
Symbiont Genus: Petrolisthes				
*Petrolisthes galathinus (*Bosc, 1801)	*Petrolisthes brasiliensis* *Porcellana danae; P. egragia; P. galathina; P. sexspinosa*	Echinoid *Echinometra mathaei* Free-living In the littoral, under stones and among sponges and corals.	Free-living, maybe occasionally facultative ectosymbiont of Echinoid	(Lazarus et al. 2004; Hayes 2007)
Petrolisthes sanfelipensis Glassell, 1936	*Petrolisthes felipensis*	Echinoid *Centrostephanus coronatus* *Diadema mexicanum* *Eucidaris thouarsii*	Symbiont underneath the spines of Echinoid	(Hayes et al. 2022)
Petrolisthes myakkensis Bishop & Portell, 1989 (Fossil)		Echinoid *Heliaster microbrachius*	Suggest as ecto-commensal of fossil echinoderms (the crab/asteroid association is preserved in calcareous quartzitic sandstone)	(Bishop and Portell 1989; Gašparič et al. 2015)
Petrolisthes virgatus Paulson, 1875		Echinoid *Echinometra mathaei* Free-living In corals, rock crevices in the rocky-cobble shores of the low intertidal zone	Free-living, and occasionally facultative ectosymbiont of echinoderms	(Nakasone and Miyake 1972; Limviriyakul et al. 2016)

Table S1

Symbiont Genus: Pisidia				
Pisidia serratifrons (Stimpson, 1858)	*Porcellana serratifrons; P. spinulifrons*	Crinoid *Tropiometra macrodiscus*	Symbiont (poorly studied)	(Morton and Mladenov 1992)
Pisidia dispar (Stimpson, 1858)	*Polyonyx carinatus Porcellana dispar; P. rostrata*	Crinoid *Tropiometra macrodiscus*	Symbiont (poorly studied)	(Morton and Mladenov 1992)
Symbiont Family: Portunidae Symbiont Genus: Lissocarcinus				
Lissocarcinus orbicularis Dana, 1852	*Lissocarcinus pulchellus*	Holothuroid *Actinopyga echinites; A. mauritiana; A. obesa.* *Bohadschia argus; B. subrubra; B. vitiensis* *Holothuria scabra* *Holothuria (Halodeima) atra Holothuria (Microthele) fuscogilva* *Holothuria (Mertensiothuria) isuga* *Holothuria (Metriatyla) lesson* *Holothuria (Stauropora) pervicax* *Holothuria (Microthele) whitmaei* *Stichopus chloronotus ; S. horrens* *Thelenota ananas; T.anax*	Obligate parasite or commensal	(Chopra 1931; Crosnier 1962; Trott and Garth 1970; James 1983, 1995; Jangoux 1987; Ng and Jeng 1999; Spiridonov 1999; Vannini and Innocenti 2000; Eeckhaut et al. 2003; Ayotte and DeMaintenon 2005; Caulier et al. 2012, 2013, 2014; Pinn et al. 2014; Castro 2015; Horká et al. 2016; Brasseur et al. 2018; Nigam et al. 2020; Hamel et al. 2022)
Lissocarcinus ornatus Chopra, 1931		Holothuroid *Holothuria sp.* (describe as a large black Holothurian)	Parasite or commensal	(Chopra 1931; Jangoux 1987)

Table S1

Symbiont Family: Rhynchocinetidae Symbiont Genus: Rhynchocinetes				
Rhynchocinetes brucei Okuno, 1994		Echinoid *Diadema setosum* Other *Anthozoa* Free-living In crevice	Free-living, generalist facultative ectosymbiont of echinoderms during daytime	(Dumont et al. 2010; Ory et al. 2013)
Symbiont Family: Spongicolidae Symbiont Genus: Microprosthema				
Microprosthema sp. Stimpson, 1860		Crinoid Unspecified, maybe misidentified as *Microprosthema sp.* instead of *Odontozona crinoidicola*, because of the body form	Symbiont (poorly studied)	(Deheyn et al. 2006; Saito and Fujita 2009)
Symbiont Family: Stenopodidae Symbiont Genus: Odontozona				
Odontozona crinoidicola Saito & Fujita, 2009		Crinoid *Phanogenia gracilis*	Symbiont	(Saito and Fujita 2009; Saito et al. 2017)

Table S1

Symbiont Genus: Stenopus				
Stenopus hispidus (Olivier, 1811)		Echinoid *Diadema antillarum* Free-living In crevice	Free living and occasionally associated with echinoderms	(Hayes 2007; Tavares et al. 2017; Ghory et al. 2018)
Symbiont Family: Sternostylidae Symbiont Genus: Sternostylus				
Sternostylus salvadori (Rice & Miller, 1991)	*Gastroptychus salvadori*	Asteroid *Novodinia antillensis*	Symbiont (poorly studied)	(Rice and Miller 1991)
Symbiont Family: Thoridae Symbiont Genus: Thor				
Thor amboinensis (de Man, 1888)	*Hippolyte amboinensis* *Thor discosomatis*	Others *Cnidarians (primary host)* Crinoid *Comactinia echinoptera (unusual association)*	Generalist parasite	(Criales 1984; Khan et al. 2004; Tavares et al. 2017)
Thor manningi Chace, 1972		Crinoid *Nemaster grandis* Others *Macroalgae; Hydroids; Corals; Sea anemones* Free-living In seagrass flats, dead corals, clumps of corraline algae	Free-living and facultative generalist parasite	(Criales 1984; Lindberg and Stanton 1988; Tavares et al. 2017; Romero-RodrÍguez and Álvarez 2020)

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Table S2 Summary of the symbiotic decapod crustaceans known to symbiotically interact with echinoderm hosts and their biogeographic distribution based on WORMS (WoRMS Editorial Board 2023).

Species	Distribution	Marine Regions	Climate Zone
Alpheus cristulifrons Rathbun, 1900	• Antigua and Barbuda • Barbados • Caribbean Sea • Dominica • Grenada • Gulf of Mexico • Saint Kitts and Nevis • Trinidad and Tobago • Venezuela • Other (Guana island, Lesser Antilles and Virgin Gorda)	31 34 47	Tropical and Subtropical
Alpheus serenei Tiwari, 1964	• Arabian sea • Mozambic channel • South China sea • Indonesian sea • Timor and Coral sea	51 57 71	Tropical
Alpheus splendidus Coutière, 1897	• Reunion Island • South China sea • Indonesian sea • Coral sea	51 57 71	Tropical
Arete acanthocarpus (Miya & Miyake, 1968)	Unspecified	NA	NA
Arete amboinensis de Man, 1910	Unspecified	NA	NA
Arete dorsalis Stimpson, 1860	• Indian Ocean • Mozambique Channel • South Pacific Ocean • Other (New Caledonia)	51	Tropical and South-Subtropical
Arete indicus Coutière, 1903	• Indian Ocean • Mozambique • New Zealand • Red Sea • South Pacific Ocean	51 57 71 81	Tropical and South-Subtropical
Athanas anatidactylus Anker & Marin, 2007	Unspecified	NA	NA

Athanas areteformis Coutière, 1903	• Indian Ocean • South Pacific Ocean	51 71	Tropical
Athanas ornithorhynchus Banner & Banner, 1973	Unspecified	NA	NA
Athanas parvus De Man, 1910	• Red sea • Arabian sea • Mozambic channel • South China sea • Indonesian sea • Australian sea • New Caledonia sea	51 57 71 81	Tropical and South-Subtropical
Betaeus macginitieae J.F.L. Hart, 1964	Unspecified	NA	NA
Synalpheus carinatus (de Man, 1888)	• South China sea • Indonesian sea • Coral sea	71	Tropical
Synalpheus comatularum (Haswell, 1882)	• Indian sea • Timor sea	57	Tropical
Synalpheus demani Borradaile, 1900	• New Caledonian • Indonesian sea • Coral sea	57 71	Tropical
Synalpheus odontophorus de Man, 1909	• South China sea • Indonesian sea	71	Tropical
Synalpheus stimpsoni (de Man, 1888)	• Indian Ocean • Others (New Caledonian and West Pacific) • Red sea • Mozambic Channel • South China sea • Indonesian sea • Timor and Coral sea • Hawaiian Islands	51 57 71	Tropical and South-Subtropical
Synalpheus tijou Banner & Banner, 1982	• Indonesian sea • Coral sea	71	Tropical

Synalpheus townsendi Coutière, 1909	• Antigua and Barbuda • Barbados • Caribbean Sea • Dominica • Grenada • Gulf of Mexico • Saint Kitts and Nevis • Trinidad and Tobago • Venezuela • Others (Lesser Antilles, Saint Thomas, Tortola, Virgin Gorda) • South Atlantic Ocean • Gulf of Califrornia • Indian sea	31 41 (77) (57)	Tropical and North-Subtropical
Synalpheus tropidodactylus Banner & Banner, 1975	• Indonesian sea	71	Tropical
Calcinus californiensis Bouvier, 1898	• Gulf of Califrornia	77	North-Subtropical
Uroptychodes barunae Baba, 2004	• Indonesian sea	71	Tropical
Uroptychodes grandirostris (Yokoya, 1933)	• Tosa Bay (Japan) • Timor sea	61 57	Tropical and North-Subtropical
Uroptychus babai Ahyong & Poore, 2004	• New South Wales (Australia) • Papua New Guinea • Coral sea	71 81	Tropical and North-Subtropical
Uroptychus capillatus Benedict, 1902	• Gulf of Mexico	31	Tropical
Uroptychus rugosus (A. Milne-Edwards, 1880)	• Barbados • Dominica • Gulf of Mexico • Saint Vincent and the Grenadines • Other (Lesser Antilles)	31	Tropical and North-Subtropical
Menaethius monoceros (Latreille, 1825)	• Mediterranean Sea • North Pacific Ocean • Red Sea • South Pacific Ocean • Tyrrhenian Sea • Indian Ocean • Mozambique Channel • Seychelles • South Africa • South Pacific Ocean • Others (Indo-Pacific, East Africa and New Caledonian)	37 51 57 71 81	Tropical and Subtropical

Allogalathea babai Cabezas, Macpherson & Machordom, 2011	• Pacific Ocean • Indonesian sea	61 71	Tropical
Allogalathea elegans (Adams & White, 1848)	• Indian Ocean • Madagascar • Mozambique • New Zealand • Seychelles • Others (Indo-Pacific, Mascarene and New Caledonia)	51 57 71 81	Tropical, Subtropical and North-Temperate
Allogalathea inermis Cabezas, Macpherson & Machordom, 2011	• Pacific Ocean • Coral sea • New Caledonia • Arabic sea	51 71 81	Tropical and North-Subtropical
Galathea amboinensis de Man, 1888	• Australian sea	57 71	Tropical
Galathea continua Baba & Fujita, 2008	• Papua New Guinea	71	Tropical
Galathea genkai Miyake & Baba, 1964	• Kyushu (Japan) • Red Sea • Indonesian sea	51 61 71	Tropical and South-Subtropical
Galathea inflata Potts, 1915	• Indonesian sea • New Caledonia	71	Tropical
Galathea leptocheir Baba & Fujita, 2008	• Indonesian sea	71	Tropical
Galathea orientalis Stimpson, 1858	• Sea of Japan • East China sea • South China sea • Coral sea	61 71	Tropical, North-Subtropical and North-Temperate
Hippolyte catagrapha d'Udekem d'Acoz, 2007	Unspecified	NA	NA

Hippolyte prideauxiana Leach, 1817	• Aegean Sea • Alboran Sea • Balearic Sea • Bay of Biscay • Celtic Sea • English Channel • France • Greece • Ionian Sea • Ireland • Irish Sea and St. George's Channel • Italy • Mediterranean Sea • North Atlantic Ocean • Spain • Tyrrhenian Sea • United Kingdom • Others (East Atlantic)	27 34 37 47	Tropical, North-Subtropical and North-Temperate
Latreutes parvulus (Stimpson, 1871)	• South Atlantic Ocean • Caribbean Sea • Gulf of Mexico • Venezuela • Others (Sahara Coast and Lesser Antilles)	31 34 41 47 51	Tropical and North-Subtropical
Neohymenicus orientalis (Sakai, 1932)	• Mozambique Channel • Indian Ocean • Sea of Japan	51 61	Tropical and South-Subtropical
Achaeus brevirostris (Haswell, 1879)	• Red Sea • Mozambique channel • Australian sea	51 57 71 81	Tropical and South-Subtropical
Stenorhynchus debilis (Smith, 1871)	• North Pacific Ocean • Gulf of Mexico	77	Tropical and South-Subtropical
Stenorhynchus seticornis (Herbst, 1788)	• Barbados • Caribbean Sea • Dominica • Grenada • Gulf of Mexico • Haiti • Trinidad and Tobago • Venezuela • Others (Lesser Antilles, Norman Island, Saint Croix, Saint Thomas and Statia) • South Atlantic Ocean	31 34 41	Tropical and South-Subtropical

Mithraculus sculptus (Lamarck, 1818)	• Antigua and Barbuda • Barbados • Caribbean Sea • Gulf of Mexico • Haiti • Trinidad and Tobago • Venezuela • Others (Anguilla, Aves, Lesser Antilles, Saint Croix, Saint Thomas and Statia) • South Atlantic Ocean	31 34	Tropical
Munida intermedia A. Milne-Edwards & Bouvier, 1899	• Adriatic Sea • Alboran Sea • Balearic Sea • Bay of Biscay • Celtic Sea • Ionian Sea • Mediterranean Sea • Mediterranean Sea - Eastern Basin • North Atlantic Ocean	27	North-Temperate
Munidopsis abdominalis (A. Milne-Edwards, 1880)	• Barbados • Caribbean Sea • North Atlantic Ocean • Saint Kitts and Nevis • Saint Vincent and the Grenadines • Others (Lesser Antilles)	31	Tropical and North-Subtropical
Munidopsis alaminos Pequegnat & Pequegnat, 1970	• Barbados • Caribbean Sea • Dominica • Gulf of Mexico • North Atlantic Ocean • Others (Anguilla and Lesser Antilles)	31	Tropical and North-Subtropical
Munidopsis spinifer (A. Milne-Edwards, 1880)	• Barbados • Caribbean Sea • Gulf of Mexico • North Atlantic Ocean • Saint Kitts and Nevis • Saint Vincent and the Grenadines • Others (Lesser Antilles)	31	Tropical and North-Subtropical

Actinimenes inornatus (Kemp, 1922)	• Australia • Comores • Indian Ocean • Kenya • Mozambique Channel • Red Sea • Seychelles • South Pacific Ocean • Tanzania • Others (Andaman and Nicobar Islands, Indo-Pacific, Melanesia and New Caledonian)	51 57 71	Tropical and South-Subtropical
Allopontonia alastairi Bruce, 2010	• Timor sea	57	Tropical
Allopontonia brockii (de Man, 1888)	• Australia • Indian Ocean • Kenya • Red Sea • Tanzania • Others (East Pacific and New Caledonian) • Indonesian sea	51 57 71	Tropical and North-Subtropical
Araiopontonia odontorhyncha Fujino & Miyake, 1970	• Indo-Pacific • Melanesia	71	Tropical
Bathymenes aleator (Bruce, 1991)	• South Pacific Ocean • Others (New Caledonia)	71	Tropical
Brucecaris tenuis (Bruce, 1969)	• Australia • Indian Ocean • Red Sea • Tanzania • Others (Andaman and Nicobar Islands, Indo-Pacific and Melanesia)	51 71	Tropical
Conchodytes tridacnae Peters, 1852	• Australia • Indian Ocean • Kenya • Maldives • Mozambique • Mozambique Channel • North Pacific Ocean • Red Sea • Seychelles • South Pacific Ocean • Tanzania • Others (Andaman and Nicobar Islands, Indo-Pacific, Melanesia and New Caledonian)	51 57 71	Tropical
Crinotonia anastasiae Marin, 2006	Unspecified	NA	NA

Crinotonia attenuatus (Bruce, 1971)	• Australia • Others (Indo-Pacific and Melanesia)	71	Tropical
Cristimenes brucei Park, De Grave & Kim, 2019	Unspecified	NA	NA
Cristimenes commensalis (Borradaile, 1915)	• Australia • Indian Ocean • Kenya • Mozambique • Red Sea • Tanzania • Others (Andaman and Nicobar Islands, Indo-Pacific, Melanesia and New Caledonian)	51 57 71	Tropical and South-Subtropical
Cristimenes cristimanus (Bruce, 1965)	• Australia • Others (Indo-Pacific and Melanesia • Red sea	51 71	Tropical
Cristimenes zanzibaricus (Bruce, 1967)	• Australia • Indian Ocean • Kenya • Red Sea • Seychelles • Tanzania	51 57	Tropical
Cuapetes americanus (Kingsley, 1878)	• Antigua and Barbuda • Caribbean Sea • Grenada • Gulf of Mexico • Haiti • North Atlantic Ocean • Saint Kitts and Nevis • Saint Vincent and the Grenadines • Trinidad and Tobago • Venezuela • Others (Anguilla, Guana Island, Lesser Antilles, Peter Island, Saint Croix, Saint Thomas, Statia, Tortola and Virgin Gorda) • India sea	31 41 57	Tropical and South-Subtropical
Diapontonia maranulus Bruce, 1986	• Caribbean Sea • Other (Lesser Antilles)	31	Tropical
Echinopericlimenes aurorae Marin & Chan, 2014	Unspecified	NA	NA
Echinopericlimenes calcaratus (Chace & Bruce, 1993)	• Indonesian sea	71	Tropical

Echinopericlimenes dentidactylus (Bruce, 1984)	• Indonesian sea	71	Tropical
Echinopericlimenes hertwigi (Balss, 1913)	• Australia • Others (Indo-Pacific and New Caledonian) • Indonesian sea	71	Tropical
Gnathophylloides mineri Schmitt, 1933	• Antigua and Barbuda • Barbados • Caribbean Sea • Gulf of Mexico • North Atlantic Ocean • Seychelles • South Pacific Ocean • Trinidad and Tobago • Venezuela • Others (Lesser Antilles, Marotiri and Statia) • Indonesian sea and Coral sea	31 51 71	Tropical and Subtropical
Gnathophylloides robustus Bruce, 1973	• Timor sea	57	North-Subtropical
Gnathophyllum americanum Guérin-Méneville, 1855	• Mozambique • South Africa • Antigua and Barbuda • Australia • Caribbean Sea • Gulf of Mexico • Haiti • Indian Ocean • Madagascar • Mozambique • Mozambique Channel • North Atlantic Ocean • Red Sea • Seychelles • South Pacific Ocean • Venezuela • Others (Central Atlantic Ocean, Indo-Pacific, Chilean, Lesser Antilles, Marquesas Islands, New Caledonian, Raroia Atoll, Saint Thomas, Tuamotu Islands, Ua Huka Island, Virgin Gorda and West Atlantic)	31 34 41 47 51 57 61 71 81	Tropical and Subtropical
Gnathophyllum precipuum Titgen, 1989	Unspecified	NA	NA

Gnathophyllum taylori Ahyong, 2003	• Australian sea	81	South-Subtropical
Hymenocera picta Dana, 1852	• Indo-Pacific • Indian Ocean • Madagascar • Mozambique • Mozambique Channel • North Pacific Ocean • Red Sea • Seychelles • South Pacific Ocean • Others (New Caledonia)	51 57 71 77	Tropical
Laomenes amboinensis (de Man, 1888)	• Australian sea	81	South-Subtropical
Laomenes ceratophthalmus (Borradaile, 1915)	• Australia • Indian Ocean • Kenya • Red Sea • Seychelles • Tanzania • Others (Andaman and Nicobar Islands, Indo-Pacific, Melanesia and New Caledonia) • Gulf of Mexico	31 51 71	Tropical
Laomenes clarki Marin, 2009	• Indonesian sea	71	Tropical
Laomenes cornutus (Borradaile, 1915)	• Australia • Maldives • Indonesian sea	51 71	Tropical
Laomenes holthuisi Marin & Okuno, 2010	Unspecified	NA	NA
Laomenes jackhintoni (Bruce, 2006)	Unspecified	NA	NA
Laomenes nudirostris (Bruce, 1968)	• Australia • Others (Indo-Pacific and New Calednian) • Indonesian sea	71	Tropical
Laomenes pardus Marin, 2009	• Indonesian sea	71	Tropical
Laomenes pestrushka Marin, Chan & Okuno, 2012	• Ryukyu Islands	61	North-Subtropical
Laomenes tigris Marin, 2009	Unspecified	NA	NA

*Levicaris mammillata (*Edmondson, 1931)	• East China sea	61	North-Subtropical
Lipkebe holthuisi Chace, 1969	• Gulf of Mexico • Caribbean sea	31	Tropical and North-Subtropical
Lipkemenes lanipes (Kemp, 1922)	• Australia • Indian Ocean • Kenya • Mozambique • Red Sea • Tanzania • Others (Andaman and Nicobar Islands, Indo-Pacific and New-Caledonian)	51 57 71	Tropical and Subtropical
Margitonia insolitus (Bruce, 1974)	• Australia • Indian Ocean • Kenya • Red Sea • Tanzania • Others (Andaman and Nicobar Islands, Indo-Pacific, Melanesia and New Caledonia)	51 57 71	Tropical and North-Subtropical
Palaemonella pottsi (Borradaile, 1915)	• Australia • Indian Ocean • Kenya • Red Sea • Tanzania • Others (Andaman and Nicobar Islands, Indo-Pacific, Melanesia and New Caledonia)	51 57 71	Tropical and North-Subtropical
Periclimenaeus djiboutensis Bruce, 1970	• East China sea • Australia • Indian Ocean • Red Sea • Tanzania • Others (Andaman and Nicobar Islands and Indo-Pacific)	57 71	Tropical
Periclimenella spinifera (de Man, 1902)	• Australia • Indian Ocean • Kenya • Maldives • Mozambique Channel • Red Sea • Seychelles • South Pacific Ocean • Tanzania • Others (Andaman and Nicobar Islands, Indo-Pacific, Melanesia and New Caledonia)	51 57 71	Tropical
Periclimenes affinis (Zehntner, 1894)	• Australia • Maldives • Others (Andaman and Nicobar Islands, Indo-Pacific, Melanesia and New Caledonia)	57 71	Tropical

Periclimenes albolineatus Bruce & Coombes, 1997	• Indian Ocean	51	Tropical
Periclimenes bowmani Chace, 1972	• Caribbean Sea • North Atlantic Ocean	31	Tropical
Periclimenes carinidactylus Bruce, 1969	• Australia • French Polynesia	71 81	Tropical and South-Subtropical
Periclimenes colemani Bruce, 1975	• Indonesia sea	71	Tropical
Periclimenes compressus Borradaile, 1915	• Seychelles	51	Tropical
Periclimenes crinoidalis Chace, 1969	• Caribbean Sea • Others (Lesser Antilles)	31	Tropical
Periclimenes hongkongensis Bruce, 1969	Unspecified	NA	NA
Periclimenes ingressicolumbi Berggren & Svane, 1989	• North Atlantic Ocean	31	North-Subtropical
Periclimenes maldivensis Bruce, 1969	• Gulf of Aden	51	Tropical
Periclimenes meyeri Chace, 1969	• Caribbean Sea • Other (Lesser Antilles)	31	Tropical
Periclimenes milleri Bruce, 1986	• North Atlantic Ocean	31	North-Subtropical
Periclimenes novaffinis Bruce & Coombes, 1997	Unspecified	NA	NA
Periclimenes pectiniferus Holthuis, 1952	Unspecified	NA	NA
Periclimenes pectinipes Bruce, 1991	• New Caledonia	71	Tropical
Periclimenes parvus Borradaile, 1898	Unspecified	NA	NA

Periclimenes perryae Chace, 1942	• Caribbean Sea • Gulf of Mexico • Others (Lesser Antilles and Statia)	31	Tropical and North-Subtropical
Periclimenes ruber Bruce, 1982	• Indonesia Sea • Timor and Coral sea	71	Tropical and South-Subtropical
Phyllognathia ceratophthalma (Balss, 1913)	• New Caledonia • Bay of Bengal • Indonesia sea • Timor and Coral sea	57 71	Tropical and South-Subtropical
Pontoniopsis comanthi Borradaile, 1915	• Kenya • Red Sea • Seychelles • Others (New Caledonia) • Indonesia sea • Timor and Coral sea • Pacific Ocean	51 57 71	North-Subtropical
Pseudocoutierea elegans Holthuis, 1951	Unspecified	NA	NA
Pycnocaris chagoae Bruce, 1972	• Indonesia sea • Pacific Ocean	71	Tropical
Sandimenes hirsutus (Bruce, 1971)	• Indian Ocean • Red Sea • Seychelles • Tanzania • Others (Melanesia and New Caledonian)	51 57 71	Tropical and South-Subtropical
Sandimenes rectirostris (Bruce, 1981)	• New Caledonia	71	Tropical
Stegopontonia commensalis Nobili, 1906	• Indian Ocean • Australia • Kenya • North Pacific Ocean • Red Sea • Seychelles • South Pacific Ocean • Others (West Pacific, Andaman and Nicobar Islands, Indo-Pacific, Melanesia and New Caledonia)	51 57 71	Tropical and Subtropical
Tuleariocaris sarec Berggren, 1994	Unspecified	NA	NA
Tuleariocaris holthuisi Hipeau-Jacquotte, 1965	• Australia • Indian Ocean • Kenya • North Pacific Ocean • Red Sea • South Pacific Ocean • Other (East Pacific)	51 71	Tropical

Tulеariocaris neglecta Chace, 1969	• Barbados • Caribbean Sea • Dominica • Gulf of Mexico • North Atlantic Ocean • Other (Lesser Antilles)	31 34 47	Tropical and North-Subtropical
Tulеariocaris zanzibarica Bruce, 1967	• Indian Ocean • Kenya • Red Sea • Tanzania • Others (Andaman and Nicobar Islands and Indo-Pacific)	51 71	Tropical
Unguicaris alegrias (Bruce, 1986)	• Timor Sea	57	Tropical
Unguicaris novaecaledonia e (Bruce, 1968)	• Australia • Others (Indo-Pacific and New Caledonia) • Indonesia sea	57 71	Tropical
Unguicaris panglaonis Marin & Chan, 2006	Unspecified	NA	NA
Unguicaris pilipes (Bruce & Zmarzly, 1983)	• Indonesia Sea	71	Tropical
Zenopontonia noverca (Kemp, 1922)	• Australia • Indian Ocean • Red Sea • Tanzania • Other (New Caledonia)	51 57 71	Tropical
Zenopontonia rex (Kemp, 1922)	• Indo-Pacific • Australia • Indian Ocean • Kenya • Madagascar • Mozambique • Mozambique Channel • North Pacific Ocean • Red Sea • Seychelles • South Pacific Ocean • Tanzania • Others (Andaman and Nicobar Islands, Melanesia and New Caledonian)	51 57 71 81	Tropical and Subtropical

Zenopontonia soror (Nobili, 1904)	• India • Djibouti • Indian Ocean • Kenya • Madagascar • Mozambique Channel • Seychelles • South Pacific Ocean • Tanzania • Other (New Caledonia) • Indonesia Sea • Timor and Coral Sea • Hawaiian Islands • Red Sea • Arabic sea	51 57 71	Tropical
Panulirus argus (Latreille, 1804)	• Netherlands Antilles • Barbados • Caribbean Sea • Gulf of Mexico • Haiti • Netherlands • Trinidad and Tobago • Venezuela • Others (Aves I., Lesser Antilles, Saint Thomas, Statia and West Atlantic)	31 41	Tropical, Subtropical and North-Temperate
Percnon gibbesi (H. Milne Edwards, 1853)	• Adriatic Sea • Aegean Sea • Brazil • Cape Verde • Chile • Ghana • Gulf of Guinea • Ionian Sea • Ligurian Sea • Mediterranean Sea • Mediterranean Sea - Eastern Basin • Mediterranean Sea - Western Basin • Morocco • North Atlantic Ocean • South Atlantic Ocean • Tyrrhenian Sea • United States • Antigua and Barbuda • Balearic Sea • Barbados • Caribbean Sea • Greece • Gulf of Mexico • Haiti • Portugal • Venezuela • Others (Atlantic Ocean, Coasts of America, East Atlantic, Pacific Ocean, Trindade and Martin Vaz Islands and West Atlantic)	31 34 37 41 47	Tropical, North-Subtropical and North-Temperate

Cebudonus poppeorum Ng, 2014	Unspecified	NA	NA
Ceratocarcinus frontodentata (Chen & Dai in Shen, Dai & Chen, 1982)	Unspecified	NA	NA
Ceratocarcinus longimanus White, 1847	• New Caledonia • Timor and Coral sea	57 71	Tropical
Ceratocarcinus trilobatus (T. Sakai, 1938)	Unspecified	NA	NA
Echinoecus pentagonus (A. Milne-Edwards, 1879)	• Indian Ocean • South Pacific Ocean • Red sea • Hawaiian Islands • French Polynesia	51 57 71	Tropical and North-Subtropical
Echinoecus nipponicus Miyake, 1939	Unspecified	NA	NA
Echinoecus sculptus (Ward, 1935)	• Indonesia sea	57	Tropical
Eumedonus brevirhynchus Chia & Ng, 1999	• New Caledonia	71	Tropical
Eumedonus intermedius Chia & Ng, 1999	Unspecified	NA	NA
Eumedonus niger H. Milne Edwards, 1834	• Australia	57 71 81	Tropical and South-Subtropical
Eumedonus vicinus Rathbun, 1918	• Australia	71 81	Tropical and South-Subtropical
Eumedonus zebra Alcock, 1895	• Indonesia sea • Gulf of Aden	51 71	Tropical
Gonatonotus granulosus (MacGilchrist, 1905)	• Indian Ocean • Mozambique • Seychelles • South Africa • Tanzania	51	Tropical and South-Subtropical

Gonatonotus nasutus Chia & Ng, 1999	• New Caledonia	71	Tropical
Gonatonotus pentagonus White, 1847	• Indonesian sea • Coral sea	71	Tropical
Hapalonotus pinnotheroides (Guérin-Méneville, 1838)	Unspecified	NA	NA
Harrovia albolineata Adams & White, 1849	• South China sea • Indonesia sea • India sea	57 71	Tropical
Harrovia cognata Chia & Ng, 1998	Unspecified	NA	NA
Harrovia elegans de Man, 1887	• Japan sea • Indonesian sea • China sea • Timor and Coral Sea	57 61 71	Tropical and North-Subtropical
Harrovia japonica Balss, 1921	Unspecified	NA	NA
Harrovia longipes Lanchester, 1900	• Indonesia sea • Coral sea	71	Tropical
Harrovia ngi Chen & Xu, 1992	Unspecified	NA	NA
Harrovia tuberculate Haswell, 1880	• East China Sea • Timor sea	61 71	Tropical and North-Subtropical
Permanotus purpureus (Gordon, 1934)	• Timor and Coral sea • Indonesia sea • Pacific Ocean	71 81	Tropical and South-Subtropical
Rhabdonotus pictus A. Milne-Edwards, 1879	• India sea	57	Tropical
Rhabdonotus pilipes Chia & Ng, 1995	Unspecified	NA	NA
Rhabdonotus xynon Chia & Ng, 1995	• Indonesia sea • Pacific Ocean	71	Tropical
Tauropus egeriae (Gordon, 1947)	Unspecified	NA	NA

Tiaramedon spinosum (Miers, 1879)	• Indian Ocean • Other (New Caledonia) • Red Sea • Timor sea • South China sea • Indonesia sea • Pacific Ocean	51 57 71	Tropical and North-Subtropical
Zebrida adamsii White, 1847	• New Caledonia • India sea • Indonesia sea • Japan sea • China sea • Timor and Coral sea	57 71	Tropical and North-Subtropical
Zebrida brevicarinata Ng & Chia, 1999	Unspecified	NA	NA
Zebrida longispina Haswell, 1880	• Timor sea	57	Tropical
Zebridonus mirabilis Chia, Ng & Castro, 1995	Unspecified	NA	NA
Alain crosnieri R.B. Manning, 1998	• Timor sea	57	Tropical
Alain raymondi Ahyong & P.K.L. Ng, 2008	Unspecified	NA	NA
Austrotheres holothuriensis (Baker, 1907)	• Australia	57 81	South-Subtropical and South-Temperate
Buergeres choprai Ahyong & PKL Ng, 2020	• Bismarck sea (Papua New Guinea)	71	Tropical
Buergeres deccanensis (Chopra, 1931)	• India Sea	57	Tropical
Buergeres holothuriae (Semper, 1880)	Unspecified	NA	NA
Buergeres ortmanni (Bürger, 1895)	• Banda sea (Indonesia) • Malacca Strait (Indonesia)	71	Tropical
Clypeasterophilus juvenilis (Bouvier, 1917)	• Caribbean Sea • Gulf of Mexico • Other (Lesser Antilles)	31	Tropical and North-Subtropical

Clypeasterophilus rugatus (Bouvier, 1917)	• Caribbean Sea • Dominica • Gulf of Mexico • Other (Lesser Antilles)	31	Tropical and North-Subtropical
Clypeasterophilus stebbingi (Rathbun, 1918)	• Gulf of Mexico • Venezuela • Other (Lesser Antilles)	31	Tropical and North-Subtropical
Clypeasterophilus ususfructus (H. Griffith, 1987)	Unspecified	NA	NA
Dissodactylus crinitichelis Moreira, 1901	• Barbados • Gulf of Mexico • Other (Lesser Antilles) • Caribbean sea • South Atlantic	31 41	Tropical and Subtropical
Dissodactylus glasselli Rioja, 1944	Unspecified	NA	NA
Dissodactylus latus H. Griffith, 1987	• Gulf of Mexico	31	Tropical and North-Subtropical
Dissodactylus lockingtoni Glassell, 1935	Unspecified	NA	NA
Dissodactylus mellitae (Rathbun, 1900)	• Gulf of Mexico • North Atlantic Ocean • Others (Lesser Antilles and Statia)	21 31	Tropical, North-Subtropical and North-Temperate
Dissodactylus nitidus Smith, 1870	• Gulf of California	77	North-Subtropical
Dissodactylus primitivus Bouvier, 1917	• Barbados • Caribbean Sea • Gulf of Mexico • Others (Lesser Antilles and Statia)	31	Tropical and North-Subtropical
Dissodactylus xantusi Glassell, 1936	Unspecified	NA	NA
Glassella leptosynaptae (Wass, 1968)	• Gulf of Mexico	31	Tropical and North-Subtropical
Holotheres danielae Ahyong, 2010	• Timor sea	57	Tropical
Holotheres flavus (Nauck, 1880)	Unspecified	NA	NA

Holotheres halingi (Hamel, P.K.L. Ng & Mercier, 1999)	Unspecified	NA	NA
Holotheres semperi (Bürger, 1895)	• Indonesia sea	57	Tropical
Holotheres setnai (Chopra, 1931)	Unspecified	NA	NA
Holothuriophilus pacificus (Poeppig, 1836)	Unspecified	NA	NA
Holothuriophilus trapeziformis Nauck, 1880	Unspecified	NA	NA
Opisthopus transversus Rathbun, 1894	• North Pacific Ocean	77	North-Temperate
Pinnaxodes bipunctatus (Nicolet in Gay, 1849)	Unspecified	NA	NA
Pinnaxodes chilensis (H. Milne Edwards, 1837)	Unspecified	NA	NA
Pinnaxodes floridensis H.W. Wells & M.J. Wells, 1961	• Gulf of Mexico	31	Tropical and North-Subtropical
Pinnaxodes major Ortmann, 1894	Unspecified	NA	NA
Pinnixa barnharti Rathbun, 1918	Unspecified	NA	NA
Pinnixa huffmani Glassell, 1935	Unspecified	NA	NA
Pinnixa tumida Stimpson, 1858	• Yellow sea	61	North-Temperate
Trichobezoares pilumnoides (Nobili, 1906)	Unspecified	NA	NA

Trichobezoares villosissimus (Doflein, 1904)	• Andman sea	57	Tropical
Allopetrolisthes spinifrons (H. Milne Edwards, 1837)	Unspecified	NA	NA
Clastotoechus gorgonensis Werding & Haig, 1983	• Pacific Ocean	87	Tropical
Clastotoechus vanderhorsti (Schmitt, 1924)	• Barbados • Caribbean Sea • Haiti • Netherlands • Venezuela • Others (Lesser Antilles, Saint Croix and Saint Thomas)	31	Tropical
Liopetrolisthes mitra (Dana, 1852)	• South Pacific Ocean	41	South-Temperate
Liopetrolisthes patagonicus (Cunningham, 1871)	Unspecified	NA	NA
Pachycheles biocellatus (Lockington, 1878)	• North Pacific Ocean	77	Tropical
*Petrolisthes galathinus (*Bosc, 1801)	• Barbados • Caribbean Sea • Gulf of Mexico • Haiti • Trinidad and Tobago • Venezuela • Others (Aves I., Jost Van Dyke, Lesser Antilles, Norman Island, Saint Croix, Saint John and Saint Thomas)	31 41	Tropical and North-Subtropical
Petrolisthes sanfelipensis Glassell, 1936	Unspecified	NA	NA
Petrolisthes virgatus Paulson, 1875	• Indian Ocean • Tanzania • Djibouti • Federal Republic of Somalia • Mozambique • Red Sea • Other (East Africa) • Arabic Sea	51	Tropical and Subtropical

Pisidia serratifrons (Stimpson, 1858)	• Mozambique • Indian sea • Japan sea • Yellow sea • China sea • Indonesia sea • Timor and Coral sea	51 57 61 71	Tropical, Subtropical and North-Temperate
Pisidia dispar (Stimpson, 1858)	• New Caledonia • China sea • Indonesia sea • Australia	61 71 81	Tropical and Subtropical
Lissocarcinus orbicularis Dana, 1852	• Comores • Indian Ocean • Madagascar • Mozambique • Mozambique Channel • Republic of Mauritius • Seychelles • South Africa • South Pacific Ocean • Tanzania • Indo-Pacific • Red sea • Other (New Caledonia)	51 57 61 71	Tropical and Subtropical
Lissocarcinus ornatus Chopra, 1931	• Andman sea	57	Tropical
Rhynchocinetes brucei Okuno, 1994	• New Caledonia • China sea • Australia	57 61 71	Tropical and South-Subtropical
Odontozona crinoidicola Saito & Fujita, 2009	• South Kuroshio • Solomon Archipelago	61 71	Tropical and North-Subtropical

Stenopus hispidus (Olivier, 1811)	• Antigua and Barbuda • Caribbean Sea • Comores • Gulf of Mexico • Haiti • Indian Ocean • Madagascar • Mozambique • Mozambique Channel • New Zealand • North Atlantic Ocean • South Pacific Ocean • South Africa • Trinidad and Tobago • Venezuela • Others (Chili, East Africa, Fatu Hiva, Hao, Lesser Antilles, Makatea, Mare, Marquesas Islands, New Caledonia, Nuku Hica, Raraka, Raroia Atoll, Saint Croix, Statia, Tahuata, Tuamotu Islands and Ua Pou Islands)	31 34 37 41 47 51 57 61 71 81	Tropical, Subtropical and Temperate
Sternostylus salvadori (Rice & Miller, 1991)	• North Atlantic Ocean	31	North-Subtropical
Thor amboinensis (de Man, 1888)	• Antigua and Barbuda • Barbados • Caribbean Sea • Coral Sea • Dominica • Gulf of Mexico • Indian Ocean • Mozambique • Mozambique Channel • North Atlantic Ocean • South Pacific Ocean • Venezuela • Others (Atoll Fangataufa, Bora-Bora, Chili, Eiao Island, Indo-Pacific, Lesser Antilles, Marquesas Islands, New Caledonia, Saint Thomas, Statia and Tuamotu Islands)	31 34 51 57 71 81	Tropical and Subtropical

Thor manningi Chace, 1972	• Antigua and Barbuda • Brazil • Caribbean Sea • Dominica • Gulf of Mexico • Haiti • Mexico • North Atlantic Ocean • Portugal • Saint Kitts and Nevis • Saint Vincent and the Grenadines • South Atlantic Ocean • Trinidad and Tobago • United States • Venezuela • Others (Anguilla, East Pacific, Lesser Antilles, Tortola, Virgin Gorda and West Atlantic)	21 31 34 41 47 77	Tropical, North-Subtropical and North-Temperate

Chapter I



Table SI1 Gradient timetable used for the HPLC analysis. Eluents A and B are respectively water with 0.1 % formic acid and acetonitrile.

Time (minutes)	Eluent A (%)	Eluent B (%)	Curve
0	80	20	Equilibration
0 - 15	80 → 30	20 → 70	Linear gradient
15 - 16	30	70	Isocratic
16 - 20	30 → 80	70 → 20	Linear gradient
20 - 22	80	20	Isocratic

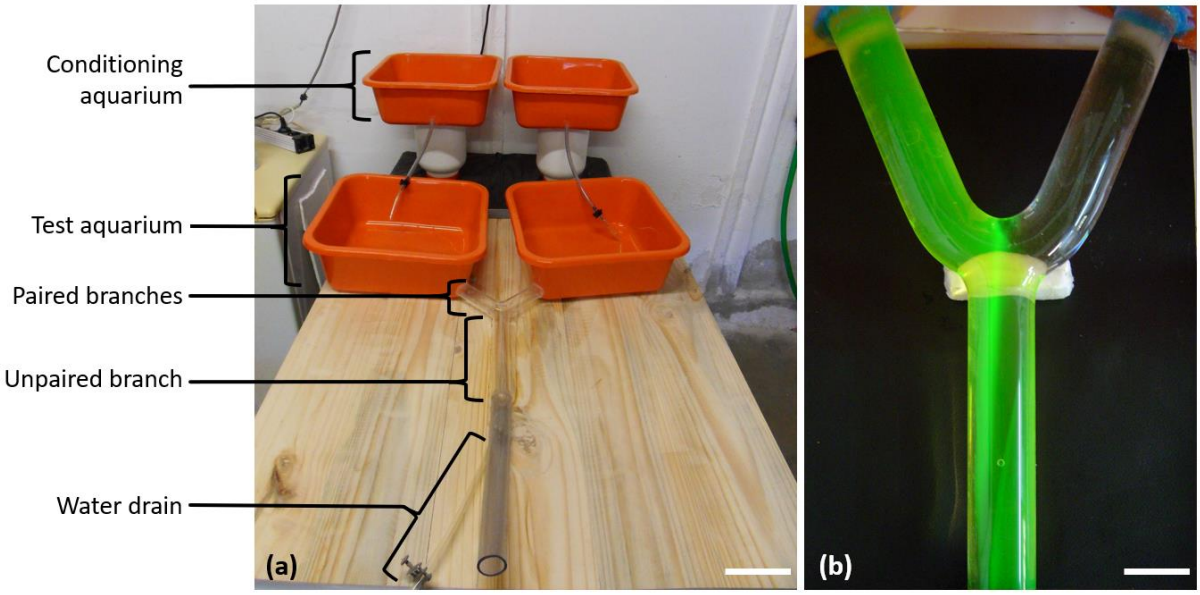


Fig.SI1 Y-tube olfactometer device (a) and laminarity dye test using fluorescein mimicking the conditioned water in the left aquarium (b). The scale bar represents 10 cm in (a) and 32 mm in (b).

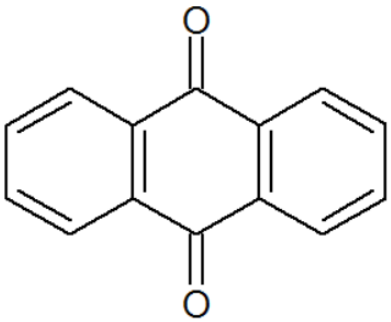


Fig.SI2 Molecular structure of the commercial anthraquinone from Alfa Aesar; CAS 84-65-1.

Videos SI1 : Chemical attraction of the symbiotic shrimp *Synalpheus stimpsonii* by water conditioned by its host crinoid *Phanogenia distincta*. The video was recorded for illustration only.
https://static-content.springer.com/esm/art%3A10.1007%2Fs00049-022-00368 6/MediaObjects/49_2022_368_MOESM1_ESM.docx

Video S2 : Chemical attraction of the symbiotic shrimp Synalpheus stimpsonii by water conditioned by anthraquinones extracted from its host crinoid Phanogenia distincta. The video was recorded for illustration only.
https://link.springer.com/article/10.1007/s00049-022-00368-6#availability-of-data-and-material

Chapter II



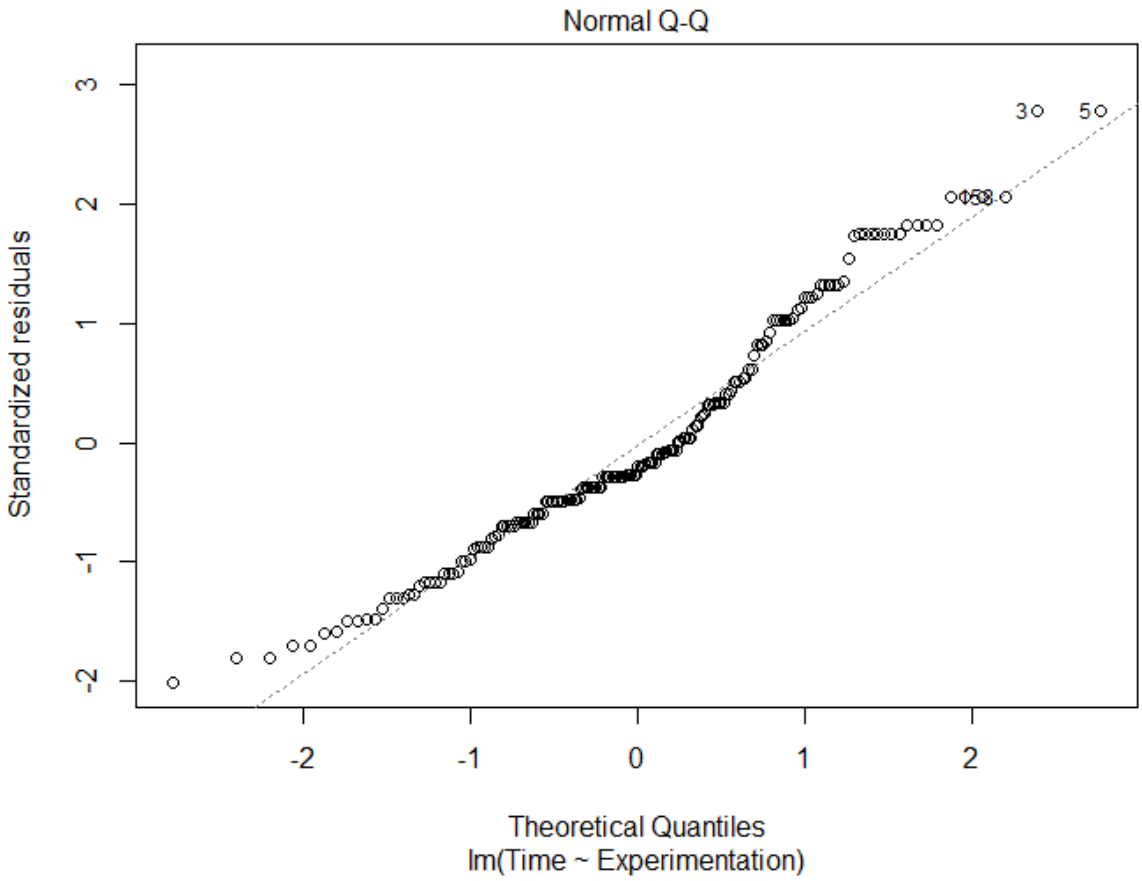
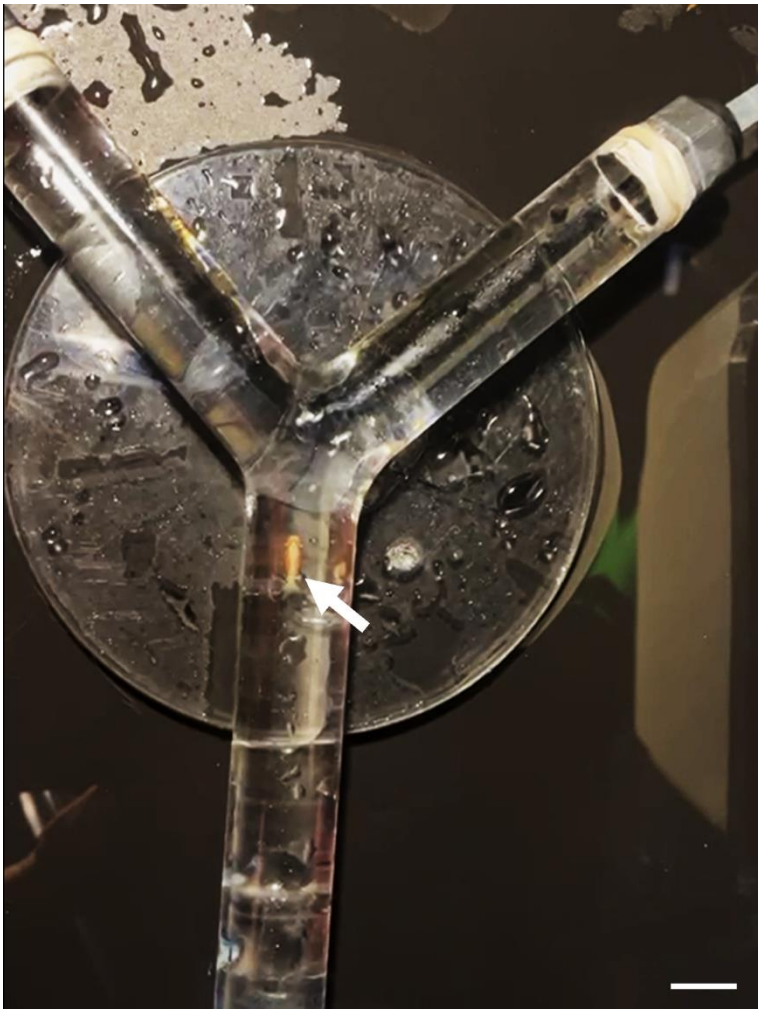
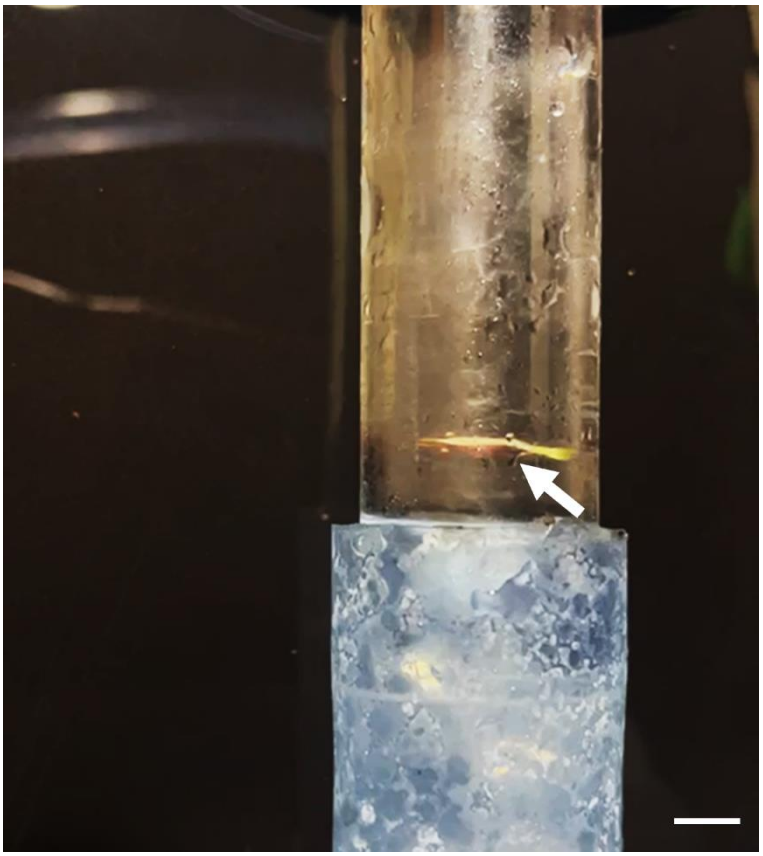


Figure SI-1 : Quantile - Quantile (Q-Q) plot representing the distribution of the residuals compared to a normal distribution prior the realization of the ANOVA analysis (see Fig. 5) comparing the mean deriving times between experimentations. X-axis = theoretical quantiles of the standard normal distribution ; Y-axis = quantiles of the residuals. The points on the plot represent the residuals from the ANOVA model. The diagonal line on the plot represents the line of equality, where the theoretical quantiles are equal to the measured quantiles. Points that correspond to this theoretical line signifies that the residuals are normally distributed. Deviations from the diagonal line indicate departures from normality.

Supplementary material



Video SI-2 : https://youtube.com/shorts/5SONg0VJaZs ; video illustrating the motion and orientation behaviour performed by an asteroid shrimp *Zenopontonia soror* (highlighted by a white arrow) into a Y-tube olfactometer. The scale bar represents 2 cm.



Video SI-2: https://youtube.com/shorts/S0wDFKK81po ; video illustrating the absence of motion and orientation behaviour (null result) performed by an asteroid shrimp *Zenopontonia soror* (highlighted by a white arrow) into a Y-tube olfactometer. The scale bar represents 0.8 cm.

Chapter III



Table S1. List of stress-related genes in crustaceans described in previous literature[34–36]. The table contain the respective genes name, the associated species and the accession ID in the NCBI database.

Gene description	Matched species	Accession ID	reference
Cytochrome P450			
Cytochrome P450	*Litopenaeus vannamei**	D4PEE6 D4PEE6_LITVA	(Armstrong et al., 2019)
Cytochrome P450 1A1-like	*Litopenaeus vannamei**	XP_027220585.1	(Armstrong et al., 2019)
Cytochrome P450 12A4 (mitochondrial)	*Litopenaeus vannamei**	XP_027222240.1	(Armstrong et al., 2019)
Cytochrome P450 15C1	*Bombyx mori*	BAH47266.1	(Armstrong et al., 2019)
Cytochrome P450 18A1-like	*Litopenaeus vannamei**	XP_027220710.1	(Armstrong et al., 2019)
Cytochrome P450 CYP2	*Portunus trituberculatus**	A0A059VQG8 A0A059VQG8_PORTR	(Armstrong et al., 2019)
Cytochrome P450 CYP2B	*Eriocheir sinensis**	A0A288UK13 A0A288UK13_ERISI	(Armstrong et al., 2019)
Cytochrome P450 CYP301A1 (mitochondrial)	*Centruroides sculpturatus*	XP_023218124.1	(Armstrong et al., 2019)
Cytochrome P450 CYP302 (fragment)	*Neocaridina denticulate**	A0A0A7EQJ8 A0A0A7EQJ8_NEODE	(Armstrong et al., 2019)
Cytochrome P450 CYP302A1	*Portunus trituberculatus*	A0A0U1XYE2 A0A0U1XYE2_PORTR	(Armstrong et al., 2019)
Cytochrome P450 306A1-like	*Litopenaeus vannamei**	XP_027215959.1	(Armstrong et al., 2019)
Cytochrome P450 315A1 (mitochondrial)	*Litopenaeus vannamei**	XP_027238669.1	(Armstrong et al., 2019)
Cytochrome P450 318A1	*Lucilia cuprina*	XP_023302463.1	(Armstrong et al., 2019)
Cytochrome P450 3A12-like	*Galendromus occidentails*	XP_003744583.1	(Armstrong et al., 2019)
Cytochrome P450 3A13-like	*Litopenaeus vannamei**	XP_027214936.1	(Armstrong et al., 2019)
Cytochrome P450 CYP330A1	*Carcinus maenas*	Q6VT82 Q6VT82_CARMA	(Armstrong et al., 2019)
Cytochrome P450 3A2-like (isoform X1)	*Drosophila serrata*	XP_020800177.1	(Armstrong et al., 2019)
Cytochrome P450 3A24-like	*Hyalella azteca**	XP_018015218.1	(Armstrong et al., 2019)
Cytochrome P450 3A29-like	*Litopenaeus vannamei**	XP_027235586.1	(Armstrong et al., 2019)

Cytochrome P450 3A41-like (isoform X1)	*Litopenaeus vannamei**	XP_027231231.1	(Armstrong et al., 2019)
Cytochrome P450 3A43-like	*Litopenaeus vannamei**	XP_027227536.1	(Armstrong et al., 2019)
Cytochrome P450 3A56	*Galendromus occidentails*	XP_027231149.1	(Armstrong et al., 2019)
Cytochrome P450 2F2-like	*Haplochromis burtoni*	XP_005952874.2	(Armstrong et al., 2019)
Cytochrome P450 2J2-like	*Hyalella azteca**	XP_018020471.1	(Armstrong et al., 2019)
Cytochrome P450 2J2-like (isoform X2)	*Stichopus japonicus*	A0A2G8L2Z2 A0A2G8L2Z2_STIJA	(Armstrong et al., 2019)
Cytochrome P450 2J3-like	*Litopenaeus vannamei**	XP_027224377.1	(Armstrong et al., 2019)
Cytochrome P450 2L1 (EC 1.14.14.1) (CYPIIL1)	*Panulirus argus*	Q27712 CP2L1_PANAR	(Armstrong et al., 2019)
Cytochrome P450 CYP44	*Litopenaeus vannamei**	XP_027213215.1	(Armstrong et al., 2019)
Cytochrome P450 4C1-like	*Litopenaeus vannamei**	XP_027231572.1	(Armstrong et al., 2019)
Cytochrome P450 4V2	*Stegastes partitus*	XP_008280300.1	(Armstrong et al., 2019)
Cytochrome P450 4V28	*Marsupenaeus japonicus**	AUB85738.1	(Armstrong et al., 2019)
Cytochrome P450 49A1	*Hyalella azteca**	XP_018009104.1	(Armstrong et al., 2019)
Cytochrome P450 CYP4V29	*Marsupenaeus japonicus**	A0A2H4W6J8 A0A2H4W6J8_MARJA	(Armstrong et al., 2019)
Cytochrome P450 6A14	*Litopenaeus vannamei**	XP_027210680.1	(Armstrong et al., 2019)
Cytochrome P450 6K1-like	*Hyalella azteca**	XP_018028068.1	(Armstrong et al., 2019)
Cytochrome P450 90A1-like	*Saccoglossus kowalevskii*	XP_002739892.1	(Armstrong et al., 2019)
Cytochrome P450 CYP9A18	*Helicoverpa armigera*	AKS48892.1	(Armstrong et al., 2019)
Cytochrome P450 9E2-like	*Hyalella azteca**	XP_018026862.1	(Armstrong et al., 2019)
Cytochrome P450 V20	*Macrobrachium nipponenseHyalella azteca**	AFA26603.1	(Armstrong et al., 2019)
Cytochrome P450 epoxidase-like protein	*Portunus trituberculatus**	A0A120GW42 A0A120GW42_PORTR	(Armstrong et al., 2019)
Glutathione S-transferase			

Glutathione S-transferase (EC 2.5.1.18)	*Paralichthys olivaceus*	A8VRH1 A8VRH1_PAROL	(Armstrong et al., 2019)
Glutathione S-transferase (micorsomal)	*Locusta migratoria*	V9Q323 V9Q323_LOCMI	(Armstrong et al., 2019)
Delta class glutathione S-transferase	*Fenneropenaeus chinensis**	A0A173GPI0 A0A173GPI0_FENCH	(Armstrong et al., 2019)
Glutathione S-transferase kappa	*Macrobrachium rosenbergii**	A0A0F7RQZ9 A0A0F7RQZ9_MACRS	(Armstrong et al., 2019)
Glutathione S-transferase omega-1	*Lygus Hesperus*	A0A0A9ZAS6 A0A0A9ZAS6_LYGHE	(Armstrong et al., 2019)
Glutathione S-transferase theta-1	*Larimichthys crocea*	A0A0F8AG91 A0A0F8AG91_LARCR	(Armstrong et al., 2019)
Glutathione S-transferase mu	*Procambarus clarkii**	F5A6F0 F5A6F0_PROCL	(Armstrong et al., 2019)
Glutathione S-transferase alpha	*Larimichthys crocea*	XP_019130952.1	(Armstrong et al., 2019)
Glutathione S-transferase C-terminal domain-containing protein homolog	*Hirondellea gigas**	A0A2P2I8H2 A0A2P2I8H2_9CRUS	(Armstrong et al., 2019)
Oxydative stress			
Catalase (EC 1.11.1.6)	*Marsupenaeus japonicus**	A0A0A0P850 A0A0A0P850_MARJA	(Armstrong et al., 2019)
Superoxide dismutase (EC 1.15.1.1)	*Penaeus monodon**	M9T285 M9T285_PENMO	(Armstrong et al., 2019)
Superoxide dismutase [Cu-Zn] (EC 1.15.1.1)	*Litopenaeus vannamei**	A0A2R3SK23 A0A2R3SK23_LITVA	(Armstrong et al., 2019)
Cytosolic manganese superoxide dismutase	*Penaeus monodon**	A0A1B2JLZ0 A0A1B2JLZ0_PENMO	(Armstrong et al., 2019)
Glutathione peroxidase	*Penaeus monodon**	A0A2H4GVH5 A0A2H4GVH5_PENMO	(Armstrong et al., 2019)
Glutathione peroxidase 3	*Paralichthys olivaceus*	XP_019936433.1	(Armstrong et al., 2019)
Metallothionein	*Penaeus monodon**	E5KXY4 E5KXY4_PENMO	(Armstrong et al., 2019)
Crustacean hyperglycemic hormone protein (CHH)			
Crustacean hyperglycemic hormone protein	*Penaeus monodon**	AWX63581.1	(Armstrong et al., 2019)
Heat shock protein (Hsps)			
Heat shock protein	*Cherax destructor**	A0A0E3X6C1 A0A0E3X6C1_CHEDE	(Armstrong et al., 2019)

Heat shock protein 10	*Litopenaeus vannamei**	A0A2L0HIK3 A0A2L0HIK3_LITVA	(Armstrong et al., 2019)
Heat shock protein 21	*Macrobrachium rosenbergii**	H8XYP5 H8XYP5_MACRS	(Armstrong et al., 2019)
Heat shock protein 22-like	*Litopenaeus vannamei**	XP_027228832.1	(Armstrong et al., 2019)
Heat shock protein 23-like	*drosophila suzukii*	XP_016933456.1	(Armstrong et al., 2019)
Heat shock protein 67B1-like	*Litopenaeus vannamei**	XP_027208091.1	(Armstrong et al., 2019)
Heat shock protein 67B2-like	*Hyalella azteca**	XP_018026303.1	(Armstrong et al., 2019)
Heat shock protein 68-like	*Galleria mellonella*	XP_026760322.1	(Armstrong et al., 2019)
Heat shock protein 68-like (isoform X3)	*bombyx mandarina*	XP_028044200.1	(Armstrong et al., 2019)
Heat shock protein 70	*Fenneropenaeus chinensis**	G8XRI7 G8XRI7_FENCH	(Armstrong et al., 2019)
Heat shock protein 70 kDa (fragment)	*Ocypode quadrata**	A0A1B1FH00 A0A1B1FH00_OCYQU	(Armstrong et al., 2019)
Heat shock protein 75 kDa (mitochondrial)	*Hyalella azteca**	XP_018017641.1	(Armstrong et al., 2019)
Heat shock protein 83 (isoform X1)	*Litopenaeus vannamei**	XP_027212945.1	(Armstrong et al., 2019)
Heat shock protein 90 beta	*Paralichthys olivaceus*	A5H1I1 A5H1I1_PAROL	(Armstrong et al., 2019)
Heat shock protein 90 alpha (cytosolic)	*Iconisemion striatum*	A0A1A7Y6E5 A0A1A7Y6E5_9TELE	(Armstrong et al., 2019)
Adhesive protein			
Cadherin 87	*Cephus cinctus*	XP_015588457	(Kim et al., 2017)
Galectin	*Litopenaeus vannamei**	AGV04659	(Kim et al., 2017)
Multiple PDZ domain protein isoform X2	*Acyrthosiphon pisum*	XP_008181772	(Kim et al., 2017)
Tetraspanin-5-like isoform 1	*Athalia rosae*	XP_012258733	(Kim et al., 2017)
Transglutaminase	*Pacifastacus leniusculus**	AAK69205	(Kim et al., 2017)
Histone H2A-like	*Diuraphis noxia*	XP_015363720	(Kim et al., 2017)
Histone H3.3	*Oncorhynchus mykiss*	ACO08041	(Kim et al., 2017)
Integral membrane, Ixodes scapularis	*Ixodes scapularis*	XP_002412964	(Kim et al., 2017)

Apoptosis and cell cycle			
inhibitor of apoptosis protein	*Penaeus monodon**	ABO38431	(Kim et al., 2017)
β-catenin-like protein 1	*Limulus polyphemus*	XP_013791309	(Kim et al., 2017)
Catenin delta-2	*Lasius niger*	KMQ88737	(Kim et al., 2017)
Programmed cell death protein 4	*Zootermopsis nevadensis*	KDR08650	(Kim et al., 2017)
Rab-1	*Macrobrachium rosenbergii*	AJC97112	(Kim et al., 2017)
beclin-1-like protein	*Penaeus vanname**	NW_020869448.1	(Chen et al., 2013)
nucleoside diphosphate kinase A-like	*Litopenaeus vannamei**	NW_020869393.1	(Chen et al., 2013)
prohibitin-like	*Litopenaeus vannamei**	NW_020871485.1	(Chen et al., 2013)
caspase-1-like	*Litopenaeus vannamei**	NW_020870979.1	(Chen et al., 2013)
caspase-8-like	*Litopenaeus vannamei**	NW_020872601.1	(Chen et al., 2013)
caspase-3-like	*Litopenaeus vannamei**	NW_020871382.1	(Chen et al., 2013)
ras-related Rab-32B-like	*Aplysia californica*	XP_005097933	(Kim et al., 2017)
Cellullar defense effecter			
Dual oxidase	*Marsupenaeus japonicus**	BAM76968	(Kim et al., 2017)
Fibrinogen-like protein	*Fenneropenaeus merguiensis**	AKR15662	(Kim et al., 2017)
Thioredoxin reductase 3	*Crassostrea gigas*	XP_011419860	(Kim et al., 2017)
c-X-C motif chemokine 15-like	*Bos taurus*	XP_003582386	(Kim et al., 2017)
Immune regulator			
signal sequence receptor beta-like protein	*Plectreurys tristis*	AJD25284	(Kim et al., 2017)
Translocon-associated protein subunit α	*Zootermopsis nevadensis*	KDR17543	(Kim et al., 2017)
polyubiquitin-C-like	*Litopenaeus vannamei**	NW_020870535.1	(Chen et al., 2013)
Carboxypeptidase B2	*Daphnia magna**	KZS09622	(Kim et al., 2017)
Protease and protease inhibitor			

26S protease regulatory subunit 10B X2	*Myotis lucifugus*	XP_014318322	(Kim et al., 2017)
α-2-macroglobulin 2 isoform 3	*Pacifastacus leniusculus**	AEC50083	(Kim et al., 2017)
trypsin	*Litopenaeus vannamei**	CAA75311	(Kim et al., 2017)
Cystatin 2	*Cherax quadricarinatus**	ALC79585	(Kim et al., 2017)
Cathepsin A	*Eriocheir sinensis**	ADO65982	(Kim et al., 2017)
Cathepsin B	*Fenneropenaeus chinensis**	AHA83423	(Kim et al., 2017)
Cathepsin C	*Fenneropenaeus chinensis**	ACG60902	(Kim et al., 2017)
Cathepsin D	*Palaemon carinicauda**	AGJ03549	(Kim et al., 2017)
Cathepsin L protein	*Cherax quadricarinatus**	AJS13771	(Kim et al., 2017)
inter-α-trypsin inhibitor heavy chain H6	*Monodelphis domestica*	XP_016282619	(Kim et al., 2017)
matrix metalloproteinase-14	*Papilio Xuthus*	XP_013166430	(Kim et al., 2017)
protein phosphatase	*Fenneropenaeus chinensis**	AHE40944	(Kim et al., 2017)
serine carboxypeptidase CPVL-like	*Neolamprologus brichardi*	XP_006801474	(Kim et al., 2017)
Redox			
hypothetical ferritin light-chain subunit	*Rimicaris exoculata**	ACR43472	(Kim et al., 2017)
Peroxiredoxin	*Penaeus monodon**	ABZ80828	(Kim et al., 2017)
selenium-binding protein 1-A-like	*Corvus brachyrhynchos*	XP_008627838	(Kim et al., 2017)
Signal transduction			
calmodulin-A-like isoform X1	*Microplitis demolitor*	XP_008550049	(Kim et al., 2017)
cAMP-dependent kinase	*Nasonia vitripennis*	ACH99585	(Kim et al., 2017)
Casein kinase II subunit beta	*Cerapachys biroi*	XP_011340131	(Kim et al., 2017)
COP9 signalosome complex subunit 2	*Athalia rosae*	XP_012264747	(Kim et al., 2017)
mitogen-activated protein kinase	*Fenneropenaeus chinensis**	AHA83424	(Kim et al., 2017)

tyrosine- kinase Src64B-like isoform 1	*Zootermopsis nevadensis*	KDR17588	(Kim et al., 2017)
RING-box protein 1A	*Litopenaeus vannamei**	NW_020870413.1	(Chen et al., 2013)
RING-box protein 2-like	*Litopenaeus vannamei**	NW_020870312.1	(Chen et al., 2013)
Caprin-1	*Harpegnathos saltator*	NW_020229952.1	(Chen et al., 2013)
Protein vav	*Neodiprion lecontei*	XP_015521070	(Kim et al., 2017)
Stress protein			
ATP synthase F0 subunit 6	*Aradacanthia heissi*	ADQ64032	(Kim et al., 2017)
heat shock factor binding 1-like	*Trachymyrmex zeteki***	KYQ58790	(Kim et al., 2017)
melanoma-associated antigen G1	*Myotis davidii*	XP_006771818	(Kim et al., 2017)
CD63 antigen	*Lepeophtheirus salmonis**	ACO12374	(Kim et al., 2017)
B-cell lymphoma leukemia 11B-like	*Zootermopsis nevadensis*	KDR23016	(Kim et al., 2017)
Transferrin	*Zootermopsis nevadensis*	KDR19744	(Kim et al., 2017)
ubiquitin specific peptidase 25	*Astyanax mexicanus*	XP_007244811	(Kim et al., 2017)
LITAF	*Litopenaeus vannamei**	AEK86526	(Kim et al., 2017)
Acute phase response/in¯ammation			
heme oxygenase 1-like isoform X2	*Apis dorsata*	XP_006620087	(Kim et al., 2017)
MAPK signaling pathways			
Protein max	*Apis mellifera*	NC_037645.1	(Chen et al., 2013)
antigen presentation pathway			
legumain-like	*Litopenaeus vannamei**	NW_020872195.1	(Chen et al., 2013)
gamma-interferon-inducible lysosomal thiol reductase-like	*Litopenaeus vannamei**	NW_020871011.1	(Chen et al., 2013)

Table S2. Description of the trinity assembly output for *A. indicus* and *T. holthuisi* transcriptomes. Conditions were "CC: Control condition (with host)"; "IC: Isolation condition"; "IC+S: Isolation condition + spinochromes". R = Replicate, M=Million, Gb = Giga bases.

Sample	Total Number	Total Length	Mean Length	N50
A. indicus CC, R1	74,245	49,579,055	667	1,185
A. indicus CC, R2	85,438	54,456,023	637	1,111
A. indicus CC, R3	81,523	55,803,555	684	1,267
A. indicus IC, R1	67,738	46,554,192	687	1,238
A. indicus IC, R2	68,876	43,338,915	629	1,075
A. indicus IC, R3	70,175	45,750,523	651	1,124
A. indicus IC+S, R1	71,171	45,981,251	646	1,103
A. indicus IC+S, R2	73,000	49,114,962	672	1,217
A. indicus IC+S, R3	65,648	42,191,479	642	1,091
T. holtuisi CC, R1	68,341	47,280,884	691	1,289
T. holtuisi CC, R2	87,823	60,290,678	686	1,290
T. holtuisi CC, R3	97,931	61,625,853	629	1,072
T. holtuisi IC, R1	71,797	51,476,390	716	1,356
T. holtuisi IC, R2	115,118	70,857,982	615	1,014
T. holtuisi IC, R3	50,056	29,462,930	588	925
T. holtuisi IC+S, R1	96,132	66,418,977	690	1,307
T. holtuisi IC+S, R2	84,885	67,689,108	797	1,649
T. holtuisi IC+S, R3	77,766	53,357,585	686	1,259

Table S3. Description of unigenes for *A. indicus* and *T. holthuisi* transcriptomes. Conditions were "CC: Control condition (with host)"; "IC: Isolation condition"; "IC+S: Isolation condition + spinochromes". R = Replicate, bp = Base pair.

Sample	Total unigene number	Mean Length (bp)	N50 (bp)
A. indicus CC, R1	46,600	818	1,445
A. indicus CC, R2	52,453	783	1,370
A. indicus CC, R3	50,229	850	1,561
A. indicus IC, R1	43,481	828	1,480
A. indicus IC, R2	42,791	782	1,352
A. indicus IC, R3	44,421	801	1,387
A. indicus IC+S, R1	45,038	794	1,362
A. indicus IC+S, R2	46,757	821	1,476
A. indicus IC+S, R3	41,768	782	1,350
T. holtuisi CC, R1	44,316	837	1,518
T. holtuisi CC, R2	53,385	839	1,522
T. holtuisi CC, R3	64,324	765	1,305
T. holtuisi IC, R1	47,830	856	1,590
T. holtuisi IC, R2	61,948	822	1,415
T. holtuisi IC, R3	32,751	707	1,115
T. holtuisi IC+S, R1	55,500	865	1,583
T. holtuisi IC+S, R2	54,738	977	1,894
T. holtuisi IC+S, R3	48,282	838	1,492
All unigenes	217,832	1,033	2,061

Table S4. BLASTx-based annotation of the 10 most expressed unigenes in the shrimp transcriptomes *A. indicus* and *T. holthuisi*. For all unigenes, the associated FPKM value and the annotation in the reference public databases.

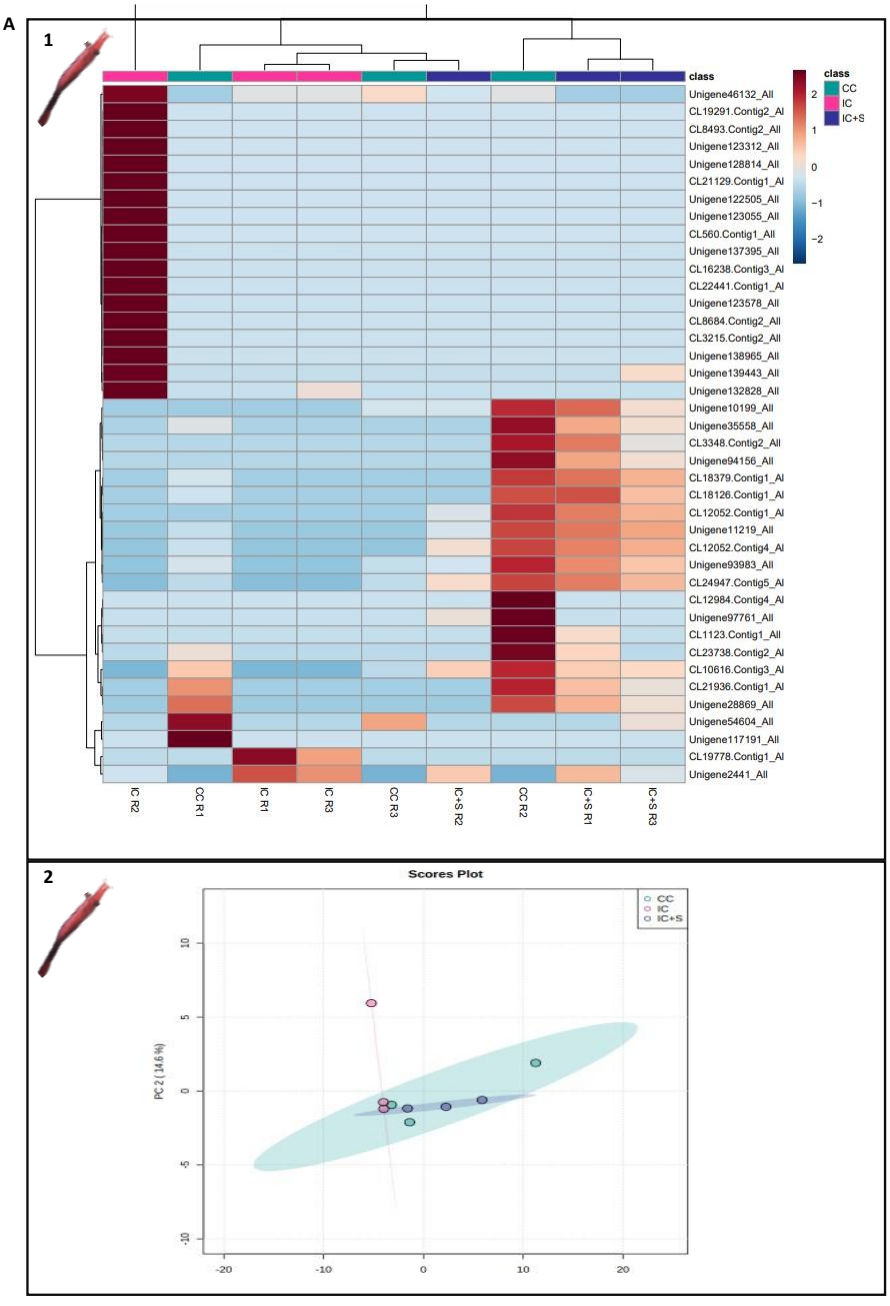
Table S5. Differentially expressed genes (DEGs) with their corresponding annotation in N National Center for Biotechnology Information (NCBI), non-redundant proteins database (Nr), non-reductant nucleotides (Nt), Swiss-Protein (SwissProt), Kyoto Encyclopedia of Genes and Genomes (KEGG), InterPro protein families and domains (InterPro), Genes Ontology (GO) and Clusters of Orthologous Genes (COG).

Table S6. BLASTx-based annotation of the 40 most differentially expressed unigenes in the shrimp transcriptomes *A. indicus* and *T. holthuisi* (20 up-regulated, 20 down-regulated). For all unigenes, the associated $\log_2$(FPKM) value, the probability value, and the annotation in National Center for Biotechnology Information (NCBI), non-redundant proteins database (Nr), non-reductant nucleotides (Nt), Swiss-Protein (SwissProt), Kyoto Encyclopedia of Genes and Genomes (KEGG), InterPro protein families and domains (InterPro), Genes Ontology (GO) and Clusters of Orthologous Genes (COG).

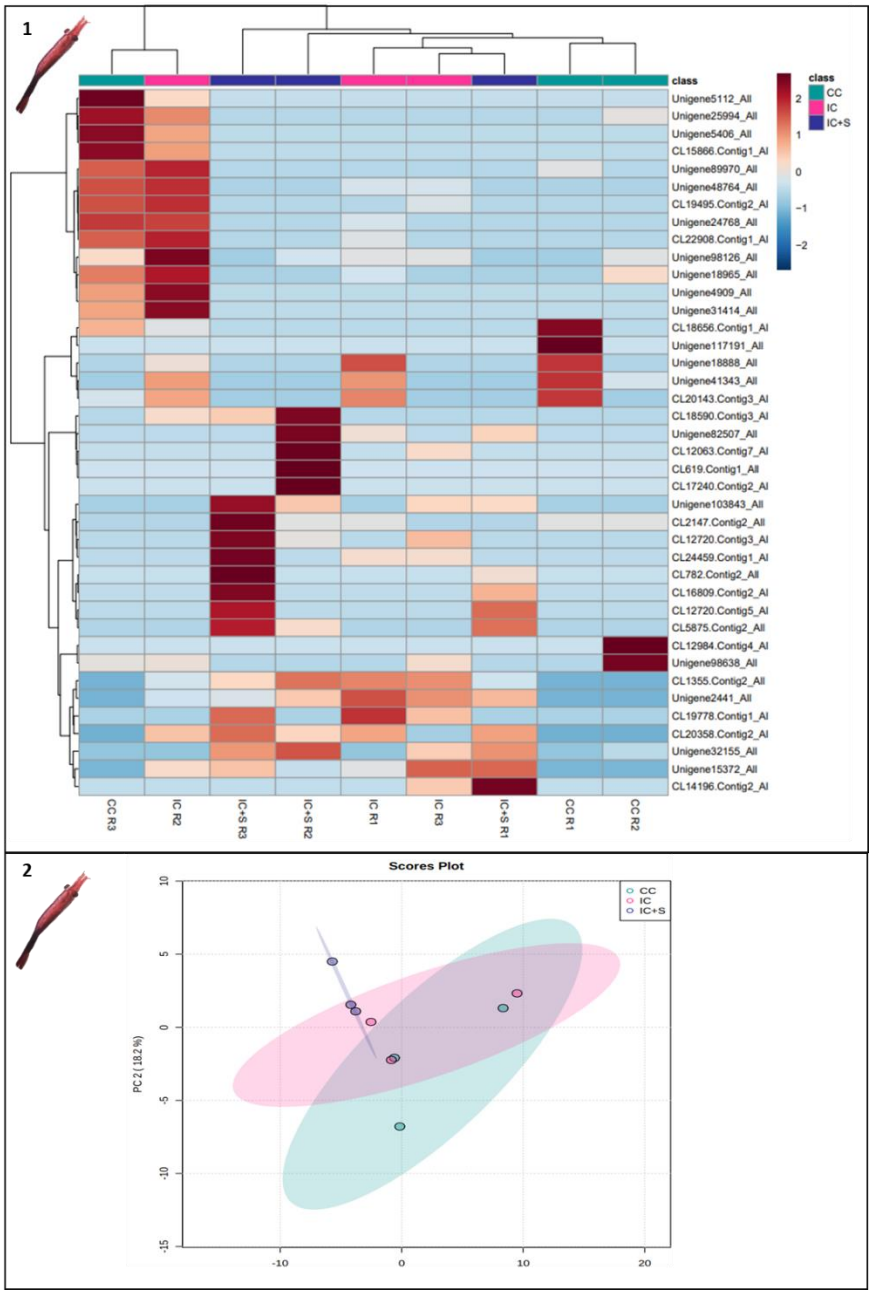
Table S7. BLASTx of the stress-related unigenes in the shrimp transcriptomes *A. indicus* and *T. holthuisi* with their level of expression.

As these tables are too large, they were not add in this version. But they are available and can be available on request to the author (alexia.lourtie@umons.ac.be).

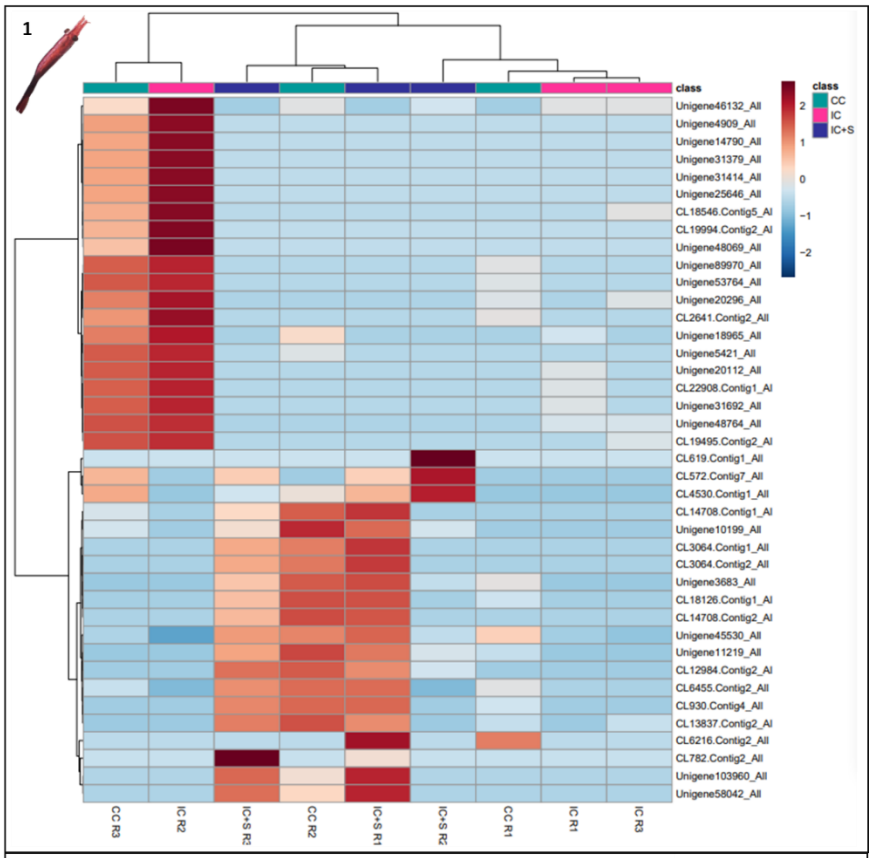
Figure S1. Differentially expressed unigenes clustering heatmaps (1) and ACP visualisation (2) of the 40 most differentially expressed unigenes (20 up-regulated, 20 down-regulated) in the shrimp transcriptomes *T. holthuisi* (A-C) and *A. indicus* (D-F). Both species were exposed to three conditions: "CC: Control condition (with host)"; "IC: Isolation condition"; "IC+S: Isolation condition + spinochromes"). 1) Heatmaps of differential unigenes expressions related host separation and chemicals dependency for *Tulearіocaris holthuisi* (A, B and C) and *Arete indicus* (D, E and F). The expression levels of statistically significant differentially expressed genes are shown for all biological replicates (shown as columns of the heatmaps). The heatmaps are based on all differentially expressed genes for every comparison (shown as row of the heatmaps). The similarity of the gene expression patterns is indicated by the Pearson's distance dendrogram at the top (sample level) and left side (unigene level). The colours of the heatmap indicate unigenes expression level. For every comparison (A and D = IC/CC; B and E= IC+S/CC and C and F =IC/IC+S), if fold change > 1 = gene(s) is upregulated the first condition compared to the second condition and appears in red, fold change < -1 = gene(s) is downregulated the first condition compared to the second condition and appear in blue, and if fold change is comprised between -1 and +1 = gene(s) is no differentially expressed in the corresponding comparison and appear in gray. The heatmap was generated using $\log_{10}$ (FPKM+ 0.001) values.

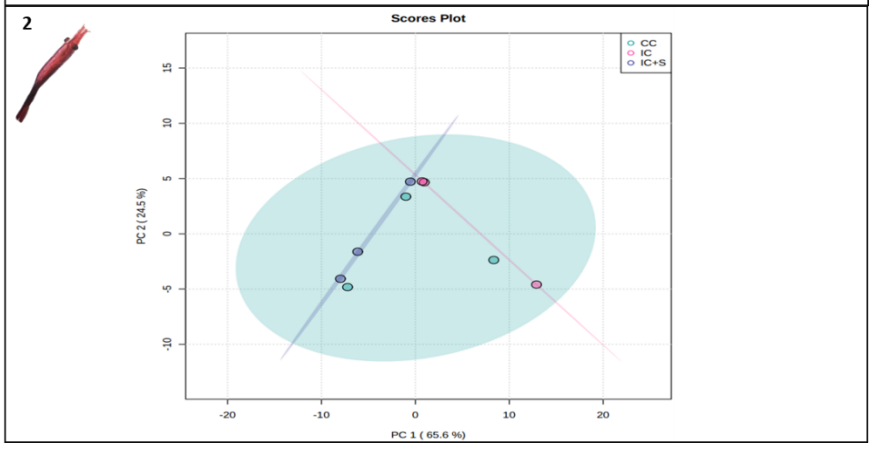


B

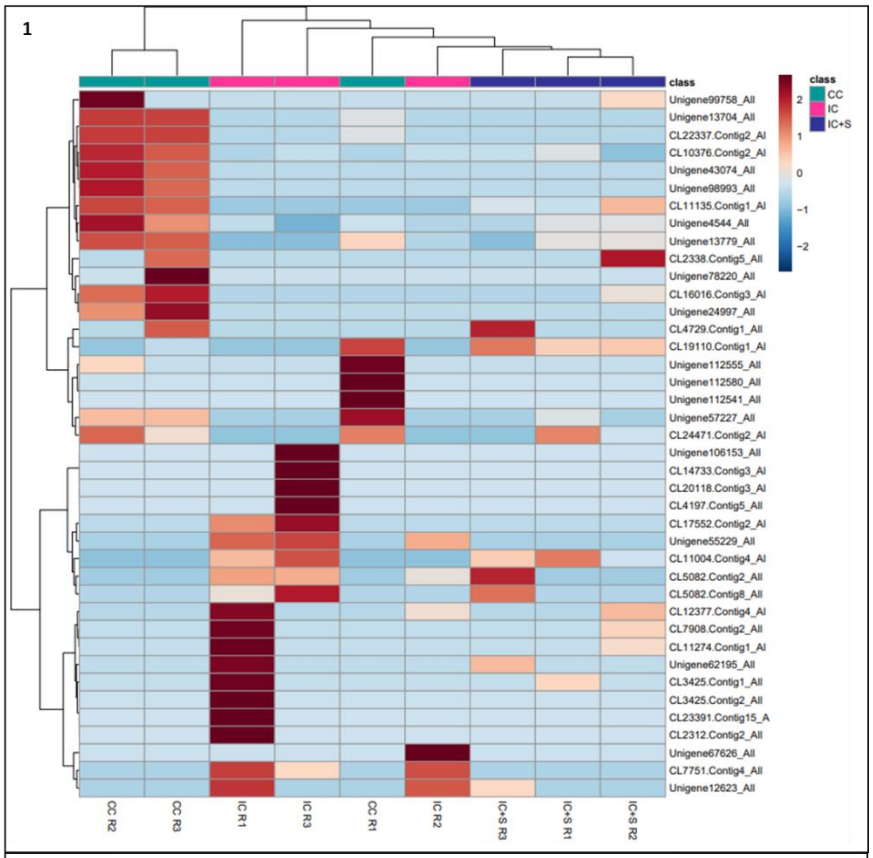

1
class
CC
IC
IC+S
2
1
0
−1
−2
Unigene5112_All
Unigene25994_All
Unigene5406_All
CL15866.Contig1_All
Unigene89970_All
Unigene48764_All
CL19495.Contig2_All
Unigene24768_All
CL22908.Contig1_All
Unigene98126_All
Unigene18965_All
Unigene4909_All
Unigene31414_All
CL18656.Contig1_All
Unigene117191_All
Unigene18888_All
Unigene41343_All
CL20143.Contig3_All
CL18590.Contig3_All
Unigene82507_All
CL12063.Contig7_All
CL619.Contig1_All
CL17240.Contig2_All
Unigene103843_All
CL2147.Contig2_All
CL12720.Contig3_All
CL24459.Contig1_All
CL782.Contig2_All
CL16809.Contig2_All
CL12720.Contig5_All
CL5875.Contig2_All
CL12984.Contig4_All
Unigene98638_All
CL1355.Contig2_All
Unigene2441_All
CL19778.Contig1_All
CL20358.Contig2_All
Unigene32155_All
Unigene15372_All
CL14196.Contig2_All
CC R3
IC R2
IC+S R3
IC+S R2
IC R1
IC R3
IC+S R1
CC R1
CC R2
2
Scores Plot
CC
IC
IC+S
PC 2 (18.2 %)
10
5
0
-5
-10
-15
-10
0
10
20

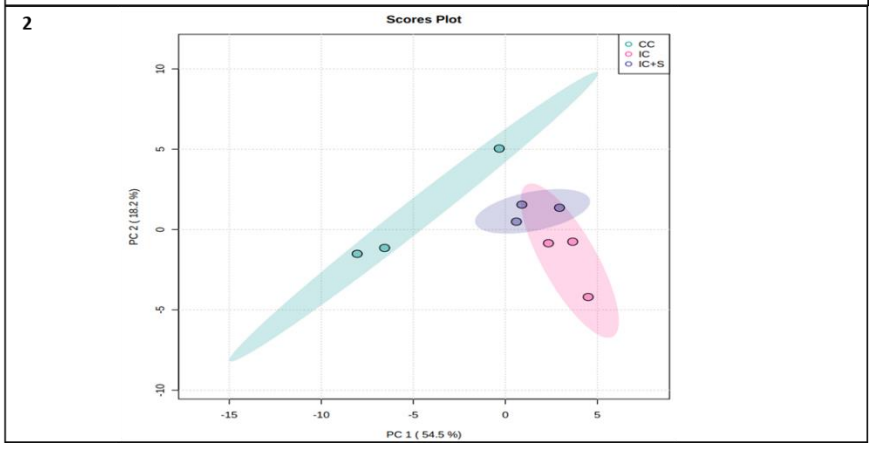
C


1
class
CC
IC
IC+S
Unigene46132_All
Unigene4909_All
Unigene14790_All
Unigene31379_All
Unigene31414_All
Unigene25646_All
CL18546.Contig5_All
CL19994.Contig2_All
Unigene48069_All
Unigene89970_All
Unigene53764_All
Unigene20296_All
CL2641.Contig2_All
Unigene18965_All
Unigene5421_All
Unigene20112_All
CL22908.Contig1_All
Unigene31692_All
Unigene48764_All
CL19495.Contig2_All
CL619.Contig1_All
CL572.Contig7_All
CL4530.Contig1_All
CL14708.Contig1_All
Unigene10199_All
CL3064.Contig1_All
CL3064.Contig2_All
Unigene3683_All
CL18126.Contig1_All
CL14708.Contig2_All
Unigene45530_All
Unigene11219_All
CL12984.Contig2_All
CL6455.Contig2_All
CL930.Contig4_All
CL13837.Contig2_All
CL6216.Contig2_All
CL782.Contig2_All
Unigene103960_All
Unigene58042_All
CC R3
IC R2
IC+S R3
CC R2
IC+S R1
IC+S R2
CC R1
IC R1
IC R3

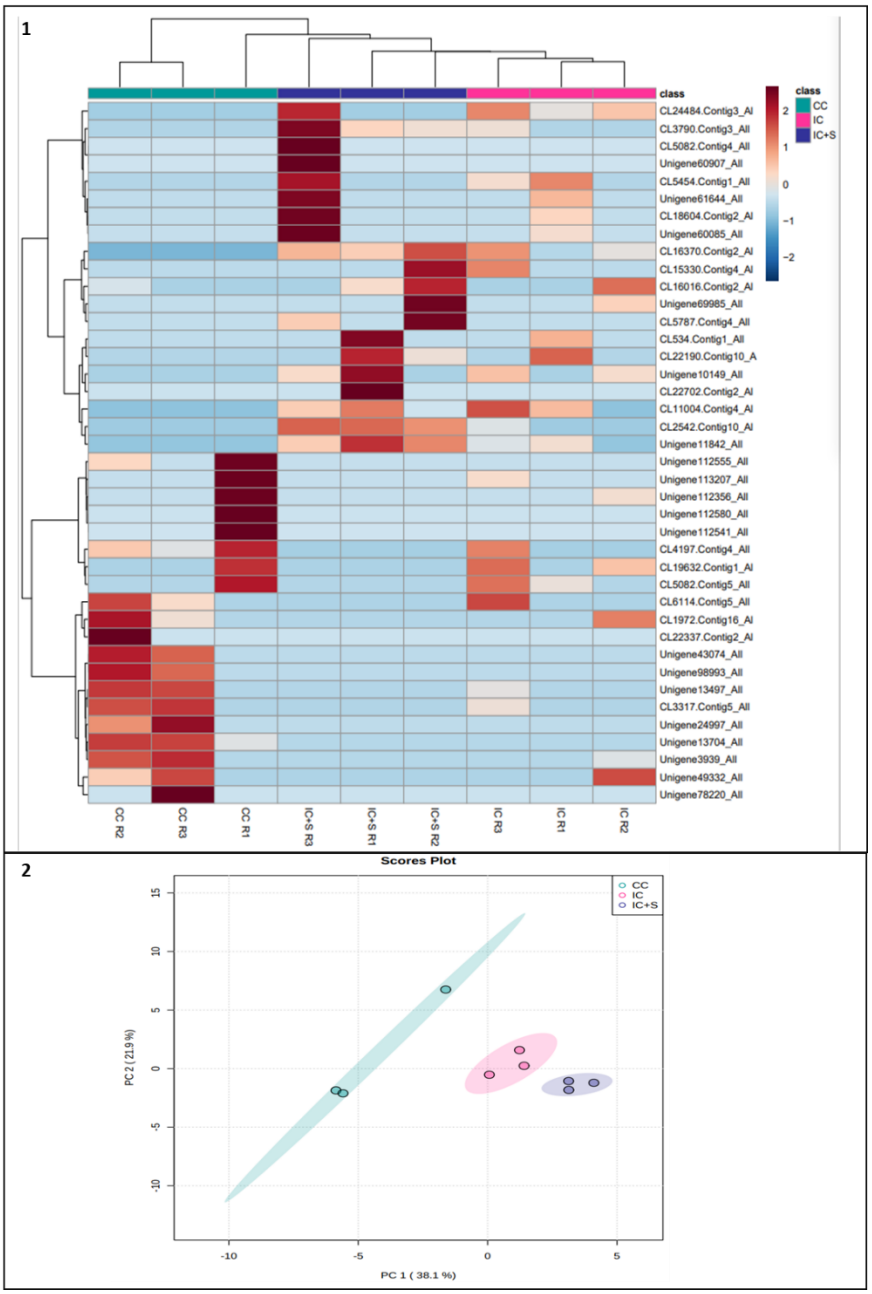

2
Scores Plot
CC
IC
IC+S
PC 2 (24.5 %)
PC 1 (65.6 %)

D


1
class
Unigene99758_All
Unigene13704_All
CL22337.Contig2_Al
CL10376.Contig2_Al
Unigene43074_All
Unigene98993_All
CL11135.Contig1_Al
Unigene4544_All
Unigene13779_All
CL2338.Contig5_All
Unigene78220_All
CL16016.Contig3_Al
Unigene24997_All
CL4729.Contig1_All
CL19110.Contig1_Al
Unigene112555_All
Unigene112580_All
Unigene112541_All
Unigene57227_All
CL24471.Contig2_Al
Unigene106153_All
CL14733.Contig3_Al
CL20118.Contig3_Al
CL4197.Contig5_All
CL17552.Contig2_Al
Unigene55229_All
CL11004.Contig4_Al
CL5082.Contig2_All
CL5082.Contig8_All
CL12377.Contig4_Al
CL7908.Contig2_All
CL11274.Contig1_Al
Unigene62195_All
CL3425.Contig1_All
CL3425.Contig2_All
CL23391.Contig15_A
CL2312.Contig2_All
Unigene67626_All
CL7751.Contig4_All
Unigene12623_All
CC R2
CC R3
IC R1
IC R3
CC R1
IC R2
IC+S R3
IC+S R1
IC+S R2
class
CC
IC
IC+S
2
1
0
−1
−2


2
Scores Plot
CC
IC
IC+S
PC 2 (18.2 %)
PC 1 (54.5 %)
10
5
0
-5
-10
-15
-10
-5
0
5

E


1
class
CL24484.Contig3_Al
CL3790.Contig3_All
CL5082.Contig4_All
Unigene60907_All
CL5454.Contig1_All
Unigene61644_All
CL18604.Contig2_Al
Unigene60085_All
CL16370.Contig2_Al
CL15330.Contig4_Al
CL16016.Contig2_Al
Unigene69985_All
CL5787.Contig4_All
CL534.Contig1_All
CL22190.Contig10_A
Unigene10149_All
CL22702.Contig2_Al
CL11004.Contig4_Al
CL2542.Contig10_Al
Unigene11842_All
Unigene112555_All
Unigene113207_All
Unigene112356_All
Unigene112580_All
Unigene112541_All
CL4197.Contig4_All
CL19632.Contig1_Al
CL5082.Contig5_All
CL6114.Contig5_All
CL1972.Contig16_Al
CL22337.Contig2_Al
Unigene43074_All
Unigene98993_All
Unigene13497_All
CL3317.Contig5_All
Unigene24997_All
Unigene13704_All
Unigene3939_All
Unigene49332_All
Unigene78220_All
CC R2
CC R3
CC R1
IC-S R3
IC-S R1
IC-S R2
IC R3
IC R1
IC R2
class
CC
IC
IC+S
2
1
0
−1
−2
2
Scores Plot
CC
IC
IC+S
PC 2 (21.9 %)
PC 1 (38.1 %)
15
10
5
0
-5
-10
-10
-5
0
5

F

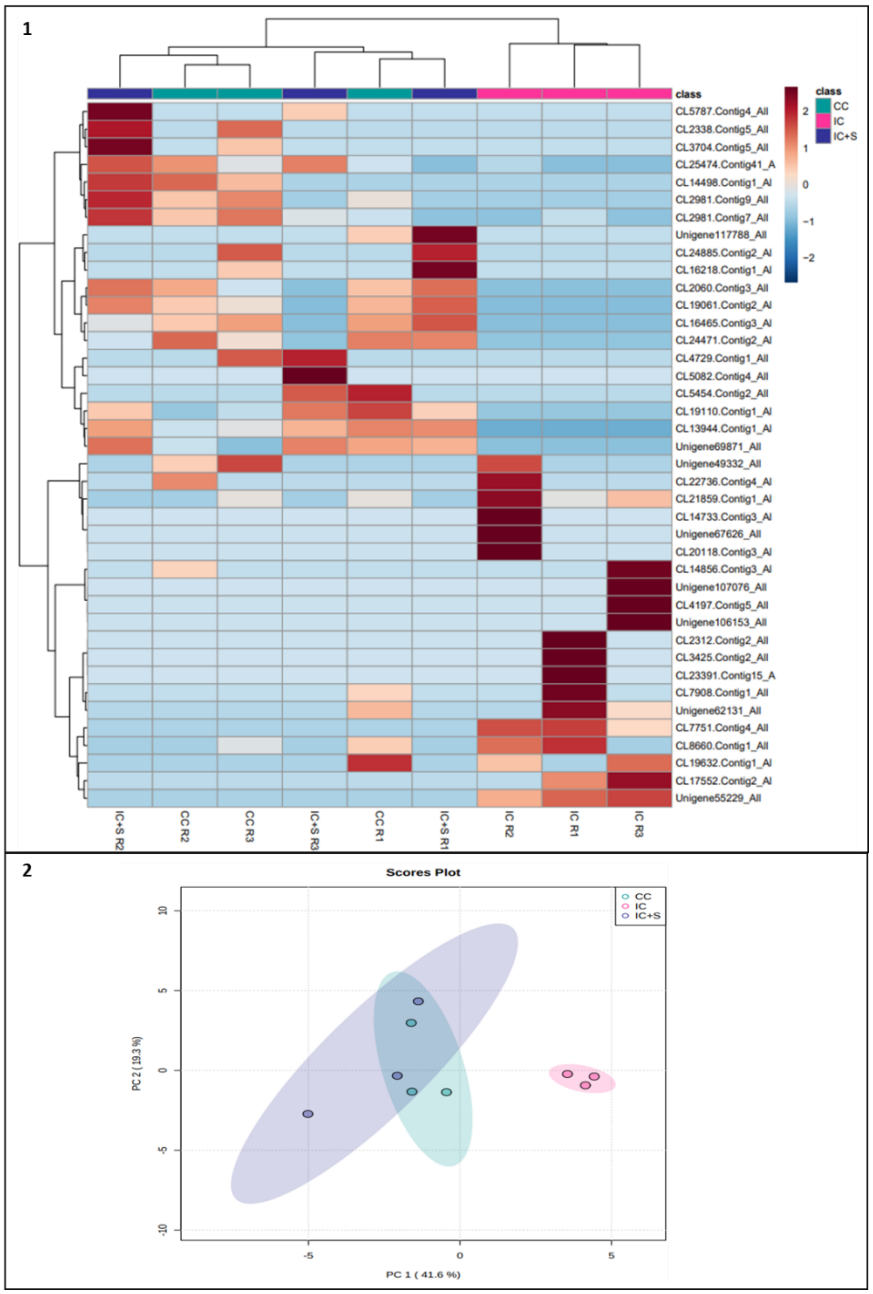
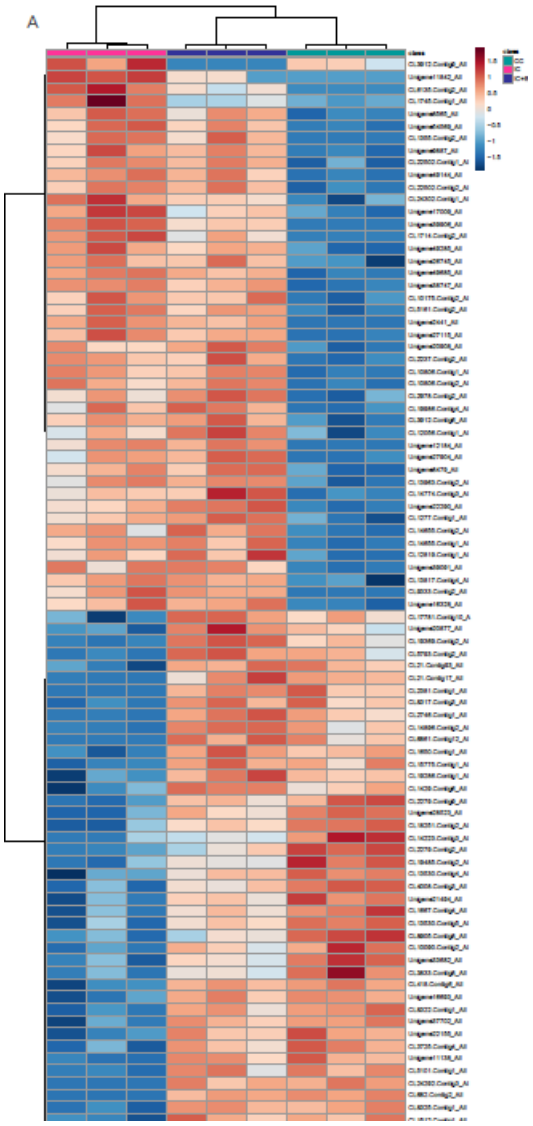
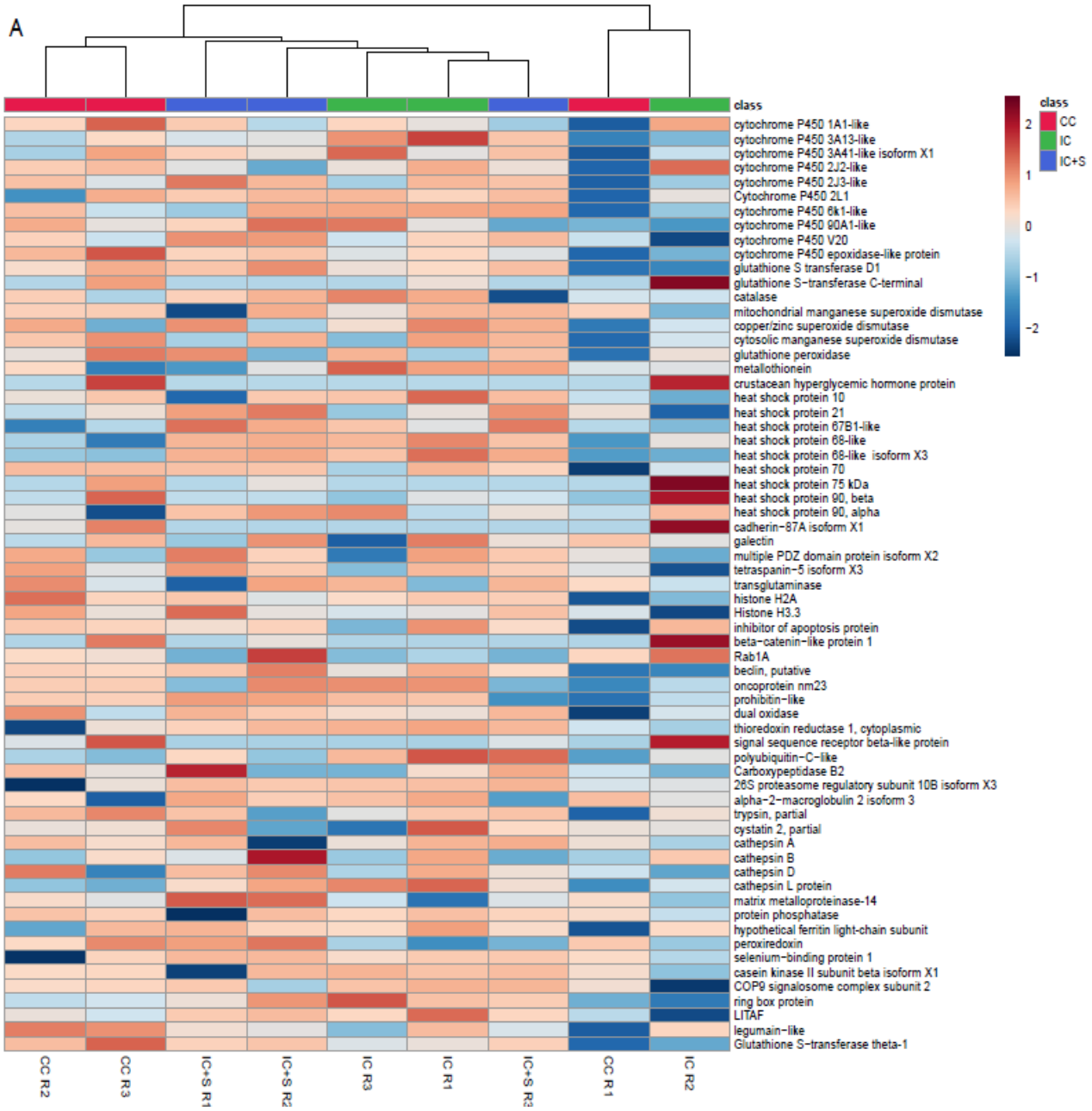

1
class
CC
IC
IC+S
2
1
0
−1
−2
CL5787.Contig4_All
CL2338.Contig5_All
CL3704.Contig5_All
CL25474.Contig41_A
CL14498.Contig1_Al
CL2981.Contig9_All
CL2981.Contig7_All
Unigene117788_All
CL24885.Contig2_Al
CL16218.Contig1_Al
CL2060.Contig3_All
CL19061.Contig2_Al
CL16465.Contig3_Al
CL24471.Contig2_Al
CL4729.Contig1_All
CL5082.Contig4_All
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CL19110.Contig1_Al
CL13944.Contig1_Al
Unigene69871_All
Unigene49332_All
CL22736.Contig4_Al
CL21859.Contig1_Al
CL14733.Contig3_Al
Unigene67626_All
CL20118.Contig3_Al
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Unigene107076_All
CL4197.Contig5_All
Unigene106153_All
CL2312.Contig2_All
CL3425.Contig2_All
CL23391.Contig15_A
CL7908.Contig1_All
Unigene62131_All
CL7751.Contig4_All
CL8660.Contig1_All
CL19632.Contig1_Al
CL17552.Contig2_Al
Unigene55229_All
IC+S R2
CC R2
CC R3
IC+S R3
CC R1
IC+S R1
IC R2
IC R1
IC R3
Scores Plot
CC
IC
IC+S
PC 2 (19.3 %)
PC 1 (41.6 %)
10
5
0
-5
-10
-5
0
5

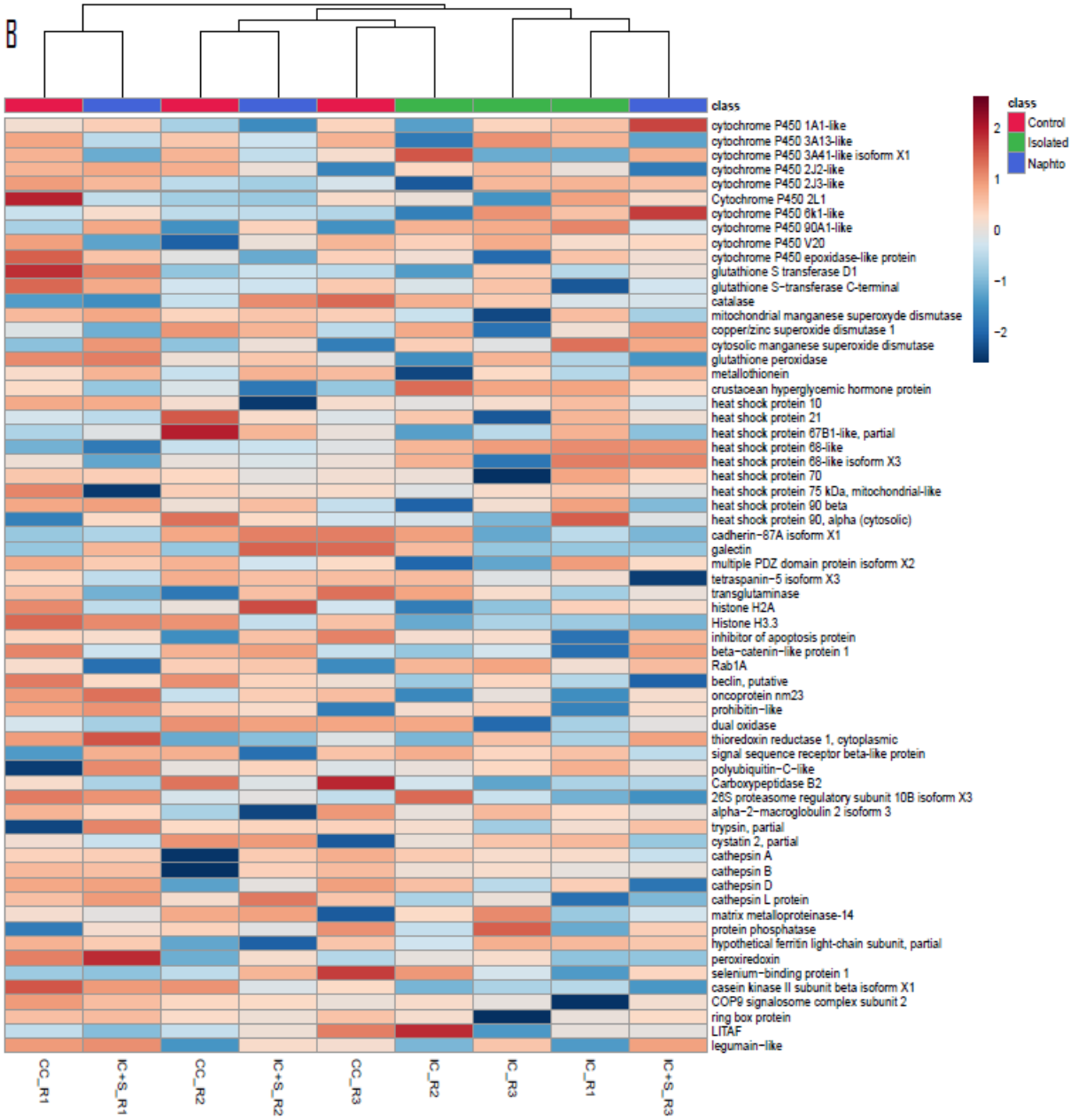
Figure S2. Clustering heatmap of the top 100 differentially expressed unigene expression in the context of host separation and chemical dependency for *Tulеariocaris holthuisi* (A-C) and *Arete indicus* (D-F). Every unigene ID is showed in the right side of heapmaps. Both species were exposed to three conditions: "CC: Control condition (with host)"; "IC: Isolation condition"; "IC+S: Isolation condition + spinochromes". The differentially expressed unigenes were determined in 3 independent replicates (shown as columns of the heatmaps). The heatmaps are based on all differentially expressed unigenes for every comparison (shown as row of the heatmaps). The similarity of the gene expression patterns is indicated by the Pearson's distance dendrogram at the top and left side. The colours of the heatmap indicate unigene expression level. For every comparison (A and D = IC/CC; B and E= IC+S/CC and C and F =IC/IC+S), if the fold change is > 1, the unigene is up-regulated in the first condition compared to the second condition and appears in red. If the fold change is < -1, the unigene is down-regulated in the first condition compared to the second condition and appears in blue. If the fold change is comprised between -1 and +, the unigene is not differentially expressed in the corresponding comparison and appears in gray. The heatmap was generated using $\log_{10}$ (FPKM+ 0.001) values.



As this Figure can not fit A4 format and remained visible, it was not add in this version. But they are available and can be requested.

Figure S3. Stress related genes expression in (A) *Tulearіocaris holtuisi* and (B) *A. indicus* subject to host separation syndrome. Every unigenes ID is showed in right side of heapmaps. There are three conditions: “CC: Control condition (with host)”; “IC: Isolation condition”; “IC+S: Isolation condition + spinochromes”. The differentially expressed genes were determined in 3 independent replicates (shown as columns of the heatmaps). The heatmaps are based on HSP genes expression (shown as row of the heatmaps). The similarity of the gene expression patterns is indicated by the Pearson’s distance dendrogram at the top and left side. The colours of the heatmap indicate the relative gene regulation level above (red) or below (blue) the average for each row. The heatmap was generated using $\log_{10}$ (FPKM+ 0.001) values.




B
class
cytochrome P450 1A1-like
cytochrome P450 3A13-like
cytochrome P450 3A41-like isoform X1
cytochrome P450 2J2-like
cytochrome P450 2J3-like
Cytochrome P450 2L1
cytochrome P450 6k1-like
cytochrome P450 90A1-like
cytochrome P450 V20
cytochrome P450 epoxidase-like protein
glutathione S transferase D1
glutathione S−transferase C-terminal
catalase
mitochondrial manganese superoxyde dismutase
copper/zinc superoxide dismutase 1
cytosolic manganese superoxide dismutase
glutathione peroxidase
metallothionein
crustacean hyperglycemic hormone protein
heat shock protein 10
heat shock protein 21
heat shock protein 67B1-like, partial
heat shock protein 68-like
heat shock protein 68-like isoform X3
heat shock protein 70
heat shock protein 75 kDa, mitochondrial-like
heat shock protein 90 beta
heat shock protein 90, alpha (cytosolic)
cadherin−87A isoform X1
galectin
multiple PDZ domain protein isoform X2
tetraspanin−5 isoform X3
transglutaminase
histone H2A
Histone H3.3
inhibitor of apoptosis protein
beta−catenin−like protein 1
Rab1A
beclin, putative
oncoprotein nm23
prohibitin−like
dual oxidase
thioredoxin reductase 1, cytoplasmic
signal sequence receptor beta-like protein
polyubiquitin−C−like
Carboxypeptidase B2
26S proteasome regulatory subunit 10B isoform X3
alpha−2−macroglobulin 2 isoform 3
trypsin, partial
cystatin 2, partial
cathepsin A
cathepsin B
cathepsin D
cathepsin L protein
matrix metalloproteinase-14
protein phosphatase
hypothetical ferritin light-chain subunit, partial
peroxiredoxin
selenium−binding protein 1
casein kinase II subunit beta isoform X1
COP9 signalosome complex subunit 2
ring box protein
LITAF
legumain−like
CC_R1
IC+S_R1
CC_R2
IC+S_R2
CC_R3
IC_R2
IC_R3
IC_R1
IC+S_R3
class
Control
Isolated
Naphto
2
1
0
−1
−2

Chapter IV



Table S1. Carotenoid pigments separated and highlighted using the HPLC system in all sample investigated

#	Time	Area	Height	Width	Sample
BetaApo_Standard 20µg	22,924	10444,1	1283,7	0,1356	BetaApo_Standard 20µg
BetaApo_Standard 5µg	22,917	5670,8	700,4	0,1349	BetaApo_Standard 5µg
BetaApo_Standard 1µg	22,922	430,3	53,4	0,1344	BetaApo_Standard 1µg
Astaxanthine_Standard 0,5µg	17,812	154,8	3,2	0,8112	Astaxanthine_Standard 0,5µg
Astaxanthine_Standard 2µg	17,772	520	11,1	0,7843	Astaxanthine_Standard 2µg
Astaxanthine_Standard 5µg	17,783	674,4	14,5	0,7748	Astaxanthine_Standard 5µg
Astaxanthine_Standard 10µg	17,712	2365,4	53,6	0,7356	Astaxanthine_Standard 10µg
Zeaxanthine-like1	14,992	168,7	9,6	0,2935	Culcita_Teg_Etoile3
Zeaxanthine-like2	15,512	928,8	76,8	0,2015	Culcita_Teg_Etoile3
Zeaxanthine-like3	16,354	615,4	63,7	0,161	Culcita_Teg_Etoile3
Zeaxanthine-like4	16,795	1484,2	98,9	0,2501	Culcita_Teg_Etoile3
Astaxanthine_Trans	17,611	2969,2	85,5	0,5785	Culcita_Teg_Etoile3
Astaxanthine_Cis	18,437	1831,4	68,9	0,4433	Culcita_Teg_Etoile3
Zeaxanthine-like5	19,066	365	38,7	0,157	Culcita_Teg_Etoile3
Lutéine	19,629	324,1	32,9	0,1643	Culcita_Teg_Etoile3
Zeaxanthine	19,93	2313,4	244,9	0,1574	Culcita_Teg_Etoile3

Zeaxanthine-like_cis1	20,256	570,6	48	0,1982	Culcita_Teg_Etoile3
Zeaxanthine-like_cis2	20,589	641,1	65,6	0,1629	Culcita_Teg_Etoile3
BetaApo_Standard	22,924	571,3	51,7	0,1842	Culcita_Teg_Etoile3
Zeaxanthine-like6	26,116	69,9	9,6	0,1211	Culcita_Teg_Etoile3
Zeaxanthine-like1	14,971	72,6	7,6	0,1596	Culcita_Teg_Etoile4
Zeaxanthine-like2	15,521	170,5	13,9	0,2048	Culcita_Teg_Etoile4
Zeaxanthine-like3	16,353	368,5	34,3	0,1793	Culcita_Teg_Etoile4
Zeaxanthine-like4	16,789	1376,1	105,1	0,2183	Culcita_Teg_Etoile4
Astaxanthine_Trans	17,6	3771,1	106,5	0,59	Culcita_Teg_Etoile4
Astaxanthine_Cis	18,267	2259,7	84,4	0,446	Culcita_Teg_Etoile4
Zeaxanthine-like5	19,077	492,6	54,7	0,1501	Culcita_Teg_Etoile4
Lutéine	19,637	285,1	35,3	0,1345	Culcita_Teg_Etoile4
Zeaxanthine	19,935	2055,5	227,3	0,1507	Culcita_Teg_Etoile4
Zeaxanthine-like_cis1	20,26	385,9	39,3	0,1636	Culcita_Teg_Etoile4
Zeaxanthine-like_cis2	20,594	449,7	53	0,1415	Culcita_Teg_Etoile4
BetaApo_Standard	22,927	477,8	43,8	0,1817	Culcita_Teg_Etoile4
Zeaxanthine-like6	26,117	282	32,1	0,1465	Culcita_Teg_Etoile4

Zeaxanthine-like1	14,969	59	5,8	0,1698	Culcita_Teg_Etoile5
Zeaxanthine-like3	16,354	333	32,7	0,1696	Culcita_Teg_Etoile5
Zeaxanthine-like4	16,782	1441,7	146,7	0,1638	Culcita_Teg_Etoile5
Astaxanthine_Trans	17,581	9175,8	270,2	0,5659	Culcita_Teg_Etoile5
Astaxanthine_Cis	18,418	4703,1	173,1	0,4527	Culcita_Teg_Etoile5
Zeaxanthine-like5	19,073	267,6	28,2	0,1584	Culcita_Teg_Etoile5
Lutéine	19,637	112,1	14,5	0,1286	Culcita_Teg_Etoile5
Zeaxanthine	19,935	1280,2	146,6	0,1455	Culcita_Teg_Etoile5
Zeaxanthine-like_cis1	20,263	197,1	20,9	0,1574	Culcita_Teg_Etoile5
Zeaxanthine-like_cis2	20,59	265,6	32	0,1385	Culcita_Teg_Etoile5
Zeaxanthine-like6	26,12	104,5	13,5	0,1286	Culcita_Teg_Etoile5
BetaApo_Standard	22,928	492,8	47,1	0,1744	Culcita_Teg_Etoile5
Zeaxanthine-like1	14,974	248,9	30,4	0,1365	Culcita_Teg_Etoile6
Unknow2	15,235	15,7	2,6	0,1021	Culcita_Teg_Etoile6
Zeaxanthine-like2	15,508	116	11,9	0,1625	Culcita_Teg_Etoile6
Unknow3	15,752	16,4	3,1	0,0874	Culcita_Teg_Etoile6
Unknow1	16,071	155,4	19,5	0,1326	Culcita_Teg_Etoile6

Zeaxanthine-like3	16,356	279,2	38,6	0,1204	Culcita_Teg_Etoile6
Zeaxanthine-like4	16,785	1998	183,9	0,1811	Culcita_Teg_Etoile6
Astaxanthine_Trans	17,58	10757,9	328,5	0,5458	Culcita_Teg_Etoile6
Astaxanthine_Cis	18,405	5876	222,4	0,4403	Culcita_Teg_Etoile6
Zeaxanthine-like5	19,053	637,2	69,9	0,152	Culcita_Teg_Etoile6
Lutéine	19,639	283,8	36,3	0,1303	Culcita_Teg_Etoile6
Zeaxanthine	19,937	3216,3	352,6	0,152	Culcita_Teg_Etoile6
Zeaxanthine-like_cis1	20,259	724,2	87,4	0,1381	Culcita_Teg_Etoile6
Zeaxanthine-like_cis2	20,593	638,7	82,7	0,1288	Culcita_Teg_Etoile6
BetaApo_Standard	23,299	431,9	50	0,144	Culcita_Teg_Etoile6
Zeaxanthine-like6	26,118	198	30,1	0,1098	Culcita_Teg_Etoile6
Zeaxanthine-like1	14,978	74	8,5	0,1447	Culcita_Teg_Etoile7
Zeaxanthine-like2	15,523	275,7	24,7	0,1859	Culcita_Teg_Etoile7
Zeaxanthine-like3	16,36	424,1	46,2	0,1529	Culcita_Teg_Etoile7
Zeaxanthine-like4	16,787	1802,4	152,3	0,1973	Culcita_Teg_Etoile7
Astaxanthine_Trans	17,601	3676,1	116,9	0,5243	Culcita_Teg_Etoile7
Astaxanthine_Cis	18,419	2113,8	82,3	0,428	Culcita_Teg_Etoile7

Zeaxanthine-like5	19,06	246,5	27,2	0,1512	Culcita_Teg_Etoile7
Lutéine	19,627	99,2	30,1	0,055	Culcita_Teg_Etoile7
Zeaxanthine	19,939	1270,1	250,1	0,0846	Culcita_Teg_Etoile7
Zeaxanthine-like_cis1	19,94	1265,6	250	0,0844	Culcita_Teg_Etoile7
Zeaxanthine-like_cis2	20,594	457,1	54,3	0,1402	Culcita_Teg_Etoile7
Zeaxanthine-like5	20,598	172,1	54,5	0,0527	Culcita_Teg_Etoile7
BetaApo_Standard	22,926	419,6	43,7	0,16	Culcita_Teg_Etoile7
Zeaxanthine-like6	26,119	246,1	36,5	0,1125	Culcita_Teg_Etoile7
Beta-carotène	30,05	27,9	1,7	0,2783	Culcita_Teg_Etoile7
Zeaxanthine-like1	14,981	115,3	14,2	0,1352	Culcita_Teg_Etoile8
Zeaxanthine-like2	15,525	480,5	43,4	0,1846	Culcita_Teg_Etoile8
Zeaxanthine-like3	16,36	731,3	72,9	0,1671	Culcita_Teg_Etoile8
Zeaxanthine-like4	16,788	2229,7	172,2	0,2158	Culcita_Teg_Etoile8
Astaxanthine_Trans	17,584	7412,7	252,1	0,49	Culcita_Teg_Etoile8
Astaxanthine_Cis	18,415	4067,2	161,9	0,4187	Culcita_Teg_Etoile8
Zeaxanthine-like5	19,073	786,4	86,9	0,1508	Culcita_Teg_Etoile8
Lutéine	19,64	388,5	51,8	0,125	Culcita_Teg_Etoile8
Zeaxanthine	19,938	4612,7	514,3	0,1495	Culcita_Teg_Etoile8

Zeaxanthine-like_cis1	20,26	875,1	101,3	0,1583	Culcita_Teg_Etoile8
Zeaxanthine-like_cis2	20,593	985,5	127,2	0,1292	Culcita_Teg_Etoile8
BetaApo_Standard	22,926	982,7	73,8	0,222	Culcita_Teg_Etoile8
Zeaxanthine-like6	26,118	189,8	26,1	0,1211	Culcita_Teg_Etoile8
NA	0	0	0	0	Zenopontonia_Trans1
BetaApo_Standard	22,928	492,8	47,1	0,1744	Zenopontonia_Trans1
NA	0	0	0	0	Zenopontonia_Trans2
BetaApo_Standard	22,926	982,7	73,8	0,222	Zenopontonia_Trans2
NA	0	0	0	0	Zenopontonia_Trans3
BetaApo_Standard	22,926	419,6	43,7	0,16	Zenopontonia_Trans3
Astaxanthine_Trans	17,88	390,8	10,5	0,6185	Zenopontonia_FullColored1
Astaxanthine_Cis	18,455	297	9,2	0,538	Zenopontonia_FullColored1
BetaApo_Standard	22,928	412,6	51	0,1347	Zenopontonia_FullColored1
Astaxanthine_Trans	17,768	223,1	6,2	0,5974	Zenopontonia_FullColored2
Astaxanthine_Cis	18,401	185,4	7,3	0,4208	Zenopontonia_FullColored2
Zeaxanthine	19,899	2,4	3.1E-1	0,1265	Zenopontonia_FullColored2
BetaApo_Standard	22,893	376,3	44,2	0,1418	Zenopontonia_FullColored2
Zeaxanthine-like5	26,088	28,3	1,3	0,3579	Zenopontonia_FullColored2

Unknow6	28,593	18,1	2,3	0,1294	Zenopontonia_FullColored2
Unknow7	28,734	13,5	1,4	0,163	Zenopontonia_FullColored2
Unknow8	28,945	6,6	1,3	0,0869	Zenopontonia_FullColored2
Beta-carotène	29,993	10,6	1,4	0,1268	Zenopontonia_FullColored2
Zeaxanthine-like4	16,774	11,8	1	0,1886	Zenopontonia_FullColored3
Astaxanthine_Trans	17,883	299,5	8,5	0,5867	Zenopontonia_FullColored3
Astaxanthine_Cis	18,441	236,1	7,5	0,5263	Zenopontonia_FullColored3
Zeaxanthine	19,921	9,6	1,1	0,144	Zenopontonia_FullColored3
BetaApo_Standard	22,924	401,7	47,8	0,1399	Zenopontonia_FullColored3
Zeaxanthine-like5	26,111	49,9	3,2	0,2613	Zenopontonia_FullColored3
Unknow5	27,689	73,4	3,7	0,3315	Zenopontonia_FullColored3
Unknow6	28,615	69,9	3,5	0,3303	Zenopontonia_FullColored3
Beta-carotène	30,031	57,6	3,6	0,2664	Zenopontonia_FullColored3
Astaxanthine_Trans	17,921	109,4	3,2	0,5737	Zenopontonia_stripedColored1
Astaxanthine_Cis	18,451	111,9	4	0,4699	Zenopontonia_stripedColored1
Zeaxanthine	19,928	1,2	1.2E-1	0,1143	Zenopontonia_stripedColored1

BetaApo_Standard	22,915	356,5	40,9	0,1453	Zenopontonia_stripedColored1
Zeaxanthine-like5	26,116	20,8	8.9E-1	0,3893	Zenopontonia_stripedColored1
Unknow6	28,608	13,3	2	0,1091	Zenopontonia_stripedColored1
Unknow7	28,747	12,2	1,4	0,1432	Zenopontonia_stripedColored1
Unknow8	28,96	5,9	1,1	0,0926	Zenopontonia_stripedColored1
Beta-carotène	30,015	5,7	7.6E-1	0,125	Zenopontonia_stripedColored1
Astaxanthine_Trans	17,881	206,7	6,2	0,5569	Zenopontonia_stripedColored2
Astaxanthine_Cis	18,445	168	5,8	0,4817	Zenopontonia_stripedColored2
Zeaxanthine	19,915	1,9	2.3E-1	0,1409	Zenopontonia_stripedColored2
BetaApo_Standard	22,913	390,5	46,6	0,1397	Zenopontonia_stripedColored2
Zeaxanthine-like5	26,106	23,8	1,3	0,3034	Zenopontonia_stripedColored2
Unknow6	28,608	8,2	9.9E-1	0,139	Zenopontonia_stripedColored2
Unknow7	28,743	14,8	1,6	0,1577	Zenopontonia_stripedColored2
Unknow8	28,899	2,2	5.4E-1	0,0664	Zenopontonia_stripedColored2
Beta-carotène	30,029	9,9	1,3	0,125	Zenopontonia_stripedColored2
Astaxanthine_Trans	17,898	230,8	6,9	0,5569	Zenopontonia_stripedColored3

Astaxanthine_Cis	18,456	214,8	7,7	0,4625	Zenopontonia_stripedColored3
Zeaxanthine	19,927	7,9	1,1	0,1234	Zenopontonia_stripedColored3
BetaApo_Standard	22,924	362,1	42,7	0,1412	Zenopontonia_stripedColored3
Zeaxanthine-like5	26,115	39,9	3	0,2239	Zenopontonia_stripedColored3
Unknow6	28,614	17,4	2,4	0,1182	Zenopontonia_stripedColored3
Unknow7	28,751	20,6	2,4	0,1437	Zenopontonia_stripedColored3
Unknow8	28,966	5,7	9.9E-1	0,0958	Zenopontonia_stripedColored3
Beta-carotène	30,035	14,7	2	0,1255	Zenopontonia_stripedColored3
Unidentified (probably anthraquinones)	5,363	4096,3	215,4	0,3169	Phanogenia distincta 2
Unidentified (probably anthraquinones)	5,7	4046,2	169	0,3991	Phanogenia distincta 2
Trans-Astaxanthin	18,432	41,7	8.4E-1	0,8286	Phanogenia distincta 2
Cis-Astaxanthin	18,731	26,5	1,2	0,3749	Phanogenia distincta 2
Trans-Canthaxanthin	21,129	109,1	14,8	0,1228	Phanogenia distincta 2
Cis-Canthaxanthin	21,667	26,6	2,9	0,1533	Phanogenia distincta 2
BetaApo_Standard	23,305	390,4	47,3	0,1377	Phanogenia distincta 2

Unidentified (probably anthraquinones)	5,375	1445,2	70,8	0,3402	Phanogenia distincta 4
Unidentified (probably anthraquinones)	5,562	1451,5	58,8	0,4116	Phanogenia distincta 4
Trans-Astaxanthin	18,131	53,9	1,2	0,7478	Phanogenia distincta 4
Cis-Astaxanthin	18,733	36,8	1,7	0,3654	Phanogenia distincta 4
Trans-Canthaxanthin	21,132	165,2	22,4	0,1227	Phanogenia distincta 4
Cis-Canthaxanthin	21,668	47,5	4,8	0,1638	Phanogenia distincta 4
BetaApo_Standard	23,302	390,8	47	0,1385	Phanogenia distincta 4
Unidentified (probably anthraquinones)	5,352	1679,6	66,3	0,422	Phanogenia distincta 5
Unidentified (probably anthraquinones)	5,558	733,3	45,1	0,2708	Phanogenia distincta 5
Zeaxanthin	20,208	13,6	1,8	0,1263	Phanogenia distincta 5
Canthaxanthin-like 1	20,796	4,6	5.1E-1	0,1505	Phanogenia distincta 5
Trans-Canthaxanthin	21,129	49,8	6,7	0,1239	Phanogenia distincta 5
Cis-Canthaxanthin	21,669	7,4	7.4E-1	0,1658	Phanogenia distincta 5
BetaApo_Standard	23,301	353,5	43,3	0,136	Phanogenia distincta 5

Trans-Beta-carotène	30,271	16,4	2,2	0,1224	Phanogenia distincta 5
Unidentified (probably anthraquinones)	5,283	2491	80,7	0,5143	Phanogenia distincta 6
Unidentified (probably anthraquinones)	5,698	1604,3	70,8	0,3776	Phanogenia distincta 6
Trans-Canthaxanthin	21,135	229	31,4	0,1215	Phanogenia distincta 6
Cis-Canthaxanthin	21,671	26,6	2,7	0,1616	Phanogenia distincta 6
BetaApo_Standard	23,305	434,1	54,2	0,1336	Phanogenia distincta 6
Zeaxanthine-like	26,701	6,5	9.3E-1	0,1152	Phanogenia distincta 6
Trans-Beta-carotène	30,273	10,8	9.8E-1	0,1839	Phanogenia distincta 6
Unidentified (probably anthraquinones)	5,355	2391,2	105,6	0,3773	Phanogenia distincta 7
Unidentified (probably anthraquinones)	5,588	1530,6	73	0,3497	Phanogenia distincta 7
Trans-Canthaxanthin	21,134	77,9	10,4	0,1243	Phanogenia distincta 7
Cis-Canthaxanthin	21,673	15,2	1,6	0,1539	Phanogenia distincta 7
BetaApo_Standard	23,306	385,7	46,4	0,1385	Phanogenia distincta 7
Unidentified (probably anthraquinones)	5,365	927,7	40,8	0,3786	Phanogenia distincta 11

Unidentified (probably anthraquinones)	5,551	524	36,9	0,237	Phanogenia distincta 11
Trans-Astaxanthin	18,085	30	8.1E-1	0,6179	Phanogenia distincta 11
Cis-Astaxanthin	18,72	19,4	1	0,321	Phanogenia distincta 11
Trans-Canthaxanthin	21,136	372,1	50	0,124	Phanogenia distincta 11
Cis-Canthaxanthin	21,672	89,5	9,1	0,1641	Phanogenia distincta 11
BetaApo_Standard	23,305	367,7	44	0,1394	Phanogenia distincta 11
Unidentified (probably anthraquinones)	5,34	3302,8	149,4	0,3684	Phanogenia distincta 17
Unidentified (probably anthraquinones)	5,578	1313,3	73	0,2998	Phanogenia distincta 17
Trans-Astaxanthin	18,187	54,7	1,4	0,4513	Phanogenia distincta 17
Cis-Astaxanthin	18,458	19,9	9.9E-1	0,3351	Phanogenia distincta 17
Trans-Canthaxanthin	21,137	278,2	37,4	0,1241	Phanogenia distincta 17
Cis-Canthaxanthin	21,674	38,3	3,4	0,1853	Phanogenia distincta 17
BetaApo_Standard	23,306	420	51,7	0,1353	Phanogenia distincta 17
Unidentified (probably anthraquinones)	5,345	5036,9	227,7	0,3687	Phanogenia distincta 18

Unidentified (probably anthraquinones)	5,594	2007,8	113,7	0,2944	Phanogenia distincta 18
Trans-Astaxanthin	18,183	23	5.5E-1	0,702	Phanogenia distincta 18
Cis-Astaxanthin	18,709	15	7.4E-1	0,3352	Phanogenia distincta 18
Trans-Canthaxanthin	21,133	109,9	14,3	0,1281	Phanogenia distincta 18
Cis-Canthaxanthin	21,673	33,2	2,9	0,1889	Phanogenia distincta 18
BetaApo_Standard	23,301	344,2	38,8	0,1479	Phanogenia distincta 18
Unidentified (probably anthraquinones)	5,353	1931,1	83	0,3876	Phanogenia distincta 15
Unidentified (probably anthraquinones)	5,568	846,2	53,7	0,2628	Phanogenia distincta 15
Trans-Canthaxanthin	21,118	95,1	12,5	0,1269	Phanogenia distincta 15
Cis-Canthaxanthin	21,654	12,4	1,2	0,1775	Phanogenia distincta 15
BetaApo_Standard	23,29	491,5	59,6	0,1374	Phanogenia distincta 15
Lutein-like1	17,402	29,2	3,5	0,1372	Synalpheus stimpsonii 2
Trans-Astaxanthin	18,222	193,9	4,7	0,488	Synalpheus stimpsonii 2
Zeaxanthin-like 1	18,534	99,4	13,9	0,1193	Synalpheus stimpsonii 2
Cis-Astaxanthin	18,778	29,7	2,8	0,179	Synalpheus stimpsonii 2

Zeaxanthin-like 2	19,366	530	66,2	0,1335	Synalpheus stimpsonii 2
Lutein	19,919	63,3	10,4	0,1017	Synalpheus stimpsonii 2
Zeaxanthin	20,204	1250,7	156,1	0,1336	Synalpheus stimpsonii 2
Zeaxanthin-like 3	20,548	26,4	3,5	0,1256	Synalpheus stimpsonii 2
Canthaxanthin-like 1	20,875	182,8	23,9	0,1275	Synalpheus stimpsonii 2
BetaApo_Standard	23,315	277,9	33,9	0,1368	Synalpheus stimpsonii 2
Zeaxanthin-like 5	26,394	45,7	5,5	0,1377	Synalpheus stimpsonii 2
Zeaxanthin-like 6	28,195	29,3	4,7	0,1049	Synalpheus stimpsonii 2
Trans-Beta-carotène	30,313	377,5	38,6	0,163	Synalpheus stimpsonii 2
Cis-Beta-carotène	30,736	83,6	9,1	0,1539	Synalpheus stimpsonii 2
Lutein-like1	17,407	8,3	1,2	0,1205	Synalpheus stimpsonii 4.1
Trans-Astaxanthin	18,261	79,4	2,4	0,4072	Synalpheus stimpsonii 4.1
Cis-Astaxanthin	18,759	27,2	2,2	0,2056	Synalpheus stimpsonii 4.1
Zeaxanthin-like 2	19,369	29,3	3,9	0,1252	Synalpheus stimpsonii 4.1
Lutein	19,922	11,1	1,8	0,106	Synalpheus stimpsonii 4.1
Zeaxanthin	20,211	148,7	18,9	0,1309	Synalpheus stimpsonii 4.1

Canthaxanthin-like 1	20,882	18,3	3,1	0,0981	Synalpheus stimpsonii 4.1
BetaApo_Standard	23,323	317,5	37,3	0,1417	Synalpheus stimpsonii 4.1
Zeaxanthin-like 4	23,857	25,9	4,2	0,1016	Synalpheus stimpsonii 4.1
Zeaxanthin-like 6	28,195	3,9	6.6E-1	0,0985	Synalpheus stimpsonii 4.1
Trans-Beta-carotène	30,31	48	4,8	0,165	Synalpheus stimpsonii 4.1
Cis-Beta-carotène	30,732	10,2	1,1	0,1601	Synalpheus stimpsonii 4.1
Lutein-like1	17,408	17,2	2,4	0,1192	Synalpheus stimpsonii 4.2
Trans-Astaxanthin	18,242	204,3	4,6	0,5431	Synalpheus stimpsonii 4.2
Zeaxanthin-like 1	18,537	11,2	2	0,0941	Synalpheus stimpsonii 4.2
Cis-Astaxanthin	18,765	51,1	4,3	0,1963	Synalpheus stimpsonii 4.2
Zeaxanthin-like 2	19,375	58,4	7,7	0,1257	Synalpheus stimpsonii 4.2
Lutein	19,927	16	2,5	0,1055	Synalpheus stimpsonii 4.2
Zeaxanthin	20,216	236,8	30,2	0,1309	Synalpheus stimpsonii 4.2
Zeaxanthin 3	20,555	8,8	1,2	0,1204	Synalpheus stimpsonii 4.2
Canthaxanthin-like 1	20,886	46,3	6,3	0,1227	Synalpheus stimpsonii 4.2
BetaApo_Standard	23,324	286,6	32,7	0,1461	Synalpheus stimpsonii 4.2
Zeaxanthin-like 4	23,855	12,8	2,1	0,1031	Synalpheus stimpsonii 4.2

Zeaxanthin-like 5	26,388	10	1,4	0,1169	Synalpheus stimpsonii 4.2
Zeaxanthin-like 6	28,193	4,6	8.3E-1	0,0909	Synalpheus stimpsonii 4.2
Trans-Beta-carotène	30,305	46,7	4,8	0,1609	Synalpheus stimpsonii 4.2
Cis-Beta-carotène	30,734	11,2	1,2	0,1582	Synalpheus stimpsonii 4.2
Lutein-like1	17,413	29,2	3,9	0,1246	Synalpheus stimpsonii 5
Lutein-like2	17,963	682,9	26,1	0,4363	Synalpheus stimpsonii 5
Zeaxanthin-like 1	18,547	15	2,8	0,0882	Synalpheus stimpsonii 5
Cis-Astaxanthin	18,758	138,1	12	0,1925	Synalpheus stimpsonii 5
Zeaxanthin-like 2	19,381	184,1	22,8	0,1346	Synalpheus stimpsonii 5
Lutein	19,936	33	5	0,1096	Synalpheus stimpsonii 5
Zeaxanthin	20,223	437,2	54,4	0,1338	Synalpheus stimpsonii 5
Zeaxanthin 3	20,564	25	3,3	0,1255	Synalpheus stimpsonii 5
Canthaxanthin-like 1	20,893	99,1	13,2	0,1246	Synalpheus stimpsonii 5
BetaApo_Standard	23,326	313,8	34,6	0,151	Synalpheus stimpsonii 5
Zeaxanthin-like 4	23,858	57,2	9	0,1059	Synalpheus stimpsonii 5
Zeaxanthin-like 5	26,391	17,1	2,3	0,1261	Synalpheus stimpsonii 5

Zeaxanthin-like 8	28,818	11,6	1,6	0,1189	Synalpheus stimpsonii 5
Trans-Beta-carotène	30,313	61,7	8,5	0,1211	Synalpheus stimpsonii 5
Cis-Beta-carotène	30,744	14,4	1,9	0,1257	Synalpheus stimpsonii 5
Lutein-like1	17,411	10,8	1,4	0,1312	Synalpheus stimpsonii 6.1
Lutein-like2	17,949	275,8	10	0,4584	Synalpheus stimpsonii 6.1
Zeaxanthin-like 1	18,548	44,1	6,7	0,1094	Synalpheus stimpsonii 6.1
Zeaxanthin-like 2	19,376	192,4	24,3	0,1321	Synalpheus stimpsonii 6.1
Lutein	19,929	55,3	8,5	0,1083	Synalpheus stimpsonii 6.1
Zeaxanthin	20,217	1655,7	208,2	0,1325	Synalpheus stimpsonii 6.1
Zeaxanthin 3	20,546	24,5	3,4	0,1195	Synalpheus stimpsonii 6.1
Canthaxanthin-like 1	20,886	190,5	25,5	0,1243	Synalpheus stimpsonii 6.1
BetaApo_Standard	23,32	334,4	43,4	0,1285	Synalpheus stimpsonii 6.1
Zeaxanthin-like 5	26,398	51,5	6,4	0,1344	Synalpheus stimpsonii 6.1
Zeaxanthin-like 6	28,194	17,1	3	0,0953	Synalpheus stimpsonii 6.1
Zeaxanthin-like 7	28,315	22,4	3,7	0,1008	Synalpheus stimpsonii 6.1
Trans-Beta-carotène	30,308	588,1	80,7	0,1214	Synalpheus stimpsonii 6.1
Cis-Beta-carotène	30,739	157,1	18,9	0,1389	Synalpheus stimpsonii 6.1

Astaxanthin-like 1	16,935	6,2	1,2	0,0853	Synalpheus stimpsonii 6.2
Lutein-like2	17,949	822	27,8	0,4936	Synalpheus stimpsonii 6.2
Cis-Astaxanthin	18,732	82	6,2	0,2198	Synalpheus stimpsonii 6.2
Zeaxanthin	20,214	73,8	9,5	0,1297	Synalpheus stimpsonii 6.2
BetaApo_Standard	23,321	381	45,4	0,1399	Synalpheus stimpsonii 6.2
Echinenone-like 1	25,658	125,1	8,2	0,254	Synalpheus stimpsonii 6.2
Echinenone-like 1	25,848	132,6	7,4	0,2513	Synalpheus stimpsonii 6.2
Astaxanthin-like 2	27,847	76,7	4,1	0,313	Synalpheus stimpsonii 6.2
Zeaxanthin-like 6	28,192	22,5	2,7	0,1398	Synalpheus stimpsonii 6.2
Canthaxanthin-like 1	28,812	59,3	9,4	0,1053	Synalpheus stimpsonii 6.2
Astaxanthin-like 3	29,188	11,4	2	0,0936	Synalpheus stimpsonii 6.2
Trans-Beta-carotène	30,311	111,8	11,5	0,1616	Synalpheus stimpsonii 6.2
Lutein-like2	17,918	46,3	3,3	0,2305	Synalpheus stimpsonii 7
Cis-Astaxanthin	18,718	9,9	1	0,1599	Synalpheus stimpsonii 7
Zeaxanthin	20,198	20,2	2,6	0,1312	Synalpheus stimpsonii 7
BetaApo_Standard	23,308	384,9	44,8	0,1433	Synalpheus stimpsonii 7

Zeaxanthin 4	23,843	46,3	7	0,1096	Synalpheus stimpsonii 7
Trans-Beta-carotène	30,301	23,2	2,1	0,1817	Synalpheus stimpsonii 7

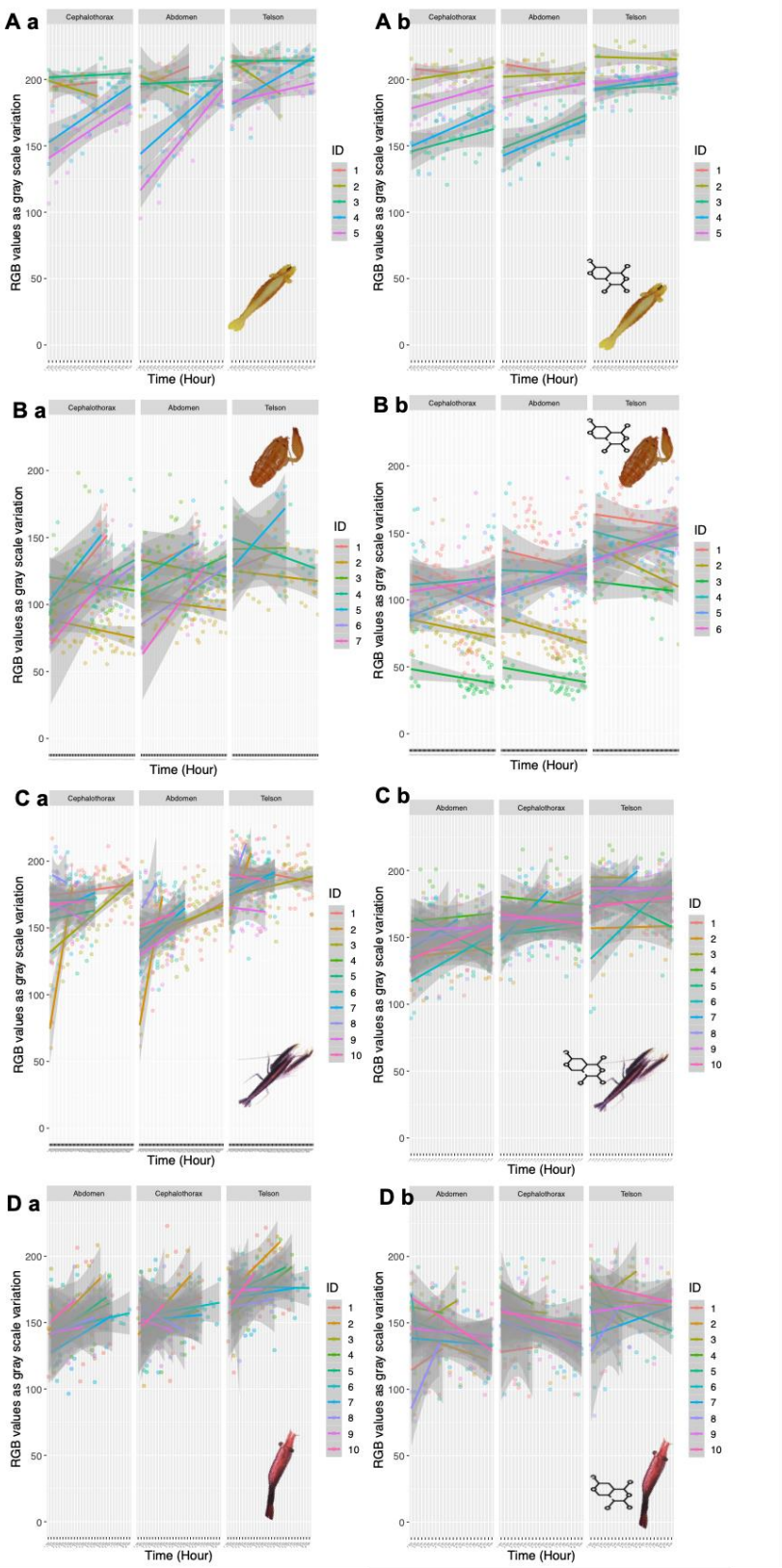


Figure S1. Shrimp coloration variation when individuals are isolated (Aa, Ba, Ca, Da) or conditioned with a host-chemical environment **(**Ba, Bb, Bc, Bd) Each graph represents gray values from transformed RGB values obtained with the photos taken during the time of the experiments on *Z. soror* individuals (A, B), 7 (C) and 6 (D) *S. stimpsonii*, 10 *A. indicus T. holthuisi*. Each colored line represents the variation in gray values of one individuals. The gray variations are represented for the cephalothorax, abdomen and telson separately. Lines extending to the end of the graph represent individuals who survived throughout the experiment, while lines that fail to reach the end of the graph indicate that these individuals did not survive to the end of the experiment. These trajectories were generated from the linear regression of grey variation values, represented by the points on the graph.

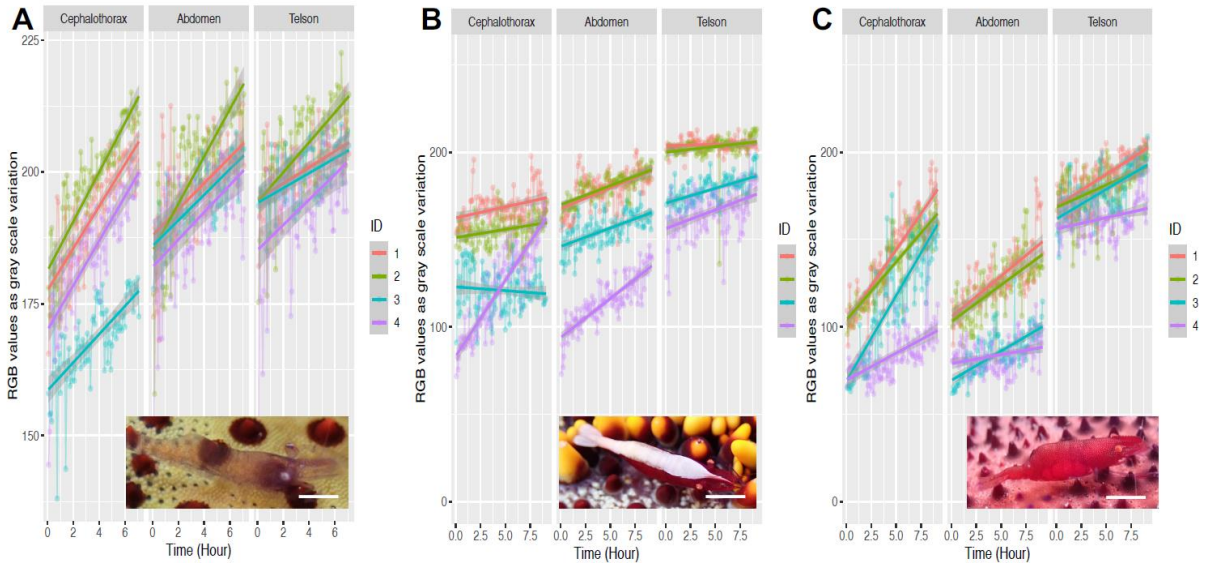


Figure S2. Variation in *Z. soror* coloration when individuals are isolated. Each graph represents the grey values of the transformed RGB values obtained with the photos taken at the time of the experiments. Each colored line represents the variation in gray values for an individual. Gray variations are shown for the cephalothorax, abdomen and telson separately. Lines that extend to the end of the graph represent individuals that survived throughout the experiment, while lines that do not reach the end of the graph indicate that these individuals did not survive to the end of the experiment. These trajectories were generated from the linear regression of grey variation values, represented by the points on the graph. (A) represents the colored morphotype, (B) represents the stripe-colored morphotype and (C) represents the translucent morphotype.

Table S2: Table summarizing raw data concerning carotenoid analysis by High Pressure Liquid Chromatography HPLC.

Sample	Sum Area	BetaApo_ Standard (20µg)	Sample weight (mg)	Pigments weight in all the sample (µg)	Pigment weight (mg) per sample g	Mean	SD
Phanogenia distincta 2	203,9	390,4	13,44	10,44569672	0,777209577	1,549781119	0,800008548
Phanogenia distincta 4	303,4	390,8	10,4	15,52712385	1,492992678		
Phanogenia distincta 5	141	353,5	5,74	7,977369165	1,389785569		
Phanogenia distincta 6	272,9	434,1	7,01	12,57313983	1,793600546		
Phanogenia distincta 7	93,1	385,7	6,26	4,827586207	0,771179905		
Phanogenia distincta 11	511	367,7	8,6	27,79439761	3,231906698		
Phanogenia distincta 17	391,1	420	8,15	18,62380952	2,285130003		
Phanogenia distincta 18	181,1	344,2	8,25	10,52295177	1,275509306		
Phanogenia distincta 15	107,5	491,5	4,7	4,374364191	0,930715785		
Synalpheus stimpsonii 2	2941,5	277,9	3,73	211,6948543	56,75465262	97,70536533	23,47247424
Synalpheus stimpsonii 4.1	410,3	317,5	10,35	25,84566929	2,497166115		
Synalpheus stimpsonii 4.2	677,5	286,6	12,58	47,27843685	3,758222325		
Synalpheus stimpsonii 5	1805,6	313,8	10,65	115,0796686	10,80560268		
Synalpheus stimpsonii 6.1	3285,3	334,4	3,91	196,4892344	50,25300114		
Synalpheus stimpsonii 6.2	1523,4	381	4,84	79,96850394	16,52241817		
Synalpheus stimpsonii 7	145,9	384,9	11,06	7,581189919	0,68546021		
Culcita_Teg_Etoile3_2022	12281,8	571,3	67,8	429,9597409	6,341589099	64,61666667	9,664246824
Culcita_Teg_Etoile4_2022	11969,3	477,8	62,5	501,017162	8,016274592		
Culcita_Teg_Etoile5_2022	11969,3	492,8	65,4	485,7670455	7,427630664		
Culcita_Teg_Etoile6_2022	17939,7	431,9	70,1	830,7339662	11,85069852		
Culcita_Teg_Etoile7_2022	12150,7	419,6	46,9	579,1563394	12,34874924		
Culcita_Teg_Etoile8_2022	11379,7	491,85	75	462,7305073	6,169740097		
Z.soror_Colorée3_2022	807,8	401,7	9,2	40,21906896	4,37163793	7,116666667	2,226581835

Z.soror_Colorée1_2022	687,8	412,6	7,5	33,33979641	4,445306188
Z.soror_Must3_2022	551,8	362,1	9	30,47776857	3,38641873
Z.soror_Must1_2022	280,4	356,5	4,2	15,73071529	3,745408402
Z.soror_Must2_2022	198,626	390,5	4,5	10,17290653	2,260645896
Z.soror_Colorée2_2022	488	376,3	8,3	25,93675259	3,124909951