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COLOUR ADAPTATION MECHANISMS OF THE SEA STAR SYMBIOTIC SHRIMP *ZENOPONTONIA SOROR* DURING HOST SHIFT EXPERIMENTS

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ABSTRACT

Mimetic colouration is a widespread survival strategy that allows organisms to reduce predation by blending into their environment through adaptations in colour, texture, or shape. Symbiotic crustaceans are no exception and frequently exhibit host-specific morphological traits, including mimetic pigmentation. In some cases, however, ecological pressures force symbionts to switch hosts. When the new host exhibits a different chromotype (i.e., colour morphotype), the symbiont may adjust its colouration accordingly to maintain effective camouflage. This study investigates whether the symbiotic shrimp *Zenopontonia soror* can adapt its colouration to that of its host sea star, *Culcita novaeguineae*. An experimental host chromotype switch was conducted, and changes in shrimp colouration were monitored over a 12-day period using a standardized photography protocol. To explore potential pigment acquisition pathways, the diet of the symbiotic pair was examined through direct observation of the stomach contents using optical and scanning electron microscopy, complemented by stable isotope ratio analyses. The results demonstrate a clear chromatic adaptation of the symbiont to its new host following the host switch: yellow shrimp individuals progressively acquired the purple colouration of their new host within 12 days. Stable isotope data and stomach content analyses both indicate food sharing and direct feeding on host tegument by the symbiont.

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These results suggest that *Z. soror* could acquire pigments from its host by consuming its tissues and stealing some of its food intake, which would allow colour adaptation and question its symbiotic status as a commensal.

Key words. — Asteroidea, colouration, mimicry, symbiotic association, trophic ecology

INTRODUCTION

In tropical coral ecosystems, colours play a fundamental role in mediating interactions between living organisms along with their external environment (Hutton et al., 2015). Colouration is involved in a variety of crucial transfer of biological information allowed by photoreception, including trophic interactions (e.g. predation), sexual interactions (e.g. courtship), as well as defence mechanisms (e.g. visual aposematism, camouflage and mimicry) (Latscha, 1989). Crustaceans are characterized by a large morphological diversity presenting different types of pigmentation and ornamentation of their body wall (Briones-Fourzan & Hendrickx, 2022). This is particularly the case for shrimps displaying various colour patterns which are often a camouflage adaptation (Duarte et al., 2016). In colour variable habitats, disruptive colour patterns and spots disguise the shrimps outline from visual predators (Bauer, 2023). When considering symbiotic associations, crypsis (concealment) of the symbiont may not only encompass colour patterns but also shape, body structure, and behaviour of their hosts (Merilaita et al., 2017).

Zenopontonia soror (Nobili, 1904) (Crustacea, Decapoda, Paelaemonidae) is a symbiotic shrimp known for its remarkable camouflage abilities, closely mimicking the appearance of its host (Olliff, 2013; Antokhina & Britayev, 2020). Also known as the sea star shrimp, this species maintains an obligate ectosymbiotic relationship with 27 different species belonging to the class Asteroidea (Echinodermata) (e.g. *Acanthaster planci*, *Linckia laevigata*, *Choriaster granulatus*) (Antokhina &. Britayev, 2020; Lourtie et al., 2023). This shrimp has developed specific adaptations to thrive within this symbiotic association, including its ability to adjust its colouration to its host, thus achieving appropriate camouflage (Olliff, 2013). This species presents three different chromotypes (i.e., different colouration patterns): (i) a full-coloured chromotype with vivid colours (ii) a striped-coloured chromotype presenting a dorsal white stripe and (iii) a translucent chromotype in juveniles (Antokhina and Sorokin, 2010; Fernandez-Lereé et al., 2024; Lourtie et al., 2023 and 2024). *Z. soror* is commonly found under the "cushion sea star" *Culcita novaeguineae* which also presents a large number of colours morphotypes, called chromotypes (i.e. from light-yellow to deep red through purple-blue colour). This variability is potentially associated to its diet which is based on colourful prey such as scleractinian corals (Glynn & Kurpp, 1986; Tseng et al., 2022). Fernandez-Lereé et al. (2024) showed that *Z. soror* displays at least 18 distinct colours aiming

at mimicking the variety of colours of *C. novaeguineae*. The prevalence of this symbiotic association in Moorea (French Polynesia) is high (i.e., 69%), with a symbiotic load ranging from 1 to 14 shrimps per sea star (Lourtie et al., 2023). The shrimps are primarily located around the oral region or in proximity to the ambulacral grooves on their host, but they may hide in the mouth of their hosts when stressed (Lourtie et al., 2023).

With the exception of coloured physical phenomena like iridescence, most colours in the biosphere are made possible by the presence of pigments that absorb specific wavelengths of the electromagnetic spectrum in the visible range corresponding to the vision capacities of a given organism (Delgado-Vargas et al., 2000; Cuthill et al., 2017). The colours harboured by living organisms can be due to different types of pigments such as carotenoids, flavins or melanins (Lederer, 1939). Those chemicals show a diverse distribution among organisms, some being shared between different taxa, while others are specific to certain taxonomic groups (Brasseur et al., 2018a; Caulier et al., 2022). For example, within the phylum Echinodermata, anthraquinone pigments are restricted to the class Crinoidea (i.e. feather stars), while naphthoquinones are specific to Echinoidea (i.e., sea urchins) (Kornprobst, 2005). In crustaceans, the most widespread and abundant pigments are carotenoids (Babin et al., 2019). These molecules contribute to the yellow, orange or pink/red colouration (Wang et al., 2023). However, de novo synthesis of carotenoids is not possible in animals and only occurs in photosynthetic organisms (Cazzonelli, 2011). Animals show different main strategies to obtain carotenoid pigments (McGraw et al., 2001): (i) direct acquisition through their diet, (ii) partial metabolization of pigments obtained from their diet (Maoka, 2020), (iii) association with symbiotic bacteria (Cobbs et al., 2013), or (iv) a horizontal gene transfer (Moran & Jarvik, 2010).

Consequently, an organism diet plays a central role in determining its pigmentation. This influence of diet is well established in many organisms, as demonstrated by numerous studies on the supplementation of carotenoids in the diet of farmed shrimps (Wade et al., 2017a, b). Sea stars, in general, may also acquire their carotenoids through their diet: *Acantaster planci* obtains those pigments (e.g. astaxanthine or peridininol) from the scleractinian corals on which it feeds (Maoka et al., 2011) and *Pisaster ochraceus* recovers its pigments from bivalves (Taylor, 2012).

Some crustaceans such as the symbiotic snapping shrimp *Synalpheus stimpsonii* (Caulier et al., 2022; Lourtie et al., 2024) and the Harlequin crab *Lissocarcinus orbicularis* (Ayotte, 2005; Caulier et al., 2013), the chameleon prawn *Hippolyte varians* (Gamble and Keeble, 1900), can adapt their colouration to blend into their environment or mimic their hosts. These phenomena can appear when the host is attacked or when the host moves to another environment (Ayotte, 2005).

The first objective of this study is to quantify the extent to which the symbiotic shrimp *Z. soror* adjusts its colouration to match that of a new host, *C. novaeguineae*, following an experimental host chromotype switch. Changes in shrimp colouration were monitored over time using a standardized photographic protocol combined with RGB colour analyses (Lourtie et al., 2024).

The second objective is to investigate whether this colour adjustment is mediated by dietary pigment acquisition. Because animals are unable to synthesize carotenoids de novo and must obtain them through their diet (Maoka, 2020), and because this symbiotic pair shares several carotenoids (Lourtie et al., 2024), we hypothesize that trophic interactions may play a key role in the shrimp's colour adaptation. To test this hypothesis, the diet of both symbiotic partners is examined using several complementary approaches, including in situ behavioural observations, stomach content analyses, and measurements of carbon (δ^{13}C) and nitrogen (δ^{15}N) stable isotope ratios. Stomach content analyses performed using light microscopy and scanning electron microscopy provide a detailed snapshot of recently ingested food items, whereas stable isotope analyses offer integrated information on assimilated dietary sources over longer temporal scales (Caulier et al., 2013; Terrana et al., 2024).

The overall aim is to determine whether the carotenoids shared by the shrimp and its host originate from direct feeding of the symbiont on host tissues, from the consumption of a common food source, or from a combination of both mechanisms. Ultimately, this study seeks to clarify the trophic processes potentially underlying the ability of *Z. soror* to adjust its colouration to that of its host (Fig. 1).

MATERIALS AND METHODS

The collection of organisms, preparation of samples, host change experiment, and photographic monitoring were carried out between July and August 2022 at the Island Research Center and Environmental Observatory (CRIOBE) located in Moorea, French Polynesia. For the study of stable isotopes, individuals from previous trip to the same location and the same season in 2021 were included for comparative purposes and to allow for an increase in replicates.

Sampling and organism maintenance

Culcita novaeguineae hosting *Z. soror* were hand-collected by free-diving in depths ranging from 1 to 5 m on the northern coast of Moorea near Opunohu Bay (17°28′50″S 149°50′00″W). To ensure the preservation of all symbionts during the collection and transportation process, all collected host organisms, and their

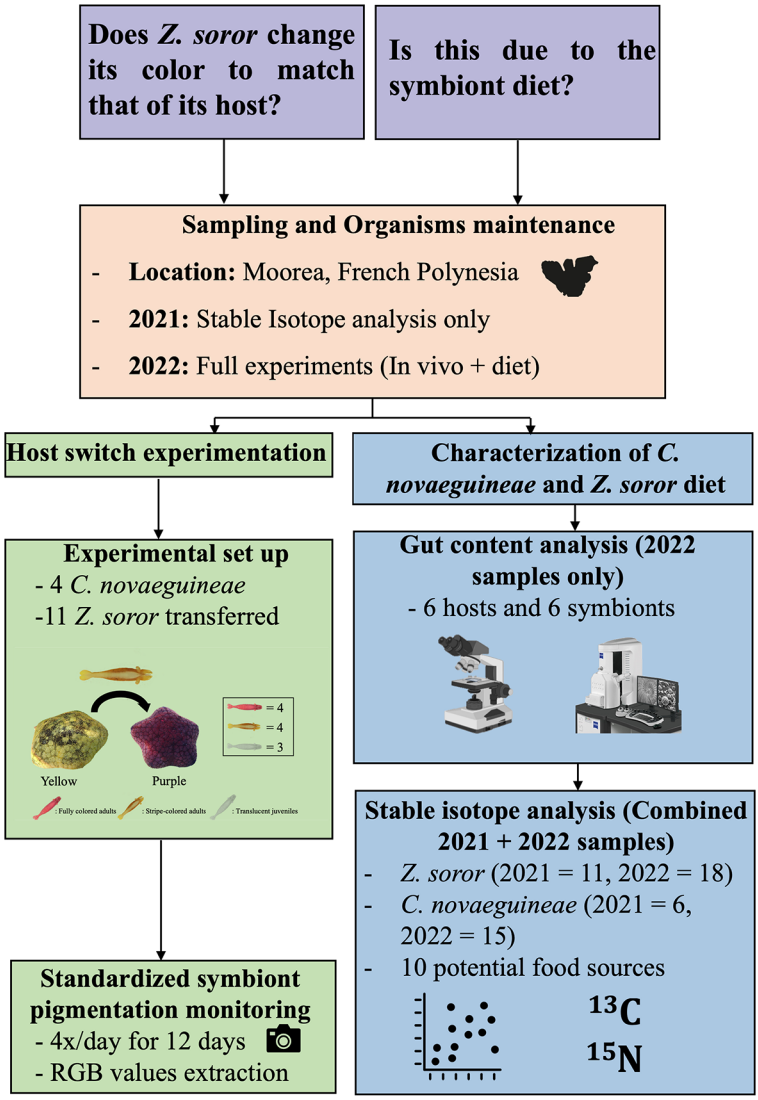

Fig. 1. Experimental design used to investigate how colour adaptation in *Z. soror* is mediated by dietary pigment acquisition. It details the biological replicates used in our integrated methodology, including host-switching experiments, pigmentation monitoring, gut content analysis, and stable isotope characterization.

entire associated fauna were carefully and temporarily stored in individual 3-litre airtight plastic bags filled with seawater for a maximum of 2 h. Upon arrival at the laboratory, an inventory and characterization of the symbiotic organisms was conducted, including the enumeration of symbionts per host and the identification of their chromotypes and colours (for hosts and symbionts). Each shrimp was

photographed upon arrival, affording the reference picture at time point T_0, using the standardized photography setup detailed below. Subsequently, the specimens were maintained in 20-litre open water aquaria continually supplied with seawater sourced directly from the sea. The tanks were aerated with an air bubbler and naturally maintained at a constant temperature of 28-29°C, a salinity of 35 ppt and a pH of 8. For each experiment, different individuals were selected.

Host switching experimentation

Experimental set up

In order to investigate the phenotypic plasticity of *Z. soror*, two distinct chromotypes of the sea star *C. novaeguineae* ($n = 2$ host individuals per chromotype) were selected due to their contrasting RGB value profiles, corresponding to two chromotypes: yellow and purple (Fig. 1). The two individuals of the same chromotype were placed together in a 20-litre aquarium, allowing several symbiotic shrimps to be housed at a density compatible with a natural symbiotic load (5-6 individuals for two sea stars) (Lourtie et al., 2023). The host transfer experiment was conducted on 11 individuals of the symbiont *Z. soror*, including three translucent juveniles, four striped adults, and four fully coloured adults. The symbionts initially associated with yellow chromotype hosts were manually transferred to purple chromotype hosts after an initial cohabitation period of 6 days. Subsequently, the colouration of the shrimp was assessed four times a day during the post-transfer period. The experiment lasted a total of 12 days (Fig. 1).

Standardized symbiont pigmentation monitoring

In order to objectively monitor the colour variation induced by the host switch, symbionts were collected on their host four times a day at 6.00 am, 12.00 am, 6.00 pm and 12.00 pm during 12 days. They were directly transferred into 30 ml vials containing seawater and placed in a light-sealed photographic chamber equipped with a ColourChecker (ColourChecker® MSCC model). Imaging was performed under LED illumination (1300 lumen) using an Olympus TG-6 camera in RAW format with BKT focus stacking. Colour analysis consisted in extracting Red-Green-Blue (RGB) values from the abdomen. A camera colour profile was first generated from RAW images using ColourChecker Camera Calibration V2.2.0 (X-Rite, 2010-2020). Images were then standardized, and RGB values were extracted using Adobe Photoshop 2022 (v25.5.2). Data visualization and statistical analyses were performed using Studio software (v2022.12.0), based on temporal changes in RGB values (Lourtie et al., 2024).

Characterization of *C. novaeguineae* and *Z. soror* diet

Gut content analysis

In order to determine whether the potential phenotypic plasticity of *Z. soror* is due to its diet, six individuals of *C. novaeguineae* along with their six biggest *Z. soror* symbiotic shrimps were selected for gut content analysis; the dried weight of the samples being a limitation for stable isotope characterization but the size being also limiting for dissection of the shrimps. Six sea stars were dissected, their entire cardiac and pyloric stomachs removed and preserved in formaldehyde (4% diluted in seawater) while the other tissues were kept for further stable isotope analyses (see below). Whole bodies of *Z. soror* were preserved in the same fixative solution and were micro dissected under a microscope at the University of Mons (Belgium). Stomachs were isolated and gut contents were observed and photographed under light microscopy (CX41, Olympus) and scanning electron microscopy (SEM), requiring the following preparation. Samples were dehydrated in seven successive ethanol baths of increasing concentration (3 baths at 70% for 30 min, 1 night at 70% for 30 min; 2 baths at 90% for 30 min and 1 bath at 100% for 1 h) and chemically dried in six successive baths of hexamethyldisilazane (HMDS) of increasing concentration of HMDS diluted in ethanol 100% (1 bath at 33%; 1 bath at 50%; 1 bath at 66% and 3 baths at 100%), the last bath being allowed to evaporate overnight in a fume hood. Once dried, samples were coated with a mix of gold and palladium (40% and 60%, respectively; JFC-1100E metalliser, JEOL) and observed and photographed with a scanning electron microscope (JSM-168 7200F, JEOL). The samples were placed inside the SEM observation chamber, and the working distance (WD) was set to 10 mm. The voltage of the electron beam was set to 10kV with average beam width. The SEM preparation methodology is derived from Lourtie et al. (2022).

Stable isotope analysis

Measurements were performed on both symbionts (29 individuals of Z. *soror*) and 21 individuals of *C. novaeguineae*, including individuals collected in 2021) and their potential food sources. Those food sources were selected where sea stars *C. novaeguineae* were collected and sometimes observed eating (Hawkins, 2006). These included five morphotypes of macroalgae: *Turbinaria ornata* ($n = 6$), *Padina broyana* ($n = 6$), *Halimeda heteromorpha* ($n = 6$), *Ceratodictyon sp.* ($n = 6$), one undetermined Phaeophyceae ($n = 6$), one species of coralline algae ($n = 6$), the scleractinian corals *Pocillopora acuta* ($n = 6$) and *Porites* sp. ($n = 6$). For both coral species, soft tissue was recovered from the calcareous skeleton using an air compressor. The microphytobenthos ($n = 5$), covering the substrate in the vicinity of *C. novaeguinea* was removed in the field by scalpel

scraping. Some other consumers were added for comparative purposes: the sea urchin *Echinometra mathaei* ($n = 6$), found eating in the same location, and two symbiotic organisms found on (or in) *C. novaeguinae*: carapid fishes *Encheliophis* sp. ($n = 2$) discovered during dissection of a host seastar, and polychaetes *Asterophila* sp. ($n = 11$) (probably *A. culcitae*). All samples were rinsed with distilled water to remove any salts or potential contaminants. All samples were then oven-dried at 60°C for 72 h immediately upon returning to the station.

For stable isotope analysis, polychaetes (*Asterophila* sp.) and *Z. soror* samples were fully consumed due to their small size. For the other samples, between 20 and 200 mg were ground using a mortar and pestle to obtain a homogeneous fine powder. Then, all samples were acidified with fuming HCl (37%) for at least 48 h in order to remove any carbonates from skeletal structures (e.g. cuticles, skeleton, ossicles). Isotopic ratios and elemental compositions were determined and analysed with an isotopic ratio mass spectrometer (IRMS, PrecisION, Elementar) coupled to an elemental analyser (EA, Vario MICRO cube, Elementar). Elemental concentrations are expressed in percent relative to dry weight (% DW). Isotopic ratios are expressed in δ notation (‰) following the equation:

$$\delta\,\mathrm{X} = \frac{R_{\mathrm{sample}} - R_{\mathrm{standard}}}{R_{\mathrm{standard}}} \times 1000$$

where X represents the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio of the sample to be studied and R_{standard} the isotopic ratios of international standards: the vPDB (Vienna Peedee Belemnite) and atmospheric N_2, respectively, for carbon and nitrogen. Certified reference materials were IAEA-N1 ($\delta^{15}N = 0.4 \pm 0.2$‰) and IAEA CH-6 (sucrose) ($\delta^{13}C = -10.4 \pm 0.2$‰), for nitrogen and carbon respectively. The standard deviation observed on the replicated measurements of the sample, intercalated every 15 samples, is 0.3‰ for both $\delta^{15}N$ and $\delta^{13}C$ values.

In order to improve the representation and interpretation of isotopic data, Student t-tests were performed on the $\delta^{13}C$ and $\delta^{15}N$ ratios of several biological groups to assess any significant differences between them (Table A1 in the Appendix). First, the isotopic values of the different algae species were compared. The results indicate that *H. heteromorpha*, *T. ornata* and *P. broyana* did not show any isotopic significant differences. They were therefore grouped together in the analyses under the name "Benthic Algal Complex." For a comprehensive analysis of the symbiotic pair over the two years, both host and symbionts were grouped together by year. Finally, the two genera of corals studied, *Porites* and *Pocillopora*, did not show any isotopic significant difference. They were therefore combined into a single "Corals" group for further analysis.

Individual $\delta^{13}C$ and $\delta^{15}N$ values were used to describe and compare the isotopic niches of *Z. soror* and *C. novaeguineae* using the SIBER ver. 2.0 package (Jackson

et al., 2011) implemented in R (R Development Core Team, 2024). Isotopic niche metrics were computed from Bayesian standard ellipses derived from the individual δ^{13}C and δ^{15}N measurements. For each group, bivariate standard ellipses (SEA) were calculated to represent the core isotopic niche space containing approximately 40% of the observations while excluding extreme outliers. When sample sizes were below 30 individuals, small-sample corrections (SEAc) were applied instead of SEA. In addition, Bayesian posterior distributions of ellipse areas (SEAb) were additionally generated through Markov Chain Monte Carlo simulations to obtain credibility intervals and enable robust comparisons between groups. Niche overlap analyses were based on SEAb. Pairwise niche overlap was quantified using the 'bayesianOverlap' function in SIBER, which estimates the shared area between the two Bayesian ellipses and expresses the overlap relative to each group's niche area (i.e., the fraction of one group's standard ellipse area that is shared with the other). Only groups containing at least five individuals were included in the overlap analyses.

The SIMMR isotopic mixing model (Parnell et al., 2013) was used to estimate the relative contribution of different potential food sources to the diet of *Z. soror* (years 2021 and 2022). The individual isotopic values of consumers (δ^{13}C and δ^{15}N) were incorporated into the model, while sources were summarized as averages and standard deviations calculated for each trophic category. Trophic Enrichment Factors (TEFs) specific to the symbiotic pair were incorporated into the model, including their means and standard deviations for δ^{13}C and δ^{15}N (mean δ^{13}C $\pm$ SD $= 0.5 \pm 1.2$, Mean δ^{15}N $\pm$ SD: 2.3 ± 1.61), in accordance with the recommendations of McCutchan et al. (2003). A Bayesian MCMC model was then applied with two configurations: a short chain (10 000 iterations) used to verify the diagnostics, followed by a long chain (100 000 iterations, 4 chains, burn-in of 10 000, thinning of 100) used for the final inference. The model outputs include correlation matrices, posterior densities, a posteriori predictive tests, and box plots representing the distributions of estimated contributions for each group. Source contributions are presented as credibility intervals derived from the probability distributions obtained by the Bayesian model.

RESULTS

Symbiotic population description and chromotypes inventory

A total of 67 *C. novaeguineae* sea stars were collected, ranging in size from 12 to 19 cm in diameter (mean$\pm$SD $= 15 \pm 1.5$ cm). These individuals hosted a total of 150 *Z. soror* shrimps, which were primarily aggregated around the buccal region

and along the ambulacral grooves of their hosts. The prevalence of the symbiotic association reached 64.2% (43 out of 67 sea stars).

The number of shrimps per host varied considerably, ranging from 1 to 14 individuals. Most infested sea stars harboured between one and four shrimps, with one shrimp (10/67 hosts), two shrimps (8/67), and three shrimps (8/67) representing the most common occurrences. In contrast, only a single sea star hosted as many as fourteen shrimps (1/67).

The sea star population exhibited remarkable colour variability (Fig. 2Da-Di), with individuals displaying either homogeneous primary colouration (e.g., a single dominant colour) (Fig. 2Db-Dh) or heterogeneous primary patterns (e.g., a primary colour with secondary spots of another hue) (Fig. 2A). Additionally, less predominant colours were systematically observed, particularly along the ambulacral grooves, and on the small spines or large conical tubercles found on both the oral and aboral surfaces. Each sea star displays a nearly unique colour pattern. This variability allows these brightly coloured shrimp to blend in perfectly with their host environment. These symbionts can therefore camouflage themselves either by adapting to the dominant colouration of their hosts or by mimicking secondary, less widespread patches of colour (for example, Fig. 2Ba-Bc and Ca-Cc).

The most common host chromotype consisted of yellow sea stars covered with dark spots (28/67; e.g. Fig. 3Da), followed by uniformly yellow individuals (13/67; e.g. Fig. 2Db), uniformly purple individuals (6/67; e.g. Fig. 2Dc), and uniformly orange/red individuals (5/67; e.g. Fig. 2Dd). Less frequent chromotypes included orange/red individuals with darker spots (3/67; e.g. Fig. 2De), grey individuals with red spots (1/67; e.g. Fig. 2Df), white-bluish individuals (1/67; e.g. Fig. 2Dg) and brownish individuals (1/67; e.g. Fig. 2Dh), among other rare variants (e.g. Fig. 2Di). In total, 12 sea stars displayed unique colouration patterns that were observed only once throughout the sampling period.

Shrimps exhibited three distinct chromotypes: translucent (108/150) (Fig. 2Aa-Ac), stripe-coloured (22/150) (Fig. 2Ba-Cc) and fully coloured (20/150) (Fig. 2Ca-Cc). Within the translucent individuals, forty-four were entirely transparent, whereas the remaining 64 displayed slight pigmentation through which the host's colouration remained visible. This subtle pigmentation varied among individuals, with red/purple shades being the most common (37/64), followed by pink (17/64), orange (9/64) and yellow (1/64). The translucent chromotype had a mean body size of 0.8 ± 0.2 cm, with individuals ranging from 0.4 to 1.2 cm in length. Notably, no ovigerous females were observed within this morphotype.

Striped-coloured individuals were distinguished by a striking dorsal band consistently exhibiting white colouration (Fig. 1). The remaining parts of their

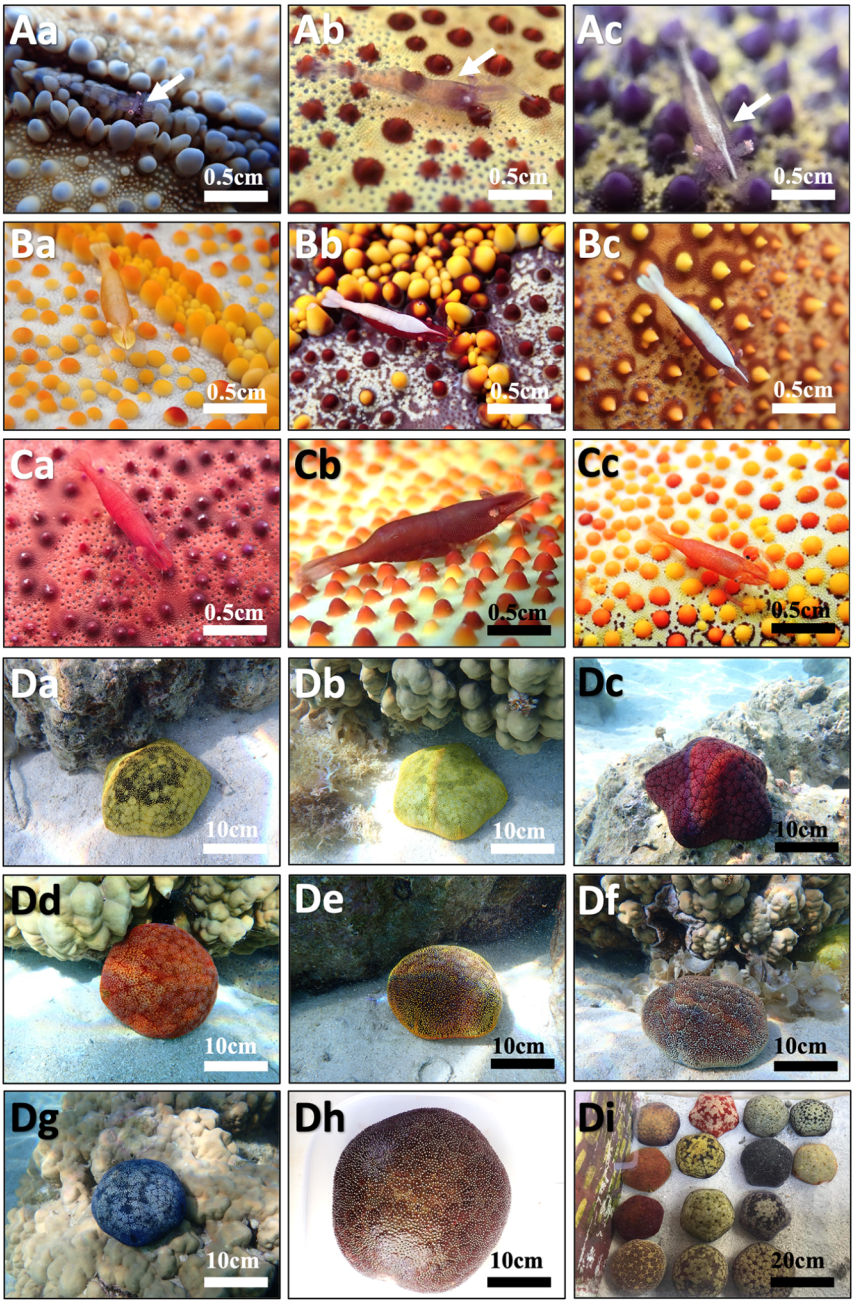

Fig. 2. Different colour morphotypes (chromotypes) of *Zenopontonia soror* and *Culcita novaeguineae* collected in the north of Moorea. Aa-Ac represent three colourations of the transparent morphotype of *Z. soror*; Ba-Bc represent three colour variants of the striped morphotype of *Z. soror*; Ca-Cc represent three colour variants of the multicoloured morphotype of *Z. soror*; Da-Di represent various colour variants of *C. novaeguineae*.

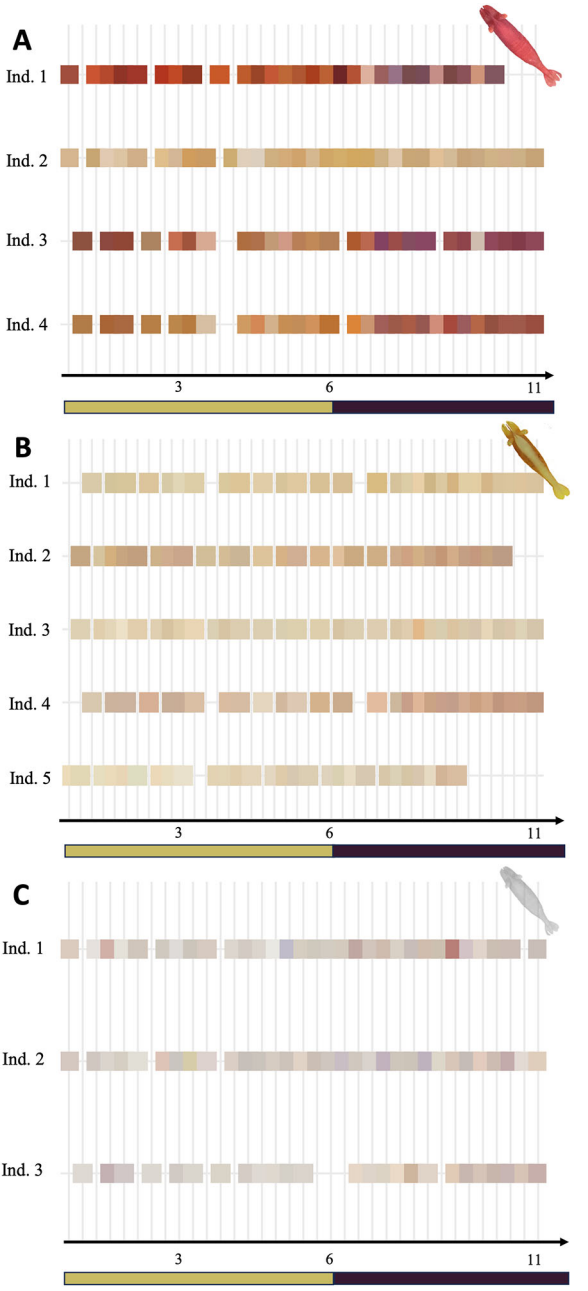

Fig. 3. Colour variations of the three *Z. soror* chromotypes over time (days). A, RGB colour variations on 4 individuals of the coloured chromotype; B, on 5 individuals of the stripped-coloured chromotype; C, on 3 individuals of the translucent chromotype during a host exchange. The coloured bar represents the colours of the two hosts on which the exchange was made. The *x*-axis represents the total experimental time in days. Colours were created from sampled RGB values.

anatomy displayed vibrant hues, resembling the full-coloured chromotype. Individuals from both coloured chromotypes predominantly showcased dark red/purple (28/42; e.g. Fig. 2Bc and Cb), others harbouring orange (6/42; e.g. Fig. 2Cc), yellow (5/42; e.g. Fig. 2Ba), red (4/42 Fig. 2Bb) or pink (2/42; e.g Fig. 2Ca) pigmentation. These chromotypes exhibited similar sizes, with striped-coloured individuals measuring 1.2 ± 0.2 cm, ranging from 0.9 to 1.5 cm, and full-coloured individuals measuring 1.1 ± 0.2 cm (0.6-1.5 cm). Moreover, both chromotypes were observed to carry eggs, with 55% of stripe-coloured individuals and 75% of full-coloured individuals being gravid.

Host-switch experiment

All individuals were transferred from a yellow host to a purple host. For individuals with a fully coloured chromotype, the RGB values reveal a variation in hue in response to the change in host chromotype (Fig. 3). For example, individual 1 had a reddish hue before the host change, but after 8 days, its RGB values indicated a hue tending towards purple. A similar trend was observed for individuals 3 and 4. By contrast, individual 2 maintained constant RGB values throughout the experiment, despite the change of host (Fig. 3A). These colour changes in this chromotype appear to occur progressively rather than abruptly, as suggested by individuals 1 and 3. Variations are less pronounced in striped chromotypes. For individuals 2 and 4, the RGB values indicate a transition to purple tones, which also appear around the eighth day of the experiment, two days after the host chromotype changes. In individuals 1 and 3, no colour change can be observed despite the equivalent experiment time (Fig. 3B). Finally, for the transparent morphotype concerned, variations between red and purple are visible, but are not consistent with the change of host. These variations also appear to be very sporadic and independent of the change in host chromotype or the timing of the experiment.

Due to the limited number of replicates, resulting from compliance with the 3Rs principle and handling-related mortality, as well as the high inter-individual variability observed within the species, colouration changes were evaluated qualitatively based on visual observations. Therefore, the observed patterns should be considered as indicative trends rather than statistically supported differences.

Characterization of *C. novaeguineae* and *Z. soror* diet

Gut content analysis under microscopic observations

In total, for both symbionts and seastars, 411 different items were found in the investigated stomachs. However, *Z. soror* demonstrated a higher diversity in the microstructures of the particles (Fig. 4A) compared to its host, *C. novaeguineae*

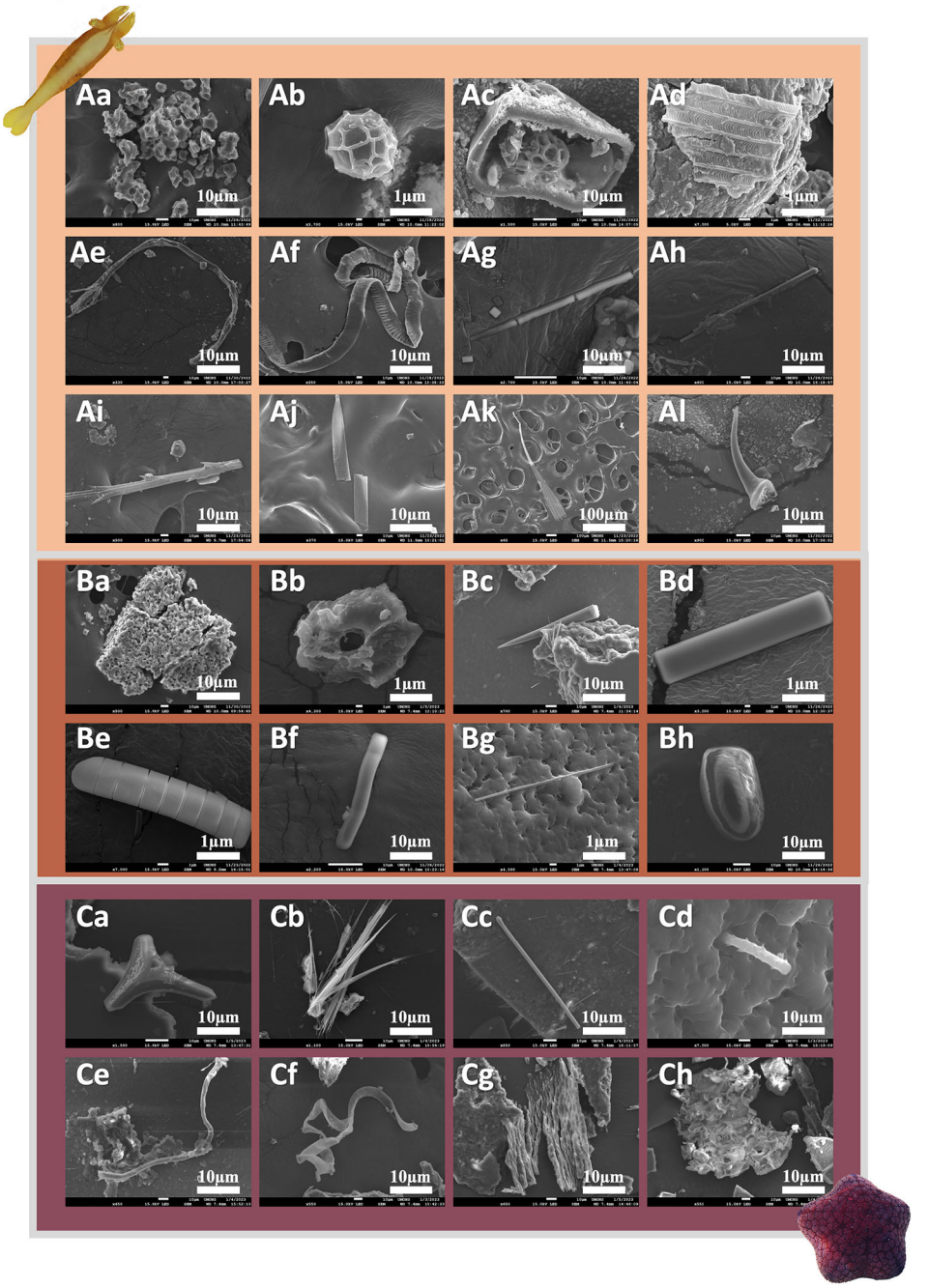

Fig. 4. Potential food particles observed in the gut content of the *Zenopontonia soror* (Aa-Al; in light orange), *Culcita novaeguineae* (Ba-Bh; in purple), or that were found in both symbiotic partners (Ca-Ch; dark orange).

(Fig. 4C), and 14.59% of the particles were found in both host and symbionts stomachs (Fig. 4C). Particles found in the host averaged 104.7 ± 87.33 μm, while symbiont particles averaged 93.17 ± 160 μm (photos of the particles can be found in Fig. A1 in the Appendix). Two kinds of particles were clearly identified: (i) host integument ossicles (Fig. 4Aa), that were found uniquely in half of *Z. soror* stomachs and were identified by comparison with ossicles directly extracted from the host teguments (Fig. A1A-B) and (ii) particles of corals (Fig. 4Ba), found in both species and identified based on Mansur et al. (2005) (Fig. A1C-D). For the others, only hypotheses could be made but their description provides an overview of the diverse dietary components observed in both *Z. soror* and *C. novaeguineae*.

In the host *C. novaeguineae*, the gut contents included triangular particles (Fig. 4Ca), elongated spicule-like structures (Fig. 4Cb-Cd), worm-like particles (Fig. 4Ce-Cf), and unstructured organic-tpe particles (Fig. 4Cg-Ch). In addition to coral skeleton particles (Fig. 4Ba), elongated and structured formations (Fig. 4Bb-Bg) and rounded structures resembling sand or sedimentary particles (Fig. 4Bh) were present in both sets of gut contents.

In *Z. soror*, the gut contents revealed several distinct particles, including diatom-like or organisms with intricate skeleton structures (Fig. 4Ab-Ad), worm-like particles (Fig. 4Ae-Af), elongated spicule-like structures with or without segmentation (Fig. 4Ag-Ah), as well as fragments resembling algae (Fig. 4Ai-Ak) and chetae-like particles typical of Polychaeta (Fig. 4Ai-Ak).

Stable isotopes analysis

Isotopic data showed three distinct groups:

(i) the first group, characterized by a maximum δ^{15}N value of 5‰, correspond to samples of the various species of algae and coral, which potentially represent the main food sources of the symbiotic couple;
(ii) the second group, with δ^{15}N values between 5 and 11‰, encompasses both hosts, shrimp symbionts and polychaetes;
(iii) the third group with a δ^{15}N value between 12 and 15‰ is include carapidae fish *E. chardewalli*.

A one-way ANOVA revealed significant differences in δ^{15}N among the three groups ($p = 2.1\times 10^{-16}$), whereas no significant differences were observed for δ^{13}C ($p = 0.055$). Algae and corals exhibited δ^{13}C values between −17 and −3‰, the symbiotic group (i.e., *Z. soror* of 2021 and 2022 and *C. novaeguineae* of 2021 and 2022) and *Asterophila* ranged from −15 to −3‰, and Carapidae showed a wider δ^{13}C range (−22 to −3‰) (Fig. 5A). All the means and standard deviations for every sample are listed in Table 1.

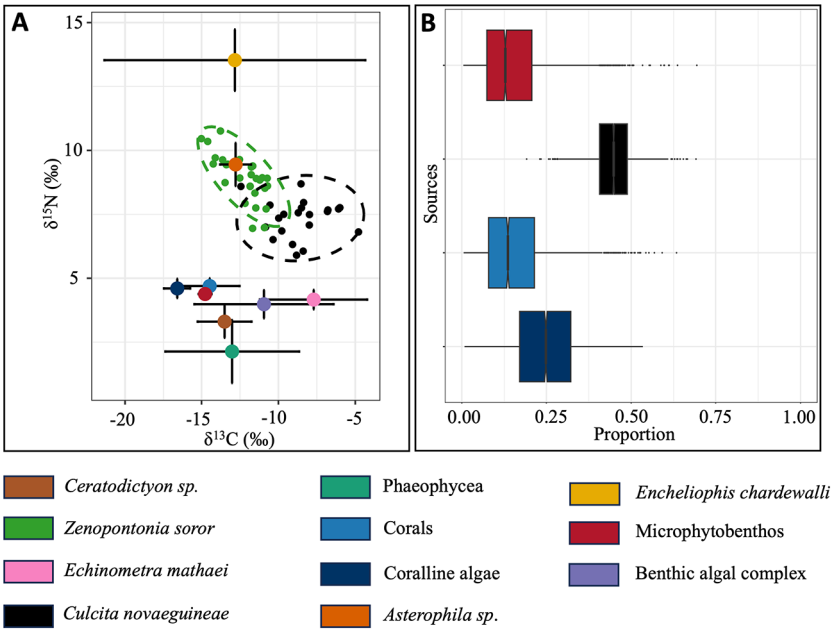

Fig. 5. A, δ^{13}C vs δ^{15}N values of *Z. soror*, their sea star host *C. novaeguineae*, two other symbionts and their potential food sources (mean±SD); B, mixed model created using the SIMMR package to estimate the relative contributions of different potential sources in the diet of *Z. soror*; the least abundant sources were excluded. The *x*-axis indicates the estimated proportion of each source in the diet of *Z. soror*. The line in the centre of the box plot represents the median and most probable value of this proportion, while the horizontal bars represent the 95% confidence interval for these proportions. The colour legend is the entire figure.

Standardized elliptical areas (SEAb) indicated that *Z. soror* occupied a niche of 16.5‰ while *C. novaeguineae* had a broader isotopic niche of 26.4‰. Bayesian niche analyses revealed an average overlap zone of 6.3‰ (median: 6.1‰) between the isotopic niches of *C. novaeguineae* and *Z. soror*, with a 95% credibility interval between 3.0 and 11.0‰. Expressed as relative proportions, 38.3% of the isotopic niche of *Z. soror* overlaps with that of its host, while only 23.9% of the host niche overlaps with the isotopic niche of the shrimp. The isotopic mixing model (SIMMR) (Fig. 5B) reveals that *C. novaeguineae* could be the dominant food source for *Z. soror*, with a median contribution of 44.8% (95% CI: 32.4-56.7%). Coralline algae represent 24.4% of the diet (95% CI: 5.3-43.9%), followed by corals (14.0%, 95% CI: 2.4-40.4%) and microphytobenthos (13.0%, 95% CI: 2.2-39.8%). A difference in δ^{13}C is observed between *C. novaeguineae* individuals from 2021 (-7.2 ± 1.3) and 2022 (-9.6 ± 0.8). This difference was not observed within the *Z. soror* groups for either year (-12.3 ± 1.4) for samples collected in 2021 and (-12.3 ± 1.2) for samples collected in 2022).

Table 1
Mean and standard deviation of $\delta^{13}C$ and $\delta^{15}N$ values for each studied species

Species	Number of tested individuals	Isotopic ratios measurements	
		$\delta^{15}N$	$\delta^{13}C$
Symbiont			
Z. soror 2021	12	8.7 (±1.2)	−12.3 (±1.4)
Z. soror 2022	17	9.1 (±0.5)	−12.3 (±1.2)
Host			
C. novaeguineae 2021	9	7.7 (±0.5)	−7.2 (±1.3)
C. novaeguineae 2022	12	7.1 (±0.8)	−9.6 (±0.8)
Control symbioses			
Asterophila sp.	12	9.4 (±0.8)	−12.8 (±1.0)
E. chardewalli	3	13.5 (±1.2)	−12.8 (±8.5)
E. mathaei	3	4.2 (±0.4)	−7.7 (±3.5)
Potential food sources			
P. acuta	5	4.7 (±0.3)	−15.5 (±0.9)
Porites sp.	6	4.89	−13.6 (±2.2)
Coralline algae	6	4.6 (±0.4)	−16.6 (±0.9)
Microphytobenthos	6	4.1 (±0.8)	−20.2 (±13.3)
Ceratodictyon sp.	12	2.1 (±1.2)	−13 (±4.4)
H. heteromorpha	12	4.1 (±0.4)	−13.2 (±6.9)
Pheophycea	6	3.3 (±0.6)	−13.5 (±1.7)
P. broyana	6	3.9 (±0.8)	−8.7 (±2)
Potential food sources grouped			
Complexe algal benthique	12	3.9 (±0.6)	−9.7 (±1.9)
Corals	11	4.7 (±0.3)	−14.5 (±1.9)
Z. soror	29	8.9 (±0.9)	−12.3 (±1.3)

No standard deviation is reported for *Porites sp.*, as only a single sample that worked during the analysis for $\delta^{15}N$. Black lines represent groups of samples belonging to the same species and year. Blue bold lines indicate groups formed by pooling species that showed no significant isotopic differences (see Materials and Methods).

DISCUSSION

The results of the host-switch experiment demonstrate that *Z. soror* is capable of adjusting its colouration to match that of a host exhibiting a contrasting chromotype. When shrimps initially associated with yellow hosts were transferred to purple hosts, gradual chromatic changes became apparent within a few days, with RGB values shifting progressively toward the purple range. These observations are consistent with those reported by Fernandez-Lere et al. (2024), who documented comparable chromatic shifts occurring within 24-36 h following a host switch. Taken together, these findings suggest that the dynamics of colour change may vary as a function of environmental conditions and/or the physiological state of the individuals at the time of the transfer. Relative to this earlier study, the present work

provides a complementary analytical dimension by coupling the characterization of chromatic plasticity with trophic analyses, stomach content examination and stable isotope measurements thereby enabling investigation of the underlying pigment acquisition mechanism. The influence of diet on colouration thus appears central, as does the phenotypic plasticity of crustaceans more broadly, as has been documented in other marine organisms where pigment acquisition is directly linked to feeding behaviour (de Bruyn & Gosselin, 2014; Siegenthaler et al., 2018; Plitcha et al., 2021).

The progressive, rather than instantaneous, nature of the colour change observed here is consistent with the slow chromatic adjustment mechanisms described in other shrimp species (Duarte et al., 2016; Siegenthaler et al., 2018), and stands in contrast to the rapid physiological colour changes characteristic of cephalopods, which rely on contractile chromatophores capable of producing colour shifts within fractions of a second (Mäthger et al., 2019; Williams et al, 2019). Crustaceans lack such specialized organs, and pigment-based colouration in this group is therefore expected to change on slower timescales, likely tied to the incorporation of dietary pigments during the cuticle renewal process at moulting (Duarte et al., 2016). This type of trophically mediated chromatic plasticity remains comparatively understudied in symbiotic crustaceans, despite documented examples in the chameleon prawn *Hippolyte varians* (Gamble & Keeble, 1900), the snapping shrimp *Synalpheus stimpsonii* (Caulier et al., 2022), and the harlequin crab *Lissocarcinus orbicularis* (Ayotte, 2005; Caulier et al., 2013). These cases collectively illustrate that colouration in marine invertebrate symbionts can function as a dynamic phenotypic trait shaped jointly by predation pressure and trophic interactions with the host.

Substantial inter-individual variability was observed in both the rate and extent of colour change. Certain individuals showed no detectable chromatic modification over the course of the experiment despite undergoing an equivalent host transfer. This variability may reflect differences in body size, moulting stage at the time of the switch, or individual stress responses to handling (Lourtie et al., 2024). Smaller individuals may be disproportionately sensitive to handling stress, potentially resulting in slower colour adaptation or mortality prior to the completion of the discolouration process. Due to the limited number of replicates, a consequence of both handling-related mortality and adherence to the 3Rs principles of animal experimentation, the patterns described here should be regarded as indicative trends rather than statistically robust conclusions, and their confirmation will require experiments with larger sample sizes.

Because carotenoids (i.e., the principal pigments in crustaceans; Babin et al., 2019; Maoka et al., 2020) cannot be synthesized de novo by animals and must be acquired through diet (Cazzonelli, 2011), trophic interactions are expected

to play a central role in driving the chromatic plasticity observed in *Z. soror*. This expectation is further supported by the fact that *Z. soror* and its host *C. novaeguineae* share a similar carotenoid profile (Lourtie et al., 2024), suggesting that the pigments responsible for the yellow-to-purple colour range exhibited by the shrimp are of dietary origin. Possible acquisition pathways in crustaceans include direct ingestion of intact pigments through the diet, partial metabolization of dietary precursors, or association with symbiotic bacteria (McGraw et al., 2001; Cobbs et al., 2013). The combination of approaches employed here allows these hypotheses to be evaluated.

Stomach content analyses conducted by light and scanning electron microscopy revealed a diverse diet in *Z. soror*, including fragments of coral skeleton, diatoms, coralline algal material, and critically ossicles originating from the host tegument. The latter were identified in half of the shrimp stomachs examined, but were absent from those of the host, providing direct evidence of symbiont feeding on host tissues. Furthermore, 14.59% of the particles identified were common to both partners, indicating a degree of dietary overlap consistent with resource sharing rather than mere cohabitation. These results are in agreement with previous descriptions of *C. novaeguineae* as a predominantly corallivorous species, feeding mainly on *Porites*, *Pocillopora* and *Acropora* (Glynn & Krupp, 1986; Hawkins, 2006).

Stable isotope analyses corroborates as well as quantifies further these findings. The δ^{15}N values of *Z. soror* (8.9 $\pm$ 0.9‰) were consistently higher than those of *C. novaeguineae* (7.4 $\pm$ 0.7‰), placing the shrimp at a higher trophic level than its host, while similar δ^{13}C values between the two partners indicate shared carbon sources (Bunn et al., 1995). The Bayesian isotopic mixing model (SIMMR) estimated that *C. novaeguineae* represents the dominant dietary source for *Z. soror*, with a median contribution of 44.8% (95% CI: 32.4-56.7%), followed by coralline algae (24.4%), corals (14.0%), and microphytobenthos (13.0%). It should be noted that when isotopic signatures of potential food sources overlap substantially, mixing models may overestimate contributions from isotopically similar sources and exhibit reduced resolution; these estimated contributions should therefore be interpreted with appropriate caution. The interannual variation observed in the δ^{13}C values of *C. novaeguineae* between 2021 and 2022 may reflect natural environmental variability, such as fluctuations in sea surface temperature, nutrient availability, or shifts in the composition of benthic primary producers known to influence the isotopic baseline of coral reef food webs at Moorea (Post, 2002), although the precise driver remains speculative in the absence of concurrent environmental data.

Taken together, the stomach content analysis and the isotopic data strongly supports the conclusion that *Z. soror* acquires carotenoid pigments both by

ingesting food shared with its host and by directly consuming host tissues. The frequent observation of the symbiont in close proximity to, or directly within, the mouth of *C. novaeguineae* (Lourtie et al., 2024) provides a behavioural context consistent with this dual feeding strategy, granting the shrimp access to both prey items ingested by the host and perioral host tissues. This mechanism offers a proximate explanation for the progressive colour shift observed in the host-switch experiment: by feeding on a new host and its food sources, *Z. soror* gradually incorporates the carotenoids characteristic of the new host's chromotype, thereby tracking it chromatically over time.

These results fit within a growing body of literature documenting trophic mediated chromatic plasticity in marine invertebrate symbionts. The harlequin crab *L. orbicularis*, a symbiont of various holothurian genera, consumes both host-shared resources and host tissues, and is capable of adjusting its colouration to match that of its host (Ayotte, 2005; Caulier et al., 2013). Similarly, the shrimps *Arete indicus* and *Tulеariocaris holthuisi*, symbionts of the sea urchin *Echinometra mathaei*, depend trophically on their host for both nutrition and the maintenance of their colouration (Brasseur et al., 2018b). Beyond crustaceans, the principle that pigmentation can directly reflect trophic position is well illustrated by flamingos (*Phoenicopterus roseus*), which acquire their distinctive colouration by consuming carotenoid-rich brine shrimp (Yim et al., 2015). In the context of echinoderms, *C. novaeguineae* is itself thought to acquire carotenoids through its corallivorous diet, while *Acanthaster planci* obtains pigments such as astaxanthin from scleractinian coral prey (Maoka et al., 2011). By feeding on both its host and the host's prey, *Z. soror* thus participates in a trophic cascade of pigment transfer tracing back to the photosymbiotic microalgae of coral tissues from zooxanthellae to corals, from corals to sea stars, and from sea stars to the shrimp constituting an elegant mechanism through which chromatic mimicry is dynamically maintained.

The isotopic position of *Z. soror*, closely matched that of *Asterophila* sp., a polychaete symbiont of *C. novaeguineae* described as feeding directly on its host (Britayev & Fauchald, 2005). This suggests that phylogenetically distant symbionts sharing the same host may converge on similar trophic niches. This pattern is not unique to this system, as Cannona & Jenning (1987) demonstrated that several symbiont species, including flatworms, feed exclusively on the resources of their freshwater crayfish hosts, highlighting the generality of host-dependent trophic strategies across symbiotic systems.

The combination of direct tissue ingestion and exploitation of the host's food intake documented here invites reconsideration of the current classification of *Z. soror* as a commensal (Olliff, 2013; Antokhina & Britayev, 2020; Lourtie et al.,

2023). These behaviours are more consistent with a mild form of trophic parasitism, or cleptoparasitism, as defined by Iyengar (2008), and parallel the behaviour of *Asterophila* sp. in the same host system (Britayev & Fauchald, 2005). Nevertheless, in the absence of data demonstrating measurable negative effects on host condition or fitness, any reclassification should be approached with caution. Symbiotic relationships are best understood as occupying a continuum between mutualism, commensalism, and parasitism, with the nature of the interaction potentially shifting depending on environmental conditions, symbiont density, or resource availability. Rather than redefining the association, these results primarily underscore the complexity and phenotypic plasticity of the *Z. soror-C. novaeguineae* relationship and emphasize the central role of diet in shaping both the ecological interaction and the visual phenotype of the symbiont. Ultimately, the capacity of *Z. soror* to adaptively track the colours of successive hosts through trophically acquired pigments represents a sophisticated mechanism linking feeding behaviour, pigment biochemistry, and crypsis one whose molecular and physiological bases, particularly the metabolic fate of carotenoids across moulting cycles, warrant further investigation.

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AUTHOR CONTRIBUTIONS

L.M. performed, contributed, analysed and interpreted all the results. A.L. and G.C. conceived the scientific strategy and methodology pipeline, collected and gathered samples during field trips, including all the photography used to illustrate the article. L.M. and A.L. drafted figures and performed the analyses. L.H. provided the scientific material in the CRIOBE station and advised A.L. and G.C. for the selection of accurate sampling areas. G.L. carried out the stable isotope analyses and contributed to their statistical analysis. A.L., G.C., I.E. and P.G. supervised the study. A.L., G.L., G.C., I.E., L.H. and P.G corrected the manuscript.

CONFLICT OF INTEREST

All authors state no conflict of interest.

DATA AVAILABILITY

Data are available upon request.

ETHICAL AND LEGAL DECLARATIONS

All specimens were collected, processed, and preserved in accordance with DIREN regulations and ethical guidelines; the number of individuals used was kept to the minimum required for meaningful analysis in accordance with the 3Rs principles of animal experimentation.

REFERENCES

Antokhina, T.I. & Britayev, T.A. (2020). Host recognition behaviour and its specificity in pontoniine shrimp *Zenopontonia soror* (Nobili, 1904) (Decapoda: Caridea: Palaemonidae) associated with shallow-water sea stars. *Journal of Experimental Marine Biology and Ecology* 524, 151302. DOI: 10.1016/j.jembe.2019.151302

Antokhina, T.I. & Sorokin, P.A. (2010). Molecular genetic analysis of the two morphs of sea star shrimp *Periclimnes soror* Nobili, 1904, the symbionts of tropic sea stars. *Russian Journal of Genetics* 46, 855-860. DOI: 10.1134/S1022795410070100

Ayotte, P. (2005). *The relationship between holothurians and the Portunid crab Lissocarcinus orbicularis*. Ph. D. Thesis. University of Hawaii, Honolulu, HI.

Babin, A., Moreau, J. & Moret, Y. (2019). Storage of carotenoids in Crustaceans as an adaptation to modulate immunopathology and optimize immunological and life-history strategies. *BioEssays* 41, 1800254. DOI: 10.1002/bies.201800254

Bauer, R.T. (2023). Shrimp hygiene: antifouling adaptations. In: *Shrimps: their diversity, intriguing adaptations and varied lifestyles*, pp. 195-245. Cham, Springer.

Brasseur, L., Caulier, G., Flammang, P., Gerbaux, P. & Eeckhaut, I. (2018a). Mapping of spinochromes in the body of three tropical shallow water sea urchins. *Natural Product Communications* 13. DOI: 10.1177/1934578X1801301222

Brasseur, L., Caulier, G., Lepoint, G., Gerbaux, P. & Eeckhaut, I. (2018b). *Echinometra mathaei* and its ectocommensal shrimps: the role of sea urchin spinochrome pigments in the symbiotic association. *Scientific Reports* 8, 17540. DOI: 10.1038/s41598-018-36079-8

Brasseur, L., Demeyer, M., Decroo, C., Caulier, G., Flammang, P., Gerbaux, P. & Eeckhaut, I. (2018c). Identification and quantification of spinochromes in body compartments of *Echinometra mathaei's* coloured types. *Royal Society Open Science* 5, 171213. DOI: 10.1098/rsos.171213

Briones-Fourzán, P. & Hendrickx, M.E. (2022). Ecology and diversity of marine decapod Crustaceans. *Diversity* 14, 614. DOI: 10.3390/d14080614

Britayev, T.A. & Fauchald, K. (2005). New species of symbiotic scaleworms *Asterophilia* (Polychaeta, Polynoidae) from Vietnam. *Invertebrate Zoology* 2, 15-22.

Bunn, S.E., Loneragan, N.R. & Kempster, M.A. (1995). Effects of acid washing on stable isotope ratios of C and N in penaeid shrimp and seagrass: implications for food-web studies using multiple stable isotopes. *Limnology and Oceanography* 40, 622-625. DOI: 10.4319/lo.1995.40.3.0622

Cannona, L.R.G. & Jenning, J.B. (1987). Occurrence and nutritional relationships of four ectosymbiotes of the freshwater crayfishes *Cherax dispar* Riek and *Cherax punctatus* Clark (Crustacea: Decapoda) in Queensland. *Australian Journal of Marine and Marine Freshwater Research* 38, 419-427. DOI: 10.1071/MF9870419

Caulier, G., Lepoint, G., Van Nedervelde, F. & Eeckhaut, I. (2014). The diet of the Harlequin crab *Lissocarcinus orbicularis*, an obligate symbiont of sea cucumbers (holothuroids) belonging to the genera *Thelenota*, *Bohadschia* and *Holothuria*. *Symbiosis* 62, 91-99. DOI: 10.1007/s13199-014-0274-2

Caulier, G., Lourtie, A., Brasseur, L., Mallefet, J., Gerbaux, P., Flammang, P. & Eeckhaut, I. (2022). Crinoid anthraquinones as kairomones allowing host selection for the symbiotic snapping shrimp *Synalpheus stimpsonii*. *Chemoecology* 32, 95-104. DOI: 10.1007/s00049-022-00368-6

Cazzonelli, C.I. (2011). Carotenoids in nature: insights from plants and beyond. *Functional Plant Biology* 38, 833-847. DOI: 10.1071/FP11192

Cobbs, C., Heath, J., Stireman III, J.O. & Abbot, P. (2013). Carotenoids in unexpected places: gall midges, lateral gene transfer, and carotenoid biosynthesis in animals. *Molecular Phylogenetics and Evolution* 68, 221-228. DOI: 10.1016/j.ympev.2013.03.012

Cuthill, I.C., Allen, W.L., Arbuckle, K., Caspers, B., Chaplin, G., Hauber, M.E., Hill, G.E., Jablonski, N.G., Jiggins, C.D., Kelber, A., Mappes, J., Marshall, J., Merrill, R., Osorio, D., Prum, R., Roberts, N.W., Roulin, A., Rowland, H.M., Sherratt, T.N., Skelhorn, J., Speed, M.P., Stevens, M., Caswell Stoddard, M., Stuart-Fox, D., Talas, L., Tibbetts, E. & Caro, T. (2017). The biology of colour. *Science* 357, eaan0221. DOI: 10.1126/science.aan0221

de Bruyn, R.A. & Gosselin, L.A. (2014). Prevalence of ontogenetic changes in colour brightness among benthic invertebrates and their association with microhabitat shifts. *Marine Ecology Progress Series* 498, 147-159. DOI: 10.3354/meps10626

Delgado-Vargas, F., Jiménez, A.R. & Paredes-López, O. (2000). Natural pigments: carotenoids, anthocyanins, and betalains — characteristics, biosynthesis, processing, and stability. *Critical Reviews in Food Science and Nutrition* 40, 173-289. DOI: 10.1080/10408690091189257

Duarte, R.C., Stevens, M. & Flores, A.A. (2016). Shape, colour plasticity, and habitat use indicate morph-specific camouflage strategies in a marine shrimp. *BMC Evolutionary Biology* 16, 218. DOI: 10.1186/s12862-016-0796-8

Fernández-Lereé, C.G., Ávila-García, A., Sánchez, C., Borda, E., López-Vivas, J.M., Huato-Soberanis, L. & Gómez-Gutiérrez, J. (2024). *Zenopontonia soror* caridean shrimp exhibits dynamical mimicry with sea star host switching. *Journal of Experimental Marine Biology and Ecology* 568, 151936. DOI: 10.1016/j.jembe.2023.151936

Gamble, F.W. & Keeble, F.W. (1900). *Hippolyte varians*: a study in colour-change. *Journal of Cell Science* 2, 589-698. DOI: 10.1242/jcs.s2-43.172.589

Glynn, P.W. & Krupp, D.A. (1986). Feeding biology of a Hawaiian sea star corallivore, *Culcita novaeguineae* Muller & Troschel. *Journal of Experimental Marine Biology and Ecology* 96, 75-96. DOI: 10.1016/0022-0981(86)90014-6

Hawkins, S.V. (2006). *Feeding preference of the cushion star, Culcita novaeguineae in Mo'orea*. Student research paper. University of California, Berkeley, CA.

Hutton, P., Seymoure, B.M., McGraw, K.J., Ligon, R.A. & Simpson, R.K. (2015). Dynamic colour communication. *Current Opinion in Behavioral Sciences* 6, 41-49. DOI: 10.1016/j.cobeha.2015.08.007

Iyengar, E.V. (2008). Kleptoparasitic interactions throughout the animal kingdom and a re-evaluation, based on participant mobility, of the conditions promoting the evolution of kleptoparasitism. *Biological Journal of the Linnean Society* 93, 745-762. DOI: 10.1111/j.1095-8312.2008.00954.x

Jackson, A.L., Inger, R., Parnell, A.C. & Bearhop, S. (2011). Comparing isotopic niche widths among and within communities: SIBER–Stable Isotope Bayesian Ellipses in R. *Journal of Animal Ecology* 80, 595-602. DOI: 10.1111/j.1365-2656.2011.01806.x

Kornprobst, J.M. (2005). *Substances naturelles d'origine marine: chimiodiversité, pharmacodiversité, biotechnologies, Vol. 1*. Paris, Editions Tec & Doc.

Latscha, T. (1989). The role of astaxanthin in shrimp pigmentation. In: *Advances in Tropical Aquaculture, Workshop at Tahiti, French Polynesia, 20 Feb-4 Mar 1989*.

Lederer, P.E. (1940). Les pigments des invertébrés:(À l'exception des pigments respiratoires). *Biological Reviews* 15, 273-306. DOI: 10.1111/j.1469-185X.1940.tb00759.x

Lourtie, A., Duchatelet, L., Straube, N., Puozzo, N., Grace, M.A., Naylor, G.J. & Delroisse, J. (2022). Placoid scales in bioluminescent sharks: scaling their evolution using morphology and elemental composition. *Frontiers in Marine Science* 9, 908237. DOI: 10.3389/fmars.2022.908237

Lourtie, A., Eeckhaut, I., Mallefet, J., Savarino, P., Isorez, M., Mussoi, L., Bischoff, H., Delroisse, J., Hédouin, L., Gerbaux, P. & Caulier, G. (2023). Species-specific metabolites mediate host selection and larval recruitment of the symbiotic sea star shrimp. *Scientific Reports* 13(1), 12674. DOI: 10.1038/s41598-023-39527-2

Lourtie, A., Mussoi, L., Caulier, G., Isorez, M., Mahavory, H., Tolodraza, T., Engels, G., David, F., Eeckhaut, I. & Mallefet, J. (2024). Exploring the mimetic pigmentation of symbiotic shrimps associated with echinoderms. *Symbiosis* 94(1), 107-127. DOI: 10.1007/s13199-024-01018-x

Mansur, H.S., Mansur, A.A. & Pereira, M. (2005). XRD, SEM/EDX and FTIR characterization of Brazilian natural coral. *Key Engineering Materials* 284, 43-46. DOI: 10.4028/www.scientific.net/KEM.284-286.43

Maoka, T. (2011). Carotenoids in marine animals. *Marine Drugs* 9, 278-293. DOI: 10.3390/md9020278

Maoka, T. (2020). Carotenoids as natural functional pigments. *Journal of Natural Medicines* 74, 1-16. DOI: 10.1007/s11418-019-01364-x

Maoka, T. (2021). Carotenoids as natural functional pigments. *Journal of Natural Medicines* 74, 1-16. DOI: 10.1007/s11418-019-01364-x

Mäthger, L.M., Denton, E.J., Marshall, N.J. & Hanlon, R.T. (2019). Mechanisms and behavioural functions of structural colouration in cephalopods. *Journal of the Royal Society Interface* 6(Suppl. 2), S149-S163. DOI: 10.1098/rsif.2008.0366.focus

McCutchan Jr, J.H., Lewis Jr, W.M., Kendall, C. & McGrath, C.C. (2003). Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos* 102, 378-390. DOI: 10.1034/j.1600-0706.2003.12098.x

McGraw, K.J., Hill, G.E., Stradi, R. & Parker, R.S. (2001). The influence of carotenoid acquisition and utilization on the maintenance of species-typical plumage pigmentation in male American goldfinches (*Carduelis tristis*) and northern cardinals (*Cardinalis cardinalis*). *Physiological and Biochemical Zoology* 74, 843-852. DOI: 10.1086/323797

Merilaita, S., Scott-Samuel, N.E. & Cuthill, I.C. (2017). How camouflage works. *Philosophical Transactions of the Royal Society London Series B: Biological Sciences* 372, 20160341. DOI: 10.1098/rstb.2016.0341

Moran, N.A. & Jarvik, T. (2010). Lateral transfer of genes from fungi underlies carotenoid production in aphids. *Science* 328, 624-627. DOI: 10.1098/rsif.2008.0366.focus

Olliff, E.R. (2013). Symbiosis of the sea star shrimp, *Periclimenes soror* nobili, 1904 (Decapoda, Palaemonidae), and cushion star, *Culcita novaeguineae* Müller & Troschel, 1842 (Echinodermata, Asteroidea, Oreasteridae): host finding and benefits. *Crustaceana* 86, 564-577. DOI: 10.1163/15685403-00003192

Parnell, A.C., Phillips, D.L., Bearhop, S., Semmens, B.X., Ward, E.J., Moore, J.W., Jackson, A.L., Grey, J., Kelly, D.J. & Inger, R. (2013). Bayesian stable isotope mixing models. *Environmetrics* 24, 387-399. DOI: 10.1002/env.2221

Plichta, Z., Kobak, J., Maciaszek, R. & Kakareko, T. (2021). All shades of shrimp: preferences of colour morphs of a freshwater shrimp Neocaridina davidi (Decapoda, Atyidae) for substrata of different colouration. *Animals* 11, 1071. DOI: 10.3390/ani11041071

Post, D.M. (2002). Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* 83, 703-718. DOI: 10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2

Siegenthaler, A., Mastin, A., Dufaut, C., Mondal, D. & Benvenuto, C. (2018). Background matching in the brown shrimp Crangon crangon: adaptive camouflage and behavioural-plasticity. *Scientific Reports* 8, 3292. DOI: 10.2307/1543609

Taylor, K.R. (2012). *Diet manipulations and colour changes in* Pisaster ochraceus. Student research paper, Oregon State University, Corvallis, OR.

Terrana, L., Lepoint, G., Lienard, N. & Eeckhaut, I. (2024). The bed and board services of crinoids to their associated fauna: a case study from the Great Reef of Toliara, SW Madagascar. *Community Ecology* 25, 267-280. DOI: 10.5281/zenodo.10972757

Tseng, L.C., Limviriyakul, P. & Hwang, J.S. (2022). Macrosymbionts of sea star *Echinaster luzonicus* (Gray, 1840) in the waters of a volcanic western Pacific island. *PLoS ONE* 17, e0278288. DOI: 10.1371/journal.pone.0278288

Wade, N.M., Cheers, S., Bourne, N., Irvin, S., Blyth, D. & Glencross, B.D. (2017a). Dietary astaxanthin levels affect colour, growth, carotenoid digestibility and the accumulation of specific carotenoid esters in the Giant Tiger Shrimp, Penaeus monodon. *Aquaculture Research* 48, 395-406. DOI: 10.1111/are.12888

Wade, N.M., Gabaudan, J. & Glencross, B.D. (2017b). A review of carotenoid utilisation and function in crustacean aquaculture. *Reviews in Aquaculture* 9, 141-156. DOI: 10.1111/raq.12109

Wang, Y., Hao, Y., Zhou, D., Pan, L. & Tu, K. (2023). Differences in commercial quality and carotenoids profile of yellow-and white-fleshed nectarine fruit during low temperature storage and the regulation of carotenoids by sugar. *Postharvest Biology and Technology* 197, 112206. DOI: 10.1016/j.postharvbio.2022.112206

Williams, T.L., Senft, S.L., Yeo, J., Martín-Martínez, F.J., Kuzirian, A.M., Martin, C.A., DiBone, C.W., Chen, C., Dinneen, S.R., Nguyen, H.T., Gomes, C.M., Rosenthal, J.J.C., MacManes, M.D., Chu, F., Buehler, M.J., Hanlon, R.T. & Deravi, L.F. (2019). Dynamic pigmentary and structural colouration within cephalopod chromatophore organs. *Nature Communications* 10, 1004. DOI: 10.1038/s41467-019-08891-x

Yim, K.J., Kwon, J., Cha, I.T., Oh, K.S., Song, H.S., Lee, H.W., Rhee, J., Song, E., Rho, J.R., Seo, M.L., Choi, J., Choi, H., Lee, S., Nam, Y. & Roh, S.W. (2015). Occurrence of viable, red-pigmented haloarchaea in the plumage of captive flamingoes. *Scientific Reports* 5, 16425. DOI: 10.1038/srep16425

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Table A1
The various *p*-values after the Student's *t*-test in order to group the various food sources

Comparison	Variable	*p*-value
P. broyana vs *Ceratodictyon* sp.	$\partial^{13}C$	0.0014835018
T. ornata vs *Ceratodictyon* sp.	$\partial^{13}C$	0.0066984520
Paeophycea vs *P.broyana*	$\partial^{13}C$	0.0122581900
P. broyana vs *T. ornata*	$\partial^{13}C$	0.0535766929
Paeophycea vs *T. ornata*	$\partial^{13}C$	0.1244141882
P. broyana vs *H. heteromorpha*	$\partial^{13}C$	0.1755777011
H. heteromorpha vs *T. ornata*	$\partial^{13}C$	0.4458733049
Paeophycea vs *Ceratodictyon* sp.	$\partial^{13}C$	0.7368603575
H. heteromorpha vs *Ceratodictyon* sp.	$\partial^{13}C$	0.9310831621
Paeophycea vs *H. heteromorpha*	$\partial^{13}C$	0.9445448066
Paeophycea vs *H. heteromorpha*	$\partial^{15}N$	0.0006845755
Paeophycea vs *T. ornata*	$\partial^{15}N$	0.0009171214
Paeophycea vs *P. broyana*	$\partial^{15}N$	0.0041382882
Paeophycea vs *Ceratodictyon* sp.	$\partial^{15}N$	0.0227251346
H. heteromorpha vs *Ceratodictyon* sp.	$\partial^{15}N$	0.0251065186
T. ornata vs *Ceratodictyon* sp.	$\partial^{15}N$	0.0430731843
P. broyana vs *Ceratodictyon* sp.	$\partial^{15}N$	0.1905762089
P. broyana vs *H. heteromorpha*	$\partial^{15}N$	0.6146301352
P. broyana vs *T. ornata*	$\partial^{15}N$	0.6835151698
H. heteromorpha vs *T. ornata*	$\partial^{15}N$	0.9158852990
Porites sp. vs *P. acuta*	$\partial^{13}C$	0.09035

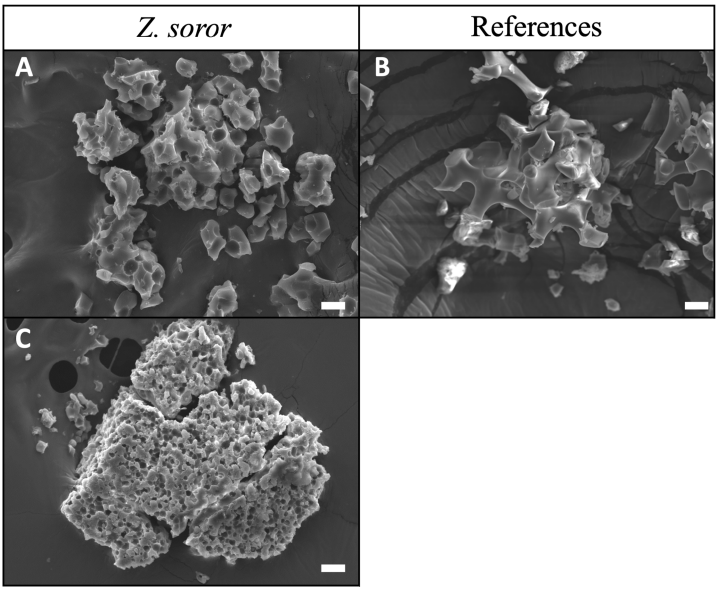

Fig. A1. A, Potential starfish ossicles found in the stomach contents of *Z. soror*; B, ossicle of the seastar *C. novaeguineae* observed under SEM; C, potential coral fragment found in the stomach contents of *Z. soror* and *C. novaeguineae* (Mansur et al., 2005). Each scale bar represents 10 μm.