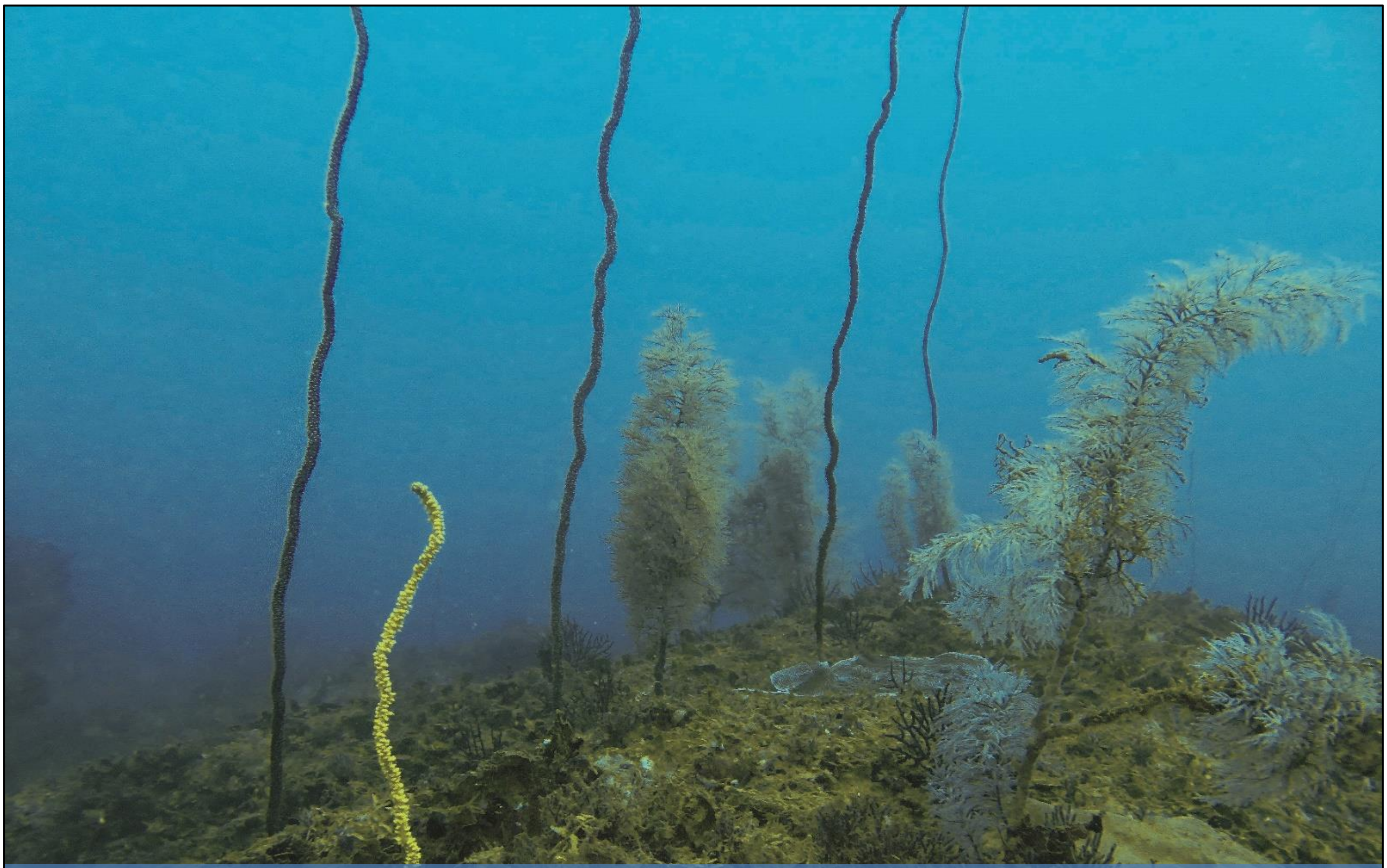
Université de Mons
Institut des Biosciences
Laboratoire de Biologie des Organismes
Marins et Biomimétisme



Biology and ecology of the shallow-water black corals (Cnidaria: Hexacorallia: Antipatharia) from the southwest of Madagascar

A dissertation submitted to the University of Mons for the degree of
Doctor of Science
presented by
Lucas Terrana

Supervisor
Prof. Igor Eeckhaut (UMONS, Belgium)
Committee
Prof. Annie Mercier (MUN, Canada)
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Université de Mons
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BOMB

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Voici venu le temps de rédiger les remerciements, signe que ces 4 années de doctorat se terminent bel et bien. C'est à l'heure de faire le bilan des personnes à remercier que je me rends compte à quel point j'eus la chance d'être extrêmement bien entouré et aidé durant ces années, et qu'il est pratiquement impossible d'énumérer toutes les personnes concernées. Ainsi, s'il est de coutume d'introduire le manuscrit d'une thèse de doctorat par une myriade de remerciements, c'est bien avec sincérité et non par obligation que je rédige ces lignes.

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RESUME DE LA THESE

Les coraux noirs sont des organismes marins vivant à toutes les profondeurs, dans tous les océans et les mers du globe. Madagascar demeure un endroit remarquable par la présence de nombreuses espèces regroupées en zones, appelées champs de coraux noirs, où leur abondance est impressionnante. Cependant, à l'image de ce qu'il se passe dans plusieurs îles tropicales, les coraux noirs sont massivement et illégalement collectés dans le sud de la Grande Île. Si leur présence dans cette partie du monde est connue depuis plusieurs siècles, et malgré l'enjeu environnemental et socio-économique que leurs pêcheries représentent, la biologie et l'écologie de ces organismes demeurent paradoxalement méconnues. Cette thèse a pour objectif général d'augmenter les données concernant la biologie et l'écologie des coraux noirs du sud-ouest de Madagascar. La première étape de ce travail a consisté en l'établissement d'un état des lieux des pêcheries de corail noir se déroulant dans le sud du pays, où plusieurs centaines de kilos ainsi que du matériel de plongée en grand nombre ont déjà été saisis par les autorités.

Dans le cadre de programmes de conservation et de gestion des ressources, il est nécessaire d'identifier correctement les espèces que l'on souhaite protéger. Par conséquent, le premier chapitre des résultats s'intéresse à l'identification des coraux noirs du sud-ouest de Madagascar. Nous rapportons ici 18 espèces identifiés sur la base de caractéristiques morphologiques (utilisant des observations *in situ* de l'ensemble des colonies) et des analyses microscopiques, en particulier en MEB, sur des fragments de coraux. Toutes les espèces appartiennent aux familles Antipathidae et Myriopathidae. Sur ces 18 espèces, 12 sont identifiées sur la base des descriptions de la littérature, tandis que les autres proviennent des genres *Cirrhipathes, Stichopathes* et *Cupressopathes* avec probablement de nouvelles espèces. Un champ de coraux noirs situé dans la partie nord du Grand Récif de Toliara est décrit et l'abondance des espèces est détaillée. Une clé taxonomique sous-marine est proposée pour les espèces de la côte sud-ouest de Madagascar.

Le chapitre suivant concerne le cycle et la stratégie de reproduction de *Cirrhipathes* sp., une des deux espèces les plus abondantes de Tuléar. Des échantillons de tissus ont été prélevés mensuellement à la base et au sommet de chaque colonie sur environ 10 spécimens sur une durée de 13 mois. Au total, 114 échantillons ont été observés en microscopie, représentant 1561 polypes, dont 591 provenaient de 39 colonies femelles

(dont 56% étaient matures) et 476 provenaient de 37 colonies mâles (dont 60% étaient matures). L'échantillonnage a révélé que l'espèce est gonochorique, et que les gamètes sont libérés au cours de plusieurs événements mineurs durant l'année avec une maturité maximale et une ponte majeure entre janvier et mars, lorsque les températures sont les plus hautes. Les polypes vivant dans la partie la plus élevée des colonies étaient matures toute l'année alors que les gamètes dans les polypes situés à la base de la colonie étaient presque absents pendant toute la saison froide.

Le troisième chapitre porte sur la connectivité entre les individus de l'espèce *Stichopathes* sp. aff *maldivensis* récoltés sur une distance allant de Maromena à Andavadoaka, soit 200 km de côte. Dans cette région, trois rivières déversent leur eau douce dans la mer, en particulier pendant la saison des pluies, et ces masses d'eau douce pourraient être des barrières naturelles pour la dispersion des gamètes et des larves de coraux noirs. Ainsi, nous avons étudié la variation génétique existant entre 41 spécimens collectés au niveau de 5 sites et estimé la variation génétique des deux types de couleur existants - brun et blanc - présents dans cette zone. Nous avons utilisé une approche génétique basée sur les haplowebs construits à partir des haplotypes provenant des séquences ITS1 de l'ADN ribosomal. Vingt-et-un haplotypes ont été observés dans les échantillonnages et les connexions entre ces haplotypes suggèrent qu'ils appartiennent tous à une seule population sans division en sous-populations. Certains spécimens provenant d'Indonésie, dont les séquences ont été extraites de la littérature, partagent également les mêmes haplotypes et font partie du vaste ensemble de séquences ITS1, ce qui suggère qu'ils pourraient appartenir à la même espèce et au même pool génétique.

L'âge des coraux noirs est évidemment un critère important à prendre en compte en biologie de la conservation : un profil écologique qui met cent années à se remettre suite à une surexploitation humaine est plus délicat à gérer qu'un autre profil qui reviendrait à un état de climax en une année. Dans le quatrième chapitre, les coraux noirs *Stichopathes* sp. aff. *maldivensis* (n = 2) et *Cirrhipathes* sp. (n = 3), deux espèces très récoltées par les pêcheurs, ont été échantillonnées dans les eaux peu profondes (<50 m) autour de Tuléar pour établir leur âge et leur taux de croissance. Ces colonies peuvent atteindre jusqu'à 5 mètres de hauteur et sont très abondantes dans cette région. Pour la première espèce, la longueur des deux échantillons était de 235 et 435 cm et de 186, 325 et 385 cm pour les trois spécimens de la seconde espèce. Une datation au radiocarbone fut réalisée sur la première strie de croissance à côté du canal central creux, qui est la partie la plus ancienne du squelette, ainsi qu'à des intervalles connus du centre vers le bord extérieur. Une courbe référence de datation au radiocarbone fut employée à partir du récif de Watamu au Kenya. Les résultats ont montré que *Stichopathes* sp. aff. *maldivensis* est âgé de 29-78 ans tandis que *Cirrhipathes* sp. est âgé de 29 à plus de 69 ans. Deux à six anneaux sont produits par an pour ces coraux noirs, et leurs croissance radiale varie de 157 à 400 µm/an tandis que leur croissance verticale varie de 4 à 11 cm/an. Considérant qu'il faudra une vie humaine pour que les populations de coraux noirs pêchés retrouvent un état écologique et structurel normal, les dommages causés par les pêcheries illégales de coraux noirs se traduiront sans aucun doute par une diminution importante de la biodiversité des eaux tropicales peu profondes.

Les deux chapitres suivants de la thèse se sont intéressés aux réseaux trophiques des coraux noirs et de leurs symbiotes. Pour cela, *Stichopathes* sp. aff. *madivensis*, *Cirrhipathes* sp., *Cirrhipathes spiralis, Cirrhipathes anguina, Cirrhipathes* cf. *contorsa, Cirrhipathes densiflora, Cupressopathes* sp. et *Myriopathes stechowi* ont été échantillonnés à leur base et à leur sommet. Des sources de nourriture potentielles ont aussi été collectées pendant la journée et la nuit à l'aide de filets et de pièges, il s'agissait de matière organique en suspension, de zoobenthos, de copépodes, de larves mégalopes, de microzooplancton, de mésozooplancton et de biofilms. Les approches SIBER et SIAR ont été utilisées pour évaluer les niches isotopiques ainsi que les différentes proportions des sources alimentaires dans la diète des coraux. Tous les coraux noirs se nourrissent d'une partie importante du mésozooplancton contenant des copépodes pélagiques, tandis qu'ils n'exploitent presque pas les matières organiques en suspension. Les coraux « fouet » (*whip black corals*) sont tous au même niveau trophique, mais ils sont spécialisés dans la capture de différents types de proies issues du mésozooplancton. Les coraux noirs branchus se trouvent à un niveau trophique inférieur, mais les modèles statistiques montrent également une dominance du mésozooplancton dans leur régime alimentaire. Parmi les plus grands coraux noirs de plus de 1 m de long, il y a des différences dans les valeurs de $\delta^{15}N$ entre les sommets et les bases des colonies suggérant une capture de proies différentes ou un fractionnement isotopique différent au sein de la colonie.

Les mêmes techniques ont ensuite été utilisées pour déterminer le type de relations trophiques existant entre différents organismes symbiotiques et leurs hôtes. L'étude concerne le poisson *Bryaninops yongei*, les crevettes *Pontonides unciger* et *Periclimenes* sp. qui sont ectosymbiotiques, et le myzostomide nouvellement décrit dans le dernier

chapitre de cette thèse *Eenymeenymyzostoma nigrocorallium*, qui vit au sein des cavités gastrovasculaires de certains coraux noirs. Les résultats ne montrent qu'aucun de ces organismes ne se nourrit directement des tissus de leur hôte. Ils suggèrent aussi que la morphologie des coraux noirs permet aux crevettes et aux poissons de se hisser en hauteur dans la colonne d'eau afin d'avoir un accès facilité au plancton diurne, alors que les antipathaires se nourrissent majoritairement du plancton nocturne. Le myzostomide présente un léger enrichissement en isotopes de l'azote, mais pas suffisamment pour justifier une relation proie-prédateur ce qui suggère qu'il profite uniquement du bol alimentaire de son hôte pour se nourrir. La description de ce myzostomide a fait l'objet du dernier chapitre des résultats tant il est particulier : c'est la première fois qu'un myzostomide vivant sur un corail noir est décrit en détail, les autres espèces étant des symbiotes obligatoires d'échinodermes et en particulier de crinoïdes.

Cette thèse est la première étude extensive de la biologie et de l'écologie des coraux noirs de faibles profondeurs (<50 m) de l'Océan Indien, et notamment de Madagascar. Elle apporte une meilleure compréhension de ces organismes, indispensable dans le cadre de l'établissement futur de programmes de conservation. Dans le dernier chapitre, la discussion générale, les nouvelles informations que cette thèse a mis en évidence par rapport à ce qui était connu auparavant sont reprises et discutées afin d'appuyer la création d'une nouvelle Aire Marine Protégée au niveau de Grand récif de Tuléar, le site principal de cette étude, et de dégager la meilleure option pour une gestion durable des antipathaires.

Chapitre 1

Introduction Générale

Jncola Jnſularum Vrinatorq; Accarbaar abu abu ſive Flabellum marinum cinereum majus Rumphij exhibens.

Laurent^s B Jsham, del.

Plate3.

INTRODUCTION GENERALE

1. De l'Histoire Naturelle des Coralliaires à l'établissement du groupe des Antipathaires

Depuis la nuit des temps, l'Homme s'intéresse et exploite ce qu'il est capable d'atteindre. Ainsi, les coraux précieux, c'est à-dire le corail rouge et le corail noir actuels, n'échappent pas à la règle et se voient collectés dans tout le bassin Méditerranéen à des fins commerciales, et ce depuis l'Antiquité. Jusqu'au XVIII^{ème} siècle, les coralliaires, c'est-à-dire les coraux anthozoaires actuels possédant un squelette dont la nature importe peu, sont encore considérés comme des pierres. Les naturalistes de l'époque essaient tant bien que mal d'en discuter l'origine, mais leurs affirmations, souvent basées sur des hypothèses peu robustes, tombent rapidement. Et pour cause, les observateurs font bien souvent face aux organismes secs, dépourvus de tout tissu, et possédant l'allure d'une plante mais rappelant la dureté de la pierre (Fig. 1, 2). Dans son ouvrage *Histoire Naturelle du Corail* (1864), Lacaze-Duthiers, brillant biologiste français du XIX^{ème} siècle, dresse dans ce sens un état des lieux de leur classification au cours du temps. Durant l'Antiquité, les savants n'hésitent pas à employer la poésie pour imager leurs observations scientifiques, mêlant parfois réalité et mythologie. De fait, tout le monde possède à l'esprit les fameuses fables mythologiques relatant de Persée vainquant la Gorgone Méduse, dont le regard pétrifiait quiconque osait le croiser. Mais pourtant les pensées divergent, et si Ovide dans *Métamorphoses* considère les coraux comme des pierres, Théophraste (±300 av. J–C), Dioscoride (40), ou Pline (200) n'hésitent pas à les caractériser comme des plantes, ce dernier les nommant d'ailleurs « lithophytes », littéralement plantes-cailloux (voir Lacaze-Duthiers 1864 pour plus de précision sur les œuvres de ces auteurs). Les naturalistes de la Renaissance laissent étonnamment un grand vide scientifique du V^{ème} au XVI^{ème} siècle, où les découvertes relatives aux coraux se font très discrètes, non aidées par l'omniprésence de l'Eglise et la répression religieuse de l'époque. C'est aux botanistes des XVII^{ème} et XVIII^{ème} siècles que l'on doit la description de nouvelles espèces mais surtout de nouvelles hypothèses quant à leur origine. La plupart des scientifiques de cette époque s'accordent sur l'origine végétale des coralliaires (Lobel 1591 ; Imperato 1599 ; Tournefort 1700 ; Ray 1704 ; voir Milne-Edwards 1857 les extraits de ces œuvres souvent en latin), notamment Réaumur (1727) qui, bien que reprenant d'abord l'idée générale de l'époque attestant que les coraux soient des végétaux, déclare que l'écorce vivante, comme il l'appelle, est responsable du dépôt de couches inertes, comparable à croissance de la coquille des mollusques.

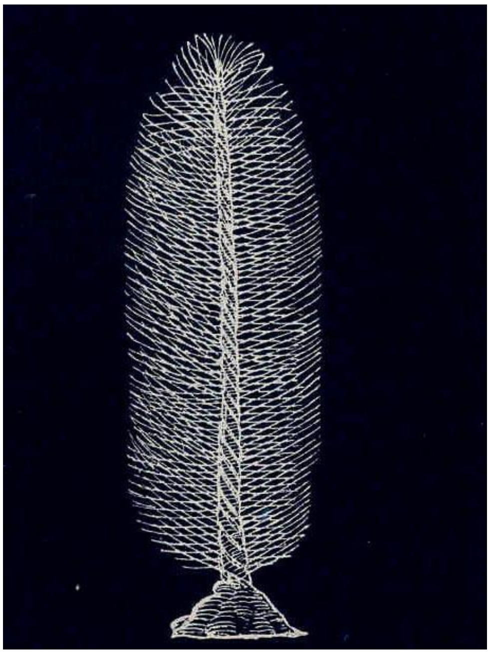
Jusqu'au jour où le comte Marsigli, dans son ouvrage *Histoire physique de la mer* (1725) et grâce au développement des premiers microscopes, prétend avoir observé des fleurs à la surface des coraux, qui sont en réalité leurs polypes. Paradoxalement, pensant valider l'hypothèse végétale, Marsigli ouvre ainsi la voie aux naturalistes qui en doutaient et regardaient plutôt les coraux comme des animaux. Rumphius (1750) est un des premiers de son époque à publier ses doutes quant à la nature végétale des coraux, et les rapproche des étoiles de mer, des holothuries, des actinies et d'autre radiaires sous le nom de zoophytes, mais ne fournit aucune preuve du caractère animal des coralliaires. Malheureusement son opinion passe inaperçue de la communauté scientifique de l'époque, même si Boccone (1671) publie également ses doutes et critique l'existence de fleurs à la surface du corail :

> « *Une chose qui fortifie les conjectures que j'ay, qu'il peut estre mis au rang des plantes, est que l'on ne trouve aucune semence dans le Corail qui puisse servir à la production, ny de vaisseaux qui la puissent contenir ; car, quoy que veuillent dire des apoticaires de Marseille de leurs fleurs de Corail, ce ne sont, selon ma pensée et mon observation, que les extrémités de cette pierre qui sont arrondies et percées de plusieurs pores étoilez. Il n'y a dans le Corail ny fleurs, ny feuilles, ny chair, ny graine, ny racine, et cela posé, je crois qu'il est bien éloigné du genre des plantes.* »

Son prédécesseur Peyssonnel (1751) avait déjà soulevé que les auteurs antiques désignaient les anémones de mer comme des animaux, appelés animaux-plantes ou zoophytes, et fit rapidement le lien entre ces zoophytes et les prétendues fleurs du corail. A travers ses expériences, il observa notamment ces fleurs se contracter ou encore pondre des œufs :

> « *Je fis fleurir le Corail, dans des vases pleins d'eau de mer, et j'observai que ce que nous croyons être la fleur de cette prétendue plante n'était, au vrai, qu'un insecte semblable à une petite Ortie ou Poulpe… J'avais le plaisir de voir remuer les pattes ou pieds de cette Ortie, et ayant mis le vase plein d'eau où le Corail était à une douce chaleur auprès du feu, tous les petits insectes*

> *s'épanouirent… L'Ortie sortie étend les pieds, et forme ce que Marsigli et moi avions pris pour les pétales de la fleur. Le calice de cette prétendue fleur est le corps même de l'animal avancé et sorti hors de sa cellule. »*



▲ Figure 1. Planche de Pona datée de 1617 illustrant pour la toute première fois une colonie de corail noir dont l'espèce ne fait aucun doute. Celle-ci est actuellement nommée *Paranthipathes larix* (Esper, 1788).

▲ Figure 2. Illustration d'un corail noir par Elizabeth Blackwell (1737) dans son ouvrage intitulé "*A curious herbal containing five hundred cuts, of the most useful plants, which are now used in the practice of physick engraved on folio copper plates, after drawings taken from the life, by Elizabeth Blackwell. To which is added a short description of ye plants and their common uses in physick*". On y voit la dénomination *Corallium nigrum* et la description s'y rapportant mentionne des propriétés médicinales équivalentes au corail rouge et au corail blanc. Ces coraux permettent de fluidifier le sang, de lutter contre les remontées acides et tout type de pertes de fluides, ou de servir de solution alcaline. Il est mentionné la rareté des coraux noirs dans les boutiques, et qui est donc par la force des choses rarement prescrit. Il est difficile d'établir si l'auteur fait référence à quelconque espèce de gorgone où s'il s'agit réellement d'un antipathaire, les deux possédant un squelette d'apparence noire mais de composition différente.

Peyssonnel étendit alors ses recherches à ce qu'ils appelaient alors à l'époque les litophytons, corps soutenus par un axe corné reprenant les gorgones et antipathes. Ainsi, ces derniers sont caractérisés par Peyssonnel comme ayant « *Un corps souple et pliant, qualités différentes du corail qui est pierreux, dur, inflexible.* » Par rapport au squelette : « *Leur substance propre est d'une nature approchante de celle de la corne, ou, pour mieux dire, semblable à l'écaille de torture ou du carret.* ».

Cependant la vision de Peyssonnel est vivement rejetée et combattue à l'époque, et il faut attendre que les travaux de Trembley (1744) sur les hydres d'eau douce soit réévalués pour remettre en cause les convictions des zoologistes. Après avoir réitéré les expériences et observations de ce dernier, Réaumur et Jussieu (1747) finissent par nommer ces fameuses fleurs « polypes », justifiant ainsi « *leurs cornes analogues aux bras de l'animal de mer qui est en possession de ce nom* », c'est-à-dire le poulpe, et « polypier » la partie solide qui les compose. C'est de la sorte que Réaumur, d'abord combattant les idées de Peyssonnel, finit par se ranger à son avis. A partir de ce moment, le caractère animal des coralliaires ne fut plus remis en doute. Ainsi Linné, bien que classifiant d'abord les coralliaires parmi les plantes dans sa première version de *Systema Naturae*, finit par rapidement classifier les coralliaires dans le règne animal d'où ils n'en sortiront plus jamais (Linné 1758).

Les antipathaires ont évidemment suivi l'histoire de l'origine des coralliaires en étant repris dans les ouvrages de plantes médicinales ou dans les récits d'expéditions, notamment celle de Rumphius qui publie *Herbarium Amboinse* à la suite de périple à Ambon en Indonésie (1750) où les antipathaires sont appelés « accarbaar » et « kalbahaar » (Fig. 3). Parmi le groupe des coralliaires tel qu'il est défini au XVIII^{ème} siècle dans le règne animal, les antipathaires sont regroupés avec les gorgones, qui reprennent tous les coraux au squelette d'apparence noire décrits à cette époque sous le nom de genre *Gorgonia*. Ceci n'est pas étonnant à une époque où rien n'est connu quant à la nature du squelette et la structure des polypes. C'est d'ailleurs ce que Linné (1758) publie en classifiant dans sa 10ème édition de *Systema Naturae* deux espèces d'antipathaires sous les noms de *Gorgonia abies* (maintenant *Cupressopathes abies*) et *Gorgonia spiralis* (maintenant *Cirrhipathes spiralis*). Il existe de nombreuses confusions entre gorgones et antipathaires dans la littérature de cette époque, certains critères morphologiques des antipathaires n'étant pas encore pris en compte, mais celles-ci proviennent également de la mauvaise identification de colonies ramenées à la surface, comme le rapporte Lacaze-Duthiers (1864) en faisant référence à l'existence de bijoux noirs à Naples :

> « *J'ai eu en main du corail noir, très noir, mais ce n'est pas une espèce, c'est un accident, une altération : la couleur est due à une transformation, et paraît être la conséquence d'une sorte de réaction chimique [...]. On peut maintenant se rendre un compte exact de ce qu'est le corail noir connu, dans le commerce, sous le nom de corail mort, de corail noir, de corail pourri.* »

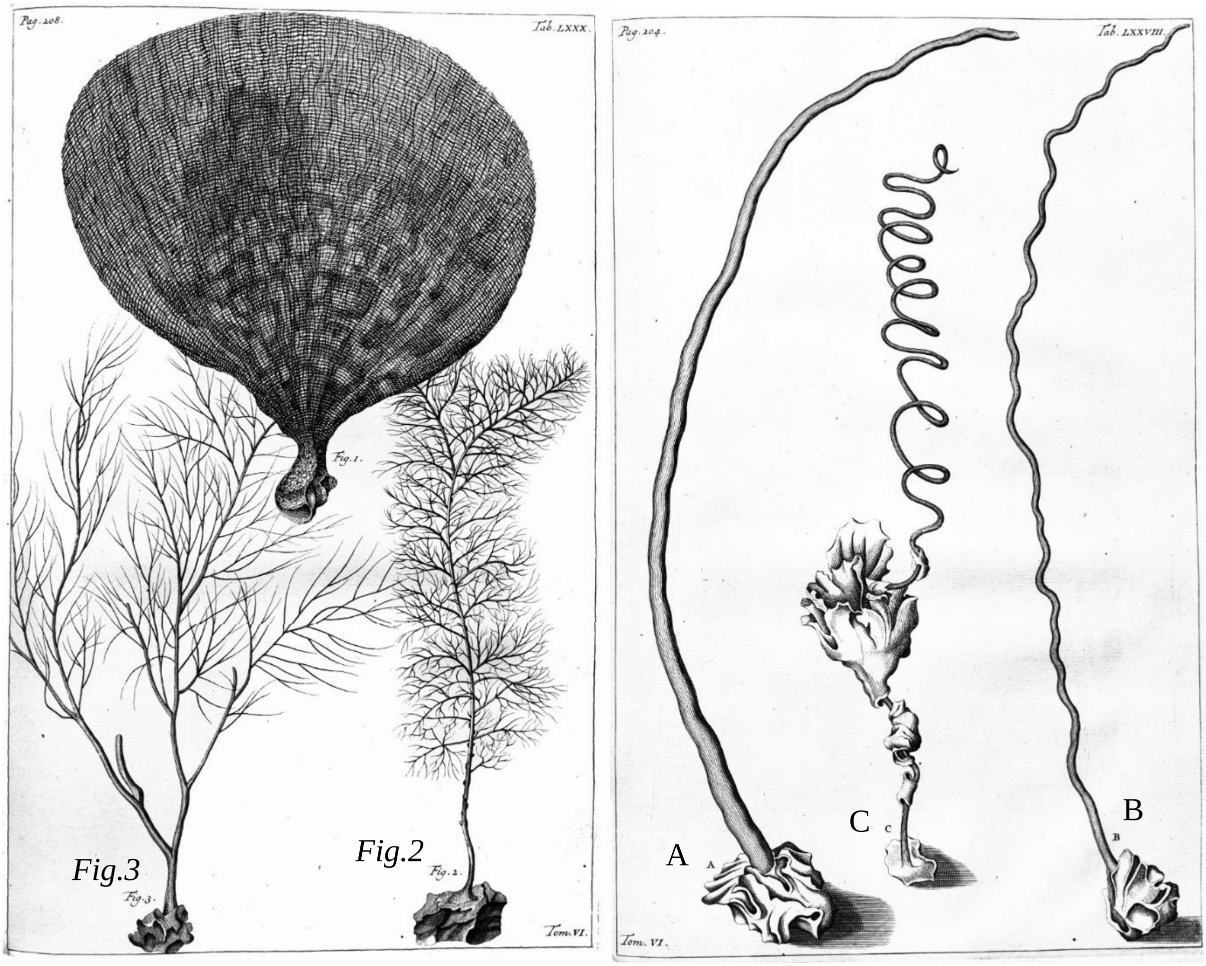


Figure 3. Antipathaires décrits lors du voyage de Rumphius à Ambon (1750) où il nomme les antipathaires sous les noms locaux de « Accarbaar » et « Kalbahaar ». Ils sont considérés par les autochtones comme ayant des pouvoirs magiques et vertus médicinales. L'œuvre de Rumphius concernant les alcyonaires et les antipathaires est revue par Bayer en 1959 où il établit la correspondance avec les espèces actuelles *Cirrhipathes rumphii* (planche droite, A), *Cirrhipathes anguina* (planche droite, B), *Cirrhipathes spiralis* (planche droite, C), *Cupressopathes abies* (planche gauche, fig. 2) et *Antipathes dichotoma* (planche gauche, fig.3).

Si la présence d’épines chez les antipathaires avait déjà été mise en évidence pour la toute première fois par Marsigli (1725, Fig. 4), il faut attendre les travaux remarquablement précis et détaillés de Pallas en 1776 qui se basent sur leur présence pour voir la séparation du genre *Gorgonia*.

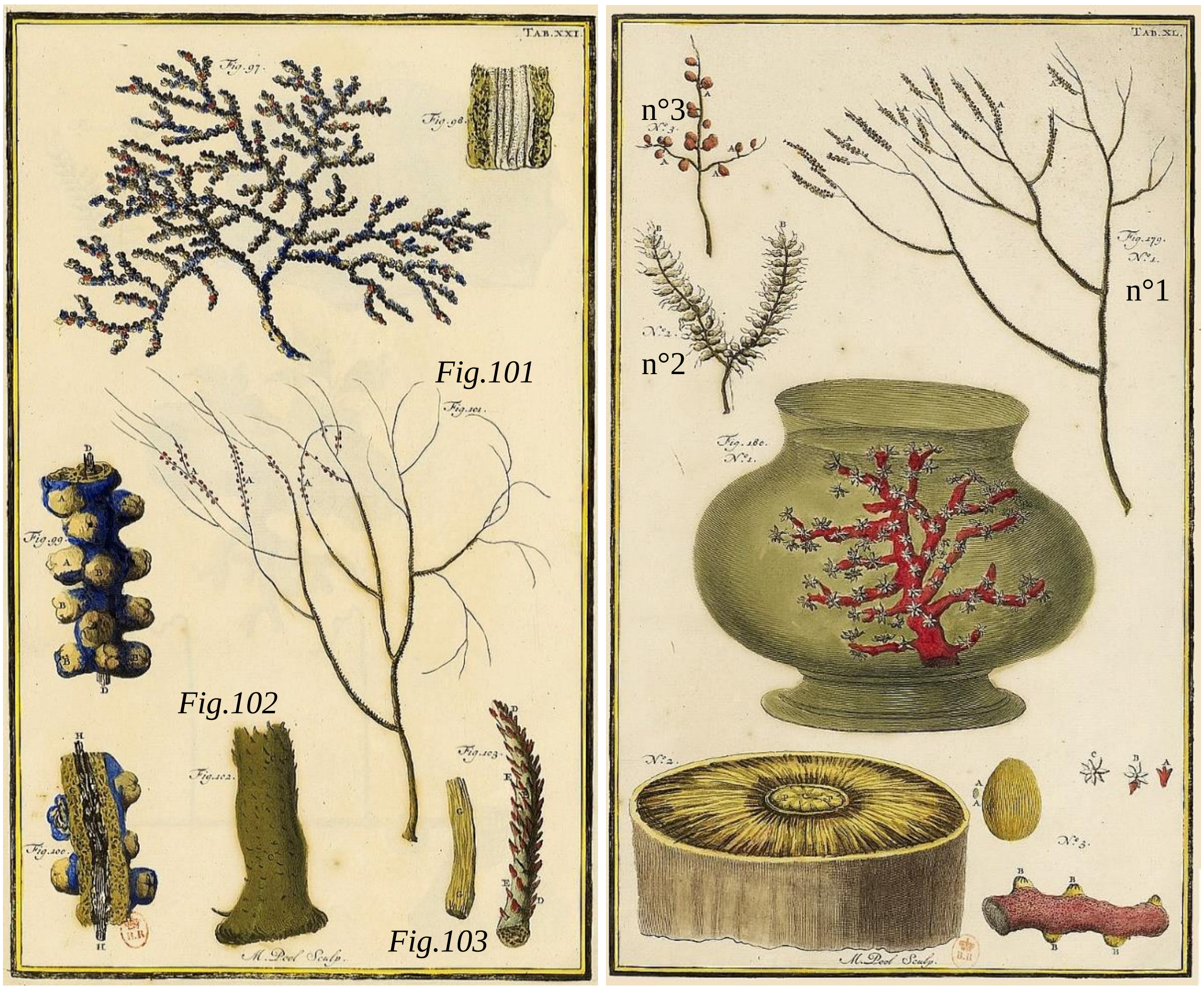


Figure 4. Planches dessinées par le comte Marsigli (1725) illustrant pour la toute première fois les épines du squelette d’un corail noir ; ici représentés sur les figures 101, 102 et 103 et repris sous le nom de lithophyte, car encore considéré comme appartenant aux plantes marines par cet auteur, cette idée transparaissant dans l’apparence des polypes grossièrement représentés. Sur la planche de droite, les « fleurs » du corail du dessin n°1 sont représentées en n°2 et n°3.

Bien que se trouvant toujours dans le même ordre, les coraux noirs sont cependant rassemblés dans un nouveau genre appelé *Antipathes*, qui tire son origine des mots « *anti* » – contre, et « *pathos* » la maladie. Pallas définit un groupe composé d’organismes ayant une tige kératinisée, une surface rugueuse pourvue d’épines, étroite, et une écorce gélatineuse. Ainsi débute véritablement le développement des connaissances relatives aux antipathaires et l'histoire naturelle de ces derniers à proprement parler, bien que la première représentation d'un corail noir date de 1617. La séparation des antipathaires et des gorgones est néanmoins timide, en témoignent Ellis & Solander (1786) qui approuvent l'établissement du nouveau genre en soulignant les différences morphologiques mais restent persuadés de la grande affinité avec les gorgones. En plus de remarquer les particularités du squelette, c'est-à-dire la présence de l'emplacement des épines à l'intérieur du squelette, vestige de la croissance antérieure du corail noir, ils sont également les premiers à publier une illustration détaillée des polypes possédant 6 tentacules (Fig. 5).

Des travaux de Pallas en 1766 jusqu'en 1889, date de publication de l'œuvre de Brook dont il sera question ci-après, la taxonomie des antipathaires ne se base que sur la morphologie du squelette en lui-même, le pauvre état de conservation des colonies rendant l'observation des polypes et des tissus impossibles, qui de plus ne résistent bien souvent pas aux conditions de dragage et de chalutage. Durant cette centaine d'année seront établis 5 genres distincts regroupant environ 60 espèces. En 1857, Milne-Edwards est le premier auteur à élever les antipathaires au rang d'ordre et à établir une classification intrinsèque à ce dernier (Fig. 6), ayant les Antipathidae comme seule famille érigée quelques années auparavant par Ehrenberg (1834). Milne-Edwards (1857) défend cependant la ressemblance avec les gorgones en mentionnant que le squelette de ces derniers est composé de la même matière que celui des antipathaires. Ces coraux sont considérés comme des zoanthaires sclérobasiques dont le squelette ne se solidifie pas et constitue seulement un tissu coriace parsemé de spicules, et qui est composé de couches superposées qui forment une tige solide. S'il admet que les épines aident à l'identification, il ne reconnaît néanmoins pas la généralité que certains auteurs y attribuent. Bien que sa classification soit simple et basée sur la morphologie générale des colonies, elle possède le mérite de clarifier la situation d'un groupe baignant dans la confusion depuis des siècles. Ainsi, elle sépare les « divisae », c'est-à-dire les colonies branchues, des « indivisae », les colonies non branchues. Elle permet d'identifier les 5 genres suivants :

–*Antipathes* établi par Pallas en 1766 et qui regroupe les colonies arbustives ;

–*Cirrhipathes* établi par Blainville en 1834 qui regroupe les colonies non branchues ;

–*Leiopathes* établi par Haime en 1849 qui regroupe les coraux dont le squelette ne présente pas d'épines ;

–*Arachnopathes* établi par Milne-Edwards en 1857 qui regroupe les coraux anastomosés ;

–*Rhipidipathes* également établi par Milne-Edwards en 1857 qui regroupe les coraux en forme d'éventail.

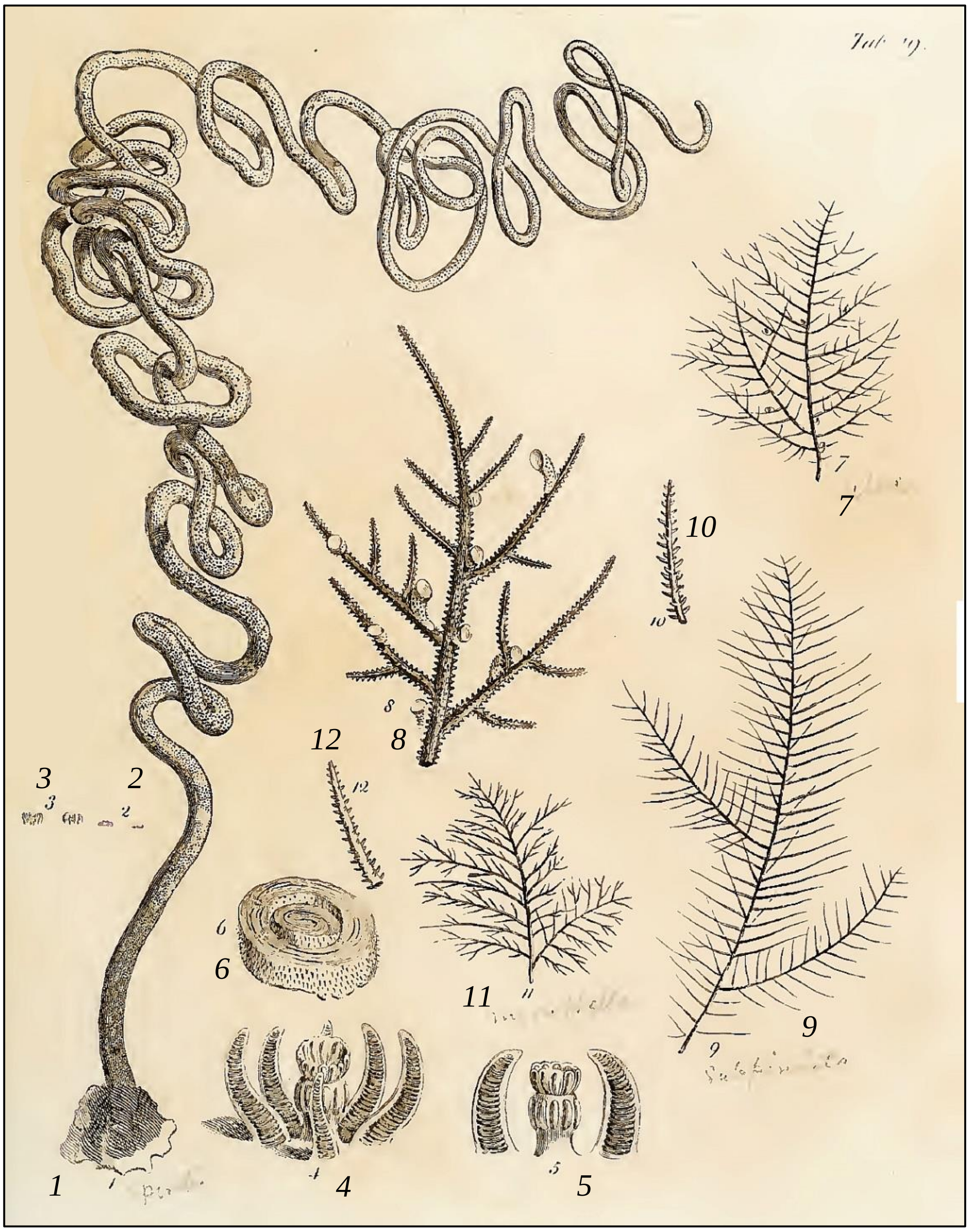
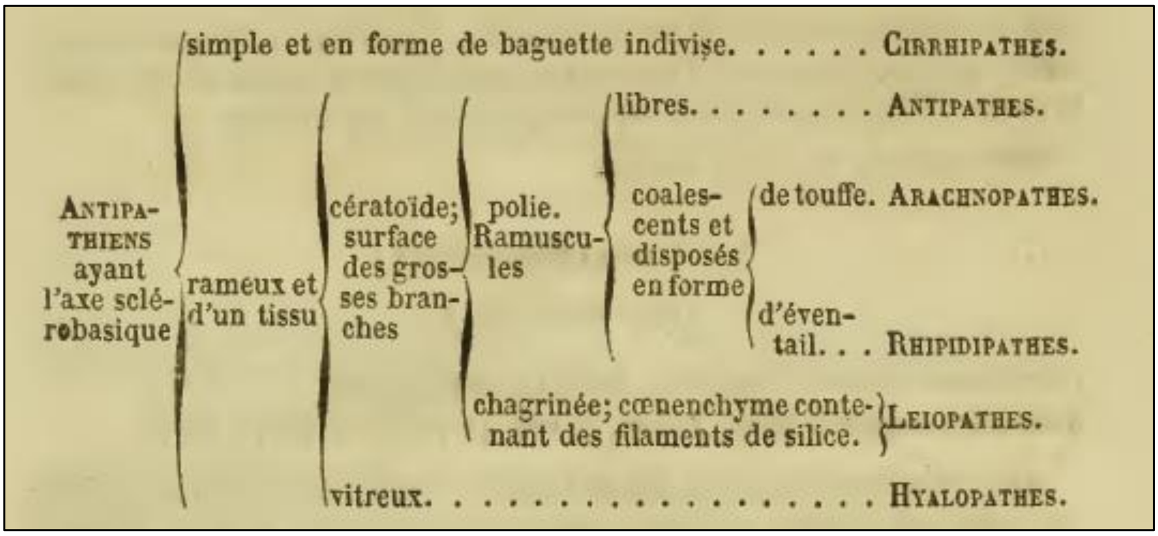


Figure 5. Planche tirée d'Ellis & Solander (1786) illustrant pour la première fois les polypes d'antipathaires détaillés avec 6 tentacules. Les figures 1 à 6 représentent *Antipathes spiralis*, 7–8 *Antipathes ulex*, 9–10 *Antipathes subpinnata*, et 11–12 *Antipathes myriophylla*. Il est important de souligner que ces noms d'espèces ont été modifiés depuis la publication originale.

Figure 6. Classification des antipathaires tirée de Milne-Edwards (1857).



Antipathiens ayant l'axe sclérobasique
- simple et en forme de baguette indivise. . . . . . Cirrhipathes.
- rameux et d'un tissu
  - cératoïde; surface des grosses branches
    - polie. Ramuscules
      - libres. . . . . Antipathes.
      - coalescents et disposés en forme
        - de touffe. Arachnopathes.
        - d'éventail. . . Rhipidipathes.
    - chagrinée; cœnenchyme contenant des filaments de silice. Leiopathes.
  - vitreux. . . . . Hyalopathes.

Quelques années plus tard, Brook (1889) marque l'histoire naturelle des antipathaires en publiant un ouvrage remarquable sur les antipathaires collectés durant l'expédition du *HMS Challenger*. Dans son œuvre, il révise entièrement la classification et les espèces décrites jusqu'alors, en plus d'ériger 11 nouveaux genres et de décrire 41 nouvelles espèces. Pour la première fois, les descriptions se basent non seulement sur l'aspect général de la colonie mais également sur la morphologie interne et externe des polypes, comme leur arrangement autour de l'axe squelettique, leur taille, la position des tentacules par rapport à la bouche et le nombre de mésentères. L'importance du nombre de ses derniers est mise en avant par Brook, qui affirme que pour établir une classification correcte des antipathaires il est absolument nécessaire d'analyser les polypes. Mais il doit rapidement faire face aux limites de la taxonomie qui se présentent à lui, à cause d'un grand nombre de descriptions vagues et de spécimens incomplets, alors que l'ordre en tant que tel est déjà vieux d'une centaine d'année. Avant lui, seules 3 espèces ont vu leurs polypes observés : *Savaglia lamarcki* –un zoanthidé à l'époque mal classifiée dans le groupe des antipathaires–, *Antipathella subpinnata* et *Parantipathes larix*. Il précise d'ailleurs dans son ouvrage que l'établissement de certaines espèces est impossible à résoudre. Néanmoins, il porte le nombre d'espèces connues à 98 et publie sa propre classification, différente de son prédécesseur Milne-Edwards, laquelle se base principalement sur la morphologie des polypes. Brook ne considère pas l'aspect des épines comme étant un critère taxonomique valable, mais les jugent utiles dans le processus d'identification (Fig. 7). Il divise ces dernières en 3 types : les épines triangulaires, les cylindriques et les tuberculées. En conséquence, il établit 3 familles dans l'ordre

Antipatharia : la famille Savagliidae, qui ne comprend que le genre *Savaglia* et qui sont en fait des parazoanthidés; la famille Dendrobachiidae qui ne comprend que le genre *Dendrobrachia* et qui sont en fait des alcyonaires; et la famille Antipathidae, qui se subdivise elle-même en sous-familles Antipathinae et Schizopathinae.

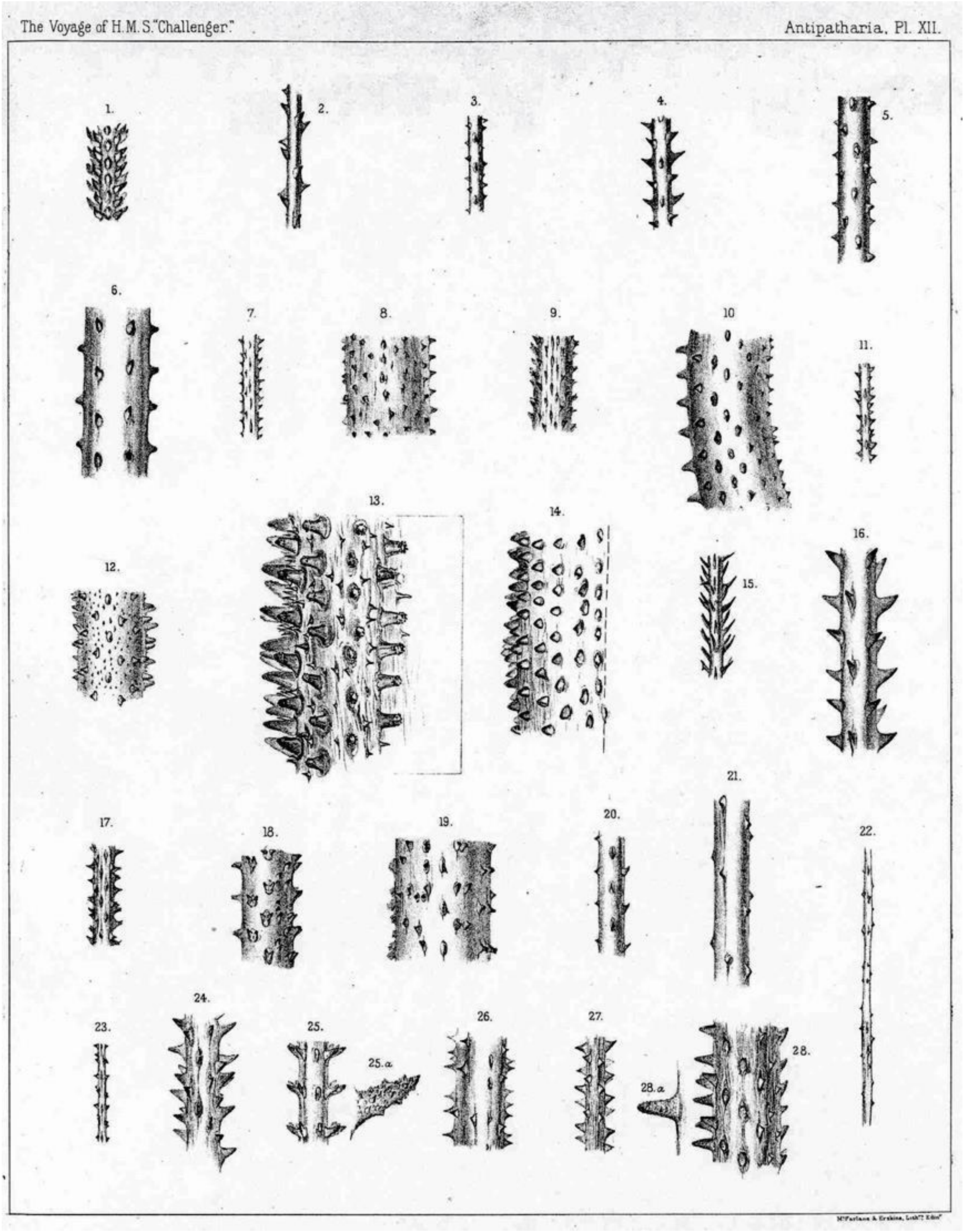


Figure 7. Exemple d’une planche tirée de Brook (1889) illustrant les épines squelettiques de différentes espèces, qui sont représentées avec une précision rarement vue jusqu’alors.

La sous-famille Antipathinae comprend les coraux qui possèdent des polypes n'étant pas dimorphiques, elle regroupe 9 genres dont 5 sont nouveaux pour la Science. Au contraire, la sous-famille Schizopathinae reprend les coraux possédant des polypes dimorphiques – gastérozoïdes et gonozoïdes– et comprend 4 genres, tous nouvellement établis.

La taxonomie continuera d'évoluer durant les années qui suivirent le travail exceptionnel de Brook. Peu de temps après, Schultze (1896) publiera également sa propre classification il divise la famille Antipathidae en trois sous-familles : les Dodékamérotes dont les polypes sont munis de 12 cloisons et qui possèdent donc trois paires de mésentères secondaires, reprenant le genre *Leiopathes* ; les Dékamérotes pourvus de 10 cloisons et qui possèdent eux deux paires de mésentères secondaires, dont les principaux genres sont *Antipathes*, *Stichopathes*, *Cirrhipathes*, *Aphanipathes* et *Parantipathes* ; et les Hexamérotes pourvus de 6 cloisons n'ayant aucune paire secondaire et qui comprennent le genre *Cladopathes*. Carlgren (1895) soustrait la famille des Savagliidae des antipathaires pour la replacer auprès des *Parazoanthus*. Deux ans plus tard, van Beneden (1897) publie un ouvrage sur les Anthozoaires de la PLANKTON-Expedition consacré principalement aux cérianthaires. Il associe ces derniers aux antipathaires et crée de l'ordre des Cérianthipathaires parmi les Anthozoaires, contenant ainsi les antipathaires et les cérianthaires.

Paradoxalement, si la taxonomie et la classification des antipathaires bouge beaucoup à la fin du XIX^{ème}–début du XX^{ème} siècle, chaque auteur y va de sa propre méthode de classification, ce qui n'aide pas à résoudre la confusion qui caractérise ce groupe depuis sa création. À la suite des collectes d'échantillons de l'expédition en Atlantique nord du Prince Souverain de Monaco, Roule (1905) publie la description des antipathaires et cérianthaires issus de cette campagne. Il donne un grand soin à la description la plus détaillée possible des antipathaires, en ne négligeant aucun aspect, bien qu'à nouveau certaines espèces sont impossibles à classer dans certains genres étant donné le manque d'informations concernant la diagnose de ces derniers. Il est surprenant alors que l'espèce est établie avec certitude quand le genre ne l'est pas. Comme déjà souligné auparavant par Brook (1889), Roule (1905) mentionne qu'il est difficile de discriminer les espèces entre elles, certaines étant bien souvent confondues avec des variations d'une seule et même espèce. A cette époque a déjà lieu le problème de réunir des types appartenant à la même espèce plutôt que d'en décrire de nouvelles, en insistant sur les ressemblances et les différences.

Même si l'importance des épines est déjà établie, Roule (1905) ne se contente pas de dire qu'elles sont longues ou courtes, mais insiste sur le fait qu'il faut mesurer leurs dimensions exactes en microns, et remarque déjà que cela varie intraspécifiquement selon la localisation sur l'axe squelettique. S'il y a bien une chose sur laquelle insiste Roule (1905), c'est la capacité de variation considérable des antipathaires. Pour déterminer une espèce, il est impératif d'observer plusieurs spécimens de la même espèce et de décrire les critères suivants :

- La dimension des colonies comparée à celle du squelette et des épines ;
- L'éloignement mutuel des polypes sur les branches qui les porte, exprimée en tant que distance intertentaculaire ;
- Le nombre de mésentères.

Roule (1905) discute également de la phylogénie des antipathaires, où il considère les colonies non branchues comme étant primitives par rapport à celles qui se divisent car elles ne possèdent qu'un seul bourgeonnement. Quant aux colonies branchues, il les définit selon un ordre de complexité croissant : les polypes entiers subissent progressivement une fragmentation de plus en plus prononcée, le groupe le plus évolué étant par conséquent celui des Schizopathidae dont les polypes possèdent des tentacules éloignés les uns des autres (Fig. 8).

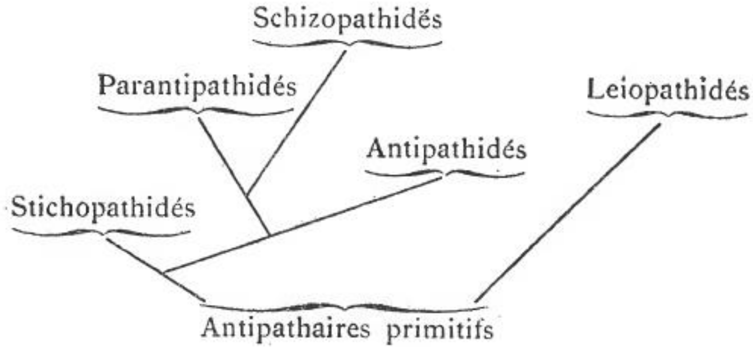


Figure 8. Phylogénie des antipathaires publiée par Roule en 1905.

Par rapport à ses prédécesseurs, Roule (1905) discute en plus de l'origine des antipathaires, qu'il place proche des cérianthaires à cause de leur ressemblance avec le développement embryonnaire de ces derniers. Il reprend les travaux du zoologiste van Beneden (1897) qui affirme qu'il existe certaines similarités entre la larve cérinule des cérianthaires et les polypes d'antipathaires : « *Un polype d'antipathe est conformé quant à ses cloisons principales et aux loges qu'elles délimitent, comme une cérinule.* » Cet état est permanant chez les uns, passager chez les autres. L'organisation du polype est donc « du type primitif » et non pas « évolué », car l'individu n'a que 6 tentacules et les cloisons supplémentaires ne sont que des néo-formations. Sans rentrer dans les détails des groupes ancestraux, Roule (1905) considère que les antipathaires possèdent l'organisation la plus simple et donc la plus ancienne, et pense que la cause de cette simplicité est à chercher dans l'apparition du bourgeonnement responsable de l'établissement colonial, stoppant ainsi l'évolution du polype, chose qui n'arrive que bien plus tard dans les autres groupes (Fig. 9). Il décrète donc que l'évolution des polypes s'est poursuivie dans le temps chez les autres anthozoaires, entrainant ainsi la production de cloisons et de tentacules supplémentaires.

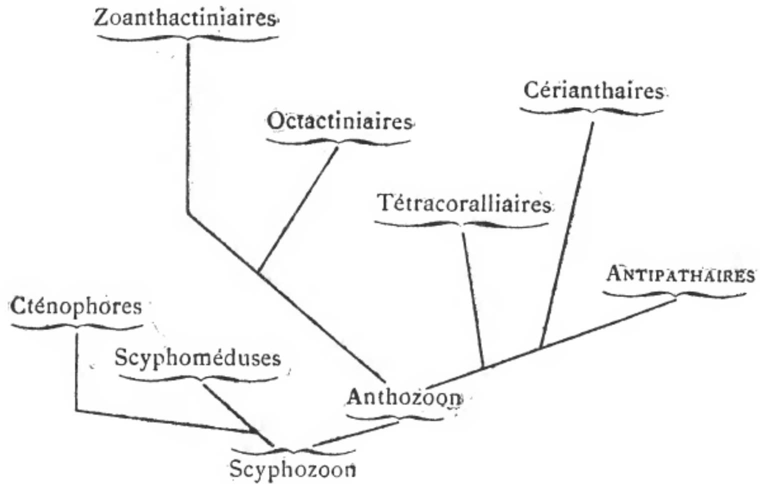


Figure 9. Phylogénie relative au groupe des antipathaires, considérés comme les anthozoaires les plus primitifs par Roule (1905).

Enfin, le dernier ouvrage majeur de ce début du $XX^{ème}$ siècle est le travail de van Pesch (1914), qui publie son rapport sur l'expédition *Siboga* ayant eu lieu entre 1899 et 1900 dans les eaux indonésiennes. Dans celui-ci, il discrédite le dimorphisme des polypes utilisés jusque-là pour classifier les antipathaires. En outre, il divise la famille Antipathidae en deux sous-familles basées sur la présence ou l'absence de mésentères secondaires, appelées respectivement Heterotaeniales et Homoeotaeniales. A ce moment, la famille comprend 190 espèces pour 9 genres, dont 2 comprennent à eux seuls près de 150 espèces. Suite à cela, l'ordre des Antipatharia n'évoluera plus pendant près d'un demi-siècle.

2. Taxonomie et morphologie des antipathaires

Il faut attendre la fin des années 1960 et le début des travaux remarquables d'Opresko pour voir le groupe des antipathaires réévalué. Aujourd'hui, la taxonomie et la nomenclature modernes des antipathaires doivent presque tout à cet auteur, qui reprend et réinterprète les travaux de ses prédécesseurs (Pallas 1766 ; Esper 1788 ; Milne-Edwards 1857 ; Verrill 1864 ; Gray 1868 ; de Pourtalès 1868, 1871, 1874, 1878, 1880 ; Duchassaing 1870 ; Brook 1889 ; Schultze 1896 et van Pesch 1914). Pendant près de 50 ans, Opresko publie des révisions d'espèces, de genres, de familles, mais aussi des nouvelles descriptions (Opresko 1972, 1974, 1976, 1993, 1996, 1997, 1998, 1999, 2001a, 2001b, 2002, 2003a, 2003b, 2004, 2005a, 2005b, 2006, 2009, 2011 ; Opresko & Genin 1990 ; Opresko & Bayer 1991 ; Opresko & Cairns 1992, 1994 ; Opresko & Baron-Szabo 2001a, 2001b ; Opresko & de Laia Loiola 2008 ; Opresko & Breedy 2010 ; Opresko et al. 2012 ; Bo & Opresko 2015 ; Wagner & Opresko 2015 ; Molodtsova & Opresko 2017). Il a toujours existé au sein des antipathaires un manque cruel de traits taxonomiques clairs et précis pour distinguer les familles et les genres, et c'est en grande partie dû à l'appartenance erronée de certains groupes tels que le zoanthidé *Savalia* qui possède un axe ressemblant à celui des antipathaires (Brito & Ocana 2004), l'octocoralliaire *Dendrobrachia fallax* qui possède des épines squelettiques (Opresko & Bayer 1991) et l'hydroïde *Tropidopathes saliciformis* dont les apophyses des hydrocauli furent mal interprétées en tant qu'épines squelettiques d'antipathaire (Opresko & Baron-Szabo 2001b). Pour réviser entièrement l'ordre des antipathaires, Opresko conserve la division de Schultze (1896) en définissant les familles sur base du nombre de mésentères des polypes (6 chez les Cladopathidae, 10 dans les familles Aphanipathidae, Myriopathidae, Schizopathidae et Stylopathidae et 12 chez les Leiopathidae), mais rajoute de nouvelles informations sur la morphologie des polypes et la corrélation existante entre celle-ci et celle des épines squelettiques, et enfin utilise la morphologie générale de la colonie en tant que critère générique. Bien entendu, ces progrès vont de pair avec le développement des moyens technologiques, et notamment du microscope électronique à balayage qui permet de révéler le véritable aspect des épines, dont la surface peut considérablement varier en fonction de l'espèce. Enfin, ce n'est que depuis très récemment que l'apparence des polypes vivants et non plus fixés commence à être prise en compte (Opresko 2005b ; Wagner et al. 2010).

Aujourd'hui le groupe des antipathaires comprend 7 familles, dont 3 ont été nouvellement établies par Opresko et 2 entièrement révisées (Opresko 2001b, 2002, 2003b, 2004, 2005b, 2006). De façon générale, il existe une répartition des familles en fonction de la bathymétrie :

- Antipathidae Ehrenberg, 1834 en cours de révision complexe car historiquement considérée comme un groupe « fourre-tout » ; généralement entre 10 et 200 mètres de profondeur ;
- Aphapnipathidae Opresko, 2004 nouvellement établie, trouvée entre 50 et 400 mètres ;
- Cladopathidae Kinoshita, 1910 révisée et comprenant des coraux vivants entre 400 et 2000 mètres ;
- Leiopathidae Haeckel, 1896 monogénérique et en cours de révision, de 100 à plus de 1000 mètres de profondeur ;
- Myriopathidae Opresko, 2001 nouvellement établie, entre 10 et 200 mètres ;
- Schizopathidae Brook, 1889 révisée, entre 400 et 5000 mètres
- Stylopathidae Opresko, 2006 nouvellement établie, entre 200 et 1000 mètres.

Comme on peut le voir, les antipathaires forment un groupe dont la majorité des familles vivent à grandes profondeurs (>200 m) ce qui est une des raisons pour lesquelles les connaissances relatives à ces organismes ont lentement évolué au cours de leur histoire, étant donné la difficulté d'échantillonnage et d'observation des colonies. Cependant, les espèces d'eaux peu profondes, et notamment celles de l'Océan Indien restent très peu connues. Majoritairement membres de la famille Antipathidae, ces espèces sont toujours en cours de révision. A l'heure actuelle, il est donc difficile de les identifier avec certitude étant donné le manque d'informations détaillées dans les descriptions originales ou encore la perte de nombreuses espèces types.

Il est néanmoins possible de dresser les caractères généraux des antipathaires et des familles qui les composent. Les polypes possèdent toujours 6 tentacules groupés en trois paires : deux sont situés à l'extrémité du polype pointant vers le sommet de la branche et sont appelés tentacules distaux latéraux, deux sont à l'autre extrémité pointant vers la base de la branche ou de l'axe et sont appelés tentacules proximaux latéraux, et enfin les deux restants sont situés au milieu du polype de part et d'autre de la bouche et portent le nom de tentacules sagittaux (Fig. 10, 11). Ces tentacules ne sont jamais branchus et ne peuvent pas se rétracter, bien qu'ils puissent se contracter à différents degrés. Il n'existe pas non plus de disque oral au sein du groupe, c'est-à-dire que les tentacules ne sont jamais parfaitement disposés de façon circulaire autour de la bouche, qui est souvent située sur un cône oral (Fig. 10, 11). Le polype possède deux axes, le premier étant dans la direction de la branche qui le porte (axe transverse) et le second dans un plan perpendiculaire à celle-ci (axe sagittal). La taille du polype se mesure entre les limites externes des tentacules distaux latéraux et proximaux latéraux, on parle alors du diamètre transverse (Fig. 10, 11) ; quant à la distance interpolypaire elle se mesure entre le centre des bouches de deux polypes adjacents (Wagner et al. 2010). Ce diamètre peut être sujet à la variation au sein d'une seule et même colonie, au point que certains polypes peuvent être totalement dégénérés sur certaines tiges en ne possédant plus de tentacules.

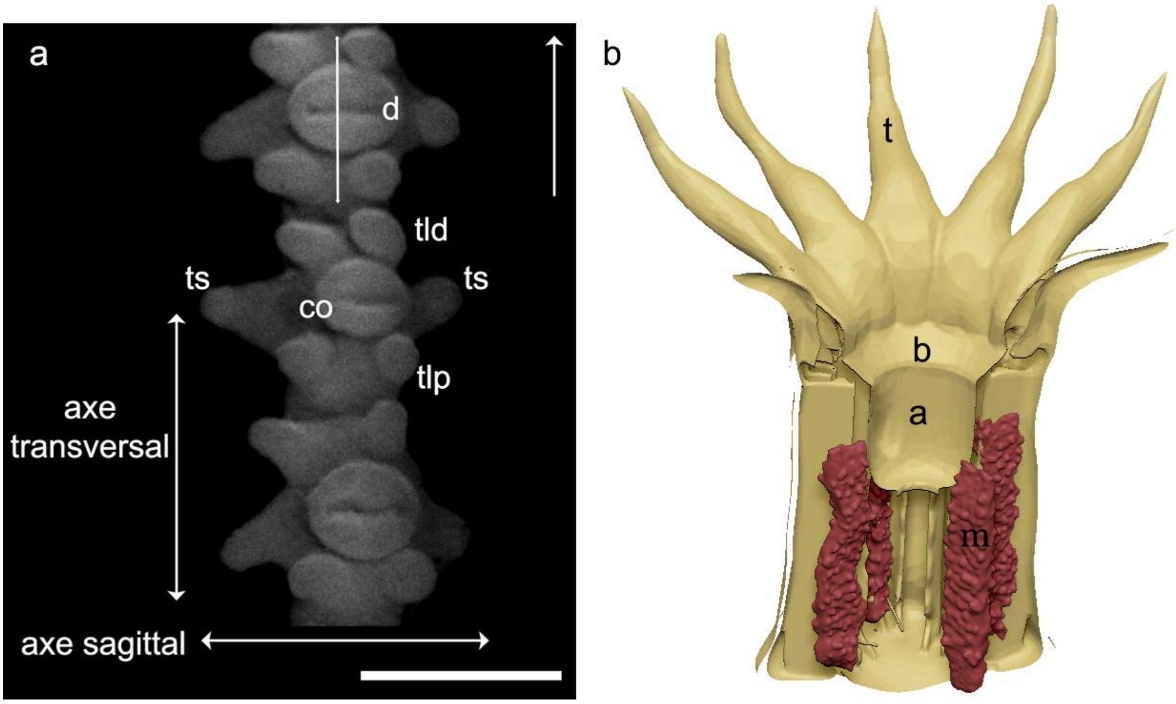


Figure 10. (a) Photo des polypes situés sur une branche de *Myriopathes ulex*, la flèche indiquant le sens vers l'extrémité de la branche portant les polypes (illustration personnelle). (b) Schéma général de l'organisation d'un polype d'anthozoaire, auquel se rapporte le polype d'antipathaire mais qui possède 6 tentacules et un cône oral supportant la bouche (illustration de Nathan Puozzo, adapté de l'application MetaMorphos). Echelle (a) : 1 mm. *a – actinopharynx ; b – bouche ; cg – cavité gastrovasculaire ; co – cône oral ; d – diamètre du polype ; m – mésentère ; t – tentacule ; tlp – tentacules latéraux proximaux ; tls – tentacules latéraux distaux ; ts – tentacules sagittaux.*

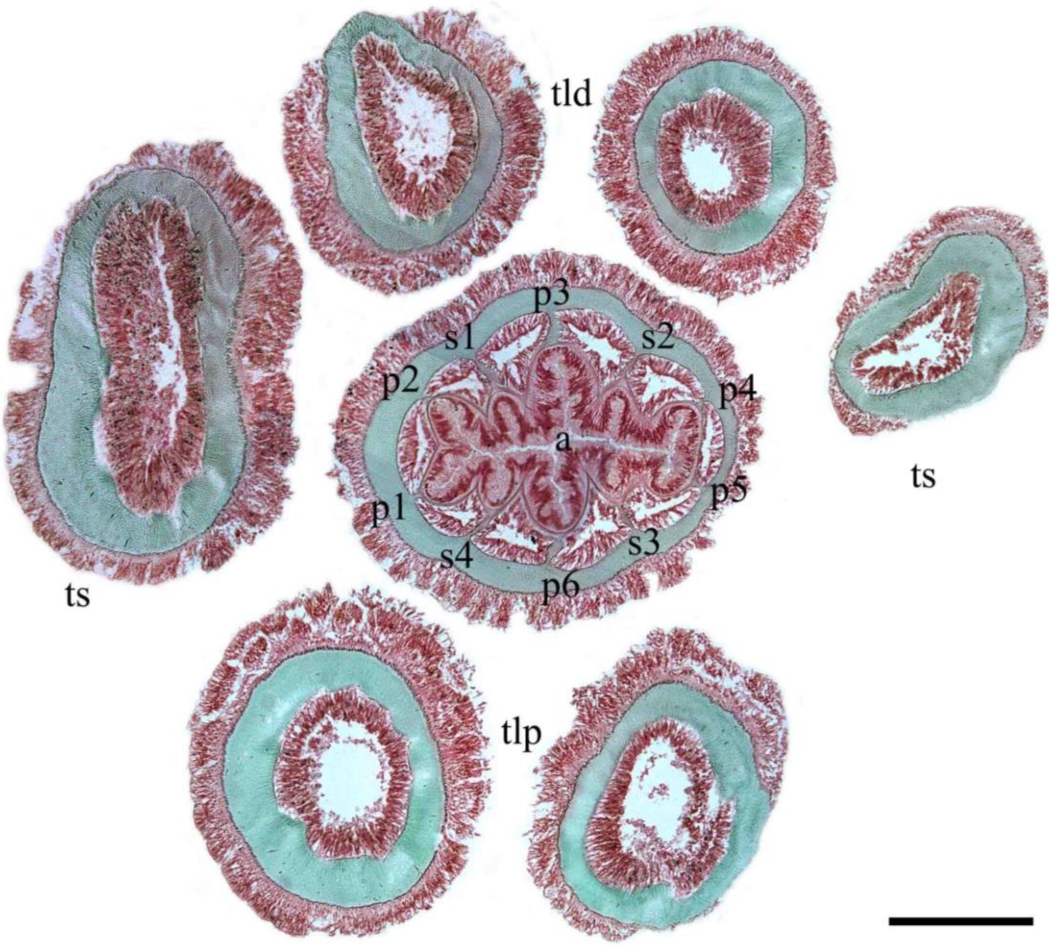


Figure 11. Coupe histologique transversale d'un polype de *Cirrhipathes* sp membre de la famille Antipathidae et possédant 10 mésentères. Echelle : 100 µm. *a – actinopharynx ; p – mésentère primaire de 1 à 6 ; s – mésentère secondaire de 1 à 4 ; tld – tentacules latéraux distaux ; tlp – tentacule latéraux proximaux ; ts – tentacules sagittaux*. Illustration personnelle.

Ces caractères variables impliquent l'observation de plusieurs colonies dans leur entièreté lorsqu'il est question de décrire une espèce. De cette manière, les polypes permettent de définir la famille auxquelles appartiennent les espèces (Brugler et al. 2013) alors que leur disposition au sein de la colonie est parfois utile à la détermination du genre :

- Chez les Antipathidae, Aphanipathidae et Leiopathidae les polypes sont ronds, ovales, légèrement compressés ou allongés transversalement, les tentacules sont longs et possèdent une forme pointue ;
- Chez les Myriopathidae et Stylopathidae les polypes sont légèrement allongés transversalement, ne dépassent pas 1 mm et possèdent des tentacules subégaux, courts et ronds à leurs extrémités ;

- Chez les Schizopathidées les polypes sont allongés transversalement, sont assez grands en dépassant toujours 3 mm et les tentacules sont longs et étroits ;
- Chez les Cladopathidae les polypes sont allongés transversalement et souvent plus grands que 3 mm en ayant des tentacules courts, subégaux et ronds.

Comme vu précédemment, les mésentères primaires sont toujours au nombre de 6 au sein du polype et sont toujours complets, c'est-à-dire qu'ils relient l'actinopharynx qui possède lui-même un siphonoglyphe. Ce sont dans les mésentères transverses situés dans l'axe transversal du polype que les gamètes se développent.

Il n'est pas nécessaire de revenir sur les aspects généraux des colonies tels que mentionnés précédemment, car ils ont été pour la plupart été utilisés dans le passé par les naturalistes pour classifier les antipathaires. Cependant, la taxonomie moderne de ce groupe considère toujours la morphologie du squelette pour l'identification des espèces ou des genres tout en apportant son lot de révisions, c'est pourquoi il en sera question dans ce paragraphe. Avant d'identifier un corail noir, il est impératif d'avoir à l'esprit que ces coraux possèdent à la fois un grand éventail de morphologies différentes ainsi qu'une plasticité phénotypique intraspécifique assez large, en plus de varier en fonction de la taille globale et de l'âge absolu de la colonie (Fig. 12). Il arrive également que certaines colonies non branchues le deviennent à la suite de fractures naturelles. D'autres facteurs environnementaux tels que la force des courants ou la disponibilité des sources de nourriture influencent également la densité, la forme et la taille des épines et des branches (Warner 1981). De plus, il est à présent bien connu que certaines espèces symbiotiques influencent le développement du corail en modifiant localement les épines, les branches ou les polypes, comme chez certains polychètes des familles Polynoidae et Eunicidae (Molodtsova & Budaeva 2007). Un autre exemple de cette plasticité provient de certaines espèces de la famille des Stylopathidae qui fusionnent carrément leurs branches pour former des tunnels cylindriques empruntés par certains vers marins (Opresko 2006), dans lesquels les polypes sont dégénérés au point de ne plus posséder qu'un cône oral. Une fois ces considérations minutieusement prises en compte, le risque de délimiter de nouvelles espèces de façon erronée en est considérablement réduit et l'observation du corallum se fait de manière réfléchie. Si la colonie est non branchue, son apparence droite, sinueuse ou spiralée aura de l'importance. En revanche il existe deux types de colonies ramifiées, qui peuvent présenter une pinnulation ou non. Les pinnules sont des ramifications simples ou branchues symétriquement arrangées et de tailles égales se trouvant sur l'axe principal et les branches du corail et dont l'arrangement est crucial pour l'identification d'un taxon. Dans les deux cas, l'angle d'insertion des branches est à prendre en compte en plus de leur épaisseur et de leur degré d'anastomose.

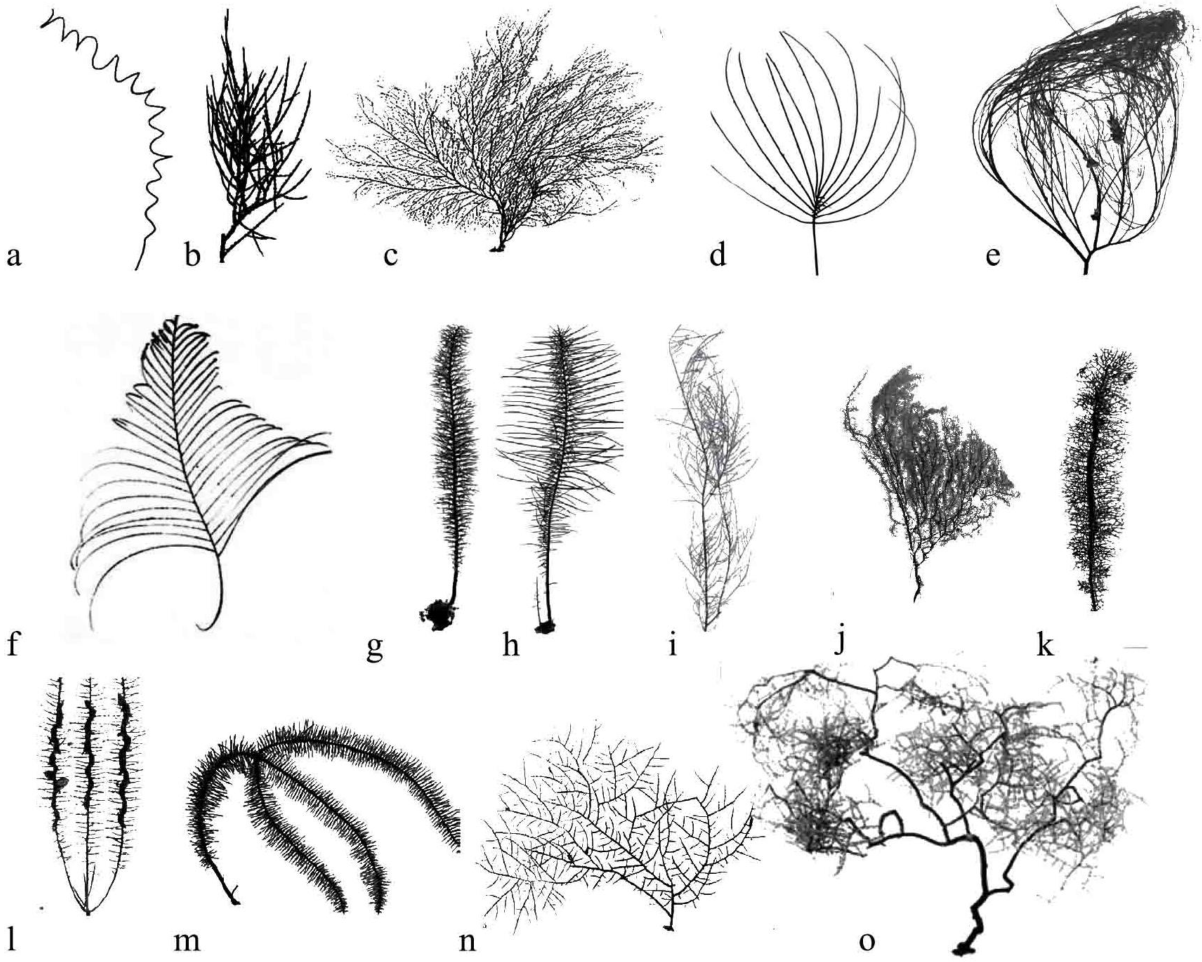


Figure 12. Aperçu des différentes morphologies rencontrées au sein de l'ordre des antipathaires, montrant la grande diversité caractérisant ce groupe (adapté de Brugler et al. 2013). Antipathidae : a, b, c, e. Cladopathidae : d. Aphanapathidae : l, m, n. Myriopathidae : g, h, i. Stylopathidae : j, k. Schizopathidae : f. Leiopathidae : o.

Enfin, sur le squelette se trouvent les épines dont l'apparence joue un rôle décisif dans la majorité des cas, excepté dans la famille Myriopathidae où le branchement des colonies constitue le critère primordial. A nouveau, le critère branchu ou non de la colonie aura son importance dans l'observation des épines, car l'apparence de ces dernières varie en fonction de la localisation et de la branche qui les porte. Ces dernières sont de deux types, les épines primaires qui se retrouvent partout sur le squelette et les épines secondaires qui sont facultatives et qui sont généralement plus petites et plus éparses. La taille des épines primaires est fonction de l'âge et de l'épaisseur de la colonie, il n'est pas non plus rare de voir de nouvelles épines se développer sur d'anciennes parties. Elles peuvent être triangulaires, latéralement compressées, coniques, cylindriques, en forme d'aiguille ou de massue, ou encore en forme d'ampoule (Opresko 2001, 2002, 2003b, 2004, 2005b, 2006). Quant à leur surface, elle peut être lisse, légèrement ou fortement striée, légèrement ou fortement papilleuse, ou tuberculée ; tandis que leur sommet peut être pointu, boutonneux, fourchu ou ramifié (Fig. 13). Les épines secondaires sont d'apparence plus aléatoire, souvent triangulaires ou cylindriques, mais parfois sans véritable forme pouvant être caractérisée. De manière générale, les épines s'organisent en rangées le long de l'axe transversal alors qu'au sein du genre *Pseudocirrhipathes* les épines se groupent en verticilles (Bo et al. 2009).

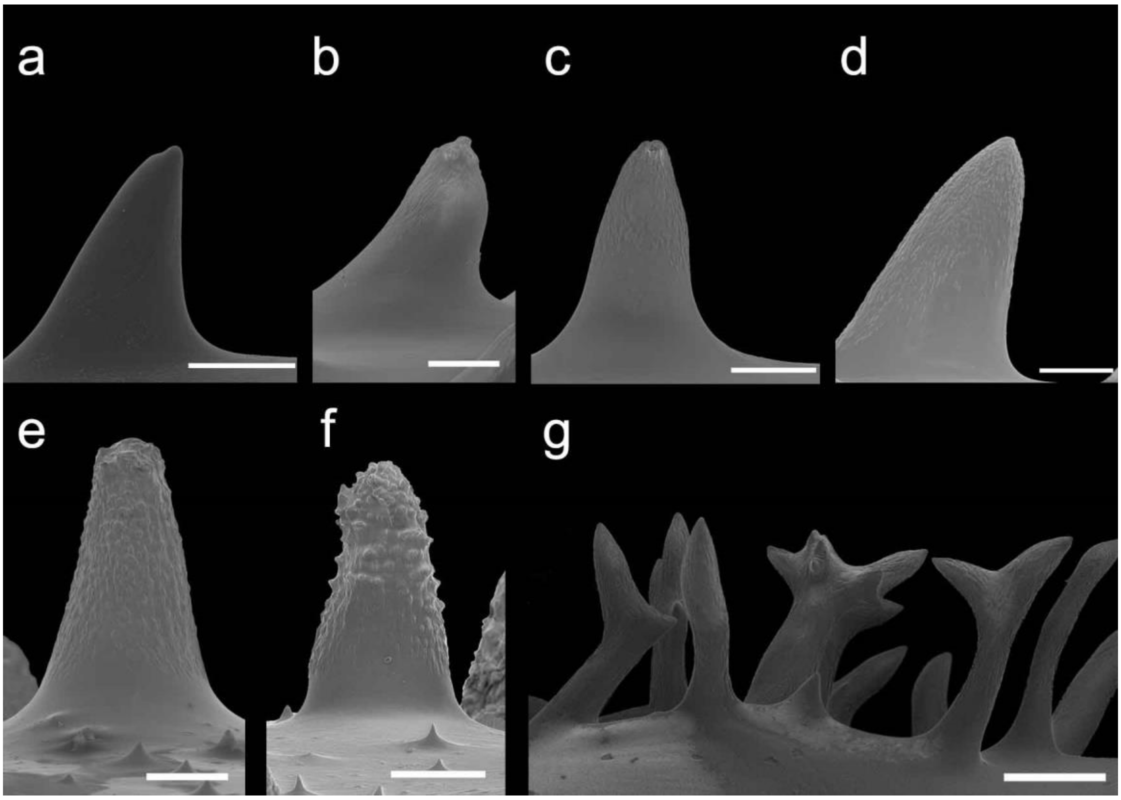


Figure 13. Exemples d'épines retrouvées chez des espèces des familles Antipathidae et Myriopathidae. Echelles : 100 µm. a : épine lisse et triangulaire, b : épine conique et tuberculée au sommet, c : épine conique, striée et tuberculée au sommet, d : épine triangulaire et finement granuleuse, e : épine conique, ronde au sommet et tuberculée sur sa surface, f : épine conique et épaisse, très papilleuse, g : épines dendritiques et finement striées. Illustrations personnelles.

La taille et l'apparence des épines varie chez certaines espèces en fonction de l'emplacement du polype. Dans le genre *Stichopathes* par exemple, qui regroupe des coraux n'ayant qu'une seule rangée de polype d'un côté de l'axe squelettique, les épines sont toujours plus longues du côté de ces derniers, on parle alors d'épines polypaires et d'épines abpolypaires pour les plus petites situées du côté opposé. C'est encore plus complexe au sein de certains genres appartenant à la famille Aphanipathidae où les épines diffèrent fortement sous et autour des polypes, les plus petites étant les épines hypostomales situées sous la bouche du polype, les plus longues étant les épines circumpolypaires situées en périphérie du polype, et celles de taille intermédiaire étant situées entre les polypes appelées épines interpolypaires (Opresko 2004).

La position phylogénétique des antipathaires en relation avec les autres anthozoaires est restée la même jusqu'à l'avènement des techniques modernes de génétiques. Comme mentionné précédemment, van Beneden (1897) regroupait les antipathaires et les cérianthaires dans la sous-classe des Ceriantipatharia sur base des ressemblances morphologiques entre la larve cérinule des cérianthes et les polypes d'antipathaires (van Beneden 1897). Ce n'est que près d'un siècle plus tard que France et al. (1996), Berntson et al. (1999) et Won et al. (2001) réévalueront leur origine phylogénétique grâce à différents marqueurs génétiques mitochondriaux et nucléaires. Ces auteurs arriveront à la conclusion que les Antipatharia et Ceriantharia ne forment pas un groupe monophylétique, l'ordre des Antipatharia étant compris au sein de la sous-classe des Hexacorallia. Quelques années plus tard, Brugler & France (2007) supportèrent cette hypothèse en séquençant l'entièreté du génome mitochondrial de *Chrysopathes formosa*. Cependant, le groupe frère des antipathaires au sein des Hexacoralliaires demeure encore incertaint, comme en témoignent les arbres phylogénétiques présentés Fig. 14. Soit les antipathaires seraient être le groupe frère du clade comprenant les scléractiniaires et les Corallimorphes (Fig. 14A) ; soit ils seraient compris dans le même clade que les Actiniaires, et ce clade serait le groupe frère du clade comprenant les Scléractiniaires et les Corallimorphes (Fig. 14B). Dans tous les cas, les Antipathaires ne sont pas groupés dans le même clade que les Cérianthaires, ces derniers étant soit (i) le groupe frère des Octocoralliaires (Fig. 14A), soit (ii) le groupe frère du clade comprenant tous les hexacoralliaires (Fig. 14B).

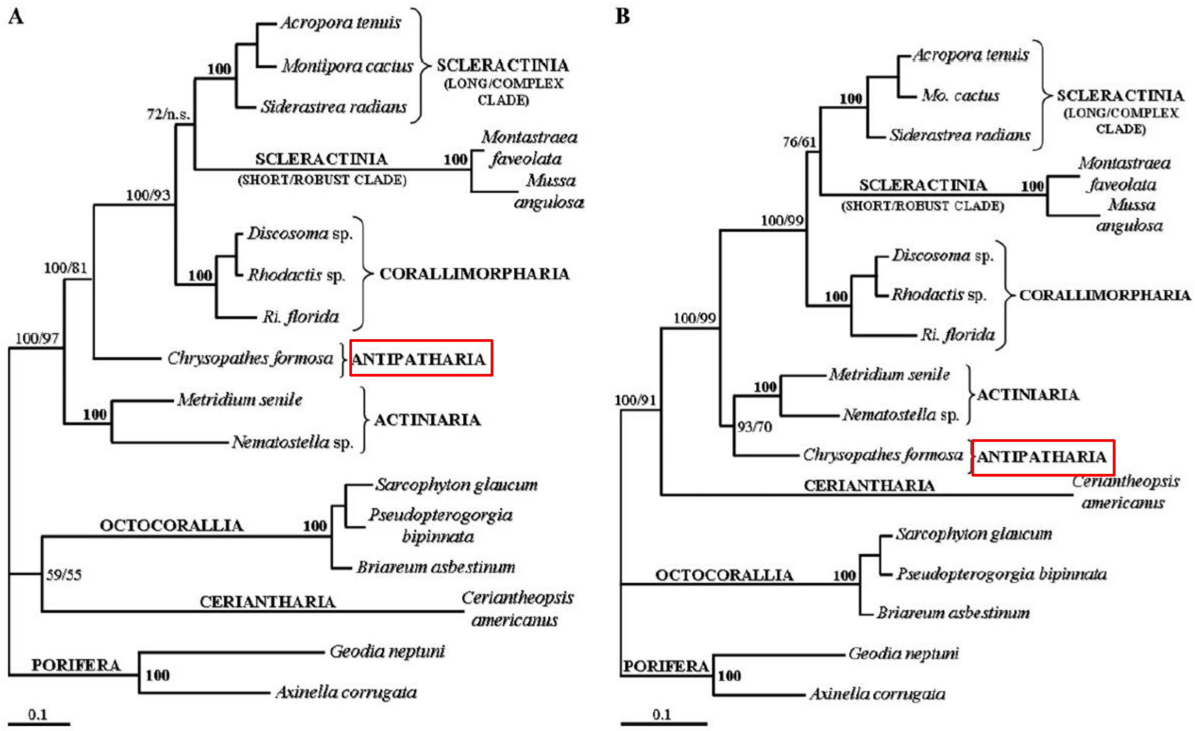


Figure 14. Position la plus récente de l'Ordre Antipatharia par rapport aux autres anthozoaires. Obtenus par les méthodes de maximum de vraisemblance et d'analyse bayésienne, ces arbres se basent sur l'alignement soit (A) des séquences nucléotidiques soit (B) des séquences protéiques de trois gènes mitochondriaux concaténés (*cox1*, *cox2*, *nad1*). Sur chaque nœud sont représentés les valeurs de probabilités postérieures bayésiennes ainsi que les valeurs de bootstrap, respectivement. Les mêmes valeurs ne sont représentées qu'une fois. Tirés de Brugler & France (2007).

3. Distribution et habitat

Le groupe des antipathaires comprend à l'heure actuelle environ 250 espèces reprises dans 44 genres (Brugler et al. 2013 ; Molodtsova & Opresko 2018). Bien entendu, pour les raisons mentionnées ci-dessus, ce nombre est en constante évolution en fonction des révisions de synonymies et de descriptions de nouvelles espèces. Avec plus de 75% des espèces de ce groupe vivant à plus de 50 mètres de profondeur, ce sont paradoxalement ces derniers qui sont les mieux connus d'un point de vue taxonomique. Au contraire, les espèces vivant à faibles profondeurs ont souffert d'un manque d'intérêt jusqu'aux publications récentes de nouvelles études (Opresko & Sanchez 2005 ; Molodtsova 2007 ; Tazioli et al. 2007 ; Bo et al. 2008 ; Bo et al. 2009 ; Opresko et al. 2014 ; Wagner 2015 ; Todinanahary et al. 2016 ; Terrana & Eeckhaut 2017). Cependant il reste encore de très nombreuses espèces dont l'identification est difficile, car originellement décrites depuis des spécimens incomplets combiné au manque de traits taxonomiques clairs au moment de leur description. Un grand nombre de spécimens types de l'Océan Indien sont perdus ou introuvables (Molodtsova, communication personnelle) ce qui rend encore plus complexe la réévaluation des groupes concernés, même si la famille Antipathidae –la plus représentée en eaux peu profondes– est actuellement en cours de révision par Marzia Bo (communication personnelle). En outre, certaines analyses moléculaires récentes montrent que les coraux noirs contiennent des écotypes et des caractères morphologiques plastiques compliquant ainsi le regroupement ou la délimitation d'espèces (Lapian et al. 2007 ; Brugler et al. 2013).

Si les antipathaires de l'Océan Pacifique et surtout des îles Hawaïennes sont très étudiés (Grigg 1965, 1974, 1984, 1989, 1993, 2001, 2002, 2003, 2004, 2010 ; Wagner et al. 2010, 2011 ; Wagner 2015), les connaissances modernes relatives aux espèces de l'Indo-Pacifique ne se focalisent exclusivement que sur la région Indonésienne, principalement le Bunaken Marine Park dans les Sulawesi. Cette zone est la plus riche et la plus diversifiée en coraux de faible profondeur (<50 m) décrite à ce jour, avec 16 espèces rapportées (Tazioli et al. 2007 ; Bo et al. 2008). Au contraire, mis à part quelques notes sur leur présence, les antipathaires de l'Océan Indien et particulièrement de Madagascar sont totalement inconnus (Thomson & Simpson 1902 ; Cooper 1909, Summers 1910 ; Pichon 1972 ; Terrana & Eeckhaut 2017 ; Fig. 15).

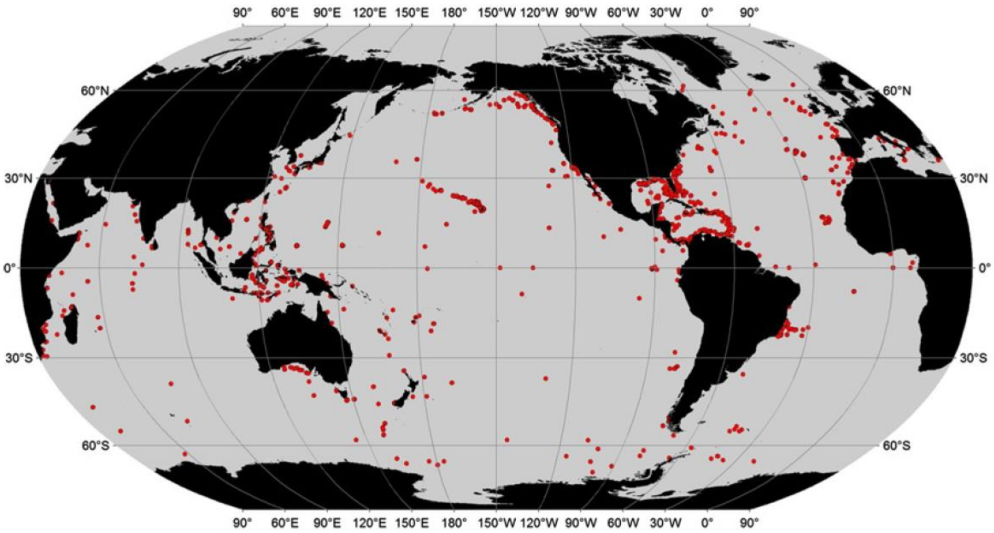


Figure 15. Distribution des antipathaires selon Wagner et al. (2012) basée sur la littérature et sur la collection du National Museum of Natural History of Smithsonian Institution (USA). Seules 4 occurrences sont rapportées pour Madagascar.

Les travaux datant du début du XX^ème^ siècle sont les derniers en date auxquels il faut se référer pour identifier les espèces de cette zone, ce qui renvoie une fois encore à tous les problèmes taxonomiques expliqués précédemment. En se référant à la révision de Wagner et al. (2012), le constat suivant peut être dressé (Tableau 1) :

Tableau 1. Compte rendu des études publiées sur différents thèmes concernant les antipathaires dans le monde et dans l'Océan Indien.

Type d'étude	Monde	Océan Indien
Structure des champs de coraux	20 articles	Néant
Reproduction	24 articles	Néant
Croissance et longévité	23 articles	Néant
Connectivité entre populations	6 articles	Néant
Identification des symbiotes	148 symbioses	25 symbioses
Données sur les relations trophiques	7 articles	Néant

Il existe donc clairement un manque d'informations et d'études concernant les coraux de l'Océan Indien, qui se rencontrent pourtant très fréquemment en plongée et qui sont la cible de collectes sauvages et illégales dont il sera question plus loin dans cette introduction.

Les antipathaires sont un des rares groupes à vivre à presque toutes les profondeurs possibles, de 4 mètres (*Antipathella aperta*, Grange 1985) à 8900 mètres (*Schizopathes affinis*, Molodtsova 2006), et dans presque toutes les mers et les océans. Bien qu'ils vivent des pôles à l'équateur, ils sont plus abondants sous les tropiques où ils sont parfois tellement denses qu'ils forment ce que l'on appelle des champs de coraux noirs (Tazioli et al. 2007 ; Wagner et al. 2011 ; Bo et al. 2014 ; Bo et al. 2015 ; Ingrassia et al. 2016). Il est cependant difficile d'établir avec exactitude l'aire de répartition des espèces, car un grand nombre d'entre elles ne sont connues que par leur type. De plus, il y a encore et toujours ce problème de taxonomie qui empêche l'unification de certains spécimens et l'établissement exact de biogéographies, surtout au niveau des espèces vivant en eaux peu profondes (<50 m). Par conséquent, les documents les plus précis géographiquement sont souvent les récits d'expéditions océanographiques ou plus récemment d'explorations mettant en œuvre de gros moyens technologiques tels que l'utilisation de robots sous-marins télécommandés, évidemment toujours utilisés pour les études à grandes profondeurs (Tableau 2).

Tableau 2. Liste des principales expéditions de fin du XIX^{ème}/ début du XX^{ème} siècle ayant mené à la description, l'identification et/ou la découverte d'antipathaires.

Auteur	Année	Expédition	Localisation
Dana	1846	United States Exploring Expedition	Amérique du Nord, Amérique du Sud, Madère, Cap de Bonne Espérance, Fidji, Samoa, Australie, Hawaii, Antarctique, Asie du sud-est
de Pourtalès	1874	Hassler expedition	Amérique du Nord et du Sud
de Pourtalès	1878	Blake expedition	Floride, Golfe du Mexique, Caraïbes, Golfe de Maine, Atlantique est
Brook	1889	HMS Challenger	Monde entier
Schultze	1896	Valdivia	Atlantique est, Afrique, océan Indien
Thomson	1905	Scottish Antartic Expedition	Antarctique
Cooper	1909	Percy Sladen Trust Expedition	Océan Indien
van Pesch	1914	Siboga Expeditie	Asie du sud-est
Totton	1923	British Antarctic Terra Nova Expedition	Antarctique
Thomson & Rennet	1931	Australian Antarctic Expedition	Antarctique, Océanie

Wagner et al. (2012) dressent un résumé des localités où un inventaire des espèces d'antipathaires a été effectué : Mozambique et Archipel Mergui (Summers 1910), îles Maldives et Laccadive (Cooper 1903), Diego Garcia et la partie nord de l'Océan Indien (Cooper 1909), Golfe de Manaar (Thomson & Simpson 1905), Japon (Siberfeld 1909 ; Pax 1932), Corée (Moon & Song 2005, 2008a, b), Chine (Zou & Zhou 1982, 1984 ; Zhou & Zou 1984, 1987, 1992), Indonésie (Bo 2008), îles Moluques (Schultze 1896), Antarctique (Thomson 1905 ; Thomson & Rennet 1931), îles Mariannes (Paulay et al. 2003 ; Parrish & Baco 2007), l'archipel d'Hawaï (Grigg & Opresko 1977 ; Parrish & Baco 2007 ; Wagner 2015), îles alouétiennes (Heifetz et al. 2005), Alaska (Heifetz 2002), Golfe du Mexique (Opresko 2009), Caraïbes (Opresko & Sanchez 2005), Brésil (Echeverria 2002, Loiola & Castro 2005), Madère (Johnson 1899), îles Canaries (Brito & Ocaña 2004), Golfe de Gascogne (Roule 1905 ; Grasshoff 1981), Jamaïque (Warner 2005), et la Méditerranée (Bo et al. 2008).

Depuis cette publication, d'autres études sont venues compléter la liste avec la description de champs d'antipathaires autour de Malte (Deidun et al. 2015), des Açores (Matos et al. 2014), des îles Pontines (Ingrassia et al. 2016), l'établissement d'inventaires en Nouvelle-Zélande (Opresko et al. 2014) ou aux Bermudes (Wagner & Shluer 2017), l'étude de la distribution de *Leiopathes glaberrima* en Méditerranée (Massi et al. 2018) et l'étalement de l'aire de distribution du genre *Heteropathes* (De Matos et al. 2014 ; Molodtsova 2017). A nouveau, l'exploration ciblée de failles sous-marines ou le chalutage profond révèlent l'existence d'antipathaires, que ce soit dans l'Océan Pacifique (Araya & Aliaga 2016 ; Molodtsova & Opresko 2017), le Groenland (Jorgensen et al. 2013), le Canada (Macisaac et al. 2013) ou l'Alaska (Stone & Cairns 2017). Enfin, depuis une dizaine d'années les études se concentrant sur les environnements mésophotiques (50-100 m) se multiplient et entrainent par conséquent une augmentation de la présence des antipathaires à différentes régions du globe (USA, Hourigan et al. 2017 ; Hawaï, Wagner et al. 2010, Wagner 2015 ; Australie, Bridge et al. 2010 ; Golfe du Mexique, Silva & MacDonald 2017 ; Johnson Atoll, Wagner et al. 2014 ; Brésil, Cordeiro et al. 2015). Si la répartition des coraux noirs est la mieux connue dans le Pacifique et l'Atlantique, notamment au niveau du Golfe du Mexique ou de l'Archipel d'Hawaï, c'est parce que les moyens mis en œuvre pour étudier ces zones sont considérables, et toujours concentrées aux mêmes endroits. Ceci n'est pas dû au hasard mais représente la conséquence de l'accroissement de la vulnérabilité des coraux formant les Ecosystèmes Marins Vulnérables (EMV) profonds face aux chalutages, dragages ou forages miniers les détruisant (Yesson et al. 2017). Finalement, le constat demeure identique : les études publiées ces 15 dernières années ne concernent presque qu'exclusivement les espèces vivant à grandes profondeurs (>100 m à >1000 m), délaissant de ce fait les antipathaires d'eaux peu profondes, et particulièrement celles de l'Océan Indien. Yesson et al. (2017) modélisent la distribution globale des antipathaires vivants à grandes profondeurs sur base de paramètres et de facteurs environnementaux favorisant l'habitat idéal de ces coraux. De cette manière, il devient possible de prédire les zones adéquates où croissent les antipathaires profonds dans le monde entier. Ils soulignent de cette façon l'existence vraisemblable d'autres zones marines où les coraux devraient se retrouver de manière abondante, montrant ainsi que seuls 7 à 8% des zones prédites se retrouvent dans des aires marines protégées. Cette étude résume également le problème du manque de données sur les espèces de l'Océan Indien : sur 1362 recensements bibliographiques utilisés dans leur modèle de prédiction, seuls 122 proviennent de cet océan, soit à peine 9%.

Si les récifs coralliens de Madagascar sont connus des navigateurs européens dès le 16ème siècle, il faut attendre la construction de la station marine de Tuléar (à présent appelée Institut Halieutique et des Sciences Marines) dans le sud-ouest en 1961 pour voir apparaître les premières études intensives des récifs coralliens de cette zone. En conséquence, les antipathaires d'eaux peu profondes de Madagascar ne sont répertoriés qu'exclusivement et indirectement à travers les travaux de Humes (1967, 1969) et de Pichon (1972), le premier se focalisant sur les copépodes inféodés aux antipathaires dans la région de Nosy Be au nord du pays, le second aux scléractiniaires formant les récifs coralliens du sud-ouest de la Grande Île (Fig. 15). Le tableau suivant (Tableau 3) résume les espèces potentiellement présentes dans les eaux malgaches de la côte ouest entre 15 et 50 mètres de profondeur, telles que rapportées par Humes (1967, 1969) et Pichon (1972):

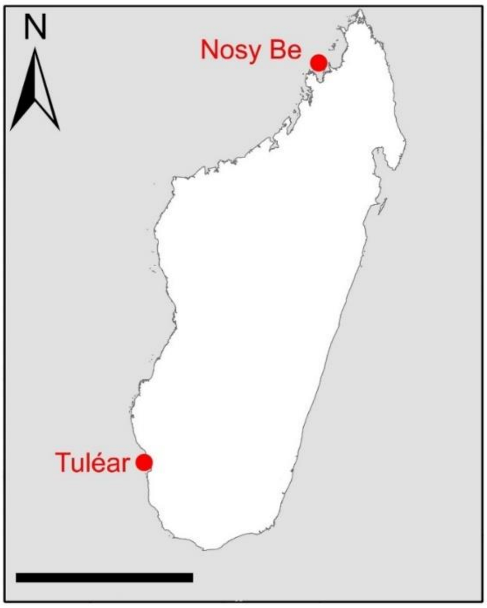
Tableau 3. Résumé des espèces potentiellement retrouvées à Nosy Be et à Tuléar, d'après Humes (1965, 1969) et Pichon (1972).

Espèce rapportée	Nom actuel	Lieu	Prof. (m)	Référence
Antipathes abies Linnaeus, 1758	*Cupressopathes abies* (Linnaeus, 1758)	Nosy Be	25	Humes 1969
Antipathes abies Linnaeus, 1758	*Cupressopathes abies* (Linnaeus, 1758)	Tuléar	25-35	Pichon 1972
Antipathes cf. spinescens Gray, 1857	*Tanacetipathes spinescens* (Gray, 1857)	Nosy Be	25	Humes 1969
Antipathes dichotoma Pallas, 1766	*Antipathes dichotoma* Pallas, 1766	Tuléar	25-35	Pichon 1972
Antipathes ericoides Pallas, 1766	*Arachnopathes ericoides* (Pallas, 1766)	Nosy Be	25	Humes 1969
Antipathes ericoides Pallas, 1766	*Arachnopathes ericoides* (Pallas, 1766)	Tuléar	15-20	Pichon 1972
Antipathes longibrachiata van Pesch, 1914	*Antipathes longibrachiata* van Pesch, 1914	Nosy Be	23	Humes 1969
Antipathes myriophylla Pallas, 1766	*Myriopathes myriophylla* (Pallas, 1766)	Nosy Be	24	Humes 1969
Antipathes pennacea Pallas, 1766	*Plumapathes pennacea* (Pallas, 1766)	Tuléar	25-35	Pichon 1972
Antipathes reticulata Esper, 1795	*Rhipidipathes reticulata* (Esper, 1795)	Tuléar	25-35	Pichon 1972
Antipathes ulex Ellis & Solander, 1786	*Myriopathes ulex* (Ellis & Solander, 1786)	Tuléar	15-20	Pichon 1972
Cirrhipathes anguina (Dana, 1846)	*Cirrhipathes anguina* (Dana, 1846)	Tuléar	15-50	Pichon 1972
Stichopathes echinulata Brook, 1889	*Stichopathes echinulata* Brook, 1889	Nosy Be	30	Humes 1967

Puisqu'il n'existe à l'heure actuelle aucune étude entièrement dédié à cette faune marine centrée sur Madagascar, il faut strictement se référer aux identifications des antipathaires rapportés par ces deux auteurs pour avoir une idée de leur diversité dans cette région du monde. En connaissant les problèmes liés à la taxonomie des antipathaires, il faut garder un œil critique sur les identifications effectuées dans ces études, qui mis à part le nom de l'espèce ne donnent véritablement aucune information permettant de les déterminer avec certitude. Certaines espèces mentionnées par Humes et Pichon n'ont jamais été rapportée dans l'Océan Indien en dehors de leurs observations : *Tanacetipathes spinescens* présent en Atlantique (Opresko 2001b) ; *Antipathes dichotoma* en Méditerranée (Bo et al. 2011) et en Chine (Qi et al. 2009) ; *Plumapathes pennacea* dans le Golfe du Mexique (Boland & Sammarco 2005), la Jamaïque (Warner 2005), les Caraïbes (Opresko & Sanchez 2005) et la Corée (Moon & Song 2008) ; et *Myriopathes ulex* à Hawaii (Wagner et al. 2011) et la Corée (Moon & Song 2008). Au contraire, d'autres ont déjà été rapportées au moins dans l'Indo-Pacifique voire ailleurs dans le monde : *Cupressopathes abies* (Opresko 2001b) ; *Arachnopathes ericoides* (van Pesch 1914 ; Opresko 1976, Grigg 1964) ; *Myriopathes myriophylla* (Opresko 2001b) ; *Rhipidipathes reticulata* (Gaino & Scoccia 2010) ; *Cirrhipathes anguina* (Grigg 2001 ; Gaino & Scoccia 2008) ; et *Stichopathes echinulata* (Summers 1910 ; Wagner 2015). Enfin, en dehors des travaux de Humes et Pichon, *Antipathes longibrachiata* n'est connu que par l'ouvrage de van Pesch (1914) qui observe cette espèce en Indonésie. Il est donc nécessaire de réévaluer ces observations vieilles de près d'un demi-siècle avec un œil empreint de la taxonomie moderne des antipathaires. Seul Pichon (1972) donne des informations détaillées quant à la distribution locale des antipathaires au niveau du Grand Récif barrière de Tuléar, qui se retrouvent en abondance au niveau de la pente externe et du plateau continental jusque 53 mètres de profondeur, mais qui sont presque tous absents sur la pente interne et le lagon. En revanche, il n'évoque pas la présence massive d'un champ de corail noir au niveau de la passe Nord du récif, qui sera la zone principale d'étude de cette thèse (Fig. 16, 17).

Quelle que soit la profondeur à laquelle ils se trouvent, les antipathaires croissent toujours sur des substrats durs, sur lesquels ils synthétisent une plaque basale, à l'exception notable des genres *Bathypathes* et *Schizopathes* qui fabriquent une attache en forme de crochet pour s'ancrer dans les sédiments mous (Grigg & Opresko 1977 ; Opresko

1997, 2002). Ils privilégient les zones fortement exposées aux courants ainsi qu'à faible luminosité (Grigg 1984, 1993 ; Opresko & Genin 1990 ; Tazioli et al. 2007).



◄ **Figure 16.** Carte de Madagascar montrant la localisation de Nosy Be et Tuléar, qui sont jusqu'à présent les seuls endroits où la présence d'antipathaires vivants jusque 50 mètres de profondeur est confirmée. Echelle : 500 km. Illustration personnelle.

▼ **Figure 17.** Image satellite du Grand Récif de Tuléar (GRT), récif barrière long de 18 kilomètres bordé au nord par le fleuve Fiherenana et au sud par le fleuve Onilahy. Il représente le lieu principal d'étude cette thèse, où les recherches ont majoritairement été effectuées au niveau de la passe nord. Celle-ci se situe approximativement à 6 kilomètres de navigation depuis le port de Tuléar. Adapté de Google Earth. Echelle : 10 km.

4. Marqueurs moléculaires et génétique des populations

La distribution des antipathaires est globale d'un point de vue mondial, comme mentionné précédemment. Il n'est pas rare d'observer des canyons sous-marins au-delà de 1000 mètres abritant des populations entières de coraux noirs, parfois monospécifiques, et de tels assemblages impliquent naturellement des échanges génétiques entre populations plus ou moins distantes. A l'heure actuelle, les études relatives à ces échanges génétiques entre populations sont peu nombreuses, autant sur les espèces bathyales (Thoma et al. 2009 ; Miller et al. 2010) que sur les espèces tropicales d'eaux peu profondes (Miller 1997). Les espèces abyssales formant des populations distantes de plusieurs centaines voire de plus de 1000 km peuvent partager les mêmes haplotypes de certains gènes, signifiant qu'il existe une connectivité entre ces populations.

Lapian et al. (2007) et Lapian (2009) se sont intéressés à la phylogénie moléculaire des antipathaires et se basent sur les régions variables et non codantes ITS (*Internal Transcribed Spacers*) de l'ADN ribosomal pour inférer les relations de parenté dans l'ordre des antipathaires. En outre, Brugler et al. (2013) discutent de l'histoire évolutive de l'ordre basée sur des gènes mitochondriaux et nucléaires et l'implication de ces résultats sur la taxonomie. A l'heure actuelle, il n'existe qu'un seul génome mitochondrial complet, provenant de *Chrysopathes formosa* (Brugler et France 2007). Si l'utilisation des régions ITS a montré ses limites concernant la résolution des liens évolutifs au sein des antipathaires lorsqu'ils sont utilisés seuls (Lapian et al. 2007), ces gènes se sont cependant avérés informatifs une fois utilisés en complément d'autres gènes mitochondriaux (Brugler et al. 2013). Ensemble, ils ont rendu la taxonomie plus compliquée que ce qu'elle n'était déjà en révélant la polyphylie de familles et de genres, ainsi que du caractère morphologique non branchu. Ceci implique naturellement de considérer les variabilités génétiques intra- et interspécifiques dans les futures révisions taxonomiques, jusqu'à présent basées uniquement sur des critères morphologiques (Brugler et al. 2013). Les gènes ITS sont extrêmement variables au sein des anthozoaires, notamment chez les scléractiniaires, avec une variabilité intraspécifique très importante ainsi qu'un taux d'hétérozygotie élevé (Marti-Puig et al. 2014). Néanmoins cette variabilité s'avère informative et utile afin de délimiter les espèces là où la morphologie n'est plus suffisante (Flot & Tillier 2006) ou d'établir la connectivité entre différentes populations grâce à l'établissement de réseaux d'haplotypes (Flot et al. 2010 ; Adjeroud et al. 2014). Au contraire, les gènes mitochondriaux sont de manière générale extrêmement peu variable chez les anthozoaires, avec des taux d'évolution allant de 50 à 100 fois plus lentement que chez d'autres métazoaires : ceci serait du soit à des mécanismes de réparation de l'ADN très efficace, soit à une évolution lente de l'ADNm qui serait ancestrale, soit à une évolution lente qui serait apparue une seconde fois chez les anthozoaires (France & Hoover 2002 ; Huang et al. 2008 ; Shearer & Coffroth 2008). Ceci implique que le gène COI (codant pour le cytochrome oxydase I) traditionnellement utilisé en tant que « code barre du vivant » ne convient pas dans ce cas pour discriminer les espèces, du moins lorsqu'il est utilisé seul (McFadden et al. 2011). Néanmoins, au même titre que les régions nucléaires non codantes ITS, il en existe également au sein du génome mitochondrial appelées IGRs (*Intergenic Regions*) supposées varier intraspécifiquement. Ce sont précisément ceux-là que Brugler et al. (2013) ont utilisés en combinaison avec les gènes nucléaires 18S et 28S pour retracer l'histoire évolutive des antipathaires et de leur phylogénie intrinsèque. Cette étude reste de loin la plus complète et la plus détaillée à ce jour. L'étude révèle la polyphylie du genre *Stichopathes* ainsi que des 3 familles Antipathidae, Cladopathidae et Aphanipathidae. Ces analyses moléculaires soulignent également que la classification basée sur les caractéristiques microscopiques du squelette est plus fiable que celle basée sur l'aspect général des colonies.

5. Reproduction, croissance et longévité

Les plus vieilles mentions concernant la reproduction des antipathaires remontent aux descriptions de coupes histologiques par van Pesch (1914) où le développement des gamètes était visible. A défaut de suivre des colonies marquées pendant de longues périodes ou d'avoir suffisamment de matériel tout au long de l'année, ces observations ne donnent pour seule information que le sexe du polype et/ou de la colonie si celle-ci était entièrement disponible. Aujourd'hui, il n'existe qu'une quinzaine d'espèces pour lesquelles on connait le type et la période de reproduction (Wagner et al. 2011 ; Wagner et al. 2012 ; Rakka et al. 2017 ; Laurette et al. 2018). En outre, il existe un large écart géographique et bathymétrique dans l'information disponible à l'heure actuelle : sur les 56 études liées à la reproduction des antipathaires disponibles dans la littérature scientifique, seules 12 ont été réalisées en collectant des spécimens en eaux peu profondes (moins de 50 m, tableau 4) ; aucune ne se focalisant sur des spécimens de l'Océan Indien. De ces 13 études seuls *Cirrhipathes* sp., *Stichopathes* sp. et *Antipathella fiordensis* ont été observés sur des périodes suffisamment longues pour établir la saisonnalité de leur reproduction et le sexe des polypes et/ou des colonies au cours du temps (Goenaga 1977 ; Miller 1996 ; Parker et al. 1997 ; Bo 2008). La saisonnalité de la reproduction ou l'existence d'un éventuel hermaphrodisme successif des autres espèces étudiées n'a pas été déterminée et les connaissances se limitent à des descriptions morphologiques de polypes ou gamètes.

Tableau 4. Liste des études disponibles à ce jour donnant des informations sur la reproduction des antipathaires d'eaux peu profondes (<50 m). Le sexe fait référence à la colonie entière.

Espèce	Localisation	Prof. (m)	Sexe	Références
Arachnopathes ericoides	Indonésie	5-34	Gonochorique	van Pesch 1914
Cirrhipathes anguina	Indonésie	36	Gonochorique	van Pesch 1914
Cirrhipathes cf. *anguina*	Indonésie	25-35	Gonochorique	Gaino et al. 2008, Gaino & Scoccia 2008
Cirrhipathes contorta	Indonésie	9-45	Gonochorique	van Pesch 1914
Cirrhipathes propinqua	Nouvelle-Guinée	7	Gonochorique	van Pesch 1914
Pseudocirrhipathes mapia	Indonésie	17-32	Gonochorique	Bo et al. 2009a
Cirrhipathes rumphii	Indonésie	30-113	Gonochorique	van Pesch 1914
Cirrhipathes spiralis	Indonésie	32-469	Gonochorique	van Pesch 1914
Stichopathes sp.	Porto Rico	15	Gonochorique	Goenaga 1977
Antipathella fiordensis	Nouvelle-Zélande	9-22	Gonochorique	Miller 1996, Parker et al. 1997
Cupressopathes pumila	Indonésie	30	Gonochorique	Gaino & Scoccia 2009
Myriopathes lata	Corée	10-45	Gonochorique	Moon & Song 2008b

Par extension, les espèces supposées malgaches *Arachnopathes ericoides* et *Cirrhipathes anguina* rapportées par Pichon (1972) et Humes (1969) pourraient présenter les mêmes caractéristiques reproductives que rapportées par van Pesch (1914) et Gaino et al. (2008), Gaino & Scoccia (2008) et Scoccia et Gaino (2010) en Indonésie.

De façon générale chez tous les antipathaires, les gamètes se développent exclusivement au sein des mésentères primaires transverses, atteignant parfois la base de la cavité des tentacules (Fig. 18). Il n'existe à l'heure actuelle aucune donnée mentionnant l'existence de polypes portant à la fois des gamètes mâles et femelles, par conséquent il est communément admis que les polypes sont toujours gonochoriques chez les antipathaires (Wagner et al. 2011), de même que les colonies entières sont soit mâles soit femelles sur base des données histologiques disponibles à ce jour.

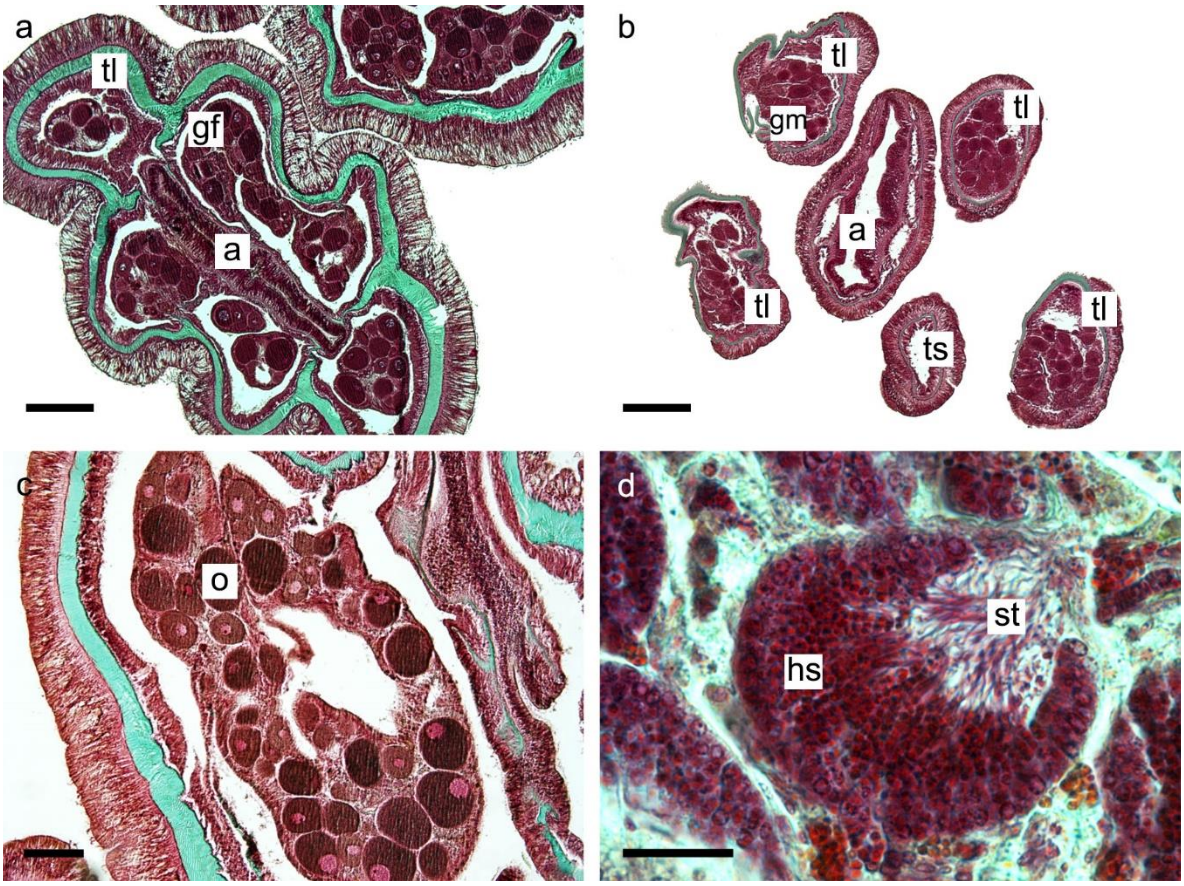


Figure 18. a – Polype femelle ayant les gamètes se développant dans les mésentères primaires transverses. b – Polype mâle où les mésentères portant les gamètes se prolongent jusque dans la cavité des tentacules latéraux. c – Vue rapprochée d'un mésentère contenant des oocytes matures. d – Vue détaillée d'un spermatocyste contenant des spermatozoïdes. Echelles : a, 200µm ; b, c 100µm ; d, 20µm. *a – actinopharynx ; gf – gamètes femelles ; gm – gamètes mâles ; hs – têtes des spermatozoïdes; o – oocytes ; st – queues des spermatozoïdes; tl – tentacules latéraux ; ts – tentacules sagittaux*. Illustrations personnelles.

Les antipathaires peuvent se reproduire annuellement avec une seule période de ponte (Grigg 1976 ; Parker et al. 1997), avec plusieurs périodes de ponte (Bo 2008) ou toute l'année durant (Goenaga 1977), les pontes étant généralement liées à l'augmentation de température (Schmidt & Zissler 1979 ; Gaino & Scoccia 2010). Dans tous les cas, la fécondation est supposée être externe étant donné que jusqu'à présent aucune larve n'a été observée en développement au sein des polypes.

L'ultrastructure des gamètes ou des groupes gamétiques qu'ils forment a été étudiée chez *Cirrhipathes* cf. *anguina* (Gaino & Scoccia 2008 ; pour rappel, *C. anguina* est supposé présent à Madagascar), *Cirrhipathes* sp. (Gaino et al. 2008), *Cupressopathes pumila* (Gaino & Scoccia 2009) et *Rhipidipathes reticulata* (Gaino & Scoccia 2010b), toutes les 3 trouvées en Indonésie entre 25 et 30 m ; *Antipathella subpinnata* (Gaino & Scoccia 2010a) en Méditerranée, et *Dendrobathypathes grandis* (Lauretta & Penchaszadeh 2017) en Atlantique.

Comme chez de nombreux cnidaires, les antipathaires peuvent également se reproduire par bourgeonnement (Pax et al. 1987), par fracture où la colonie mère regénère de nouvelles branches (Grigg 1964, Bo et al. 2009 ; Fig. 19) et par production de larves asexuées en conditions de stress (Miller & Grange 1995 ; Parker et al. 1997).

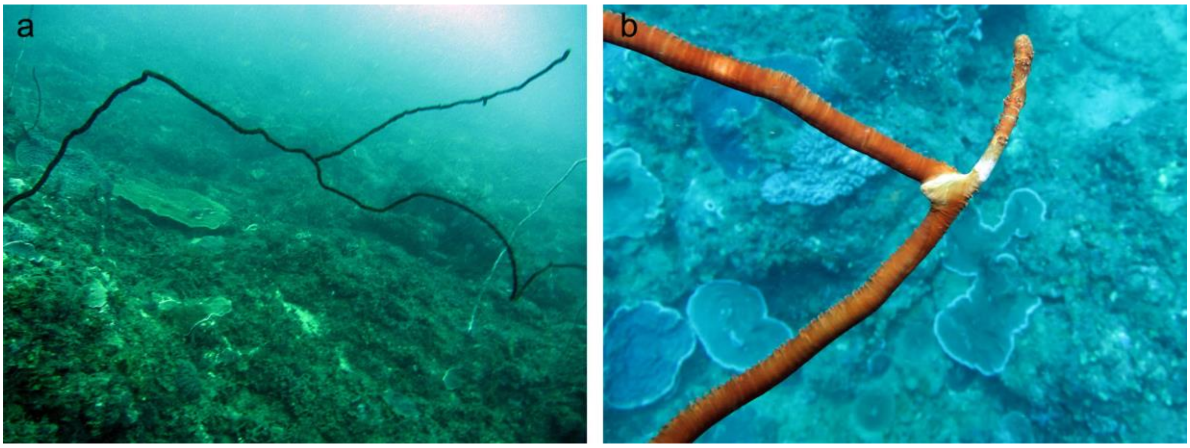


Figure 19. Vue générale et détaillée d'une fracture survenue sur une colonie normalement non branchue de *Stichopathes* sp. à Tuléar (Madagascar). Cet évènement a dans ce cas entrainé l'apparition d'une dichotomie donnant ainsi un aspect de colonie branchue. Les colonies photographiées mesurent 3 à 4 mètres de longueur. Illustrations personnelles.

Il n'est également pas rare d'observer des polypes se diviser par bourgeonnement à certains endroits de la colonie, même au niveau des parties les plus anciennement formées (Pax et al. 1987 ; Wagner et al. 2010 ; Fig. 20).

Figure 20. Photo *in situ* d'une colonie de *Cirrhipathes* sp. à Tuléar (Madagascar), montrant la présence de polypes de très petites tailles parmi de plus larges (flèches rouges). Si la division n'est pas prouvée sur cette photo, elle illustre l'apparence et la taille que pourraient avoir des polypes nouvellement formés. Illustration personnelle. Echelle : 1 cm.

Le squelette des antipathaires est composé d'antipathine, qui est un mélange de chitine et de scléroprotéines. Dans une colonie, le squelette croît en longueur (verticalement par rapport au substrat) et en épaisseur (croissance radiale) donnant naissance à des stries de croissance (Goldberg 1976, Goldberg et al. 1994 ; Fig. 21). Cette croissance radiale, au même titre que l'accroissement vertical, varie énormément au sein de l'ordre des antipathaires et dépend de la profondeur ainsi que des conditions environnementales (voir Wagner et al. 2012 pour une liste exhaustive des taux de croissance). Les espèces tropicales possèdent les taux de croissance verticale les plus rapides connus à ce jour, *Cirrhipathes* cf. *anguina* croissant jusqu'à 159 centimètres par an en Indonésie à 20 mètres de profondeur (Bo et al. 2009). Concernant la croissance radiale, de façon générale les antipathaires profonds (>200 m) possèdent les taux les plus lents, comme par exemple chez le genre *Leiopathes* retrouvé à plus de 300 mètres et dont la croissance radiale du squelette ne dépasse pas 22 µm/an (Prouty et al. 2011). Il est communément accepté que ces stries se forment de façon annuelle (Grigg 1976 ; Grange & Goldberg 1993 ; Williams et al 2006 ; Love et al 2007 ; Risk et al 2009 ; Prouty et al 2011). Dans la majorité des cas, ces mesures de croissance sont simplement effectuées *in situ* sur des colonies marquées ou sur des colonies se développant sur des structures artificielles monitorées. Dans d'autres cas, ces données sont obtenues par utilisation de radioisotopes qui permettent également d'obtenir des informations sur la longévité des colonies étudiées, le plus utilisé étant bien entendu le radiocarbone (^{14}C). L'utilisation de cette technique met en avant l'âge extrêmement ancien que certaines colonies peuvent avoir, le record étant à nouveau dans le genre *Leiopathes* avec une colonie datée de plus de 4200 ans (Roark et al. 2009). Les antipathaires abyssaux possèdent toujours une très longue longévité (de quelques centaines à plusieurs milliers d'année) par rapport aux antipathaires tropicaux d'eaux peu profondes dont l'âge le plus ancien connu est de 81 ans chez *Antipathes* sp. en Mer Rouge (Risk et al. 2009). Parmi les 13 espèces d'eaux peu profondes pour lesquelles il existe des informations quant à la croissance et/ou la longévité, il n'en existe aucune collectée dans l'Océan Indien.

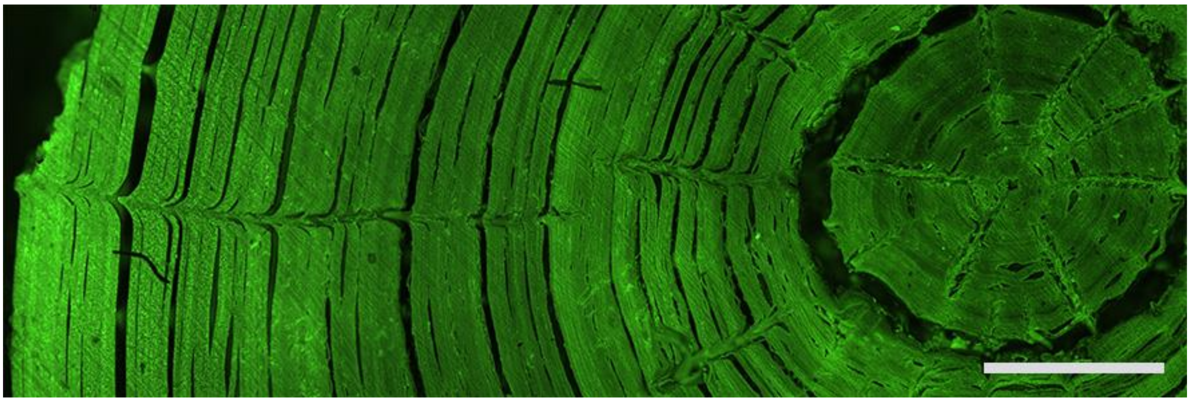


Figure 21. Aperçu d'une coupe transversale de squelette de *Cirrhipathes* sp., observé sous lumière réfléchie verte. Le squelette a subi un traitement de KOH afin de délaminer les différentes stries de croissance et de pouvoir mieux les observer. Echelle : 1 mm. Illustration personnelle.

6. Nutrition

En l'état actuel des connaissances, il n'est pas directement établi si les vitesses de croissance sont dépendantes des types de ressources disponibles pour les antipathaires. Les antipathaires sont des suspensivores capturant le plancton (phyto et/ou zooplancton) passant directement aux alentours de la colonie, ce qui va de pair avec leur habitat où les courants sont généralement puissants (Warner 1981 ; Genin et al. 1986). Le plancton est capturé grâce à la sécrétion de mucus ou grâce aux nématocytes et spyrocystes des tentacules ; et la bouche peut s'élargir en fonction du type de proie (Grigg 1964 ; Goenaga 1977 ; Lewis 1978). Les antipathaires se nourrissent soit le jour (Grigg 1964 ; Lewis 1978) soit la nuit (Goenaga 1977 ; Tazioli et al. 2007), ce qui se traduit par une contraction clairement visible des tentacules lors des périodes d'inactivité (Fig. 22).

Les observations directes ne permettent pas d'établir la proportion de phytoplancton ou de matière organique particulaire dans le régime alimentaire des coraux noirs. Les études mentionnées ci-dessus ne concernent que des espèces dont les polypes sont relativement gros et la bouche facilement discernable, et les observations in situ effectuées sur ces colonies à gros polypes deviennent plus compliquées pour des espèces dont les polypes mesurent moins de 1mm. Dès lors, les rapports isotopiques de l'azote, du soufre et du carbone s'avèrent des outils indispensables pour comprendre et inférer les réseaux trophiques liés aux antipathaires et aux organismes qui leur sont associés. En effet, les rapports isotopiques d'un consommateur sont directement liés à leur diète (DeNiro &

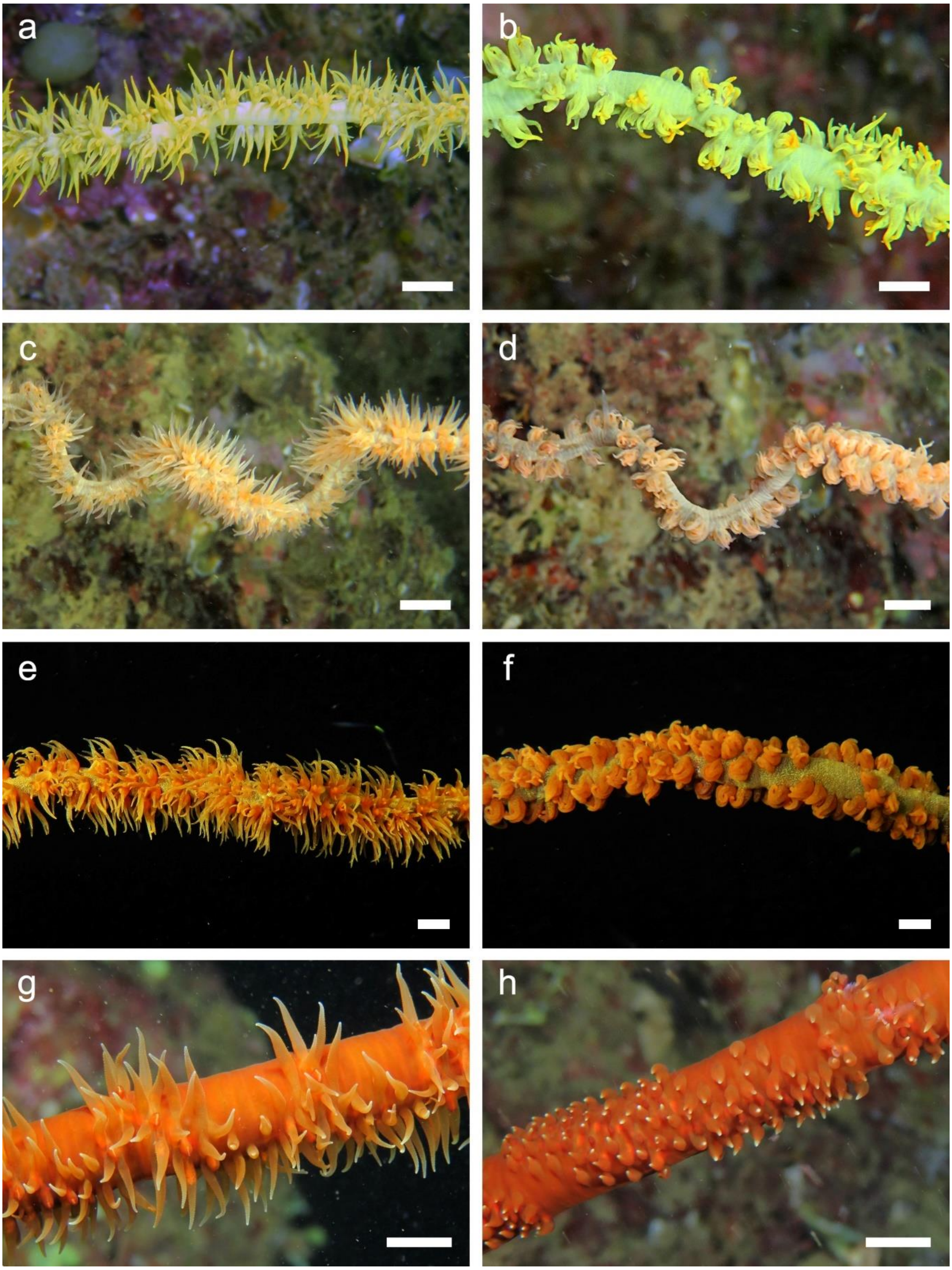


Figure 22. Comparaison des états épanouis (colonne de gauche, a, c, e, g) et des états contractés (colonne de droite, b, d, f, h) des polypes d'antipathaires. a, b – *Cirrhipathes anguina* ; c, d – *Cirrhipathes spiralis* ; e, f – *Cirrhipathes densiflora* ; g, h – *Stichopathes* sp. aff. *maldivensis*. Echelles : 1 cm. Illustrations personnelles.

Epstein 1981), et leurs tissus sont généralement enrichis en isotopes les plus lourds (McCutchan et al. 2003). De cette manière, les valeurs isotopiques du carbone renseignent sur la source de nourriture utilisée, ceux de l'azote sur la position trophique du consommateur, tandis que ceux du soufre renseignent sur l'origine de la source, provenant du lagon ou de la colonne d'eau océanique, ou encore ayant une origine terrigène, les cycles du soufre dépendant de leur origine (McCutchan et al. 2003). Dans le cas des antipathaires, ce type de données existent à la fois pour les espèces abyssales *Leiopathes glaberrima* (Williams et al. 2006, 2007 ; Carlier et al. 2009 ; Roark et al. 2009) et *Bathypathes arctica* (Sherwood et al. 2008) et pour les espèces peu profondes *Antipathes* sp. (Risk et al. 2009, Williams & Grottoli 2010) et *Rhipidipathes* sp. (Williams & Grottoli 2010).Les rapports isotopiques de l'azote ($\delta^{15}N$) de ces espèces varient de ± 4.5 à ± 11 ‰, signifiant qu'il y a une grande variabilité trophique au sein des antipathaires en considérant qu'un niveau trophique correspond à un accroissement d'environ 2.3 ‰ (McCutchan et al. 2003). En fonction du type de tissus employé pour l'analyse – c'est-à-dire le squelette ou les tissus vivants – et de la localisation, il est possible d'obtenir des informations autres que relatives à la nutrition. En effet, via l'analyse du squelette, Risk et al. (2009) ont mis en évidence que les antipathaires « capturaient » la dégradation de la qualité des eaux au cours du temps en voyant leurs rapports $\delta^{15}N$ varier en fonction du largage des eaux usées côtières sur une durée de 60 ans. A l'heure actuelle, il n'existe aucune information quant aux rapports isotopiques des antipathaires au niveau de l'Océan Indien, et ce quelle que soit la profondeur.

7. Organismes associés aux antipathaires

Quelle que soit la profondeur à laquelle ils se situent, les antipathaires forment des habitats pour de nombreux organismes (Fig. 23). Ceux-ci appartiennent aussi bien aux annélides qu'aux arthropodes, cnidaires, échinodermes, mollusques, urochordés, ou encore vertébrés (une liste exhaustive des symbioses décrites est disponible dans Wagner et al. 2012). Ces interactions peuvent être obligatoires ou transitoires, les coraux noirs fournissant protection, substrat, ou nourriture aux organismes qu'ils abritent, comme par exemple dans le cas de l'association entre *Bassozetus* sp. (Ophidiidae) et *Schizopathes* sp. entre 1300 et 2600 mètres de profondeur (Gates et al. 2017), l'utilisation des branches de *Leiopathes glaberrima* par les requins *Scyliorinus canicula* pour y pondre leurs œufs

(Cau et al. 2017), ou l'existence de plus de 2500 organismes vivants sur une colonie morte d'*Antipathes dendrochristos* (Love et al. 2007). Qu'ils soient symbiotiques ou non, les organismes associés aux coraux noirs tirent profit de la surface des branches ou de l'espace entre celles-ci, ou encore des cavités au sein des tissus ou du squelette (Buhl-Mortensen & Mortensen 2004 ; Gates et al. 2017 ; Terrana & Eeckhaut 2017). A leur mort, les colonies fournissent toujours une protection ou un substrat sur lequel s'installer pour de nombreux organismes, qui croissent alors le long du squelette de l'antipathaire (Love et al. 2007 ; Tazioli et al. 2007 ; Bo et al. 2009b). A Madagascar, seuls les travaux de Bruce (1982), Spotte et al. (1994) et Humes (1969, 1979, 1992) identifient des symbiontes d'antipathaires peu profonds, représentant un total de 4 espèces de crevettes Pontoniidae et 6 espèces de copépodes (Tableau 5). Aucune autre étude ne s'est intéressée à la faune associée à ces coraux dans cette région du globe.

Longtemps considérés comme azooxanthellés, certaines espèces possèdent bien des zooxanthelles dans leur gastroderme. Wagner et al. (2011) les identifie grâce au gène ITS-2 sur des espèces retrouvées entre 10 et 396 mètres de profondeur à Hawaii. Avec une densité de zooxanthelles rapportée entre 0 et 92 cellules par mm^3, elles sont présentes chez *Cirrhipathes* cf. *anguina*, *Antipathes griggi*, *Antipathes grandis*, *Aphanipathes* sp., *Myriopathes ulex*, *Stichopathes* cf. *echinulata*, *Stichopathes* sp., *Acanthopathes undulata*, *Bathypathes* sp. et *Myriopathes* sp. Les zooxanthelles des antipathaires proviennent des clades C et D, dont certaines séquences sont identiques à celles des zooxanthelles présentes chez les scléractiniaires *Porites* et *Montipora* (Lajeunesse et al. 2004 ; Lajeunesse 2005 ; Stat et al. 2009). Le clade D contient des zooxanthelles opportunistes et thermo-tolérantes qui ont une très large distribution géographique et qui forment des blooms après des épisodes blanchissement sévères chez les scléractiniaires.

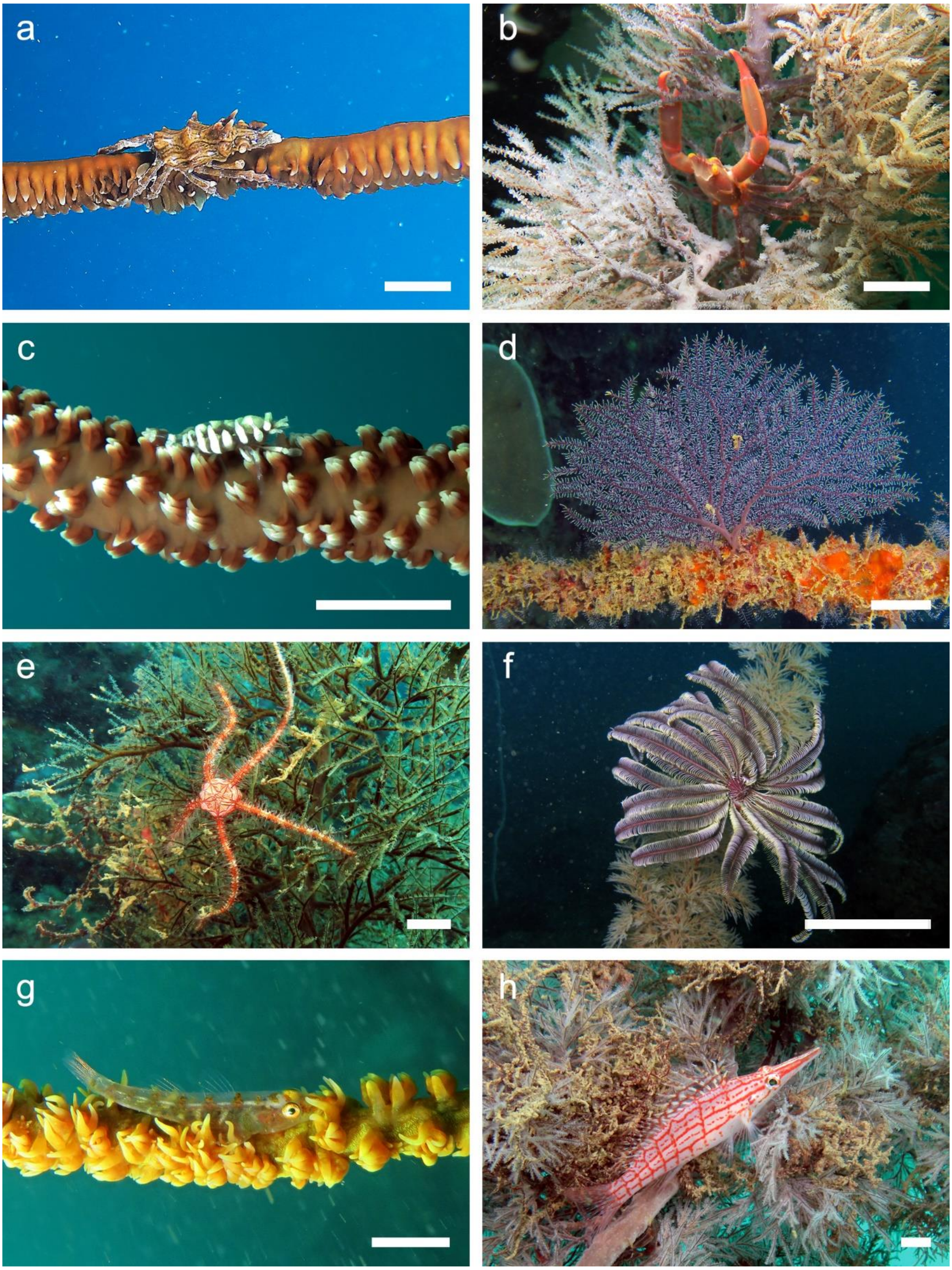


Figure 23. Faune associée aux antipathaires à Tuléar (Madagascar). Ces organismes peuvent être symbiotiques (a, b, c, e, g), ou profiter d'un substrat pour s'installer (d, f, h). Ils sont très diversifiés, ici présentés comme des crustacés décapodes (a, b, c), des échinodermes (e, f) ou encore de poissons (g, h). Le squelette mort d'antipathaire peut également servir de substrat pour la croissance d'organismes tels que des gorgones ou des hydrozoaires (d). Echelles : a,b,c,d,e,g,h :1 cm ; f :10 cm. Illustrations personnelles.

Tableau 5. Symbiotes décrits à Madagascar vivant avec les antipathaires d'eaux peu profondes.

Symbiote	Groupe	Hôte	Références
Periclimenes granulimanus	Décapodes	Antipathaires	Bruce (1982), Spotte et al. 1994
Periclimenes lepidus	Décapodes	Antipathaires	Spotte et al. (1994)
Periclimenes nilandensis	Décapodes	Antipathaires	Spotte et al. (1994)
Periclimenes sp.	Décapodes	Antipathaires	Spotte et al. (1994)
Asteropontius bandicola	Copépodes	*Cupressopathes abies*, *Tanacetipathes* cf. *spinescens*	Humes (1992)
Orecturus grandisetiger	Copépodes	*Antipathes* sp., *A. longibrachiata*, *Arachnopathes ericoides*, *Rhipidipathes reticulata*	Humes (1992)
Paramolgus constrictus	Copépodes	*Cupressopathes abies*, *Arachnopathes ericoides*	Humes (1969, 1979)
Paramolgus insectus	Copépodes	*Antipathes* sp., *Tanacetipathes* cf. *spinescens*, *Myriopathes myriophylla*, *Cupressopathes abies*	Humes (1969, 1979, 1992)
Thamnomolgus robustus	Copépodes	*Antipathes* sp., *Cupressopathes abies*, *Myriopathes myriophylla*, *Tanacetipathes* cf. *spinescens*, *Arachnopathes ericoides*	Humes (1969, 1979, 1990, 1992)
Vahinius petax	Copépodes	*Stichopathes echinulata*, *Antipathes longibrachiata*	Humes (1967, 1969, 1979, 1992)

8. Menaces et collectes illégales de corail noir

Il existe des preuves matérielles et littéraires attestant que les coraux noirs sont connus et collectés depuis l'Antiquité, comme le rapporte Brook (1889). Ce dernier mentionne un commerce existant dans le sud de l'Italie destiné à des personnes ayant un statut social important, alors que dans certaines civilisations ils sont utilisés comme amulettes, sceptres ou objets de croyance. Si la collecte destinée à la bijouterie est toujours d'actualité aux XX^{ème} et XXI^{ème} siècles, les principaux problèmes contemporains des antipathaires sont la conséquence d'activités sous-marines tels que la pêche industrielle, l'exploitation minière et pétrolière, et la construction de pipelines ou de câbles sous-marins (Freiwald et al. 2004 ; Rogers & Gianni 2010). Probert et al. (1997) rapportent que les antipathaires sont majoritaires au sein des prises accessoires résultant des chalutages et dragages situés entre 750 et 1500 m de profondeur en Nouvelle-Zélande. Face à ces problèmes environnementaux majeurs, les antipathaires ne possèdent malheureusement pas une grande marge de rétablissement, étant donné leur croissance lente et leur maturité tardive. Ces destructions massives entrainent évidemment des dommages collatéraux sur la faune associée à ces coraux, qui sont menacés à l'instar de leurs hôtes ou de l'écosystème formé par ces derniers. A une moindre échelle, mais pourtant tout aussi impactante, le commerce de corail noir trouve toujours sa clientèle, bien que l'ordre Antipatharia soit classé sous l'Appendice II de la Convention sur le commerce international des espèces de faune et de flore sauvages menacées d'extinction (CITES). Excepté à Hawaii où s'est développé une pêcherie durable (Grigg 1974, 1976), leur collecte s'effectue depuis toujours sans le moindre contrôle ni la moindre gestion. C'est le cas dans de nombreuses îles tropicales, et plus particulièrement à Madagascar où le commerce illégal se développe de plus en plus (Todinanahary et al. 2016). Depuis 2011, un trafic se déroule dans les principales villes côtières du sud, Ambovombe et Tolagnaro. La première saisie de corail noir illégallement pêché fut effectuée par le Centre de Surveillance des Pêches en 2014. Celle-ci comprenait près de 180 kg de squelettes de coraux ainsi qu'un grand nombre de matériel de plongée et de collecte. Depuis, d'autres saisies eurent lieu, portant à plusieurs centaines de kilos la quantité de coraux noirs saisis. Si le braconnage reste difficile à éradiquer, toute forme d'exploitation ou de collecte de corail noir sur le territoire malgache demeure néamoins totalement interdite par un décret ministériel publié en 2014. Pour d'avantage d'informations concernant la pêche illégale de coraux noirs à Madagascar, se réferer à Todinanahary, Terrana et al. (2016) en annexe de cette thèse.

BIBLIOGRAPHIE – INTRODUCTION GENERALE

Adjeroud M., Guérécheau A., Vidal-Dupiol J., Flot J.F., Arnaud-Haond S., Bonhomme F. (2014) Genetic diversity, clonality and connectivity in the scleractinian coral Pocillopora damicornis: a multi-scale analysis in an insular, fragmented reef system. Marine biology, 161(3): 531-541.

Araya J.F., Aliaga J.A., Araya M.E. (2016). First record of *Lillipathes ritamariae* Opresko and Breedy, 2010 (Cnidaria: Antipatharia) from the southeastern Pacific Ocean. Marine Biodiversity, 1-5.

Berntson E.A., France S.C., Mullineaux L.S. (1999) Phylogenetic relationships within the class Anthozoa (phylum Cnidaria) based on nuclear 18S rDNA sequences. Molecular Phylogenetics and Evolution, 13 : 417–433.

Blainville H.M.D. (1834) Manuel d'Actinologie ou de Zoophytologie: viii + 1-694, pis 1-102, Paris.

Bo M. (2008) Taxonomy and ecology of antipatharians, p. 212. Ph.D. Dissertation in Marine Biology and Ecology, Universita Politecnica Delle Marche, Ancona, Italy.

Bo M., Barucca M., Biscotti M.A., Canapa A., Lapian H.F.N., Olmo E., Bavestrello G. (2009a) Description of *Pseudocirrhipathes* (Cnidaria: Anthozoa: Hexacorallia: Antipathidae), a new genus of whip black coral from the Indo-Pacific. Italian Journal of Zoology, 76: 392–402.

Bo M., Di Camillo C.G., Addamo A.M., Valisano L., Bavestrello G. (2009b) Growth strategies of whip black corals (Cnidaria: Antipatharia) in the Bunaken Marine Park (Celebes Sea, Indonesia). Marine Biodiversity Records 2: 1–6.

Bo M., Bavestrello G., Canese S., Giusti M., Angiolillo M., Cerrano C., Salvati E., Greco S. (2011) Coral assemblage off the Calabrian Coast (South Italy) with new observations on living colonies of *Antipathes dichotoma*. Italian Journal of Zoology, 78(2): 231-242.

Bo M., Canese S, Bavestrello G. (2014) Discovering Mediterranean black coral forests: *Parantipathes larix* (Anthozoa: Hexacorallia) in the Tuscan Archipelago, Italy. Italian journal of zoology, 81(1): 112-125.

Bo M. & Opresko D.M. (2015) Redescription of *Stichopathes pourtalesi* Brook, 1889 (Cnidaria: Anthozoa: Antipatharia: Antipathidae). Breviora, 1-18.

Bo M., Bavestrello G., Angiolillo M., Calcagnile L., Canese S., Cannas R., Cau A., D'Elia M., D'Oriano F., Follesa M.C., Quarta G., Cau A. (2015) Persistence of Pristine Deep-Sea Coral Gardens in the Mediterranean Sea (SW Sardinia). PLoS ONE 10(3): e0119393.

Boccone P. (1671) Recherches et observations curieuses sur la nature du corail blanc & rouge, vray de Dioscoride. Ed. Claude Barbin, Paris.

Boland G.S. & Sammarco P.W. (2005) Observations of the antipatharian" black coral" *Plumapathes pennacea* (Pallas, 1766) (Cnidaria: Anthozoa), northwestern Gulf of Mexico. Gulf of Mexico Science, 23(1): 127-132.

Bridge T.C., Done T.J., Friedman A., Beaman R.J., Williams S.B., Pizarro O., Webster J.M. (2011) Variability in mesophotic coral reef communities along the Great Barrier Reef, Australia. Marine Ecology Progress Series, 428: 63-75.

Brito A. & Ocaña A. (2004) Corales de las islas Canarias.- La Laguna: FranciscoLemus Editor, 477 pp.

Brook G. (1889). Report on the Antipatharia Collected by HMS Challenger During the Years 1873-76. Johnson Reprint Corporation.

Brugler M.R. & France S.C. (2007) The complete mitochondrial genome of the black coral *Chrysopathes formosa* (Cnidaria: Anthozoa: Antipatharia) supports classification of antipatharians within the subclass Hexacorallia. Molecular Phylogenetics and Evolution, 42: 776–788.

Brugler M.R., Opresko D.M., France S.C. (2013) The evolutionary history of the order Antipatharia (Cnidaria: Anthozoa: Hexacorallia) as inferred from mitochondrial and nuclear DNA: implications for black coral taxonomy and systematics. Zoological Journal of the Linnean Society, 169(2), 312-361.

Buhl-Mortensen L. & Mortensen P.B. (2004) Symbiosis in deep-water corals. Symbiosis, 37: 33–61.

Carlgren O. (1895) Ueber die G. *Gerardia* Lacaze-Duthiers. Öfvers. Kongl. Vetensk. Akad. Förh. No.5, 319-334, figs 1-7.

Carlier A., Le Guilloux E., Olu K., Sarrazin J., Mastrototaro F., Taviani M., Clavier J. (2009) Trophic relationships in a deep Mediterraean cold-water coral bank (Santa Maria di Leuca, Ionian Sea). Marine Ecology Progress Series, 397: 125–137.

Cooper C.F. (1903) Antipatharia. In "The Fauna and Geography of the Maldive and Laccadive Archipelagoes", (J. S. Gardiner, ed.). Vol. 2, pp. 791–796. University Press, Cambridge.

Cooper C.F. (1909) Reports of the Percy Sladen Trust expedition on the Indian Ocean, 1905. Antipatharia. Transactions of the Linnean Society of London, 12 (4), 301–321.

Cordeiro R.T., Neves B.M., Rosa-Filho J.S., Pérez C.D. (2015) Mesophotic coral ecosystems occur offshore and north of the Amazon River. Bulletin of Marine Science, 91(4): 491-510.

Dana J.D. (1846) United States Exploring Expedition: During the Years 1838, 1839, 1840, 1842 Under the Command of Charles Wilkes, USN. Ethnography and philology (Vol. 6). Lea and Blanchard.

De Matos V., Braga-Henriques A., Santos R.S., Ribeiro P.A. (2014) New species of *Heteropathes* (Anthozoa: Antipatharia) expands genus distribution to the NE Atlantic. Zootaxa, 3827(2): 293-300.

DeNiro M.J. & Epstein S. (1981) Influence of diet on the distribution of nitrogen isotopes in animals. Geochimica et cosmochimica acta, 45(3): 341-351.

Duchassaing P. (1870) Revue des Zoophytes et des Spongiaires des Antilles. Masson, Paris, 55 pp.

Echeverria C A. (2002) Black corals (Cnidaria: Anthozoa: Antipatharia): First records and a new species from the Brazilian coast. Revista de Biologı´a Tropical 50: 1067–1077.

Ellis J. & Solander D.C. (1786) The Natural History of Many Curious and Uncommon Zoophytes: Collected... by the Late John Ellis... Systematically Arranged and Described by the Late Daniel Solander. London, B. White and Son.

Ehrenberg C.G. (1834) Die Corallenthiere des rothen Meeres: 1-156, Berlin

Esper E.J.C. (1788). Die Pflanzenthiere in Abbildungen nach der Natur mit Farben erleuchtet nebst Beschreibungen. [Theilen 1-3 (Lieferungen 1-17)]. Raspe, Nürnberg.

Flot J.F. & Tillier S. (2006) Molecular phylogeny and systematics of the scleractinian coral genus *Pocillopora* in Hawaii. In: Proceedings of the 10th International Coral Reef Symposium, Vol. 1, pp. 24-29.

Flot J.F., Couloux A., Tillier S. (2010) Haplowebs as a graphical tool for delimiting species: a revival of Doyle's" field for recombination" approach and its application to the coral genus *Pocillopora* in Clipperton. BMC Evolutionary Biology, 10(1): 372.

France S.C., Rosel P.E. Agenbroad J.E., Mullineaux L.S., Kocher T.D. (1996) DNA sequence variation on mitochondrial large-subunit rRNA provides support for a two-subclass organization of the Anthozoa (Cnidaria). Molecular Marine Biology and Biotechnology, 5: 15–28.

France S.C. & Hoover L.L. (2002) DNA sequences of the mitochondrial COI gene have low levels of divergence among deep-sea octocorals (Cnidaria: Anthozoa). Hydrobiologia, 471(1-3): 149-155.

Freiwald A., Fossa J.H., Grehan A., Koslow T., Roberts J.M. (2004) Cold water coral reefs: out of sight-no longer out of mind. UNEP-WCMC Biodiversity Series 22.

Gaino E., Bo M., Boyer M., Scoccia F. (2008) Sperm morphology in the black coral *Cirrhipathes* sp. (Anthozoa, Antipatharia). Invertebrate Biology, 127: 249–258.

Gaino E. & Scoccia F. (2008) Female gametes of the black coral *Cirrhipathes cfr. Anguina* (Anthozoa, Antipatharia) from the Indonesia Marine Park of Bunaken. Invertebrate Reproduction and Development, 51: 119–126.

Gaino E. & Scoccia F. (2009) Release of sperm clusters in spheres by the black coral *Cupressopathes pumila* (Anthozoa, Antipatharia). Coral Reefs, 28: 851–857.

Gaino E. & Scoccia F. (2010a) Gamete spawning in *Antipathella subpinnata* (Anthozoa, Antipatharia): A structural and ultrastructural investigation. Zoomorphology, 129: 213–219.

Gaino E. & Scoccia F. (2010b) Sperm ultrastructure of a member of the black coral family Aphanipathidae: *Rhipidipathes reticulata* (Anthozoa, Antipatharia). Tissue and Cell, 42(6): 391-394.

Gates A.R., Morris K.J., Jones D.O., Sulak K.J. (2017) An association between a cusk eel (*Bassozetus* sp.) and a black coral (*Schizopathes* sp.) in the deep western Indian Ocean. Marine Biodiversity, 47(3): 971-977.

Genin A., Dayton P.K., Lonsdale P.F., Spiess F.N. (1986) Corals on seamount peaks provide evidence of current acceleration over deep-sea topography. Nature, 322(6074): 59.

Goenaga C. (1977) Two new species of *Stichopathes* (Zoantharia; Antipatharia) with observations on aspects of their biology. Thesis in Marine Sciences., p. 101. University of Puerto Rico, Puerto Rico.

Goldberg W M. (1976) Comparative study of the chemistry and structure of gorgonian and antipatharian coral skeletons. Marine Biology, 35: 253–267.

Goldberg W.M., Hopkins T.L., Holl S.M., Schaefer J., Kramer K.J., Morgan T D., Kim K. (1994). Chemical composition of the sclerotized black coral skeleton (Coelenterata: Antipatharia): A comparison of two species. Comparative Biochemistry and Physiology 107B, 633–643.

Grange K.R. (1985) Distribution, standing crop, population structure, and growth rates of black coral in the southern fiords of New Zealand. New Zealand journal of marine and freshwater research, 19(4): 467-475.

Grange K.R. & Goldberg W.M. (1993) Chronology of black coral growth bands: 300 years of environmental history. In "Proceedings of the Second International Temperate Reef Symposium." (C. N. Battershill et al. eds), pp. 169–174. NIWA Marine, Wellington.

Grasshoff M. (1981). Die Gorgonaria, Pennatularia und Antipatharia des Tiefwassers der Biskaya (Cnidaria, Anthozoa). Ergebnisse der franzo¨sichen Expedition Biogas, Polygas, Geomanche, Incal, Noratlante und Fahrten der "Thalassa". I. Allgemeiner Teil. Bulletin du Muse´um national d'histoire naturelle, Section A: Zoologie, Biologie, et Ecologie Animales 3 : 731–766.

Gray J.E. (1868) Descriptions of new genera and species of Alcyonoid corals in the British museum. The Annals and Magazine of Natural History (Series 4) 2: 441–445.

Grigg R.W. (1964) A contribution to the biology and ecology of the black coral, *Antipathes grandis* in Hawai'i. MS Thesis in Zoology, p. 74. Hawai'i., Honolulu.

Grigg R.W. (1965) Ecological studies of black coral in Hawaii. Pacific Science 19: 244–260.

Grigg R.W. (1974) Distribution and abundance of precious corals in Hawaii. In A.M. Cameron et al. (editors), Proceedings of the 2nd International Coral Reef Symposium, 2, Great Barrier Reef Community, Australia, p. 235-240.

Grigg R.W. (1976) Fishery Management of Precious and Stony Corals in Hawaii. University of Hawaii, Honolulu. UNIHI-SEAGRANT-TR-77-03 p. 48.

Grigg R.W. (1984) Resource management of precious corals. A review and application to shallow water reef building corals. Marine Ecology, 5: 57–74.

Grigg R.W. (1989) Precious coral fisheries of the Pacific and Mediterranean. In: Marine Invertebrate Fisheries: Their Assessment and Management. J. F. Caddy (ed.), pp 637–645. John Wiley and Sons, New York.

Grigg R.W. (1993) Precious coral fisheries of Hawaii and the US Pacific Islands. Marine Fisheries Review, 55(2): 50-60.

Grigg R.W. (2001) Black coral: History of a sustainable fishery in Hawai'i. Pacific Science, 55(3): 291-299.

Grigg R.W. (2002) Precious corals in Hawaii: discovery of a new bed and revised management measures for existing beds. Marine Fisheries Review, 64(1): 13-20.

Grigg R.W. (2003) Invasion of a deep black coral bed by an alien species, Carijoa riisei, off Maui, Hawaii. Coral Reefs, 22(2): 121-122.

Grigg R.W. (2004) Harvesting impacts and invasion by an alien species decrease estimates of black coral yield off Maui, Hawai'i. Pacific Science, 58(1): 1-6.

Grigg R.W. (2010) The Precious Corals Fishery Management Plan of the Western Pacific Regional Fishery Management Council. Western Pacific Regional Fishery Management Council, Honolulu.

Grigg R.W. & Opresko D.M. (1977) Order Antipatharia, black corals. In “Reef and Shore Fauna of Hawaii. Section 1: Protozoa through Ctenophora”. (D. M. Devaney and L. G. Eldredge, eds), pp. 242–261. Bishop MuseumPress, Honolulu.

Haime J. (1849) Sur le polypieroide d’un *Leiopathes glaberrima*. Annales des Sciences Naturelles, 12, 224–226.

Heifetz J. (2002) Coral in Alaska: Distribution, abundance, and species associations. Hydrobiologia 471, 19–28.

Heifetz J., Wing B.L., Stone R.P., Malecha P.W., Courtney D.L. (2005) Corals of the Aleutian Islands. Fisheries Oceanography 14, 131–138.

Hourigan T.F., Cairns S.D., Reed J., Ross, S.W. (2017) Deep-Sea Coral Taxa in the US Southeast Region: Depth and Geographical Distribution. Online resource: https://deepseacoraldata.noaa.gov/.

Huang D., Meier R., Todd P.A., Ming Chou L. (2008) Slow mitochondrial COI sequence evolution at the base of the Metazoan tree and its implications for DNA barcoding. Journal of Molecular Evolution, 66: 167–174.

Humes A.G. (1967) *Wahinius petax* n. gen., n. sp., a cyclopoid copepod parasitic in an antipatharian coelenterate in Madagascar. Crustaceana, 12: 233–242.

Humes A.G. (1969) Cyclopod copepods associated with antipatharian coelenterates in Madagascar. Zoologische Mededelingen, 44: 1–30.

Imperato F. (1599) Dell’Historia Naturale. In: Napoli: Nella stamparia à Porta Reale per Constantino Vitale, MDIC, 1599.

Ingrassia M., Macelloni L., Bosman A., Chiocci F.L., Cerrano C., Martorelli E. (2016) Black coral (Anthozoa, Antipatharia) forest near the western Pontine Islands (Tyrrhenian Sea). Marine Biodiversity, 46(1), 285-290.

Johnson J.Y. (1899) Notes on the antipatharian corals of Madeira, with descriptions of a new species and a new variety, and remarks on a specimen from the West Indies in the British Museum. Proceedings of the Zoological Society of London 53: 813–824.

Jørgensen O.A., Tendal O.S., Arboe N.H. (2013) Preliminary mapping of the distribution of corals observed off West Greenland as inferred from bottom trawl surveys 2010-2012. NAFO SCR Doc, 13(007): 1-10.

Jussieu B. (1747) Examen de quelques productions marines qui ont été mises au nombre des plantes, et qui sont l'ouvrage d'une sorte d'insectes de mer. Mémoires de l'Académie des sciences, p.290.

Lacaze-Duthiers H.D. (1864) Histoire naturelle du corail. JB Bailiere et fils, Paris, 371.

Lajeunesse TC (2005) 'Species' radiations of symbiotic dinoflagellates in the Atlantic and Indo-Pacific since the Miocene-Pliocene transition. Molecular Biology and Evolution 22, 570–581.

Lajeunesse TC, Thornhill DJ, Cox EF, Stanton FG, Fitt WK, Schmidt GW (2004) High diversity and host specificity observed among symbiotic dinoflagellates in reef coral communities from Hawai'i. Coral Reefs 23, 596–603.

Lapian H.F.N. (2009) Biodiversity study of black coral (order: Antipatharia) collected from Manado, Indonesia based on rDNA internal transcribed spacer (ITS) sequences analysis. Biodiversitas, 10(1): 1-5.

Lapian H.F.N., Barucca M., Bavestrello G., Biscotti M.A., Bo M., Canapa A., Tazioli S., Olmo E. (2007) A systematic study of some Black Coral species (Antipatharia, Hexacorallia) based on rDNA internal transcribed spacers sequences. Marine Biology 151: 785–792.

Lauretta D, & Penchaszadeh P.E. (2017) Gigantic oocytes in the deep sea black coral *Dendrobathypathes grandis* (Antipatharia) from the Mar del Plata submarine canyon area (southwestern Atlantic). Deep Sea Research Part I: Oceanographic Research Papers, 128: 109-114.

Lauretta D., Wagner D., Penchaszadeh P.E. (2018). First record of a trophonema in black corals (Cnidaria: Antipatharia). Coral Reefs, 1-4.

Lewis J.B. (1978) Feeding mechanisms in black corals (Antipatharia). Journal of Zoology, 186(3): 393-396.

Linné C.V. (1758) Systema naturae, 10th Edition. Ex Typographia Juntiniana. Lucae.

Lobel M. (1591) Icones stirpium, seu plantarum tam exoticarum, quam indigenarum, in gratiam rei herbariae studiosorum in dus partes digestae. Cum septem linguarum indicibus, ad diversarum nationum usum. Anvers, Ex officina Plantiana, Eds. Kruydtboeck, Plantin.

Loiola L.L. & Castro C.B. (2005) *Tanacetipathes* Opresko, 2001 (Cnidaria: Antipatharia: Myriopathidae) from Brazil, including two new species. Zootaxa 1081, 1–31.

Love M.S., Yoklavich M.M., Black B.A., Andrews A.H. (2007) Age of black coral (*Antipathes dendrochristos*) colonies, with notes on associated invertebrate species. Bulletin of Marine Science, 80: 391–400.

MacIsaac K.G., Best M., Brugler M.R., Kenchington E.L.R., Anstey L.J., Jordan T. (2013) *Telopathes magna* gen. nov. spec. nov.(Cnidaria: Anthozoa: Antipatharia: Schizopathidae) from deep waters off Atlantic Canada and the first molecular phylogeny of the deep-sea family Schizopathidae. Zootaxa, 3700(2): 237-258.

Marsigli L.F. (1725) Histoire physique de la Mer.

Marti-Puig P., Forsman Z.H., Haverkort-Yeh R.D., Knapp I.S., Maragos J.E., Toonen R.J. (2014) Extreme phenotypic polymorphism in the coral genus *Pocillopora*; micro-morphology corresponds to mitochondrial groups, while colony morphology does not. Bulletin of Marine Science, 90(1): 211-231.

Massi D., Vitale S., Titone A., Milisenda G., Gristina M., Fiorentino F. (2018). Spatial distribution of the black coral *Leiopathes glaberrima* (Esper, 1788)(Antipatharia: Leiopathidae) in the Mediterranean: a prerequisite for protection of Vulnerable Marine Ecosystems (VMEs). The European Zoological Journal, 85(1) : 170-179.

McCutchan J.H., Lewis W.M., Kendall C., McGrath C.C. (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos, 102(2): 378-390.

McFadden C.S., Benayahu Y., Pante E., Thoma J.N., Nevarez P.A., France S.C. (2011) Limitations of mitochondrial gene barcoding in Octocorallia. Molecular Ecology Resources, 11: 19–31.

Milne-Edwards H. (1857) Histoire naturelle des Coralliaires ou Polypes proprement dits, Tome Premier. Librairie Encyclopédique de Roret, Paris, 372.

Miller K. (1996) Piecing together the reproductive habits of New Zealand's endemic black corals. Water & Atmosphere 4, 18–19.

Miller K.J. (1997) Genetic structure of black coral populations in New Zealand's fiords. Marine Ecology Progress Series, 161: 123–132.

Miller K. & Grange K.R. (1995) Population genetic studies of antipatharian black corals from Doubtful and Nancy Sounds, Fiordland, New Zealand. In “Proceedings of the 6th International Conference on Coelenterate Biology.” pp. 353–363.

Miller K., Williams A., Rowden A.A., Knowles C., Dunshea G. (2010) Conflicting estimates of connectivity among deep-sea coral populations. Marine Ecology, 31(s1): 144-157.

Molodtsova T.N. (2006) Black corals (Antipatharia: Anthozoa: Cnidaria) of the north-eastern Atlantic. Biogeography of the Atlantic Seamounts, edited by: Mironov, AN, Gebruk, AV, and Southward, AJ, KMK Scientific Press, Moscow, 141-151.

Molodtsova,T. (2007) Black corals (Antipatharia) of New Caledonia. In: Payri, C.E. & Richer de Forges B. (Eds.), Compendium of marine species of New Caledonia, Documents Scientifiques et Technique 117, IRD Nouméa, 119–125.

Molodtsova T.N. (2017) New records of *Heteropathes* Opresko, 2011 (Anthozoa: Antipatharia) from the Mid-Atlantic Ridge. Marine Biodiversity, 47(1): 179-186.

Molodtsova T. & Budaeva N. (2007) Modifications of corallum morphology in black corals as an effect of associated fauna. Bulletin of Marine Science 81: 469–479.

Molodtsova T.N. & Opresko D.M. (2017) Black corals (Anthozoa: Antipatharia) of the Clarion-Clipperton Fracture Zone. Marine Biodiversity, 47(2), 349-365.

Molodtsova T. & Opresko D.M. (2018) World List of Antipatharia. Antipatharia. Accessed through: World Register of Marine Species at: http://marinespecies.org/aphia.php?p=taxdetails&id=22549 on 2018-05-04

Moon H.W. & Song J.I. (2005) Three new records of Antipatharia (Anthozoa: Ceriantipatharia) from Korea. Korean Journal of Systematic Zoology 21: 259–272.

Moon H.W. & Song J.I. (2008a) Taxonomy of the black coral family Antipathidae (Anthozoa: Antipatharia) from Korea. Korean Journal of Systematic Zoology 24: 209–214.

Moon H.W. & Song J.I. (2008b) Taxonomy of the black coral family Myriopathidae (Anthozoa: Antipatharia) from Korea. Korean Journal of Systematic Zoology 24: 251–263.

Opresko D.M. (1972) Redescriptions and reevaluations of the antipatharians described by L.F. de Pourtalès. Bulletin of Marine Science 22: 950–1017.

Opresko D.M. (1974) A study of the classification of the Antipatharia (Coelenterata: Anthozoa) with redescriptions of eleven species. Dissertation, University of Miami, Coral Gables, Florida, USA.

Opresko D.M. (1976) Redescription of *Antipathes panamensis* Verrill (Coelenterata, Antipatharia). Pacific Science, 30: 235–240.

Opresko D.M. (1993) A new species of *Sibopathes* (Cnidaria: Anthozoa: Antipatharia: Antipathidae) from the Gulf of Mexico. Proceedings of the Biological Society of Washington, 106: 195–203.

Opresko D.M. (1996) New species of black corals (Cnidaria: Anthozoa: Antipatharia) from the Caribbean. Bulletin of Marine Science 58: 289–300.

Opresko D.M. (1997) Review of the genus *Schizopathes* (Cnidaria: Antipatharia: Schizopathidae) with a description of a new species from the Indian Ocean. Proceedings of the Biological Society of Washington, 110: 157–166.

Opresko D.M. (1998) Three new species of *Leiopathes* (Cnidaria: Anthozoa: Antipatharia) from southern Australia. Records of the South Australian Museum 31: 99–111.

Opresko D.M. (1999) New species of *Antipathes* and *Parantipathes* (Cnidaria: Anthozoa: Antipatharia) from coastal waters of south Australia and Tasmania. Records of the South Australian Museum 32: 143–154.

Opresko D.M. (2001a) New species of antipatharians (Cnidaria: Anthozoa) from Madeira, with the establishment of a new genus. Proceedings of the Biological Society of Washington 114: 349–358.

Opresko D.M. (2001b) Revision of the Antipatharia (Cnidaria: Anthozoa). Part I. Establishment of a new family, Myriopathidae. Zoologische Mededelingen, Leiden 75: 343–370.

Opresko D.M. (2002) Revision of the Antipatharia (Cnidaria: Anthozoa). Part II. Schizopathidae. Zoologische Mededelingen, Leiden 76: 411–442.

Opresko D.M. (2003a). Redescription of *Antipathes dichotoma* Pallas, 1766 (Cnidaria: Anthozoa: Antipatharia). Zoologische Mededelingen, Leiden 77: 481–493.

Opresko D.M. (2003b). Revision of the Antipatharia (Cnidaria: Anthozoa). Part III. Cladopathidae. Zoologische Mededelingen, Leiden 77: 495–536.

Opresko D.M. (2004) Revision of the Antipatharia (Cnidaria: Anthozoa). Part IV. Establishment of a new family, Aphanipathidae. Zoologische Mededelingen, Leiden 78: 209–240.

Opresko D.M. (2005a) A new species of antipatharian coral (Cnidaria: Anthozoa: Antipatharia) from the southern California Bight. Zootaxa 852: 1–10.

Opresko D.M. (2005b). New genera and species of antipatharian corals (Cnidaria: Anthozoa) from the North Pacific. Zoologische Mededelingen, Leiden 79-2: 129–165.

Opresko D.M. (2006) Revision of the Antipatharia (Cnidaria: Anthozoa). Part V. Establishment of a new family, Stylopathidae. Zoologische Mededelingen, Leiden 80-4: 109–138.

Opresko D.M. (2009) A new name for the Hawaiian antipatharian coral formerly known as *Antipathes dichotoma* (Cnidaria: Anthozoa: Antipatharia). Pacific Science 63: 277–291.

Opresko D.M. (2011) Nomenclatural note: *Heteropathes* (Cnidaria: Anthozoa: Antipatharia: Cladopathidae): a new name for *Heliopathes* Opresko, 2003. Zootaxa 2782: 67–68.

Opresko D.M. & Genin A. (1990) A new species of antipatharian (Cnidaria: Anthozoa) from seamounts in the eastern north Pacific. Bulletin of Marine Science 46: 301–310.

Opresko D.M. & Bayer F.M. (1991) Rediscovery of the enigmatic coelenterate *Dendrobrachia*, (Octocorallia: Gorgonacea) with descriptions of two new species. Transactions of the Royal Society of South Australia 115: 1–19.

Opresko D.M. & Cairns S.D. (1992) New species of black coral (Cnidaria: Antipatharia) from the northern Gulf of Mexico. Northeast Gulf Science 12: 93–97.

Opresko D.M. & Cairns S.D. (1994) Description of the new genus *Allopathes* (Cnidaria: Antipatharia) and its type species *Cirripathes desbonni*. Proceedings of the Biological Society of Washington 107: 185–192.

Opresko D.M. & Baron-Szabo R.C. (2001a). Re-descriptions of the antipatharian corals described by E.J.C. Esper, with selected English translations of the original German text. Senckenbergiana Biologica 81: 1–21.

Opresko D.M. & Baron-Szabo R.C. (2001b). Reevaluation of *Tropidopathes saliciformis* Silberfeld: a hydroid originally identified as an antipatharian coral. Proceedings of the Biological Society of Washington 114: 788–793.

Opresko D.M. & Sanchez J.A. (2005) Caribbean shallow–water black corals (Cnidaria: Anthozoa: Antipatharia). Caribbean Journal of Science, 41 (3): 492–507.

Opresko D.M. & de Laia Loiola L. (2008) Two new species of *Chrysopathes* (Cnidaria: Anthozoa: Antipatharia) from the Western Atlantic. Zootaxa 1707: 49–59.

Opresko D.M. & Breedy O. (2010) A new species of antipatharian coral (Cnidaria: Anthozoa: Antipatharia: Schizopathidae) from the Pacific coast of Costa Rica. Proceedings of the Biological Society of Washington 123: 234–241.

Opresko D.M., Wagner D., Montgomery A.D. Brugler M.R. (2012) Discovery of *Aphanipathes verticillata* (Cnidaria: Anthozoa: Antipatharia) in the Hawaiian Islands. Zootaxa 3348: 24–39.

Opresko D.M., Tracey D., Mackay E. (2014) Antipatharia (black corals) for the New Zealand region. A field guide of commonly sampled New Zealand black corals including illustrations highlighting technical terms and black coral morphology. New Zealand.

Probert P.K., McKnight D.G., Grove S.L. (1997) Benthic invertebrate bycatch from a deep-water trawl fishery, Chatham Rise, New Zealand. Aquatic Conservation: marine and freshwater ecosystems, 7(1), 27-40. Aquatic Environment and Biodiversity Report No. 131. 20 p.

Pallas P.S. (1766) Elenchus Zoophytorum Sistens Generum Adumbrationes GeneraKores et Specierum Cognitarum Succinctas Descriptiones cum Selectis Auctorum Sjmonymis: i-xvi, l 28, l 451. Hagae-Comitum.

Parker N.R., Mladenov P.V., Grange K.R. (1997) Reproductive biology of the antipatharian black coral *Antipathes fiordensis* in Doubtful Sound, Fiordland, New Zealand. Marine Biology, 130: 11–22.

Parrish F.A. & Baco A.R. (2007) State of Deep Coral Ecosystems in the U.S. Pacific Islands Region: Hawaii and the U.S. Pacific Territories. pp. 155–194. In “The State of Deep Coral Ecosystems in theUnited States” (S. E.Lumsden,T. F.Hourigan,A.W.Bruckner and G. Dorr, eds), p. 365. NOAA Technical Memorandum CRCP-3, Silver Spring, MD.

Paulay G., Puglisi M.P., Starmer J.A. (2003) The non-scleractinian Anthozoa (Cnidaria) of the Mariana Islands. Micronesica 35–36: 138–155.

Pax F. (1932) Beitrag zur Kenntnis der japanischen Do¨rnchenkorallen. Zoologische Jahrbu¨- cher. Abteilung fu¨r Systematik, O ¨ kologie und Geographie der Thiere 63: 407–450.

Pax F., Van-Praët M., Doumenc D. (1987) Ordre des antipathaires. In “Traite´ de zoologie—anatomie, systématique, biologie. Vol. 3 (Fasc. 3), Cnidaires anthozoaires,” Masson, Paris.

Peyssonnel J.A. (1751). Traité du Corail. Philosophical Transactions of the Royal Society, 1752, 445-469.

Pichon M. (1972) The coral reefs of Madagascar. In: Battistini, R. & Richard-Vindard, G. (Eds.), Biogeography and Ecology in Madagascar. Springer Netherlands, pp. 367-410.

de Pourtalès L.F. (1868) Contributions to the fauna of the Gulf Stream at great depths. Bulletin of the Museum of Comparative Zoology of Harvard 1: 121–142.

de Pourtalès L.F. (1871) Deep-sea corals. III. Catalogue of the Museum of Comparative Zoology of Harvard 4: 1–93.

de Pourtalès L.F. (1874) Crinoids and corals. Zoological results of the 'Hassler' expedition. III. Catalogue of the Museum of Comparative Zoology of Harvard 8: 25–50.

de Pourtalès L.F. (1878) Report on the corals of the 'Blake' expedition. Bulletin of the Museum of Comparative Zoology of Harvard 5: 197–212.

de Pourtalès L.F. (1880) Zoological results of the 'Blake' expedition to the Caribbean Sea. Bulletin of the Museum of Comparative Zoology of Harvard 6: 113–118.

Prouty N.G., Roark E.B., Buster N.A., Ross S.W. (2011) Growth rate and age distribution of deep-sea black corals in the Gulf of Mexico. Marine Ecology Progress Series, 423: 101–115.

Qi S.H., Su G.C., Wang Y.F., Liu Q.Y., Gao C.H. (2009) Alkaloids from the South China Sea black coral *Antipathes dichotoma*. Chemical and Pharmaceutical Bulletin, 57(1): 87-88.

Rakka M., Orejas C., Sampaio I., Monteiro J., Parra H., Carreiro-Silva M. (2017) Reproductive biology of the black coral *Antipathella wollastoni* (Cnidaria: Antipatharia) in the Azores (NE Atlantic). Deep Sea Research Part II: Topical Studies in Oceanography, 145: 131-141.

Ray J. (1704) Historia Plantarum. Vol.3, Mariae Clark: prostant apud Henricum Faithorne regiae Societatis typographum, ad Insigne Rosae in Coemeterio D. Pauli, 1704.

Réaumur R.A.F.D. (1727) Observations sur la formation du Corail, & des autres productions appellées plantes pierreuses. Histoire et Mémoires de l'Académie Royale des Sciences, pour l'Année,1727, 269-81.

Risk M.J., Sherwood O.A., Nairn R., Gibbons C. (2009) Tracking the record of sewage discharge off Jeddah, Saudi Arabia, since 1950, using stable isotope records from antipatharians. Marine Ecology Progress Series, 397: 219–226.

Roark E.B., Guilderson T.P., Dunbar R.B., Fallon S.J., Mucciarone D.A. (2009) Extreme longevity in proteinaceous deep-sea corals. Proceedings of the National Academy of Sciences of the United States of America, 106: 5204–5208.

Rogers A.D. & Gianni M. (2010) The Implementation of UNGA Resolutions 61/105 and 64/72 in the Management of Deep-Sea Fisheries on the High Seas. Report Prepared for the Deep-Sea Conservation Coalition. International Programme on the State of the Ocean, London, UK.

Roule L. (1905) Description des antipathaires et cérianthaires recueillis par SAS le Prince de Monaco dans l'Atlantique Nord: 1886-1902. Imprimerie de Monaco.

Rumphius G.E. (1750) Herbarium Amboinense. Joannis Burmanni, Meinardum Uytwerf, Amstelaedami (Amsterdam), The Netherlands.

Schultze L.S. (1896) Beitrag zur Systematik der Antipatharien. Abh. Senckenberg. naturforsch. Ges. 23: 1-40.

Schmidt H. & Zissler D. (1979) Die Spermien der Anthozoen und ihre phylogenetische Bedeutung. Zoologica, 44: 1–97.

Shearer T.L. & Coffroth M.A. (2008) DNA BARCODING: Barcoding corals: limited by interspecific divergence, not intraspecific variation. Molecular Ecology Resources, 8(2): 247-255.

Sherwood O.A., Jamieson R.E., Edinger E.N., Wareham V.E. (2008) Stable C and N isotopic composition of cold-water corals from the Newfoundland and Labrador continental slope: Examination of trophic, depth and spatial effects. Deep-Sea Research I, 55: 1392–1402.

Silberfeld E. (1909) Diagnosen neuer japanischer Antipatharien aus der Sammlung von Herrn Prof. Doflein (Mu¨nchen). Zoologischer Anzeiger 34: 760–763.

Silva M. & MacDonald I.R. (2017) Habitat suitability modeling for mesophotic coral in the northeastern Gulf of Mexico. Marine Ecology Progress Series, 583:121-136.

Stat M, Pochon X, Cowie ROM, Gates RD (2009) Specificity in communities of Symbiodinium in corals from Johnston Atoll. Marine Ecology Progress Series 386, 83–96

Stone R.P. & Cairns S.D. (2017) Deep-sea coral taxa in the Alaska Region: depth and geographical distribution. Online resource: https://deepseacoraldata.noaa.gov/.

Summers S.L. (1910) VIII.–Antipatharians from the Indian Ocean. Journal of the Royal Microscopical Society, 30 (3), 273–281.

Tazioli S., Bo M., Boyer M., Rotinsulu H., Bavestrello G. (2007) Ecological observations of some common antipatharian corals in the Marine Park of Bunaken (North Sulawesi, Indonesia). Zoological Studies, 46 (2), 227–241.

Terrana L. & Eeckhaut I. (2017) Taxonomic description and 3D modelling of a new species of myzostomid (Annelida, Myzostomida) associated with black corals from Madagascar. Zootaxa, 4244 (2), 277–295.

Thoma J.N., Pante E., Brugler M.R., France S.C. (2009) Deep-sea octocorals and antipatharians show no evidence of seamount-scale endemism in the NW Atlantic. Marine Ecology Progress Series, 397: 25-35.

Thomson J A. (1905) Scotia collections: Scottish Antarctic expedition—Report on the Antipatharians. Proceedings of the Royal Physical Society of Edinburgh 16: 76–79.

Thomson J.A. & Simpson J.J. (1905) Report of the Antipatharia collected by Prof. Herdman at Ceylon, 1902. Ceylon Pearl Oyster Report, 4, 93–106.

Thomson J.A. & Rennet N. (1931) Alcyonaria, Madreporaria, and Antipatharia. Australasian Antaractic Expedition 1911-14. Scientific Reports.Series C-Zoology and Botany 9, 46.

Todinanahary G., Terrana L., Lavitra T., Eeckhaut I. (2016) First records of illegal harvesting and trading of black corals (Antipatharia) in Madagascar. Madagascar Conservation & Development, 11 (1), 6–11. http://dx.doi.org/10.4314/mcd.v11i1.5

Trembley A. (1744) Mémoires pour servir à l'histoire d'un genre de polypes d'eau douce, à bras en forme de cornes. Jean & Herman Verbeek, The Netherlands.

Totton A.K. (1923) Coelenterata. Part III-Antipatharia (and their cirripede commensals). British Antarctic (“Terra Nova”) Expedition, 1910, Natural History Report. Zoology 5, 97–120.

Tournefort J.P. (1700) Institutiones rei herbariae. Aubriet, Claude, 1665-1742, Typographia regia, Paris.

Van Beneden E. (1897) Les Anthozoaires de la “Plankton-Expedition”. Ergebn. Plankton-Exped.. Humboldt Stift: 485–521.

Van Pesch A.J. (1914) The Antipatharia of the Siboga Expedition. Siboga- xpeditie Monographe 17: 1-258.

Verrill A.E. (1864) Revision of the Polypi of the eastern coast of the United States. Memoirs of the Boston Society of Natural History 1: 1–45.

Wagner D. (2015) The spatial distribution of shallow-water (< 150 m) black corals (Cnidaria: Antipatharia) in the Hawaiian Archipelago. Marine Biodiversity Records, 8.

Wagner D., Brugler M.R., Opresko D.M., France S.C., Montgomery A.D., Toonen R.J. (2010). Using morphometrics, in situ observations and genetic characters to distinguish among commercially valuable Hawaiian black coral species; a resdescription of *Antipathes grandis* Verrill, 1928 (Antipatharia: Antipathidae). Invertebrate Systematics 24: 271–290.

Wagner D., Papastamatiou Y.P., Kosaki R.K., Gleason K.A., McFall G.B., Boland R.C., Pyle R.L., Toonen R.J. (2011) New records of commercially valuable black corals (Cnidaria: Antipatharia) from the Northwestern Hawaiian Islands at mesophotic depths. Pacific Science: 65(2), 249-255.

Wagner D., Luck D.G., Toonen R.J. (2012) The biology and ecology of black corals (Cnidaria: Anthozoa: Hexacorallia: Antipatharia). In: Advances in Marine Biology, Vol. 63, pp. 67-132, Academic Press.

Wagner D., Kosaki R.K., Spalding H.L., Whitton R.K., Pyle R.L., Sherwood A.R., Tsuda R.T., Calcinai B. (2014). Mesophotic surveys of the flora and fauna at Johnston Atoll, Central Pacific Ocean. Marine Biodiversity Records, 7.

Wagner D. & Opresko D.M. (2015) Description of a new species of *Leiopathes* (Antipatharia: Leiopathidae) from the Hawaiian Islands. Zootaxa, 3974(2), 277-289.

Wagner D. & Shuler A. (2017) The black coral fauna (Cnidaria: Antipatharia) of Bermuda with new records. Zootaxa, 4344(2): 367-379.

Warner G.F. (1981) Species descriptions and ecological observations of black corals (Antipatharia) from Trinidad. Bulletin of Marine Science 31: 147–163.

Warner G.F. (2005) The occurrence of black corals in Jamaican reef environments, with special reference to *Stichopathes lutkeni* (Antipatharia: Antipathidae). Revista de Biologı´a Tropical 53: 61–66.

Williams B., Risk M.J., Ross S.W., Sulak K.J. (2006) Deep-water antipatharians: Proxies of environmental change. Geology, 34: 773–776.

Williams B., Risk M.J., Ross S.W., Sulak K.J. (2007) Stable isotopes data from deep-water antipatharians: 400-year records from the southeastern coast of the United States of America. Bulletin of Marine Science, 81: 437–447.

Williams B. & Grottoli A.G. (2010) Stable nitrogen and carbon isotope ($\delta^{15}N$) and ($\delta^{13}C$) variability in shallow tropical Pacific soft coral and black coral taxa and implications for paleoceanographic reconstructions. Geochimica et Cosmochimica Acta, 74: 5280–5288.

Won J.H., Rho B.J., Song J.I. (2001) A phylogenetic study of the Anthozoa (phylum Cnidaria) based on morphological and molecular characters. Coral Reefs 20: 39–50.

Yesson C., Bedford F., Rogers A.D., Taylor M.L. (2015) The global distribution of deep-water Antipatharia habitat. Deep Sea Research Part II: Topical Studies in Oceanography, 145: 79-86.

Zhou J. & Zou R. (1984) Studies on the antipatharians of China. II. The genus *Antipathes*. Tropic Oceanology 3: 56–61.

Zhou J. & Zou R. (1987) Studies on the antipatharians of China. III. The genus *Stichopathes*. Tropic Oceanology 6: 63–70.

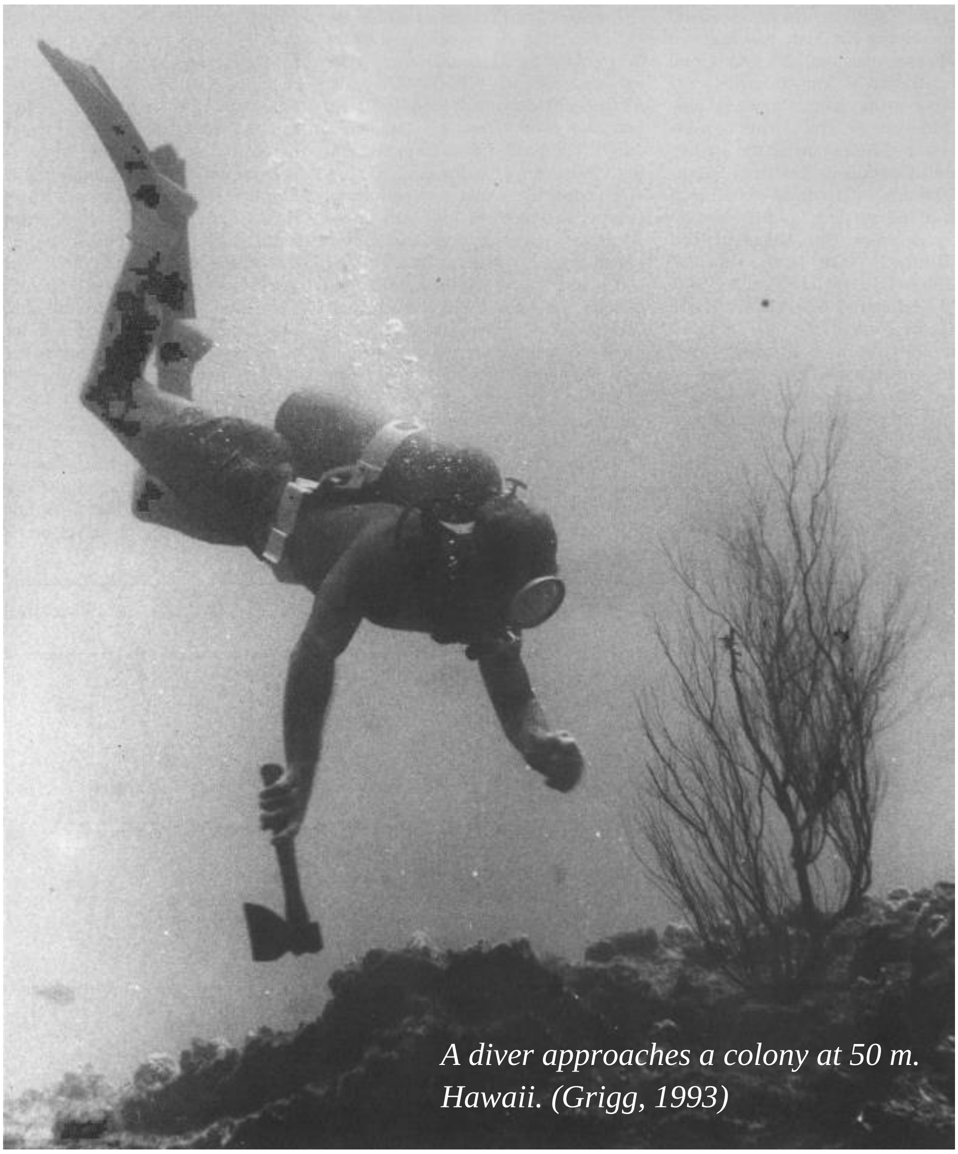
Zhou J. & Zou R. (1992) Studies on the antipatharians from Zhongsha Islands waters. Tropic Oceanology 11: 45–52.

Zou R. & Zhou J. (1982). Studies on the antipatharians of China. I. The genus *Cirrhipathes* with the description of a new species. Tropic Oceanology 1: 82–91.

Zou R. & Zhou J. (1984) Antipatharians from Hong Kong waters with a description of a new species. Asian Marine Biology 1: 101–105.

Chapitre 2

Objectifs de la thèse



A diver approaches a colony at 50 m. Hawaii. (Grigg, 1993)

Objectifs de la thèse

Les coraux noirs, ou antipathaires, peuvent se rencontrer à faible profondeur dans tous les océans et les mers du globe. Ils se retrouvent parfois en densité élevée et forment alors des écosystèmes particuliers, appelés champs de coraux noirs, qui abritent de nombreuses autres espèces. Paradoxalement, les coraux noirs mésophotiques, bathyaux et abyssaux sont mieux connus que les espèces de faibles profondeurs (< 50m) . Les études biologiques et écologiques concernant ces dernières se cantonnent principalement à deux endroits de l'Océan Pacifique en Indonésie et à Hawaii. Si leur présence dans l'Océan Indien est connue depuis plus d'un siècle au travers quelques descriptions taxonomiques, les recherches sur ces organismes dans cette partie du monde sont presque inexistantes. Pourtant, à l'image de ce qui se passe dans plusieurs îles tropicales pacifiques, les coraux noirs sont massivement et illégalement collectés dans l'Océan Indien et en particulier à Madagascar, pays où se situe un laboratoire de terrain géré par mon université d'accueil. Pour assurer une protection et une gestion de collecte adéquates de ces coraux noirs, il est indispensable de connaître leur biologie et leur écologie à l'endroit des collectes. Ainsi, l'objectif général de cette thèse a été d'apporter ces connaissances et donc d'analyser les paramètres biologiques et écologiques indispensables pour permettre dans un second temps (hors cadre de cette thèse) une mise au point d'une protection des coraux noirs du Sud-Ouest de Madagascar. Dans ce but, les principaux points auquel cette thèse se sont intéressés sont: (i) la biodiversité des coraux noirs et leur abondance, (ii) leur reproduction et (iii) la connectivité génétique des individus, (iv) leur âge et (v) les ressources trophiques qu'ils exploitent ainsi que (vi) celles de leur faune associée.

i) Quelles sont les espèces présentes au niveau du sud-ouest de Madagascar ?

L'identification des antipathaires au niveau de l'Océan Indien se résume actuellement aux descriptions originales, souvent peu informatives et peu précises. Dans cette littérature datée de plus d'un siècle, les eaux peu profondes du sud-ouest malgache (< 50 m) n'ont fait l'objet d'aucune étude descriptive et détaillée. De plus, la taxonomie moderne des antipathaires implique l'utilisation de techniques de microscopie électronique pour discriminer les espèces. Par conséquent, nous avons réalisé un inventaire des espèces rencontrées dans le sud-ouest malgache en collectant et décrivant chaque morphotype rencontré jusqu'à 50 mètres de profondeur. Plus particulièrement, il existe un champ d'antipathaires situé dans la passe nord du Grand Récif de Tuléar dans lequel nous avons décrit l'abondance et la diversité.

ii) Comment se déroule la reproduction sexuée des antipathaires ?

Après avoir déterminé quelles étaient les espèces les plus abondantes du Sud-Ouest, nous avons choisi l'une d'entre elles comme espèce modèle pour étudier sa reproduction sexuée. Les quelques études concernant la reproduction de ces organismes se focalisent sur des espèces de l'Océan Pacifique. Celles-ci sont généralement gonochoriques et présentent un cycle souvent lié aux fluctuations de température. Il est essentiel de connaître ces caractéristiques biologiques notamment pour déterminer les périodes de reproduction : c'est lors de ces périodes que se déroule les flux géniques entre différents individus plus ou moins éloignés grâce à la dispersion des gamètes et grâce à la dispersion des larves pélagiques.

iii) Est-ce que les antipathaires du Sud-Ouest appartiennent à une seule et unique population, ou forment-ils des populations distinctes ?

Comprendre la structure des populations est important en biologie de la conservation car le flux génétique entre individus maintient une grande hétérogénéité du pool génique des espèces, favorisant ainsi leur pérennité au travers du temps et des stress environnementaux ou anthropiques. Cela permet également d'étudier le pouvoir de dispersion de l'espèce et sa capacité à éventuellement passer des barrières naturelles. Une des méthodes employées pour déterminer la structure génétique des populations est l'étude des régions non-codantes ITS (Internal Transcribed Spacers) situés entre les régions codantes des gènes ribosomiaux. Ces régions hypervariables sont répétées de nombreuses fois au sein du génome et varient intra- et inter-spécifiquement. Bien souvent ces gènes sont hétérozygotes et l'établissement de réseaux d'haplotypes, appelés haplowebs, permet d'identifier d'éventuels échanges géniques entre organismes de la même espèce. Pour répondre à cette question, l'étude dans ce chapitre s'est focalisée sur une espèce abondamment retrouvée sur 200 km de côtes, afin d'avoir des zones géographiques à comparer. Si celle-ci présente des variations de couleur, l'étude permettra également de montrer que cette dernière n'est seulement qu'une variation phénotypique.

iv) Quel âge atteignent les antipathaires d'eaux peu profondes (<50m) ?

Les antipathaires sont des organismes qui possèdent généralement de longues espérances de vie associées à des vitesses de croissance lentes. Si la croissance et l'âge des coraux noirs abyssaux et/ou pêchés sont connus pour certaines espèces, ce n'est pas le cas pour celles vivant à faibles profondeurs dans l'Océan Indien. Parmi les techniques existant pour la datation (mesures *in situ*, marquage isotopique ou mesures de rapports isotopiques), celle utilisée pour établir l'âge des colonies dans cette thèse s'est basée sur la mesure du carbone radioactif naturellement présent dans le squelette des antipathaires. Elle se fonde dans le cas présent sur l'augmentation considérable de Carbone^{14} dans l'atmosphère au début des années 1950 suite aux nombreux essais et tirs nucléaires terrestres et sous-marins. Grâce à un étalon établi sur un récif kényan suivi durant une cinquantaine d'année, il est possible de déterminer l'âge des antipathaires étudiés. En parallèle, ces derniers croissent en développant des stries de croissance à l'image de ce que l'on trouve chez les plantes ligneuses. Il devient alors possible de mettre en relation l'âge de la colonie avec la production de ces stries au cours de la vie du corail.

v) Est-ce que les antipathaires du champ de Tuléar partagent les mêmes ressources trophiques ?

Le champ d'antipathaires de Tuléar possède l'avantage d'avoir en son sein différentes espèces présentant des morphologies différentes. Ces colonies se trouvent au même endroit et sont donc globalement soumises aux mêmes conditions environnementales et hydrodynamiques, en plus d'être potentiellement exposées aux mêmes sources trophiques. La diversité trophique sera étudiée grâce aux isotopes stables du carbone, de l'azote et du soufre. Nous avons utilisé dans ce chapitre le concept de niche écologique qui est un hypervolume mathématique dans un espace de *n* dimensions où chacun des axes représente un paramètre environnemental. Les isotopes stables du soufre sont utilisés pour déterminer une influence plutôt terrigène, pélagique ou du lagon au niveau des sources trophiques, étant donné que le cycle du soufre diffère en fonction de sa provenance.

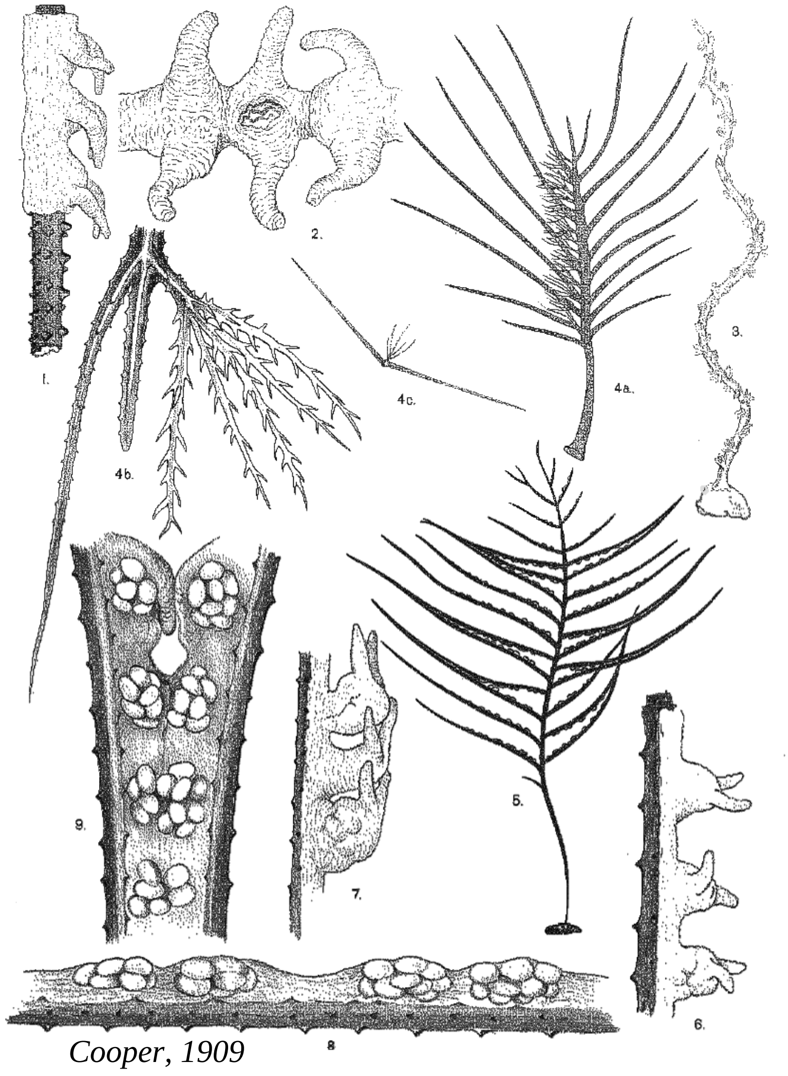
vi) Existe-t-il un lien trophique entre les coraux noirs et leurs symbiontes les plus fréquents?

Les coraux noirs sont connus pour être le siège d'une grande biodiversité. Morts ou vivants, les antipathaires abritent parfois jusqu'à plusieurs milliers d'organismes sur une seule et même colonie à la recherche d'un abri, d'un substrat ou de nourriture. En outre, ils forment des refuges pour de nombreuses espèces de poissons ou d'invertébrés, jouant ainsi un rôle clé dans le maintien de la biodiversité. Afin de mettre en évidence l'importance des antipathaires en tant qu'hôte et déterminer leur rôle dans les réseaux trophiques qui leurs sont associés, les mêmes techniques d'étude de rapports isotopiques telles qu'exposées au point précédent ont été utilisées sur différents symbiontes fréquemment retrouvés sur les colonies de coraux noirs.

Enfin, la discussion générale de cette thèse met en évidence les nouvelles informations apportées par ce travail en rapport à ce qui était connu auparavant. Les résultats sont discutés dans leur ensemble en voyant comment les réponses apportées à chaque question peuvent se complémenter les unes aux autres. En guise de perspectives, les résultats obtenus sont discutés afin d'appuyer la création d'une Aire Marine Protégée au niveau du Grand Récif de Tuléar et de dégager la meilleure option pour une protection durable des antipathaires.

Chapitre 3

Biodiversity of the shallow-water black corals (Hexacorallia: Antipatharia) from the southwest of Madagascar



Cooper, 1909
Antipatharia from the Indian Ocean

Biodiversity of the shallow-water black corals (Hexacorallia: Antipatharia) from the southwest of Madagascar

ABSTRACT

Antipatharians, also known as black corals, have been harvested for hundreds of years, especially in the tropical regions for jewellery. With the exception of a few reports on their presence and incomplete identifications, shallow-water black corals from the Indian Ocean and especially those from Madagascar are completely unknown. We here report on 18 species living in the south-west of Madagascar based on morphological features using *in situ* observations of the whole colonies, and optical microscopy and scanning electron microscope analyses on fragments from the top of the corals to avoid collecting entire colonies. All the species belongs to the Antipathidae and Myriopathidae families. Out of these 18 species, 12 are identified based on the descriptions from literature. The other undescribed species are from the genera *Cirrhipathes*, *Stichopathes* and *Cupressopathes*. The first two genera comprise species having different morphotypes: *Cirrhipathes* sp2 encompasses 5 morphotypes and *Stichopathes* sp. aff. *maldivensis* includes 2 morphotypes. They differ slightly, either on their colour, the polyps or the secondary spines of the skeleton. A black coral bed located in the north part of the Great Reef of Toliara is described, the abundance of the species is detailed, and an underwater taxonomic key is proposed for the species from the Southwestern coast of Madagascar.

This manuscript is the draft of the paper that will be submitted with the following co-authors and order:

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INTRODUCTION

Antipatharians, also known as black corals, are colonial organisms characterized by non-retractile polyps with six unbranched tentacles and a black spiny flexible proteinaceous skeleton (Opresko *et al.* 2014). With 7 families (Antipathidae, Aphanipathidae, Cladopathidae, Leiopathidae, Myriopathidae, Schizopathidae and Stylopatidae) and around 247 species (Brugler *et al.* 2013), they have different morphologies. Some species are branched with a bush-like shape, a fan-shape, a feather-like shape or a bottle-brush shape, while unbranched species can be straight, or whip-like, with regular or irregular curves or coils (Wagner *et al.* 2012). The spines on the skeleton are one of the main taxonomic features to identify species and may be triangular, conical, horn-shaped, cylindrical, bifurcated or dendritic with a surface that is smooth, papillose, or covered with tubercules (Opresko 2001; 2002; 2003b; 2004; 2006).

Taxonomy of the shallow-water species have suffered from a lack of interest but since a few years new studies have been published (Opresko & Sanchez 2005; Molodtsova 2007; Tazioli *et al.* 2007; Bo *et al.* 2008; Bo *et al.* 2009; Opresko *et al.* 2014; Todinanahary *et al.* 2016; Terrana & Eeckhaut 2017). Numerous species have been described from incomplete specimens or because of the lack of clear taxonomic traits until the revision works of Opresko began (1972; 2001; 2002; 2003b; 2004; 2006 and references therein). A lot of type species of the Indian Ocean is lost or missing (Molodtsova, personal communication), making more difficult the identification of the species. In parallel, the redescription of some types specimens is still carried out (Grange 1988; Opresko & Genin 1990; Opresko & Cairns 1994; Opresko 1997; Opresko & Baron-Szabo 2001; Opresko 2003a; Molodtsova 2005; Opresko & Sanchez 2005; Ocana *et al.* 2006; Opresko 2009; Wagner *et al.* 2010; Bo & Opresko 2015). Moreover, recent molecular analyses have shown that black corals may contain ecotypes and that some genera are polyphyletic (Lapian 2007, Brugler et al. 2013). A large part of the current classification is polyphyletic, and the number of species may be overestimated while many genera may be polyphyletic (Lapian *et al.* 2007; Lapian 2009; Brugler *et al.* 2013).

Studies on shallow black corals in the Indo-Pacific are focused on some restricted regions and the area of the Bunaken Marine Park (Sulawesi, Indonesia) hosts the richest and most diversified shallow-water antipatharians ever described to date, with 16 species reported (Tazioli *et al.* 2007; Bo *et al.* 2008). Besides a few reports on their presence and incomplete identifications, shallow-water black corals from the Indian Ocean and especially those from Madagascar are completely unknown (Thomson & Simpson 1902; Cooper 1909; Summers 1910; Pichon 1972; Terrana & Eeckhaut 2017). As they are the target of many illegal fisheries despite being crucial habitat for many organisms, more attention needs to be addressed to the antipatharians of this region. The aim of this paper is to describe for the first time the biodiversity of the shallow black corals found in the southwestern coast of Madagascar, one of the regions targeted by illegal harvests (Todinanahary et al. 2016).

MATERIAL & METHODS

Sampling

Black corals were collected between December 2014 and December 2016 in the southwestern coast of Madagascar from 10 to 52 meters depth by scuba-diving (Fig. 25, Table 6). The living colonies were labelled and photographed before being cut into small fragments with clippers to avoid killing the entire colonies. They were then stored in individuals bags filled with seawater before being fixed in Bouin's fluid for microscopy, in formalin 10% buffered with sea water for long term storage, in 100% ethanol for molecular analyses and air dried for skeleton observation. Samples from new species were deposited in the Museum of Natural History in Brussels, Belgium (see the species description for the IG numbers).

Field description

Study sites where black coral were supposed to be present were chosen depending on reef topography and currentology (areas with high currents were favoured). Fishermen were also asked for the presence of black corals. The study area is comprised between two coastal villages, from Andavadoaka (22°04.214'S, 43°14.332'E) to Maromena (23°48.476'S, 43°39.477'E), which represents almost 200 km of shore (Table 6).

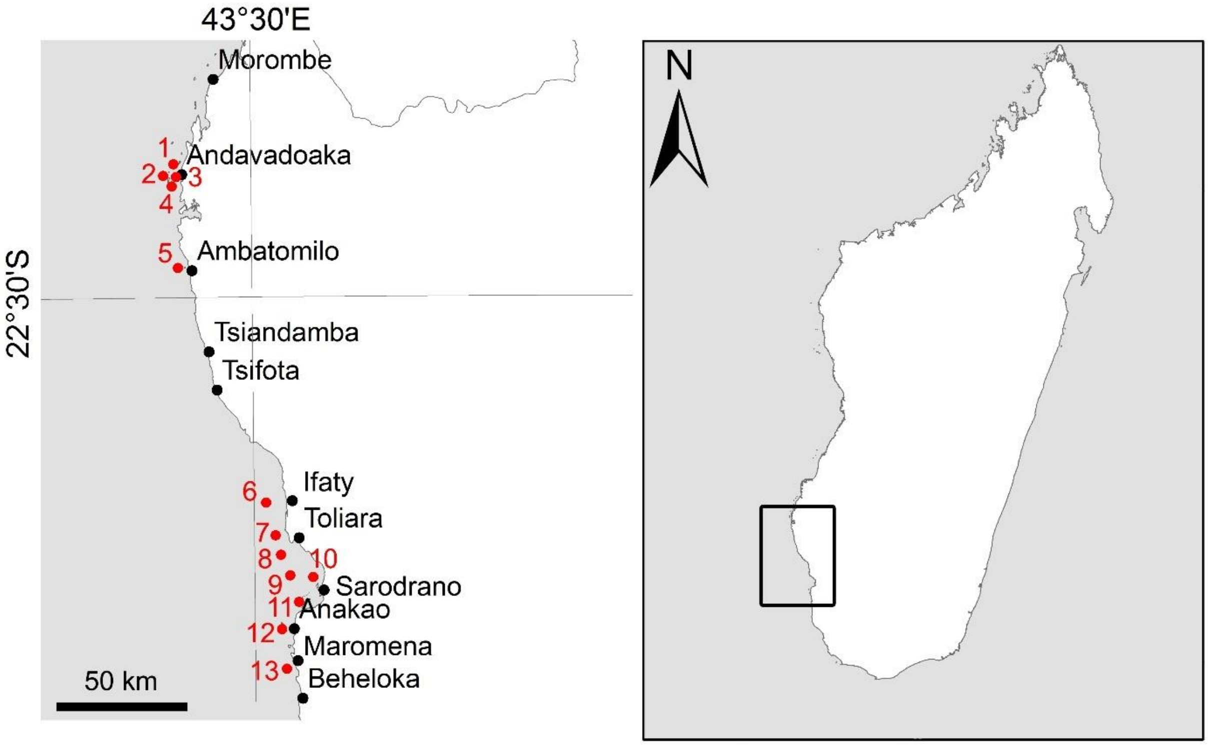

Figure 25. Sampling sites in the southwest of Madagascar (red dots). Scale in kilometers.

Table 6. Coordinates of the different locations examined.

Station Number	Station name	Coordinates		Max Depth (m)
1	Nosy Fasy, Andavadoaka	21°58.674'S	43°11.653'E	10
2	Nosy Hao, Andavadoaka	22°04.742'S	43°10.944'E	10
3	Fringing reefs, Andavadoaka	22°00.530'S	43°12.960'E	20
4	Patch reefs, Andavadoaka	22°07.518'S	43°11.886'E	20
5	Ambatomilo	22°30.941'S	43°14.934'E	20
6	Ifaty barrier reef	23°09.089'S	43°33.883'E	25
7	GRT, north channel	23°21.040'S	43°36.944'E	30
8	GRT, Grande Vasque	23°22.976'S	43°38.152'E	15
9	GRT, south channel	23°30.051'S	43°41.555'E	10
10	Sarodrano reefs	23°29.695'S	43°43.549'E	10
11	Soalara	23°35.259'S	43°42.531'E	14
12	Anakao	23°39.306'S	43°36.541'E	10
13	Maromena	23°48.113'S	43°38.252'E	22

The abundance of the different black coral species was assessed in a black coral bed found at the north extremity of the Great Reef of Toliara. It is located at the north extremity of the Great Reef of Toliara and was found at about 1.7 km from the shore. It extends on about 500 m in length and 300 m in width with the black corals that live at depths ranging between 10 to 30 m. The bed is in front of the mouth of the Fiherenana river and particulate organic matter regularly flows in abundance. A few meters deeper in the West direction, small black coral beds were also found on the sea floor between 45 and 52 m depth (Table 7). A total of 10 transects were made at the north extremity of the Great Reef of Toliara by scuba-diving using linear transects of 60 m long. The length was chosen to optimize dive time and air supply. Each transect was made by two divers recording each black coral encountered in a wideness of 3 m from each side of the rope. Each diver was filming with a camera equipped with a red filter and/or strobes. Records were then analysed on a computer and species counted.

Table 7. GPS data for the black coral bed of Toliara (WGS84).

Number	Coordinates		Max Depth (m)
1	23°21.093'S	43°36.661'E	27.7
2	23°21.063'S	43°36.648'E	29.7
3	23°21.019'S	43°36.693'E	27.5
4	23°21.022'S	43°36.697'E	24.4
5	23°20.986'S	43°36.807'E	26
6	23°20.959'S	43°36.837'E	28.7
7	23°20.978'S	43°36.885'E	20.3
8	23°21.000'S	43°36.830'E	24.5
9	23°21.003'S	43°36.836'E	14
10	23°21.030'S	43°36.789'E	19.6
11	23°21.028'S	43°36.883'E	16.3
12	23°21.053'S	43°36.812'E	18
13	23°21.105'S	43°36.853'E	21
14	23°21.090'S	43°36.923'E	23.6
15	23°22.986'S	43°38.353'E	14
16	23°22.973'S	43°38.419'E	13.1
17	23°21.012'S	43°36.835'E	19.6
18	23°21.277'S	43°36.346'E	45
19	23°21.343'S	43°36.468'E	33
20	23°21.362'S	43°36.404'E	35
21	23°21.325'S	43°36.367'E	53

Measurements

Different measurements were made on specimens fixed following Wagner *et al.* (2010). Size of the skeleton spines, spine spacing, polyp size, polyp spacing and skeleton diameter (Fig. 26) were measured with ImageJ (Schneider *et al.* 2012).

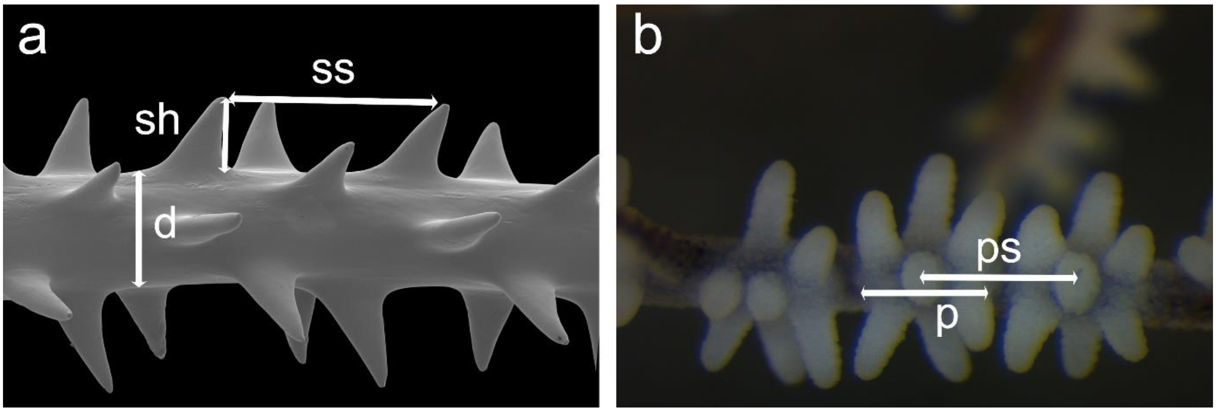

Figure 26. Measurements made on black coral species. (a) skeleton measurements. (b) polyp measurements. d: skeleton diameter; p: polyp size (diameter); ps: polyp spacing (interpolypar space); sh: spine height; ss: spine spacing.

Scanning electron microscopy

Polyps were carefully removed under a dissection microscope. Residual tissues were then cleaned by spraying some bleach before being immediately rinsed with clean water to avoid denaturing the skeleton. To remove remaining tissue, the skeletons were sonicated. They were then allowed to dry at room temperature before being mounted on aluminium stubs and coated with gold in a JEOL JFC-1100E sputter coater. Samples were observed with a JEOL JSM-7200F scanning electron microscope.

RESULTS

Species description

The following descriptions are based on observations of whole colonies *in situ*, and microscopical analyses on fragments cut off from the top of the corals to avoid killing entire colonies. Using morphological characters, we distinguish 18 species, some of them being composed of different morphotypes. All of them belong to the Antipathidae and Myriopathidae families. The description of 12 species match with 12 original descriptions from the literature. The other undescribed species are from the genera *Cirrhipathes*, *Stichopathes* and *Cupressopathes*. The first two genera comprise species having different morphotypes: *Cirrhipathes* sp2 encompasses 5 morphotypes and *Stichopathes* sp. includes 2 morphotypes. They differ slightly, either on their colour, the polyps or the secondary spines of the skeleton. An underwater taxonomic key of the species is presented in Table 12.

Family Antipathidae Ehrenberg, 1834

This family is the first one to have been established in the history of the antipatharian taxonomy and it contains the highest number of species. The family is characterized by branched and unbranched colonies that can have a single stem, be bushy or fan-shaped. When the branches are present, they are not pinnulate and the spines are smooth or papillose, and they can be simple, forked or multi-lobed. The polyps have always 10 mesenteries and range from 1 to 3 mm in size. They are not elongated in transverse plane and generally the sagittal tentacles are longer than the lateral ones (Opresko 2005b; Bo 2008; Wagner 2015a). To date, there are currently 8 genera within the Antipathidae: *Antipathes*, *Arachnopathes*, *Hillopathes*, *Pteropathes*, *Stichopathes*, *Cirrhipathes*, *Pseudocirrhipathes* and *Allopathes*. They typically occur at depths between 10 and 400 m. Historically, this family has always been a dumping ground, consequently resuting in species wrongly gathered together. Revisions have been published to clarify the identification (Opresko 2001; 2002; 2003b; 2004; 2005a; 2006), but the group still needs a major revision since colony and skeleton morphologies are very heterogeneous.

Genus *Antipathes* Pallas, 1766

The genus *Antipathes* is the oldest to have been established. It is characterized by colonies that are branched, bramble-like, bushy or fan-shaped. The branches are not pinnulate and the polyps occur in a single row on the smaller branches. The spines are smooth, papillose, simple, forked, multilobed or knobbed at the apex. There are currently 35 nominal species from the Pacific and Indian Oceans, but for 13 of them the type species are lost. In addition to original descriptions often incomplete, it is difficult to determine the exact number of species in the genus *Antipathes*.

Antipathes pseudodichotoma Silberfeld, 1909

Antipathes pseudodichotoma: Silberfeld 1909, p.27, Pl.2, fig.4.

Biogeography. Japan Silberfeld (1909); Madagascar (present paper).

Localisation. Colony found in July 2016 at 14 m depth in Soalara. Rare southerly of Toliara, never observed northerly of Toliara.

Material examined. Entire colony *in situ*, fragment of 10 cm in formol 10% (M210716-M026-4), 2 fragments of 4 cm in absolute ethanol (M210716-M026-1), SEM sample of the skeleton (M210716-M026-S), dry part of the colony of about 7 cm (M210716-M026-S2).

Description. A sparsely branched colony measuring about 25 cm in height (Fig. 27, a, b). The colony is pseudo-dichotomous and the branches are not restricted to a two-dimensional plan (Fig. 27, c). The angles of the branches measure between 21° and 95°. The basal diameter is nearly 1 mm and it reaches 0.54 mm on the upper branches. The black skeleton is visible when the coral is alive due to the transparency of the tissues, and the polyps are light grey (Fig. 27, a, b). They are located on a single side of the branches, but it can turn around the other side along the branch (Fig. 27, d). Smaller polyps are inserted between larger ones and sometimes two rows of polyps are present. On thicker branches, the polyps are sometimes found on different sides. They measure 0.57-1.70 mm and they are spaced by 1.0-2.4 mm. There are 8 polyps per cm along the branches. When the polyps are extended, the tentacles are long and thin with acute tips (Fig. 27, b). When contracted, they appear thick and rounded (Fig. 27, d). The spines are arranged in spirals on the skeleton but also in longitudinal rows, 5-6 of which can be seen from one aspect (Fig. 27, e). The spines are smooth, triangular and the distal edge is acute and forms a right angle to the corallum (Fig. 27, f). They measure 0.06-0.15 mm and the interspine spaces are 0.17-0.39 mm between spines from a spiral or 0.29-0.49 mm between spines of a row.

Remarks. This species has been originally described from Japan. The present description is similar to the original diagnosis made by Silberfeld (1909) which is incomplete by lacking information about the spine morphology and the size of the polyps. Silberfeld (1909) reported a diameter of 0.355 mm, a spine size of 0.128 mm, a spine spacing varying between twice or three times the length of the spines, and a polyp spacing of 1-1.5 mm. The original description states that the spines are arranged in spirals and longitudinal rows, 5-6 of which can be seen from one aspect. The spines are wide, their tip curved slightly upwards with the distal edge forming a right angle to the axis. The type specimen is lost and thus the identification cannot be totally confirmed.

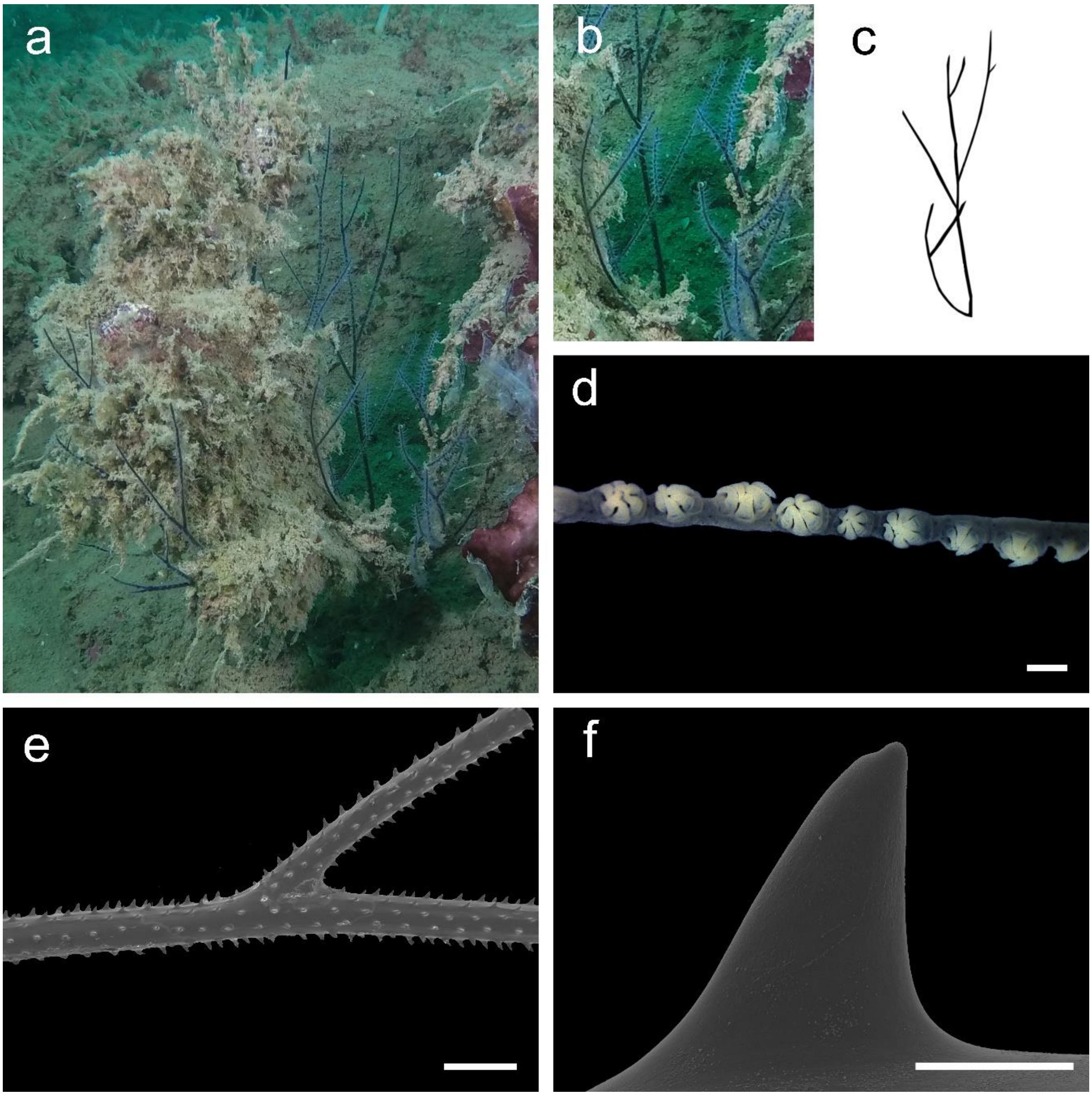

Figure 27. *Antipathes pseudodichotoma*. (a) Entire colonies measuring about 25 cm in height. (b) Picture showing the extended polyps. (c) Schema showing the pseudo-dichotomous branching. (d) Polyps when contracted. (e) Skeleton view by SEM. (f) Spine morphology. Scales: d, e: 1 mm, f: 50 µm.

Antipathes flabellum Pallas, 1766

Antipathes flabellum: Pallas 1766, p.211; Lamarck 1815, p.476; Lamouroux 1816, p.382, 1824, p.73; Deshayes & Edwards 1836; Dana 1846, p.579; Gray 1857; Schultze 1896, p.29.

Rhipidipathes flabellum: Milne-Edwards & Haime 1857, p.321.

Tylopathes? flabellum: Brook 1889, p.137.

Biogeography. Indian Ocean (Pallas 1766; Lamarck 1815; Lamouroux 1816; 1824; Deshayes & Edwards 1836; Dana 1846; Milne-Edwards & Haime 1857), Indonesia (Schultze 1896), Madagascar (Brook 1889, present paper).

Localisation. Colony found in December 2015 in the black coral bed of Toliara at 22 m depth. A rare encounter within the bed, and never observed on other localities.

Material examined. Entire colony *in situ*, fragments from the top of the anastomose colony (M041215-M017-1, M041215-M017-2, M041215-M017-3) and dry parts of the same part of the colony (M041215-M017-S, M041215-M017-S2, M041215-M017-S3).

Description. A branched, flabellate and anastomosed colony with a brownish colour (Fig. 28, a, b). It measures 45 cm wide and 11 to 25 cm high. It has the appearance of a flattened bush but at some point it has grown out horizontally of the general plane of growth of the colony to form a plate-like portion. The corallum is reticulated; the branches grow almost vertically and bear primary pinnules which are biserial, slightly bent upwards and very often fused with the adjacent primary pinnules (Fig. 28, c). They measure 1.0-6.0 mm and don't seem to be regularly arranged, as they sometimes alternate, or they are sub-opposite or opposite with varying spaces between them. The secondary pinnules are rarely present and measure 0.8-2.0 mm with a uniserial arrangement. The pinnules are growing out of the plan of the branches but all are on the same side of the colony. The polyps are located to this side and slightly elongated along the axis of the branch (Fig. 28, b). They are small and measure 0.61-0.90 mm and are spaced by 0.60-1.70 mm. Their tentacles are small, thick and rounded at the tip. They are disposed on a single row except for thicker branches. The spines of the skeleton are smooth, conical with a sharp apex and stand at right angle to the corallum, but they are sometimes slightly hooked (Fig. 28, d, e). They measure 0.03-0.09 mm and the inter spine space is very irregular. It varies from 0.06 to 0.31 mm. On smaller branches, the spines seem to be arranged in spirals but this arrangement tends to be lost at some point. Despite this, 5-6 longitudinal rows of spines can be seen along the corallum.

Remarks. This species was originally described from the Indian Ocean by Pallas (1766). In the literature, most of the descriptions of this species have been made during the XIXth century, with a specimen from Madagascar described from a museum by Brook (1889). Since that time, no description of *A. flabellum* has been recorded. Within the Antipathidae, the genus *Arachnopathes* regroups species with anastomosed corallum. However, the spines of the present specimen are similar to those of some *Antipathes* species while their arrangement is similar to those of the genus *Tylopathes* from the Stylopathidae family. The latter contain anastomosed colonies, as well as the genus *Arachnopathes* from the Antipathidae family, but they differ in their spines and their polyps. *Tylopathes* species are usually found deeper than 100 m and have similar polyps to the present specimens, but redescriptions of the type material are lacking. Pending a revision of the groups and anastomosed colonies, the name *Antipathes flabellum* is assigned to the present specimen, as the type specimen is lost.

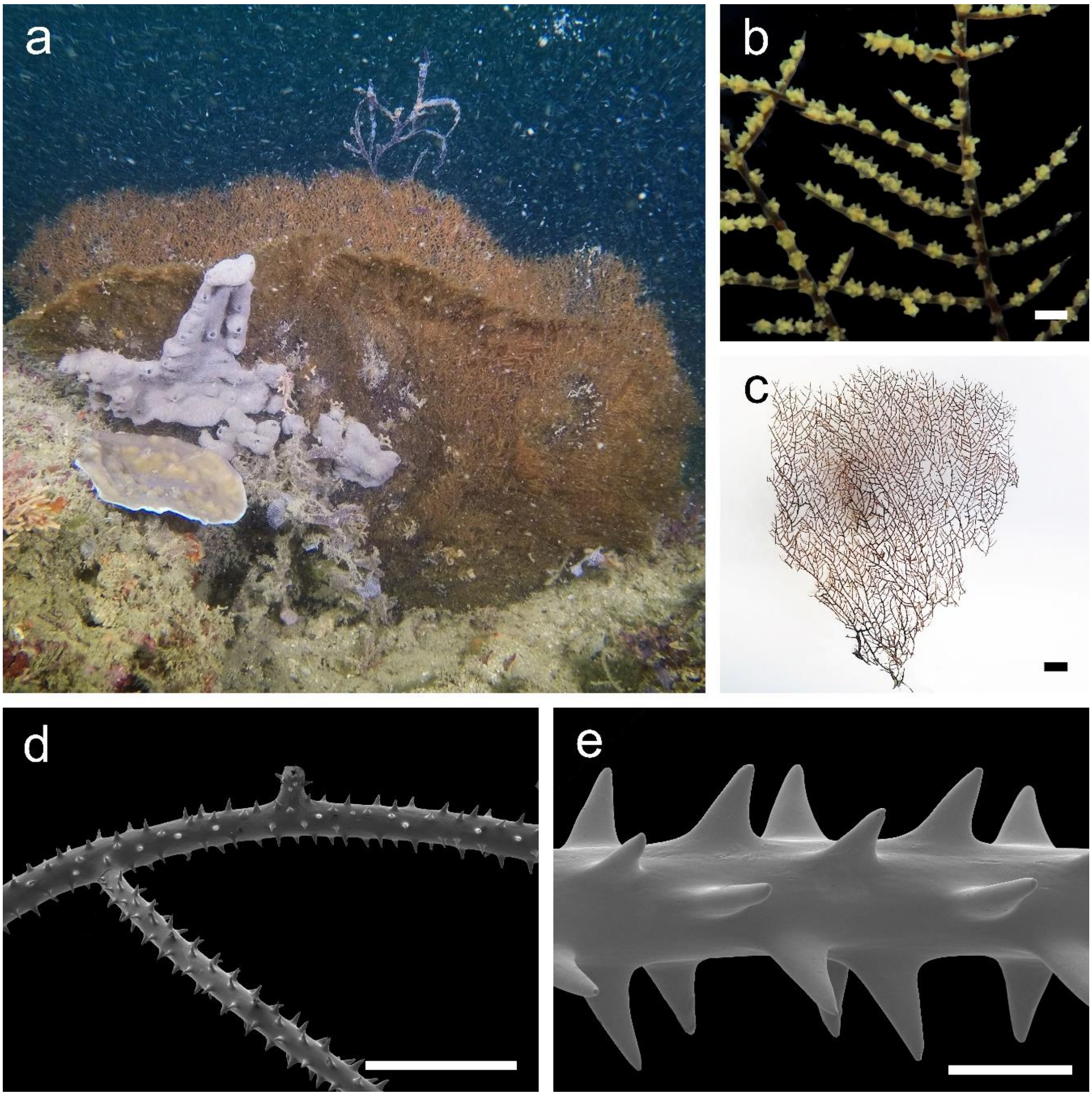

Figure 28. *Antipathes flabellum*. (a) Entire colony where the plate-like structure is visible. It measures 45 cm width. (b) Polyps of a portion of the upper corallum. (c) The anastomosed corallum. (d) General view of the skeleton. (e) Spine morphology. Scales: b, d: 1 mm; e: 100 µm.

Genus *Cirrhipathes* (Blainville, 1834)

The genus *Cirrhipathes* is characterized by corals being unbranched with polyps irregularly found on all the sides of the corallum and spines not arranged in verticils (Brook 1889; Bo *et al*. 2009; Wagner 2015a). *Cirrhipathes* species can be straight, slightly to highly contorted, curved or coiled. The spines are papillose and may be lobed at the apex.

The specimens of the genus *Cirrhipathes* collected in the southwestern coast of Madagascar are very abundant and have different sizes and shapes. The corals are straight or sinuous, or they can form spirals more or less tightened. The polyps are never found in a single row; they are distributed all around the stem or gathered on the same side. The spines are cylindrical or conical, with thick rounded or acute apex. Their tip can be slightly knobbed or multi lobed. They are always papillose either if it is slightly nor coarsely (Figs. 32, 33). Some of the specimen description are not corresponding to the literature, and they are very similar in their skeletons between each other, mainly differing by their colours. They are referred below as *Cirrhipathes* sp.X with X being a number associated to the species variation.

Cirrhipathes anguina (Dana, 1846)

Antipathes anguina: Dana 1846, p.576.

Cirripathes anguina: Brook 1889, p.84-85; Siberfeld 1909b, p.10; Pax 1932, p.407-408, 444-445; Wagner 2015, p. 7, 10-11, fig.3.

Cirripathes propingua Brook 1889, p.82-83, pl.10, figs.9-13, pl.12, fig.14, pl.14, fig.7.

Cirripathes densiflora Silberfeld 1909a, p.762; 1909b, p.10; Carlgren 1940, p.15.

Cirripathes (Eucirripathes) anguina: Van Pesch 1914, p.146-153, figs.203-205, pl.8, figs.3-4, 7.

Cirrhipathes anguinus: Bayer 1959, p.229.

Biogeography. Fiji islands (Dana 1846), Maldives (Cooper 1903), Red Sea (Cooper 1903), Seychelles (Cooper 1903), Ceylon (Cooper 1903), New Guinea (van Pesch 1914), Cape Moreseby (van Pesch 1914), Korea (Song 1987), Japan (Okiyama & Tsukamoto 1989), Indonesia (Rumphius, see Bayer 1959), Andaman & Nicobar islands (Kumar *et al*. 2012), Hawaii (Grigg 2001), Madagascar (present paper).

Localisation. Specimen M271115-M010 found in November 2015 at 23 m depth, in the bed of Toliara. Specimen M041215-M015 found in December 2015 at 22 m depth, in the

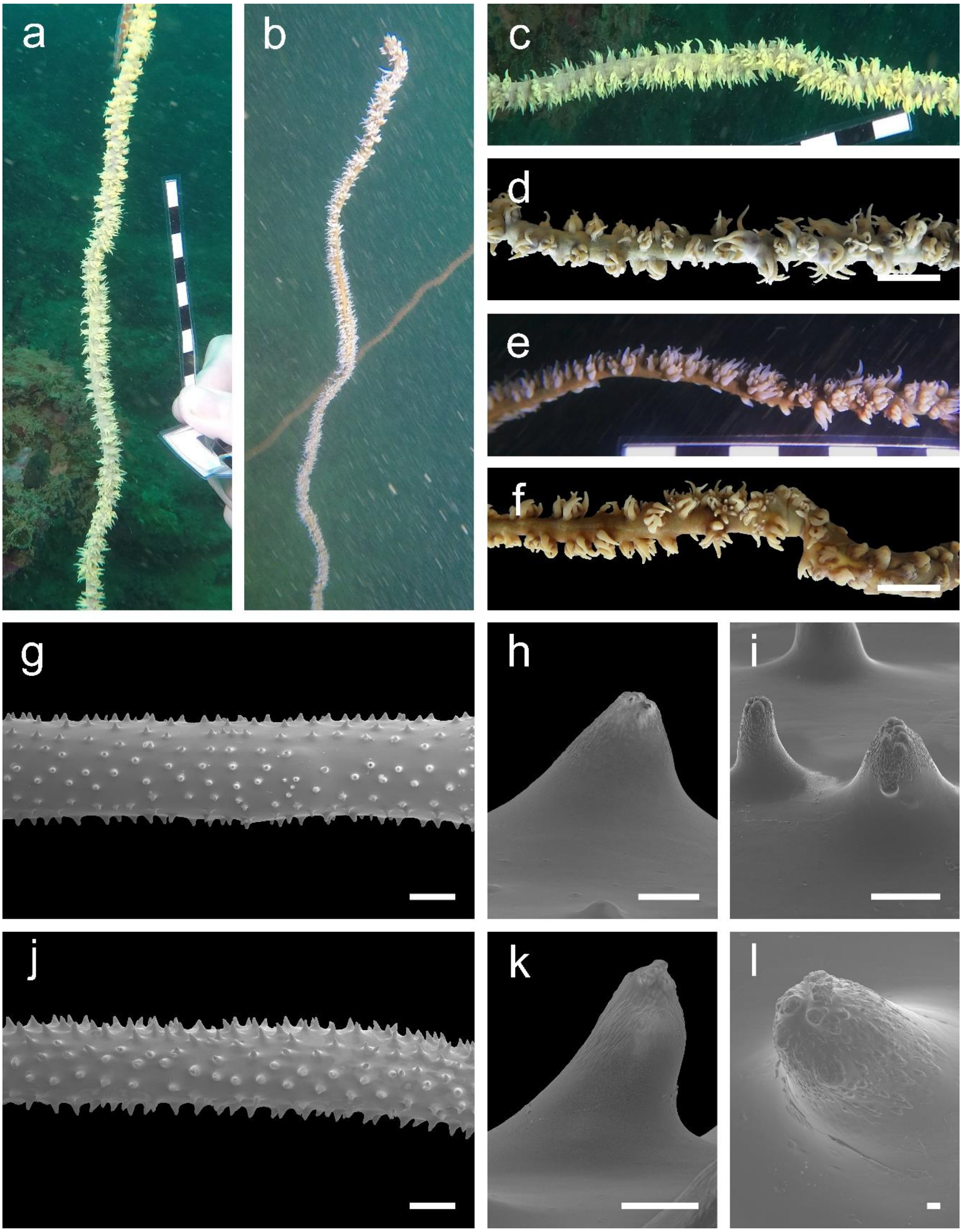

Figure 29. (**a, c, d, g, h**) *Cirrhipathes anguina* morphotype 1. (**b, e, f, i, j**) *Cirrhipathes anguina* morphotype 2, measuring 75 cm in length. (**a-b**) Entire colonies. (**c-f**) Views of the polyps. (**g, j**) Spine arrangement on the corallum. (**h, i, k, l**) Spine morphologies. Scales: a, c-f: 1 cm; g, j: 1 mm; h, i, k, l: 100 µm.

bed of Toliara. These specimens are common in the southwestern coast of Madagascar, and especially near Toliara.

Material examined. Morphotype 1 colony *in situ*, fixed fragments (M271115-M010-1, M271115-M010-2) and dry fragments (M271115-M010-S, M271115-M010-S2). Morphotype 2 colony *in situ*, fixed fragment (M041215-M015-1) and dry fragments (M041215-M015-S, M041215-M015-S2).

Descriptions. Morphotype 1 (Fig. 29, a). A single stem colony, sinuous but not curved. It measures 120 cm in length with a basal diameter of about 6.0 mm tapering upwards until about 2.0 mm at the top. The entire colony is light yellow and the polyps are located all around the corallum in an irregular way, sometimes leaving one side without any polyp (Fig. 29, c). Small and large polyps are found alternating along the colony, but not in a defined order (Fig. 29, d). The oral cones are prominent and clearly visible when the polyps are expanded with the mouth appearing like a slit. The tentacles are thick, with rounded tips. The polyps measure 1.5-4.2 mm, and they are spaced by varying distances between 1.6 and 7.0 mm. Between them, sagittal and longitudinal grooves are clearly visible (Fig. 29, c, d). The skeleton spines are conical, blunt, papillose and with a knobbed tip (Fig. 29, g, h). They are sometimes more cylindrical with a narrower basis. They measure up to 0.21 mm. The interspine space is variable and range from 0.15 to 0.70 mm. There are 9-10 longitudinal rows and a subtle spiral arrangement of the spines can be figured out. The spine density is 5/mm². The core of the skeleton is small with a size of about 0.45 mm representing about 20% of the total diameter.

Morphotype 2 (Fig. 29, b). An unbranched colony which is sinuous but not curved. It measures 75 cm in length with a basal diameter of 2.5 mm. It tapers upwards until it reaches 1.6 mm. The colony is brown with white polyps located all around the stem, sometimes leaving one side of the colony devoid of any polyp (Fig. 29, e, f). Smaller polyps are often inserted between larger ones. The oral cones are prominent and appear like a slit. The tentacles are thick with a rounded tip. The polyps measure 1.0-4.4 mm and they are spaced by 1.5-7.0 mm. Longitudinal and cross grooves are visible between the polyps (Fig. 29, e, f). The spines are papillose, with a knobbed tip (Fig. 29, i, j). They are conical, and slightly inclined in different directions (Fig. 29, i). They measure 0.2-0.3 mm in height and are spaced by 0.15-0.75 mm. They are disposed in 7-8 longitudinal rows, and the density of the spines is 5/mm². The core of the skeleton measures about 0.65 mm which represents 29% of the total diameter.

Remarks. The type specimen of this species is lost. Pending the description of a neotype, the name *Cirrhipathes anguina* is used here. This species has been previously recorded in Korea, Japan, Fiji, New Guinea, Ambon, Anchorage, Seychelles, Nilanda, Billiton, Ceylon, Cape Moresby, Suvadiva Atoll, the Red Sea and Hawaii. The present morphotypes are different in their general appearance and their skeleton but both correspond to the diagnoses of *Cirrhipathes anguina*. Van Pesch (1914) describes blunt and conical spines at right angle to the corallum as well as spines more acute and distally inclined with a rough surface. Song (1987) describes the same kind of morphology and observe a slightly spiral arrangement of the spines which have a granulated top. The spine sizes reported in these diagnoses are similar to the present ones, as they report a height of 0.18 mm for Van Pesch (1914) and 0.198-0.365 mm for Song (1987). They differ from the present specimens in having a different number of rows, which is 12-14 for both authors. The spine spacing is also similar with 450 µm for Van Pesch (1914) and 336-720 µm for Song (1987). With respect to the polyps, these authors describe polyps arranged all around the colony with varying interpolypar spaces and sizes reaching 4 mm, which is consistent with the present diagnoses. They also report about the beak appearance of the mouth and oral cones, which are found here. All the differences could simply be intraspecific variation or environmental effects on the shape and size of the colony. The present description is similar to the one made by Wagner (2015).

Cirrhipathes contorta van Pesch, 1910

Cirripathes contorta: van Pesch 1910.

Eucirripathes contorta: van Pesch 1914, Pl. VIII, Fig.9.

Biogeography. Indonesia (Van Pesch 1910), Madagascar (present paper).

Localisation. Colony found in November 2015 at 17 m depth in the black coral bed of Toliara. Common on this location but never seen elsewhere.

Material examined. Colony *in situ*, fixed fragments (M261115-M008-1, M261115-M008-3) and dry fragments (M261115-M008-S, M261115-M008-S2).

Description. The colony is very sinuous and ends by a small loop of about 4 cm in diameter (Fig. 30, a). The whole colony measures about 30 cm in height and the basal diameter is 8 mm. The polyps are found on all sides of the stem but they tend to gather on the same side, leaving parts of the corallum lacking polyps. The polyps measure 1.49-4.20 mm and are spaced by 1.12-4.66 mm (Fig. 30, b, c). The cross grooves are clearly visible behind

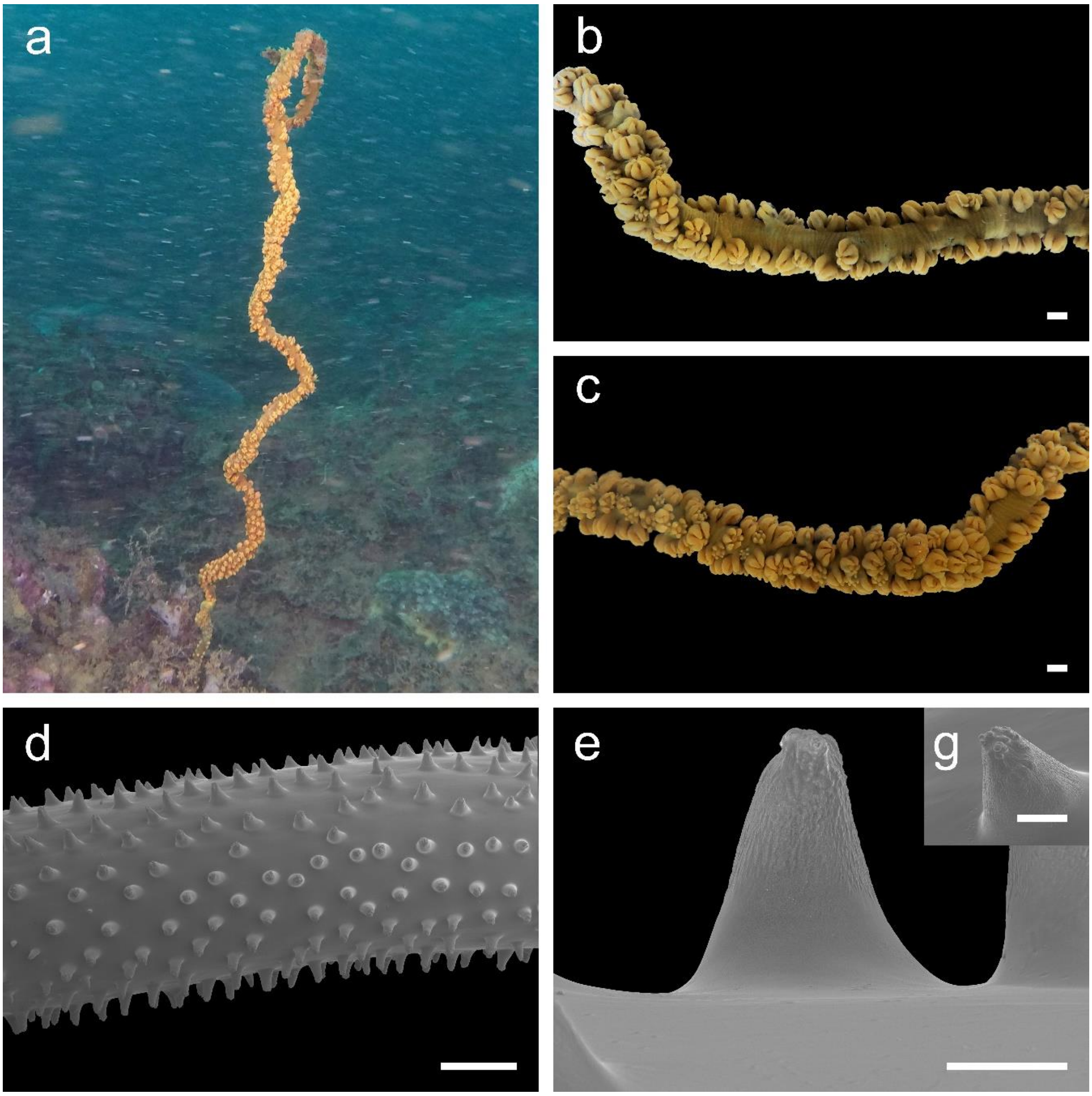

Figure 30. *Cirrhipathes contorta*. (**a**) General view of the entire colony measuring 30 cm in height. (**b, c**) Zoom on the polyps. (**d**) General view of the skeleton. (**e**) Side view of the spine. (**g**) View of the top of the spine. Scales: b, c, d: 1 mm; g, e: 100 µm.

the polyps. The spines stand at right angle to the skeleton, they measure 0.16-0.31 mm and are spaced by 0.15-0.69 mm. They are arranged in about 10 longitudinal rows in quincunx; a spiral arrangement might be present but irregular (Fig. 30, d). The spines are conical, slightly papillose and are knobbed at the apex (Fig. 30, e). There are 5-6 spines per mm^2. Secondary spines are very few.

Remarks. Within the *Cirrhipathes* species, only *C. contorta* and *C. indica* have similar papillose spines without the presence of secondary spines. However, only the original description of *C. contorta* states about a contorted and twisted appearance like the present specimen. The latter is distinguished of *C. contorta* by having spines with knobbed at the tip, and the original description lacks details of the spines to confirm the identification. The table 10 compares the different measurements of these *Cirrhipathes* species.

Cirrhipathes densiflora Silberfeld, 1909

Cirrhipathes densiflora: Silberfeld 1909a, p.762; 1909b, p.10; Carlgren 1940, p.15.

Biogeography. Japan (Silberfeld 1909a), Madagascar (present paper).

Localisation. Colony found in November 2015 at 22 m depth in the black coral bed of Toliara. Frequent in this location, but very rare on the southwestern coast.

Material examined. Colony *in situ*, fixed fragments (M241115-M003-1, M241115-M003-2, M241115-M003-3) and dry fragments (M241115-M003-S, M241115-M003-S2).

Description. The colony is crooked and measure 83 cm but larger specimens have been observed at more than 1.7 m in length (Fig. 31, a). The basal diameter is 4 mm and it slightly tapers upwards to reach 2.5 mm at the top. The tissues are grey with orange polyps which are located all around the stem, sometimes leaving one side of the colony lacking polyps (Fig. 31, b, c). The polyps are thick and appear like buds along the coral. They measure 1.3-4.9 mm and the interpolypar space varies between 1.3-9.3 mm (Fig. 31, d). The grooves are not visible, but the whole surface between the polyps is covered by small white spots (Fig. 31, c). The tentacles are thick, with rounded tips and the sagittal ones appear of the same length than the lateral ones. The oral cone is prominent with a visible aperture when the mouth is fully opened. There is a single type of spine on the corallum (Fig. 31, e). The spines are conical, covered with papillae and knobbed at the apex (Fig. 31, g). They seem to be arranged in steep spirals but this arrangement is lost at some point. They measure 0.2-0.3 mm and they stand at right angle to the corallum. The interspine distance range from 0.25 to 0.95 mm, with a spine density of 3-4 spines/mm^2. The core is small compared to the diameter of the skeleton. In a fragment of 3.2 mm in diameter, it measures 0.44 mm which represents only 13% of the surface (Fig. 31, f).

Remarks. Originally described from Japan by Siberfeld (1909a) at an unknown depth. Since that time, no description has been published.

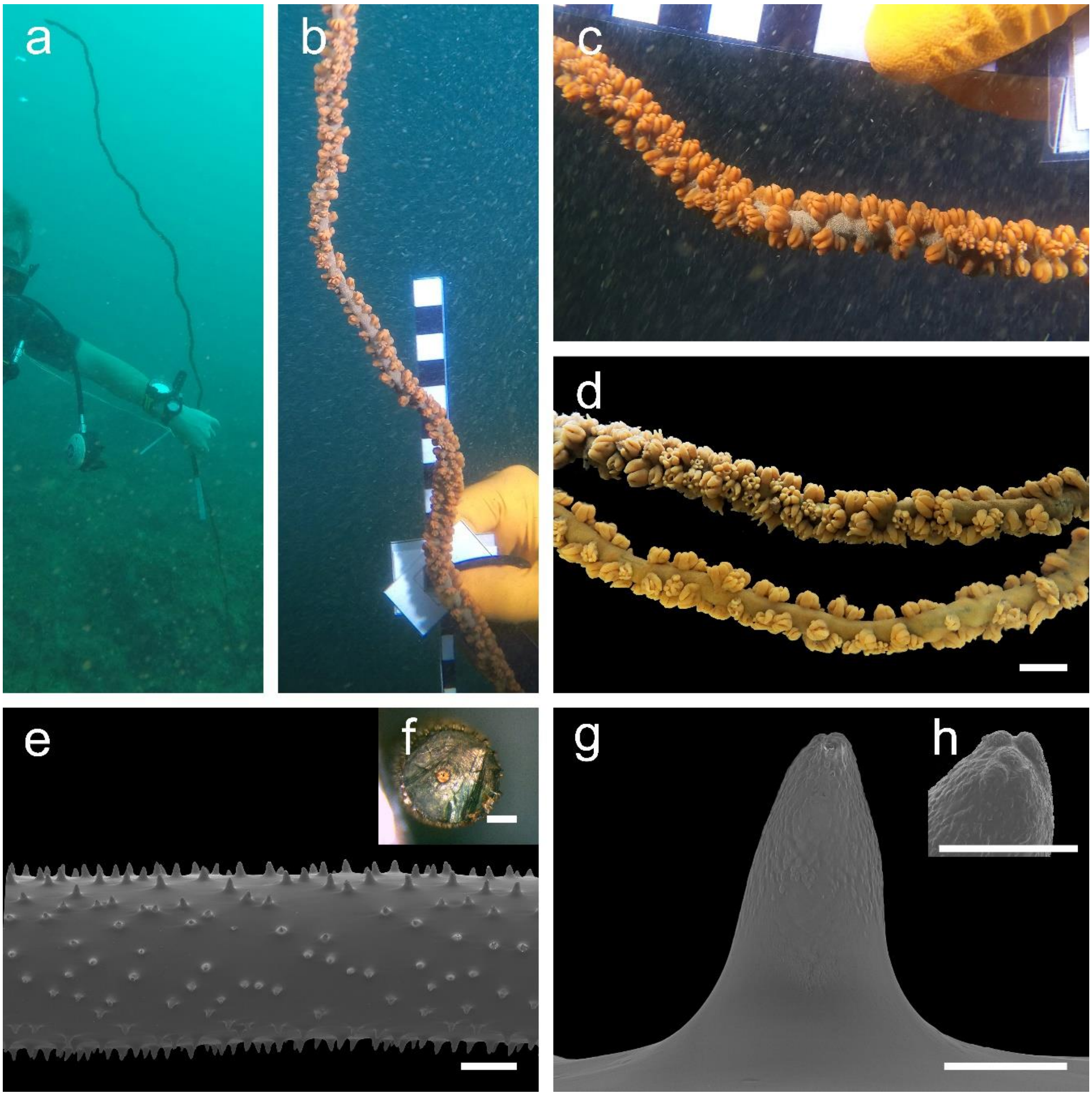

Figure 31. *Cirrhipathes densiflora*. (**a**) Picture of the entire colony. (**b, c**) Pictures showing the polyp arrangement along the colony. (**d**) Polyps appearance showing the arrangement and the large mouth. (**e**) General view of the skeleton by SEM. (**f**) Transverse section showing the small hollow core. (**g**) Side view of the spine. (**h**) View of the top of the spine. Scales: a: 10 cm; b, c, d: 1 cm; e, f: 1 mm; g, h: 100 µm.

Cirrhipathes rumphii van Pesch, 1910

Cirripathes ? n.sp.: Thomson & Simpson 1905, p.95, fig.8.

Eucirripathes Rumphii: van Pesch 1914, p.170-174, figs.245-249.

Cirrhipathes rumphii: Zou & Zhou 1982, p.84, pl.15-5, figs.3-5.

Biogeography. Indonesia (Rumphius, see Bayer 1959), Ceylon (Thomson & Simpson 1905), China (Zou & Zhou 1982), Madagascar (present paper).

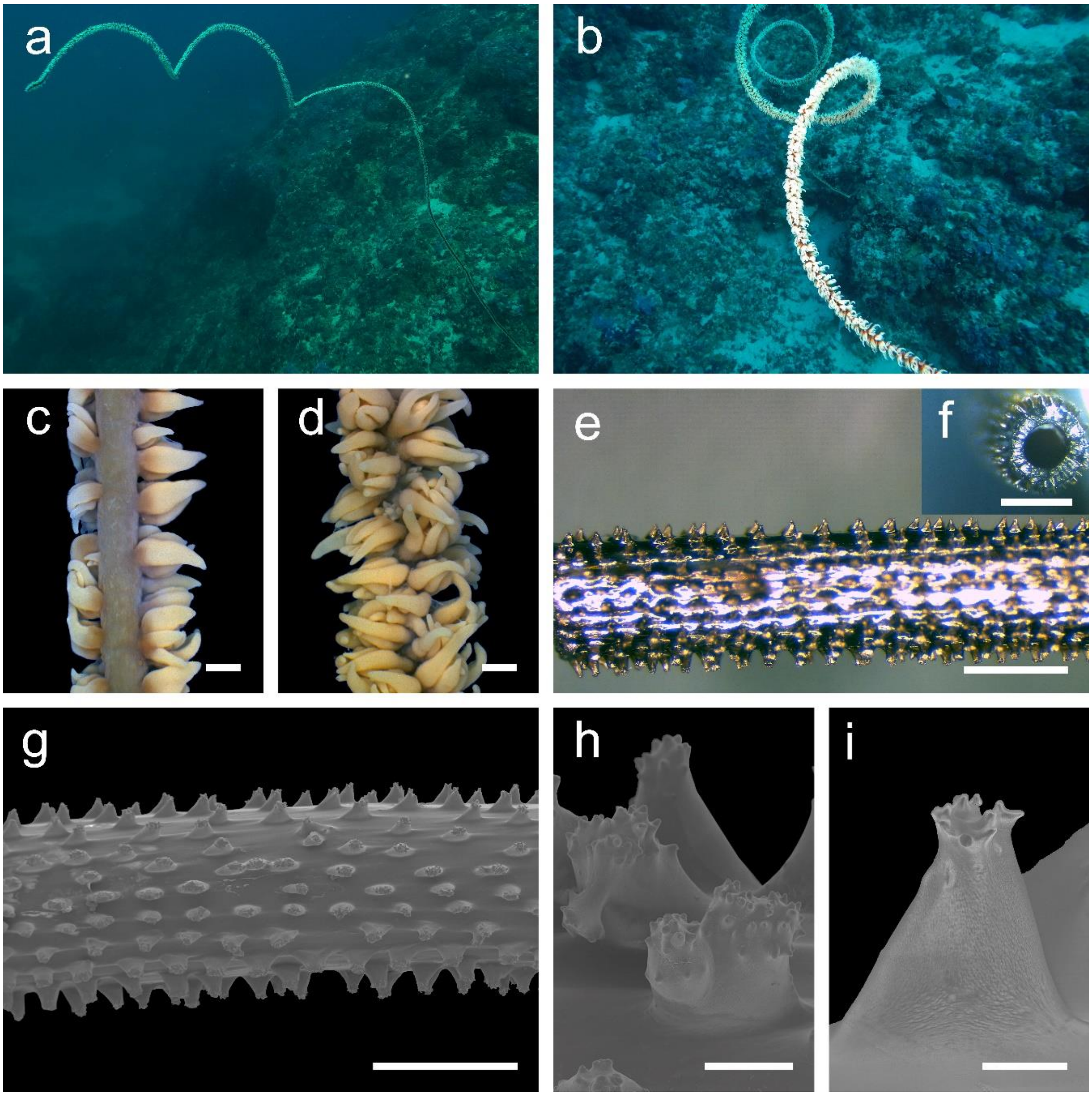

Figure 32. *Cirrhipathes rumphii*. (**a, b**) Underwater views of the entire colony measuring 1.8 m. (**c, d**) Views of the polyp arrangement along the corallum, with the polyps located on one side. (**e**) Corallum viewed with a stereomicroscope showing the arrangement of spines. (**f**) View of the large hollow core. (**g**) SEM view of the skeleton. (**h**) Abpolypar spines. (**i**) Polypar spines. Scales: c, d, e, f, g: 1 mm; h: 100 µm, i: 50 µm.

<u>Localisation.</u> Colony found in July 2016 in Ifaty at 20 m deep. Colony absent from the bed of Toliara, but present deeper than 45 m. Absent from the southern part of the bed of Toliara, and not frequent on the northern stations.

<u>Material examined.</u> Colony *in situ*, fixed fragment (M150716-M023-4), dry fragments (M150716-M023-S, M150716-M023-S2).

<u>Description.</u> A thin, single stem colony with the upper part having 3 large dextral coils (Fig. 32, a, b). It measures about 1.8 m in height and starts to coil at about 50 cm from the seafloor. The coils are spaced by about 40 cm and have a diameter of about 20 cm. The

skeleton is 1.5 mm in diameter. The coral is brown with white polyps disposed only one side of the colony (Fig. 32, c, d). They are not in a single row, they measure 1.2-2.5 mm and the calculated interpolypar distance is 1.3-3.15 mm, but the polyps are all very close to each other and they are not flattened. They are 2-4 on the same side, with smaller polyps occurring very often between the larger ones (Fig. 32, d). When expanded, the tentacles are long with round tips, and they are placed transversally to the corallum (Fig. 32, b). The skeleton has a large hollow core of about 0.65 mm and one type of spines (Fig. 32, e, f). The spines are disposed in quincunx and 11-12 rows can be seen from one aspect (Fig. 32, g). On the polypar side, the spines are conical, have a large basis with a roughly blunt apex, and measure 0.15-0.23 mm (Fig. 32, h). They are very finely papillose with the tip having many tubercles, and sometimes they can be bifid at the top. The abpolypar spines have the same shape but they differ in being sharper at the tip and smaller (Fig. 34, i), with a size of 0.1-0.16 mm. The interspine space seems to be regular, but vary between 0.25-0.6 mm.

Remarks. This species was previously recorded in the Indo-Pacific region at similar depths than the present species. This description corresponds to the one made by van Pesch (1910), which still stands the most complete description of *C. rumphii* to date. Because of that, many characters are not reliable such as the shape and size of the tentacles, since it strongly depends on the preservative and the carefulness used to collect and observe the specimen.

Cirrhipathes spiralis (Linnaeus, 1758)

Gorgonia spiralis: Linnaeus 1758, p.800.

Antipathes spiralis: Pallas 1766, p.217.

Cirripathes spiralis: Brook 1889, p.85, pl.7, fig.10; Silberfeld 1909b, p.18; Pax 1932, p.407; Opresko 1972, p.961.

Cirripathes (Eucirripathes) spiralis: van Pesch 1914, p.158, figs 216-241.

Cirrhipathes spiralis: Utinomi 1956, p.181, fig.3, Bayer 1959, p.229; Opresko 1974, p.14; Moon & Song 2008, p.210, fig.1.

Biogeography. Indian Ocean (Pallas 1766), Indonesia (Ellis & Solander 1786; Wagner *et al.* 2011), Korea (Moon & Song 2008), Hawaii (Clarke *et al.* 2015), Madagascar (present paper).

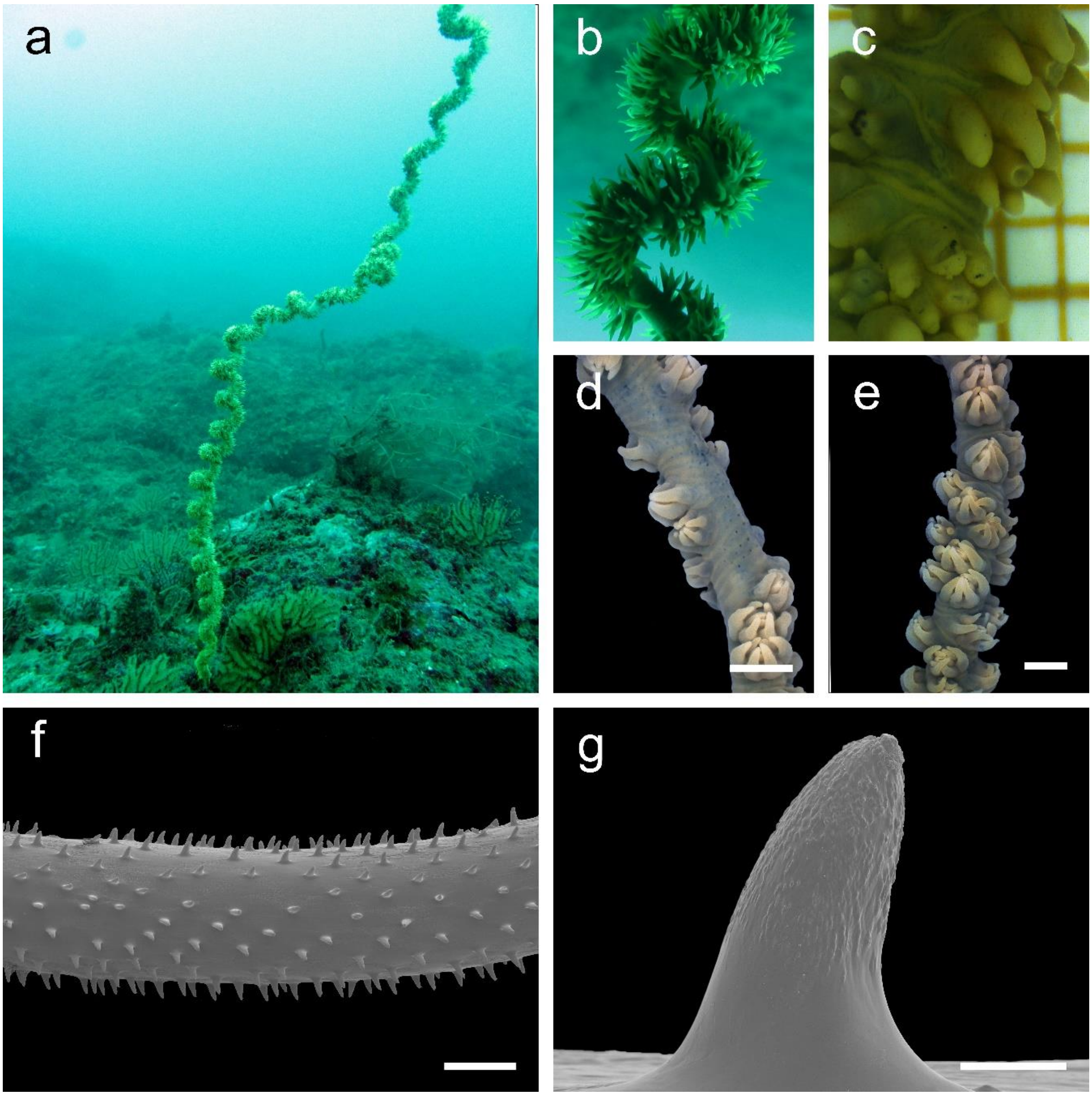

Figure 33. *Cirrhipathes spiralis*. (**a**) Entire colony measuring 50 cm in height. (**b**) Polyps fully extended. (**c**) Close-up view of the younger and older polyps. (**d, e**) Contracted polyps showing the varying interpolypar spaces. (**f**) General view of the skeleton spines. (**g**) Side view of the spine. Scales: c, d, e, f: 1 mm; g: 10µm.

Localization. Colony found at 25 m depth in December 2014 in the black coral bed of Toliara, not deeper. Not a frequent encounter. Species never seen on other locations.

Material examined. Colony *in situ*, dry fragment (M261114-PN005-1).

Description. The colony has tight dextral spirals which start coiling at about 3 cm from the base before being spaced by an average of 1.2 cm (Fig. 33, a). The colony is yellow and measures about 50 cm in height and the coils are about 1 cm in diameter. The skeleton diameter is about 3 mm and tapers slightly upwards. The polyps are found all around the colony and measures from 0.5 to 2 mm. They are irregularly spaced and the interpolypar distance can reach 3.5 mm, sometimes showing large gaps in the colony (Fig. 33, d, e). They have a prominent oral cone and the transverse canals are clearly visible (Fig. 33, c). Smaller polyps are often located between older ones (Fig. 33, c). The tentacles appear stocky when contracted, but they are long and their tip is acute when fully extended (Fig. 33, b, c). Because of the great variation in the interpolypar distance, the number of polyps per cm is different across the colony. The spines of the skeleton are conical, slightly tapering with a blunt apex (Fig. 33, f, g). They are papillose with fine knobs at the tip, and they are arranged in spirals (Fig. 33, f, g). Viewed from one aspect, one may figure out that spines are arranged in about ten rows, but the linearity could depend of the part of the observed colony. The outer spines measure 0.22-0.26 mm and are longer than the inner spines which are 0.17-0.21 mm in height. The spine spacing range from 0.15 to 0.7 mm. Remarks. Historically, this species has been described for the first time in the 18th century, in Norway, the Mediterranean Sea and the Indian Ocean. As mentioned by Lamouroux (1824), the two first locations might be from other species misidentified. The number of coils, their diameter and spacing in addition to the number of spine rows or spines spirals could depend on the age and height of the colony, making the present description different to the original descriptions in the literature. Other colonies from the same species could have loose coils with higher spaces between them.

Cirrhipathes sp. 1

Biogeography. Madagascar (present paper).

Localization. Colony found in Anakao in July 2016 at 10 m depth. Very rare as it is the only specimen found on all our observations.

Material examined. Colony *in situ*, fixed fragment (M200716-M025-3) and dry fragments (M200716-M025-S, M200716-M025-S2).

Description. A simple, very thin stem laying horizontally on a rock (Fig. 34, a, b). The coral is about 30 cm long, and it is naturally bent but not sinuous. The diameter of the skeleton is 0.76 mm including the spines and stays relatively constant along the colony. The colony is white with orange/brown polyps which stand in two distinct rows on a single side (Fig. 34, a, b). The polyps sit close together, and smaller polyps are often inserted between larger ones. They measure 0.5-1.5 mm and the interpolypar distance varies between 1 and 2 mm, depending on the size of the successive polyps (Fig. 34, c). The tentacles appear thick with a blunt apex. The oral cone is prominent and sometimes it can be as long as the

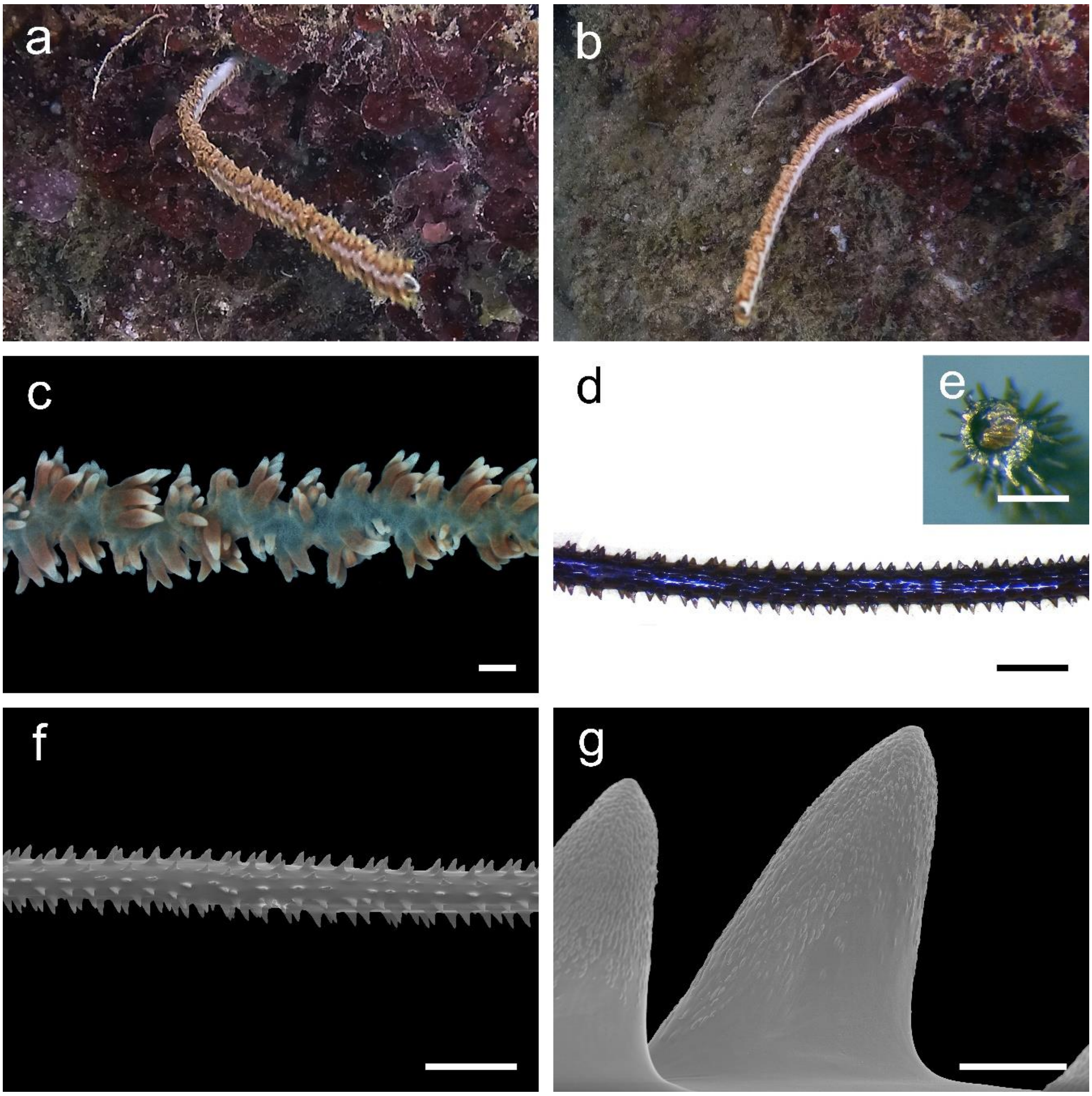

Figure 34. *Cirrhipathes* sp.1. (a, b) General views of the entire colony, showing the arrangement of the polyps and measuring 30 cm. (c) Zoom on the polyps. (d) Skeleton viewed with a stereomicroscope. (e) Transverse section of the skeleton showing the large core. (f) SEM view of the arrangement of the spines. (g) Side view of the spine. Scales: c, d, e, f: 1 mm; g: 10 µm.

lateral tentacles on the smaller polyps. On the corallum, there is a single type of spines which are conical, with a large basis and an acute tip (Fig. 34, d). They are clearly papillose on two thirds of their surface and slightly directed upwards (Fig.34, f, g). The spines are longer on the polypar side where they measure 0.15-0.2 mm, while on the abpolypar side they measure 0.10-0.17 mm. A spiral arrangement of the spines is not visible, but they are disposed in quincunx and 5-6 rows are visible from one aspect. The skeleton has a large hollow core of about 0.31 mm which is constant and represents about two thirds of the diameter (Fig. 34, e).

Remarks. This species is unusual by having two regular rows of polyps on a single side of the corallum. None of the descriptions of the *Cirrhipates* species match with the present diagnosis. The present diagnosis was compared to two species from the genus *Stichopathes* which have very similar spines, *S. dissimilis* Roule, 1902 and *S. echinulata* Brook, 1889, but the colonies differ in the shape and arrangement of the polyps, both having flattened polyps in a single row. Due to the small size of the whole colony, the coral may be young and thus explaining the morphological particularities.

Cirrhipathes sp. 2

Biogeography. Madagascar (present paper).

Localization. Morphotype 1. Colony found in Toliara in December 2015 at 17 m depth. One of the most abundant in Toliara and Sarodrano, rarer in northern locations from Toliara. Morphotype 2. Colony found in November 2015 in Toliara at 20 m depth. Abundant from Sarodrano to Ifaty, but rare in other locations. Morphotype 3. Colony found in July 2017 in Anakao at 11 m depth. A few colonies observed on this station, but never seen elsewhere. Morphotype 4. Colony found in November 2015 at 20 m depth in the black coral bed of Toliara. Abundant in Toliara and Sarodrano, but less common in other locations. Morphotype 5. Colony found in November 2015 in the black coral bed of Toliara at 18 m depth. Abundant in Toliara and Sarodrano.

Material examined. Morphotype 1. Colony *in situ*, fixed fragments (M261115-M007-1, M261115-M007-2), dry fragments (M261115-M007-S, M261115-M007-S2). Morphotype 2. Colony *in situ*, fixed fragments (M281115-M012-1, M281115-M012-3, M281115-M012-S, M281115-M012-S2, M281115-M012-S3). Morphotype 3. Colony *in situ*, fixed fragments (M200716-M024-3), dry fragments (M200716-M024-S, M200716-M024-S2). Morphotype 4. Colony *in situ*, fixed fragments (M281115-M011-1, M281115-M011-3), dry fragments (M281115-M011-S, M281115-M011-S2). Morphotype 5. Colony *in situ*, fixed fragments (M241115-M004-1, M241115-M004-2, M241115-M004-4), dry fragments (M241115-M004-S, M241115-M004-S2).

Description. Morphotype 1. The colony is straight, slightly sinuous, not forming any loop or spiral (Fig. 35, a). The colony is measuring 1.40 m in height, with a basal diameter of 4 mm. It can reach up to 3 m in height. The colony is brown with white polyps. The latter measure 1.11-2.92 mm and they are found all around the corallum (Fig. 35, f). They are spaced by 1.23-4.55 mm. The spines are slightly inclined (Fig. 35, k), they are conical

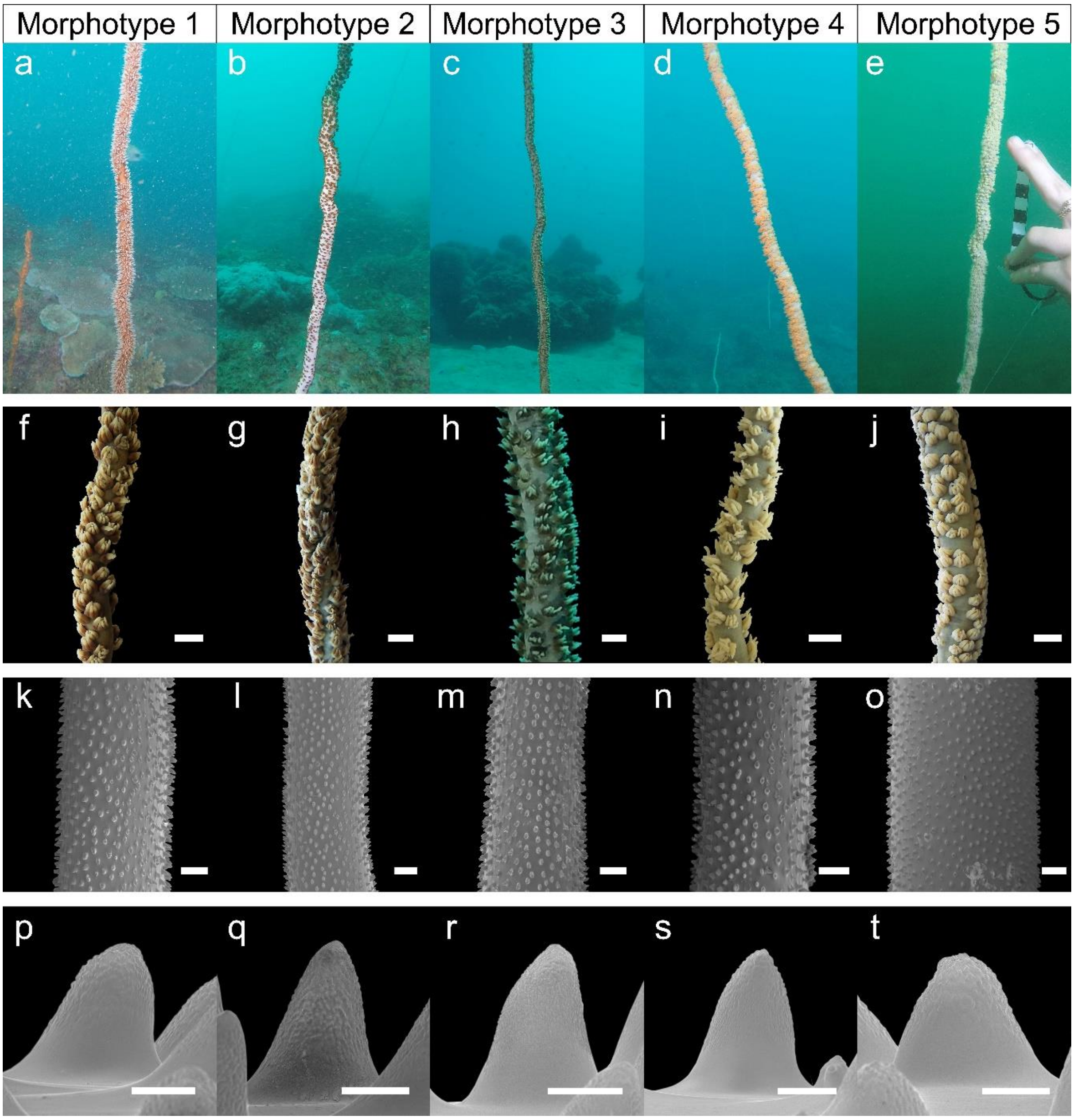

Figure 35. *Cirrhipathes* sp.2 morphotypes. (**a**-**e**) General views of the colonies measuring 1.4 m, 2.1 m, 2.0 m, 2.3 m and 1.8 m respectively. (**f**-**j**) Close-up views of the polyps. (**k**-**o**) General views of the skeletons. (**p**-**t**) Side views of the spines. Scales: f, g, h, i, j: 5 mm; k, l, m, n, o: 1 mm; p, q, r, s, t: 100 µm.

with a rounded apex and papillose on almost their whole surface (Fig. 35, p). They measure 0.10-0.32 mm and are spaced by 0.10-0.66 mm. They are arranged in about 20 irregular rows which might also be in irregular spirals. There are 7 spines per mm². The spines are sometimes different in their shapes, as they are very large and bifid at some places, but still having rounded apex and papillae on their surface. Secondary spines are present, they are minute, triangular or cylindrical, at right angle to the corallum or slightly inclined, and have papillae on their surface.

Morphotype 2. The colony is straight, slightly sinuous, not forming any loop or any spiral (Fig. 35, b). It measures 2.10 m in height and 5 mm in basal diameter, but it can reach up to 3 m. The colony is white with dark brown polyps (Fig. 35, g). The polyps are found all around the corallum with varying interpolypar spaces. The polyps measure 1.22-2.92 mm and are spaced by 1.07-3.88 mm. The spines are irregularly arranged and some of them are inclined in different ways (Fig. 35, l). They are conical, with a rounded apex and they are papillose of two third of their surface (Fig. 35, q). They measure 0.18-0.28 mm and are spaced by 0.07-0.74 mm. About 7-12 spines are counted per mm². About 20 rows may be counted from one aspect. The secondary spines are present, they are small, conical, papillose and similar to the primary spines.

Morphotype 3. The colony is straight, slightly sinuous, not forming any spiral or any loop (Fig. 35, c). It measures about 2 m in height and 8 mm in basal diameter. The colony is grey with brown polyps (Fig. 35, h). The polyps are distributed all around the stem; they measure 1.21-2.91 mm and are spaced by 1.35-6.78 mm. The spines are irregularly arranged and some of them are inclined in different ways (Fig. 35, m). They are conical, with a rounded apex and they are papillose of two third of their surface (Fig. 35, r). They measure 0.09-0.22 mm and are spaced by 0.05-0.39 mm. There are 6-8 spines per mm² and they are arranged in about 14 irregular rows. Secondary spines are present, they are either minute, smooth triangular spines, or cylindrical and papillose spines.

Morphotype 4. The colony is a straight and unbranched filament, slightly sinuous but not making any spiral or loop, and measuring 2.30 m long and 5 mm in diameter at the basis (Fig. 35, d). Sometimes these corals can measure up to 5 m. The colony is white with orange polyps which are irregularly distributed all around the stem (Fig. 35, i). They measure 0.71-2.30 mm and are spaced by 1.05-7.04 mm. The interpolypar distance can be high because of their random distribution on the corallum, sometimes showing large parts of the corallum without any polyp. The spines are subequal on the whole skeleton and measure 0.18-0.31 mm. They are spaced by 0.13-0.71 mm and are arranged in about 20 irregular rows (Fig. 35, n). The spines are thick, cylindrical, with a rounded or acute apex (Fig. 35, s). They are coarsely papillose on two thirds of their surface. There are 7-9 spines per mm². Secondary spines are present; they are minute, cylindrical, with an acute tip and have a papillose surface.

Morphotype 5. The colony is straight, very slightly sinuous, not forming any loop or spiral (Fig. 35, e). It is measuring 1.80 m in height, 1 cm in basal diameter and 4.5 mm in top

Table 8. Summary of the measurements made on the *Cirrhipathes* species. The last revision of the genus *Cirrhipathes* was made by van Pesch (1914) who only recognized 10 species and synonymized some descriptions. The genus *Cirrhipathes* contains 16 species: *C. anguina*, *C. contorta*, *C. densiflora*, *C. diversa*, *C. flagellum*, *C. gardinieri*, *C. hainanensis*, *C. musculosa*, *C. nana*, *C. propinqua*, *C. rumphii*, *C. secchini*, *C. sieboldin*, *C. sinensis*, *C. spiralis* and *C. translucens*. Most of these species were described between 1834 and 1910; some of the descriptions are poor and lack information while 3 type species are lost. Within these species, only *C. contorta, C. indica, C. propinqua, C. secchini* and *C. sinensis* have similar rounded and papillose spines than the malagasy species.

		Cirrhipathes sp.2									
	Cirrhipathes contorta	M1	M2	M3	M4	M5	*Cirrhipathes propinqua* Brook 1889	*Cirrhipathes secchini* Echeveria 2002	*Cirrhipathes sinensis* Zou & Zhou 1984	*Cirrhipathes contorta* Van Pesch 1910	*Cirrhipathes indica* Summers 1910
Author	This paper	This paper					Brook 1889	Echeveria 2002	Zou & Zou 1984	van Pesch 1910	Summers 1910
Polyp sizes (mm)	1.49-4.20	1.11-2.92	1.22-2.89	1.21-2.91	0.71-2.30	1.0-2.9	1.5	2.0-3.0	/	/	/
Interpolypar distances (mm)	1.12-4.66	1.23-4.55	1.07-3.88	1.35-6.78	1.05-7.041	1.08-4.10	/	~2.50	/	3	/
Spine sizes (mm)	0.16-0.31	0.10-0.32	0.18-0.28	0.09-0.22	0.18-0.31	0.06-0.23	irregular?	0.14-0.32	0.30-0.64	0.11-0.15	/
Interspine distances (mm)	0.15-0.69	0.10-0.66	0.07-0.74	0.05-0.39	0.13-0.71	0.14-0.62	/	0.36-0.59	0.34-0.74	0.52	/
Spine density	5-6/mm^2	7/mm^2	7-12/mm^2	6-8/mm^2	7-9/mm^2	5-7/mm^2	/	/	/	/	/
Number of spine rows	~11	~20	~20	~14	~20	~26	14-16	/	15	10-15	24-30
Diameter (mm)	2.62	3.99	3.49	3.33	3.54	6	3.75-4.25	/	4.00-4.40	2.25-3.50	2
Secondary spines	No	Yes	Yes	Yes	Yes	Yes	/	Yes	Yes	/	/

diameter. It is completely white. The polyps are arranged all around the stem, they measure 1.00-2.90 mm and are spaced by intervals of 1.08-4.10 mm (Fig. 35, j). The spines are conical, rounded and papillose on almost their whole surface (Fig. 35, o). They measure 0.06-0.23 mm and they are spaced by 0.14-0.62 mm. There are 5-7 spines per mm². About 26 irregular rows can be seen. Secondary spines are present, either they are very cylindrical and papillose or they are minute, triangular and slightly papillose (Fig. 35, t).

Remarks. All these specimens characterized by an unbranched skeleton with polyps all around the axis, with conical and papillose spines on the skeleton. None original description of the *Cirrhipathes* species is matching with the present specimens (see Table 8). Within the 16 *Cirrhipathes* species described to date, the original description of *C. propinqua* appears to be the most similar to the present species, but it is impossible to identify it as *C. propinqua* as the authors did not mention spine sizes and density or interspine spaces, and the original drawing do not allow any comparison. The colour and the polyps discriminate them on the field.

Genus *Stichopathes* Brook, 1889

The genus *Stichopathes* encompasses unbranched corals but they differ from previous genera in having polyps arranged in a single row on one side of the corallum. Even if the homology of this character has been questioned by several authors (Pasternak 1977; Bo 2008; Bo *et al*. 2012), it remains the only character for the distinction of this group. Like *Cirrhipathes* species, *Stichopathes* species can be straight, slightly to highly contorted, curved or coiled. The spines are smooth, papillose or with tubercles.

Stichopathes alcocki Cooper, 1909

Cirrhipathes diversa: Brook 1889, p.77, 82, 85, 87-88, 184, Pl.XII, fig.12.

Stichopathes alcocki: Cooper 1909, p.305-306, fig.3-4.

Stichopathes diversa: Summers 1910, p.275-276.

Biogeography. Ceylon (Brook 1889), Mozambique (Summers 1910), Maldives (Cooper 1903), Madagascar (present paper).

Localization. Colony found at 52 m depth in July 2016. Off the black coral bed of Toliara. This species is found between 45 and 52 m depth, but never in shallower waters. Only recorded in deep beds (>45 m) off the reef of Toliara.

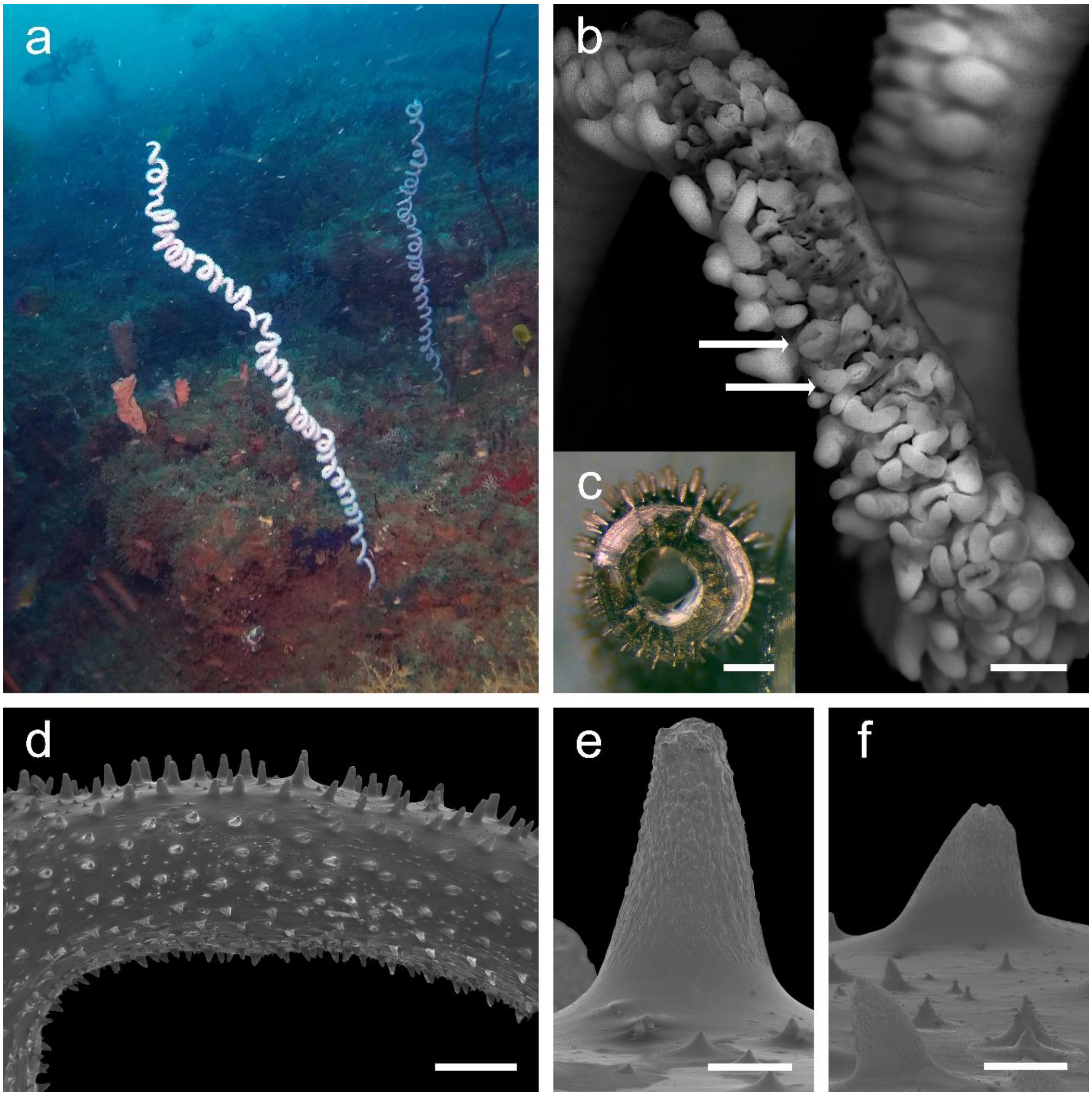

Figure 36. *Stichopathes alcocki*. (**a**) General view of the colony measuring 35 cm. (**b**) Close-up view of the polyps which are found on the external side of the coils. The white arrows are showing the alternation between bigger and smaller polyps. (**c**) Skeleton under stereomicroscope showing the large hollow core of the skeleton. (**d**) SEM which shows the size difference between polypar spines (the external ones) and abpolypar spines (the internal ones). (**e**) Side view of the blunt papillose polypar spines knobby at the tip with the secondary spines all around. (**f**) Side view of the abpolypar spine. Scales: b, c, d: 1 mm, e, f: 100 µm.

Material examined. Colony *in situ*, fixed fragments (M270716-PN282-1, M270716-PN282-3, M270716-PN282-4), dry fragments (M270716-PN282-S, M270716-PN282-S2).

Description. The colony is highly coiled with dextral spirals that are regularly spaced by an average of 1.85 cm, unless in case of a breaking event which can eventually leads to the restart of the coiling process in a different direction (Fig. 36, a). The spirals start to coil at about 3 to 4 cm from the base and have an average diameter of about 1.5 cm. The corallum is measuring about 35 cm with a basal diameter of 4.4 mm including the spines. It becomes slightly tapered from the base upwards.

The colony appears very white when lighted, and the polyps are found on a single row on the outer side of corallum (Fig. 36, a). The row is not perfect since sometimes the mouths are not perfectly aligned. On different locations on the colony, smaller polyps are inserted between bigger ones (Fig. 36, b). There are crowded (5-6 polyps per cm) and laterally flattened, with no interpolypar spaces since the tentacles of each polyp are touching the next one. Polyps measure from 0.6 mm to 1.2 mm, have a prominent oral cone with their sagittal tentacles bigger than the lateral ones. On the skeleton, there are two kinds of spines (Fig. 36, d, e, f). The larger spines are blunt, papillose and knobby at the apex. These spines are longer on the outer side of the corallum and disposed at a right angle to the corallum. They correspond to the polypar spines and measure up to 0.4 mm (Fig. 36, e). The inner spines (abpolypar) measure up to 0.2 mm. All these spines are separated by a second type of spines, which are very small, smooth and triangular spines (Fig. 36, f). The primary spines density is 7-10/mm², and there are 4-7 primary spines per mm when looking at the edge of the coral when the corallum is oriented to see both polypar and abpolypar spines. The species has a large hollow core (Fig. 36, c).

Remarks. The species has been previously observed in Mozambic. The description of the present specimen corresponds to those of Brook 1889, Cooper 1903, Cooper 1909 and Summers 1910. The species corresponds to the "Clade C" of the complex formed by *Stichopathes* species as described by Bo *et al*. 2012.

Stichopathes papillosa Thomson & Simpson, 1905

Stichopathes papillosa: Thomson & Simpson 1905, p.100, figs.6-13.

Stichopathes papillosa: Cooper 1909, p.304.

Biogeography. Ceylon (Thomson & Simpson 1905), Andamanislands (Cooper 1909), Madagascar (present paper).

Localization. Colony found at 27m depth in December 2014 in Toliara. Rarely found in the black coral bed of Toliara. The species was never seen on other locations.

Material examined. Colony *in situ*, dry fragments (M281114-PN014, M281114-PN014S).

Description. The colony is coiled with destral spirals that are regularly spaced by an average of 1 cm. (Fig. 37, a). The spirals start to coil at about 3 to 4 cm from the base and have

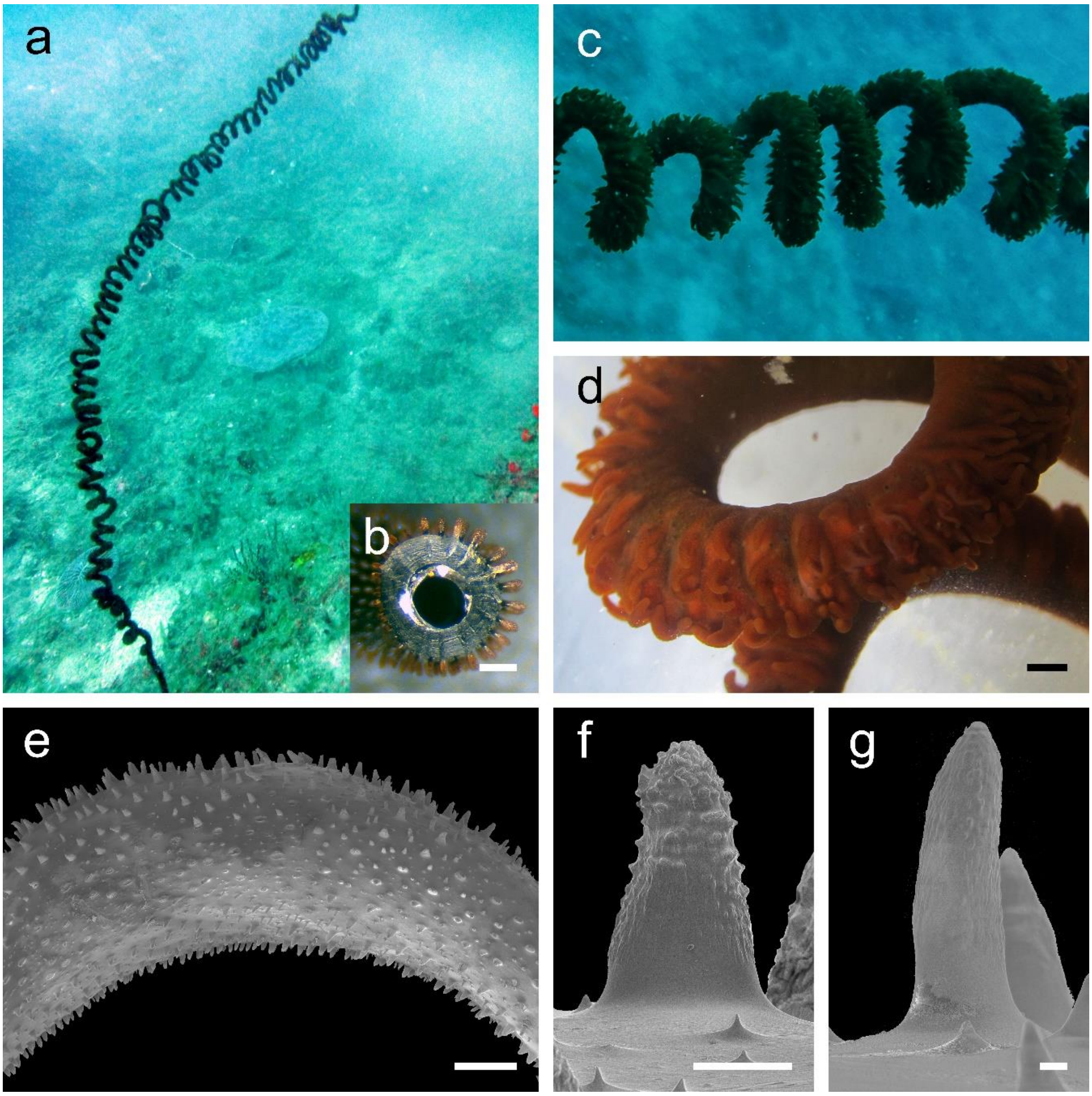

Figure 37. *Stichopathes papillosa*. (a) General view of the colony measuring 57 cm. (b) Large hollow core of the corallum. (c, d) Close-up views of the polyps. (e) General view of the spines arrangement. (f) Side view of the polypar spine which is tuberculated, small triangular spines can be seen. (g) Side view of the abpolypar spines, which are finely papillose and smaller than the polypar ones. Scales: b, d, e: 1 mm, f: 100 µm, g: 10 µm.

an average diameter of 1.5 cm. About 6 spirals are seen every 5 cm (Fig. 37, c). The corallum is measuring about 57 cm in length with a basal diameter of 4 mm including the spines. The skeleton diameter is constant upwards. The colony is entirely dark brown with the polyps arranged on a single row on the outer side of the corallum. The polyps are crowded and laterally flattened. Sometimes, a smaller polyp can be seen between two bigger ones although it does not seem to be disposed in a regular manner with 7-8 polyps per cm (Fig. 37, c, d). The tentacles are blunt and the lateral ones are of the same size with sagittal ones being bigger, though it could depend of the state of contraction of the polyp. They measure about 1 mm and the separation between 2 polyps is sometimes visible by the presence of a groove (Fig. 37, d). Three kinds of spines are present at right angle to the corallum (Fig. 37, e). The primary spines are blunt, conical, papillose and knobby at the apex, they are longer on the polypar side of the skeleton and measure up to 0.31 mm (Fig. 37, f). The second ones are smaller, elongated and finely papillose (Fig. 37, g). They vary in size but reach 0.2 mm. The third type of spines is small, smooth and triangular and is disposed between all the other spines on all the corallum. The primary spine density is 11-13/mm² and there are 7-13 spines per mm when looking at the edge of the coral when the corallum is oriented to see both polypar and abpolypar spines. The species has a large hollow core (Fig. 37, b).

Remarks. This species was previously described in the Indian Ocean (Sri Lanka and Andaman Islands). It is very similar to the previous *Stichopathes alcocki*, and despite the colour, it is almost impossible do discriminate both underwater without looking at the spines. They mainly differ in spines density, which are more numerous in *S. papillosa*. The latter has also a different type of spines which are finely papillose but smaller and not conical. The present species also corresponds to the "Clade C" of the complex formed by *Stichopathes* species as described by Bo *et al*. 2012.

Stichopathes sp. aff. *maldivensis*

Stichopathes maldivensis: Cooper 1903, pl. XLV, fig. 4.

Stichopathes cf. *maldivensis*: Bo et al. 2012, figs. 3, 5.

Biogeography. Maldives (Cooper 1903), Indonesia (Bo et al. 2012), Madagascar (present paper).

Localization. Color type 1. Colony found at 17 m depth in November 2015 in Toliara. A common encounter in the bed of Toliara, less frequent in the whole southwestern coast. Color type 2. Colony found in November 2015 at 24 m depth in the bed of Toliara. One of the most abundant species of the whole southwestern coast.

Material examined. Color type 1. Colony *in situ*, fixed fragments (M261115-M006-1, M261115-M006-3), dry fragments (M261115-M006-S, M261115-M006-S2). Color type 2. Colony *in situ*, fixed fragments (M251115-M005-1, M251115-M005-2, M251115-M005-3, M251115-M005-4), dry fragments (M251115-M005-S, M251115-M005-S2).

Description. Color type 1. A thick and straight but sinuous colony measuring 1.6 m in length with a diameter of 7.5 mm at the basis and 4.7 mm at the top (Fig. 38, a).

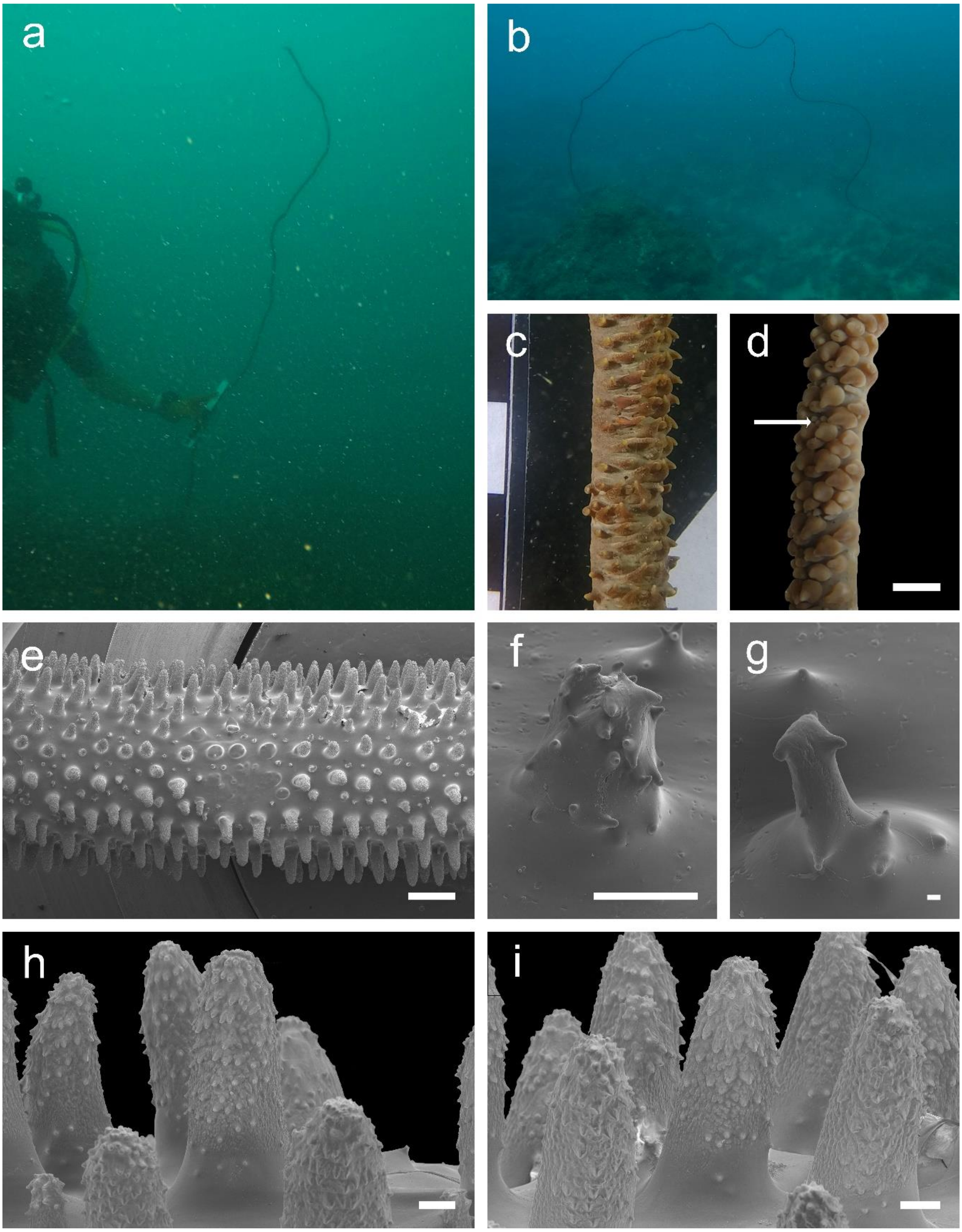

Figure 38. *Stichopathes* sp. aff. *maldivensis* colortype 1. (**a, b**) General views of the different morphologies of the species, the one examined (a) measuring 1.6 m. (**c**) Close-up view of the contracted polyp underwater. (**d**) Polyps seen under a dissecting microscope, showing the large tentacles and the absence of interpolypar space. The white arrow indicates the presence of a small polyp. (**e**) General view of the skeleton by SEM. (**f, g**) Secondary spines. (**h**) Side view of the polypar spines which are tuberculated on their whole surface. (**i**) Side view of the abpolypar spines. Scales: a: 10 cm; c, d: 1 cm; e: 1 mm; f, h, i: 100 µm, g: 10 µm.

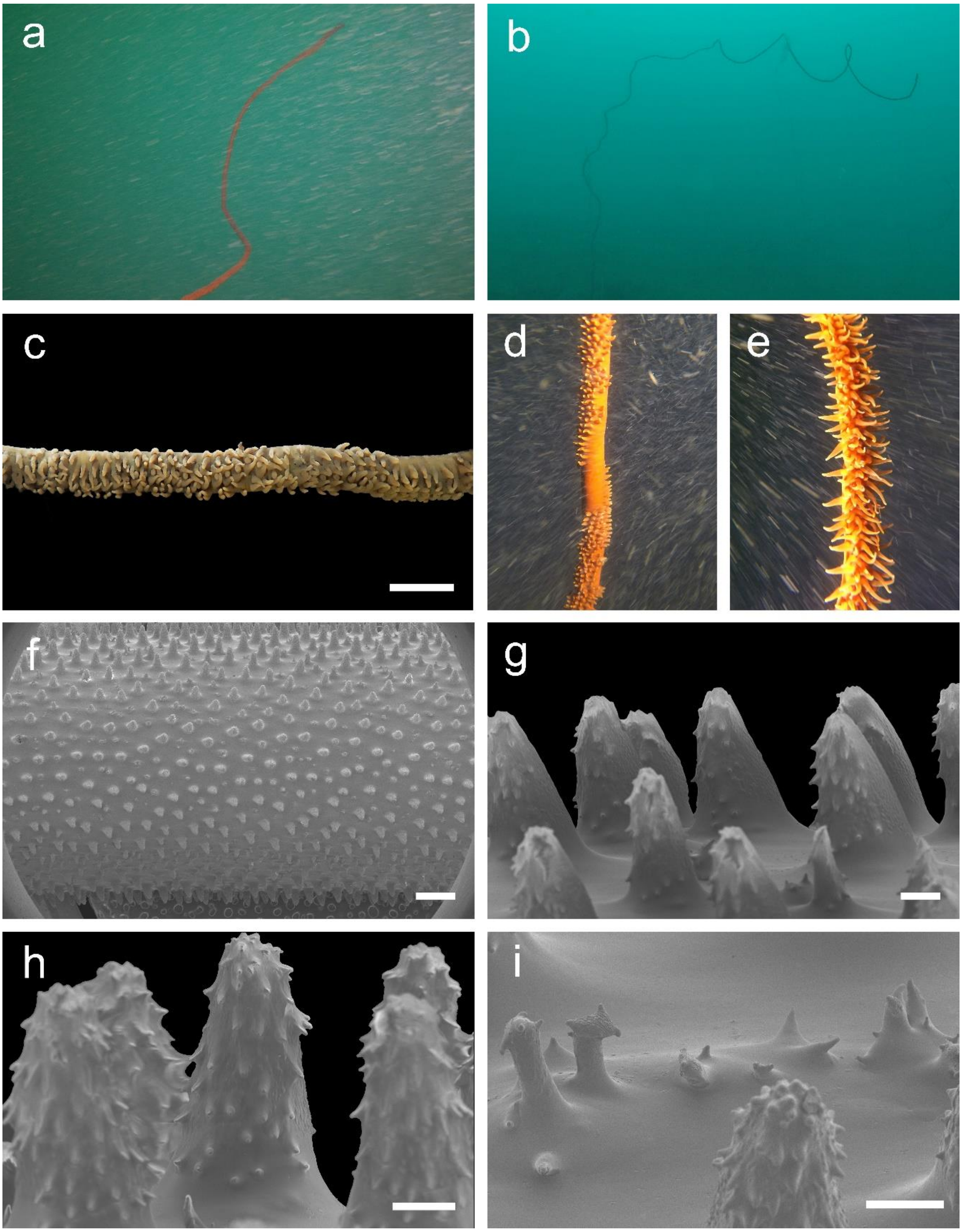

Figure 39. *Stichopathes* sp. aff. *maldivensis* colortype 2. (a) Colony collected for the diagnosis, which does not have any loop and measures 2.35 m. (b) Same species forming loose loops. (c) Contracted polyps. (d) Polyps twisting around the stem. (e) Expanded polyps captured during night time. (f) General organization of the spines. (g) Side view of the abpolypar spines. (h) Side view of the polypar spines. (i) Secondary spines. Scales: c: 1 cm; f: 1 mm; g, h, i: 100 µm.

The colony can reach several meters in length and can sometimes have curves which are not real spirals (Fig. 38, b). The coral is white with the polyps in a single row (Fig. 38, c). They are crowded and measure 2.0-4.5 mm, but very small polyps can be found between the larger ones in an irregular way (Fig. 38, d). There is no interpolypar space. The oral cone is prominent, and the tentacles are thick. The latter are

so big that sometimes the next polyp needs to be shifted beyond the alignment of the polyps.

Two types of spines are found on the corallum (Fig. 38, e, f, g, h). The primary spines are unusually long, cylindrical and disposed in quincunx nearly at right angle to the corallum (Fig. 38, e, h). There are about 9-10 rows visible from one aspect. They measure between 0.4 and 0.87 mm on the polypar side. On the abpolyar side, they have the same morphology, but they are smaller, with a size of 0.18-0.4 mm (Fig. 38, e, i). The secondary spines are small but very irregular in their shape. Either they are cylindrical, conical or triangular and have tubercles only at the tip or on their whole surface (Fig. 38, f, g).

Color type 2. The colony is a thick and straight coral measuring 2.35 m with a basal diameter of 8.5 mm and 6.6 mm at the top. It is sinuous (Fig. 39, a) but sometimes colonies forming loose loops are found (Fix. 39, b) and they can reach 5 m long. It is brownish and very similar in shape and size as the previous morphotype. The polyps are crowded and arranged in a single row (Fig. 39, c), 6 of which can be counted per cm. They can twist around the stem (Fig. 39, d). When contracted, their tentacles are small, thick, rounded and subequal; their position close to each other makes difficult to tell at which polyp the tentacles belong (Fig. 39, e) and the mouth and the tentacles are almost aligned in the same plan (Fig. 39, c). When they are expanded, they are long and finger-like (Fig. 39, e). The polyps measure 1.7-2.9 mm and the interpolypar distance was not measured as all the polyps sit next to each other. The spines of the skeleton are of two types and they are arranged in irregular spirals that are difficult to see (Fig. 39, f). The primary polypar spines are cylindrical and very tuberculate, they measure 0.42-0.43 mm and are spaced by 0.33-0.63 mm (Fig. 39, h). The primary abpolypar spines are more conical and they are tuberculate on one side and papillose on the other side (Fig. 39, g), they measure 0.27-0.41 mm and are spaced by 0.38-0.70 mm. The secondary spines are irregularly present on the whole skeleton, they have a lot of different morphologies and are only measuring a few µm (Fig. 39, i). The density of the primary spines is 4-5/mm².

Remarks. This species belongs to the “clade D” of the complex described by Bo *et al.* (2012). Despite the lack of knowledge on whip black corals taxonomy, they tentatively identify the clade as *Stichopathes* cf. *maldivensis*. It appears very similar in appearance with the present species (Bo *et al.* 2012). *S. maldivensis* was originally collected in the Maldives at 67 m. It was described by Cooper in 1903 who stated a straight, white colony with two types of spines. The secondary spines were small triangles scattered irregularly on the corallum. The present species differ by having a different type of secondary spines and much longer primary spines.

Family Myriopathidae Opresko, 2001

This family was established to differentiate branched black corals from the Antipathidae family which are closely related, but differ in various points (see Opresko 2001 for more details and comparisons between the families). The group is characterized by polyps having 10 mesenteries and measuring 0.5 to 1 mm in transverse diameter. They have short tentacles with a rounded tip, and acute, conical to blade-like spines up to 0.3 mm tall on the smallest banchlets or pinnules, and cylindrical, simple, forked or antler-like spines on the larger banches and stem (Opresko 2001). In this family, the spines cannot be used as identification criteria to distinguish the different genera, which are established based on colony morphology and pinnulation pattern.

Genus *Myriopathes* Opresko, 2001

The genus was erected as a family, the Myriopathidae, by Opresko (2001). The type species *Antipathes myriophylla* Pallas, 1766 and a neotype has been described from Ambon, Indonesia by Opresko (2001). Species from this genus are characterized by a corallum flabellate or bushy, the stem and the branches are pinnulate to the second order or more with the primary pinnules arranged biserially and alternately in two lateral or anterolateral rows. The secondary pinnules are uniserial at the base of primary pinnules but become biserial distally. The uniserial secondary pinnules usually project out of plane formed by the biserial primary pinnules (Opresko 2001).

Myriopathes ulex (Ellis & Solander, 1786)

Antipathes ulex: Ellis & Solander 1786, p.100; Brook 1889, p.167, pl.11, fig.5; Opresko 1974, p.49; 1976, p.239; Grigg & Opresko 1977, p.244, p.255, fig.15; Grigg 1993, p.50, p.56.

Antipathes (Euantipathes) ulex: van Pesch 1914, p.79, fig.62.

Myriopathes ulex: Opresko 2001, p.349, p.351-352; Bo 2008; Moon & Song 2008, p. 259-261, fig.5; Wagner *et al.* 2011a, p.249-255, fig.3; 2011b, p.1325; 2011c, p.212, p.214; 2012, p.67-132, fig.2.1b, p.796; 2015, p.6-7, p.17-18.

Biogeography. Indonesia (Ellis & Solander 1786), Indian Ocean (Lamouroux 1816), Philippines (Gray 1857), Korea (Moon & Song 2008), Hawaii (Wagner 2015a), Madagascar (present paper).

Localization. Colony found in July 2016 in Toliara at 19 m depth. Frequent in the bed of Toliara down to 50 m, very abundant in Soalara. Never observed northern to Toliara.

Material examined. Colony *in situ*, fixed fragments (M080716-M018-1, M080716-M018-3, M080716-M018-4), dry fragments (M080716-M018-S, M080716-M018-S2, M080716-M018-S3).

Description. The colony is branched and measures about 50 cm in height, but it has been observed with higher sizes (Fig. 40, a). The branching is planar and flabellate, and the colony appears dark brown. The primary pinnules measure 1.62-16.92 mm and are inclined 39-85° to the branch. The secondary pinnules are inserted uniserially, measure 0.38-5.00 mm and are inclined 20-90° to the primary pinnule. When inserted biserially, they become primary pinnules and the primary pinnule bearing the secondary pinnules becomes a branch. Tertiary pinnules are not present (Fig. 40, c). The polyps are located on a single side of the pinnules, except on thicker branches where they can be all around (Fig. 40, b). They are not elongated and measure 0.43-0.92 mm. They are spaced by 0.41-1.34 mm and about 10-12 are found per cm. The tentacles are short, thick and rounded at the apex. The mouth is prominent and very large compared to the tentacles (Fig. 40, b). The spines of the skeleton are needle-like and slightly papillose (Fig. 40, d, e, f, g). On thicker branches, they are forked either at the apex or the basis (Fig. 40, e). They have sometimes a larger width near the top than near the basis (Fig. 40, f). They measure 0.10-0.27 and are spaced by 0.05-0.40 mm. Depending on the diameter of the branch, 4-7 rows of spines can be seen. On smaller pinnules, the spines are more cylindrical, measure

0.07-0.16 and are spaced by 0.06-0.23 mm (Fig. 40, g, h). The tip of the spines is very variable as it can be completely rounded, sharp or forked.

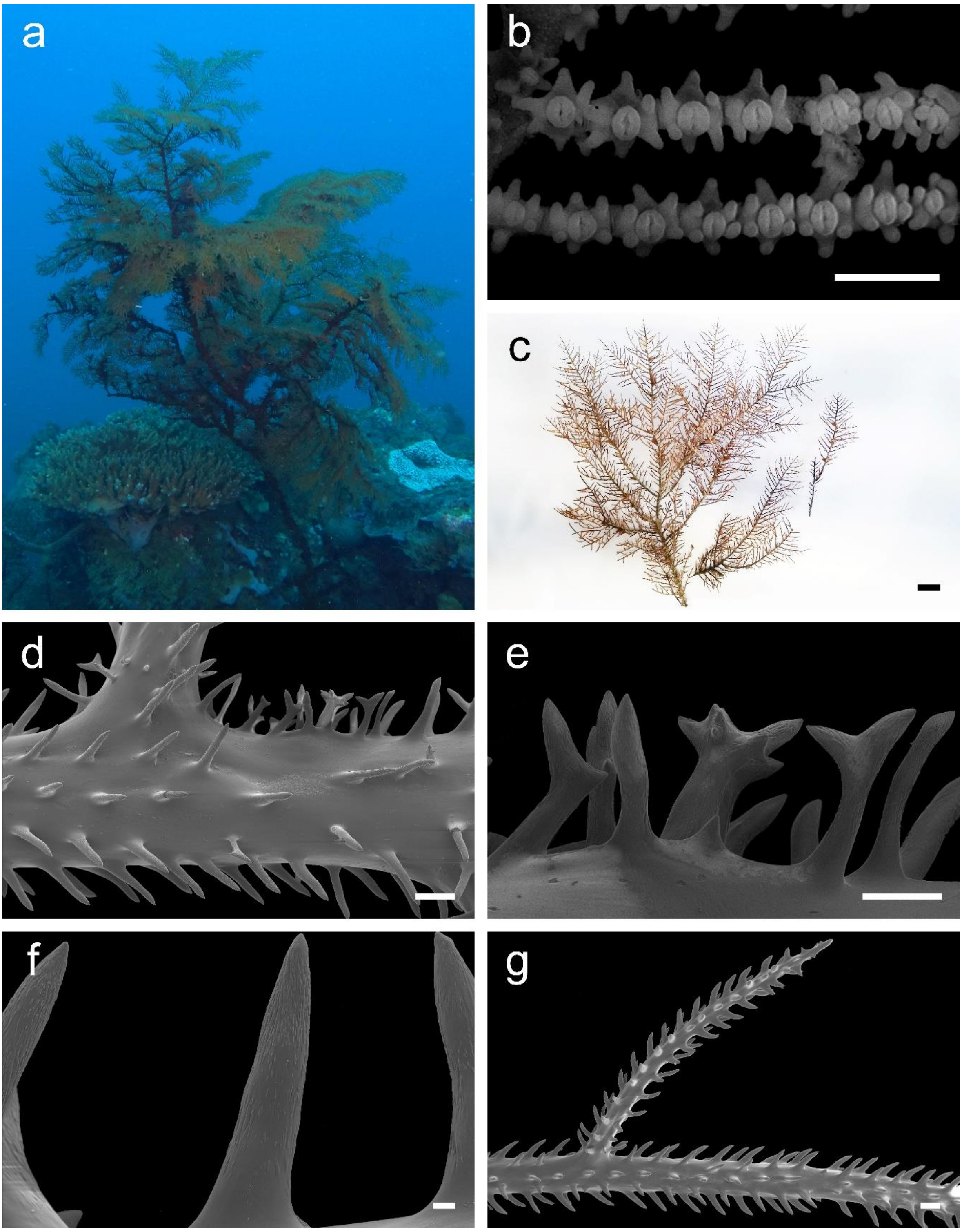

Figure 40. *Myriopathes ulex*. (a) Entire colony measuring 50 cm. (b) Polyps. (c) Piece of the corallum. (d) Skeleton of a primary pinnule. (e) Side view of the spines morphologies on a primary pinnule. (f) Side view of a spine on the primary pinnule. (g) Side view of secondary and tertiary pinnules. Scales: b: 1 mm; d: 100 µm; e, f, g, h: 10 µm.

Remarks. The type specimen is lost, but the present description is similar to those of Moon & Song (2008) and Wagner (2015a). *Myriopathes ulex* is one of those harvested in Hawaii (Grigg 1993; Wagner *et al*. 2011a). It was originally described from Indonesia and then reported throughout the Indo-West Pacific between 25 and 364 m depth (Blainville 1834; Gray 1857; Brook 1889; Van Pesch 1914; Grigg & Opresko 1977; Bo 2008; Moon & Song 2008).

Myriopathes stechowi (Pax, 1932)

Aphanipathes stechowi: Pax 1932, p.436, fig.16; Utinomi 1965, p.300, fig.468; Opresko 1974, p.27.

Myriopathes stechowi: Opresko 2001, p.349; Moon & Song 2008, p.256, fig.4.

Biogeography. Japan (Pax 1932), Korea (Moon & Song 2008), Madagascar (present paper).

Localization. Colony found in December 2016 in the bed of Toliara at 25 m depth. Not common.

Material examined. Colony *in situ*, fixed fragments (M041215-M014-1, M041215-M014-2, M041215-M014-3), dry fragments (M041215-M014-S, M041215-M014-S2).

Description. The colony is flabellate and pinnulate and measures about 20 cm high and 10 cm wide with a basal diameter of 2.5 mm (Fig. 41, a, b). The colony is branched in the same plan. The branches are pinnulate to the 3rd order. The primary pinnules are bilaterally alternated on the branch (Fig. 41, c). They are spaced 1.2-3.0 mm apart and 8-12 primary pinnules are counted per cm on both side. They are up to 1 cm long with a basal diameter of less than 0.1 mm. They are at 46-76° to the branch axis. The secondary pinnules, when present, are uniserial on the same primary pinnule but not regarding the whole colony. They are at 30-60° to the primary pinnule and sometimes in a perpendicular plan to the one formed by the branches and the primary pinnules. These secondary pinnules are up to 5 mm long with a basal diameter less than 0.1 mm. Tertiary pinnules are sometimes present. The polyps are disposed on a single side of the axes and they measure 0.37-0.66 mm (Fig. 41, d). They are spaced by 0.60-1.60 mm and about 9-10 polyps are counted per cm. They are not elongated, the mouth is large and the tentacles appear thick and blunt. The sagittal tentacles are slightly longer than the lateral ones when the polyp is contracted.

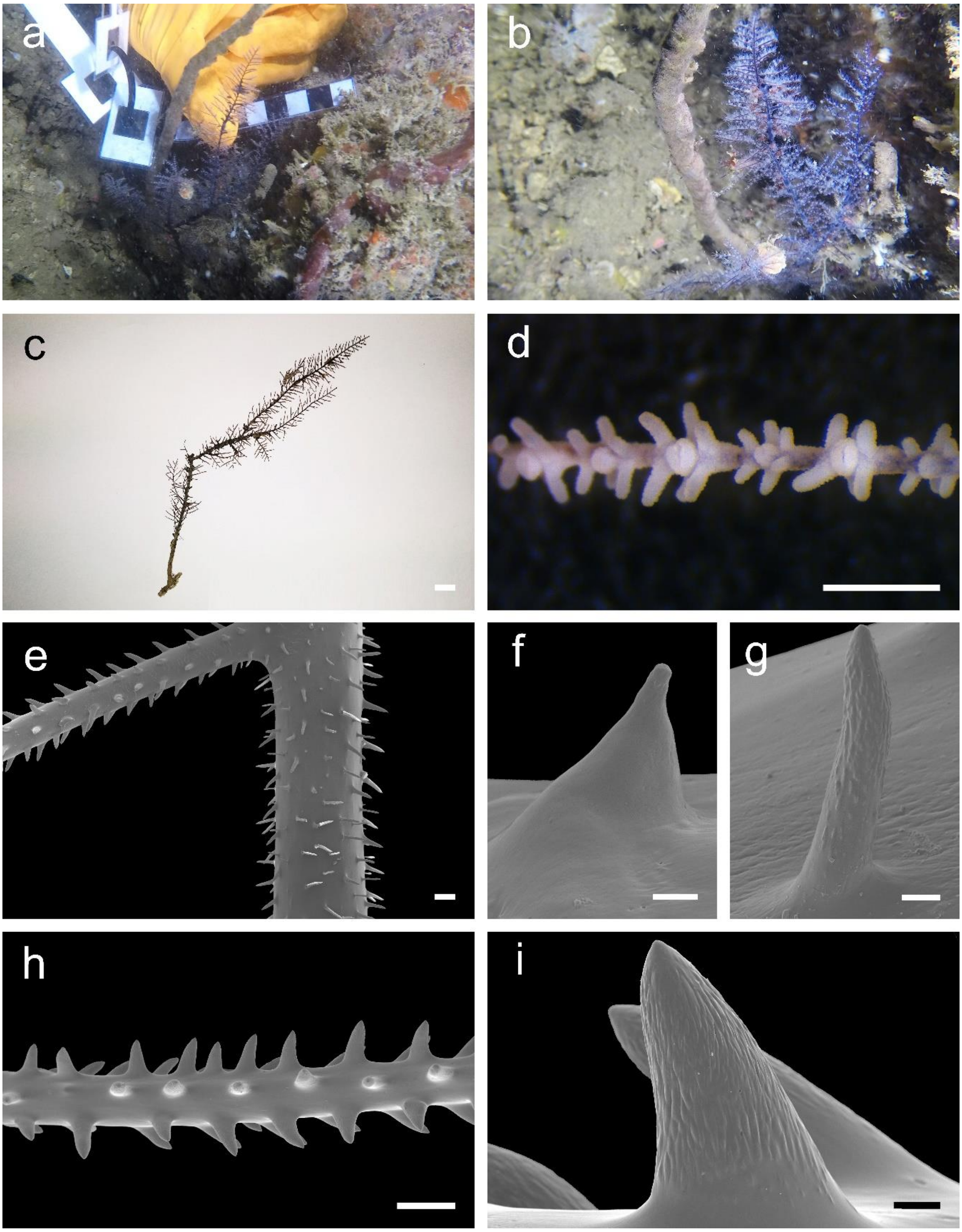

Figure 41. *Myriopathes stechowi*. (**a, b**) Entire colony. (**c**) Picture of a piece of the dried skeleton showing the arrangement of the pinnules. (**d**) Polyps. (**e**) General view of the arrangement of the spines on thicker branches. (**f, g**) Spine morphologies on thicker branches. (**h**) Spine arrangement on the pinnules. (**i**) Side view of the spine on the pinnules. Scales: a: 1 cm; d: 1 mm; e, h: 0.1 mm. f, g, i: .0.01 mm.

The spines of the pinnules are conical, horn-shaped with an acute tip (Fig. 41, h, i). They are papillose and they have different inclinations (Fig. 41, h). They measure 0.04-0.18 mm and 5 rows can be viewed from one aspect. They are spaced by 0.04-0.21 mm. On the thicker branches, their shape and size are different but they are also papillose (Fig. 41, e, f, g). There are needle-like spines with a basal width constant upwards and more conical spines width a larger basis and an acute tip. They measure up to 0.18 mm and are spaced by 0.03-0.22 mm. These spines are also inclined in different ways. Sometimes, triangular spines can be seen (Fig. 41, f).

Remarks. This diagnosis is similar to the description of *Myriopathes stechowi* made by Moon & Song (2008), while the original description made by Pax (1932) is more difficult to compare due to the lack of information in the original paper.

Genus *Cupressopathes* Opresko, 2001

This genus is characterized by a bottle-brush pinnulation with four very irregular or quasi-spiral rows of primary pinnules, and uniserial, bilateral or irregularly arranged in higher order pinnules. In this study, one known species has been found with three others that do not match any of the existing descriptions.

Cupressopathes abies (Linnaeus, 1758)

Gorgonia abies: Linnaeus 1758, p.1290.

Antipathes: Pallas 1766; Esper 1792, 1797; Lamouroux 1816, 1824; Lamarck 1816; Dana 1846; Gray 1857; Edwards & Haime 1857; Brook 1889; Thomson & Simpson 1905; Cooper 1909; Summers 1910; van Pesch 1914.

Cupressopathes abies: Opresko 2001, p.352-358.

Biogeography. Ceylon (Thomson & Simpson 1905), Seychelles (Cooper 1903), Mozambique (Summers 1910), Indonesia (van Pesch 1914), Philippines (Opresko 2001), Madagascar (present paper).

Localization. Colony found in November 2015 at 24 m depth in the bed of Toliara. Not frequent in this location, never observed outside the bed of Toliara.

Material examined. Colony *in situ*, fixed fragments (M231115-M002-1, M231115-M002-8, M231115-M002-9, M231115-M002-11), dry fragments (M231115-M002-S, M231115-M002-S2, M231115-M002-S3, M231115-M002-S4, M231115-M002-S5).

Description. Monopodial colony with a bottle-brush shape, measuring 35 cm high and 10 cm wide (Fig. 42, a). The basal diameter is about 4.5 mm. The primary pinnules are found nearly in 4 rows along the stem. They are inserted at nearly 90° to the stem and grow horizontally by being naturally bent. The primary pinnules always have subpinnules in a quite irregular distribution. These subpinnules are always on the same side of the primary

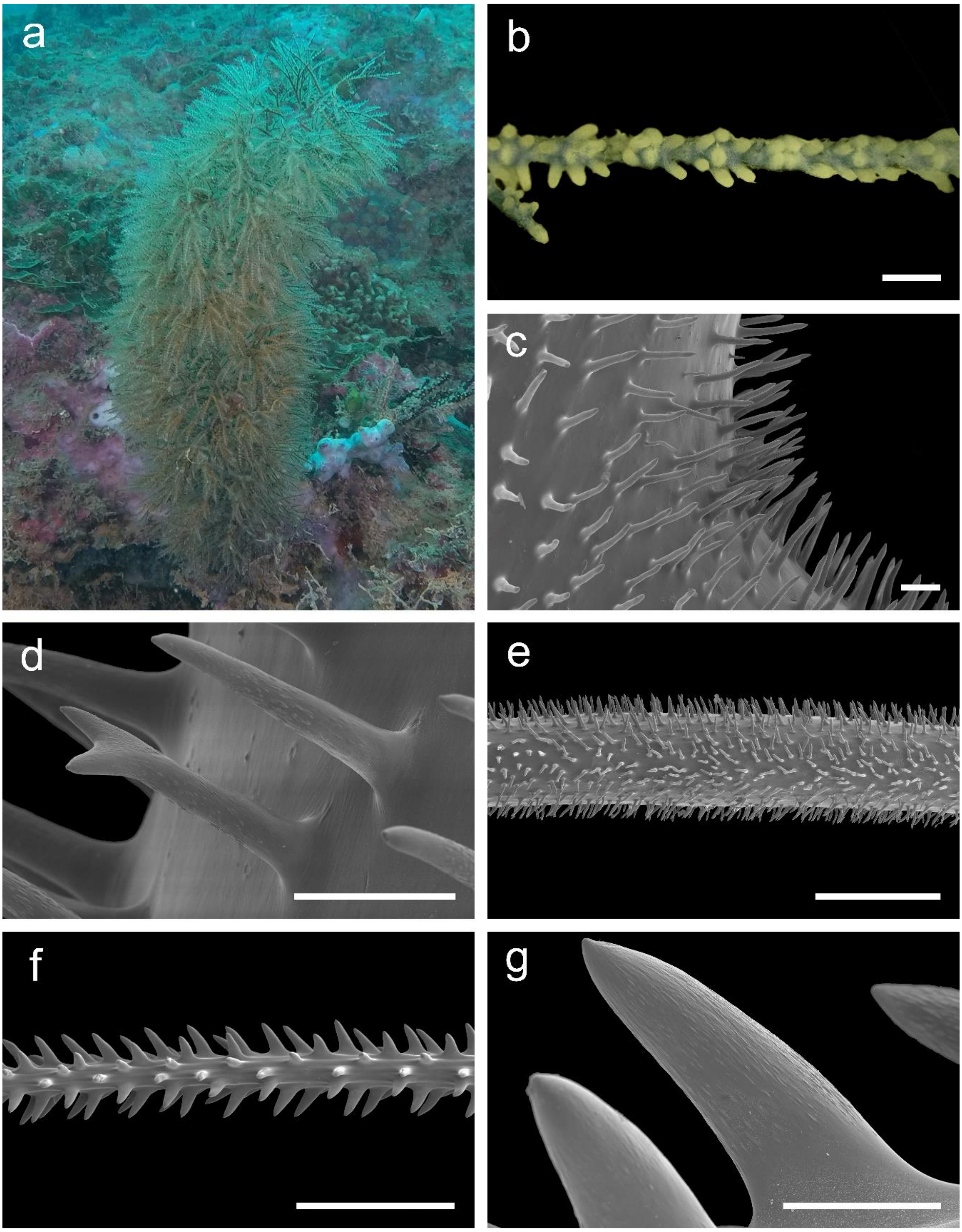

Figure 42. *Cupressopathes abies*. (a) Entire colony with a bottle-brush shape and measuring 35 cm in height. (b) Polyps. (**c, d**) Spines on a branch of 1 mm in diameter. (**e**) Spines on a branch of 0.67 mm in diameter. (**f, g**) Spines on a branch of 0.1 mm in diameter without spines. Scales: b, e, f: 1 mm; c, d: 100 µm; g: 50 µm.

pinnules and they are biserial and inclined distally. The spines are of different shape depending on the location on the corallum (Fig. 42, c, d, e, f, g). On the thicker branches, the spines are more densely and irregularly arranged than those on the pinnules, they are tall, cylindrical, needle-like and sometimes bifurcated (Fig. 42, c). They measure 0.11-0.24 mm and they are spaced by 0.04-0.18 mm. They have the same needle-like shape in the primary pinnules (Fig. 42, e) where they measure 0.95-0.16 mm and are spaced by 0.02-0.12 mm (on a pinnule of about 0.67 mm in diameter at the basis). On the subpinnules, the spines are flattened but still tall, with a basis larger than the top giving them a more conical appearance (Fig. 42, f). They are slightly inclined upwards and measure 0.07-0.12 mm with an interspine space of by 0.05-0.23 mm on a pinnule with a basal diameter of 0.1 mm. All the spines are very finely papillose (Fig. 42, g). On the thicker branch, about 11 rows of spines can be counted. This number decrease with the thickness of the branch: on a branch of 0.1 mm in diameter, about 5-6 rows are visible. The polyps are located on a single side of the branch (Fig. 42, b). Sometimes two rows of polyps can be seen. On the stem, the polyps can be distributed all around the axis. They measure 0.34-0.70 mm and they are spaced by 0.52-1.12 mm. There are 12 polyps per cm on the pinnules and subpinnules.

Remarks. This species was previously described in the Western Pacific and the Indian Ocean. The present diagnosis corresponds to the description of the neotype made by Opresko (2001) from Philippines at 40 m depth.

Cupressopathes sp. 1

Biogeography. Madagascar (present paper).

Localization. Colony found in July 2016 in Soalara at 13 m depth. Never seen on other locations.

Material examined. Colony *in situ*, fixed fragments (M210716-M027-1, M210716-M027-3, M210716-M027-4), dry fragments (M210716-M027-S, M210716-M027-S2, M210716-M027-S3).

Description. The colony is branched, not flabellate and is about 50 cm high and 40 cm wide (Fig. 43, a). The pinnules have varying lengths and seem to have no regular arrangement (Fig. 43, b). There are four irregular rows of primary pinnules of varying length, inserted at right angle to the branch. They measure 3.5 to 12.5 mm when they do not bear subpinnules. When they do, they measure 6.1-16.5 mm. About 4-6 of these

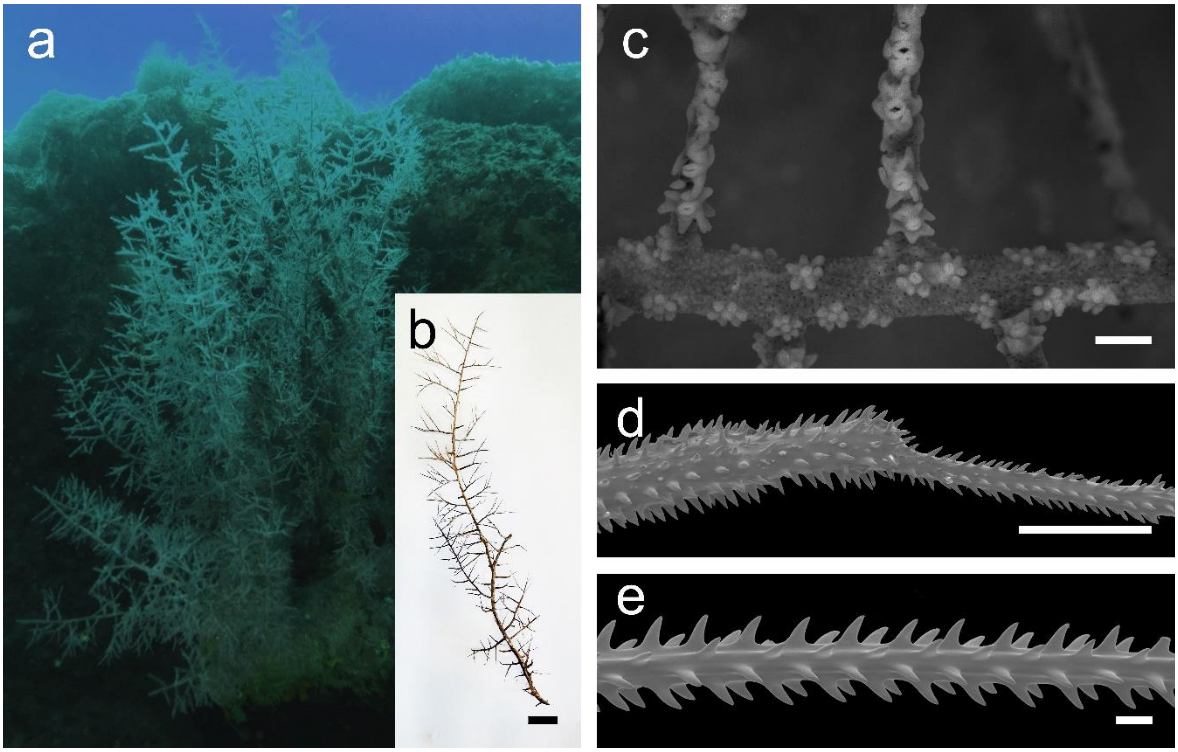

Figure 43. *Cupressopathes* morphotype 1 (**a**) Entire colony measuring 50 cm in height. (**b**) Details of the branching pattern. (**c**) Polyps. (**d,e**) Skeleton under SEM. Scales: c, d: 1 mm; e: 100 µm.

primary pinnules can be counted per cm. Secondary and tertiary pinnules are present, but not on all the primary pinnules. They are biserial or uniserial. These pinnules are inserted with an acute angle to the primary pinnules (Fig. 43, b). The secondary pinnules measure 2.5-12.0 mm and the tertiary 2.1-3.9 mm. The polyps are subequal in length; they measure 0.38-0.91 mm with an average of 0.63 mm; 9-12 can be counted per cm (Fig. 43, c). The interpolypar space is 0.51-1.30 mm with an average of 0.91 mm. The tentacles are thick and rounded, and the sagittal and lateral are subequal in size. They are located on a single side of the pinnules, but on thicker branches they are found all around with longer and varying interpolypar spaces. The spines of the pinnules are conical with an acute tip, and slightly inclined upwards (Fig. 43, d, e). On a branch of 0.23 mm of diameter (without spines), they measure 0.05-0.12 mm high and they are spaced by 0.08-0.18 mm (Fig. 43, e). On a branch of 0.36 mm, they measure up to 0.17 mm and are spaced by a distance up to 0.24 mm. There are about 7 spines per mm, and they are arranged in 6-7 rows. On thicker branches, the spines are much more elongated and more acicular, with 9-10 spines per mm (Fig. 43, d). They have a sharp apex and sometimes bifid or trifid spines are seen. They measure up to 0.20 mm and are spaced by 0.10-0.20 mm.

Secondary spines are sometimes present but they are not found regularly on the corallum (Fig. 43, d). All the spines have a smooth surface.

Remarks. The present description is not matching any of the description of *Cupressopathes* species to date, though it presents the characteristics of the genus. In his papers, Lapian *et al.* (2007) and Lapian (2009) described different morphotypes of *Cupressopathes* specimens in addition to genetic analyses. Their results suggested high hybridization processes between species from the genera *Myriopathes* and *Cupressopathes*, which could be a possibility for the present specimen.

Cupressopathes sp. 2

Biogeography. Madagascar (present paper).

Localization. Colony found in July 2016 at 16 m depth in the black coral bed of Toliara. Frequent on this location, but never seen elsewhere.

Material examined. Colony *in situ*, fixed fragments (M080716-M019-1, M080716-M019-3, M080716-M019-4), dry fragments (M080716-M019-S3, M080716-M019-S2, M080716-M019-S, M080716-M019-S4).

Description. A bottle-brush colony subdivided in five main stems that bear four rows of primary pinnules each (Fig. 44, a). It measures about 70 cm high and each bottle brush is about 10 cm wide. The primary pinnules are up to 5 cm in length and bear a lot of subpinnules which are mostly on the upper part of the primary pinnules (Fig. 44, b). They are inserted more or less at right angle to the branch, but sometimes the angle is acute. About 5 primary pinnules can be counted per cm. The subpinnules are most of the time biserial but they can be found uniserial, and they are always growing upwards (Fig. 44, b). The branches are pinnulate to the 5th order. These pinnules are inserted with an acute angle to the primary pinnules (Fig. 44, b). The polyps are subequal in length; they measure 0.37-0.89 mm with an average of 0.65 mm (Fig. 44, c). The interpolypar space is 0.60-1.28 mm with an average of 0.95 mm. The tentacles are cylindrical and rounded, and the sagittal and lateral are subequal in size. They are located on a single side of the pinnules, but on thicker branches they are found all around with longer and varying interpolypar spaces. The spines of the thicker branches are acicular, sharp, and slightly inclined upwards (Fig. 44, d). They are papillose on two thirds of their height (Fig. 44, e). On a branch of 0.55 mm in diameter, they measure 0.14-0.25 mm and are spaced by 0.08-0.30 mm. There are 7-8 spines per mm. On the subpinnules, the spines are more conical

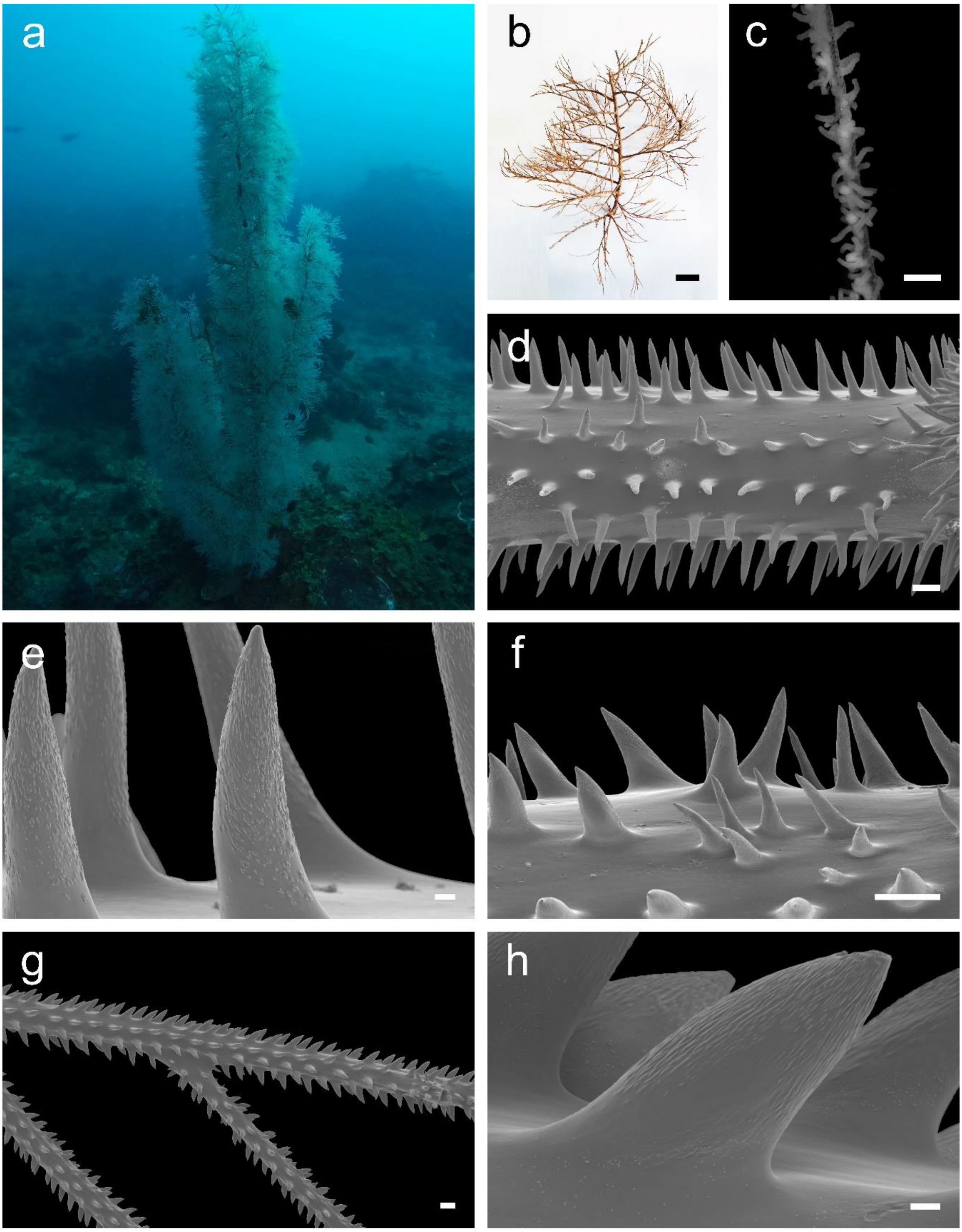

Figure 44. *Cupressopathes* morphotype 2 (a) Entire colony measuring 70 cm in height. (b) Polyps. (c) Arrangement of the spines on thicker branches. (d-e) Spine morphologies on thicker branches. (f) Arrangement of the spines on subpinnules. (g) Spine morphology on subpinnules. (h) Side view of the spine of the subpinnules. Scales: c: 1 mm; d, f, g: 100 µm; e, h: 10 µm.

and less needle-like (Fig. 44, f, g). They measure 0.02-0.13 mm and are spaced by 0.06-0.20 mm. There are 6-7 spines per mm and they are arranged in 4-5 longitudinal rows.

These spines have a sharp apex and they are also papillose on two thirds of their height (Fig. 44, h).

Remarks. The present diagnosis does not match with any description of *Cupressopathes* to date, though it presents the characteristics of the genus. This specimen is similar to the previous one in being probably hybridization between a *Myriopathes* and a *Cupressopathes* species. It is notable by its spine surfaces which are papillose, while they are usually smooth in *Cupressopathes* species.

Cupressopathes sp. 3

Biogeography. Madagascar (present paper).

Localization. Colony found on November 2015 in the black coral bed of Toliara at 24 m depth. Not rare in this location, but never seen elsewhere.

Material examined. Colony *in situ*, fixed fragments (M231115-M001-1, M231115-M001-2, M231115-M001-3, M231115-M001-5), dry colony and fragment (M231115-M001-S, M231115-M001-S2).

Diagnosis. The colony is about 40 cm high and 22 cm wide (Fig. 45, a). The basal diameter of the main stem is 6.5 mm and it tapers and becomes slightly flattened upwards. The main stem is dividing into thick branches which all bear four irregular rows of primary pinnules. This arrangement of the primary pinnules does not give the appearance of a bottle-brish though (Fig. 45, b). They appear as branchlets because of the great variability in their length, pinnulation and arrangement. The primary pinnules are measuring up to 5.0 mm. They are inserted with an angle slightly lower than 90 degrees to the branch. They are pinnulated to the 5th order and the subpinnules are numerous. About 5 primary pinnules can be counted per cm. The subpinnules are most of the time biserial but they can be found uniserial. These pinnules are inserted with an acute angle to the primary pinnules. The polyps are subequal in length; they measure 0.36-0.99 mm with an average of 0.62 mm (Fig. 45, c). The interpolypar space is 0.42-1.12 with an average of 0.71 mm. The tentacles are cylindrical and rounded, and the sagittal and lateral are subequal in size. They are located on a single side of the pinnules, but sometimes they can twist. On thicker branches, they are found all around with longer and varying interpolypar spaces. The spines of the thicker branches are acicular, needle-like and slightly inclined upwards (Fig. 45, d). They are very finely striated. On a branch of 344µm in diameter, they measure 0.11-0.21 mm and are spaced by 0.07-0.17 mm. There are 10-11 spines per mm, and they are arranged in 5 very distinct and regular rows (Fig. 45, e). On the subpinnules, the spines are more cylindrical. On a branch of 155 µm in diameter, they measure 0.08-0.14 mm and are spaced by 0.06-0.18 mm. There are 8 spines per mm and they are arranged in 6 longitudinal rows (Fig. 45, f)

Remarks. The present specimen has similar characters to both *Cupressopathes* species and *Myriopathes* species but does not correspond to any description to date. It might be a hybrid between *Myriopathes* and *Cupressopathes* species as seen before.

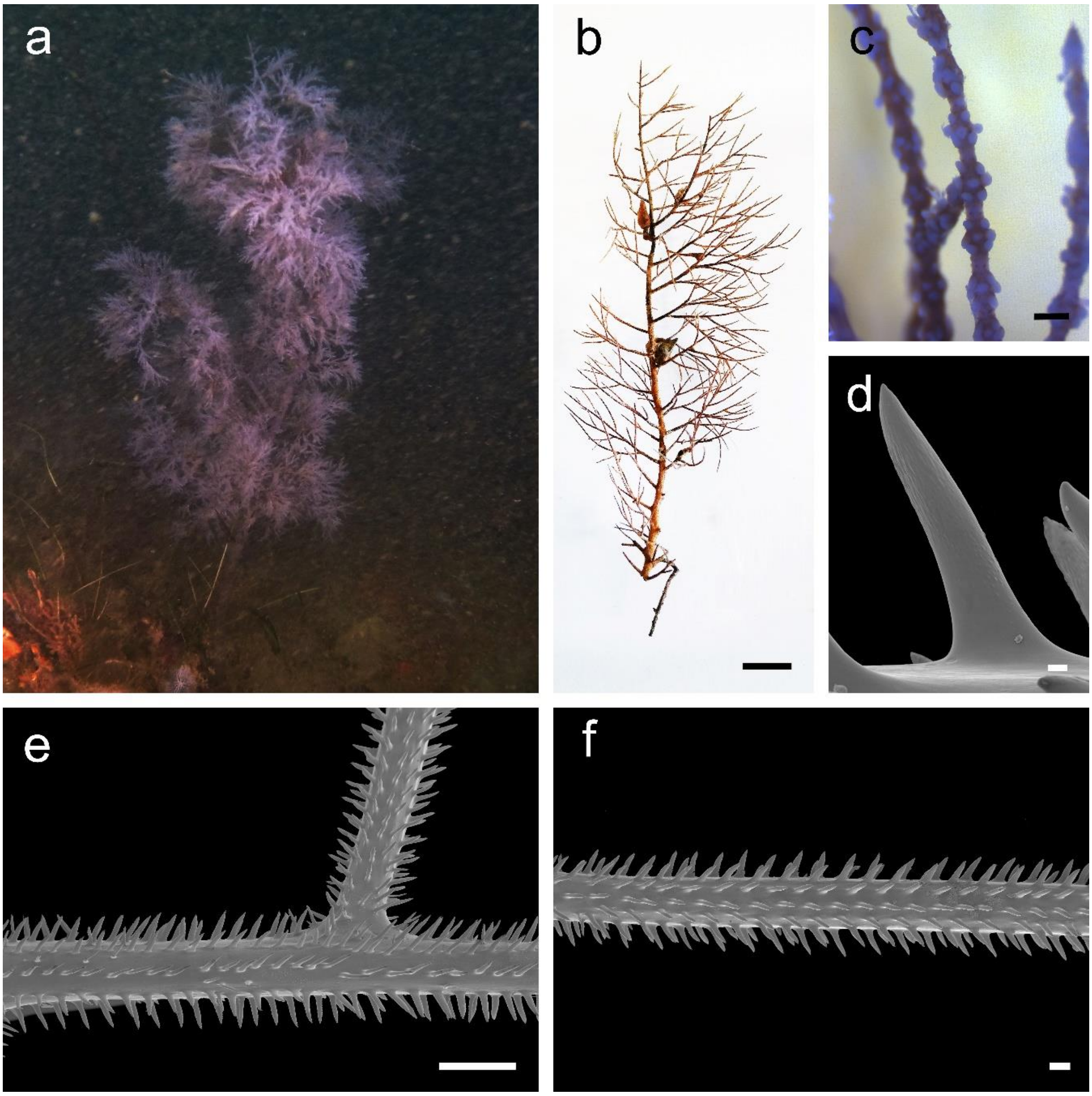

Figure 45. *Cupressopathes* sp.3. (a) Entire colony measuring 40 cm in height. (b) Arrangement of the pinnules. (c) Polyps. (d) Spine morphology. (e) Acicular spines arranged in longitudinal rows. (f) Spines on subpinnules. Scales: c: 1 mm; d: 10 µm; e: 500 µm; f: 100 µm.

Abundance of black corals in the main bed of Toliara

A total of 1201 corals was observed within the 10 transects made in the main black coral bed of Toliara located at the North tip of the Great Reef (Table 11). From this number, 199 colonies were dead (ca 16%). Dead black corals were always covered by a lot of different invertebrates such as sponges, anemones, bivalves, or algae. *Cirrhipathes* sp.2 and *Stichopathes* sp. were the most observed colonies while *Cupressopathes* sp.1, *Myriopathes ulex* and *Cirrhipathes rumphii* were the less abundant (Fig. 48). *Antipathes flabellum*, *Antipathes pseudodichotoma*, *Cirrhipathes* sp.1, *Stichopathes alcocki* and *Stichopathes papillosa* were not observed in the stations chosen for the transects.

Table 9. Number of colonies of each species observed within the black coral bed of Toliara.

Species	Number of colonies observed
Cirrhipathes sp.2	416
Stichopathes sp.aff. maldivensis	221
Dead black coral	199
Cirrhipathes anguina	137
Cupressopathes sp.2	55
Cirrhipathes densiflora	53
Cirrhipathes contorta	44
Cupressopathes sp3	27
Cirrhipathes spiralis	16
Cupressopathes abies	11
Myriopathes stechowi	11
Cupressopathes sp1	4
Myriopathes ulex	4
Cirrhipathes rumphii	3

DISCUSSION

On the west coast of Madagascar, coral reefs are well represented in the north-west and south-west area, especially between the Mangoky delta and Androka (Pichon 1972; Nadon *et al*. 2005; McClanahan *et al*. 2009; Todinanahary 2016). Our study area, from Andavadoaka to Maromena, represents more than 200 km of shore, with a reef composed of fringing reefs, barrer reefs and patch reefs (Pichon 1972; Nadon *et al*. 2005; McClanahan *et al*. 2009; Todinanahary 2016). The most important coral ecosystem of this area is the Great Reef of Toliara which is bordered at the north by the Fiherenana river and at the South by the Onilahy river. Black corals are commonly found on rocky substrates and their abundance is variable but they always live where currents are strong. They are particularly abundant in front of rivers where they can form beds of thousands of corals. This is the case around Toliara where 16 of the 18 antipatharian species described here were found in shallow waters. This abundance is probably due to the availability of organic particles brought by the rivers as the terrestrial runoffs are important -especially between December and April- and with a high organic content (Sheridan *et al.* 2014). It is however well known that terrestrial runoffs are not favourable to hard corals (Risk 2014) but the morphology of black corals, especially the unbranched ones, in addition to the fact that they do not need zooxanthellae to grow may enable them to live in these regions where turbidity is relatively high.

If black coral populations have been described elsewhere in the world, *i.e.* in the Bunaken Marine Park in Indonesia (Tazioli *et al.* 2007), the Hawaiian Islands (Wagner 2015a), the Caribbean (Opresko & Sanchez 2005) or the New Zealand region (Opresko *et al.* 2014), the populations in the southwestern coast of Madagascar are remarkable because of their amount of species and abundance. The 18 species of this study belong to 5 genera and 2 families. In comparison, Wagner (2015a) found 8 species from 6 genera and 3 families in the shallow waters of Hawaii until 150 m deep, and Tazioli *et al.* (2007) observed 16 species for 6 genera and 3 families in the Bunaken Marine Park until 45 m deep. Compared to these areas, none species of the family Aphanipathidae was observed in this study. In contrary, species of the genus *Cirrhipathes* are uncommonly very diverse compared to other regions of the world, in addition to the high abundance of *Stichopathes* corals.

The most common shallow-water black corals are almost exclusively from Antipathidae and Myriopathidae, with the most common genera being *Antipathella, Antipathes, Cirrhipathes, Cupressopathes, Myriopathes* and *Stichopathes* (Opresko *et al.* 2014). They generally form dense beds on areas with strong currents with unbranched whip corals or bushy colonies as it is the case in the southwestern coast of Madagascar. In this study, 6 species descriptions did not match any existing one from literature. Paradoxically to the deep species, taxonomy and identification of the shallow-water black corals have always been challenging. Even if a considerable work of taxonomic revision has been undertaken on black corals by Opresko and other authors since more than 40 years (*i.e.* Opresko 1972; 1974; 1997; 2001; 2002; 2003b; 2004; 2006; Opresko *et al.* 2001; Bo & Opresko 2015), species from shallow waters remain the most difficult to identify. This is due to the loss of a lot of type specimens, the frequently elusive original descriptions which often lacks crucial information such as the spine morphology –the latter being one of the most important criteria for identification– and the fact that some species have never been described so far. In the present study, only the top parts of the corals were sampled to avoid killing entire corals. Skeleton and spines morphology, but also polyps and overall aspects may vary depending on the location, the depth, the height or the age of the coral. This is also explaining why it remains challenging to identify shallow-water black corals, and whole colonies are more suitable for new species description.

In conclusion, this study is a step forward regarding the identification of the shallow-water species and can be used as a basis for new studies about intraspecific and interspecific morphological variation and genetics. One may be advised that due to the harvesting fisheries, the diversity and distribution of the black corals from the region described here may rapidly change over time.

ACKNOWLEDGMENTS

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SUPPLEMENTARY MATERIAL

Identification key for the black corals from the southwestern coast of Madagascar

1. Colony branched, monopodial or sympodial with pinnules, branchlets or intermediates forms.. 2
- Colony unbranched, whip-like, straight, sinuous, or with spirals or coils, sometimes a breaking event can lead to a branch developing on the colony 4
2. Primary pinnules branched in 4 irregular rows around the corallum…………………………………………………………......................5. *Cupressopathes*
- Primary pinnules branched in a bi-dimensional plan 3
3. Corallum anastomosed or pseudodichotomous...................................6. Antipathes
- Corallum flabellate, stems and branches pinnulate to the second order or more, primary pinnules biserial ....7. *Myriopathes*
4. Polyps found only on one side of the corallum, on a single row……...8. *Stichopathes*
- Polyps found in two rows or all around the corallum.........................10. *Cirrhipathes*
5. Colony generally monopodial, bottle-brush like, not exceeding 50 cm in height, diameter of the colony constant upwards, always dark colour, primary pinnules at right angles to the corallum, secondary pinnules biserial and alternating…..………………………………………………….*Cupressopathes abies*
- Corallium monopodial or sparsely branched, primary pinnules of very irregular lengths, not always at right angle to the corallum, a few secondary pinnules...*Cupressopathes* sp.
6. Colony small, pseudodichotomous, not branched .... *Antipathes pseudodichotoma*
- Colony anastomosed, bush-like, brown in colour, sometimes some parts growing out of the growth plan ....*Antipathes flabellum*
7. Large tree-like brownish colony, main stems generally large and clearly visible, primary pinnules can exceed 1 cm in length, stems can grow out of the plan of the colony .... *Myriopathes ulex*
- Small colony, pinnulate to the 3rd order, primary pinnules bilaterally alternated and measuring max. 1 cm, secondary pinnules not always present, the whole colony is branched in a bi-dimensional plan, light grey/white in colour... *Myriopathes stechowi*
8. Colony growing with tight spirals...9

- Colony whip-like, sometimes developing large and lose coils..*Stichopathes* sp. aff. *maldivensis*

9. Colony found above 30 meters, brown in colour....................*Stichopathes papillosa*

- Colony found below 45 meters, white in colour ....*Stichopathes alcocki*

10. Colony developing spirals..11

- Colony straight, sinuous, contorted, but not developing spirals 12

11. Colony with tight spirals, sometimes loser but still close to each other, large polyps with visible gastrovascular canals, polyps clearly spaced from each other, yellowish colour, about 50 cm in height..*Cirrhipathes spiralis*

- Colony forming large, regular coils at the top, polyps crowded on one side and sitting close from each other, interpolypar space not visible, large band of tissue lacking polyp on the other side, more than 1 meter in height ....*Cirrhipathes rumphii*

12. Colony with two well defined rows of polyps .... *Cirrhipathes* sp.1

- Polyps all around the corallum, without a defined order 13

13. Colony contorted and twisted ....*Cirrhipathes contorta*

- Colony straight, slightly sinuous, not making coils 14

14. Polyps with long tentacles, which tend to be gathered on one side of the colony making some tissues lacking polyps, brown or yellow in colour............ *Cirrhipathes anguina*

- Polyps all around the corallum, with varying interpolypar spaces...........................15

15. Polyps rounded appearing like buds on the colony, mouths wide, tentacles thick, grooves visible .... *Cirrhipathes densiflora*

- Polyps slightly flattened of varying sizes, colour varying from white to brown, grooves not visible.. *Cirrhipathes* sp.2

LITERATURE

Bayer, F. M. (1959) The Alcyonarian and Black Corals (Anthozoa; Octocorallia and Antipatharia) described and figured by G.E. Rumphius. *In*: de Wit, H.C.D (Eds.), *Rumphius Memorial Volume*, Baarn, Hollandia, pp. 225–247.

Blainville, H. M. D. DE. (1834) *Manuel d'Actinologie ou de Zoophytologie*. Levrault F.G., Paris, viii, 694 pp. http://dx.doi.org/10.5962/bhl.title.8768

Bo, M. (2008) *Taxonomy and Ecology of Antipatharians*. Ph.D. dissertation, Universita Politecnica Delle Marche, Ancona, Italy, 212 pp.

Bo, M., Barucca, M., Biscotti, M.A., Canapa, A., Lapian, H.F.N., Olmo, E. & Bavestrello, G. (2008) Description of *Pseudocirrhipathes* (Cnidaria: Anthozoa: Hexacorallia: Antipathidae), a new genus of whip black corals from the Indo–Pacific. *Italian Journal of Zoology*, 76 (4), 392–402. http://dx.doi.org/10.1080/11250000802684104

Bo, M., Di Camillo, C.G., Addamo, A.M., Valisano, L. & Bavestrello, G. (2009) Growth strategies of whip black corals (Cnidaria: Antipatharia) in the Bunaken Marine Park (Celebes Sea, Indonesia). *Marine Biodiversity Records*, 2, e54. https://doi.org/10.1017/S1755267209000712

Bo, M., Bavestrello, G., Barucca, M., Makapedua, D.M., Poliseno, A., Forconi, M., Olmo, E. & Canapa, A. (2012) Morphological and molecular characterization of the problematic whip black coral genus *Stichopathes* (Hexacorallia: Antipatharia) from Indonesia (North Sulawesi, Celebes Sea). *Zoological Journal of the Linnean Society*, 166, 1–13. https://doi.org/10.1111/j.1096-3642.2012.00834.x

Bo, M. & Opresko, D.M. (2015) Redescription of *Stichopathes pourtalesi* Brook, 1889 (Cnidaria: Anthozoa: Antipatharia: Antipathidae). *Breviora*, 1–18.

Brook, G. (1889) Report on the Antipatharia collected by HMS Challenger during the years 1873-1876. Report on the Scientific Results of the Voyage of HMS Challenger During the Years 1873-76. *Zoology*, 32 (1), 222 pp.

Brugler, M. R., Opresko, D. M. & France, S. C. (2013) The evolutionary history of the order Antipatharia (Cnidaria: Anthozoa: Hexacorallia) as inferred from mitochondrial and nuclear DNA: implications for black coral taxonomy and systematics. *Zoological Journal of the Linnean Society*, 169 (2), 312–361. https://doi.org/10.1111/zoj.12060

Capella–Gutiérrez, S., Silla–Martínez, J.M. & Gabaldón, T. (2009) trimAl: a tool for automated alignment trimming in large–scale phylogenetic analyses. *Bioinformatics*, 25 (15), 1972–1973. https://doi.org/10.1093/bioinformatics/btp348

Carlgren, O. H. (1940) *A contribution to the knowledge of the structure and distribution of the cnidae in the Anthozoa*. Lunds Universitet, Sweden, 36, 62 pp.

Clarke, M.E., Whitmire, C.E. & Yoklavich, M.M. (2015) State of Deep–Sea Coral and Sponge Ecosystems of the U.S. West Coast: 2015. *In*: Hourigan T.F., Etnoyer P.J., Cairns S.D. (Eds.), *The State of Deep–Sea Coral and Sponge Ecosystems of the United States*, NOAA Technical Memorandum X, 42 pp.

Cooper, C. F. (1903) Antipatharia. *In*: Gardiner, J.S. (Ed.), *Fauna and Geography of the Maldive and Laccadive Archipelagos*, Cambridge University Press, 791–796.

Cooper, C. F. (1909) Reports of the Percy Sladen Trust expedition on the Indian Ocean, 1905. Antipatharia. *Transactions of the Linnean Society of London*, 12 (4), 301–321.

Dana, J.D. (1846) Zoophytes. *In*: Sherman, C. (Ed.), *United States Exploring Expedition during the years 1838, 1839, 1840, 1841, 1842. Under the command of Charles Wilkes, U.S.N.*, Lea & Blanchard, Philadelphia, (7), 740 pp.

Deshayes, G. P., & Milne–Edwards, H. (1836) *Histoire naturelle des animaux sans vertèbres, par JBPA de Lamarck*. J.B. Baillière, Paris, 440 pp. http://dx.doi.org/10.5962/bhl.title.63986

Ehrenberg, C.G. (1834) Beiträge zur physiologischen Kenntnifs der Corallenthiere im allgemeinen, und besonders des rothen Meeres, nebst einem Versuche zur physiologichen Systematik derselben. *Abhandlungen der Königlich Preussischen Akademie der Wissenschaften, Berlin*, 225–380.

Ellis, J. & Solander, D. (1786) *The natural history of many curious and uncommon zoophytes collected by the late John Ellis, systematically arranged and described by the late Daniel Solander*. White, B. & Son, London, 208 pp. http://dx.doi.org/10.5962/bhl.title.2145

Esper, E.J.C. (1788-1830) Die Pflanzenthiere in Abbildungen nach der Natur mit Farben erleuchtet nebst Beschreibungen. [Theilen 1–3 (Lieferungen 1-17)]. Raspe, Nürnberg.

Flot, J. F., Blanchot, J., Charpy, L., Cruaud, C., Licuanan, W. Y., Nakano, Y., Payri, C. & Tillier, S. (2011) Incongruence between morphotypes and genetically delimited species in the coral genus *Stylophora*: phenotypic plasticity, morphological convergence, morphological stasis or interspecific hybridization? *BMC Ecology*, 11 (1), 11–22.

Grange, K.R. (1988) Redescription of *Antipathes aperta*, Totton, (Coelenterata: Antipatharia), an ecological dominant in the southern fiords of New Zealand. *New Zealand Journal of Zoology*, 15, 55–61. http://dx.doi.org/10.1080/03014223.1988.10422609

Grigg, R.W. (1993) Precious coral fisheries of Hawai'I and the U.S. Pacific Islands. *Marine Fisheries Review*, 55, 50–60.

Grigg, R. W. (2001) Black coral: History of a sustainable fishery in Hawai'i. *Pacific Science*, 55, 291–299. https://dx.doi.org/ 10.1353/psc.2001.0022

Grigg, R. & Opresko, D.M. (1977) The Antipatharia. *In*: Edmondson, C. (Ed.), *Reef and Shore Fauna of Hawaii*, B.P.Bishop Museum, Honolulu, 64 (1), 242–262.

Gray, J.E. (1857) On the animal and bark of Antipathes. *Proceedings of the Zoological Society of London*, 1857. 113 pp.

Helaers, R. & Milinkovitch, M. (2010) MetaPIGA v3.1: maximum likelihood large phylogeny estimation using the metapopulation genetic algorithm and other stochastic heuristics. *BMC Bioinformatics,* 11, 379. https://dx.doi.org/10.1186/1471-2105-11-379

Kumar, J.S.Y., Raghunathan, C. and Venkataraman, K. (2012) Studies on newly recorded antipatharian corals from Andaman and Nicobar islands. *Records of the Zoological Survey of India*, 112 (1), 1–10.

Lamarck, J.B. (1815) Sur les Polypiers corticifères. *Mémoires du Muséum d'Histoire Naturelle*, Paris, 1, 401–476.

Lamarck, J.B. (1816) Histoire Naturelle des Animaux sans Vertèbres, 2. Paris, 568 pp.

Lamouroux, J.V.F. (1816) Histoire des polypiers coralligènes flexibles, vulgairement nommés zoophytes. Imprimerie de Poisson, Caen.

Lamouroux, J.V.F., Bory de Saint–Vincent & Deslongchamps, J.A.E. (1824) Histoire Naturelle des Zoophytes ou Animaux rayonnés. *Encyclopédie Méthodique* 2, Paris, 819 pp.

Lapian, H.F.N, Barucca, M., Bavestrello, G., Biscotti, M.A., Bo, M., Canapa, A., Tazioli, S. & Olmo, E. (2007) A systematic study of some black coral species (Antipatharia, Hexacorallia) based on rDNA internal transcribed spacers sequences. *Marine Biology*, 151, 785–792. https://doi.org/10.1007/s00227-006-0525-8

Lapian, H.F.N (2009) Biodiversity study of black coral (Order: Antipatharia) collected from Manado, Indonesia based on rDNA Internal Transcribed Spacer (ITS) sequences analysis. *Biodiversitas*, 10 (1), 1–5.
https://doi.org/10.13057/biodiv/d100101

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J. & Higgins, D.G. (2007) Clustal W and Clustal X version 2.0. *Bioinformatics*, 23, 2947–2948. https://doi.org/10.1093/bioinformatics/btm404

Linnaeus, C., 1758. Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Editio decima, reformata. Laurentii Salvii, Holmiae, Stockholm, 824 pp.

McClanahan, T.R., Graham, N.A.J., Wilson, S.K., Letourneur, Y. & Fisher, R. (2009) Effects of fisheries closure size, age, and history of compliance on coral reef fish communities in the western Indian Ocean. *Marine Ecology Progress Series*, 369, 99–109. https://doi.org/10.3354/meps08279

Milne Edwards, H & Haimes, J. (1857) *Histoire Naturelle des Coralliaires ou Polypes Proprement Dits*. La Librairie Encyclopédique de Roret, Paris, 326 pp.

Molodtsova, T. (2005) Redescription of *Parantipathes euantha* (Pasternak, 1958) (Anthozoa: Antipatharia) from Kurile–Kamchatka Trench. *Invertebrate Zoology*, 2 (2), 169–179.

Molodtsova, T. (2007) Black corals (Antipatharia) of New Caledonia. *In*: Payri, C.E. & Richer de Forges B. (Eds.), *Compendium of marine species of New Caledonia*, Documents Scientifiques et Technique 117, IRD Nouméa, 119–125.

Moon, H. W., & Song, J. I. (2008) Taxonomy of the black coral family Myriopathidae (Anthozoa: Antipatharia) from Korea. *Korean Journal of Systematic Zoology*, 24, 251–263. http://dx.doi.org/10.5635/kjsz.2008.24.3.251

Montgomery, A. D. (2002) The feasibility of transplanting black coral (Order Antipatharia). *Hydrobiologia*, 471 (1), 157–164.

Nadon, M.–O., Griffiths, D. & Doherty, E. (2005) The coral reefs of Andavadaoaka, southwest Madagascar. Blue Ventures, London, UK.

Nylander, J.A.A. (2002) MrModeltest, version 1.1b. Available from the authors at: http://www.ebc.uu.se/systzoo/staff/nylander.html.

Ocaña, O., Opresko, D. M., & Brito, A. (2006) First record of the black coral *Antipathella wollastoni* (Anthozoa: Antipatharia) outside of Macaronesian waters. *Revista de la Academia Canaria de Ciencias*, 18 (4), 125–138.

Okiyama, M. & Tsukamoto, Y. (1989) Sea whip goby, *Bryaninops yongei*, collected from outer shelf off Miyakojima, East China Sea. *Japanese Journal of Ichthyology*, 36 (3), 369–370.

Opresko, D.M. (1972) Redescriptions and reevaluations of the antipatharians described by L.F. de Pourtales. *Bulletin of Marine Science*, 22, 950–1017.

Opresko, D.M (1974). A study of the classification of the Antipatharia (Coelenterata:Anthozoa) with redescriptions of eleven species. Ph.D. Dissertation, University of Miami, Miami, 193 pp. http://dx.doi.org/10.11646/zootaxa.852.1.1

Opresko D.M. (1976) Redescription of *Antipathes panamensis* Verrill (Coelenterata, Antipatharia). *Pacific Science*, 30 (3), 235–240.

Opresko, D.M. (1997) Review of the genus *Schizopathes* (Cnidaria: Antipatharia: Schizopathidae) with a description of new species from the Indian Ocean. *Proceedings of the Biological Society of Washington*, 110 (2), 157–166.

Opresko, D.M. (2001) Revision of the Antipatharia (Cnidaria: Anthozoa). Part I. Establishment of a new family, Myriopathidae. *Zoologische Mededelingen*, 75, 343–370.

Opresko, D.M. (2002) Revision of the Antipatharia (Cnidaria: Anthozoa) .Part II. Schizopathidae. *Zoologische Mededelingen*, 76, 411–442.

Opresko, D.M. (2003a) Redescription of *Antipathes dichotoma* Pallas, 1766 (Cnidaria: Anthozoa: Antipatharia). *Zoologische Mededelingen*, 77, 481–493.

Opresko, D.M. (2003b) Revision of the Antipatharia (Cnidaria: Anthozoa). Part III. Cladopathidae. *Zoologische Mededelingen*, 77, 495–536.

Opresko, D.M (2004) Revision of the Antipatharia (Cnidaria: Anthozoa). Part IV. Establishment of a new family, Aphanipathidae. *Zoologische Mededelingen*, 78, 209–240.

Opresko, D.M. (2005b) A new species of antipatharian coral (Cnidaria:Anthozoa: Antipatharia) from the southern California Bight. *Zootaxa* 852, 1–10. http://dx.doi.org/10.11646/zootaxa.852.1.1

Opresko, D.M (2006) Revision of the Antipatharia (Cnidaria: Anthozoa). Part V. Establishment of a new family, Stylopathidae. *Zoologische Mededelingen,* 80–4, 109–138.

Opresko, D.M. (2009) A New Name for the Hawaiian antipatharian coral formerly known as *Antipathes dichotoma* (Cnidaria: Anthozoa: Antipatharia). *Pacific Science*, 63 (2), 277–291.

Opresko, D. M. & Genin, A. (1990) A new species of antipatharian (Cnidaria: Anthozoa) from seamounts in the eastern North Pacific. *Bulletin of marine science*, 46 (2), 301–310.

Opresko, D. M. & Cairns, S. D. (1994) Description of the new genus *Allopathes* (Cnidaria: Antipatharia) and its type species *Cirripathes desbonni*. *Proceedings of the Biological Society of Washington*, 107 (1), 185–192.

Opresko, D. M. & Baron–Szabo, R. C. (2001) Re–descriptions of the antipatharian coral described by EJC Esper with select English translations of the original German text. *Senckenbergiana biologica*, 81, 1–21.

Opresko, D.M. & Sanchez, J.A. (2005) Caribbean shallow–water black corals (Cnidaria: Anthozoa: Antipatharia). *Caribbean Journal of Science*, 41 (3), 492–507.

Opresko, D.M., Tracey, D. & Mackay, E. (2014) Antipatharia (black corals) for the New Zealand region. A field guide of commonly sampled New Zealand black corals including illustrations highlighting technical terms and black coral morphology. *New Zealand Aquatic Environment and Biodiversity Report* No. 131. 20 p.

Pallas, P.S. (1766) *Elenchus Zoophytorum Sistens Generum Adumbrationes Generaliores et Specierum Cognitarum Succinctas Descriptiones Cum Selectis Auctorum Synonymis*. Hagae–Comitum, Frankfurt, 451 pp. http://dx.doi.org/10.5962/bhl.title.6595

Pasternak, F.A. (1977) Antipatharia. Galathea Report. *Scientific Results of the Danish Deep-Sea Expedition around the world 1950-52*, 14, 157–164.

Pax, F. (1932) Beiträge zur Kenntnis der Japanischen Dörnchenkorallen. *Zoologische Jahrbucher* 9, 63, 407–450.

Pichon, M. (1972) The coral reefs of Madagascar. *In*: Battistini, R. & Richard-Vindard, G. (Eds.), *Biogeography and Ecology in Madagascar*. Springer Netherlands, pp. 367-410. http://dx.doi.org/10.1007/978-94-015-7159-3_11

R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from: http://www.R–project.org/.

Risk, M.J (2014) Assessing the effects of sediments and nutrients on coral reefs. *Current Opinion in Environmental Sustainability*, 7, 108–117. http://dx.doi.org/10.1016/j.cosust.2014.01.003

Ronquist, F. & Huelsenbeck, J.P. (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics,* 19, 1572–1574. http://dx.doi.org/10.1093/bioinformatics/btg180

Silberfeld, E. (1909a) Diagnosen neuer japanischer Antipatharien aus der Sammlung von Herrn Prof.Doflein (München). *Zoologischer Anzeiger*, 34, 760–763.

Silberfeld, E. (1909b) Japanische Antipatharien. Abhandlungen der math.–phys. Klasse der K. Bayer. *Akademie der Wissenschaften* I. Suppl.–BD 7. Abhandlg. pp. 1–30.

Schneider, C. A., Rasband, W. S. & Eliceiri, K. W. (2012) NIH Image to ImageJ: 25 years of image analysis. *Nature methods,* 9 (7), 671–675. http://dx.doi.org/10.1038/nmeth.2089

Schultze, L.S. (1896) Beitrag zur Systematik der Antipatharien. *Abhandlungen der Senckenbergischen naturforschenden Gesellschaft,* 23, 1–40.

Sheridan, C., Baela, J.M., Kushmaro, A., Fréjaville, Y. & Eeckhaut, I. (2014) Terrestrial runoff influences white syndrome prevalence in SW Madagascar. *Marine Environmental Research*, 101, 44–54. http://dx.doi.org/10.1016/j.marenvres.2014.08.003

Summers, S. L. (1910) VIII.–Antipatharians from the Indian Ocean. *Journal of the Royal Microscopical Society*, 30 (3), 273–281. http://dx.doi.org/10.1111/j.1365-2818.1910.tb01715.x

Tazioli, S., Bo, M., Boyer, M., Rotinsulu, H. & Bavestrello, G. (2007) Ecological observations of some common antipatharian corals in the Marine Park of Bunaken (North Sulawesi, Indonesia). *Zoological Studies*, 46 (2), 227–241.

Terrana, L. & Eeckhaut, I. (2017) Taxonomic description and 3D modelling of a new species of myzostomid (Annelida, Myzostomida) associated with black corals from Madagascar. *Zootaxa*, 4244 (2), 277–295. http://dx.doi.org/10.11646/zootaxa.4244.2.9

Thomson, J. A. & Simpson, J. J. (1905) Report of the Antipatharia collected by Prof. Herdman at Ceylon, 1902. *Ceylon Pearl Oyster Report*, 4, 93–106.

Todinanahary, G., Terrana, L., Lavitra, T. & Eeckhaut, I. (2016) First records of illegal harvesting and trading of black corals (Antipatharia) in Madagascar. *Madagascar Conservation & Development*, 11 (1), 6–11. http://dx.doi.org/10.4314/mcd.v11i1.5

Todinanahary, G. (2016) Evaluation du potential biologique, économique et social de la coralliculture dans le sud–ouest de Madagascar. PhD Dissertation, University of Mons, Belgium, 253 pp.

Utinomi, H. (1956) Colored illustrations of seashore animals of Japan. *Fauna and Flora of Japan*, 8 (2). 105 pp.

van Pesch, A. J. (1910) Beiträge zur Kenntnis der Gattung *Cirrhipathes*. Leiden, 96 pp.

van Pesch, A. J. (1914) The Antipatharia of the Siboga Expedition. *Siboga Expeditie Monographie*, 17, 1–258.

Wagner, D. (2015a). A taxonomic survey of the shallow–water (< 150 m) black corals (Cnidaria: Antipatharia) of the Hawaiian Islands. *Frontiers in Marine Science*, 2, 24.

Wagner, D. (2015b) The spatial distribution of shallow–water (< 150 m) black corals (Cnidaria: Antipatharia) in the Hawaiian Archipelago. *Marine Biodiversity Records*, 8, e54.

Wagner, D., Brugler, M.R., Opresko, D.M., France, S.C., Montgomery A.D. & Toonen, R.J. (2010) Using morphometrics, *in situ* observations and genetic characters to distinguish among commercially valuable Hawaiian black coral species; a redescription of *Antipathes grandis* Verrill, 1928 (Antipatharia: Antipathidae). *Invertebrate Systematics*, 24, 271–290. http://dx.doi.org/10.1071/is10004

Wagner, D., Papastamatiou, Y. P., Kosaki, R. K., Gleason, K.A., McFall, G.B., Boland, R.C., Pyle, R.L. & Toonen, R.J. (2011a) New records of commercially valuable black corals (Cnidaria: Antipatharia) from the Northwestern Hawaiian Islands at mesophotic depths. *Pacific Science*, 65, 249–255. http://dx.doi.org/10.2984/65.2.249

Wagner, D., Pochon, X., Irwin, L., Toonen, R. J. & Gates, R. D. (2011b) Azooxanthellate? Most Hawaiian black corals contain *Symbiodinium*. *Proceedings of the Royal Society B* 278, 1323–1328. http://dx.doi.org/10.1098/rspb.2010.1681

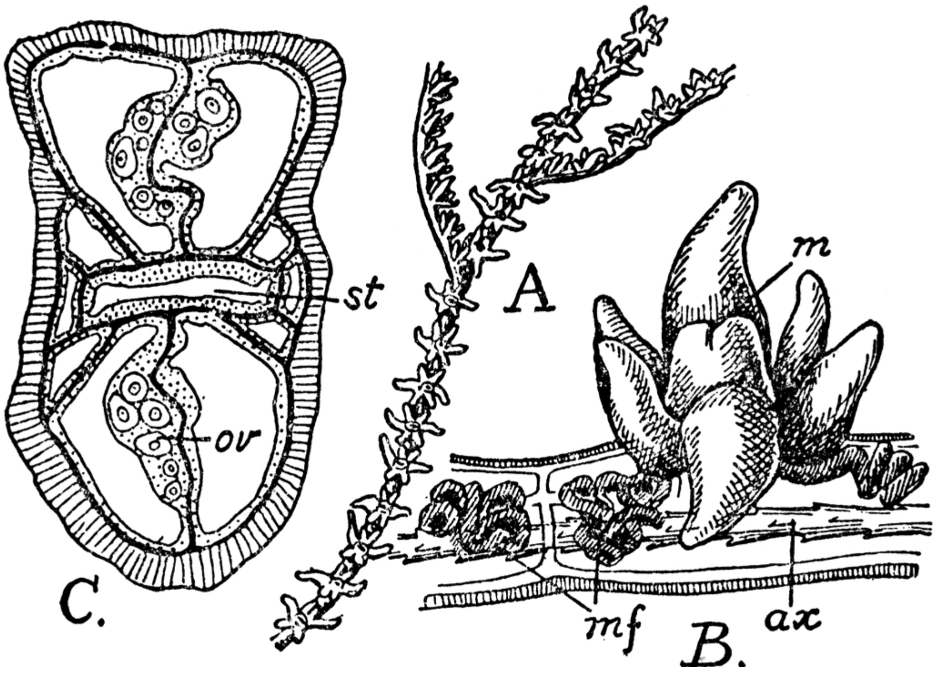
Wagner, D., Waller, R. G. & Toonen, R. J. (2011c). Sexual reproduction of Hawaiian black corals, with a review of reproduction of antipatharians (Cnidaria: Anthozoa: Hexacorallia). *Invertebrate Biology*, 130, 211–225. http://dx.doi.org/10.1111/j.1744-7410.2011.00233.x

Wagner, D., Luck, D.G. & Toonen, R.J. (2012) The biology and ecology of black corals (Cnidaria: Anthozoa: Hexacorallia: Antipatharia). *Advances in Marine Biology*, 63, 67–132. http://dx.doi.org/10.1016/b978-0-12-394282-1.00002-8

Zou, R. & Zhou, J. (1982) Studies on the antipatharians of China. I. The genus Cirrhipathes with the description of a new species. *Tropic Oceanology* 1, 82–91.

Chapitre 4

Sexual reproduction of the shallow-water black coral Cirrhipathes sp. from Madagascar



Brook, 1889

Sexual reproduction of the shallow-water black coral *Cirrhipathes* sp. from Madagascar

ABSTRACT

Random sampling of untagged shallow-water black corals *Cirrhipathes* sp. was carried out over a period of 13 months near the Great Reef of Toliara in southwest Madagascar. Small pieces of tissue were collected monthly from the base and apex of each colony. A total of 114 sets of upper and lower sections were observed. There were 37 males and 39 females for a sex ratio of 0.95, which was not significantly different from 1:1. On female and male colonies, 56% and 60% of the polyps were showing developing gametes respectively. Based on these samples, the species was determined to be gonochoric with broadcast spawning. The development of female and male gametes was divided into 3 stages, with stage 0 referring to unsexable colonies, and stage 2 referring to a high abundance of fully developed gametes inside the reproductive mesenteries. Gametes are released several times a year, as mature colonies were found each month. There was a major increase in the maturity and oocyte size when the temperature and the precipitation were at their highest, suggesting a major spawning event. Intra-colonial differences were observed regarding maturing polyps. Polyps living on the highest part of the colonies were showing gametes all year long, while gametes in the polyps located at the base of the colony were absent for most of the cold season. This could be the result of the highest polyps being in a better position to receive more food, which itself might influence the energy allocated to sexual reproduction.

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INTRODUCTION

Antipatharians, commonly known as black corals, are hexacorallian corals, 75% of the ~247 described species of which thrive below 50 m (Wagner et al. 2012; Molodtsova & Opresko 2017). They have non-retractile polyps with six unbranched tentacles and a flexible, spiny, black, proteinaceous, chitinous skeleton (Opresko et al. 2014). They are found worldwide, from abyssal depths to shallow warm tropical waters (Wagner et al. 2012).

Black corals have attracted people for long time. They have been used for religious and medical purposes (Bruckner et al. 2008), but nowadays they are mainly fished as a precious coral for jewelry in several regions around the globe (Noome & Kristensen 1976; Castorena & Metaca 1979; Kenyon 1984; Romero 1997; Grigg 2001; Padilla & Lara 2003; Huang & Ou 2010; Tsounis et al. 2010; Todinanahary et al. 2016). Black corals from the Hawaiian Islands remain the most well-known, and the best surveyed, populations on the globe to date, with quotas, fishery licenses, nursery areas, and above all good knowledge about the reproduction and the population stocks of commercially harvested species (Grigg 1976, 1993, 2001; Wagner et al. 2012). On the other hand, there is an illegal trade of shallow-water black corals on the island of Madagascar (Todinanahary et al. 2016) with a complete lack of information about the basic biology and ecology of these corals.

The sexual reproduction of antipatharians has only been investigated in the field for a few species (Wagner et al. 2011). A lot of the information available to date comes from scarce anatomy notes from original species descriptions, or centenarian reports on black corals (see Wagner et al. 2011 for an exhaustive list of the references). Apart from these descriptions, there is a large bathymetric and geographic gap in the remaining studies. Among them, only 11 refer to shallow-water species, and none of them come from the Indian Ocean (Wagner et al. 2011). After the large review by Wagner et al. (2011), two studies were carried out on the reproduction of deep corals from the Atlantic Ocean (Rakka et al. 2016; Lauretta & Penchaszadeh 2017) and one on a shallow-water species from Hong Kong (Lau 2011).Very few of these works analyzes the reproduction of colonies over multiple reproductive seasons, and most are focused on deep-water species (Grigg 1976; Goenaga 1977; Schmidt & Zissler 1979; Parker et al. 1997; Gaino & Soccia 2010; Lau 2011; Rakka et al. 2016).

In Madagascar, a black coral bed lies north of the Great Reef of Toliara, with more than 15 species occurring at the same place, between 15 and 40 meters deep (Terrana & Eeckhaut, pers. comm.). Within this bed, whip corals are the most abundant and the most harvested. As reproduction habits are crucial for establishing sustainable fisheries, and for assessing population stock management, the present work aims to analyze the seasonal reproduction of the most abundant species of shallow-water black coral from the southwestern coast of Madagascar, *Cirrhipathes* sp., which may belong to a new species (Terrana & Eeckhaut, pers. comm.).

MATERIAL & METHODS

Sampling

Black coral colonies of Cirrhipathes sp. were sampled monthly, between December 2014 and December 2015, by scuba-diving to a depth of 20-25m in the north channel of the Great Reef of Toliara (Madagascar, 23°21'05.1"S–43°36'53.2"E). The colonies in question are unbranched, straight or slightly sinuous, and can measure up to 3 m in height. Colonies have, on average, a 1 cm diameter along their whole length. Polyps are found all around the axis with 5-6 polyps per cm² and an average size of 1.9 mm. Their color varies from white to brown. On the skeleton, a high density of conical spines is found all around the axis with no clear organization; all of these spines are papillose from the base to the apex and measure up to 170 µm in height. The width of the conical spines varies, and narrower spines can also be found. In addition, small, sharp spines are scarcely present, they measure about 60 µm and have a sharp tip which is the only slightly papillose part, while the rest is smooth.

Samples of about 2-3 cm² were dissected with a sharp blade and removed from the skeleton underwater. The samples collected corresponded to a range of 1–31 polyps, depending on the sample. This technique was chosen as it allows the coral to recover and avoids having to sacrifice entire colonies. Basal and apical polyps were sampled for each colony to assess any maturity variation between parts with different accessibility to the water column. About 5-15 colonies were sampled around the 25th of every month, depending on the dive time and the weather conditions. The colonies were scattered around the black coral bed of Toliara, which is about 500 m in length and 300 m in width. The sizes ranged between 70 cm and 3 m in height. Using already sampled colonies might lead to the collection of potentially stressed colonies, where recovered tissues might be of different maturity states than the rest of the colony, therefore leading to a misinterpreted result. As the antipatharian colonies were not tagged, any colonies which showed any sign of healing or recovery were not sampled, in order to avoid resampling the same colonies. The regeneration process was not quick enough to collect from the same colony every month. Colonies measuring less than 70 cm were not collected to avoid sampling immature corals (see Parker et al. 1997; Rakka et al. 2016).

Histology and developmental stages

Samples were directly fixed in Bouin's fluid for 48h before being rinsed and stored in 70° ethanol. After dehydration, they were embedded in liquid paraffin, and thin sections of 7-8µm were made using a Zeiss Microm HM 340E microtome. These were then stained with Masson's trichrome stain and observed with an Axioscope A1 (Zeiss) light microscope. To avoid missing gametes, both transverse and longitudinal sections were examined, and all the polyps were taken into account, as long as their gastrovascular cavity was visible. Each colony was sexed, and the developmental stage of each specimen was established based on the development of the gametes.

Female mesentery development was divided into 3 stages. Stage 0 refers to unsexable colonies with polyps with no developing gametes. Stage 1 refers to colonies with early developmental stages of gametes scattered among a few maturing oocytes, found in the gastrodermis of the primary mesenteries. Finally, stage 2 refers to mesenteries with large numbers of fully grown oocytes laying close together, with folding of mesenteries, ooplasm of uniform appearance, and a few developing gametes observed (Fig. 46). Male mesentery development was also divided into 3 stages. Stage 0 refers to unsexable colonies with polyps with no developing gametes. Stage 1 refers to colonies with maturing gametes. For this stage, there is an external layer of lightly stained maturing spermatic cysts containing spermatozoa with darkly stained heads located more centrally. The spermatozoa are clustered together and their tails are oriented in the same direction. Lastly, stage 2 corresponds to fully developed spermatic cysts with mature spermatozoa, and the number of packed spermatozoa is high (Fig. 47). In both sexes, spent stages as described by Parker et al. (1997) were not observed.

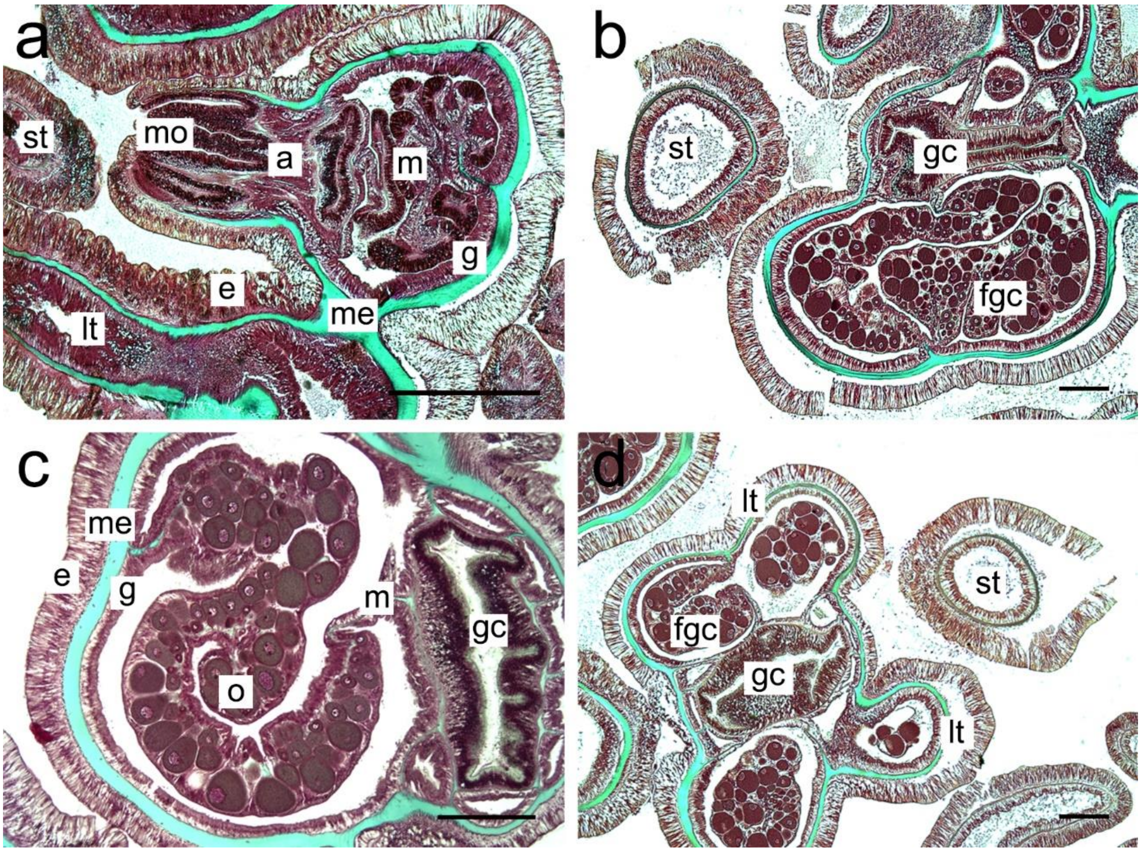

Figure 46. (a) Longitudinal section of an immature polyp. (b) Female polyp in stage 1 development with growing gametes and a few large oocytes. The folded mesentery is visible. (c) Female polyp in stage 2 showing fully grown oocytes packed together. (d) Section showing the gametes expanding into the cavity of the lateral tentacles. Scales: a: 500 µm; b-c-d: 200 µm. a actinopharynx; e ectoderm; fgc female gamete cells; g gastroderm; gc gastrovascular cavity; lt lateral tentacles; m mesentery; me mesoglea; mo mouth; o oocytes; st sagittal tentacles.

Data analysis

Corals were considered maturing when at least one of their polyps was showing developing gametes. Maturity at the top and the bottom of the colonies was calculated as a percentage, representing the total number of mature polyps (i.e. showing developing gametes) divided by the total number of polyps observed at each position for each colony. The monthly percentage of maturity was then presented as a mean percentage of all the colonies of the same sex and from the same collection period. Oocyte size frequencies were established for the mature female colonies using the Ferret diameter measured with the free software ImageJ (Schneider et al. 2012). They were then sorted in 10µm increments. The maximum number of measured oocytes was not fixed, in order to capture the maximum size range. Only oocytes sectioned through the nucleus were measured so

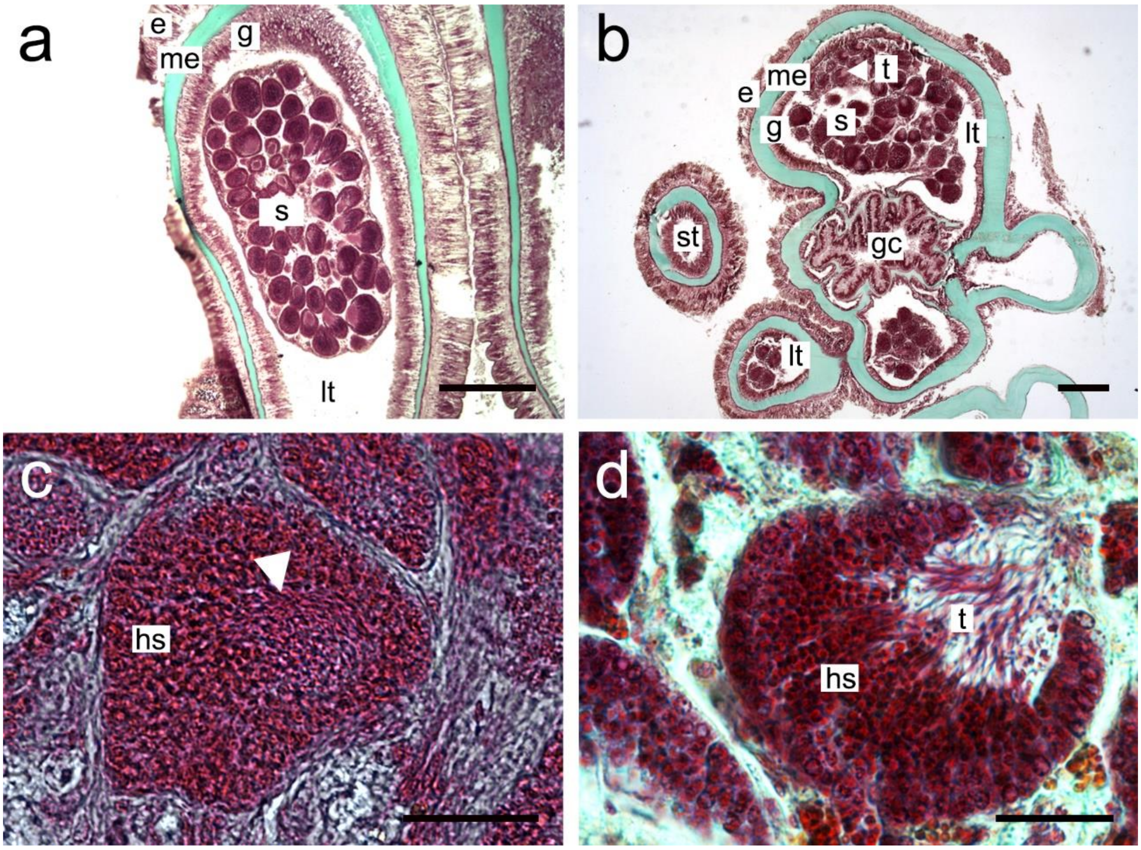

Figure 47. (**a**) Developmental stage 1 of a male polyp. The spermatozoa develop in drop-like spermatic cysts where the head of the spermatozoa are stained darker in the central part. (**b**) Developmental stage 2 of a male polyp where the spermatozoa are developed in fully grown spermatocysts, the spermatozoa appear dark and crowded with the tails going in the same direction (showed by the white arrow). (**c**) Close view of a developing spermatocyst where the tails are in formation (showed by the white arrow). The developing cyst has not reached the spherical shape yet. (**d**) Close view of a fully developed spermatocyst with a spherical shape. All the tails of the spermatozoa are directed in the same direction. The heads of the spermatozoa are organized in rows and appear darkly stained. Scales: a-b: 200 µm; c-d: 20 µm. e ectoderm; g gastroderm; gc gastrovascular cavity; hs heads of the spermatozoa; lt lateral tentacles; me mesoglea; s spermatocysts, st sagittal tentacles; t tails of the spermatozoa.

that maximum diameters were obtained. The sex ratio was established for the whole dataset.

Sea surface temperature data for the Great Reef of Toliara were obtained from the NASA Ocean Biology Processing Group (available at https://oceancolor.gsfc.nasa.gov) and analyzed. Since the black coral bed is in front of the mouth of the Fiherenana river (Terrana & Eeckhaut,pers. comm.), precipitation data for the Toliara region was also considered as a constraining factor and retrieved from Harris et al. (2014).

All the data were processed with R software (R Development Core Team 2008) using a chi-squared test for the sex ratio, correlation tests to check the relationship between temperature/precipitations and maturity, and variance analyses to compare the monthly mean sizes of the oocytes and to establish how they varied.

RESULTS

A total of 114 sets of upper and lower sections were observed for 13 months. Seventy-six of the colonies were mature and 38 unsexable. There were 37 males and 39 females for a sex ratio of 0.95, which was not significantly different from 1:1 (X^2=0.05, p=0.82), but the colonies were not tagged, meaning that a re-sampling of the same colony, once the traces of recovery had totally disappeared, cannot be excluded. On the mature colonies, polyps were either female, male or immature, but never both sexes at the same time (Fig. 46, 47). No larvae or incubating embryos were observed in the histological sections (Fig. 46, 47). Unsexable colonies were more numerous between April 2015 and August 2015, while all the collected corals were developing gametes between December 2014 and February 2015 (Table 10). The percentage of polyps developing gametes was the highest between January and March for the female colonies, and between December and and March for the male colonies (Table 10).

Polyps of both sexes had a lower maturity rate at the base of the colony the whole year round (Fig. 48). Basal polyps of male colonies did not show any gamete between April and October (excepted in September), while those of female colonies did not show any gamete between May and October (excepted in June).

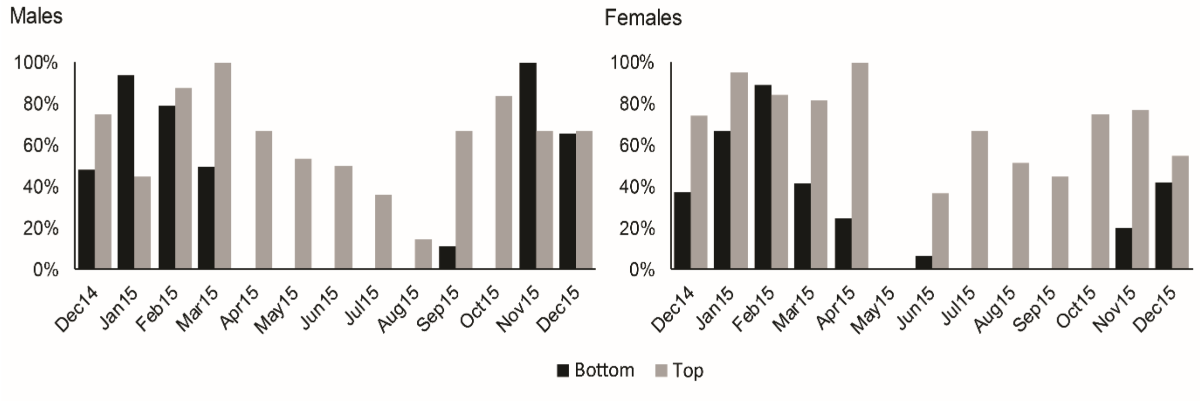

Figure 48. Percentage of mature polyps of both sexes depending on the sampling position. Observations per month between December 2014 and December 2015.

Table 10. Summary of the total number of mature and immature polyps observed every moth for both sexes.

		Male colonies			Female colonies		
Month	Total colonies sampled	Number of male colonies	Total polyps observed	% of polyps developing gametes	Number of female colonies	Total polyps observe	% of polyps developing gametes
Dec 14	8	2	21	62%	6	119	46%
Jan 15	5	2	30	87%	3	44	91%
Feb 15	9	4	58	83%	5	95	84%
Mar 15	10	5	97	79%	4	75	72%
Apr 15	7	1	10	20%	1	12	75%
May 15	9	2	20	20%	0	0	0%
Jun 15	10	1	10	30%	2	32	16%
Jul 15	10	1	20	25%	1	18	33%
Aug 15	7	1	19	5%	2	30	17%
Sep 15	10	5	97	46%	2	64	31%
Oct 15	5	4	32	44%	1	7	43%
Nov 15	15	4	20	95%	8	38	37%
Dec 15	9	5	42	67%	3	57	72%

When considering both sexes, the percentage of mature polyps was correlated to the sea surface temperature recorded in Toliara (Pearson's correlation=0.8645; $p<0.001$). Maturity was the highest between January and March, which also corresponds to the hottest months of this year (Fig. 49). On the contrary, maturity was the lowest between May and August, which corresponds to the coldest months (Fig. 49). The percentage of mature polyps was also correlated to the precipitation (mm) in Toliara (Pearson's correlation=0.8480; $p<0.001$). Rainfall was highest in January and lowest between April and September, which corresponds to the maturity peaks of this species (Fig. 50).

There were no external morphological differences between males and females. In all the specimens, male and female gametes were always located in the primary transversal mesenteries, sometimes reaching the cavities of the proximal and/or distal lateral tentacles. Figure 6 shows the female oocyte size frequencies sorted in 10µm increments and observed over 13 months. In female colonies, gametes were always observed except in May when no females were observed. Female gamete sizes ranged from about 8 µm (small oogonia) and 166 µm (mature oocyte). Monthly mean sizes were significantly different (Fig. 51, Anova, F= 29.134, $p<0.001$), with the minimum average in July 2015 (29.56 ±

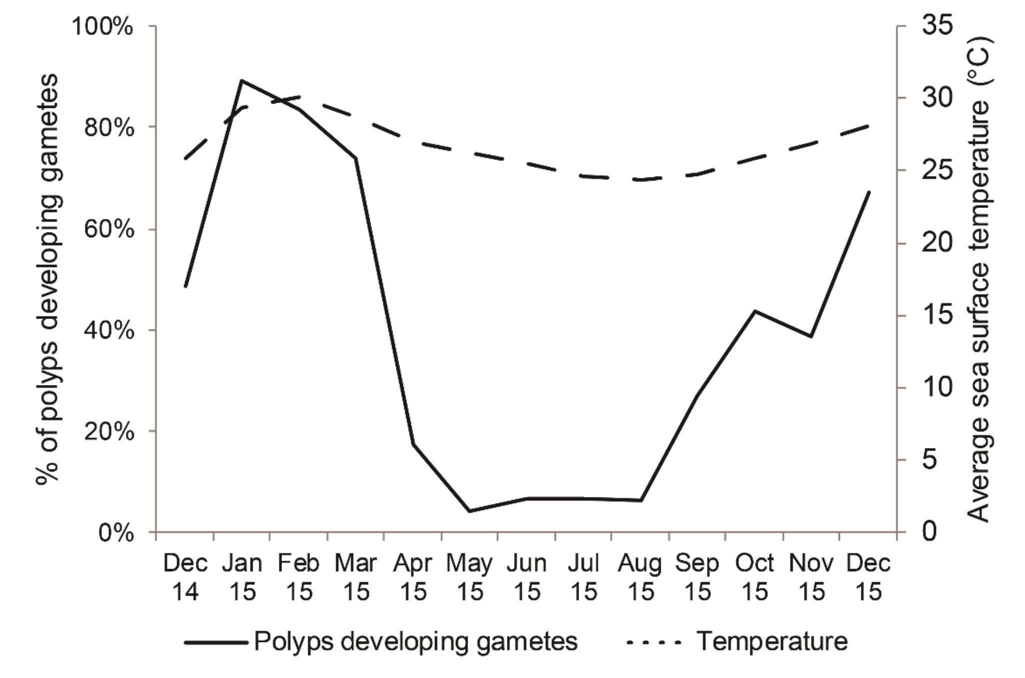

Figure 49. Monthly sea surface temperatures in Toliara plotted against the percentage of polyps developing gametes of all the collected corals between December 2014 and December 2015. Temperature data is obtained from Ocean Biology Processing Group from the NASA (https://oceancolor.gsfc.nasa.gov).

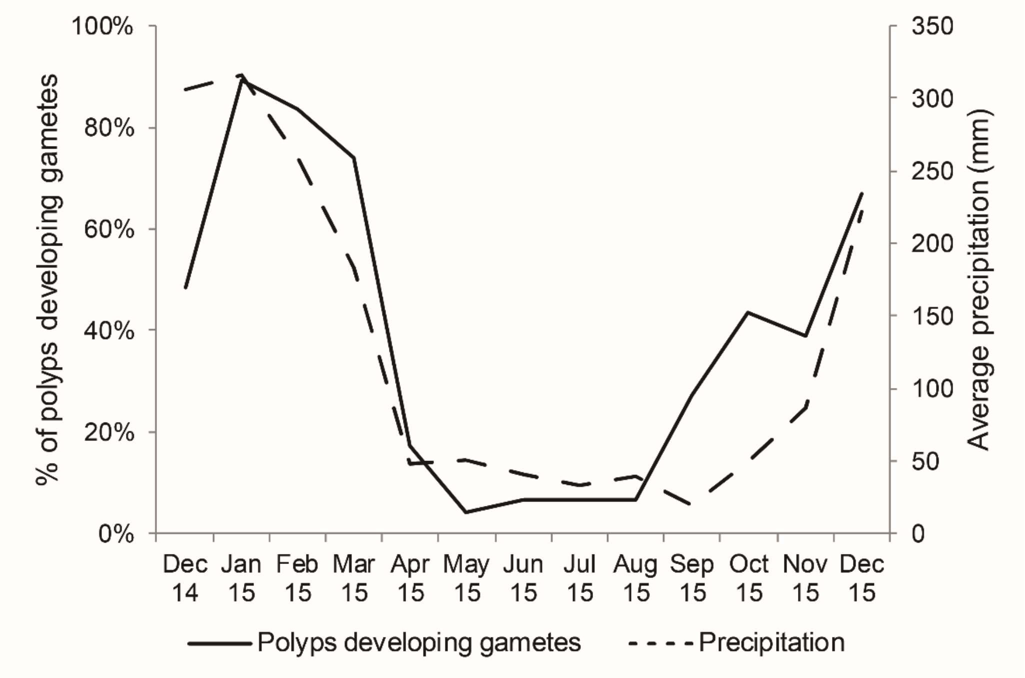

Figure 50. Monthly precipitations in Toliara region plotted against the percentage of polyps developing gametes of all the collected corals between December 2014 and December 2015. Precipitation data is obtained from Harris et al. (2014).

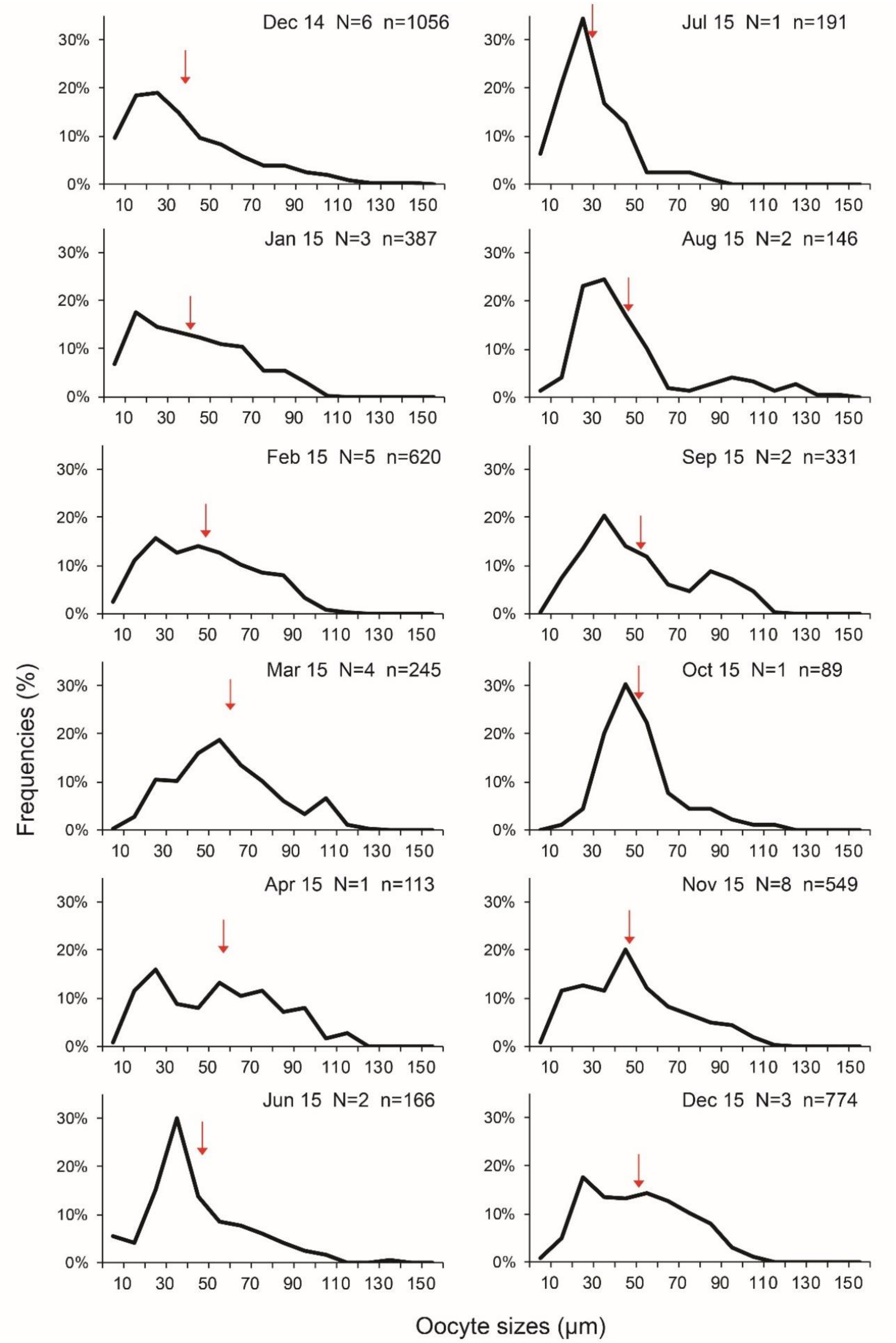

Figure 51. Oocyte-sizes frequencies sorted in 10µm increments observed across 13 months. None mature female colony was sampled in May. The monthly mean oocyte size is showed by a red arrow, where the highest mean is in March and the lowest mean is in July. N: number of female colonies collected for the given month. n: number of oocytes measured for the given month.

15.79 µm) which corresponded to stage 1, and the maximum average in March 2015 (57.92 ± 24.06 µm) which corresponded to stage 2.

There was a significant difference between mean female gamete sizes, depending on the month and colony (AOV, F = 21.242, p<0.001), while the position of the polyp collection had no effect on the average monthly size (AOV, F=0.011, p=0.92).

The development of female gametes was lowest between April and July, as stage 0 was the most abundant (Fig. 52), corresponding to unsexable corals. Stage 2 was the most represented in February and March (Fig. 52). In February 2015, stage 2 was the only one observed, while stage 1 was the only one observed in October (Fig. 52). The development of male gametes followed the same pattern (Fig. 52), as the development was lowest from April to August (stage 0), while the gametes were most developed from December to March (stage 1 and 2, Fig. 52). Mature spermatic cysts were observed all year long (Fig. 52), even if there was less than 25% in proportion between April and August (Fig. 52).

DISCUSSION

Reproductive strategy

Data regarding the occurrence of gametes in the studied species confirms that this species is gonochoric, which is consistent with previous species analyzed (Wagner et al. 2011). This study indicates that the present species, Cirripathes sp., is most probably gonochoric because the reproductive cycle observed in one year followed seasonality (sequential hermaphroditism, if it exists, would occur over periods of longer than one year). However, sequential hermaphroditism cannot be excluded as the colonies were not tagged. Sequential hermaphroditism has already been observed in Cirrhipathes sp. in the Indonesian shallow waters, where the colonies monitored for two years changed their sex over the time of the study (Bo 2008), but it remains the only known case among antipatharians. In the same way, long-time studies have reported gonochoric strategies in two Stichopathes species from Puerto Rico (Goenaga 1977), Antipathes sp. and Stichopathes sp., in Cirrhipathes sp. from the Indo-Pacific (Schmidt & Zissler 1979), and in Antipathella fiordensis (Grange, 1990) from New Zealand (Miller 1996; Parker et al. 1997). Numerous other antipatharians have been observed as being gonochoric, but the observations were limited in time or only based on a few specimens (Wagner et al. 2011). More recently, Antipathes griggi (Opresko, 2009) and Antipathella wollastoni (Gray, 1857)

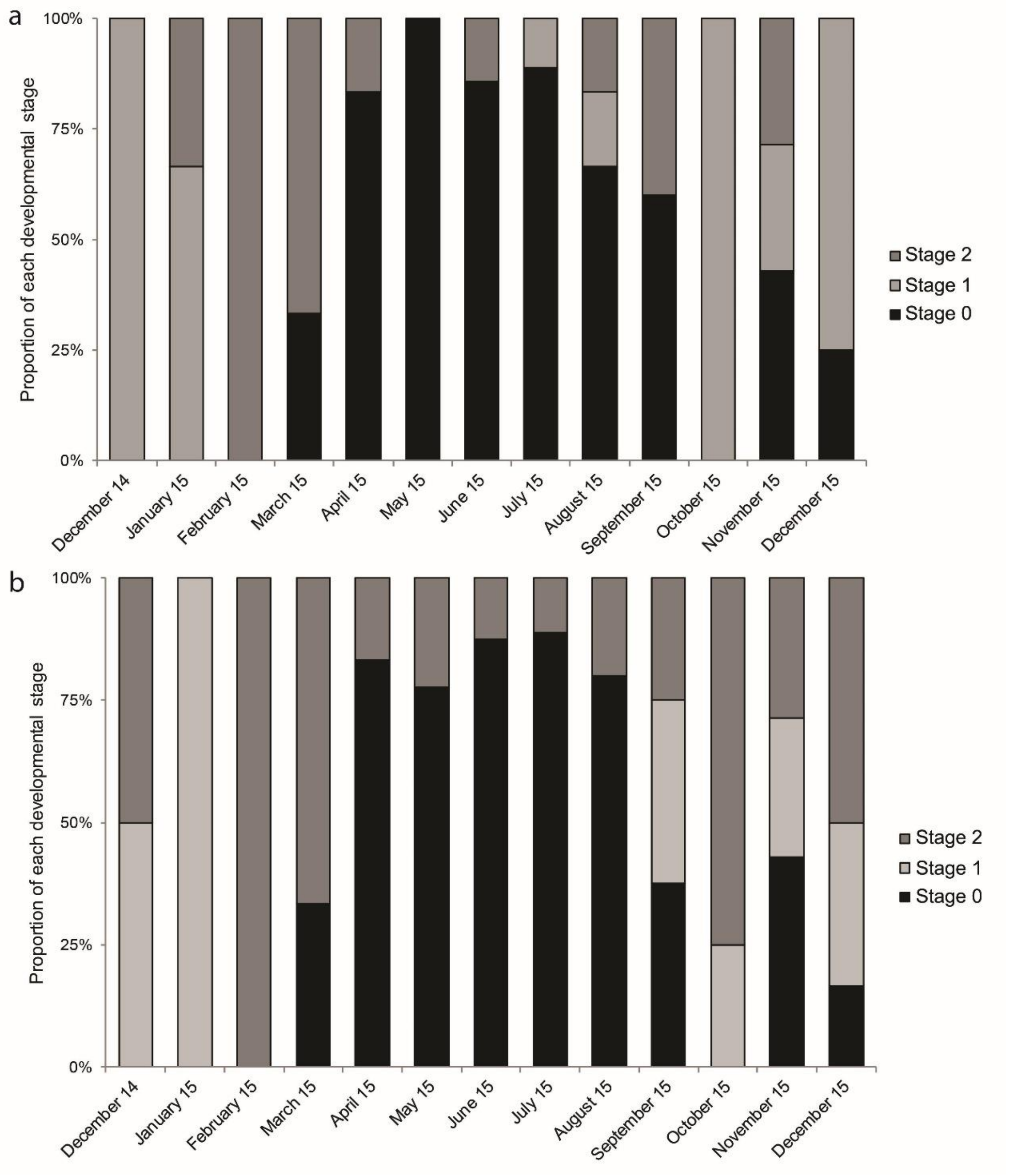

Figure 52. (a)Trend of the developmental stages of the female gametes in the study period. (b) Trend of the developmental stages of the male gametes in the study period.

were monitored over one year, and both species appeared to be gonochoric colonies (Wagner et al. 2012 and Rakka et al. 2016 respectively). Tagged colonies of Antipathes curvata (van Pesch, 1914) collected over 30 months in the shallow waters of Hong Kong were gonochoric and did not change sex over time (Lau 2011).

The female/male ratio observed for Cirripathes sp. was 1:1, which corresponds to the optimal sex allocation in a population with random mating (Maynard-Smith 1978), as highlighted in A. griggi or A. wollastoni (Wagner et al 2012; Rakka et al. 2016).

Reproductive cycle

Mature colonies were found all year at different developmental stages, but maturity peaks clearly occurred in January and February, suggesting an annual gametogenic cycle with a major spawning event. Among shallow-water antipatharians, such seasonality has already been observed, as well as continuous spawning events (Grigg 1976; Goenaga 1977; Schmidt & Zissler 1979; Parker et al. 1997; Gaino & Scoccia 2008, 2010; Lau 2011; Wagner et al. 2012; Rakka et al. 2016). In Cirripathes sp., the period of the year when maturity peaks (expressed as percentage of mature polyps) occur corresponds to the summer and the wet season, with the highest monthly temperatures recorded on the sea surface and the highest average precipitation recorded in Toliara. Such a positive correlation between sea water temperature variations and fecundity have also been observed in other shallow-water black coral species, and temperature has already been identified as a triggering environmental factor for gamete development (Schmidt & Zissler 1979; Parker et al. 1997; Gaino & Scoccia 2010; Lau 2011; Wagner et al. 2012; Rakka et al. 2016). The present reproductive pattern is very similar to those of Antipathes griggi and Antipathella wollastoni, where both male and female colonies contain an increasingly larger proportion of final developmental stage gametes as the warm season approaches, then followed by an off-season with the absence of any gametes when the temperatures are coldest (Wagner et al. 2012; Rakka et al. 2016). For Cirripathes sp., the percentage of mature polyps was lowest between May and August when the coldest temperatures were recorded for the year in question.

The correlation between the average precipitation and the percentage of mature polyps may be explained by the massive supply in organic matter flowing directly into the black coral bed area during the wet season, as the mouth of the Fiherenana river is in front of the coral bed. The resulting development of phyto- and zooplankton may increase the availability of food sources for black corals, and thus directly increase the energy input dedicated to reproduction. The gametogenic cycle may be fast, as the early developmental stages and spent stages were not observed as in Antipathella fiordensis (Parker et al. 1997).

Gamete spawning

As no larvae or developing embryos were found in the polyps, Cirripathes sp. may be either a broadcast spawner, with fertilization occurring in the water column, or female colonies may retain oocytes until they are internally fertilized and then spawn fertilized eggs very quickly after gamete fusion. However, it is more likely that the present species is a broadcast spawner as no signs of fertilization were ever observed in the histological section throughout the whole study period. In addition, the spermatozoa of Cirrhipathes species are considered primitive as they have pro-acrosomal vesicles and a (9+2) flagellum (Gaino et al. 2008), which is adapted to external fecondation (Reunov 2005). Wagner et al. (2012) suggested that Antipathes griggi oocytes could be retained for a brief period following internal fertilization as no male polyps were collected for this species over several months, while there was a small number of female polyps still containing oocytes. This was not the case for the present species as both sexes were observed every month, except in May, when no female colonies were collected.

Despite no observations of gamete spawning or planula larvae in situ, several observations support that external fertilization occurs in many black corals: (i) Miller (1996) observed that the resulting larvae of Antipathella fiordensis were negatively buoyant, weak swimmers and most likely non-feeding; (ii) oocyte release may be responding to male pheromones as demonstrated in aquaria after the release of male gametes (Goenaga 1977); (iii) polyps of Cupressopathes pumila (Brook, 1889) and Antipathella subpinnata (Ellis & Solander, 1786) release buoyant spheres from their mouth (Gaino & Scoccia 2009; Gaino & Scoccia 2010); (iv) histological sections of Cirrhipathes cf. anguina revealed lysed cells bordering the mesenteries and sperm accumulating in the gastric cavity, suggesting sperm release from the mouth (Gaino et al. 2008); and (v) the ultrastructure of the spermatozoa supports external fertilization, as stated before (Gaino et al. 2008).

It is possible to hypothesize that the annual reproductive cycle of Cirrhipathes sp. includes a peak of polyp maturity between January and March (triggered by temperature rise), followed by a major spawning event at the end of summer (March) and, consequently, a sharp decrease in the frequency of fertile polyps. Nonetheless, minor spawning events may occur throughout the year, as demonstrated by the occurrence of fertile colonies all year round. The occurrence of minor spawning events is also supported by the presence of different cohorts of gametes in the same reproductive mesentery, as observed for A. griggi and Cirrhipathes cf. anguina (Wagner et al. 2012; Gaino & Scoccia 2008). Multiple spawning has also been observed in tagged colonies of Cirrhipathes sp. from the Indonesian shallow waters (Bo 2008), in which gametes disappeared between successive events. Wagner et al. (2012) highlighted that spawning over multiple episodes could preserve the reproductive effort if a catastrophic event occurs but may decrease the fertilization success due to lower gamete concentrations per event (Levitan et al. 1991, 1992). These authors suggested that colonies of A. griggi may overcome this issue by relying on dense aggregations of colonies (Grigg 1976, 2001, 2004). The same strategy can be attributed to Cirripathes sp. as it is the most abundant species of the black coral bed of Toliara (Terrana and Eeckhaut, pers. comm.).

Male and female gametes

There were no morphological differences between male and female colonies as reported for many other species (Parker et al. 1997; Gaino & Scoccia 2008; Wagner et al. 2011, 2012; Rakka et al. 2016). In a few species a difference in coloration between gravid female and male polyps has been reported, but this is due to the orange coloration of the oocytes rather than a true sexual dimorphism (Parker et al. 1997; Gaino & Scoccia 2008; Bo et al. 2015). In Cirrhipathes sp. male and female gametes always developed in the primary transverse mesenteries, sometimes expanding into the cavity of the lateral tentacles. This gamete location is a general feature among the order Antipatharia, as it is always reported for mature colonies (Wagner et al. 2011; Lau 2011; Wagner et al. 2012; Rakka et al. 2016; Lauretta & Penchaszadeh 2017).

The female gamete sizes in Cirripathes sp. had a maximum diameter of 167 µm, which is comparable to other Antipathidae species, e.g. 125 µm in Cirrhipathes cf. anguina (Gaino & Scoccia 2008), 140 µm in Antipathella fiordensis (Parker et al. 1997), 125 µm in Antipathes curvata (Lau 2011) and 150 µm in Antipathes griggi (Wagner et al. 2012). In some cases, gametes can cluster into egg masses, e.g. in oocytes of 150 µm in Antipathella subpinnata (Gaino & Scoccia 2010), or into spheres, e.g. sperm clusters in Cupressopathes pumila (Gaino & Scoccia 2009), before being released. In Cirripathes sp., there was no evidence of such a grouping of gametes, either for female or male polyps. Other species develop larger oocytes, such as Antipathella wollastoni, with about 350 µm (Rakka et al. 2016), or the exceptionally gigantic oocytes of the deep-sea black coral Dendrobathypathes grandis (Opresko, 2002), with a diameter of about 1400 µm (Lauretta & Penchaszadeh 2017). In the latter species, only 9 oocytes per polyp have been recorded. This reproductive strategy decreases the number of offspring that can be produced but produces oocytes with a high amount of yolk. They generally develop into non-feeding lecithotrophic larvae that have a large oceanic dispersion (Lauretta & Penchaszadeh 2017).

In this case, Cirrhipathes sp. has a different strategy with the presence of small oocytes that could result in short-lived larvae and a smaller dispersion.

Intracolonial comparisons

There was an asynchronization in maturity between the top and the base of the colonies, as mature polyps were found all year long for polyps living on the highest part of the colonies. Mature polyps were almost always absent at the base of the coral during the cold season, between April and October, while the top polyps just showed a decrease in the percentage of mature polyps at this time. Such a form of inertia in maturity in different parts of the coral has already been observed for Antipathella wollastoni, where the middle parts of the branched colonies were mature for a longer time than the other parts (Rakka et al. 2016). A longer reproductive cycle in the apical portion of the unbranched colonies of Cirripathes sp. might be due to a different energy allocation in reproduction in this specific area: the upper polyps may have a better exposure to planktonic food as they are more exposed in the water column thanks to the size and shape of the colony. While reports of intra-colonial differences in reproductive parameters among black corals are scarce, it has been reported many times in other anthozoans, especially within octocorals (Orejas et al. 2002, 2007; Beazley & Kenchington 2012; Baillon et al. 2014).

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LITERATURE

Baillon S, Hamel JF, Wareham VE, Mercier A. 2014. Seasonality in reproduction of the deep-water pennatulacean coral *Anthoptilum grandiflorum*. Marine Biology 161: 29–43. https://doi.org/10.1007/s00227-013-2311-8

Beazley LI, Kenchington EL. 2012. Reproductive biology of the deep-water coral *Acanella arbuscula* (Phylum Cnidaria: Class Anthozoa: Order Alcyonacea), Northwest Atlantic. Deep Sea Research Part I. Oceanography Research Papers 68: 92–104. https://doi.org/10.1016/j.dsr.2012.05.013

Bo M. 2008. Taxonomy and ecology of antipatharians. PhD dissertation, Universita Politecnica Delle Marche, Ancona, Italy. 212 pages.

Bo M, Bavestrello G, Angiolillo M, Calcagnile L, Canese S, Cannas R, Cau A, D'Elia M, D'Oriano F, Follesa MC, Quarta G, Cau A. 2015. Persistence of Pristine Deep-Sea Coral Gardens in the Mediterranean Sea (SW Sardinia). PLoS ONE 10(3): e0119393. https://doi.org/10.1371/journal.pone.0119393

Bruckner A, de Angelis P, Montgomery T. 2008. Case Study for Black Coral from Hawaii. International Expert Workshop on CITES Non-Detriment Findings, Cancun.

Brugler MR, Opresko DM, France SC. 2013. The evolutionary history of the order Antipatharia (Cnidaria: Anthozoa: Hexacorallia) as inferred from mitochondrial and nuclear DNA: implications for black coral taxonomy and systematics. Zoological Journal of the Linnean Society 169: 312–361. https://doi.org/10.1111/zoj.12060

Castorena V, Metaca M. 1979. El coral negro, una riqueza en peligro. Tecnica Pesquera 139:22–27.

Coma R, Zabala M, Gili JM. 1995. Sexual reproductive effort in the Mediterranean gorgonian *Paramuricea clavata*. Marine Ecology Progress Series 117: 185–197.

Gaino E, Scoccia F. 2008. Female gametes of the black coral *Cirrhipathes* cfr. *anguina* (Anthozoa, Antipatharia) from the Indonesia Marine Park of Bunaken. Invertebrate Reproduction and Development 51: 119–126. https://doi.org/10.1017/S1755267209000712

Gaino E, Scoccia F. 2009. Release of sperm clusters in spheres by the black coral *Cupressopathes pumila* (Anthozoa, Antipatharia). Coral Reefs 28: 851–857. https://doi.org/10.1007/s00338-009-0525-5

Gaino E, Scoccia F. 2010. Gamete spawning in *Antipathella subpinnata* (Anthozoa, Antipatharia): a structural and ultrastructural investigation. Zoomorphology 129: 213–219. https://doi.org/10.1007/s00435-010-0112-x

Goenaga C. 1977. Two new species of *Stichopathes* (Zoantharia; Antipatharia) with observations on aspects of their biology. MS thesis, University of Puerto Rico, Puerto Rico. 101 pp.

Grigg R. 1976. Fishery management of precious and stony corals in Hawai'i. Sea Grant Technical Report, UNIHISEAGRANT-TR-77-03. 48 pp.

Grigg RW. 1984. Resource management of precious corals: a review and application to shallow water reef building corals. Marine Ecology 5: 57–74. https://doi.org/10.1111/j.1439-0485.1984.tb00307.x

Grigg RW. 1993. Precious coral fisheries of Hawai'i and the U.S. Pacific Islands. Marine Fisheries Review 55: 50–60.

Grigg RW. 2001. Black coral: History of a sustainable fishery in Hawai'i. Pacific Science 55: 291–299. https://dx.doi.org/ 10.1353/psc.2001.0022

Grigg RW. 2004. Harvesting impacts and invasion by an alien species decrease estimates on black coral yield off Maui, Hawai'i. Pacific Science 1: 1–6. https://doi.org/10.1353/psc.2004.0006

Harris I, Jones PD, Osborn TJ, Lister DH. 2014. Updated high-resolution grids of monthly climatic observations – the CRU TS3.10 Dataset. International Journal of Climatology 34(3): 623–642. https://doi.org/10.1002/joc.3711

Huang MH, Ou CH. 2010. Precious coral fisheries management in Taiwan - Past, present & future. Marine Policy 34: 1002–1009. https://doi.org/10.1016/j.marpol.2010.02.007

Kenyon J. 1984. Black coral off Cozumel. Sea Front 30:267–272

Lapian HFN, Barucca M, Bavestrello G, Biscotti MA, Bo M, Canapa A, Tazioli S, Olmo E. 2007. A systematic study of some black coral species (Antipatharia, Hexacorallia) based on rDNA internal transcribed spacers sequences. Marine Biology 151: 785–792. https://doi.org/10.1007/s00227-006-0525-8

Lau PL. 2011. Reproductive biology and steroidal levels in black coral *Antipathes curvata* in Hong Kong. PhD dissertation, The Chinese University of Hong Kong, 115 pages.

Lauretta D, Penchaszadeh PE. 2017. Gigantic oocytes in the deep-sea black coral *Dendrobathypathes grandis* (Antipatharia) from the Mar del Plata submarine canyon area (southwestern Atlantic). Deep Sea Research Part I: Oceanographic Research Papers 128: 109-114. https://doi.org/10.1016/j.dsr.2017.08.011

Maynard-Smith J. 1978. The evolution of sex. Evolutionary Biology. Cambridge University Press. Gambridge University Press, NewYork, 236 pages.

Miller K, Grange KR. 1995. Population genetic studies of antipatharian black corals from Dubtful and Nancy Sounds, Fiordland, New Zealand. Proceedings of 6th International Conference on Coelenterate Biology, Noordwijkerhout, The Netherlands, pp. 353–363.

Miller K. 1996. Piecing together the reproductive habits of New Zealand's endemic black corals. Water Atmosphere 4: 18–19.

Miller K. 1997. Genetic structure of black coral populations in New Zealand's fiords. Marine Ecology Progress Series 161: 123–132. https://doi.org/10.3354/meps161123

Miller K. 1998. Short-distance dispersal of black coral larvae: inference from spatial analysis of colony genotypes. Marine Ecology Progress Series 163: 225–233. https://doi.org/10.3354/meps163225

Noome C, Kristensen I. 1976. Necessity of conservation of slow growing organisms like black coral. CCA Ecology Conference Bonaire 11:76–77.

Opresko DM, Tracey D, Mackay E. 2014. Antipatharia (black corals) for the New Zealand region. A field guide of commonly sampled New Zealand black corals including illustrations highlighting technical terms and black coral morphology. New Zealand Aquatic Environment and Biodiversity Report No. 131. 20 pages.

Orejas C, López-González PJ, Gili JM, Teixidó N, Gutt J, Arntz WE. 2002. Distribution and reproductive ecology of the Antarctic octocoral *Ainigmaptilon antarcticum* in the Weddell Sea. Marine Ecology Progress Series 231: 101–114. https://doi.org/10.3354/meps231101

Orejas C, Gili JM, López-González PJ, Hasemann C, Arntz WE. 2007. Reproduction patterns of four Antarctic octocorals in the Weddell Sea: an inter-specific, shape, and latitudinal comparison. Marine Biology 150: 551–563. https://doi.org/10.1007/s00227-006-0370-9

Padilla C, Lara M. 2003. Banco Chinchorro: the last shelter for black coral in the Mexican Caribbean. Bulletin of Marine Science 73: 197–202.

Parker NR, Mladenov PV, Grange KR. 1997. Reproductive biology of the antipatharian black coral *Antipathes fiordensis* in Doubtful Sound, Fiordland, New Zealand. Marine Biology 130: 1–22. https://doi.org/10.1007/s002270050220

R Development Core Team. 2008. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0.

Rakka M, Orejas C, Sampaio I, Monteiro J, Parra H, Carreiro-Silva M. 2016. Reproductive biology of the black coral *Antipathella wollastoni* (Cnidaria: Antipatharia) in the Azores (NE Atlantic). Deep-Sea Research Part II 145: 131–141. https://doi.org/10.1016/j.dsr2.2016.05.011

Reunov AA. 2005. Problem of terminology in characteristics of spermatozoa of Metazoa. Russian Journal of Developmental Biology 36: 335–351. https://doi.org/10.1007/s11174-005-0050-6

Romero XM. 1997. Ecuador's vanishing black corals. Aquaticus: Journal of the Shedd Aquarium 26: 21–25.

Schmidt H, Zissler D. 1979. Die Spermien der Anthozoen und ihre phylogenetische Bedeutung. Zoologica 44: 1–97.

Schneider CA, Rasband WS, Eliceiri KW. 2012. NIH Image to ImageJ: 25 years of image analysis. Nature methods 9: 671–675. https://doi.org/10.1038/nmeth.2089

Todinanahary G, Terrana L, Lavitra T, Eeckhaut I. 2016. First records of illegal harvesting and trading of black corals (Antipatharia) in Madagascar. Madagascar Conservation & Development 11: 6–11. http://dx.doi.org/10.4314/mcd.v11i1.5

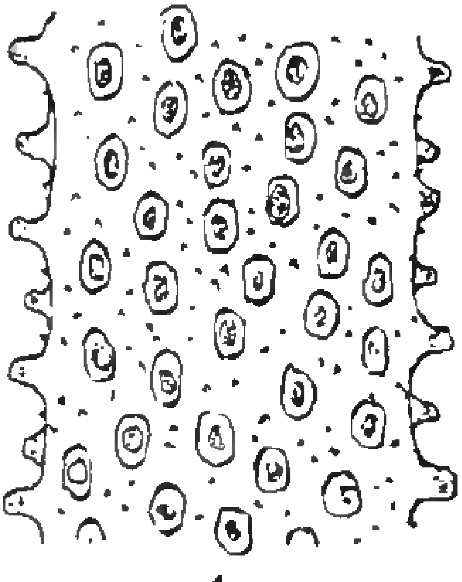
Tsounis G, Rossi S, Grigg RW, Santangelo G, Bramanti L, Gili JM. 2010. The exploitation and conservation of precious corals. Oceanography and Marine Biology – An Annual Review 48: 161–212. https://doi.org/10.1201/ebk1439821169-c3

Wagner D, Waller RG, Toonen RJ. 2011. Sexual reproduction of Hawaiian black corals, with a review of the reproduction of Antipatharians (Cnidaria: Anthozoa: Hexacorallia). Invertebrate Biology 130: 211–225. https://doi.org/10.1111/j.1744-7410.2011.00233.x

Wagner D, Luck DG, Toonen RJ. 2012. The biology and ecology of black corals (Cnidaria: Anthozoa: Hexacorallia: Antipatharia). Advances in Marine Biology 63: 67–132. https://doi.org/10.1016/b978-0-12-394282-1.00002-8

Chapitre 5

Species boundary and connectivity of the whip black coral *Stichopathes* sp. aff. *maldivensis* from the southwest of Madagascar



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Original illustration of Stichopathes maldivensis by Cooper, 1903

Species boundary and connectivity of the whip black coral *Stichopathes* sp. aff. *maldivensis* from the southwest of Madagascar

ABSTRACT

Shallow-water black corals are threatened by illegal fisheries over the world. This is especially the case in Madagascar where the total prohibition of fisheries and exploitation has been introduced in 2014 in an official statement. We recently described the black coral fauna of the South West of Madagascar and out of the 18 described species, one of the most abundant was *Stichopathes* sp. aff *maldivensis*. We here focused on the connectivity between *S*. sp. aff *maldivensis* groups in this part of Madagascar. The region from Maromena to Andavadoaka, *i.e*. 200 km of coast, was investigated. In this region, three rivers drain their fresh water into the sea, especially during the rainy season, and these fresh water masses might be natural barriers for the larval dispersal of black corals. Thus, we studied the genetic variation existing between 40 specimens of *S*. sp. aff *maldivensis* collected in 5 sites and estimated the genetic variation of the two existing colour types – brown and white– present in this area. We used a genetic approach based on haplowebs drawn with haplotypes coming from ITS1 sequences from the ribosomal DNA. Twenty-one haplotypes were observed in the samplings and connections between these haplotypes suggest that they all belong to one population with no obvious sub-populations but with individuals sharing similar haplotypes. Some specimens coming from Indonesia whose sequences were retrieved from literature were also sharing the same haplotypes and were part of the large ITS1 pool of sequences, suggesting that they could belong to the same species and gene pool.

This manuscript is the draft of the paper that will be submitted with the following co-authors and order:

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INTRODUCTION

Antipatharians, commonly called black corals, belong to the order Antipatharia which regroups approximately 240 species divided in 7 families (Wagner et al. 2012). Their classification has always been difficult because of the lack of precise traits in the beginning of their natural history, in addition to descriptions made on poorly preserved organisms. Moreover, antipatharians present a large intraspecific plasticity which might have led to the description of different morphotypes of same species as being different ones, thus overestimating the total number of species in deep waters as well as in tropical shallow waters (Lapian et al. 2007; Bo et al. 2012; Brugler et al. 2013). Bo et al. (2012) carried out a taxonomic review of the whip black coral genus *Stichopathes* from the Indonesian reefs based on both morphological and molecular traits. This genus includes species that can be straight, slightly contorted and/or forming loose spirals, or strictly helicospirals. In their analysis, the *Stichopathes* group into 4 different clades (clades A to D). These groupings suggested that single-stem colonies may be polyphyletic, and that the unbranched pattern observed in other colonies might have appeared multiple times independently during the black coral history (Bo et al. 2012; Brugler et al. 2013).

If the taxonomy of the order is still mainly based on morphological features such as skeleton spines, the shape of the colony and the polyp morphology (Brugler et al. 2013), several genetic studies have attempted to clarify the relationship between taxa. In some cases, it revealed polyphyletic genera or unexpected closely related species (Lapian et al. 2007; Lapian 2009; Wagner et al. 2010; Brugler et al. 2013), meaning that some taxa need a deep revision based on both molecular and morphological features. The first complete mitochondrial genome of a black coral was published by Brugler and France (2007) for *Chrysopathes formosa*, followed by the partial mitochondrial genome of *Leiopathes glaberrima* by Sinniger and Pawlowski (2009). Brugler et al. (2013) published the largest phylogenetic survey of antipatharians to date by inferring the evolutionary history of the whole order from multi-locus phylogenies using both mitochondrial (IGRs intergenic regions and cox3-cox1) and nuclear (ITS internal transcribed spacers, 18S and 28S) genes. Their study showed that the shape of a colony is a taxonomic character with a low phylogenetic value while the microscopic skeletal features appeared rather a better one. They showed that three families and some genera are polyphyletic, such as *Stichopathes* which belongs to two families, the Aphanipathidae and the Antipathidae, the latter being the actual, traditionally accepted family of the genus.

The mitochondrial cytochrome c oxidase subunit I (COI) gene used in barcoding shows exceptionally low rates of evolution within the Anthozoa, leading to low-to-no interspecific variation which is also the case for antipatharians (France & Hoover 2002; Shearer et al. 2002; Hellberg 2006; McFadden et al. 2010, Brugler et al. 2013). In the mitochondrial genome, the intergenic regions (IGRs) has shown their utility to solve phylogenetic relationships when combined with other nuclear markers, such as ITS1 and ITS2 regions from ribosomal DNA (Brugler et al. 2013). These non-coding regions are flanked by the highly conserved 18S, 5.8S and 28S genes and are present in numerous copies in a genome (Dover 1983). They evolve rapidly and show individual intra-genomic polymorphism (Wörheide et al. 2004) and their suitability for population genetics and phylogenetic inferences has already been proved in soft corals (Calderon et al. 2006) or scleractinian corals (Flot & Tillier 2006; Flot et al. 2010; Flot et al. 2011; Adjeroud et al. 2014). Among antipatharians, they have been used to distinguish commercially valuable black corals of Hawaiian populations (Wagner et al. 2010), to construct phylogenies of the order Antipatharia (Lapian et al. 2007; Lapian 2009) or the family Schizopathidae (MacIsaac et al. 2013), to establish the new genus *Pseudocirrhipathes* (Bo et al. 2009) and to characterize *Stichopathes* species from Indonesian reefs (Bo et al. 2012).

To date, there is only one population genetic study on antipatharian (Miller 1997) where the population structure of *Antipathes fjordensis* in New Zealand fjords was analyzed through allozyme electrophoresis. Miller (1997) found significant genetic differentiation between sampling sites within fjords. Their analyses suggested a very limited dispersal of *A. fjordensis* larvae between sites, *i.e.* over distances of tens of kilometers. On the other hand, we recently reported on the biodiversity of shallow-water black corals from the southwest of Madagascar (Chapter 3). We reported 18 species, out of which 12 were identified at the species level based on the descriptions from literature while the others were identified at the genus level. Three species were very abundant and one, identified as *Stichopathes* sp. aff *maldivensis*, was easily collected over 200 km of coast. This species corresponds to the unbranched specimens of clade D of Bo et al. (2012), which includes 8 unbranched specimens and one branched specimen. The present paper aims first at estimating the connectivity between *Stichopathes* sp. aff *maldivensis* populations in the southwest of Madagascar in order to see if it has a very limited dispersal, as it has been already seen in black corals. The area of the study is exposed to terrestrial runoffs of fresh waters coming from 3 rivers –the Onilahy, the Fiherenana and the Manombo rivers– which might be natural barrier occurring for the larval dispersal of black corals. In the same way as in Sulawesi (Bo et al. 2012), *Stichopathes* sp. aff *maldivensis* in Madagascar shows two colourations, brown and white, and the second aim of the present study is to estimate if these colour types are phenotypic variations of the same species along the southwestern coast.

MATERIAL & METHODS

Sampling

Samplings were made by SCUBA diving between 14 m and 28 m depth. in November and December 2014, in May, November and December 2015 and in July and August 2016. A total of 43 colonies of *Stichopathes* sp. aff. *maldivensis* was collected, of which 7 came from Maromena, 3 from Soalara, 21 from the north channel of Toliara, 1 from the South channel of Ifaty, 7 from the North channel of Ifaty and 4 from Andavadoaka (Fig. 53). The brown type was found in all the stations (n=37) while the white morphotype was only present in Maromena, the North channel of Toliara and Andavadoaka (n=6). The two most distant sites, Andavadoaka and Maromena, are spaced by approximately 200 km. The fresh waters of three rivers, the Onilahy, Fiherenana and Manombo (Fig. 53) run into the sea and can drop the salinity approximately to zero on the first meters of the surface (pers. obs. from authors), especially during the wet season which extends from December to March. Black corals are exceptionally abundant in the North Channel of the GRT (see Chapter 3). During the dives, pieces of 2 to 3 cm² of tissues were taken with a sharp blade at the apex of the colony and directly put into a labelled vial, before being stored in absolute ethanol. Colonies of *Stichopathes* sp. aff. *maldivensis* were always bearing a single row of crowded polyps which were sometimes turning around the axis. The color of the whole colony is either white or brown (Fig. 54). The corals are slightly sinuous, reaching up to 5 meters in length.

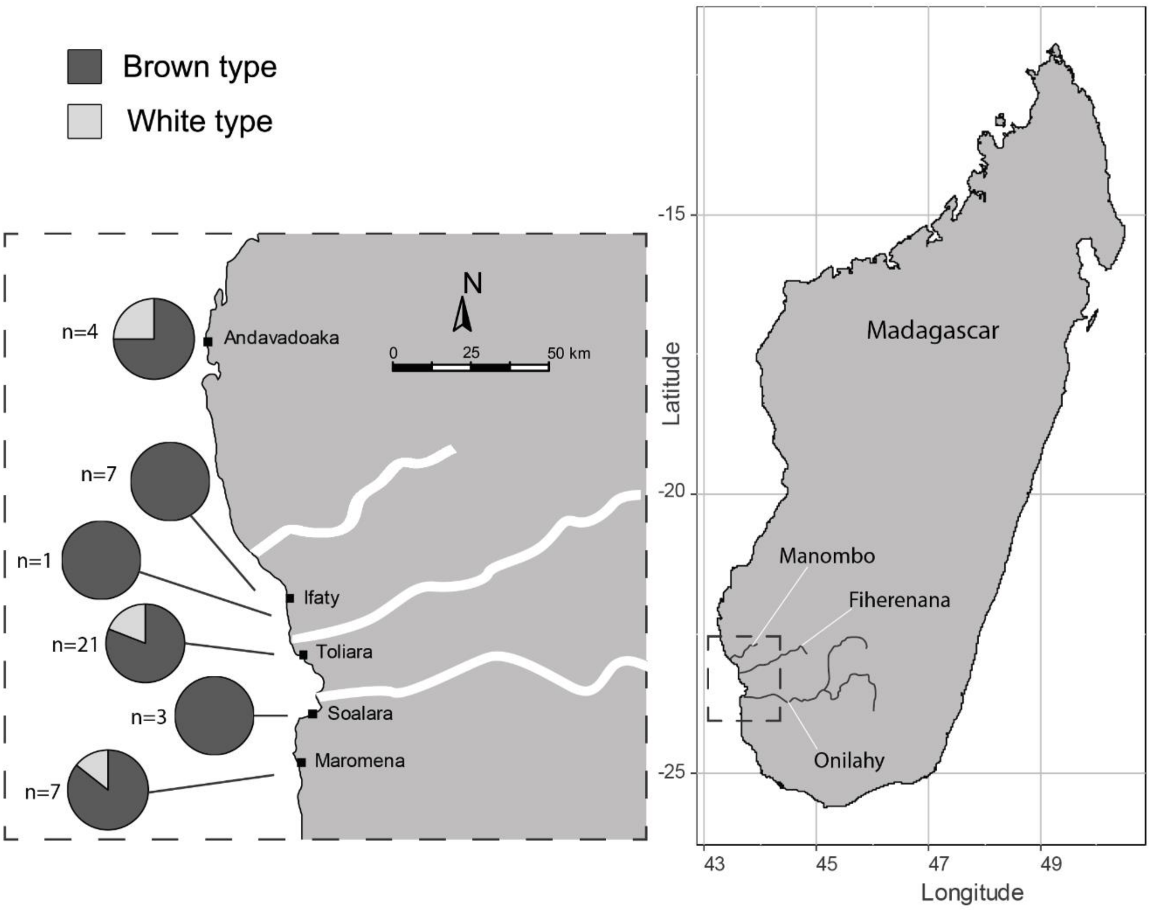

Figure 53. Sampling areas in the southwest of Madagascar. Total samples taken by location are indicated as "n".

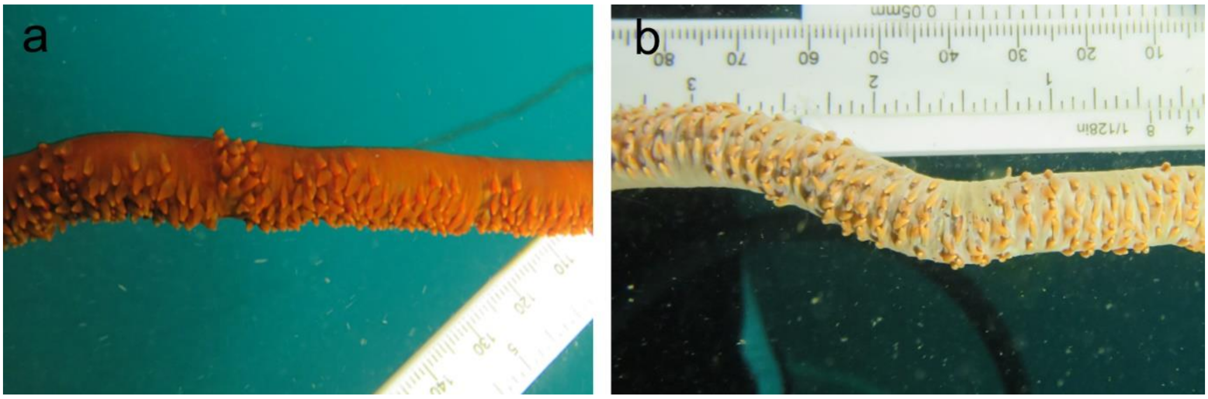

Figure 54. Color types of *Stichopathes* sp. aff. *maldivensis* found on the southwestern coast of Madagascar. a: brown type, b: white type.

DNA extraction, amplification and sequencing

Total genomic DNA was extracted using the Invisorb Spin Tissue Minikit (Invitek) according to the manufacturer's protocol and stored at -20°C. The internal transcribed spacer 1 was first amplified using the primers F18S1 (5'-CGATYGAAYGGTTTAGTGAGGC-3') with ITSc1 (5'–CATTTGCGTTCAAAGATTCG–3') of Flot et al. (2011). As the ITS did not amplified correctly in all samples, we designed the new primers T18S (5'–GGATGCCGAGAAGTTGTTCA–3') and T58S (5'– TTCACACTACTTATCGCACCTG–3'). With both pairs of primers, the amplifications were carried out in 50µl reaction mixes using the Red'y'StarMix (Eurogentec) with the following conditions: initial denaturation and polymerase activation at 95°C for 10 min; 50 cycles of denaturation at 94°C for 30 sec, annealing at 53°C for 30 sec and elongation at 72°C for 1 min; and 10°C for 5 min. Amplicons were sequenced in both directions by Sanger-sequencing (Genewiz, UK) using the same primers than the amplification.

Determination of haplotypes and data treatment (see appendix at the end of this Chapter for a more detailed description of the methodology)

Chromatograms were assembled and cleaned using Sequencher 4 (Gene Codes). For the complete detail of the following method and more explanations, please refer to the supplementary materialat the end of the chapter. Heterozygote sequences were differently treated than homozygote sequences. For heterozygotes having a single double peak, their sequences were solved manually. Heterozygotes having numerous double peaks can have the forward and the reverse sequences of equal length (without insertion or deletion) or of different length (with insertion or deletion). The haplotypes of length-variant heterozygotes were inferred from the patterns of double peaks in the forward and reverse chromatograms using the program Champuru (Flot et al. 2006; Flot 2007). Heterozygotes having haplotypes of equal lengths were phased using the programs SeqPHASE (Flot 2010) and PHASE (Stephens et al. 2001) to determine the probable haplotypes with posterior probabilities. Only unambiguous sequences were retrieved, i.e. those with the highest posterior probabilities. The sequences of all haplotypes were then aligned in MEGA7 using MUSCLE (Tamura et al. 2011). Each SNP (Single Nucleotide Polymorphisms) was checked by eye and their chromatograms reconsidered to avoid mistakes. Haplotype networks were built using the online software "Haploweb maker" with a median–joining method (available online at https://eeg-ebe.github.io/HaplowebMaker/). In the resulting networks, the connections represent co-occurring haplotypes in heterozygous individuals, the thickness of the lines represents the frequencies of co-occurrences between haplotypes, and the pie charts represents each haplotype with their size depending on their frequency.

Phylogenetic analyses

ITS1 sequences of black corals from different parts of the world (Table 11) were retrieved from Lapian et al. (2007), Bo et al. (2009), Wagner et al. (2010) and Bo et al. (2012) and aligned with sequences from this study using ClustalW implemented in MEGA7 (Kumar et al. 2016) with default gap opening and extension penalties. A maximum likelihood analysis was made using MEGA7 (Kumar et al. 2016) with a bootstrap method of 500 replications. The General Time Reversible model (GTR) as a substitution model with a gamma distribution with invariant sites (G+I) was proposed by Modeltest (Posada 2006) and used in the analyses. Overall mean distances for Madagascar specimens and distances between populations from Madagascar and the SE Asia were calculated for *Stichopathes* sp. using the Kimura 2-parameter model. Gap or missing data were treated as complete deletions.

Bayesian analyses were performed using MrBayes v3.0b4 (Ronquist & Huelsenbeck 2003), using the model (GTR+I+G). Four Markov chains were run simultaneously for 10^6 generations, and trees were sampled every 100 cycles for a total of 10^4 trees. The first 25.10^4 trees with pre–asymptotic likelihood scores were discarded as 'burnin'. The remaining trees were used to compute Bayesian posterior probabilities.

Table 11. Species, localities, accession numbers and references of the sequences used in this study.

Family and species	Locality	Accession #	References
	Antipathidae		
Allopathes desbonni	Louisiana, USA	FM882171	Bo et al. 2009
Antipathes atlantica	Hawai'i, USA	HM060621	Wagner et al. 2010
Antipathes caribbeana	Netherlands Antilles	GU296498	Wagner et al. 2010
Antipathes curvata	South China Sea	GU296497	Wagner et al. 2010
Antipathes elegans	Bunaken, Indonesia	AM404317	Lapian et al. 2007
Antipathes furcata	Hawai'i, USA	HM060620	Wagner et al. 2010
Antipathes grandis	Bunaken, Indonesia	AM404316	Lapian et al. 2007
Antipathes grandis	Hawai'i, USA	GU296493	Wagner et al. 2010
Antipathes griggi	Hawai'i, USA	GU296496	Wagner et al. 2010
Antipathes sp.1	Bunaken, Indonesia	AM404315	Lapian et al. 2007
Antipathes sp.2	Bunaken, Indonesia	AM404321	Lapian et al. 2007
Antipathes sp.3	Bunaken, Indonesia	HE600716	Bo et al. 2012
Cirrhipathes spiralis	Bunaken, Indonesia	AM404320	Lapian et al. 2007
Cirrhipathes sp.	Bunaken, Indonesia	AM404319	Lapian et al. 2007
Pseudocirrhipathes mapia	Bunaken, Indonesia	FM882167	Bo et al. 2009
Pseudocirrhipathes mapia	Bunaken, Indonesia	FM882168	Bo et al. 2009
Stichopathes cf. *occidentalis*	NW Atlantic Ocean	HM060618	Wagner et al. 2010

Stichopathes clade A BALA48	Bali, Indonesia	HE600722	Bo et al. 2012
Stichopathes clade A AMBA5	Ambon, Indonesia	HE600721	Bo et al. 2012
Stichopathes clade B BUK11	Bunaken, Indonesia	HE600719	Bo et al. 2012
Stichopathes clade B INDO19	Bunaken, Indonesia	HE600720	Bo et al. 2012
Stichopathes clade C AMBA6	Ambon, Indonesia	HE600717	Bo et al. 2012
Stichopathes clade C MENT52	Mentawai, Indonesia	HE600718	Bo et al. 2012
Stichopathes clade D AMBA45	Ambon, Indonesia	HE600710	Bo et al. 2012
Stichopathes clade D BALA46	Bali, Indonesia	HE600711	Bo et al. 2012
Stichopathes clade D BUK14b	Bunaken, Indonesia	HE600713	Bo et al. 2012
Stichopathes clade D BUNA14	Bunaken, Indonesia	AM404318	Bo et al. 2012
Stichopathes clade D MENT8b	Mentawai, Indonesia	HE600715	Bo et al. 2012
Stichopathes clade D RJ15	Raja Ampat, Indonesia	HE600712	Bo et al. 2012
Stichopathes clade D RJ16	Raja Ampat, Indonesia	HE600708	Bo et al. 2012
Stichopathes clade D RJ22	Raja Ampat, Indonesia	HE600709	Bo et al. 2012
Stichopathes clade D RJ35	Raja Ampat, Indonesia	HE600714	Bo et al. 2012
Stichopathes sp. PN004	North Toliara, Madagascar	na	This study
Stichopathes sp. PN015	North Toliara, Madagascar	na	This study
Stichopathes sp. PN016	North Toliara, Madagascar	na	This study
Stichopathes sp. PN028	North Toliara, Madagascar	na	This study
Stichopathes sp. PN030	North Toliara, Madagascar	na	This study
Stichopathes sp. PN033	North Toliara, Madagascar	na	This study
Stichopathes sp. PN058	North Toliara, Madagascar	na	This study
Stichopathes sp. PN061	North Toliara, Madagascar	na	This study
Stichopathes sp. PN066	North Toliara, Madagascar	na	This study
Stichopathes sp. PN068	North Toliara, Madagascar	na	This study
Stichopathes sp. PN071	North Toliara, Madagascar	na	This study
Stichopathes sp. PN073	North Toliara, Madagascar	na	This study
Stichopathes sp. PN080	North Toliara, Madagascar	na	This study
Stichopathes sp. PN086	North Toliara, Madagascar	na	This study
Stichopathes sp. PN88	North Toliara, Madagascar	na	This study
Stichopathes sp. PN090	North Toliara, Madagascar	na	This study
Stichopathes sp. PN096	North Toliara, Madagascar	na	This study
Stichopathes sp. PN102	North Toliara, Madagascar	na	This study
Stichopathes sp. PN132	North Toliara, Madagascar	na	This study
Stichopathes sp. PN136	North Toliara, Madagascar	na	This study
Stichopathes sp. PN137	North Toliara, Madagascar	na	This study
Stichopathes sp. IFPS07	South Ifaty, Madagascar	na	This study
Stichopathes sp. IF196	North Ifaty, Madagascar	na	This study
Stichopathes sp. IF197	North Ifaty, Madagascar	na	This study
Stichopathes sp. IF198	North Ifaty, Madagascar	na	This study
Stichopathes sp. IF199	North Ifaty, Madagascar	na	This study
Stichopathes sp. IF200	North Ifaty, Madagascar	na	This study
Stichopathes sp. SOA232	Soalara, Madagascar	na	This study
Stichopathes sp. SOA236	Soalara, Madagascar	na	This study
Stichopathes sp. SOA237	Soalara, Madagascar	na	This study
Stichopathes sp. MAR259	Maromena, Madagascar	na	This study

Stichopathes sp. MAR264	Maromena, Madagascar	na	This study
Stichopathes sp. MAR267	Maromena, Madagascar	na	This study
Stichopathes sp. MAR270	Maromena, Madagascar	na	This study
Stichopathes sp. MAR273	Maromena, Madagascar	na	This study
Stichopathes sp. MAR275	Maromena, Madagascar	na	This study
Stichopathes sp. AND284	Andavadoaka, Madagascar	na	This study
Stichopathes sp. AND286	Andavadoaka, Madagascar	na	This study
Stichopathes sp. AND289	Andavadoaka, Madagascar	na	This study
Stichopathes sp. AND290	Andavadoaka, Madagascar	na	This study
Aphanipathidae			
Aphanipathes cf. *sarothamnoides*	Palau, North Pacific	FM882166	Bo et al. 2009
Aphanipathes pedata	Florida, USA	FM882170	Bo et al. 2009
Rhipidipathes reticulata	Bunaken, Indonesia	AM404322	Lapian et al. 2007
Rhipidipathes reticulata	Bunaken, Indonesia	HE600723	Bo et al. 2012
Rhipidipathes reticulata	Bunaken, Indonesia	HE600724	Bo et al. 2012
Phanopathes rigida	Louisiana, USA	FM882169	Bo et al. 2009
Myriopathidae			
Antipathella subpinnata	Messina Strait, Italy	AM404329	Lapian et al. 2007
Cupressopathes abies	Bunaken, Indonesia	AM404324	Lapian et al. 2007
Cupressopathes pumila	Bunaken, Indonesia	AM404326	Lapian et al. 2007
Cupressopathes sp.1	Bunaken, Indonesia	AM404325	Lapian et al. 2007
Cupressopathes sp.2	Bunaken, Indonesia	AM404323	Lapian et al. 2007
Myriopathes myriophylla	Bunaken, Indonesia	AM404328	Lapian et al. 2007
Myriopathes sp.	Bunaken, Indonesia	AM404327	Lapian et al. 2007

RESULTS

Except in the North channel of Toliara where there is a black coral bed, black corals are rather scarce in the shallow waters of the southwestern coast of Madagascar. Nevertheless, the species *Stichopathes* sp. aff. *maldivensis* was found in all the stations. Two samples from the North Channel of Ifaty did not lead to clean sequences and one sample of Maromena was removed from the alignment because of the high occurrence of triple peaks in both forward and reverse sequences.

Unphased length sequences of specimens from Madagascar varied from 328 to 458 bases, 6 individuals were homozygotes, 14 were length variant heterozygotes and 20 were heterozygotes without length variation. A total of 12 SNPs was distinguished amongst the 331 bases of the phased alignment with 1 position having more than two different states, representing 3.62% of intra-specific variation. The maximum intra-individual variation recorded was 1.21%. Nucleotide composition was as follow: T(U)=24.1(±0.2) %; C=25.1(±0.3) %; A=25.7(±0.2) %; G=25.1(±0.1) %. The overall mean distance for the population from the SW of Madagascar was d=0.006%. Mean distances between populations were the highest between Madagascar and the NW Atlantic, d=0.129% and the lowest between Madagascar and Raja Ampat in Indonesia, d=0.008%, the other distances were of 0.022% (Bunaken), 0.015% (Mentawai), 0.075% (Bali) and 0.052% (Ambon).

The phylogenetic tree obtained by Bayesian inference led to the same topology than the Maximum Likelihood consensus tree (Fig. 55). Clades were the same than Bo et al. (2012), and the specimens from Madagascar grouped indistinctly with *Stichopathes* specimens all belonging to the Clade D (Bo et al. 2012) and coming from Ambon, Bali, Bunaken, Raja Ampat and Mentawai. All the sequences of specimens from Madagascar were grouped in the Antipathidae family. Other *Stichopathes* species grouped with other genera but all belonging to the Antipathidae. *Stichopathes* cf. *occidentalis* from the NW Atlantic grouped with *Antipathes caribbeana* and *Stichopathes* sp. AMBA5 from the Clade A grouped with *Antipathes grandis*. Specimens from clade C grouped together in a single clade. Specimens from clade B were grouped in a clade with various *Antipathes* species.

The phased alignment of *Stichopathes* sp. aff. *maldivensis* from the SW Madagascar included 87 sequences for all the specimens. It encompassed 40 individuals for a total of 21 haplotypes. The most common haplotype was shared between 25 specimens (Fig. 56). 13 haplotypes were found in unique specimens. The two colour types share 7 common haplotypes co-occurring between individuals, with 14 haplotypes not found in the white type (Fig. 56a). The white type includes the two most frequent and most co-occurring haplotypes. All the specimens and both types belong to a single pool of ITS1 sequences regardless of their location (Fig. 56b).

The phased alignment was combined with sequences of *Stichopathes* species coming from SE Asia and NW Atlantic for a total of 56 specimens. The alignment comprised 103 sequences of which 54 were different. The length of the cleaned-up alignment was 367 bases, of which 136 positions were differing. Specimens coming from all the locations from the southwestern coast of Madagascar had interconnected haplotypes, from Maromena to Andavadoaka (Fig. 57). We found 17 haplotypes in specimens from the North Channel of Toliara, all of them connected to the other locations from Madagascar. At least one haplotype from each Asian location was the same than those of Madagascar.

Figure 55. Consensus phylogenetic tree obtained by Maximum Likelihood using ITS1 sequences from specimens from the SW Madagascar, NW Atlantic and SE Asia, and rooted on the Myriopathidae family. Numbers above the nodes of the tree represent bootstrap values.

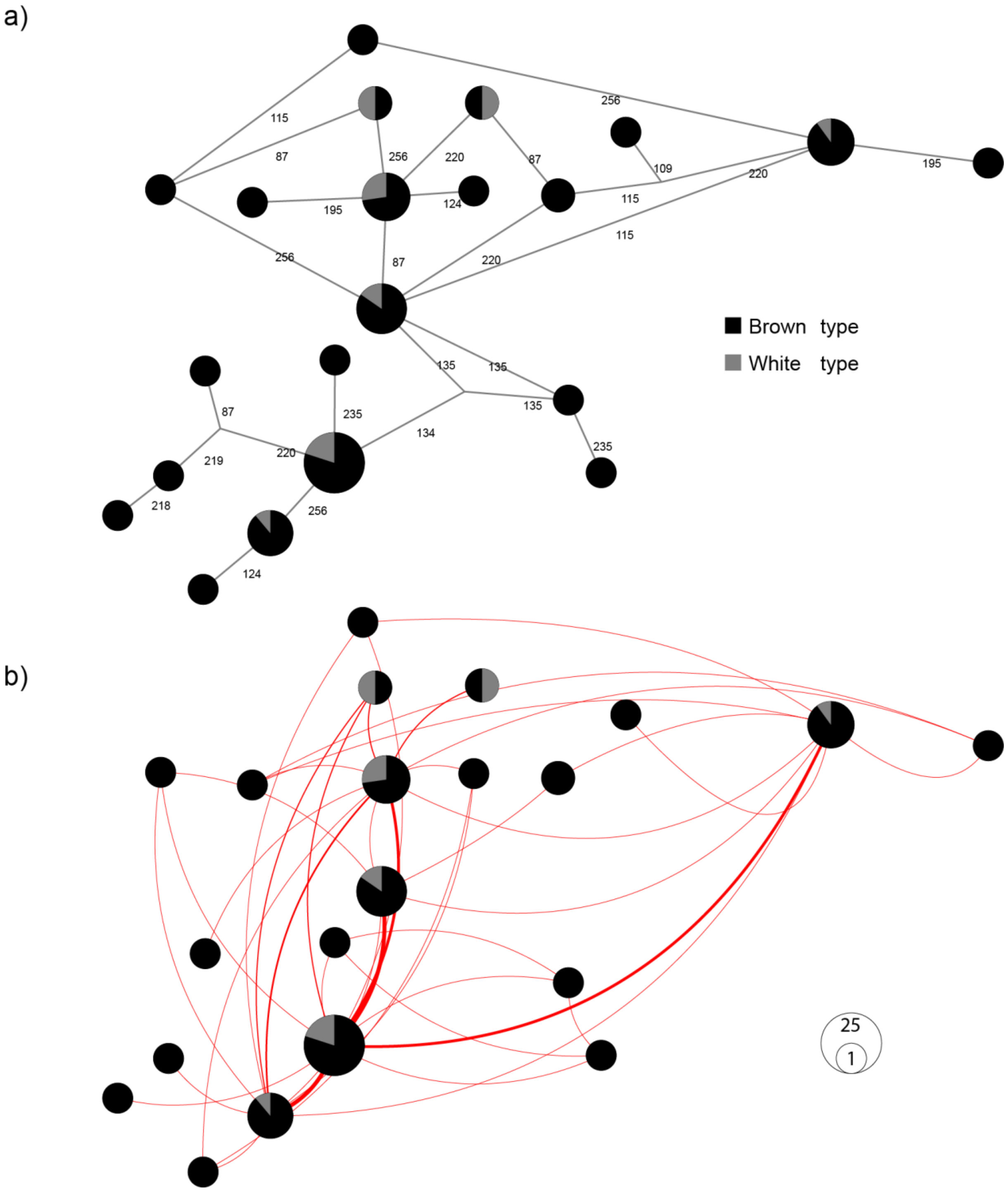

Figure 56. **(a)** Haplotype network of the ITS1 sequences of *Stichopathes* sp. aff. *maldivensis* found on the southwestern coast of Madagascar. The alignment comprised 87 sequences among which 21 haplotypes are distinguished, each represented by a circle. The circle area is proportional to the number of individuals harboring a given haplotype. Each line connecting haplotypes represent the evolutionary path with the position of the mutation in the alignment. **(b)** Haploweb of the ITS1 sequences where the red curves connect haplotypes found co-occurring in individuals, with their thickness proportional to the number of heterozygotes harboring the two haplotypes it connects. All the haplotypes are belonging to a single pool of co-occurring sequences, representing a single field for recombination.

Specimens from the South Channel of Ifaty were sharing haplotypes with specimens coming from the North Channel of Toliara, Soalara, the North Channel of Ifaty, Maromena, Andavadoaka, Mentawai and Raja Ampat. Specimen MENT52 from Mentawai was not interconnected to the ITS1 pool, as well as *Stichopathes* cf *occidentalis*, specimens RJ22 and RJ15 from Raja Ampat, specimens BUK11, INDO19 and BUK14b from Bunaken, specimens AMBA5 and AMBA6 from Ambon and specimen BALA48 from Bali. The 6 specimens from Maromena showed 7 connected haplotypes, the 3 specimens from Soalara had 4 connected haplotypes, the 4 specimens from Andavadoaka had 3 connected haplotypes, and the 5 specimens from the North Channel of Ifaty had 4 connected haplotypes. The most frequent haplotype was found in specimens coming from the North Channel of Toliara, Soalara, Raja Ampat, Andavadoaka, the North Channel of Ifaty and Maromena. Haplotypes were co-occurring between some specimens of the South East Asia and the South West Madagascar. Specimen from Bali had the same haplotype than specimens from Ambon, Bunaken, Maromena and the North Channel of Toliara. Specimen from Mentawai had the sample haplotype than the one from the South Channel of Ifaty. Specimens from Raja Ampat were sharing two common haplotypes with specimens coming from Madagascar: the most frequent was found in the North Channel of Toliara, Soalara, Andavadoaka, the North Channel of Ifaty and Maromena while the other was found in the North Channel of Toliara, Andavadoaka, Maromena and the South Channel of Ifaty.

DISCUSSION

As in Indonesia (Bo et al. 2012), the whip black corals *Stichopathes* sp. aff. *maldivensis* is one of the most abundant antipatharians in the southwestern coast of Madagascar. The Indonesian species of *Stichopathes* are divided in 4 clades both supported by molecular and morphological traits, where the clade A is characterized by helicospiral colonies, the clade B is made of contorted corals, the clade C also regroups helicospiral colonies but with different spines, and the clade D regroups straight or slightly sinuous corals (Bo et al. 2012). The present specimens from Madagascar have morphological features matching with the clade D of Bo et al. (2012), which they tentatively identified as "*Stichopathes* cf. *maldivensis*". Specimens coming from the clade D of Bo et al. (2012) are distributed throughout the Indian and Pacific Oceans and is found in the whole Indonesian Archipelago. There are currently about 35 species within the genus *Stichopathes*, with 17

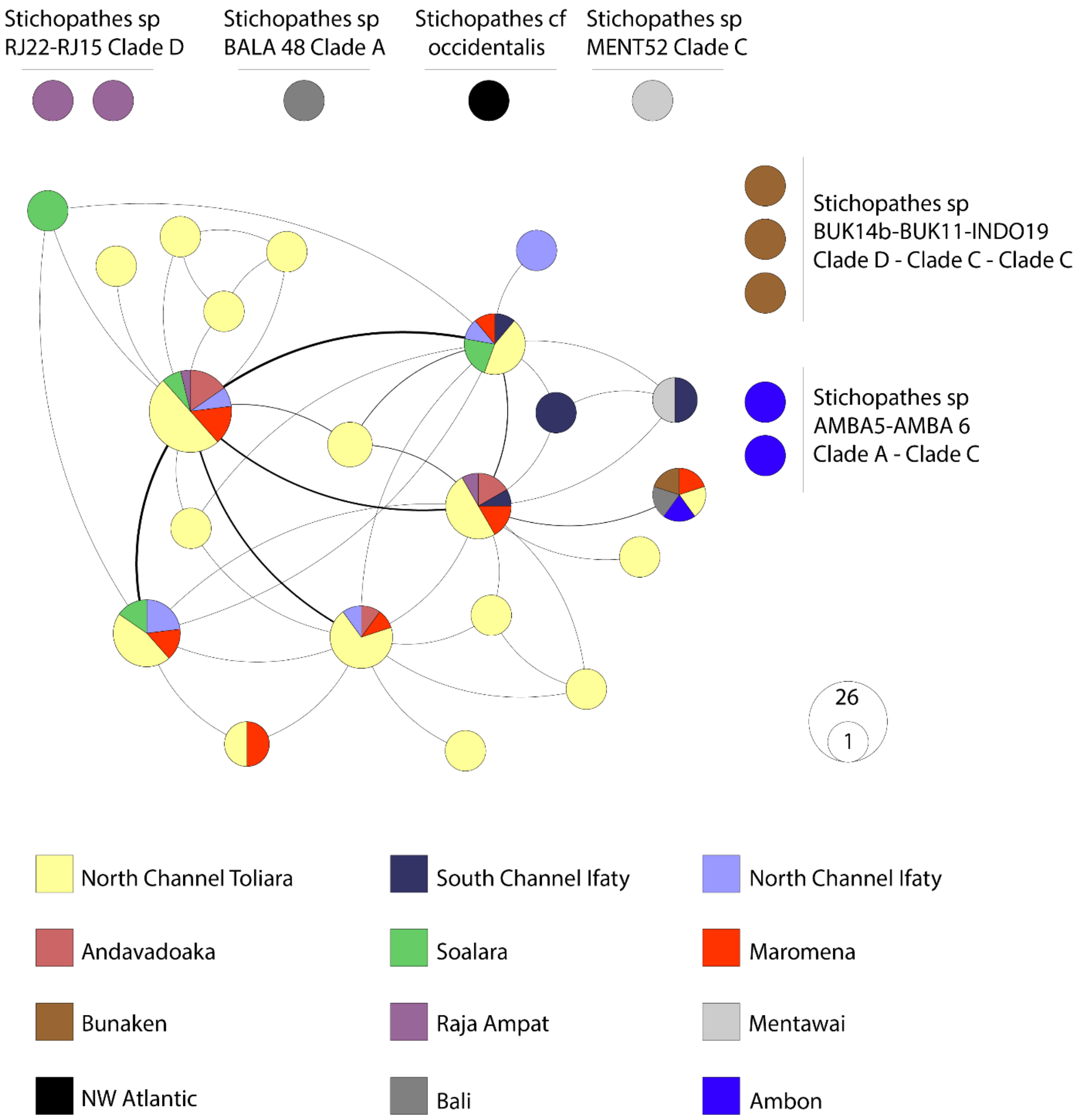

Figure 57. Haploweb of the ITS1 sequences using 40 colonies of *Stichopathes* sp. aff. *maldivensis* of Madagascar with sequences of *Stichopathes* species from other part of the world. The alignment comprised 103 sequences among which 31 haplotypes are distinguished and represented by circles. The majority of the specimens belong to a large pool of co-occurring haplotypes corresponding to a group of individuals forming a large field for recombination (FFR). Only specimens from SE Asia and NW Atlantic possess 10 isolated haplotypes. Each colour represent a location.

reported for the Indo-Pacific region, Japan and Australia (Bo et al. 2012), but many uncertainties are still existing in the species descriptions. In this clade, the *Stichopathes* sp. aff. *maldivensis* presents two colours in Indonesia and in Madagascar. In this study, we show that these two types are also coming from a single species based on the haplowebs drawn on ITS1 haplotypes, confirming that phenotypic variations exist within this genus and throughout the Indian Ocean up to the Indonesian Archipelago.

The use and utility of haplowebs based on internal transcribed spacers of the ribosomal DNA to delineate morphologically different species and their geographical distribution has already been shown in several studies about scleractinian corals (Flot & Tillier 2006; Flot et al. 2010, 2011; Adjeroud et al. 2014). In this paper, we showed that ITS markers are also informative within the order Antipatharia. Our dataset supports the intraspecific variability found in other hexacorallian corals, which is about 1% in *Montastrea* species (Lopez & Knowlton 1997), 2% in *Heliofungia actiniformis*, 15% in *Goniopora tenuidens*, up to 31% in *Stylophora pistillata* (Takabayashi et al. 1998) or 29% in *Acropora valida* (Odorico & Miller 1997). Among antipatharians, intraspecific variability of ITS1 has already been observed in some Hawaiian corals of the genera *Antipathes* and *Cirrhipathes* (Wagner et al. 2010).

In their large phylogenetic revision of the order Antipatharia, Brugler et al. (2013) found intraspecific variability in *Antipathes griggi* for the ITS1 region with single nucleotide polymorphisms and intra-individual length polymorphism but did not use this variability to infer genetic results. Despite this variability, no variation was surprisingly reported for these regions in the phylogenetic reconstructions of black corals of Lapian et al. (2007) nor Lapian (2009). In their study, they analyzed species coming from the Myriopathidae, Antipathidae and Aphanipathidae families and suggested hybridization events in the first one, because of the high similarities of the sequences belonging to specimens from the genera *Cupressopathes* and *Myriopathes*. We suggest that ITS-based haplowebs could be useful to solve species delimitation in black corals using a high number of specimens for each investigated species. In the recently described genus *Pseudocirrhipathes*, no variation has been reported neither, but it may be explained by the small number of analyzed samples for the molecular analyses (n=2, Bo et al. 2009). In the phylogeny of the deep-sea family Schizopathidae, MacIsaac et al. (2013) used ITS regions as well, but did not report any variation of the sequences, even if some nucleotides are reported as any bases (“n”) or ambiguities for some sequences of *Telopathes magna* (see accession numbers of the sequences in MacIsaac et al. 2013). In the present study, each variation is considered for the first time at a population level, and a novel approach –already used in scleractinian corals– is used for antipatharians.

In the only population genetic study of an antipatharian, *Antipathes fjordensis*, Miller (1997) found significant genetic differentiation between sampling sites within fjords suggesting that this black coral has a very limited larval dispersal, over distances of tens of kilometers only. On the 200 km of coast that were studied here, the fresh water of three rivers are carried to the sea. The Onilahy is the main river of the southwestern coast. It covers about 32,000 km^2 and its mean flow is 145 m^{3}s^{-1} (Aldegheri 1972). The river flows all year round into the sea at the village of Saint Augustin, where there is also an oceanic trench of 1,000 m depth. This river separates the sites Maromena and Soalara from all the other sites. The Fiherenana is located North from Toliara and separates the Great Reef of Toliara from the other sites located far North. The Fiherenana watershed covers about 7,500 km^2 and the average discharge is 35.4 m^{3}s^{-1} (Aldegheri 1972). Downstream, the river flow is intermittent: it only floods during the wet season, from December to March (this period corresponding to the peak of the reproductive period of at least one species in that region; see Chapter 2). The mouth of the Manombo separates the Ifaty sites from Soalary and Andavadaoka. It also floods only during the wet season. The fresh waters from the three rivers often form red masses that extend over tens of kilometers in the sea during the wet season. If *Stichopathes* sp. aff. *maldivensis* presents a limited larval dispersal that covers only distances of tens of kilometers as it is the case for *Antipathes fjordensis* and if the fresh water from the rivers are barriers for the larval dispersal, *Stichopathes* sp. aff *maldivensis* should show evident genetic differentiation between sampling sites. However, it was not the case: ITS-based haplowebs showed that malagasy specimens were sharing the same haplotypes. Since the sampling was only based on the appearance of the corals without further sampling of the skeleton, it suggests that *Stichopathes* sp. aff. *maldivensis* does not include cryptic species, nor subpopulations, with all sampled specimens having a single pool of ITS1 regions. The highest number of haplotypes was recorded in the North Channel of Toliara which is also the site with the highest black coral abundance. This black coral bed may act as a reservoir for the dispersal of the species in the southwestern coast, since haplotypes of specimens coming from that place were shared by specimens from all other malagasy locations. Interestingly, some specimens from the SE Asia shared also similar haplotypes than some of the population from the SW Madagascar: Asian specimens coming from Mentawai and Bali (Indian Ocean) had the same haplotypes than Malagasy specimens, as well as specimens coming from Raja Ampat, Bunaken and Ambon (Pacific Ocean). Because all the observed haplotypes are connected through heterozygotes, they belong to a single gene pool *sensu* Doyle (1995), meaning that all the specimens are conspecific following the criterion of mutual allelic exclusivity (Flot et al. 2010). The specimen from NW Atlantic was not part of the pool as it can be expected. Asian specimens which were not connected to the large ITS1 pool were coming from other clades than the clade D of Bo et al. (2012), which is also congruent with morphological comparisons.

In conclusion, these findings expand the spatial distribution of the shallow-water corals belonging to the genus *Stichopathes* which are classified in the clade D *sensu* Bo et al. (2012), occurring throughout the Indian Ocean and the Indonesian Archipelago. This should be a basis for further identification and redescriptions of these Indo-Pacific antipatharians considering the phenotypic variations and the geographical range. With more than 5,000 km of coasts, the marine protected areas (MPAs) of Madagascar are not sufficient to encompass all the reefs and mangroves that the country harbors (Harris 2011). If small MPAs are already established in the southwest, with temporary or permanent protected status, they remain small with a high number of fisheries (Todinanahary et al. 2016). More attention should be paid in Toliara where the black coral bed has a high diversity not seen anywhere else that act as a reservoir for the antipatharian populations.

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LITERATURE

Adjeroud M, Guérécheau A, Vidal-Dupiol J, Flot JF, Arnaud-Haond S, Bonhomme F (2014) Genetic diversity, clonality and connectivity in the scleractinian coral *Pocillopora damicornis*: a multi-scale analysis in an insular, fragmented reef system. Marine Biology 161: 531–541.

Aldegheri M (1972) Rivers and streams on Madagascar. In Biogeography and ecology in Madagascar (pp. 261-310). Springer, Dordrecht.

Bo M, Barucca M, Biscotti MA, Canapa A, Lapian HFN, Olmo E, Bavestrello G (2009) Description of *Pseudocirrhipathes* (Cnidaria: Anthozoa: Hexacorallia: Antipathidae) a new genus of whip black corals from the Indo-Pacific. Italian Journal of Zoology 76: 392–402.

Bo M, Bavestrello G, Barucca M, Makapedua DM, Poliseno A, Forconi M, Olmo E, Canapa A (2012) Morphological and molecular characterization of the problematic whip black coral genus *Stichopathes* (Hexacorallia: Antipatharia) from Indonesia (North Sulawesi, Celebes Sea). Zoological Journal of the Linnean Society, 166: 1–13.

Brugler MR, France SC (2007) The complete mitochondrial genome of the black coral *Chrysopathes formosa* (Cnidaria: Anthozoa: Antipatharia) supports classification of antipatharians within the subclass Hexacorallia. Molecular Phylogenetics and Evolution, 42: 776–788.

Brugler MR, Opresko DM, France SC (2013) The evolutionary history of the order Antipatharia (Cnidaria: Anthozoa: Hexacorallia) as inferred from mitochondrial and nuclear DNA: implications for black coral taxonomy and systematics. Zoological Journal of the Linnean Society, 169(2): 312–361.

Calderon I, Garrabou J, Aurelle D. (2006) Evaluation of the utility of COI and ITS markers as tools for population genetic studies of temperate gorgonians. Journal of Experimental Marine Biology and Ecology, 336(2): 184–197.

Dover G (1982) A molecular drive through evolution. Bioscience, 32: 526–533.

Doyle JJ (1995) The irrelevance of allele tree topologies for species delimitation, and a non-topological alternative. Systematic Botany 1995, 20:574–588.

Flot JF, Tillier S (2006) Molecular phylogeny and systematics of the scleractinian genus *Pocillopora* in Hawaii. Proceedings of the 10th Coral Reef Symposium, 24–29.

Flot JF (2007) Champuru 1.0: a computer software for unraveling mixtures of two DNA sequences of unequal lengths. Molecular Ecology Resources, 7(6): 974–977.

Flot JF (2010) SeqPHASE: a web tool for interconverting PHASE input/output files and FASTA sequence alignments. Molecular Ecology Resources, 10(1): 162–166.

Flot JF, Tillier A, Samadi S, Tillier S (2006) Phase determination from direct sequencing of length-variable DNA regions. Molecular Ecology Resources, 6(3): 627–630.

Flot JF, Couloux A, Tillier S (2010) Haplowebs as a graphical tool for delimiting species: a revival of Doyle's "field for recombination" approach and its application to the coral genus *Pocillopora* in Clipperton. BMC Evolutionary Biology, 10: 372.

Flot JF, Blanchot J, Charpy L, Cruaud C, Licuanan WY, Nakano Y, Payri C, Tillier S (2011) Incongruence between morphotypes and genetically delimited species in the coral genus *Stylophora*: phenotypic plasticity, morphological convergence, morphological stasis or interspecific hybridization? BMC Ecology, 11:22.

France SC, Hoover LL (2002) DNA sequences of the mitochondrial COI gene have low levels of divergence among deep-sea octocorals (Cnidaria: Anthozoa). Hydrobiologia, 471(1-3): 149–155.

Harris AR (2011) Out of sight but no longer out of mind: a climate of change for marine conservation in Madagascar. Madagascar Conservation & Development, 6(1): 7–16.

Helaers R, Milinkovitch MC (2010) MetaPIGA v2.0: maximum likelihood large phylogeny estimation using the metapopulation genetic algorithm and other stochastic heuristics. BMC Bioinformatics, 11: 379.

Hellberg ME (2006) No variation and low synonymous substitution rates in coral mtDNA despite high nuclear variation. BMC evolutionary biology, 6(1): 24.

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution, 33(7): 1870–1874.

Lapian HFN, Barucca M, Bavestrello G, Biscotti MA, Bo M, Canapa A, Tazioli S, Olmo E (2007) A systematic study of some black corals species (Antipatharia, Hexacorallia) based on rDNA internal transcribed spacers sequences. Marine Biology 151: 785–792.

Lapian HFN (2009) Biodiversity study of black coral (Order: Antipatharia) collected from Manado, Indonesia based on rDNA Internal Transcribed Spacer (ITS) sequences analysis. Biodiversitas, 10(1): 1–5.

Lopez JV, Knowlton N (1997) Discrimination of species in the *Montastrea annularis* complex using multiple genetic loci. In Proceedings of the 8th International Coral Reef Symposium, Vol. 2, p. 1613.

McFadden CS, Sánchez JA, France SC (2010) Molecular phylogenetic insights into the evolution of Octocorallia: a review. Integrative and Comparative Biology, 50(3): 389–410.

McIsaac KG, Best M, Brugler MR, Kenchington ELR, Anstey LJ, Jordan T (2013) *Telopathes magna* gen. nov., spec. nov. (Cnidaria: Anthozoa: Antipatharia: Schizopathidae) from deep waters off Atlantic Canada and the first molecular phylogeny of the deep-sea family Schizopathidae. Zootaxa 3700(2): 237-258.

Miller KJ (1997) Genetic structure of black coral populations in New Zealand's fiords. Marine Ecology Progress Series, 161: 123–132.

Nylander JAA (2002) MrModeltest. Version 1.1b. Available from: http://www.ebc.uu.se/systzoo/staff/nylander.html

Odorico DM, Miller DJ (1997) Variation in the ribosomal internal transcribed spacers and 5.8 S rDNA among five species of *Acropora* (Cnidaria; Scleractinia): patterns of variation consistent with reticulate evolution. Molecular Biology and Evolution, 14(5): 465–473.

Posada D (2006) ModelTest Server: a web-based tool for the statistical selection of models of nucleotide substitution online. Nucleic Acids Research, 34(suppl_2), W700-W703.

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19(12): 1572–1574.

Shearer TL, Coffroth MA (2008) DNA BARCODING: Barcoding corals: limited by interspecific divergence, not intraspecific variation. Molecular Ecology Resources, 8(2): 247–255.

Sinniger F, Pawlowski J (2009) The partial mitochondrial genome of *Leiopathes glaberrima* (Hexacorallia: Antipatharia) and the first report of the presence of an intron in COI in black corals. Galaxea, Journal of Coral Reef Studies, 11: 21–26.

Stephens M, Smith NJ, Donnelly P (2001) A new statistical method for haplotype reconstruction from population data. The American Journal of Human Genetics, 68(4): 978–989.

Takabayashi M, Carter DA, Ward S, Hoegh-Guldberg O (1998) Inter-and intra-specific variability in ribosomal DNA sequence in the internal transcribed spacer region of corals. In Proceedings of the Astralian Coral Reef Society, 75th Anniversary Conference, Vol.1, pp. 241-248.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution, 28(10): 2731–2739.

Todinanahary G, Terrana L, Lavitra T, Eeckhaut I (2016) First records of illegal harvesting and trading of black corals (Antipatharia) in Madagascar. Madagascar Conservation & Development, 11(1): 7-13.

Wagner D, Brugler MR, Opresko DM, France SC, Montgomery AD, Toonen RJ (2010) Using morphometrics, in situ observations and genetic characters to distinguish among commercially valuable Hawaiian black coral species; a redescription of *Antipathes grandis* Verrill, 1928 (Antipatharia: Antipathidae). Invertebrate Systematics 24: 271–290.

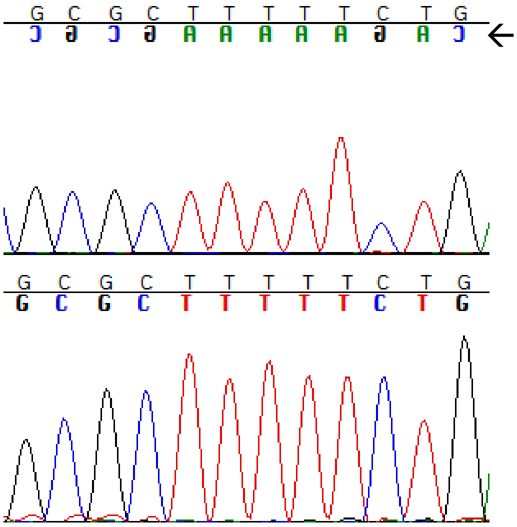
Wagner D, Luck DG, Toonen RJ (2012) The biology and ecology of black corals (Cnidaria: Anthozoa: Hexacorallia: Antipatharia). Advances in Marine Biology 63: 67–72.

Wörheide G, Nichols SA, Goldberg J. (2004) Intragenomic variation of the rDNA internal transcribed spacers in sponges (Phylum Porifera): implications for phylogenetic studies. Molecular Phylogenetics and Evolution, 33(3): 816–830.

SUPPLEMENTARY MATERIAL

Dealing with heterozygous sequences

Sequencing PCR amplicons with the Sanger's method is a good and reliable technique to obtain both forward and reverse short DNA sequences. The amplification and sequencing of single-copy genes, *i.e.* mitochondrial genes, generally gives clear and homozygous sequences. In the corresponding chromatograms, each nucleotide is represented by a peak of fluorescence whose intensity is decreasing with the length of the amplicon:



← Experimental data with Fluorescence peaks

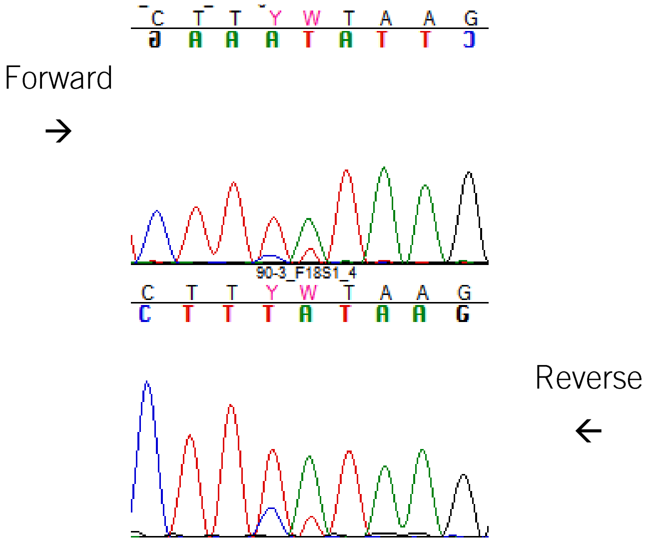
Forward →

Reverse ←

In this example, the homozygote sequence is G–C–G–C–T–T–T–T–T–C–T–G.

However, it is frequent to work with genes having a Copy Number Variation (CNV) in the genome, meaning that the number of copies of the same gene is varying through individuals from the same species, such as genes coming from the ribosomal DNA. Ribosomal DNA is made of highly conserved regions (*i.e.* 18S, 5.8S and 28S for eukaryotes) coding for ribosomal proteins involved in the translation of the messenger RNA. Non-coding regions are found between these conserved genes, called Internal Transcribed Spacers (ITS). They evolved faster than coding genes in addition to their high number of copies inside the genome. This results to an intra-individual variation where the mutations can be detected with the Sanger sequencing method.

The first case is the existence of heterozygous sequences that will be detected in the chromatograms through the appearance of double peaks in both forward and reverse sequences, they are called Single Nucleotide Polymorphisms (SNP). These SNPs follow the IUPAC Ambiguity Codes (M for A or C; R for A or G; V for A or C or G; etc…):



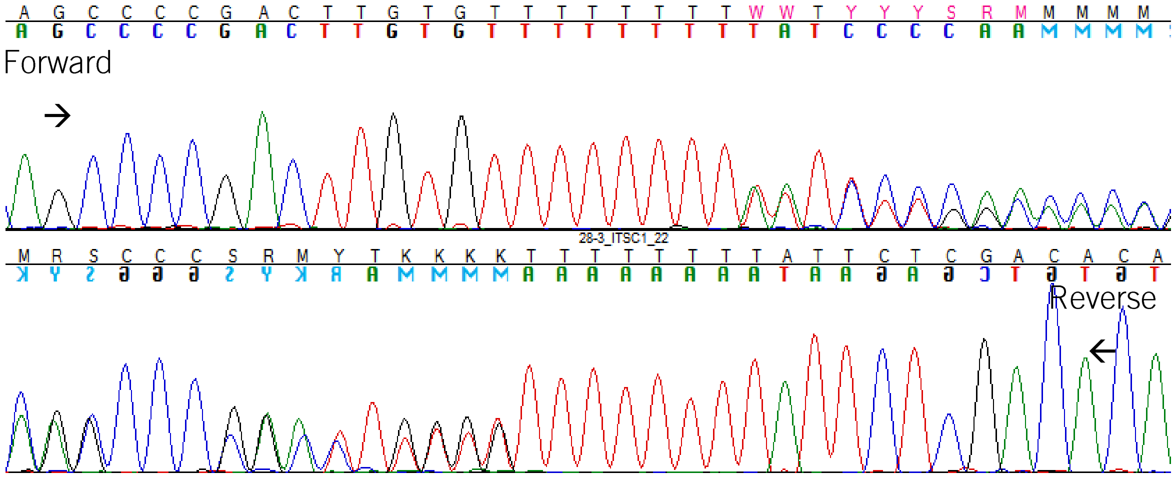
Consequently, it means that the same individual has different sequences for the same gene. Each sequence is called a haplotype, which is a sequence genetically linked to another. In this example, the consensus heterozygous sequence is:

1 2 3 4 5 6 7 8 9
C T T Y W T A A G

Where the ambiguities are found in positions 4 and 5: **Y** for C or T, and **W** for A or T.
Thus, the individual presents 4 different haplotypes for the same gene:

1) C–T–T–C–A–T–A–A–G
2) C–T–T–C–T–T–A–A–G
3) C–T–T–T–A–T–A–A–G
4) C–T–T–T–T–T–A–A–G

Sometimes, instead of having substitution mutation (*i.e.* the change of a nucleotide for another), it happens an insertion or a deletion of a nucleotide (indel). Indels are very important in coding regions because they shift the Open Reading Frame (ORF), which can have effects on the resulting coded protein. In this case for ITS regions, indels lead to the existence of Length Variant Heterozygotes (LVH) that can be also detected with the Sanger sequencing method:



LVH are detected in the chromatograms through the appearance of double peaks at some point in both forward and reverse sequences and ambiguities are solved in both of them. In this example, these sequences are the following, with the consensus sequence below:

```
Fwd   A G C C C C G A C T T G T G T T T T T T T T W W T Y Y Y S R M M M M M
Rev   M R S C C C S R M Y T K K K K T T T T T T T T A T T C T C G A C A C A
      A G C C C C G A C T T G T G T T T T T T T T A T T C T C G A C A C A
```

The presence and the number of indels can be verified by shifting the reverse sequence:

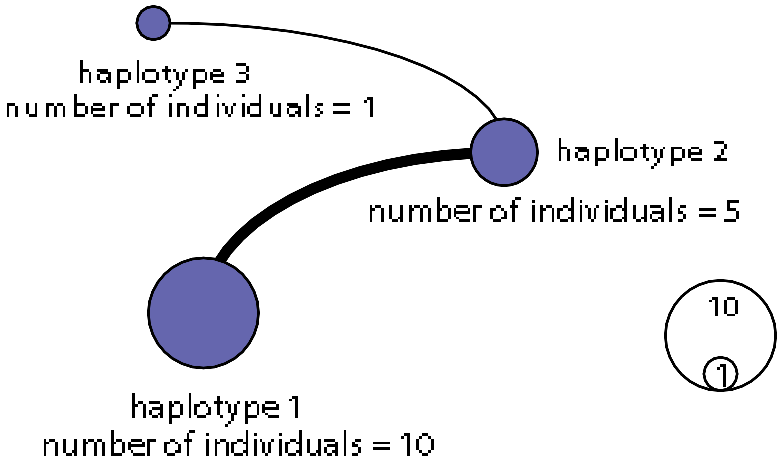
```
  A G C C C C G A C T T G T G T T T T T T T T W W T Y Y Y S R M M M M M
M R S C C C S R M Y T K K K K T T T T T T T T A T T C T C G A C A C A
M A G C C C C G A C T T G T G T T T T T T T T A T T C T C G A C A C A M
```

In this example, the consensus sequence is still solved when shifting the reverse sequence of 1 base pair, meaning that this individual is a LVH where one of the haplotypes contains 1 indel compared to the other. When the LVH is solved, both forward and reverse sequences can be submitted to Champuru V1.0, which is a computer software for unraveling mixtures of two DNA sequences of unequal lengths. It takes as input the two sequences as obtained by direct sequencing, and returns, most often after several iterations aiming at correcting basecalling errors, the two sequences present in the mixture. This provides a cheap, fast and reliable alternative to cloning that works best whenever sequences differ in length only by a small insertion/deletion. In this example, Champuru retrieves the two haplotypes, and the position of the indel is found when the sequences are aligned:

```
A G C C C C G A C T T G T G T T T T T T T T T A T T C T C G A C A C A
A G C C C C G A C T T G T G T T T T T T T T - A T T C T C G A C A C A
```

In population genetics, once all the haplotypes are solved, aligned, and analyzed, haplowebs can be drawn to assess the genetic pool of the studied gene. Haplotypes are represented by a circle where its area is proportional to the number of individual harboring this haplotype (*i.e.* individuals sharing the same sequence). When an individual has more than one haplotype for a given gene, its haplotypes are connected by a line where the thickness thickness is proportional to the number of individuals sharing the same haplotypes. In other words, the lines connect haplotypes co-occurring in the same individuals.

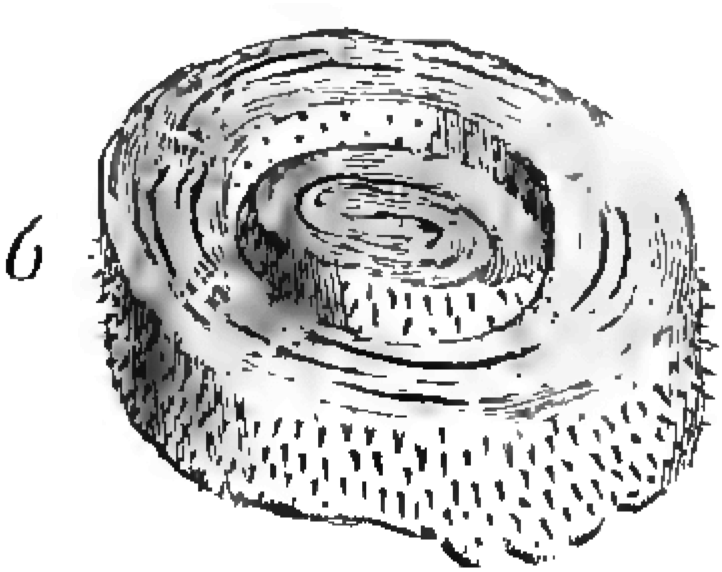
In this random example, let's consider that each individual has a maximum of two haplotypes for the gene. There are 10 individuals harboring the haplotype 1, 5 individuals have the haplotype 2, and a single individual has the haplotype 3. A single individual has the haplotype 2 and 3 co-occurring. It remains 4 individuals harboring the haplotype 1 and 2. Finally, it remains 6 individuals being homozygotes with the haplotype 1.



This technique is used and detailed in the previous chapter which focus on shallow-water populations of the black coral *Stichopathes* sp. aff. *maldivensis*.

Chapitre 6

Ages and growth rates of the harvested shallow-water whip black corals (Antipatharia) from the southwest of Madagascar



Section of the skeleton of the formerly named Antipathes spiralis showing the growth rings, by Ellis & Solander (1786)

Ages of the harvested shallow-water antipatharians from the southwest of Madagascar

ABSTRACT

In Madagascar, black corals are illegally harvested to be sold as souvenirs on the market places or exported for jewelry. In the present work, 5 whip black corals *Stichopathes* sp. aff. *maldivensis* (n=2) and *Cirrhipathes* sp. (n=3), two species highly harvested by fishermen, were collected in the shallow waters around Toliara to establish their age and their growth rates. These colonies can grow up to 5 meters in height and are very abundant in this region. For the first species, the length of the two samples were 235 and 435 cm and 186, 325 and 385 cm for the three other specimens from the second species. Two disks of 1 cm in thickness were cut at the basis of the colonies for bomb radiocarbon dating and growth ring counting. Bomn radiocarbon dating was made either on the first ring next to the central hollow core, which is the oldest part of the skeleton, or in transects at known intervals from the center to the outer edge. A reference bomb radiocarbon dating curve was used from the Watamu reef in Kenya to age black corals. Pre-bomb values were obtained and thus their age was established by a growth rate estimation. Results showed that *Stichopathes* sp. aff. *maldivensis* is 29–78 years while *Cirrhipathes* sp. is aged from 29 to at least 69 years. Basal diameters are varying from 7.25 mm to 16 mm, but all the specimens show very similar growth rates. Radial growth rate varies between 157–400 µm per year and vertical growth rate is of 4–11 cm per year. Two to six rings are produced per year. Considering that it will take a human lifetime for fished black coral populations to return to a normal ecological and structural state, the damages of illegal fisheries of black corals undoubtedly result to an important decrease in tropical shallow-water biodiversity.

This chapter is a draft of the manuscript that will be submitted with the following co-authors and order:

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INTRODUCTION

Antipatharians, also called black corals, are found in all oceans from polar latitudes to tropical waters. They are major parts of the cold-water ecosystems in the deep sea as well as shallow-water tropical ecosystems in coral reefs areas. Deep-sea black corals are generally characterized by a very slow growth and a long lifespan while both radial and vertical growth of shallow-water species tend to be faster (Roark et al. 2009; Bo et al. 2009). Several studies have focused on the age of deep-sea antipatharians which can be from centuries to thousands of years old (Roark et al. 2006, 2009; Sherwood and Edinger 2009; Prouty et al. 2011; Carreiro-Silva et al. 2013). To date, the order Antipatharia regroups one of the oldest animal living on earth: the deep-sea species *Leiopathes glaberrima* was found to be aged of more than 4,200 years old, which also had one of the slowest radial growth rates with less than 5 µm a year (Roark et al. 2009). On the other hand, there is only one record for the age of a tropical shallow-water black coral in the Red Sea: *Antipathes* sp. reached 81 years old with a radial growth of 50 $\mu m \cdot year^{-1}$ (Risk et al. 2009). While this radial growth rate never exceeds a few tens of micrometers per year, shallow-water antipatharians can have very high vertical growth rates as seen in the Indonesian shallow black corals *Stichopathes* sp. aff *maldivensis* and *Cirrhipathes* cf. *anguina* which is sometimes higher than 1 $m \cdot year^{-1}$ in monitored nubbins (Bo et al. 2009).

Radiocarbon provides a good time-varying tracer to age tropical shallow-water black corals. In the late 1950s, nuclear weapon launches increased the atmospheric radiocarbon isotope ^{14}C. The resulting $^{14}CO_2$ entered the marine food chain through the dissolution in surface sea water and disrupted the natural equilibrium in carbon isotopes, resulting in a radiocarbon peak occurring in mid-1960s. Coral reefs are incorporating dissolved inorganic carbon for their skeleton synthesis thus providing a good time-record of radiocarbon levels in the sea water (Grumet et al. 2002a). Unlike the stable isotopes ^{12}C and ^{13}C, the radiocarbon is radioactive, and its decay can be measured. Consequently, coral skeletons can be used as time tracers to establish radiocarbon levels for the past 100 years in tropical areas (Grumet et al. 2002a). In the Indian Ocean, atmospheric and surface sea water carbon are linked through the Indian-Asian monsoons (Grumet et al. 2002b). The south equatorial current brings water to the eastern coast of Africa and gives the east African coast current towards Kenya and the northeast Madagascar current towards the Mozambique Channel, both being influenced in $\Delta^{14}C$ in the same way (Grumet et al.

2002b). Therefore, the Watamu reef in Kenya for which radiocarbon measurements are known (Grumet et al. 2002b) can be used as a proxy for age calibration of bomb^{14}C data of the corals of Madagascar.

In Madagascar, black corals have been illegally harvested for many years despite being protected by international and national treaties and laws (Todinanahary et al. 2016). They are mainly fished and sold as tourist souvenirs on the market places or exported for jewelry. Whip corals are more harvested in the western coast than the eastern coast, where it is large and bushy colonies that are massively collected. These whip corals are abundant and very diverse the southwest of the country (Chapter 3) and host a diversity of symbiotic or opportunistic organisms (Terrana & Eeckhaut 2017). The illegal fisheries are destroying these ecosystems, and nothing is known about their ability to quickly recover and overcome the massive depletion of colonies. Old black coral populations may need a lot of time to counterbalance the fisheries, but their age and their growth remain unknown. In general, bomb radiocarbon dating has never been used on whip corals in shallow waters nor in the deep sea. This work aims to determine the age, the radial growth rates and the vertical growth rates of two of the most abundant shallow-water whip black corals from the southwest of Madagascar, *Stichopathes* sp. aff. *maldivensis* and *Cirrhipathes* sp. (see Chapter 3). These results will be of importance for future management programs and population stock assessments besides providing dating techniques which does not imply the collection of entire colonies.

MATERIAL & METHODS

Field collection

The most common black corals were collected in July and August 2016 in the black coral bed of Toliara, in the southwest of Madagascar. Five colonies were collected by scuba-diving at a maximum depth of 23 m, 3 were identified as an undescribed species of *Cirrhipathes* sp. and 2 as *Stichopathes* sp. aff. *maldivensis*. *Cirrhipathes* sp. has polyps all around the axis. Its color is varying from white to brown and is generally straight or slightly sinuous and measures up to 4 meters in height (Fig. 58a). *Stichopathes* sp. aff. *maldivensis* can measure up to 5 m in length and its shape is slightly sinuous sometimes forming horizontal spirals when it reaches several meters in length (Fig.58b). All colonies were photographied prior to extraction and sample preparation.

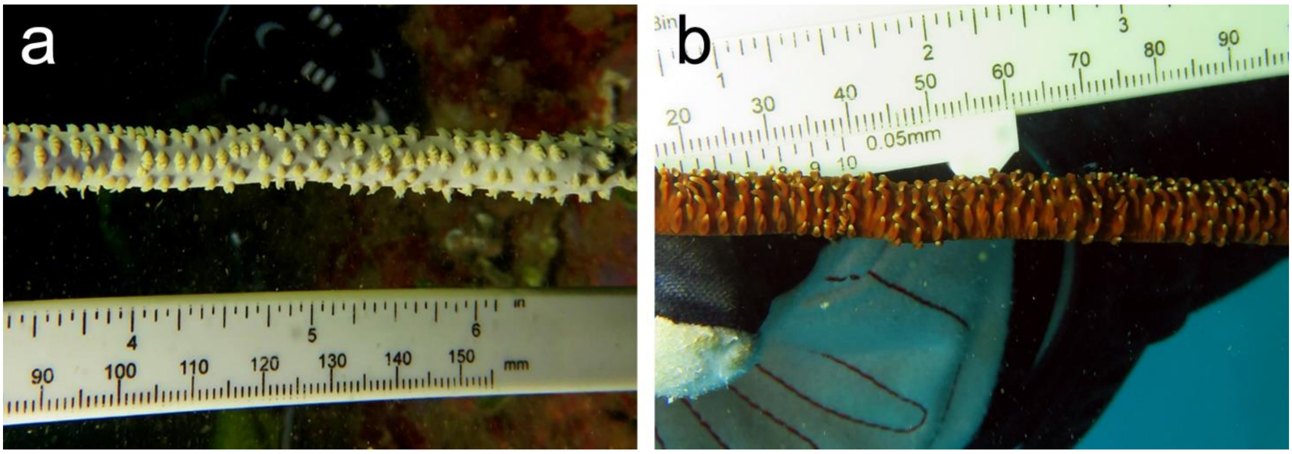

Figure 58. Underwater pictures of the black coral sampled in this study. **(a)** *Cirrhipathes* sp. **(b)** *Stichopathes* sp. aff. *maldivensis*.

Sample preparation

Underwater pictures of the whole colonies were taken before they were cut into several labelled fragments for transport. Each fragment was brought to the lab and the living tissues were removed with a sharp blade before being allowed to dry at room temperature. Fragments were photographed with a scale and measured with precision in ImageJ (Rueden et al. 2017) to determine the total length of the colony. Two small disks of 1 cm thick were cut just above the basal plate, at the basis of the colonies for bomb radiocarbon dating and growth ring counting. A petrographic thin section of 30 µm was performed on one disk before being observed under UV light. Each growth ring was then counted manually regardless of their thickness (Fig. 59). A growth ring is made of chitin and protein organized in microlayers which are tightened together by an organic cement which is opaque in section (Goldberg 1991). Consequently, the dark bands seen on the thin sections delimitate the skeletal growth rings (Fig. 59). In order to get enough material for mass spectrometry analysis and maximize the amount of powder coming from the same growth rings of the skeleton, specimens were cut longitudinally through their hollow core for powder extraction, except for *S.* sp. aff. *maldivensis* 2 which was the thickest sample.

All of the basal disk specimens were prepared for radiocarbon analyses by 1) washing with acid/base solutions, 2) rinsing in milliQ water (18 MΩ cm^{-1}), and 3) dried at 60°C in an oven overnight. Prior to sample extraction, any remaining dust was removed with compressed air and every specimen was handled with gloved hands. A computer controlled New Wave Research® (ESI-NWR Division; Freemont CA 94538 USA) micromilling machine with a 300 µm tungstic drill bit (KOMET® GROUP GmbH; 74354 Besigheim Germany) was used to extract skeleton powders. This machine allows high

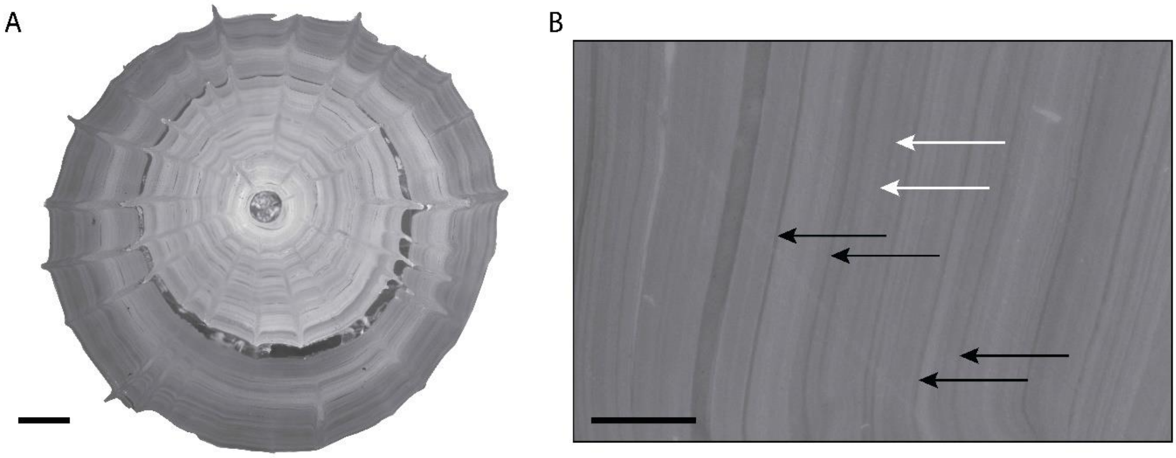

Figure 59. (A) Example of a transverse section of the basal disk showing the hollow core and all the concentric layers around, which represent the growth rings. (B) Growth rings include major rings which are the true limitations counted (black arrow) and minor rings (white arrow). Scales: A–1mm, B–400µm.

precision drilling in *x-y-z* axes and straight or curved sampling directions. Black coral disks were mounted on glass slides with commercial epoxy resin to prevent movement during the extraction process. Each specimen disk diameter and other radial measurements were made using the software ImageJ (Rueden et al. 2017). To collect enough material for accelerator mass spectrometry (AMS), extractions were performed to a depth of 200 µm inside the skeleton with the drill. Powder extractions were made from either the coral disk core on in a radial series from the core. The core was a single sampling measurement that was performed for each colony. The centre of the black coral skeleton for these species is hollow; hence, the first growth ring is posited to be the oldest. Thus, the milling was performed directly next to the hollow core to capture the oldest radiocarbon signal. To refine the age estimates for two of the colonies, extractions were made along a transect from the centre of the skeleton to the outer edge by sampling at different radial distances (Fig. 60a). In several places, the presence of the spines which are overgrown creates the superposition of several growth rings (Fig. 60b). The total mass of skeleton powder retrieved for each sample varied between 30 µg and 76 µg. Each sample was then wrapped in a tin rectangular weighing boat of 6·4·4 mm (Elementar® Analysensysteme GmbH; Langenselbold Germany) for mass spectrometry.

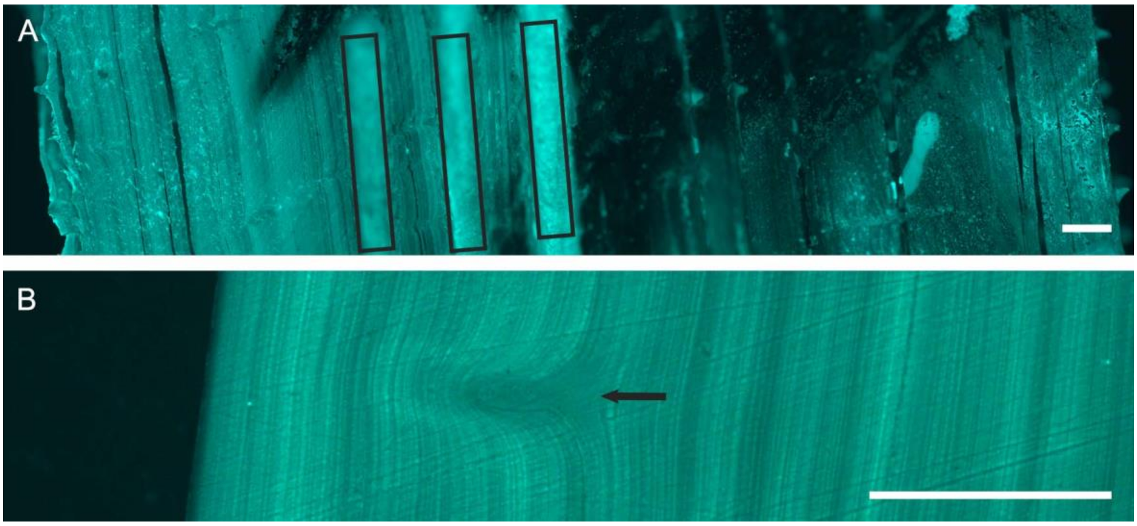

Figure 60. **(a)** Example of a radial series extraction made on a longitudinal cut. **(b)** Detailed view of the overgrowth of a spine (black arrow), which is seen through the superposition of several growth rings. Scale bars: A, B = 400 µm.

Radiocarbon analysis

Samples were combusted and reduced to graphite with iron catalyst. Graphite targets were analyzed at the Laboratory of Ion Beam Physics at the ETH Zurich (Switzerland). An organic ^{14}C-free sample for background correction was also processed. The results are reported as F^{14}C ± 1σ error according to Stuiver and Polach (1977) with a δ^{13}C correction for isotope fractionation measured at the same laboratory. Δ^{14}C values can be converted in a calendar age using a reference bomb radiocarbon time-series. In this case, we use the bomb radiocarbon measurements made on the Watamu reef in Kenya (Grumet et al. 2002a). Δ^{14}C values for the Watamu reef encompass the period from 1947 to 1987 with an absolute year-month data assignment error of less than 3-4 months. In this case, pre-bomb interval is defined as 1947 to 1955 (Grumet et al. 2002a). The atmospheric bomb radiocarbon peak is occurring in 1963 (Nydal 2000), and the maximum recorded value for the reef is in the mid-1970s (Grumet et al. 2002a).

Bomb radiocarbon data Δ^{14}C is plotted against calendar years based on this reference to estimate the age of the black corals. The first run of analyses aims at giving the absolute age of the corals. The second run based on transect sampling is aiming at capturing with more precision the bomb signal, because measurements close to the atmospheric radiocarbon peak allow to establish the annual radial growth rate of the colonies. Results are expressed in F^{14}C which represents the fraction of modern carbon and depend on the activity and the radiocarbon decay (For complete equations refers to Van der Plicht & Hogg 2006).

RESULTS

The lengths and the basal diameters of the black corals was highly varying (Table 12). All the samples showed numerous growth rings when observed under refracted light, and the delimitations between each of them were more visible when they were illuminated with UV light. Distinction between minor growth rings and major growth rings as described by Goldberg (1991) was difficult since the thickness of the opaque cement delimitating minor and major growth rings was not clearly different. To facilitate the counting, the difference of contrast was considered. For all the specimens, the appearance and disappearance of the skeleton spines was observed through the diameter of the skeleton from the centre to the outer edge. The large major growth rings were measuring 45 μm in thickness at maximum. *Stichopathes* sp. aff. *maldivensis*–1 showed 100 major rings, *Stichopathes* sp. aff. *maldivensis*–2 had 155 rings, *Cirrhipathes* sp.–1, –2, –3 had 255, 169 and 226 growth rings respectively (Table 12).

Bomb radiocarbon dating showed different absolute age depending on the colonies (Fig. 61). *Stichopathes* sp. aff. *maldivensis*–1 was aged between 29 and 46 years, *Cirrhipathes* sp.–2 was aged between 29 and 46 years, and *Cirrhipathes* sp.–3 was aged between 62 and 66 years (Table 13). The absolute age of *Stichopathes* sp. aff. *maldivensis*–2 and *Cirrhipathes* sp.–1 were estimated at a pre-bomb year and were reprocessed for transect sampling.

For the specimens with an age range captured by the bomb radiocarbon rise, the maximum and minimum radial and vertical growth rates can be calculated. Given the basal diameter, the length and the age range of *Stichopathes* sp. aff. *maldivensis*–1, the radial growth rated is estimated between 157 and 250 μm.year^{1} and the vertical growth rate between 5.10 and 8.10 cm.year^{-1}. For *Cirrhipathes* sp.–2, radial and vertical growth rates are estimated between 173.47 and 275.17 μm.year^{-1}, and between 4 and 6.41 cm.year^{-1} respectively. For *Cirrhipathes* sp.–3, radial growth rate is between 164 and 175 μm.year^{-1} and the vertical growth rate between 5.8 and 6 cm.year^{-1}. Consequently, several growth rings are synthetized every year, between ~2 and ~3.5 per year for *Stichopathes* sp. aff. *maldivensis*–1, between ~3.5 and ~6 per year for *Cirrhipathes* sp.–2 and ~3.5 per year for *Cirrhipathes* sp.–3 (Table 12).

Table 12. Summary of the basal diameter and length of the specimens, with their estimated growth rates (GR) and growth rings synthesis per year.

Specimen	Basal diameter (mm)	Length (cm)	Growth rings	Radial GR (μm year^{-1})	Vertical GR (cm year^{-1})	GR rate (number year^{-1})
S. sp. aff. *maldivensis* 1	7.25	235	100	157–250	5.10–8.10	2–3.5
S. sp. aff. *maldivensis 2*	15.53	435	155	200–400	5.6–11	2–4
Cirrhipathes sp. 1	16	325	255	233	4.7	3.7
Cirrhipathes sp. 2	7.98	186	169	173–275	4–6.41	3.5–6
Cirrhipathes sp. 3	10.85	385	226	164–175	5.8–6	3.5

Table 13. Summary of the measurements made on coral samples.

Specimen	Sample	F14C	error	Age range (years)
S. sp. aff. *maldivensis* 1	Core	1.0933	0.0092	29–46
S. sp. aff. *maldivensis 2*	Core	0.9277	0.0109	pre-bomb
S. sp. aff. *maldivensis 2*	1.8mm	0.979	0.0087	61–66
S. sp. aff. *maldivensis 2*	2.6mm	0.953	0.0097	59–61
S. sp. aff. *maldivensis 2*	3.4mm	0.94	0.0101	57–58
Cirrhipathes sp. 1	Core	0.8705	0.0137	pre-bomb
Cirrhipathes sp. 1	1mm	0.9336	0.0094	>62
Cirrhipathes sp. 1	1.7mm	0.9422	0.0114	61–65
Cirrhipathes sp. 2	Core	1.0945	0.0081	29–46
Cirrhipathes sp. 3	Core	0.9399	0.0092	62–66

Considering that the species are growing under similar environmental conditions, the average radial growth rate is estimated at 168.5–225 μm.year^{-1} for *Cirrhipathes* sp. These average growth rates can be used to infer the absolute age of the colonies for which the maximum age is before the bomb radiocarbon values. Given the basal diameters of the corals and the calculated average radial growth rates, the absolute age would be 62–99 years for *Stichopathes* sp. aff. *maldivensis*–2 and 71–95 years for *Cirrhipathes* sp.–1 (the values are obtained by dividing the basal diameter by the annual average growth rate). To confirm the utility of an average growth rate to infer the absolute age, the transect sampling aims at precisely refining the growth rate (Fig. 61).

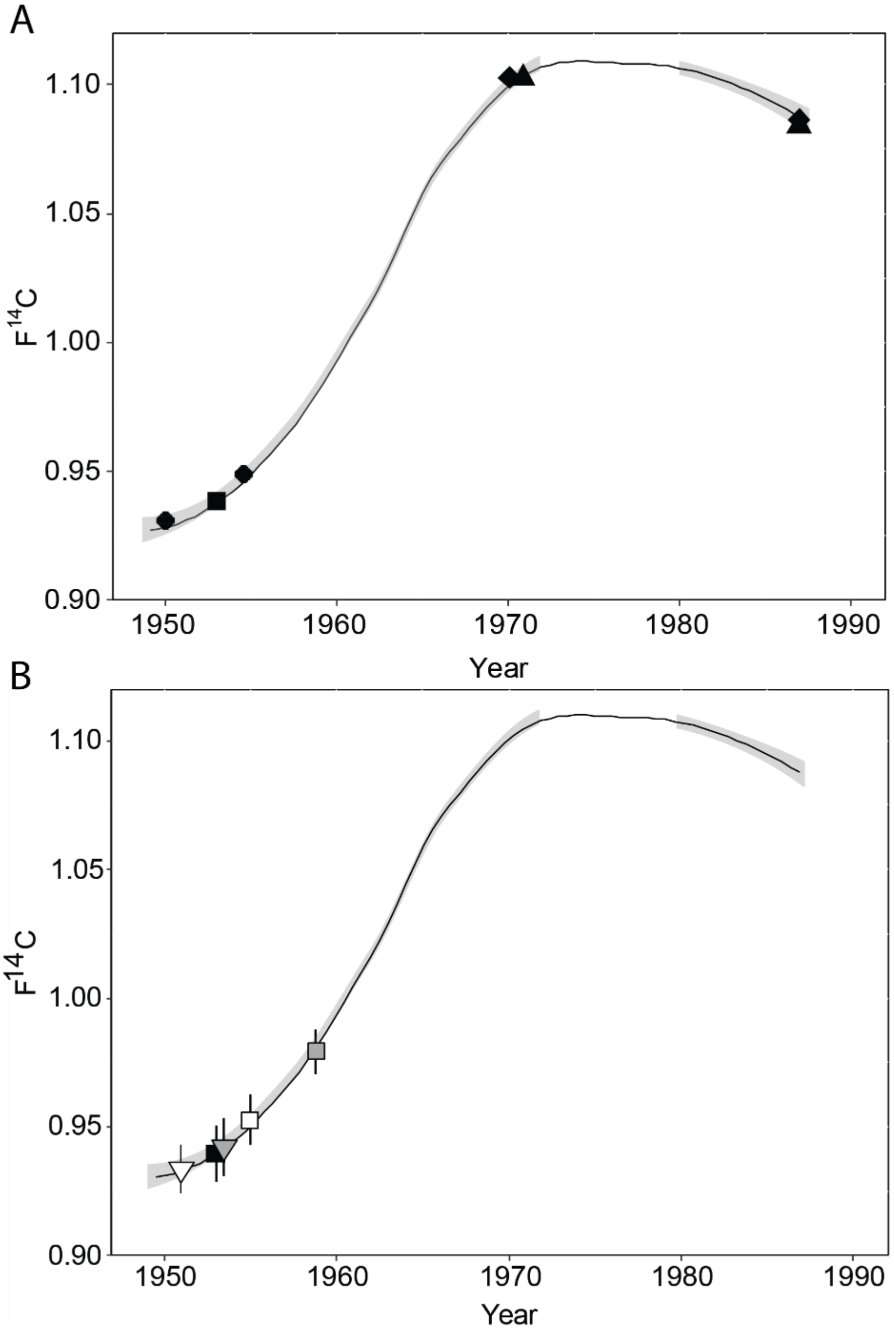

Figure 61. Data of the bomb radiocarbon $F^{14}C$ reference of the Watamu reef in Kenya with a fitted loess curve and a 95% confidence interval represented in grey areas and the year of sampling. **(A)** Age ranges inferred by $F^{14}C$ measurements made on black corals from Madagascar are plotted. Samples were collected in 2016 but they are fitted directly on the curve to get the calendar years depending on the reference. *Stichopathes* sp. aff. *maldivensis*–1 (▲), *Cirrhipathes* sp.–2 (◆) and *Cirrhipathes* sp.–3 (●) minimum and maximum values are in the range of the bomb radiocarbon reference, but *Stichopathes* sp. aff. *maldivensis*–2 (■) has a pre-bomb minimum age which is older than the first reference measure (thus not observed on the graph). **(B)** Transects values plotted on the reference curve with their error bars. All the measurements of *Stichopathes* sp. aff. *maldivensis* were captured by the reference at sampling distances of 1.8 mm (■), 2.6 mm (□) and 3.4 mm (■). For *Cirrhipathes* sp.–1, distances of 1 mm (▽) and 1.7 mm (▼) are plotted, while the core measurement was out of the range.

For *Stichopathes* sp. aff. *maldivensis*–2, the sampling was made at 1.8 mm, 2.6 mm and 3.4 mm from the centre to the outer edge. The samples were aged of 61–66 years, 59–61 years and 57–58 years respectively (Fig. 61). The 1.6 mm range measured results to a radial growth rate of 200–400 µm.year^{-1} and given the total basal diameter of the coral, the absolute age is comprised between 39–78 years. Since the core bomb radiocarbon signal was out of the reference, the oldest scenario is most likely the coral age. Considering both minimum and maximum ages, they are synthetizing ~2 to ~4 annual growth rings. The vertical growth rate is between 5.6 and 11 cm.year^{-1}.

For *Cirrhipathes* sp.–1, sampling was made a second time on the core, then at 1 mm and 1.7 mm from the center. The age ranges were out of the reference signal for the core, the sample taken at 1 mm had a minimum out of the reference and a maximum at 62 years, and the sample taken at 1.7 mm from the core was aged of 61–65 years (Fig. 61). In this case, only the youngest scenario can be inferred because of the measurements which were out of the reference. The 700µm range measured indicates a radial growth rate of 233 µm.year^{-1} resulting to an absolute age which is at least 69 years. This gives a growth rings annual production of at least ~3.7 and a vertical growth of at least 4.7cm.year^{-1}. Since there were measurements older than the reference bomb radiocarbon reference which begins at 1947, it is more likely that the coral is older than 69 years, and a maximum of 95 years using the inferred growth rate.

DISCUSSION

The two species examined in this work are both showing similar growth rates. *Stichopathes* sp. aff. *maldivensis* had and average radial and vertical growth rates of 200 µm.year^{-1} and 6.5 cm.year^{-1} respectively, with 2–3 growth rings synthetized per year. *Cirrhipathes* specimens exposed the same average radial growth rate and growth ring production than the first species, but the vertical growth rate was slightly slower with 5.4 cm.year^{-1}. The results obtained here are in accordance with previous studies and falls within the radial and vertical growth ranges of shallow-water antipatharians. In comparison, annual radial growth rates of other shallow-water antipatharians varied from 50 µm to up to 1140 µm.year^{-1} (Table 14) and vertical growth rates varied from 1.2 cm to

Table 14. Vertical and radial growth rates of shallow-water and deep-sea antipatharians with their location. Adapted from Wagner et al. 2012.

Species	Location	Depth	Radial Rate (µm year -1)	Vertical rate (cm year-1)	Reference
Antipathes dendrochristos	California	106	121	1.5	Love et al. 2007
Antipathes griggi	Hawaii	45-50	180-1140	1.2-11.6	Roark et al. 2006; Grigg 1694, 1974, 1976
Antipathes sp.	Red Sea	2-8	50	-	Risk et al. 2009
Antipathes sp.	Palau	24-34	-	4.52-9.32	Grigg 1975
Antipathes grandis	Hawaii	45-58	-	2.92-6.12	Grigg 1974, 1976
Aphanipathes salix	Virgin Islands	15-46	700	-	Olsen and Wood 1980
Antipathella fiordensis	New Zealand	5-25	100	1.3-3.9	Grange and Singleton 1988; Grange and Goldberg 1993
Cirrhipathes cf. *anguina*	Indonesia	20	-	<159	Bo et al. 2009
Leiopathes glaberrima	SE United States	307-697	14-15	-	Williams et al. 2006,2007
Leiopathes sp.	Hawaii, SE United States	304-500	5-22	-	Roark et al. 2006, 2009; Prouty et al. 2011
Plumapathes pennacea	Jamaica, Saint Croix	15-46	810-920	5.7	Olsen and Wood 1980; Oakley 1988
Stauropathes arctica	Nefoundland Labrador	812-876	33-66	1.22-1.36	Sherwood and Edinger 2009
Stichopathes gracilis	Curacao	>10	-	46.8-84.76	Noome and Kristensen 1976
Stichopathes lutkeni	Jamaica	18	-	76.65	Warner 2005
Stichopathes cf. *maldivensis*	Indonesia	20	-	<15.6	Bo et al. 2009
Stichopathes sp.	Puerto Rico	22-36	-	3-7	Goenaga 1977

84.76 cm (Table 14). In a general way, the growth of deep-sea antipatharians is much slower, with radial extensions of 5–121 µm per year (Table 14). Vertical growth rates are scarce for deep-sea antipatharians and are only known for *A. dendrochristos* which expands of 1.5 cm per year and *S. arctica* which grows of 1.22–1.36 cm per year (Love et al. 2007; Sherwood and Edinger 2009; Table 3). The vertical growth rate of the two present whip corals is lower than those reported by Bo et al. (2009) in Indonesia despite being the same species or at least the same genus. It means that shallow-water whip corals are growing at different rates depending on the species and the location over the world, where the environmental parameters such as food availability or hydrodynamic parameters vary. In contrary of the deep-sea species *Leiopathes* sp. for which the radial growth rate is consistent across oceanographic region (Carreiro-Silva et al. 2013), those of shallow-water whip corals may vary independently to their linear extension rates.

Among shallow-water species, whip corals grow faster than branched species. It has already been shown that the growth rate and the size of the colony are depending of both environmental parameters such as resource capture and physiological features such as metabolic rate (Sebens 1982; Kim and Lasker 1998) and it may become more difficult for bushy corals to catch food from the environment as the size of the colony increase: the newly formed branches shade the old ones and consequently energy allocation may be preferentially dedicated to reproduction or other physiological functions rather than growth (Kim and Lasker 1998).

In deep-sea black corals, there are evidences of different intra-individual growth rates across the lifetime: the growth rate decreases as the lifespan increases (Prouty et al. 2011). In the present study, intraspecific growth rates are very close even if the ages are different. Slight differences seen in radial and vertical growth rates may result from food availability over time, but it is more likely that the differences are due to the sampling process: skeleton powder was extracted with a drill larger than the width of a single growth ring and the bomb radiocarbon dating thus encompasses several years at a time and induces minor errors.

The average vertical and radial growth rates of a species can be used as a proxy to estimate the absolute age of a colony as long as they are influenced by the same environmental parameters. Risk et al. (2009) evaluated the lifespan of *A. dichotoma* based on the linear growth rate of monitored tagged colonies and then validated the age by bomb radiocarbon dating. They showed the utility of an average growth rate to estimate the age of these shallow-water colonies. With the growth rates of *Stichopathes* sp. aff. *maldivensis*–

1, we can estimate the age of the second specimen as between 62–99 years old while bomb radiocarbon dating estimated the absolute age between 39–78 years old. Since the core measurement was out of the reference signal, it is more likely that the coral was aged of *ca* 78 years old, which is in the middle range of the estimated age. For *Cirrhipathes* sp. the average growth rates estimated the specimen aged of 71–95 years while bomb radiocarbon dating resulted in a colony aged of at least 69 years old. For this specimen, the maximum age was not established but the minimum age was corresponding to the one estimated by the average growth rate. These results are supporting the use of growth rates to estimate shallow-water coral ages, which is very important in future stock and population assessments. After sacrificing a few specimens to confirm growth rates with radiocarbon, it would be a fast, cheap and non-invasive method to know the lifespan range of numerous colonies.

Black coral skeleton is made by concentric layers of scleroproteins and chitin (Goldberg 1991) tightly bounded together by a cement which appears opaque in microscopy and displays minor and major growth rings. It is generally assumed that black corals are producing annual growth rings (Goldberg 1991; Love et al. 2007; Sherwood and Edinger 2009). However, in some case it is challenging to distinguish between minor and major rings, a characteristic already pointed out by Goldberg (1991). The annual formation of these rings has been suggested in numerous species such as the deep-sea species *S. arctica* (Sherwood and Edinger 2009) and *A. dendrochistos* (Love et al. 2007) or the shallow-water coral *Antipathes* sp. (Risk et al. 2009). In addition, the growth ring pattern may be influenced by skeleton modification during the growing process, sometimes leading the hollow core to be off-centered, or by annual variations in food supply (Sherwood 2002). Therefore, we suggest that an average growth rate for a given species at the same locality should be used rather than growth ring counting for aging colonies, which is a more reliable method that does not imply the collection of the specimen.

It is very important to establish non-invasive methods for studying black coral, especially in developing countries such as Madagascar. In this country antipatharians are mainly collected for tourist souvenirs or jewelry (Todinanahary et al. 2016). Even if national laws prohibit any exploitation of these coral in the whole territory, illegal harvests still occur in the south. The longevities recorded here for whip black corals are almost similar to a human lifespan, and consequently their fishery cannot be sustainable. Additionally, antipatharians are associated with a lot of symbiotic organisms and are used as nurseries for many fishes and invertebrates (Tazioli et al. 2007; Terrana & Eeckhaut 2017). Considering that it will take a human lifetime for fished black coral populations to return to a normal ecological and structural state by growing such old colonies, the damages of the illegal fisheries of black corals undoubtedly result to an important decrease in tropical shallow-water biodiversity.

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LITERATURE

Bo M., Di Camillo C.G., Addamo A.M., Valisano L., Bavestrello G. (2009) Growth strategies of whip black corals (Cnidaria: Antipatharia) in the Bunaken Marine Park (Celebes Sea, Indonesia). Marine Biodiversity Records 2: 1–6.

Carreiro-Silva M., Andrews A.H., Braga-Henriques A., De Matos V., Porteiro F.M., Santos R.S. (2013) Variability in growth rates of long-lived black coral Leiopathes sp. from the Azores. Marine Ecology Progress Series, 473: 189–199.

Goenaga C. (1977) Two new species of *Stichopathes* (Zoantharia; Antipatharia) with observations on aspects of their biology. Thesis in Marine Sciences., p. 101. University of Puerto Rico, Puerto Rico.

Goldberg W.M. (1991) Chemistry and structure of skeletal growth rings in the black coral *Antipathes fiordensis* (Cnidaria, Antipatharia). In Coelenterate Biology: Recent Research on Cnidaria and Ctenophora (pp. 403-409). Springer, Dordrecht.

Grange K.R., Singleton R.J. (1988) Population structure of black corals, *Antipathes aperta*, in the southern fiords of New Zealand. New Zealand Journal of Zoology, 15: 481–489.

Grange K.R., Goldberg W.M. (1993) Chronology of black coral growth bands: 300 years of environmental history. In “Proceedings of the Second International Temperate Reef Symposium.” (C. N. Battershill et al. eds), pp. 169–174. NIWA Marine, Wellington.

Grigg R.W. (1964) A contribution to the biology and ecology of the black coral, *Antipathes grandis* in Hawai‘i. MS Thesis in Zoology, p. 74. Hawai‘i., Honolulu.

Grigg R.W. (1974) Distribution and abundance of precious corals in Hawaii. In A.M. Cameron et al. (editors), Proceedings of the 2nd International Coral Reef Symposium, 2, Great Barrier Reef Community, Australia, p. 235-240.

Grigg R.W. (1975) The commercial potential of precious corals in the western Caroline Islands, Micronesia. Sea Grant Technical, Report. UNIHI-SEAGRANT-AR-75-03 p.74.

Grigg R.W. (1976) Fishery Management of Precious and Stony Corals in Hawaii. University of Hawaii, Honolulu.

Grumet N.S., Guilderson T.P., Dunbar R.B. (2002a) Pre-bomb radiocarbon variability inferred from a Kenyan coral record. Radiocarbon, 44(2): 581–590.

Grumet N.S., Guilderson T.P., Dunbar R.B. (2002b) Meridional transport in the Indian Ocean traced by coral radiocarbon. Journal of marine research, 60(5): 725–742.

Kim K., Lasker H.R. (1998) Allometry of resource capture in colonial cnidarians and constraints on modular growth. Functional Ecology, 12(4): 646–654.

Love M.S., Yoklavich M.M., Black B.A., Andrews A.H. (2007) Age of black coral (*Antipathes dendrochristos*) colonies, with notes on associated invertebrate species. Bulletin of Marine Science, 80: 391–400.

Noome C., Kristensen I. (1976) Necessity of conservation of slow growing organisms like black coral. C.C.A Ecology Conference Bonaire, 11: 76–77.

Nydal R. (2000) Radiocarbon in the ocean. Radiocarbon, 42(1): 81–98.

Oakley S.G. (1988) Settlement and growth of *Antipathes pennacea* on a shipwreck. Coral Reefs, 7(2): 77–79.

Olsen D.A., Wood R.S. (1980) Investigations on black coral in Salt River Submarine Canyon St. Croix, US Virgin Islands. Final scientific report, 80–12.

Prouty N.G., Roark E.B., Buster N.A., Ross S.W. (2011) Growth rate and age distribution of deep-sea black corals in the Gulf of Mexico. Marine Ecology Progress Series, 423: 101–115.

Risk M.J., Sherwood O.A., Nairn R., Gibbons C. (2009) Tracking the record of sewage discharge off Jeddah, Saudi Arabia, since 1950, using stable isotope records from antipatharians. Marine Ecology Progress Series, 397: 219–226.

Roark E.B., Guilderson TP., Dunbar R.B., Ingram B.L. (2006) Radiocarbon-based ages and growth rates of Hawaiian deep-sea corals. Marine Ecology Progress Series, 327: 1–14.

Rueden C.T., Schindelin J., Hiner M.C., DeZonia B.E., Walter A.E., Arena E.T., Eliceiri K.W. (2017) ImageJ2: ImageJ for the next generation of scientific image data. BMC bioinformatics, 18(1): 529.

Roark E.B., Guilderson T.P., Dunbar R.B., Fallon S.J., Mucciarone D.A. (2009) Extreme longevity in proteinaceous deep-sea corals. Proceedings of the National Academy of Sciences of the United States of America, 106: 5204–5208.

Sebens K.P., Witting J., Helmuth B. (1997) Effects of water flow and branch spacing on particle capture by the reef coral *Madracis mirabilis* (Duchassaing and Michelotti). Journal of experimental marine biology and ecology, 211(1): 1–28.

Sherwood K. (2002) Local commitment to conservation: A Philippine success story. Status of Coral Reefs of the World: 2002, 143.

Sherwood O.A., Edinger E.N. (2009) Ages and growth rates of some deep-sea gorgonian and antipatharian corals of Newfoundland and Labrador. Canadian Journal of Fisheries and Aquatic Sciences, 66(1): 142–152.

Stuiver M., Polach H.A. (1977). Discussion reporting of 14 C data. Radiocarbon, 19(3): 355–363.

Tazioli S., Bo M., Boyer M., Rotinsulu H., Bavestrello G. (2007) Ecological observations of some common antipatharian corals in the Marine Park of Bunaken (North Sulawesi, Indonesia). Zoological Studies, 46 (2): 227–241.

Terrana L., Eeckhaut I. (2017) Taxonomic description and 3D modelling of a new species of myzostomid (Annelida, Myzostomida) associated with black corals from Madagascar. Zootaxa, 4244 (2): 277–295.

Todinanahary G., Terrana L., Lavitra T., Eeckhaut I. (2016) First records of illegal harvesting and trading of black corals (Antipatharia) in Madagascar. Madagascar Conservation & Development, 11 (1): 6–11.

Van der Plicht J., Hogg A. (2006) A note on reporting radiocarbon. Quaternary Geochronology, 1(4): 237–240.

Wagner D., Luck D.G., Toonen R.J. (2012) The biology and ecology of black corals (Cnidaria: Anthozoa: Hexacorallia: Antipatharia). Advances in Marine Biology, 63: 67–132.

Warner G.F. (2005) The occurrence of black corals in Jamaican reef environments, with special reference to *Stichopathes lutkeni* (Antipatharia: Antipathidae). Revista de Biologı´a Tropical 53: 61–66.

Williams B., Risk M.J., Ross S.W., Sulak K.J. (2006) Deep-water antipatharians: Proxies of environmental change. Geology, 34: 773–776.

Williams B., Risk M.J., Ross S.W., Sulak K.J. (2007) Stable isotopes data from deep-water antipatharians: 400-year records from the southeastern coast of the United States of America. Bulletin of Marine Science, 81: 437–447.

Chapitre 7

Trophic niches and stable isotope composition ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) reveals different resource usage by antipatharians within a shallow-water bed

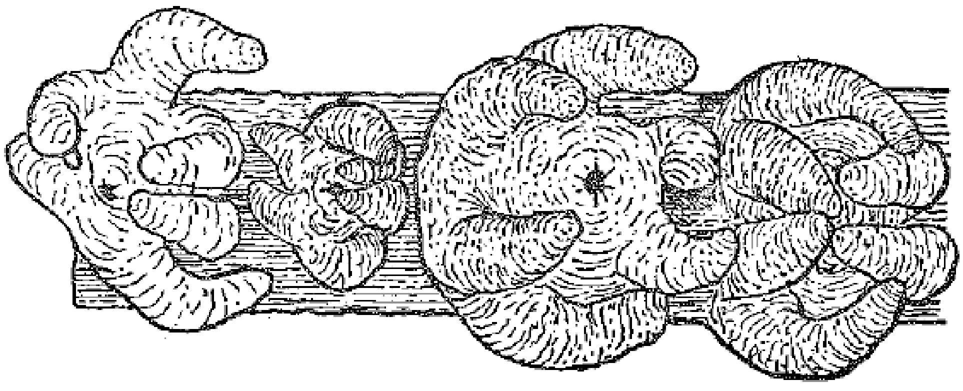

Fig. 9a.

Illustration of the polyps of Stichopathes seychellensis
by Cooper, 1909

Trophic niches and stable isotope composition ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) reveals different resource usage by antipatharians within a shallow-water bed

ABSTRACT

This work aims at investigating the trophic ecology of several shallow-water black corals from the southwest of Madagascar through their isotopic compositions of carbon, nitrogen and sulphur. Tissues of *Stichopathes* sp. aff. *madivensis*, *Cirrhipathes* sp., *Cirrhipathes spiralis*, *Cirrhipathes anguina*, *Cirrhipathes* cf. *contorta, Cirrhipathes densiflora*, and branchlets of *Cupressopathes* sp. and *Myriopathes stechowi* were sampled at their bases and apexes. Potential food sources were sampled during the day and the night using nets and traps: suspended particulate organic matter, zoobenthos, copepods, megalopa larvae, microzooplankton, mesozooplankton, and biofilms. The SIBER (Stable Isotope Bayesian Ellipses in R) approach was used to assess the isotopic niches (proxy for their trophic niches), as well as the mixing model SIAR to show the different food sources proportions in the coral diets. All the black corals feed on a significant part of the mesozooplankton which contains copepods. Suspended particulate organic matter is almost not fed on by black corals. The whip corals were all at the same trophic position, but they are specialized in catching different types of preys from the mesozooplankton. Branched black coral ellipses were totally separated from the others and were at a lower trophic position, but the mixing models also showed a dominancy of mesozooplankton in their diet. Among the biggest whip corals of more than 1 m long, there were differences in $\delta^{15}N$ values between the apexes and the bases of the colonies. Polyp size differences between species were corresponding to the trophic niche segregations between the same species.

This chapter is a draft of the manuscript that will be submitted with the following co-authors and order:

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INTRODUCTION

Antipatharians, also called black corals, are known to live from shallow waters to abyssal depths worldwide. With 7 families and around 247 species (Brugler et al. 2013), they have various sizes and different morphologies: some species are branched with a bush-like shape, a fan-shape, a feather-like shape or a bottle-brush shape, while unbranched species can be straight, or whip-like, with regular or irregular curves or coils (Wagner et al. 2012). Black corals are suspension feeders and the limited field observations and aquaria-based experiments have shown that they feed either during night-time or day-time according to the species (Grigg 1964; Lewis 1978; Goenaga 1977; Tazioli et al. 2007) and that they catch directly food, for the most part made of zooplankton, thanks to their tentacles (Grigg 1964; Goenaga 1977; Warner 1981; Ocaña et al. 2006; Carlier et al. 2009). The various sizes and morphologies observed amongst shallow-water black corals suggest that different species may have different trophic niches. A reliable and effective method to study the trophic ecology of an organism is to assess the carbon, nitrogen and sulphur stable isotope ratios ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) of their tissues. The isotopic composition of a consumer is reflecting a proportional mixture of the isotopic ratios of each of its food sources, plus a generally small difference called isotopic fractionation. Fractionation is dictated by various physical, chemical and biological factors including physiological processes (McCutchan et al. 2003, Connolly et al. 2004). Therefore, it depicts the habitat and the resource use of a species that can be specialist –with a narrow trophic niche– or generalist –with a wide trophic niche (Jackson et al. 2011). It can be used as a tool to understand how trophic interactions affect the community structure and highlight diet shifts and trophic plasticity (Layman et al. 2007; Jackson et al. 2011).

Isotopic studies about black corals are scarce and aim mainly at aging the colonies in bathyal or shallow (5-85 m) waters species, or to use them as proxy records of historical oceanographic, biogeochemical and anthropogenic changes (Williams et al. 2006; Williams et al. 2007; Sherwood et al. 2008; Carlier et al. 2009; Risk et al. 2009; Roark et al. 2009; Williams and Grottoli 2010). In contrast to deep-sea corals which directly rely on enhanced currents and are mainly fueled by surface primary production via sinking fluxes of particles (Duineveld et al 2004), shallow-water black corals and particularly those living on coral reefs may benefit of massive zoo– and phytoplankton productions. Isotopic variation of the plankton or the organic matter depends of different environmental parameters such as depth, location, temperatures, currents, seasons, rivers or sewage discharges, or even anthropogenic effects on land (Goericke and Fry 1994; Wainright and Fry 1994; Thornton and McManus 1994; Teng et al. 2014; Cram et al. 2015; Kurle and McWhorter 2017). Consequently, due to their large geographical and bathymetrical repartition, black corals are potentially exposed to isotopically different food sources.

In front of Toliara (southwest of Madagascar), a shallow-water black coral bed occurs, north to the Great Reef, and is composed of at least of 18 species with varying sizes and morphologies (Chapter 1). The black coral bed is found close to the mouth of the Fiheranana river that brings a lot of terrigenous organic matter (Pichon 1972). This work aims at investigating the trophic ecology of these shallow-water black corals. For that purpose we sampled branched and unbranched colonies and, within unbranched colonies, we collected straight and coiled species, some small and others measuring up to 5 meters long. Specifically we aimed to (i) compare the isotopic niches (i.e. proxy of the trophic niches) of the sampled species found in the same bed, (ii) test the difference between the isotopic compositions of the polyps located at the basis and the apex of the colonies, (iii) estimate the relative importance of potential food sources for black corals taking into account their morphologies and polyp sizes.

MATERIAL & METHODS

Sampling

Samples of unbranched and branched black corals were collected at the base and apex of colonies during scuba diving in May 2015 (15–28 meters) in front of Toliara (southwest of Madagascar). A total of 142 samples coming from 71 colonies was collected. They belonged to 8 species from the two families Antipathidae and Myriopathidae: *Stichopathes* sp. aff. *maldivensis* (n=13), *Cirrhipathes* sp. (n=37), *Cirrhipathes spiralis* (n=1), *Cirrhipathes densiflora* (n=6), *Cirrhipathes anguina* (n=3), *Cirrhipathes* cf. *contorta* (n=3), *Myriopathes stechowi* (n=1) and *Cupressopathes* sp. (n=7), the two latter being branched colonies while all the others were unbranched corals. *C. spiralis* is a helicospiral colony whose spirals are varying in size and diameter according to the environmental parameters. *C. densiflora* and *C. anguina* are usually slightly sinuous with a maximum height of 1.5 m. *C.* cf. *contorta* is very contorted and is never longer than 1m. Unidentified *Cirrhipathes* sp. is one of the most abundant antipatharians found at this place along with *Stichopathes* sp.

aff. *maldivensis* (see Chapter 3). It has polyps located all around the axis, it is generally straight or slightly sinuous and measures up to 4 meters in height. *Stichopathes* sp. aff. *maldivensis* is also very common in Toliara, measures up to 5 m in length and is generally slightly sinuous or forming loose horizontal spirals when measuring several meters. The species *M. stechowi* is a small, branched coral which is not exceeding 20 cm long, with all the branches being in a bidimensional plan. *Cupressopathes* sp. is also abundant in the bed. It is a branched coral which has the first level of branching disposed in four irregular rows around the axis. Sometimes the primary axis divides into a second one which becomes branched in the 4 directions as well. Pieces of tissues of 2 to 3 cm^2 were sampled with a sharp blade to avoid sacrificing the whole colony. For the branched corals, the tissues are very thin and delicate and to get enough material the branchlets have been collected too. The old skeleton material might have captured old environmental isotope records. To minimize its amount, the smallest branches at the extremities of the colony were chosen since the corallum is very thin on these branchlets.

Potential food sources were sampled in the same location during the day and the night. Plankton was collected with 50 µm nets at the surface for 15 minutes before being sieved to obtain the microzooplankton (60–200 µm, n=6) and the mesozooplankton (>200 µm, n=3). Copepods (5 pools of 100 copepods each for the day, 4 pools of 100 copepods each for the night) and crab megalopa larvae (n=6) were manually retrieved from the plankton bulks. Suspended particulate organic matter (SPOM) was accumulated by filtering 3 L of sea water on a pre-combusted 47 mm GF/F filter (n=5 for the day, n=5 for the night). Biofilm samples were also obtained thanks to 10 glass plates put underwater for one week before being retrieved, dried and scratched. Zoobenthos was captured with 10 luminescent traps fixed in the substrate close to antipatharians during the night before being retrieved the next day early in the morning, but only 4 had a sufficient mass for the analyses.

All samples were dried at 60°C for 72 hours or more. They were ground into a homogenous powder using a mortar and a pestle. As inorganic carbon present in samples can be a source of bias in carbon stable isotope ratio analysis, carbonates were removed by acidification. Therefore, samples of plankton, copepods and megalopa larvae were analysed two times. The first run of analyses was made with the native material to obtain $\delta^{15}N$ and $\delta^{34}S$ values. The second run was made on the acidified material which was exposed with HCl vapours (37%) during 24–48h and re-dried to obtain $\delta^{13}C$ values.

Stable isotope analysis

Isotopic measurements of each sample of black coral and the different potential food sources were performed by continuous flow – elemental analysis – isotope ratio mass spectrometry (CF-EA-IRMS) using a Isoprime 100 (Isoprime, UK) coupled to a Vario Micro Cube elemental analyser (Elementar, Germany). Certified reference materials from the International Atomic Energy Agency (IAEA, Vienna, Austria) used were sucrose (IAEA-C6, $\delta^{13}C$ = –10.8 ± 0.5‰; mean ± standard deviation), ammonium sulphate (IAEA-N2, $\delta^{15}N$ = 20.3 ± 0.2‰) and silver sulphide (IAEA-S1, $\delta^{34}S$ = –0.3 ± 0.3‰). Standard deviations on multibatch replicate measurements of one black coral sample were ± 0.2 ‰ both for $\delta^{13}C$ and $\delta^{15}N$, and ±0.6 ‰ for $\delta^{34}S$. Isotope ratios of carbon, nitrogen and sulphur were conventionally expressed as δ values (‰), relative to the international standards Vienna PeeDee Belemnite (vPDB), atmospheric N (Air) and Vienna Canyon Diablo Troilite (VCDT), respectively.

Polyp sizes

Because the preservation process in formaldehyde did not allow examination of the mouths, polyp diameters were measured using ImageJ (Rueden et al. 2017). Measurements were made on polyps from the apex of the colony coming from a single specimen dedicated for taxonomic purposes for each species. The size of the polyps on the branched species remain constant along the branches and thus the small branchlets used for this study does not influence polyp size measurements. For unbranched species, the colony size is not influencing the size of the polyps.

Data treatment and statistics

Isotope ratio value means of the black coral species and the potential food sources were compared with Kruskal-Wallis and Anova tests in R (R Development Core Team 2018). Values for the apex and the base of each black coral species were also compared for each element (C, N, S) with t-tests and Kruskal-Wallis tests. Means of the isotope ratio values were plotted with their standard deviations. Using each individual measurement, the SIBER package v.2.0 (Stable Isotope Bayesian Ellipses in R, Jackson et al. (2011) for R (R Development Core Team 2015) was used to assess the isotopic niche parameters. It generates bivariate standard ellipses whose areas (SEA) represent the core isotopic niche, as it does not encompass outlier individuals in the isotopic space. When dealing with sample sizes inferior to 30 individuals (Syväranta et al. 2013), a correction for small sample size can be used (SEA_C). It is an effective way to compare small and/or unbalanced samples, and it tends to converge when sample size increases (Jackson et al. 2011). Areas of the ellipses associated to each population were also estimated using Bayesian modelling (SEA_B; 10^5 iterations). SEA_B involves the use of an iterative model based on Bayesian inference to estimate the covariance matrix from the data. SEA_B takes into account variability in the data (caused by both natural variations and analytical error) more efficiently and provides a distribution of solutions rather than a single value, therefore allowing error estimates as well as pairwise comparisons. Model solutions were presented using credibility intervals of probability density function distributions. Direct pairwise comparisons were performed and were considered meaningful when probability of occurrence (i.e. number of model solutions where a given situation was found) exceeded 95%.

The stable isotope mixing model SIAR (Parnell et al. 2008) was used to estimate the relative contribution of the different potential food sources collected to the diet of all the black coral species. Isotopic compositions of the antipatharian species and means and standard deviations of the different potential food sources (i.e., night and day plankton, particulate organic matter, zoobenthos, biofilms, megalopa larvae and copepods) were fitted in R. Because studies about discrimination factors are scarce, particularly about hexacorallians, the trophic enrichment factors (TEFs) for marine invertebrates were retrieved from McCutchan et al. (2003) and were implemented in the mixing model. Solution are presented using credibility intervals of probability density function distributions. Mixing models perform better with a few sources which have not a high variance (Parnell et al. 2008). Thus, after visual observation of the means in Fig.1, biofilms and zoobenthos were removed from the dataset to run the model estimations with $5(10^5)$ iterations and a burn-in of $8(10^4)$. Finally, polyp mean sizes were compared with Kruskal-Wallis tests in R (R Development Core Team 2018).

RESULTS

Stable isotope values of food items and black coral species

The $\delta^{13}C$ values ranged between -19.0 and -20.8 ‰ for the black coral species, and -15.1 and -22.6 ‰ for the food sources (Table 15). All the corals were found in the same

$\delta^{15}N$ interval, as most values ranged from 7.5‰ to 9.8‰, except for *M. stechowi* and *Cupressopathes* sp. which ranged from 6.4‰ to 7.4‰. (Table 15). All food items presented $\delta^{15}N$ values in the same range from 4‰ to 7‰, except for biofilm which were between 1.6‰ and 3.4‰. The $\delta^{34}S$ values ranged between 17.7 and 20.2 ‰ for the black corals while it was between 15.2 and 19.2 ‰ for the food sources. These values were not obtained for the zoobenthos.

Black coral species had significant different $\delta^{13}C$ means (Fig. 62, Anova, F=31.28, p-value <0 .001) and $\delta^{15}N$ means (Fig. 62, Anova, F=31.066, p-value<0.001), but no significant $\delta^{34}S$ means (Fig. 62, Anova, F=1.40, p-value=0.20). The table 16 summarize all the p-values for significantly different means between the species.

Table 15. Ranges of the delta values for each carbon, nitrogen and sulphur isotopes.

	$\delta^{13}C$ (‰)		**$\delta^{15}N$ (‰)**		**$\delta^{34}S$ (‰)**	
	Min	Max	Min	Max	Min	Max
Species						
Cirrhipathes anguina	-19.2	-18.5	8.4	9.7	18.8	20.0
Cirrhipathes cf. *contorta*	-19.5	-17.5	8.5	9.4	19.6	21.0
Cirrhipathes densiflora	-20	-18	8.3	9.8	18.3	20.8
Cirrhipathes spiralis	-19	-18.3	8.4	9.3	20.1	20.2
Cirrhipathes sp.	-20.5	-18.9	7.5	9.7	18.1	20.7
Cupressopathes sp.	-20.8	-19.5	6.5	7.4	19.5	20.9
Myriopathes stechowi	-19.7	-19	6.4	6.5	20.2	20.2
Stichopathes sp. aff. *maldivensis*	-20.7	-19.3	7.8	9.4	17.7	20.3
Food source						
Day copepods	-21.4	-20.7	5.7	6.7	17.8	18.7
Night copepods	-22.6	-21.2	5.4	6.3	18	18.8
Day microzooplankton	-19.8	-19	5.4	6.3	17.4	18.8
Night microzooplankton	-20.6	-20.5	5.6	6.4	15.7	18.3
Day mesozooplankton	-19.7	-19.1	5.4	6.2	19.2	19.9
Megalopa larvae	-22.7	-19.5	5.3	6.1	17.5	18.4
Zoobenthos	-20.7	-14.3	5.1	7	-	-
Biofilms	-15.1	-12.3	1.6	3.4	15.2	18.3
Night POM	-20.3	-15.9	4	5.7	15.3	17.4
Day POM	-21.1	-17.8	4.3	4.7	15.7	17.0

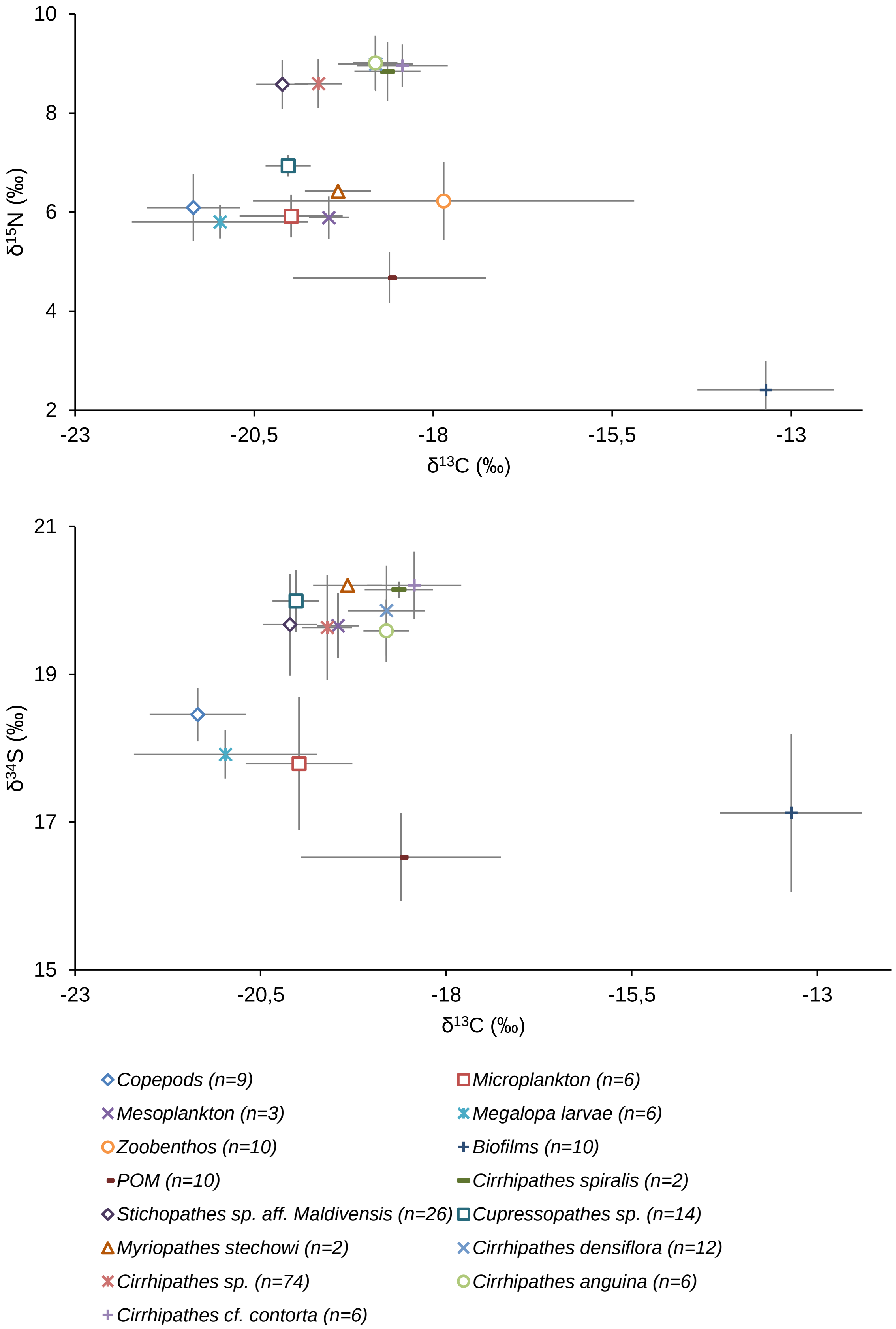

Figure 62. a) Mean values (± SD) of δ^{13}C (‰) and δ^{15}N (‰) of the antipatharians and their potential food sources. **b)** Mean values (± SD) of δ^{34}S (‰) and δ^{13}C (‰) of the antipatharians and potential food sources.

Table 16. Summary of post-hoc Tukey tests for multiple comparisons between delta values of all black coral speciesSignificant differences are underlined.

δ^{13}C	*C. anguina*	*C. cf. contorta*	*C. densiflora*	*Cirrhipathes sp.*	*C. spiralis*	*Cupressopathes sp.*	*M. stechowi*
C. cf. *contorta*	0.61						
C. densiflora	1	0.43					
Cirrhipathes sp.	<0.001	<0.001	<0.001				
C. spiralis	0.99	0.99	0.99	<0.01			
Cupressopathes sp.	<0.001	<0.001	<0.001	<0.01	<0.001		
M. stechowi	0.43	0.05	0.54	0.96	0.54	0.18	
S. sp. aff. *maldivensis*	<0.001	<0.001	<0.001	<0.001	<0.001	0.99	0.07
δ^{15}N	*C. anguina*	*C. cf. contorta*	*C. densiflora*	*Cirrhipathes sp.*	*C. spiralis*	*Cupressopathes sp.*	*M. stechowi*
C. cf. *contorta*	*1*						
C. densiflora	*1*	*1*					
Cirrhipathes sp.	*0.39*	*0.59*	*0.12*				
C. spiralis	*1*	*1*	*1*	*0.99*			
Cupressopathes sp.	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*		
M. stechowi	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	
S. sp. aff. *maldivensis*	*0.44*	*0.62*	*0.19*	*1*	*0.99*	*<0.001*	*<0.001*
δ^{34}S	*C. anguina*	*C. cf. contorta*	*C. densiflora*	*Cirrhipathes sp.*	*C. spiralis*	*Cupressopathes sp.*	*M. stechowi*
C. cf. *contorta*	*0.69*						
C. densiflora	*0.98*	*0.95*					
Cirrhipathes sp.	*1*	*0.4*	*0.94*				
C. spiralis	*0.95*	*1*	*0.99*	*0.94*			
Cupressopathes sp.	*0.88*	*0.99*	*0.99*	*0.51*	*1*		
M. stechowi	*0.93*	*1*	*0.99*	*0.91*	*1*	*1*	
S. sp. aff. *maldivensis*	*1*	*0.58*	*0.98*	*1*	*0.96*	*0.79*	*0.94*

Means were also different between the food sources for δ^{13}C (K-W, X^2=39.385, df=8, p-value<0.001), δ^{15}N (K-W, X^2=33.169, df=8, p-value<0.001) and δ^{34}S (K-W, X^2=37.88, df=7, p-value<0.001). Since we did not detect any differences between delta values of the POM samples collected during the night and day (p=0.76 for δ^{13}C, p=0.90 for δ^{15}N, p=0.71 for δ^{34}S), of the copepods collected duringthe day and the night (p=0.70 for δ^{13}C, p=0.60 for δ^{15}N, p=0.75 for δ^{34}S) and of the night and day microzooplankton (p=0.70 for δ^{13}C, p=0.89 for δ^{15}N, p=0.29 for δ^{34}S), night and day samples were considered as a whole.

Stable isotope values of the bases and the apexes of the colonies

There were significant differences for δ^{13}C and δ^{15}N values between all the black coral species when considering the position of the collected tissues at the apex of the colonies or the basis, but not for δ^{34}S values (Table 17). *Cupressopathes* sp. had significant differences in δ^{13}C between their basis and their apex (Table 17). *Cirrhipathes anguina, Cirrhipathes densiflora, Cirrhipathes* sp., and *Stichopathes* sp. aff. *maldivensis* had significant differences in δ^{15}N between their bases and their apexes (Table 17). Delta values for *C. spiralis* and *M. stechowi* were not compared statistically because there was only one colony sampled for both species (Fig. 63). In all the significant cases, it was always the apex of the colony that was depleted in the heavy isotope (Fig. 63).

Table 17. Statistical comparisons in the stable isotope compositions between tissues collected at the basis and the apex of the colonies, for each antipatharian species. Comparisons are made with Student t-tests. When notified with an "*", Kruskal-Wallis tests were performed. Underlined p-values are significant differences.

	C. anguina	*C.* cf. *contorta*	*C. densiflora*	*Cirrhipathes* sp.	*Cupressopathes* sp.	*S.* sp. aff. *maldivensis*
Carbon	p=0.17	p=0.43	p=0.08	p=0.10	p=0.01	p=0.08
Nitrogen	p<0.01	p=0.29	p<0.001	p<0.01	p=0.36	p<0.001
Sulphur	p=0.71	p=0.21	p=0.75*	p=0.74*	p=0.75*	p=0.16*

Standard Ellipses δ^{15}N (‰²) vs. δ^{13}C (‰²) and δ^{34}S (‰²) vs. δ^{13}C (‰²)

Standard Ellipses obtained with SIBER suggested separated isotopic niches between the different species when considering δ^{15}N versus δ^{13}C and δ^{34}S versus δ^{13}C (Fig. 64). When considering carbon and nitrogen, the Bayesian approach modelling the standard ellipses clearly showed 5 distinct groups: the first one is made by *Stichopathes* sp. aff. *maldivensis*, the second by *Cirrhipathes* sp., the third is composed of *Cirrhipathes anguina*, *Cirrhipathes* cf. *contorta*, *Cirrhipathes densiflora* and *Cirrhipathes spiralis*, and the two last groups are made by *Cupressopathes* sp. and *Myriopathes stechowi.*

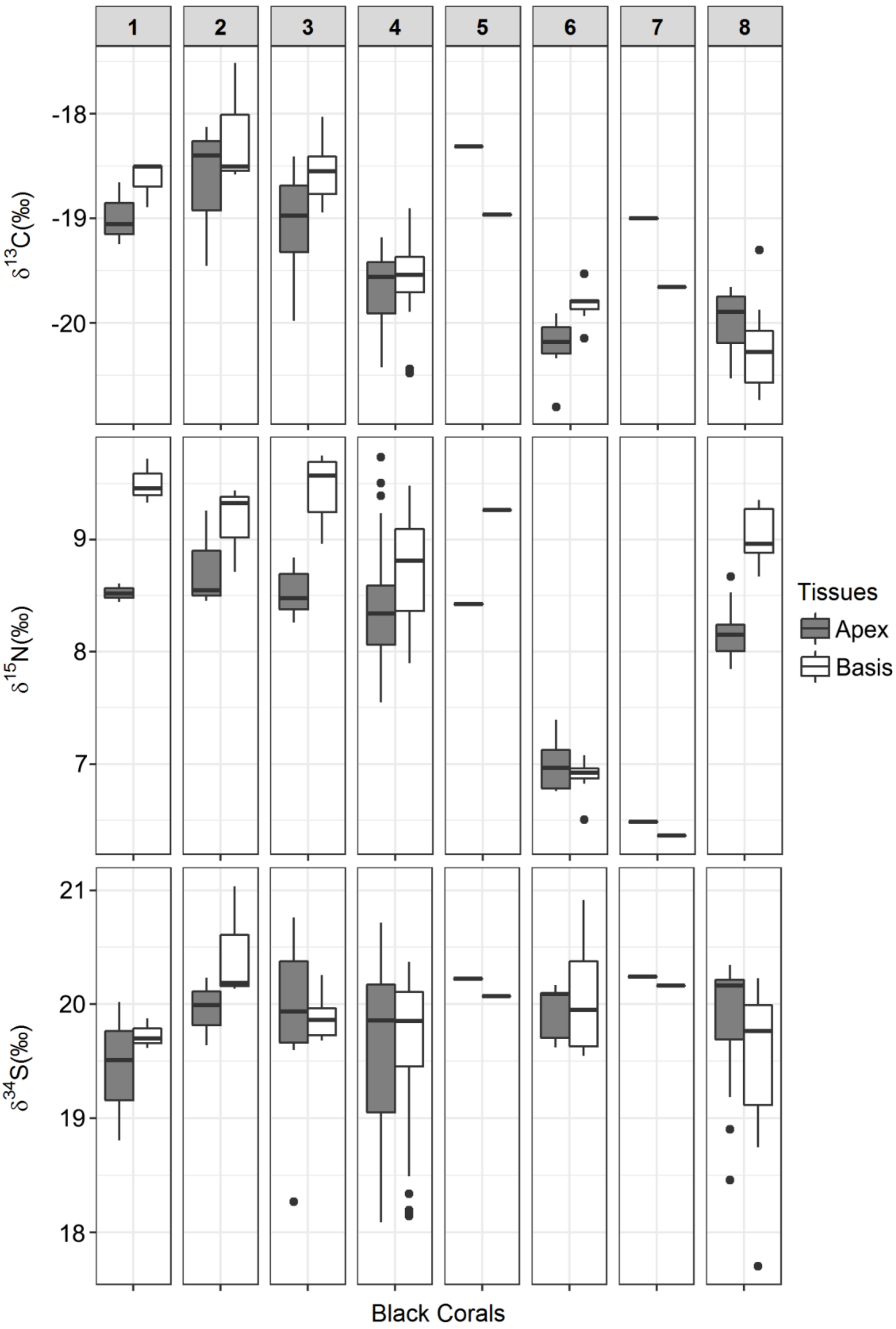

Figure 63. Boxplots of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values for each black coral species with a comparison with tissues collected at the apex of the colonies and their basis. Legend: 1–*Cirrhipathes anguina*, 2–*Cirrhipathes* cf. *contorta*, 3–*Cirrhipathes densiflora*, 4–*Cirrhipathes* sp., 5–*Cirrhipathes spiralis*, 6–*Cupresopathes* sp., 7–*Myriopathes stechowi*, 8–*Stichopathes* sp. aff. *maldivensis*.

The ellipse of *Stichopathes* sp. aff. *maldivensis* (SEAc = 0.56 ‰^{2}) was overlapping the one of *Cirrhipathes* sp. (SEA = 0.51 ‰^{2}) of 0.09 ‰^{2}, representing 17.37 % and 19.11 % of the respective ellipse areas. *C. densiflora* (SEAc = 0.87 ‰^{2}) ellipse overlapped three other species: the overlap had an area of 0.02 ‰^{2} with *Cirrhipathes* sp. (representing 5.08 % and 2.94 % of the respective ellipses), 0.55 ‰^{2} with *C. anguina* (SEAc = 0.55 ‰^{2}, representing 61.38 % and 96.50 % of the respective ellipses), and 0.58 ‰^{2} with *C.* f. *contorta* (SEAc = 1.07 ‰^{2}, representing 65.50 % and 53.45 % of the respective ellipses). The last overlap between *C. anguina* and *C.* cf. *contorta* was 0.40 ‰^{2}, representing 71.63 % and 37.18 % of the respective ellipses surfaces. *Cupressopathes* sp. (SEAc = 0.22 ‰^{2}) was not overlapping any other ellipse. Ellipses were not drawn for *C. spiralis* and *M. stechowi* as there was only one specimen sampled for both species. *C. spiralis* had one value included in the ellipses of *C. densiflora* and *C. anguina* while the other value was out of any ellipse, but close to the one of *C.* cf. *contorta* (Fig. 64a). *M. stechowi* had no values included in any ellipse, but nitrogen values were lower than those of *Cupressopathes* sp. (Fig. 64). In pairwise comparisons, the ellipse area of *Cupressopathes* sp. was always smaller than all the other species in more than 95 % of the occurrences of the model, while all the other had similar ellipse areas, *i.e.* always under 95% of occurrences in the model (Fig. 65a).

When considering carbon and sulphur, there was no ellipse completely separated from the others (Fig. 64b). *Stichopathes* sp. aff. *maldivensis* (SEAc = 0.80 ‰^{2}) had an overlap of 0.40 ‰^{2} with *Cupressopathes* sp. (SEAc = 0.44 ‰^{2}), representing 49.01 % and 88.11 % of their surfaces respectively, and an overlap of 0.14 ‰^{2} with *Cirrhipathes* sp. (SEA = 0.74 ‰^{2}) representing 16.77 % and 17.90 % of their respective ellipse areas. *Cirrhipathes* sp. was also overlapping *Cupressopathes* sp. of 0.09 ‰^{2}, representing 20.79 % and 12.34 % of their respective surfaces; and overlapping *C. densiflora* (SEAc = 1.03 ‰^{2}) by a surface of 0.02 ‰^{2}, which is 2.30 % and 1.68 % of their surfaces, respectively. *C. densiflora* was also overlapping *C. anguina* (SEA = 0.50 ‰^{2}) by a surface of 0.45 ‰^{2}, which is 43.22 % and 87.64 % of their surfaces, respectively. It was also overlapping *C.* cf. *contorta* (SEAc = 1.11 ‰^{2}) by 0.45 ‰^{2}, representing 42.60 % and 39.48 % of their respective ellipse areas. Finally, *C. anguina* overlapped *C.* cf. *contorta* of 0.09 ‰^{2}, representing 17.74 % and 8.11 % of their areas, respectively. In pairwise comparisons, the ellipse area of *Cupressopathes* sp. was smaller than all other species except *C. anguina* in more than 95 % of the occurrences of the model (Fig. 65b).

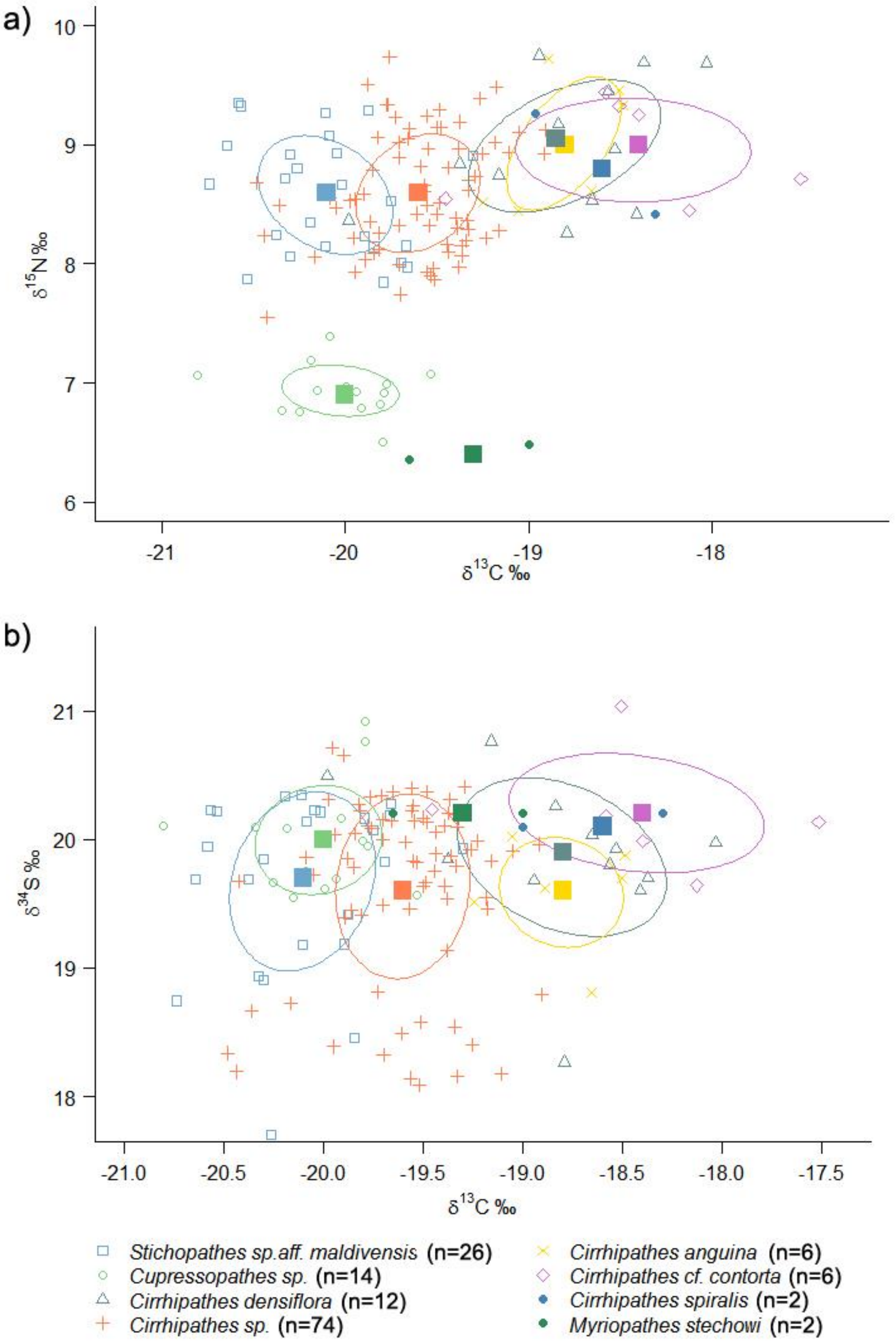

Figure 64. Standard Ellipses obtained with SIBER with a p-interval of 0.40, meaning that the ellipses contain 40% of the whole data of each group. Each species is represented by a different colour and a different symbol. The solid squares are plotted to show the bivariate means for every species. Ellipses are not shown for *C. spiralis* and *M. stechowi* since there was only one colony sampled for each. **a)** δ^{15}N (‰) values plotted against δ^{13}C (‰) values. **b)** δ^{34}S (‰) values plotted against δ^{13}C (‰) values.

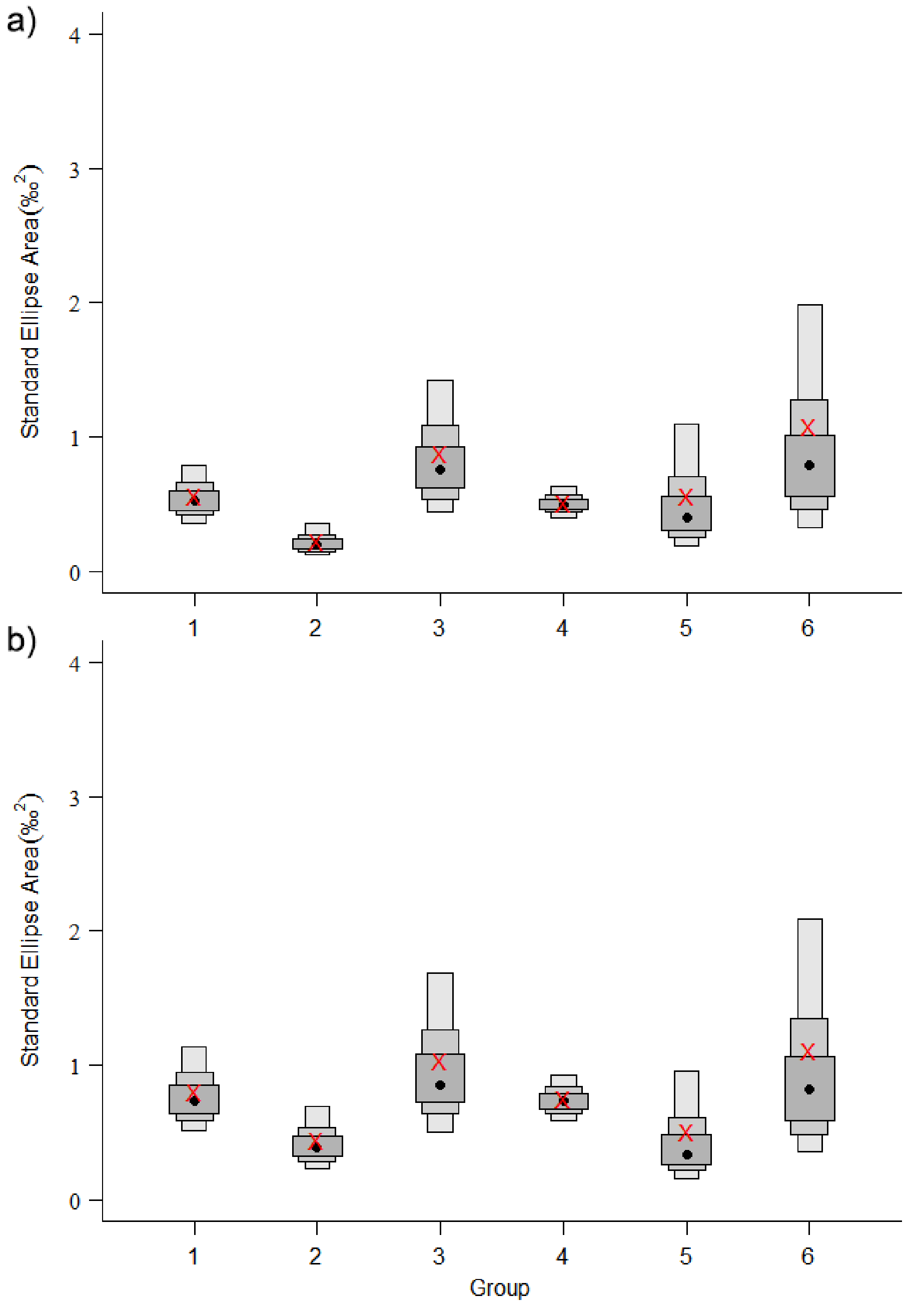

Figure 65. Estimates of standard ellipse area for each black coral species with more than 5 colonies sampled. The boxplots represent posterior probability distributions of model estimations of the standard ellipse areas (SEA_B). The boxes are the 50 %, 75 % and 95 % confidence interval from dark grey to light grey respectively. The mode of each distribution is shown by a black dot, while the red cross represents the standard ellipse area calculated using correction for small sample size (SEA_C). **a)** Ellipses inferred using carbon and nitrogen stable isotope ratios. **b)** Ellipses inferred using carbon and sulphur stable isotope ratios. 1– *Stichopathes* sp. aff. *maldivensis*, 2– *Cupressopathes* sp., 3– *Cirrhipathes densiflora*, 4– *Cirrhipathes* sp., 5– *Cirrhipathes anguina*, 6– *Cirrhipathes* cf. *contorta*.

Polyp sizes

Polyps of *C. spiralis* measured 1.33 ± 0.34 mm, *Stichopathes* sp. aff. *maldivensis* 2.47 ± 0.27 mm, *Cupressopathes* sp. 0.62 ± 0.11 mm, *M. stechowi* 0.50 ± 0.07 mm, *C. densiflora* 2.74 ± 0.64 mm, *Cirrhipathes* sp. 1.85 ± 0.36 mm, *C. anguina* 2.74 ± 0.58 mm, and *C.* cf. *contorta* 2.45 ± 0.57 mm. The Kruskal-Wallis test confirmed that at least one black coral had a significant difference in mean polyp size than the others ($\chi^2 = 560.28$, df = 7, p-value < 0.001). Comparisons were made with Dunn tests. *C. spiralis* polyp sizes were significantly different than all other species (all p-values <0.001). Polyps of *M. stechowi* and *Cupressopathes* sp. were not significantly different (p-value =0.06). *Cirrhipathes* sp. had significantly different polyp sizes than all the others (all p-values <0.001) and *Stichopathes* sp. aff. *maldivensi*, *C. densiflora*, *C. anguina* and *C.* cf. *contorta* had the same polyp sizes (all p-values > 0.05).

Food source contributions

The ranges of feasible contributions for each food source to the studied black coral species are shown in Fig. 66 (see also supplementary material). The model suggested that mesozooplankton and copepods appeared to constitute the most used food sources for both unbranched and branched corals, while megalopa larvae and POM were the less in proportions *C. spiralis* and *M. stechowi* were the corals with the most homogenous diet but it is explained by the low variance of the isotope ratios induced by the single colony sampled.

Copepods had the highest proportion in the diet of *Stichopathes* sp. aff. *maldivensis* (35–59 % for 95 % credibility intervals) and the lowest in *C. densiflora* (0–14 %). Microzooplankton had the highest proportion in *C. spiralis* (0–40 %) and the lowest in *Stichopathes* sp. aff. *maldivensis* (0–13 %). Mesozooplankton had the highest proportion in *C. densiflora* (60–91 %) and the lowest in *C. spiralis* (0–46 %). POM proportions were very low for all species, but it was the highest in *C. spiralis* (0–39 %) and the lowest in *Cirrhipathes* sp. (0–3 %). Finally, megalopa larvae were the highest in *C. spiralis* (0–37 %) and the lowest in *Cirrhipathes* sp. (0–14 %).

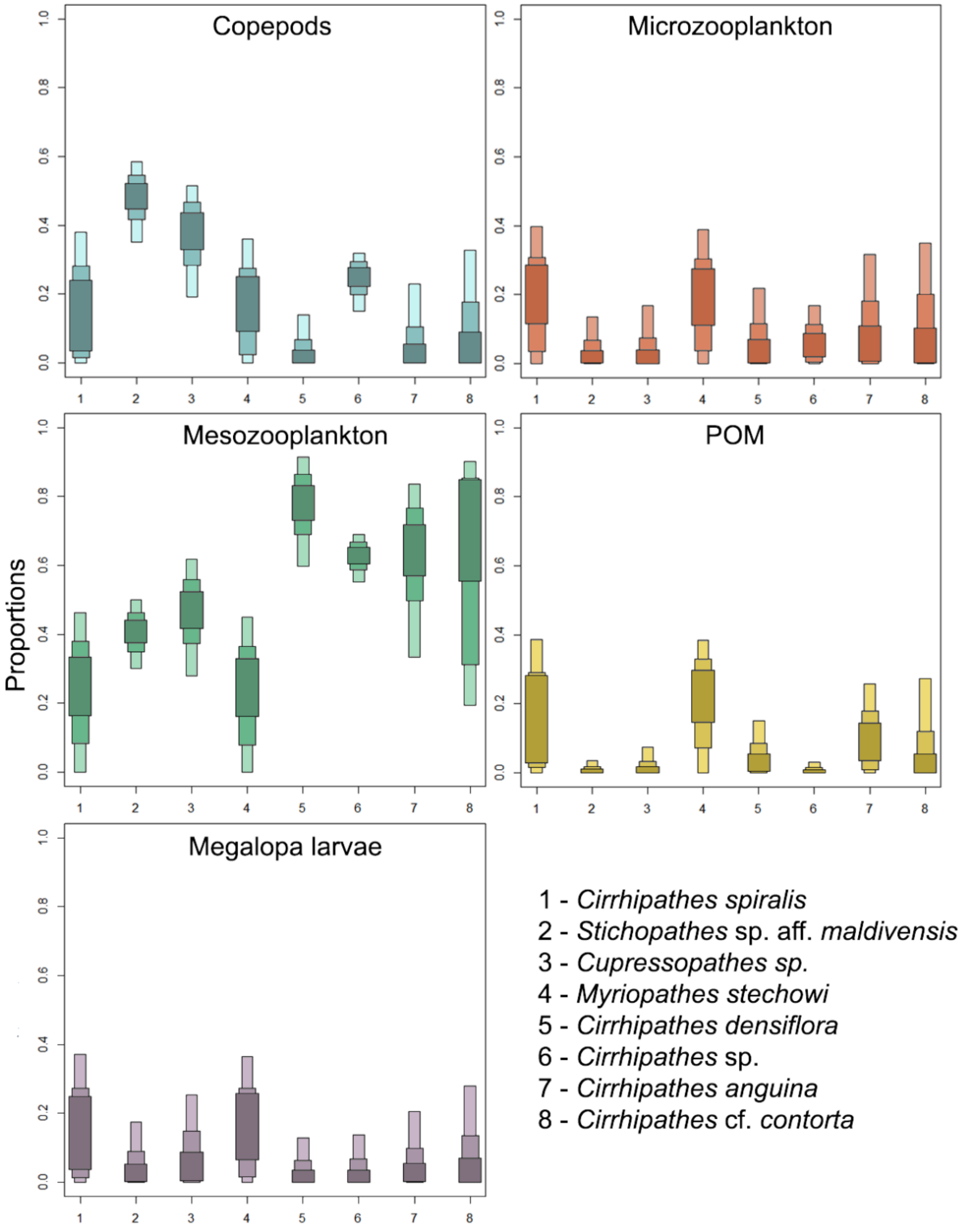

Figure 66. Boxplots of the relative contribution of the different potential food sources to the diet of each black coral species. Credibility intervals (CI): CI_{50} = dark colour, CI_{75} = medium colour, CI_{95} = light colour.

DISCUSSION

Isotopic compositions among black corals

Isotopic ratios are known to depict the diet and the food source of an organism, as well as the trophic niche which is generally modelled by an ellipse encompassing 40% of the isotopic variability (McCutchan et al. 2003; Jackson et al. 2011). To date, isotopic ratios of branched black corals are only known for carbon and nitrogen for the deep-sea species *Leiopathes glaberrima*, *Leiopathes* sp., and *Stauropathes arctica* and the shallow-water species *Antipathes* sp. and *Rhipidipathes* sp. (Williams et al. 2006; Williams et al. 2007; Carlier et al. 2009, Sherwood et al. 2008; Roark et al. 2009; Risk et al. 2009; Williams and Grottoli 2010). Carbon isotopic ratios of *Leiopathes* species are varying between -19.3 and -15.7 ‰ while nitrogen isotopic ratios range between 7.7 to 9.3 ‰ (Williams et al. 2006; Williams et al. 2007; Carlier et al. 2009; Roark et al. 2009). *S. arctica* had δ^{13}C values of -20.3 ±0.6 (SD) ‰ and δ^{15}N values of 10.8 ±1.2 (SD) ‰ (Sherwood et al. 2008). Among the shallow-water black corals of Toliara in Madagascar, mean isotopic values were varying between species. Considering the branched corals *Cupressopathes* sp. and *M. stechowi*, δ^{13}C values ranged from -20.8 ‰ to -19.0 ‰, δ^{15}N values ranged between 6.4 ‰ and 7.4 ‰, and δ^{34}S ranged from 19.6 ‰ to 20.9 ‰. The δ^{13}C values were quite similar for the present branched specimens compared to the deep species, while the δ^{15}N values were sometimes different of around 2.5 ‰ between species which is considered by McCutchan et al. (2003) as a change in a trophic level. Shallow-water *Antipathes* corals from the Red Sea had δ^{13}C values of about -18 ‰ and δ^{15}N values of about 4 ‰, but these values were influenced by sewage discharges and both isotopic values increased to more than -17 ‰ and more than 8 ‰ respectively (Risk et al. 2009). The comparison is not consistent since the depth and the environmental factors are different.

To date, there is no isotopic records for whip black corals in the deep sea as well as in shallow waters. When considering all studied species, whip black corals from Toliara had δ^{13}C values varying between -20.7 ‰ and -17.5 ‰, δ^{15}N values that ranged from 7.6 ‰ to 9.8 ‰, and δ^{34}S values that ranged from 17.7 ‰ to 21.0 ‰. The maximum difference for δ^{13}C, δ^{15}N and δ^{34}S values were corresponding to 3.2 ‰, 2.2 ‰ and 3.3 ‰ respectively. This variability suggests that unbranched black corals diet is based on a source located at the same trophic level, such as mesozooplankton, but presenting a specialization in some types of prey like copepods, even if they are all sedentary species living at the same place and being unbranched corals. Looking at theδ^{15}N values, branched black corals may be feeding at a lower trophic position than unbranched ones, suggesting that the food sources used by the two types of morphologies may be different (see section below *Trophic niches, resource use and polyp sizes*).

Apart from carbon and nitrogen, sulphur isotopes are also used to trace organic matter and discriminate food sources (Connolly et al. 2004). Pelagic producers using seawater sulphates have generally higher δ^{34}S values (~22.9 ‰) while sulphur coming from sediments have lower δ^{34}S values (~1 ‰) (Kharlamenko et al. 2001; Connolly et al. 2004; Mittermayr et al. 2014). A terrestrial runoff influence brought by the Fiherenana river would be seen through these isotopes because inorganic sulphur from the environment would contribute to the total S pool of an animal and to its sulphur isotope ratio (McCutchan et al. 2003). The sulphur isotope ranges of the studied antipatharians showed that they are feeding on a source which is using pelagic seawater sulfates and do not rely on food from the benthos. They are corresponding to those of zooplankton ranging between 17.5 and 20.3 ‰ (Mittermayr et al. 2014), supporting the carnivorous diet of the corals for all the studied species since there is no to low level of fractionation across trophic levels (McCutchan et al. 2003).

Intra-colonial differences

The unbranched black corals *C. anguina*, *C. densiflora*, *Cirrhipathes* sp. and *Stichopathes* sp. aff. *maldivensis* showed significant differences in δ^{15}N values between the bases and the apexes of the colonies. Interestingly, these species are the only ones of this study that can grow more than 1 meter in height, *Cirrhipathes* sp. and *Stichopathes* sp. aff. *maldivensis* sometimes reaching up to 5 meters in length. These colonies are thus exposing the polyps to different hydrodynamics and potential food sources with the top polyps being more exposed to currents than those located close to substrate. Consequently, the nature and quantity of food sources brought by water flows to these polyps might be different. The food access of the species having long colonies is improved and may explain why they present fast vertical growth rates as seen in the Chapter 4. and in the literature with 76 cm.y^{-1} for *Stichopathes lütkeni* and 159 cm.y^{-1} for *Cirrhipathes* sp. (Warner 2005; Bo et al. 2009). Intra-individual isotopic variation is common among vertebrates (*e.g.* Pinnegar et al. 2001) but has never been observed in diploblastic organisms. In black corals, a homogenous isotopic composition would be expected since they are colonial organisms with all the polyps communicating through the whole colony. In the studied species, it was always the apex of the colonies that was depleted in heavy isotopes which suggests that (i) the isotopic fractionation for these colonial organism is differing with the distance spacing polyps, (ii) the preys captured are different and thus have different isotopic compositions, and/or (iii) the turnover rate of the living tissues is slow, and consequently the oldest tissues are located at the bases and are capturing short-timed environmental changes through the nitrogen stable isotopes.

Cupressopathes sp. was apart from other black corals by having only the $\delta^{13}C$ values significantly different between the bases and the apexes. This difference might be the result of a different fractionation between the living tissues and the skeleton, which were both used at the same time in this case for the analysis and thus recording old environmental carbon isotopic composition in their old branches (Roark et al. 2009). Statistical analyses were not made on *M. stechowi* since only one colony was sampled, but the carbon values between the apex and the top of the colony were also varying with the same range than *Cupressopathes* sp.

Trophic niches, resource use and polyp sizes

Fractionation factors were taken from McCutchan et al. (2003) to run the mixing model since no extensive study has never been made on isotopic fractionation among hexacorallians. Consequently, the mixing model results must be taken with caution. When comparing results with other antipatharians in the world, it is assumed that deep-sea corals *Leiopathes* and *S. arctica* are mainly feeding on sinking POMcontaining a significant part of nanozooplankton (Williams et al. 2006; Williams et al. 2007; Sherwood et al. 2008; Carlier et al. 2009; Roark et al. 2009). The shallow-water black corals *Antipathes* sp. also feed on zooplanktonic preys with a small amount of POM in the diet, which is influenced by terrestrial runoffs (Roark et al. 2009), while in contrary Sherwood et al. (2008) hypothesized that *Rhipidipathes* and *Antipathes* may feed on POM rather than zooplankton. In our results, it was observed that the POM was not the major part of the diet of the shallow-water black corals, both for branched and whip coral species. The diet is instead composed of a significant amount of mesozooplankton, which favorize some types of prey such as copepods. This specialization on zooplanktonic preys is clear when looking at the isotopic niches shown by the Bayesian ellipses. The shallow-water black corals are using different resources even if they live at the same place. For the unbranched specimens, the $\delta^{15}N$ and $\delta^{13}C$ values suggested that they feed on the same type of source, but the separated isotopic niches showed a specialization on some types of prey from the mesozooplankton rather than a radically different resource. This was also shown when using S and C isotopes. Interestingly, the niche overlaps and separations were consistent with the morphological differences in polyp sizes. *Stichopathes* sp. aff. *maldivensis*, has a separated niche from all the other whip corals. Its large polyps are not significantly different from *C. densiflora*, *C. anguina* and *C.* cf. *contorta*. but *Stichopathes* sp. aff. *maldivensis* has polyps located in a single row with a regular size. On the other hand, the three species of *Cirrhipathes* have polyps varying in size along the colony, from small to large sizes. This feature may allow them to catch different types of zooplanktonic preys at the same time, a hypothesis also supported by the large ellipse areas. Polyps of *Cirrhipathes* sp. are different in size from all the other species and this species have a nearly totally isolated trophic niche which highlights again the importance of this morphological trait in the selection of food type. The standard ellipse area confidence intervals of *Cirrhipathes* sp. are very narrow suggesting a strong prey specialization for this species.

It is known that polyp size determines the size of particles that corals can feed on (Lewis 1978, 1982). Corals with large polyps would be expected to be enriched in $\delta^{15}N$ because the ratio generally increases with particle size (Wu et al. 1999). In the present study, this is observed with the branched corals being approximately at a lower trophic position and having significantly smaller polyps than all the whip species. The niche of the branched corals *Cupressopathes* sp. is clearly separated from all the others. While the isotopic niche could not be obtained for the other branched species *M. stechowi*, the values were also separated from all the other whip corals, and from *Cupressopathes* sp. Based on the mixing models, the small size of the polyps allow them nonetheless to feed on preys coming from the zooplankton rather than from the POM. The narrow standard ellipse area of *Cupressopathes* sp. indicates again a prey specialization which is a trophic position below the types of prey captured by whip corals.

In conclusion, black coral species living within the same shallow-water bed are showing different resource usages where catching the preys seems to depend on the polyp sizes, and extensive future studies are needed to understand why large whip corals have different nitrogen isotopic compositions across the colonies.

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LITERATURE

Bo M, Di Camillo CG, Addamo AM, Valisano L, Bavestrello G (2009) Growth strategies of whip black corals (Cnidaria: Antipatharia) in the Bunaken Marine Park (Celebes Sea, Indonesia). Marine Biodiversity Records, 2.

Carlier A, Le Guilloux E, Olu K, Sarrazin J, Mastrototaro F, Taviani M, Clavier J (2009) Trophic relationships in a deep Mediterraean cold-water coral bank (Santa Maria di Leuca, Ionian Sea). Marine Ecology Progress Series 397: 125–137.

Connolly RM, Guest MA, Melville AJ, Oakes JM (2004) Sulfur stable isotopes separate producers in marine food-web analysis. Oecologia, 138(2): 161-167.

Cram JA, Chow CET, Sachdeva R, Needham DM, Parada AE, Steele JA, Fuhrman JA (2015) Seasonal and interannual variability of the marine bacterioplankton community throughout the water column over ten years. The ISME journal, 9(3): 563.

Duineveld GCA, Lavaleye MSS, Berghuis EM (2004) Particle flux and food supply to a seamount cold-water coral community (Galicia Bank, NW Spain). Marine Ecology Progress Series, 277: 13-23.

Grigg RW (1964) A contribution to the biology and ecology of the black coral, *Antipathes grandis* in Hawai'i. MS Thesis in Zoology, p. 74. Hawai'i., Honolulu.

Goenaga C (1977) Two new species of *Stichopathes* (Zoantharia; Antipatharia) with observations on aspects of their biology. Thesis in Marine Sciences., p. 101. University of Puerto Rico, Puerto Rico.

Goericke R, Fry B (1994) Variations of marine plankton $\delta^{13}C$ with latitude, temperature, and dissolved CO_2 in the world ocean. Global Biogeochemical Cycles, 8(1): 85-90.

Jackson AL, Inger R, Parnell AC, Bearhop S (2011) Comparing isotopic niche widths among and within communities: SIBER - Stable Isotope Bayesian Ellipses. J Anim Ecol 3: 595–602. https://doi.org/10.1111/j.1365-2656.2011.01806.x

Kharlamenko VI, Kiyashko SI, Imbs AB, Vyshkvartzev DI (2001) Identification of food sources of invertebrates from the seagrass Zostera marina community using carbon and sulfur stable isotope ratio and fatty acid analyses. Marine Ecology Progress Series, 220: 103-117.

Kurle CM, McWhorter JK (2017) Spatial and temporal variability within marine isoscapes: implications for interpreting stable isotope data from marine systems. Marine Ecology Progress Series,568: 31-45.

Layman CA, Arrington DA, Montaña CG, Post DM (2007) Can stable isotope ratios provide for community-wide measures of trophic structure? Ecology, 88(1): 42-48.

Lewis JB (1978) Feeding mechanisms in black corals (Antipatharia). Journal of Zoology 186: 393–396.

Lewis JB (1982) Feeding behaviour and feeding ecology of the Octocorallia (Coelenterata: Anthozoa). Journal of Zoology, 196(3): 371-384.

McCutchan JH, Lewis WM, Kendall C, McGrath CC (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen and sulfur. Oikos 102:378–390. https://doi.org/10.1034/j.1600-0706.2003.12098.x

Mittermayr A, Hansen T, Sommer U (2014) Simultaneous analysis of δ^{13}C, δ^{15}N and δ^{34}S ratios uncovers food web relationships and the trophic importance of epiphytes in an eelgrass Zostera marina community. Marine Ecology Progress Series, 497: 93-103.

Ocaña O, Opresko DM, Brito A (2006) First record of the black coral *Antipathella wollastoni* (Anthozoa: Antipatharia) outside of Macaronesian waters. Revista de la Academia Canaria de Ciencias 18, 125–138.

Parnell A, Inger R, Bearhop S, Jackson AL (2008) SIAR: Stable isotope analysis in R.

Pichon M (1972) Les peuplements à base de Scléractiniaires dans les récifs de la Baie de Tuléar (Sud-Ouest de Madagascar). In Proc. 1st Int. Coral Reef Symp., Mandapan (1969) (Vol. 5, pp. 135-154).

Pinnegar JK, Campbell N, Polunin NVC (2001) Unusual stable isotope fractionation patterns observed for fish host—parasite trophic relationships. Journal of Fish Biology, 59(3): 494-503.

R Development Core Team (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. http://www.R-project.org.

Risk MJ, Sherwood OA, Nairn R, Gibbons C (2009) Tracking the record of sewage discharge off Jeddah, Saudi Arabia, since 1950, using stable isotope records from antipatharians. Marine Ecology Progress Series 397: 219–226.

Roark EB, Guilderson TP, Dunbar RB, Fallon SJ, Mucciarone DA (2009) Extreme longevity in proteinaceous deep-sea corals. Proceedings of the National Academy of Sciences of the United States of America, 106: 5204–5208.

Rueden CT, Schindelin J, Hiner MC, DeZonia BE, Walter AE, Arena ET, Eliceiri KW (2017) ImageJ2: ImageJ for the next generation of scientific image data. BMC Bioinformatics 18: 529.

Sherwood OA, Jamieson RE, Edinger EN, Wareham VE (2008) Stable C and N isotopic composition of cold-water corals from the Newfoundland and Labrador continental slope: Examination of trophic, depth and spatial effects. Deep Sea Research Part I: Oceanographic Research Papers, 55(10): 1392-1402.

Sherwood OA, Edinger EN (2009) Ages and growth rates of some deep-sea gorgonian and antipatharian corals of Newfoundland and Labrador. Canadian Journal of Fisheries and Aquatic Sciences, 66(1): 142-152.

Syväranta J, Lensu A, Markomäki TJ, Oksanen S, Jones RI (2013) An empirical evaluation of the utility of convex hull and standard ellipse areas for assessing population niche widths from stable isotope data. PLoS One 8:1–8. https://doi.org/10.1371/journal.pone.0056094

Tazioli S, Bo M, Boyer M, Rotinsulu H, and Bavestrello G. (2007) Ecological observations of some common antipatharian corals in the marine park of Bunaken (North Sulawesi, Indonesia). Zoological Studies 46: 227–241.

Teng YC, Primeau FW, Moore JK, Lomas M.W, Martiny AC (2014) Global-scale variations of the ratios of carbon to phosphorus in exported marine organic matter. Nature Geoscience, 7(12): 895.

Thornton SF, McManus J (1994) Application of organic Carbon and Nitrogen stable isotope and C/N ratios as source indicators of organic matter provenance in Estuarine systems: evidence from the Tay Estuary, Scotland. Estuarine, Coastal and Shelf Science, 38(3): 219-233.

Wainright SC, Fry B (1994) Seasonal variation of the stable isotopic compositions of coastal marine plankton from Woods Hole, Massachusetts and Georges Bank. Estuaries, 17(3): 552-560.

Warner GF (1981) Species descriptions and ecological observations of black corals (Antipatharia) from Trinidad. Bulletin of Marine Science 31: 147–163.

Warner GF (2005) The occurrence of black corals in Jamaican reef environments, with special reference to Stichopathes lutkeni (Antipatharia: Antipathidae). Revista de biología tropical, 53: 61-66.

Williams B, Risk MJ, Ross SW, Sulak KJ (2006) Deep-water antipatharians: proxies of environmental change. Geology, 34(9): 773-776.

Williams B, Risk MJ, Ross SW, Sulak KJ (2007) Stable isotope data from deep-water antipatharians: 400-year records from the southeastern coast of the United States of America. Bulletin of Marine Science, 81(3): 437-447.

Williams B, Grottoli AG (2010) Stable nitrogen and carbon isotope ($\delta^{15}N$ and $\delta^{13}C$) variability in shallow tropical Pacific soft coral and black coral taxa and implications for paleoceanographic reconstructions. Geochimica et Cosmochimica Acta, 74: 5280–5288.

Wu J, Calvert SE, Wong CS, Whitney FA (1999) Carbon and nitrogen isotopic composition of sedimenting particulate material at Station Papa in the subarctic northeast Pacific. Deep-Sea Research II, 46: 793–2832.

SUPPLEMENTARY MATERIAL

Table 18. Modes and confidence intervals of 95% for each food sources, indicating the proportions in the diet of all the black coral species.

	C. spiralis		*S.* sp. aff. *maldivensis*		*Cupressopathes sp.*	
	Mode	CI 95	Mode	CI 95	Mode	CI 95
Copepods	21	0-37.94	48.26	35.09-58.56	38.46	19.23-51.42
Microzooplankton	24.03	0-39.74	1.07	0-13.48	1.09	0-16.77
Mesozooplankton	26.29	0-46.30	40.47	30.20-49.95	47.27	27.90-61.77
POM	23.75	0-38.57	0.26	0-3.61	0.53	0-7.48
Megalopa	15.73	0-37.20	1.31	0-17.39	1.94	0-25.31

	Cirrhipathes sp.		*Cirrhipathes anguina*		*Cirrhipathes* cf. *contorta*	
	Mode	CI 95	Mode	CI 95	Mode	CI 95
Copepods	24.9	15.10-31.95	1.48	0-23.02	2.26	0-32.82
Microzooplankton	5.32	0-16.92	2.62	0-31.71	2.62	0-34.85
Mesozooplankton	62.76	55.27-68.90	64.27	33.49-83.47	75.77	19.55-90.06
POM	0.23	0-3.02	11.15	0-25.83	1.45	0-27.37
Megalopa	1	0-13.83	1.39	0-20.48	1.75	0-27.95

	M. stechowi		*C. densiflora*	
	Mode	CI 95	Mode	CI 95
Copepods	20.3	0-35.92	0.1	0-13.95
Microzooplankton	23.29	0-38.85	1.76	0-21.79
Mesozooplankton	26.05	0-44.88	78.05	59.86-91.34
POM	23.32	0-38.50	1.45	0-15.09
Megalopa	22.74	0-36.42	0.97	0-12.86

Chapitre 8

Assessing trophic relationships between shallow-water black corals (Hexacorallia: Antipatharia) and their symbionts using stable isotopes

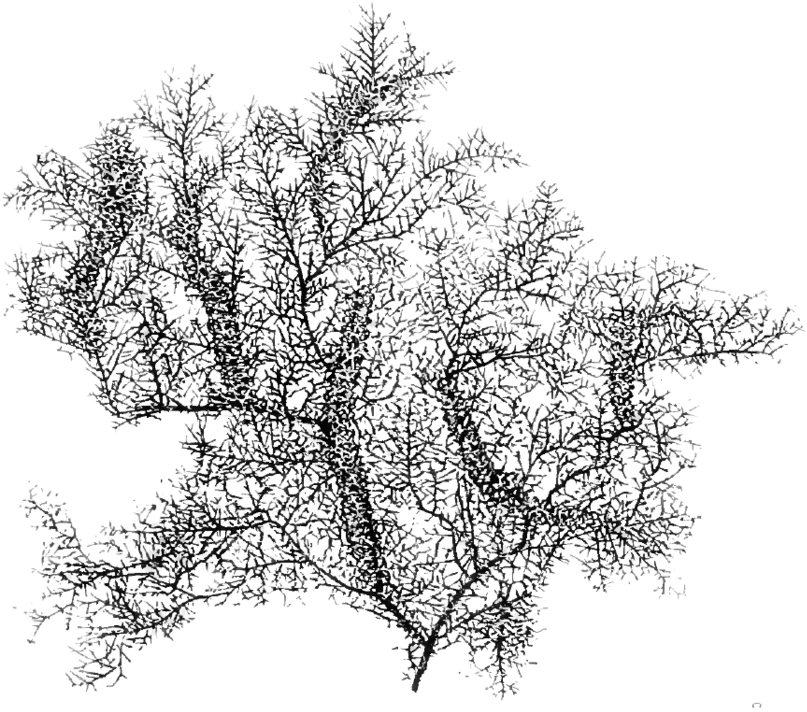

Illustration of Tylopathes crispa with tunnel-like skeletal modifications from an annelid symbiont by Brook, 1889

Assessing trophic relationships between shallow-water black corals (Hexacorallia: Antipatharia) and their symbionts using stable isotopes

ABSTRACT

Shallow-water antipatharians or black corals are shelters for many symbiotic species which spend their whole adult life with them and/or use them to have access to food. We determine the trophic relationships between the four most common macrosymbionts observed on *Cirripathes* and *Stichopathes* in the southwest of Madagascar. The four symbionts are the myzostomid *Eenymeenymyzostoma nigrocorallium,* the gobiid fish *Bryaninops yongei*, and two palaemonid shrimps, *Pontonides unciger* and an undetermined species of *Periclimenes* sp. The first is an endosymbiont living in the digestive tract, the others are ectosymbionts. The analyses show that (*i*) none of the symbionts directly feed on the host, (*ii*) antipatharians feed mainly on nocturnal plankton while the symbionts feed on diurnal plankton, (iii) the myzostomid has the narrowest trophic niche and *Periclimenes* sp. the largest, (iv) the two shrimps have distinct trophic niches and feed at lower trophic level than other symbionts. Concerning the myzostomids, they had the same $\delta^{13}C$ values but had significantly higher $\delta^{15}N$ values than the hosts. TEFs (Trophic Enrichment Factors) recorded were $\Delta^{13}C = 0.28 \pm 0.25$ ‰ and $\Delta^{15}N = 0.51 \pm 0.37$ ‰, but they were not high enough to explain a predator-prey relationship. These worms rely on the coral diet but may also ingest host fluids explaining the slight enrichment in heavier nitrogen isotopes. On the other hand, the ectosymbionts use the coral as a pathway to food from the midwater: they feed on the plankton passing nearby the black corals, but a robber behaviour may not be excluded.

This chapter is a draft of the manuscript that will be submitted with the following co-authors and order:

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INTRODUCTION

Antipatharians, also called black corals, are found in diverse habitats, from shallow waters to abyssal plains, and they house a lot of organisms, such as worms, arthropods, cnidarians, bryozoans, molluscs, echinoderms and fishes (Soule and Soule 1972; Wirtz and d'Udekem-d'Acoz 2001; Vega et al. 2002; Kirkendale and Messing 2003; Boland and Parrish 2005; Herler 2007; Love et al. 2007; Molodtsova and Budaeva 2007). These associated organisms can take advantages while hiding on the surface of the living or dead branches and/or use them as nursery or feeding areas (Buhl-Mortensen and Mortensen 2004; Wagner et al. 2012).

Amongst the black coral symbionts, decapods and gobiid fishes are commonly observed on their surface (See Wagner et al. 2012 for a detailed exhaustive list of the symbionts). Whipcoral gobies from the genus *Bryaninops* are known to live in male-female pairs of small groups (Larson 1985; Munday et al. 2002) on their antipatharian or gorgonian hosts. They never leave their host's branches and their social organisation and reproductive biology are well known (Munday et al. 2002). Shrimps are also commonly observed on black corals as well as in many other sessile organisms. Particularly, members of the Pontoniidae family are well represented (De Ridder and Holthuis 1979; Criales 1980; Marin 2007) and they obtain from black corals a protection against predators (Wirtz and d'Udekem d'Acoz 2001). They are generally found living in heterosexual pairs, even if larger groups may occur (Bruce 2005; Marin 2007).

Recently, the myzostomids *Eenymeenymyzostoma nigrocorallium* have been found into the digestive tract of black corals (Terrana and Eeckhaut 2017, see chapter 7). In general, myzostomid worms are associated with crinoids (see Eeckhaut and Lanterbecq 2005 for a review), and *E. nigrocorallium* is one ofthe first described myzostomids living on another phylum.

The characterization of symbioses involving black corals as hosts remains elusive, as it is generally based on direct observations. Also, boundaries between the different types of symbioses (*e.g.*, parasitism, commensalism or mutualism) are not rigid and interactions are much more illustrated as a large continuum (Parmentier and Michel 2013). Black coral symbionts can surely find protection and probably feeding benefits while the association, at least through general and gross observations, seems neutral for the corals. Feeding is one of the symbiont's benefit during a symbiotic relation but the ways the symbionts acquire food are varied. The symbionts in a mutualistic relationship may remove the encrusting fauna off the coral surface. In commensalism, the coral colony may act as an intermediary enabling symbionts to catch food from the water column (Davis and Cohen 1968; Sih and Chouw 2009). Finally, if they are parasites, associated organisms may directly feed on the host tissues or steal food directly from the coral polyps.

Observations made directly on black coral symbiont feeding habits are scare and have the disadvantage of giving a snapshot and not a global view of their feeding behaviour. Carbon and nitrogen stable isotopes at the opposite can give a global view of the feeding behaviour of organisms and have been used in the past as proxies to characterize symbiotic associations among marine organisms (Caulier et al. 2014; Terrana et al. 2016). Stable isotope ratios of carbon and nitrogen are powerful trophic markers, with the isotope composition of the consumer tissues being directly linked to their diet (DeNiro and Epstein 1981). These values can be used to determine food source and trophic levels (Post 2002), and thus assess the trophic relationships between symbiotic organisms. Proportions of the different food sources are reflected in the tissues of the predators, with enrichment in the heavier isotope (Mccutchan et al. 2003). Parasites are considered by some authors as micro-predators (Raffel et al. 2008), that would be expected to be enriched on heavier isotopes (Doucet et al. 1999). However, studies focusing on specific host-parasites relationships showed that they could be enriched and/or depleted in heavier isotopes with respect to their hosts (Iken et al. 2001; Deudero et al. 2002; Parmentier and Das 2004; Power and Klein 2004), and particularly for nematodes and cestodes which are known to be significantly depleted (Iken et al. 2001; Pinnegar et al. 2001).

To estimate the diet assimilated by consumers, several Bayesian mixing models have been developed (reviewed by Phillips et al. 2014). In addition, it has been proposed that the variability of isotopic composition of a population or a species (*i.e.*, its isotopic niche) can be used as a proxy to assess the trophic niche of this population or species, and/or the degree of individual specialization in the population (Bearhop et al. 2004; Jackson et al. 2011). This concept of isotopic niche has also been developed through diverse numerical methods (Jackson et al. 2011). Comparison of isotopic niches allows to compare diet of species (variability, overlap).

The aim of the present study is to determine the trophic relationships between the four most common macrosymbionts and their whip black corals host (*Cirripathes* and *Stichopathes* genera) in the southwest of Madagascar. The four symbionts are the gobiid fish *Bryaninops yongei* (Davis and Cohen 1969), the myzostomid *Eenymeenymyzostoma nigrocorallium* Terrana & Eeckhaut 2017, and two palaemonid shrimps *Pontonides unciger* Calman, 1939 and *Periclimenes* sp. All these symbionts are somehow linked to the trophic web involving their host as they are using the colony as a pathway to feed on the plankton or as a direct food source.

MATERIAL & METHODS

Sampling

All the samples were collected in November and December 2015 at the north extremity of the Great Reef of Toliara in the southwest of Madagascar (23.36°S, 43.66°E) at a maximum depth of 25 m. The fishes *Bryaninops yongei* (*n* = 14), the shrimps *Pontonides unciger* (*n* = 11) and *Periclimenes* sp. (*n* = 7) were collected at the surface of whip black corals and the myzostomid *Eenymeenymyzostoma nigrocorallium* (*n* = 83) were found in the digestive tract after dissection of 19 black corals The symbionts were all collected from 4 species of whip corals: *Cirrhipathes* cf. *anguina*, *Cirrhipathes* cf. *densiflora*, *Cirrhipathes* sp. and *Stichopathes* sp., the last two being both undescribed species. The shrimp *Periclimenes* sp. was compared to all the shrimp species associated to antipatharians listed on the exhaustive table of Wagner et al. (2012) and all the symbiotic shrimps recorded on Madagascar by Bruce (1978). However, the present specimens did not match any species description as their rostrum was smooth, very sharp and slightly curved downwards.

Surface mesozooplankton was collected during the night and the day using a 100µm net for 15 min (*n* = 10 for the night, *n* = 10 for the day). Zoobenthos (nocturnal copepods) values were used from Frédérich et al. (2017) as they were from the same collection sites at the same period.

The head and the digestive system of the fishes were removed. All sample were dried at 60 °C for 72 hours. They were ground into a homogenous powder using a mortar and a pestle. Samples of shrimps were exposed to fuming HCl (37 %) during 24 to 48h in order to remove the cuticle carbonates and then re-dried.

Stable isotope analysis

Each individual was analysed using a mass spectrometer (Isoprime 100, Isoprime, UK) fitted with an elemental analyser (VarioMicro, Elementar, Germany) for combustion and automated analysis. Carbon and nitrogen isotope ratios were expressed as δ values (‰) using ammonium sulfate IAEA-N1 ($\delta^{15}N$ = 0.4 ± 0.2 ‰; mean ± SD) and sucrose IAEA-C6 ($\delta^{13}C$ = -10.8 ± 0.5 ‰; mean ± SD) as certified reference materials for nitrogen and carbon respectively. These references are both calibrated against the international isotopic references Vienna Pee Dee Belemnite for carbon and atmospheric air for nitrogen. Standard deviations on multibatch replicate measurements of one black coral sample were ± 0.2 ‰ for carbon and ± 0.1 ‰ for nitrogen.

Data treatment and statistics

Means of the isotope ratio values were plotted with their standard deviations. Using each individual measurement, the SIBER package v.2.0 (Stable Isotope Bayesian Ellipses in R, Jackson et al. 2011) for R (R Development Core Team 2015) was used to assess the isotopic niche parameters. It generates bivariate standard ellipses whose areas (SEA) represent the core isotopic niche, as it does not encompass outlier individuals in the isotopic space. When dealing with sample sizes inferior to 30 individuals (Syväranta et al. 2013), a correction for small sample size can be used (SEA_C). It is an effective way to compare small and/or unbalanced samples, and it tends to converge when sample size increases (Jackson et al. 2011). Areas of the ellipses associated to each population were also estimated using Bayesian modelling (SEA_B; 10^5 iterations). SEA_B involves the use of an iterative model based on Bayesian inference to estimate the covariance matrix from the data. SEA_B takes into account variability in the data (caused by both natural variations and analytical error) more efficiently and provides a distribution of solutions rather than a single value, therefore allowing error estimates as well as pairwise comparisons. Model solutions were presented using credibility intervals of probability density function distributions. Direct pairwise comparisons were performed and were considered meaningful when probability of occurrence (i.e. number of model solutions where a given situation was found) exceeded 95 %.

The stable isotope mixing model SIAR (Parnell et al. 2008) was used to estimate the relative contribution of the different food sources collected to the diet of the 4 symbionts. Isotopic compositions of the 4 symbionts species and means and standard deviations of the different potential food sources (i.e., zooplankton day and night, zoobenthos and black coral tissues) were fitted in R. A concentration-dependent mixing model was used after using a Kruskal-Wallis test on C:N ratios of each food source. Trophic enrichment factors

(TEF) for marine organisms obtained from McCutchan et al. (2003) were implemented in the mixing model. Solution are presented using credibility intervals of probability density function distributions.

RESULTS

$\delta^{13}C$ values ranged from -20.0 to -18.4 ‰ for *Eenymeenymyzostoma nigrocorallium*, -20.2 to -18.8 ‰ for the black corals, -19.8 to -18.9 ‰ for *Pontonides unciger*., -20.5 to -18.7 ‰ for *Bryaninops yongei*, -19.5 to -18.6 ‰ for *Periclimenes* sp., -19.1 to -16.4 ‰ for the night plankton, and -22.4 to -17.3 ‰ for the day plankton (Fig. 67A). $\delta^{15}N$ values ranged from 7.9 to 10.1 ‰ for *E. nigrocorallium*, 7.8 to 9.6 ‰ for the black corals, 7.0 to 8.1 ‰ for *P. unciger*, 8.2 to 9.5 ‰ for *B. yongei*, 7.4 to 8.6 ‰ for *Periclimenes* sp., 5.7 to 7.0 ‰ for the night plankton, and 4.1 to 5.6 ‰ for the day plankton (Fig. 67A).

$\delta^{15}N$ values differed significantly between organism (Fig. 67A, ANOVA, $F = 349.16$, $df = 8$, $P < 0.001$) except between the fishes and the myzostomids (Tukey, $P > 0.99$), between the two shrimps (Tukey, $P > 0.13$) and between the black corals and *Periclimenes sp.* (Tukey, $P > 0.08$). $\delta^{13}C$ values showed also some significant difference between species (Fig. 67A, ANOVA, $F = 16.636$, $df = 8$, $P < 0.001$). Black corals and all the symbionts did not have significant differences in $\delta^{13}C$ values (Tukey, $P > 0.05$). Mesozooplankton collected during the day had significant differences in $\delta^{13}C$ values than black corals and the symbionts, except for *Periclimenes* sp. (Tukey, $P = 0.77$). Mesozooplankton collected during the night had significant different $\delta^{13}C$ values than the black corals and the symbionts, except for *Periclimenes* sp. and *Bryaninops yongei* (Tukey, $P = 0.947$ and $P = 0.056$ respectively). Zoobenthos differ always very significantly ($p < 0.001$) from all symbionts and corals.

E. nigrocorallium individuals were enriched in $\delta^{13}C$ in comparison to the black coral hosts (Fig. 68, mean $\Delta^{13}C = 0.28 \pm 0.25$ ‰) and in $\delta^{15}N$ (Fig. 68, mean $\Delta^{15}N = 0.51 \pm 0.37$ ‰). Isotopic niche area of corals and their symbionts ranged from 0.21 ‰² to 0.62 ‰² (SEA_C). Bivariate standard ellipses of the corals, the fishes and the myzostomids were overlapping, as well as both shrimps (Fig. 67B). Ellipses of the shrimps did not overlap

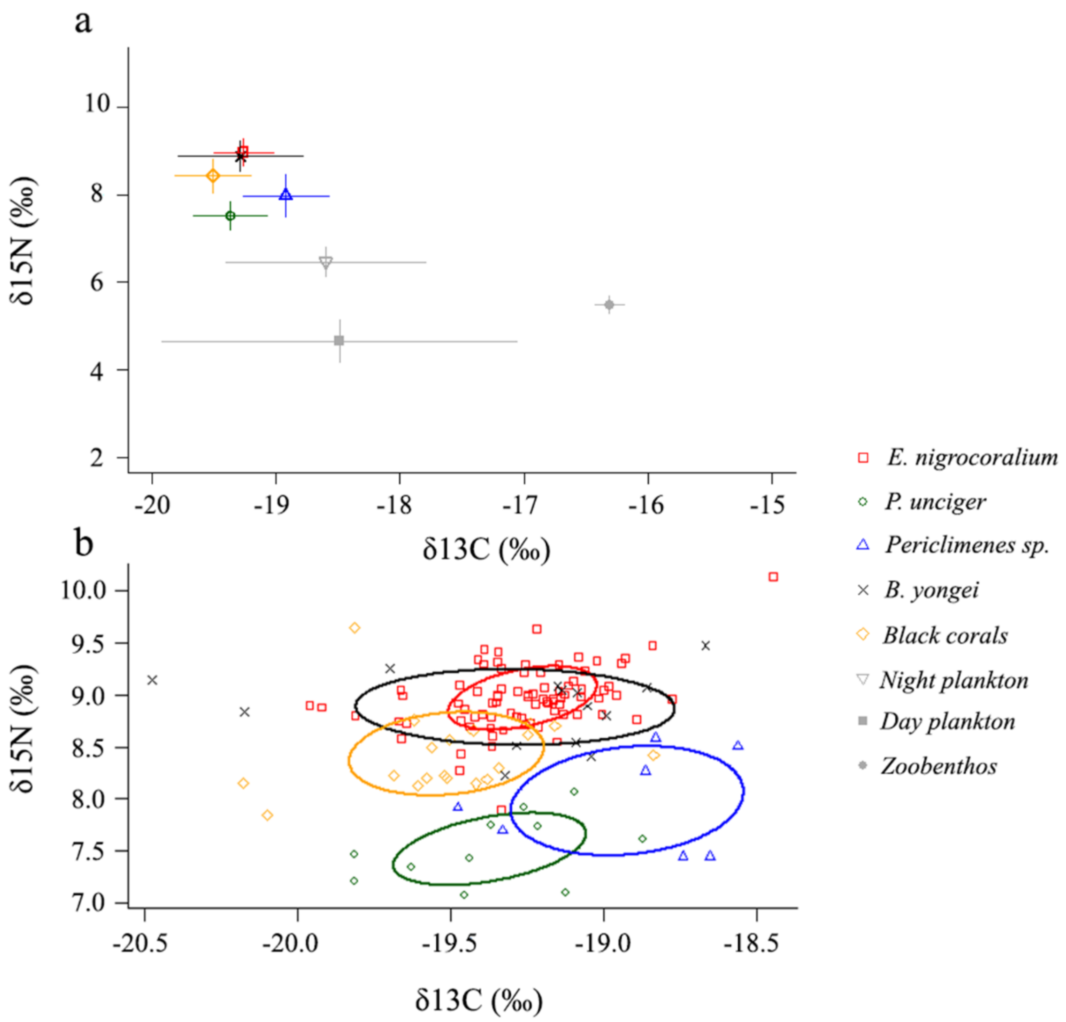

Figure 67. (A) Mean values (± SD) of δ^{13}C (‰) and δ^{15}N (‰) of the symbionts and potential food sources. (B) Isotopic niches of the symbionts and the black coral hosts represented by the bivariate standard ellipses of each group.

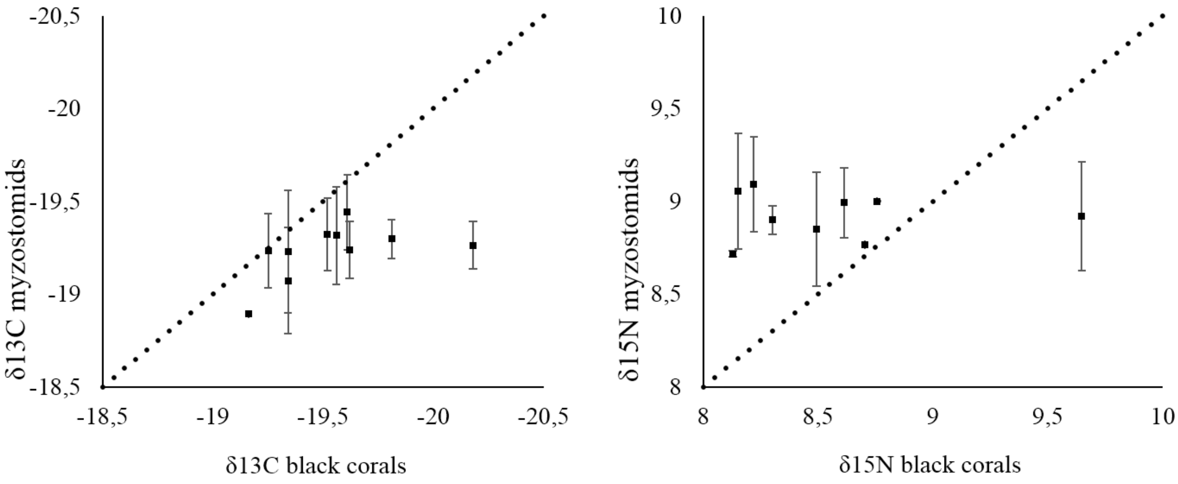

Figure 68. Stable carbon and nitrogen isotope data for *Eenymeenymyzostoma nigrocorallium* vs. their respective black coral hosts. The dashed line represents a 1:1 linear relationships. Standard deviations were calculated depending on the number of worms found on each hosts, which was variable.

any other organism. The overlap between *B. yongei* (SEA_C = 0.59 ‰²) and the black corals (SEA_C = 0.39 ‰²) was 0.11 ‰², representing 28.20 % of the ellipse area of the corals and 18.64% of the fishes. The overlap between the black corals and *E. nigrocorallium* (SEA_C = 0.21 ‰²) was 0.03 ‰², representing 7.69 % of the ellipse area of the corals and 14.28 % of the myzostomids. The overlap between the myzostomids and the fishes was 0.20 ‰², representing 95.23 % of the ellipse area of the myzostomids and 33.89 % of the fishes. The overlap between *P. unciger* (SEA_C = 0.31 ‰²) and *Periclimenes* sp. (SEA_C = 0.62 ‰²) was 0.06 ‰², representing 19.35 % of the ellipse area of *P. unciger* and 9.83 % of *Periclimenes* sp.

The niche widths of the different samples were variable as shown with pairwise comparisons of the model-estimated ellipse areas (SEA_B) and showed that the myzostomids *E.nigrocorallium* had the smallest isotopic niche width (Fig. 69, Table 19).

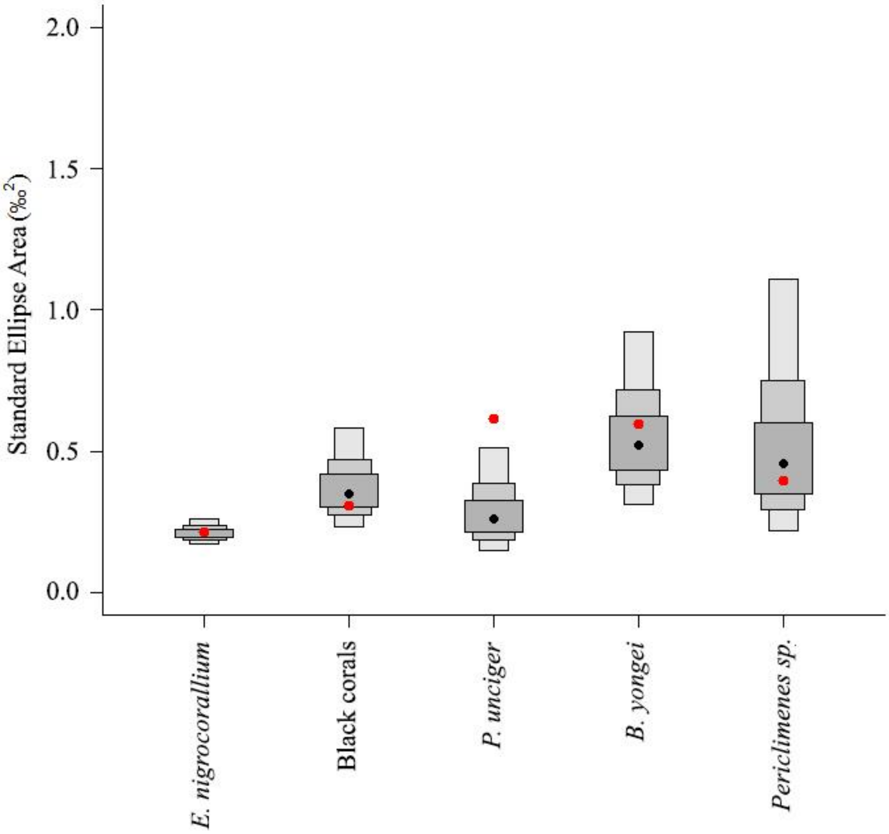

Figure 69. Estimates of standard ellipse area (SEA) for each species. The boxplots represent posterior probability distributions of model estimations of the standard ellipse areas (SEA_B). The boxes are the 50 %, 75 % and 95 % credibility interval. The mode of each distribution is shown by a black dot, while the red dot represents the standard ellipse area calculated using correction for small sample size (SEA_C).

Table 19. Pairwise comparisons of standard ellipses areas estimated using Bayesian modelling (SEA_B). The relative probabilities (%) that the standard ellipse of the group listed as line is smaller than the standard ellipse of the group listed as column are shown. Probabilities are based on 10^5 iterations. Values with an asterisk are probabilities higher than 95 %.

	B. yongei	Black corals	*E.nigrocorallium*	*Periclimenes*	*P. unciger*
B. yongei	-	0.129	<0.001	0.469	0.057
Black corals	0.871	-	0.007	0.792	0.251
E.nigrocorallium	0.999*	0.992*	-	0.99*	0.848
Periclimenes	0.53	0.207	0.003	-	0.098
P. unciger	0.942	0.749	0.151	0.901	-

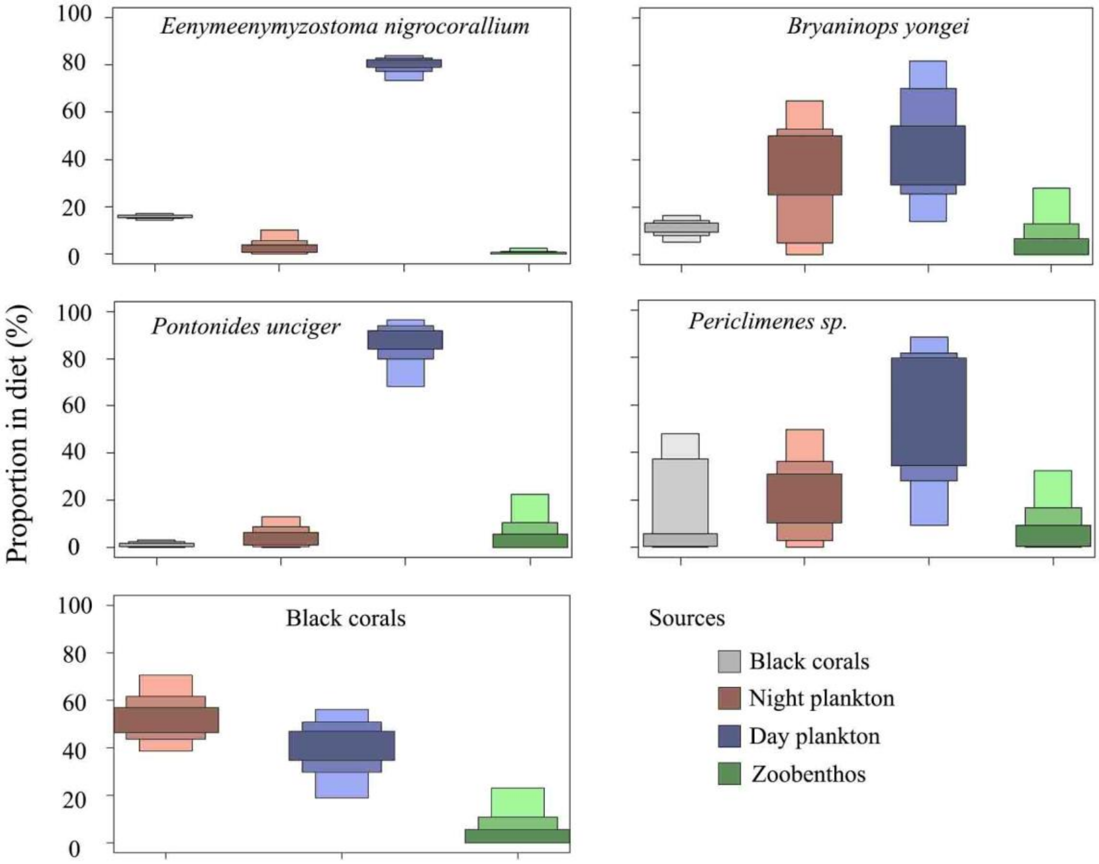

Figure 70. Boxplots of the relative contribution of the different potential food sources to the diet of the symbionts and the black corals. Credibility intervals (CI): CI_{50} = dark colour, CI_{75} = medium colour, CI_{95} = light colour. Grey: black corals; red: night plankton; blue: day plankton; green: zoobenthos.

The C:N ratios of each food source were significantly different ($X^2 = 30.458$, $p < 0.001$) and thus a concentration-dependent mixing model was used in SIAR. The estimated relative contribution of each potential food sources was varying between the symbionts as modelled by the mixing model (Fig. 70) using TEFs from McCutchan et al. (2003). Black corals were relatively low in proportions in the symbionts' diets (Table 20). They had the higher proportion in the myzostomids (14.49–17.17 % for 95 % credibility intervals) and the lowest in the *Pontonides* (0–3.40 % for 95 % credibility intervals). Mesozooplankton collected during the day was clearly the most abundant food source for all the symbionts (Table 20) with a range of 46–85 % of the diets. In contrary, zoobenthos was the less represented in the diet of all the symbionts, with an almost inexistent contribution in the myzostomid diet (range 0.01 %–9.21 % for all the symbionts). Night plankton was the most abundant food source for black corals as consumers with 38.54–70.00 % while day plankton was comprised between 19.33–56.22 %. Zoobenthos was the less represented in the black corals' diet with a range of 0-23.19 % (Fig. 70).

Table 20. Contribution of each tested food source to the diet of the four symbionts (%) as modelled by SIAR. The credibility intervals for 95 % are given (CI 95).

	E. nigrocorallium		*P. unciger*		*Periclimenes sp.*	
	Mode	CI 95	Mode	CI 95	Mode	CI 95
Black corals	15.81	14.49–17.17	0.98	0–3.40	2.77	0–47.81
Night Plankton	2.66	0–10.29	4.69	0–12.69	20.64	0–49.47
Day Plankton	79.75	73.26–83.77	87.54	70.37–96.40	72.69	9.68–89.09
Zoobenthos	0.2	0–2.37	1.88	0–22.20	2.63	0–32.33

	B. yongei		Black corals	
	Mode	CI 95	Mode	CI 95
Black corals	11.96	5.46–16.56	-	-
Night Plankton	39.2	0–63.73	52.66	38.23–70.49
Day Plankton	42.03	15.29–82.35	41.65	18.13–56.02
Zoobenthos	1.84	0–27.00	1.58	0–23.14

DISCUSSION

Our results firstly showed that black corals and their symbionts share the same $\delta^{13}C$ values which is consistent with organisms relying on plankton resources (Frédérich et al. 2017). Our mixing model indicated that none of the symbiont feeds on the surrounding zoobenthos but they are rather dependent on the water column food. However, there is an isotopic niche segregation between the hosts and the symbionts showed both by the results of the mixing models and by the small (or absent) overlapping between isotopic niches. Black corals are known to be suspension feeders that feed mainly at night (Goenaga 1977; Warner 1981; Goldberg and Taylor 1989) which is confirmed by the present results. Indeed, our mixing model indicate a major contribution of night zooplankton to black coral diet. At the contrary, the four symbionts rely globally on a diurnal planktonic source. This important finding indicate that host and symbiont trophic niches are segregated which could be a way to alleviate competition for trophic resource.

The four symbionts did not occupy exactly the same trophic level and did not share exactly the same isotopic niches. This indicated that trophic niches are, at least partly, partitioned between the different species living in association with black corals. For most of them, niche overlaps are relatively small considering they live on the same host. The two shrimps and the fishes showed the largest isotopic niches indicating a more diversified diet than their host and the myzostomids. Moreover, the two shrimps display the lowest trophic level, lower than their host suggesting that their diet relies partly on phytoplankton or algae growing on their host. In addition, night plankton is also a part of the diets of pontoniid shrimps and fishes which could therefore have a more diversified diet than their hosts.

The fish *B. yongei* certainly take advantage of the height of the whip corals to have a direct and easy access to zooplankton. This is supported by the morphology of *Bryaninops* fishes which have large mouth and specific teeth linked to a carnivorous diet (Davis and Cohen 1968; Munday et al. 2002). Also, Larson (1985) and Davis and Cohen (1968) observed these fishes darting off the coral hosts to prey on small zooplankton passing nearby the corals.

The four investigated symbionts do not feed on black coral tissues: they use their hosts as shelters and feed opportunistically on plankton and/or small organism associated to their host. In contrary to their hosts or the three other symbiont species, the myzostomid *E. nigrocorallium* has the lowest isotopic niche area. This is consistent with the localization of the parasite in the digestive tracts of its host (Terrana and Eeckhaut 2017) where the worms are totally dependent of the host diet while fishes and shrimps have an access all the food around the coral colony.

Parasites are sometimes considered as micropredators (Raffel et al. 2008) and are expected to be enriched in N or C with respect to their hosts, as it might be the case for a predator feeding on its prey (Doucett et al. 1999). However, some studies have shown that both enriched and depleted isotopic compositions occur in parasite organisms (Boag et al. 1998, Neilson and Brown 1999; Baillon et al. 2014). For instance, fish endoparasitic nematodes and cestodes are often depleted in $\delta^{15}N$ compared to their host (Iken et al. 2001; Pinnegar et al. 2001), while ectoparasites stable isotopes ratios may depend on their feeding strategy and/or life-history stage (Iken et al. 2001; Pinnegar et al. 2001). Isotopic compositions depleted in heavier isotopes are generally related to parasite that are devoted of digestive tracts. Isotopic composition enriched in heavier isotopes are generally related to parasite eating host tissues. Host-parasite trophic studies have already focused on cnidarian hosts of endoparasites (Baillon et al. 2014; Fleming et al. 2014) or polychaetes endoparasites (Becker et al. 2013), and all of them come to different trophic enrichment factors, which ranged from -2.0 ‰ to +1.08 ‰ for $\delta^{13}C$ and -1.0 ‰ to +2.62 ‰ for $\delta^{15}N$. In the present work, myzostomids were always enriched in both $\delta^{13}C$ and $\delta^{15}N$ with respect to the black corals but enrichment factors are low (∆13C = 0.28 ± 0.25 ‰ and ∆15N = 0.51 ± 0.37 ‰) and thus are not corresponding to parasite devoted of digestive tracts nor to the expected increase in a trophic level for predators (McCutchan et al. 2003). Accordingly, *E. nigrocorallium* certainly divert the coral food in the host digestive tract and process it with their own digestive system which is fully developped. Our results suggest that they feed during the day as the participation of nocturnal plankton is very low. When black corals inhabited by myzostomids feed during night time, their food intake will more likely be higher than during the day, as it will not be stolen by the worms. The TEF observed here might correspond to the same food eaten than the corals, but the slight enrichment in $\delta^{15}N$ and $\delta^{13}C$ may be due to a limited consumption of the host digestive fluids or mucus. If there is no data about the composition of the black corals mucus, scleractinian coral mucus is principally composed of glycoproteins and lipids (Meikle et al. 1988).

Feeding strategy is likely to be very similar among the Myzostomida. The endosymbiotic genera *Asteriomyzostomum* and *Notopharyngoides* living in the digestive lumen of their hosts (asteroids and crinoids, respectively) are supposed to steal particles ingested by their host in the same way as *E. nigrocorallium* (Eeckhaut and Lanterbecq 2005). Myzostomida also include ectosymbiont species that move freely at the surface of crinoids or induce cysts on their hosts. They are known to feed on particles diverted thanks to their introvert (Eeckhaut and Lanterbecq 2005). In a previous study, $\delta^{15}N$ and $\delta^{13}C$ values of the ectosymbiont *Myzostoma fissum* and their crinoid host *L. palmata* indicated that the host and the symbiont share the same food source (Caulier et al. 2014), both organsims being suspension-feeders.

In conclusion, the symbiotic assemblage showed isotopic niche segregations with the associated fauna mainly feeding on different food sources than the black coral hosts. Bearing all these symbionts may also be favorable for the black coral hosts. It is well known that some symbiotic organisms are defending their host against predators (Glynn 1987;

Pratchett 2001) or removing sediment from their surface (Grange 1991). These examples point out the importance to bring together behavioral observations with stable isotopes studies to unveil the nature of the associations between several organisms.

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LITERATURE

Baillon S, Hamel JF, Mercier A (2014) Diversity, distribution and nature of faunal associations with deep-sea pennatulacean corals in the Northwest Atlantic. PloS one 9(11):e111519. https://doi.org/10.1371/journal.pone.0111519

Bearhop S, Adams CE, Waldron S, Fuller RA, MacLeod H. (2004) Determining trophic niche width: a novel approach using stable isotope analysis. J Anim Ecol 73(5):1007–1012. https://doi.org/10.1111/j.0021-8790.2004.00861.x

Becker EL, Cordes EE, Macko SA, Lee RW, Fisher CR. (2013) Using stable isotope compositions of animal tissues to infer trophic interactions in Gulf of Mexico lower slope seep communities. PloS one 8(12):e74459. https://doi.org/10.1371/journal.pone.0074459

Boag B, Neilson R, Robinson D, Scrimgeour CM, Handley LL (1998) Wild rabbit host and some parasites show trophic-level relationships for δ13C and δ15N: a first report. Isot Environ Healt S 34(1-2):81-85. https://doi.org/10.1080/10256019708036335

Boland RC, Parrish FA (2005) A description of fish assemblages in the black coral beds off Lahaina, Maui, Hawai'i. Pac Sci 59:411–420. https://doi.org/10.1353/psc.2005.0032

Bruce AJ (1978) A report on a collection of pontoniine shrimps from Madagascar and adjacent seas. Zool J Linn Soc 62:205-290. https://doi.org/10.1111/j.1096-3642.1978.tb01039.x

Bruce AJ (2005). Pontoniine shrimps from the 2003 NORFANZ Expedition, 10 May–16 June (Crustacea: Decapoda:Palaemonidae). Zootaxa 981:1–20.

https://doi.org/10.11646/zootaxa.981.1.1

Buhl-Mortensen L, Mortensen PB (2004) Symbiosis in deep-water corals. Symbiosis 37:33–61

Calman WT (1939) Crustacea: Caridea. Scientific Reports of the John Murray Expedition, 6:183–224

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. https://doi.org/10.1007/s13199-014-0274-2

Criales MM (1980) Commensal caridean shrimps of Octocorallia and Antipatharia in Curaçao and Bonaire with description of a new species of *Neopontonides*. Studies on the Fauna of Curaçao and other Caribbean Islands 61(1):68–85

Davis WP, Cohen DM (1968) A gobiid fish and a palaemonid shrimp living on an antipatharian sea whip in the tropical pacific. B Mar Sci 18(4):749-761

DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Ac 45:341–351. https://doi.org/10.1016/0016-7037(81)90244-1

De Ridder C, Holthuis LB (1979) *Pontonides sympathes*, a new species of commensal shrimp (Crustacea, Decapoda, Pontoniinae) from Antipatharia in the Galapagos Islands. Zool Medede 54(7):101–110

Deudero S, Pinnegar JK, Polunin NV (2002) Insights into fish host-parasite trophic relationships revealed by stable isotope analysis. Dis Aquat Organ 52(1):77–86. https://doi.org/10.3354/dao052077

Doucett RR, Giberson DJ, Power G (1999) Parasitic association of *Nanocladius* (Diptera:Chironomidae) and *Pteronarcys biloba* (Plecoptera:Pteronarcyidae): insights from stable-isotope analysis. J North Am Benthol Soc, 18:514–523

Eeckhaut I, Lanterbecq D (2005) Myzostomida: a review of the phylogeny and ultrastructure. Hydrobiologia 535(1):253–275. https://doi.org/10.1007/s10750-004-5636-y

Fleming NEC, Harrod C, Griffin DC, Newton J, Houghton JDR (2014) Scyphozoan jellyfish provide short-term reproductive habitat for hyperiid amphipods in a temperate near-shore environment. Mar Ecol Prog Ser 510:229-240. https://doi.org/10.3354/meps10896

Frédérich B, Michel LN, Zaeytydt E, Bolaya RL, Lavitra T, Parmentier E, Lepoint G (2017) Comparative feeding ecology of cardinalfishes (Apogonidae) at Toliara Reef, Madagascar. Zool Stud 56:10. https://doi.org/10.6620/ZS.2017.56-10

Glynn PW (1987) Some ecological consequences of coral-crustacean guard mutualisms in the Indian and Pacific Oceans. Symbiosis 4(1-3):301-323.

Goenaga C (1977) Two new species of *Stichopathes* (Zoantharia; Antipatharia) with observations on aspects of their biology. Unpublished MS Thesis, University of Puerto Rico, Puerto Rico.

Goldberg WM, Taylor GT (1989) Cellular structure and ultrastructure of the black coral *Antipathes aperta*: 2. The gastrodermis and its collar cells. J Morphol 202(2):255-269. https://doi.org/10.1002/jmor.1052020211

Grange KR (1991) Mutualism between the antipatharian *Antipathes fiordensis* and the ophiuroid *Astrobrachion constrictum* in New Zealand fjords. Hydrobiologia 216(1):297-303. https://doi.org/10.1007/bf00026478

Herler J (2007) Microhabitats and ecomorphology of coral- and coral rock-associated gobiid fish (Teleostei: Gobiidae) in the northern Red Sea. Mar Ecol 28:82–94. https://doi.org/10.1111/j.1439-0485.2007.00165.x

Humes AG (1992) Copepoda associated with the thorny coral *Antipathes* (Antipatharia) in the Indo-Pacific. J Nat Hist 26:709–744. https://doi.org/10.1080/00222939200770461

Iken K, Brey T, Wand U, Voigt J, Junghans P (2001) Food web structure of the benthic community at the Porcupine Abyssal Plain (NE Atlantic): a stable isotope analysis. Prog Oceanogr 50(1-4):383–405. https://doi.org/10.1016/s0079-6611(01)00062-3

Jackson AL, Inger R, Parnell AC, Bearhop S (2011) Comparing isotopic niche widths among and within communities: SIBER - Stable Isotope Bayesian Ellipses. J Anim Ecol 3:595–602. https://doi.org/10.1111/j.1365-2656.2011.01806.x

Kim IH, Je JG (2000) Two new species of Asterocheridae (Copepoda, Siphonostomatoida) from Korea. Korean J Biol Sci 4:304-314. https://doi.org/10.1080/12265071.2000.9647561

Kirkendale L, Messing CG (2003) An annotated checklist and key to the Crinoidea of Guam and the Commonwealth of the Northern Marianas Islands. Micronesica 3536:523–546

Larson H (1985) A revision of the gobiid genus *Bryaninops* (Pisces), with a description of six new species. Beagle: Records of the Museums and Art Galleries of the Northern Territory, The, 2, 57

Layman CA, Allgerier JE (2012) Characterizing trophic ecology of generalist consumers: a case study of the invasive lionfish in the Bahamas. Mar Ecol Prog Ser 448:131–141. https://doi.org/10.3354/meps09511

Love MS, Yoklavich MM, Black BA, Andrews AH (2007) Age of black coral (*Antipathes dendrochristos*) colonies, with notes on associated invertebrate species. B Mar Sci 80(2):391–399

Marin I (2007) The coral-associated shrimp genus *Pontonides* (Caridea, Palaemonidae, Pontoniinae) in Nhatrang Bay, Vietnam, with description of two new species. Zootaxa 1635:1-21. https://doi.org/10.5281/zenodo.179514

McCutchan JH, Lewis WM, Kendall C, McGrath CC (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen and sulfur. Oikos 102:378–390. https://doi.org/10.1034/j.1600-0706.2003.12098.x

Meikle P, Richards GN, Yellowlees D (1988) Structural investigations on the mucus from six species of coral. Mar Biol 99(2):187-193. https://doi.org/10.1007/bf00391980

Molodtsova TN, Budaeva N (2007) Modifications of corallum morphology in black corals as an effect of associated fauna. B Mar Sci 81:469–479

Munday PL, Pierce SJ, Jones GP, Larson HK (2002) Habitat use, social organization and reproductive biology of the seawhip goby, *Bryaninops yongei*. Mar Freshwater Res 53:769–775. https://doi.org/10.1071/mf01205

Neilson R, Brown DJ (1999) Feeding on different host plants alters the natural abundances of $\delta^{13}C$ and $\delta\ ^{15}N$ in Longidoridae (Nemata). J Nematol 31(1):20

Newsome SD, Martinez del Rio C, Bearhop S, Phillips DL (2007) A niche for isotopic ecology. Front Ecol Environ 5:429–436. https://doi.org/10.1890/1540-9295(2007)5[429:anfie]2.0.co;2

Okada Y (1920) One species of Myzostomida attached to a hexactinellid sponge. Zoological Magazine Tokyo, 32:324–325

Parmentier E, Das K (2004) Commensal vs. parasitic relationship between Carapini fish and their hosts: some further insight through δ13C and δ15N measurements. J Exp Mar Bio Ecol 310(1):47–58. https://doi.org/10.1016/j.jembe.2004.03.019

Parmentier E, Michel L (2013) Boundary lines in symbiosis forms. Symbiosis 60:1–5. https://doi.org/10.1007/s13199-013-0236-0

Parnell A, Inger R, Bearhop S, Jackson AL (2008) SIAR: Stable isotope analysis in R.

Phillips DL, Inger R, Bearhop S, Jackson AL, Moore JW, Parnell AC, Semmens BX, Ward E J (2014) Best practices for use of stable isotope mixing models in food-web studies. Can J Zool 92(10):823–835. https://doi.org/10.1139/cjz-2014-0127

Pinnegar JK, Campbell N, Polunin NVC (2001) Unusual stable isotope fractionation patterns observed for fish host—parasite trophic relationships. J Fish Biol 59(3):494–503. https://doi.org/10.1006/jfbi.2001.1660

Post DM (2002) Using stable isotope to estimate trophic position: models, methods, and assumptions. Ecology 83:703–718. https://doi.org/10.2307/3071875

Power M, Klein GM (2004) Fish host–cestode parasite stable isotope enrichment patterns in marine, estuarine and freshwater fishes from northern Canada. Isotopes Environ Health Stud 40(4):257–266. https://doi.org/10.1080/10256010410001678062

Pratchett MS (2001) Influence of coral symbionts on feeding preferences of crown-of-thorns starfish *Acanthaster planci* in the western Pacific. Mar Ecol Prog Ser 214:111-119. https://doi.org/10.3354/meps214111

R Development Core Team. (2015) R: A language and environment for statistical computing. Vienna, Austria. http://www.R-project.org.

Raffel TR, Martin LB, Rohr JR (2008) Parasites as predators: unifying natural enemy ecology. Trends Ecol Evolut 23(11):610–618. https://doi.org/10.1016/j.tree.2008.06.015

Syväranta J, Lensu A, Markomäki TJ, Oksanen S, Jones RI (2013) An empirical evaluation of the utility of convex hull and standard ellipse areas for assessing population niche widths from stable isotope data. *PLoS One* 8:1–8. https://doi.org/10.1371/journal.pone.0056094

Towanda T, Thuesen EV (2006) Ectosymbiotic behavior of *Cancer gracilis* and its trophic relationships with its host *Phacellophora camtschatica* and the parasitoid *Hyperia medusarum*. Mar Ecol Prog Ser 315:221–236. https://doi.org/10.3354/meps315221

Wagner D, Luck DG, Toonen RJ (2012) The biology and ecology of black corals (Cnidaria: Anthozoa: Hexacorallia: Antipatharia). Adv Mar Biol 63:67–132. https://doi.org/10.1016/b978-0-12-394282-1.00002-8

Warner GF (1981) Species descriptions and ecological observations of black corals (Antipatharia) from Trinidad. B Mar Sci 31(1):147-163

Wirtz P, d'Udekem-d'Acoz C (2001) Decapoda from Antipatharia, Gorgonaria and Bivalvia at the Cape Verde Islands. Helgoland Mar Res 55:112–115. https://doi.org/10.1007/s101520100073

Sih J, Chouw J (2009) Fish and whips: Use of gorgonians as a habitat by the large whipcoral goby, *Bryaninops amplus* (Larson). Raffles B Zool 22:145–157

Soule DF, Soule JD (1972) Ancestrulae and body wall morphogenesis of some Hawaiian and eastern Pacific Smittinidae (Bryozoa). T Am Microsc Soc 91:251–260. https://doi.org/10.2307/3224873

Terrana L, Caulier G, Todinanahary G, Lepoint G, Eeckhaut I (2016) Characteristics of the infestation of *Seriatopora* corals by the coral gall crab *Hapalocarcinus marsupialis* Stimpson, 1859 on the great reef of Toliara, Madagascar. Symbiosis 69(2):113–122. http://doi.org/10.1007%2Fs13199-016-0391-1

Terrana L, Eeckhaut I (2017) Taxonomic description and 3D modelling of a new species of myzostomid (Annelida, Myzostomida) associated with black corals from Madagascar. Zootaxa 4244(2):277–295. https://doi.org/10.11646/zootaxa.4244.2.9

Todinanahary G, Terrana L, Lavitra T, Eeckhaut I (2016) First records of illegal harvesting and trading of black corals (Antipatharia) in Madagascar. Madag Conserv Dev 11(1):6–11. http://dx.doi.org/10.4314/mcd.v11i1.5

Vega R, Vega R, Luque AA (2002) *Coralliophila kaofitorum,* a new species (Gastropoda: Coralliophilidae) from the Canary Islands living on *Antipathes wollastoni* (Cnidaria: Anthozoa: Antipatharia). Nautilus 116:50–55

Chapitre 9

Discussion Générale

Several Stichopathes species described by Roule, 1905

DISCUSSION GENERALE

Avant le début de cette thèse, les antipathaires de Madagascar, mais aussi ceux de l'Océan Indien, n'étaient connus que par de brèves mentions dans une littérature souvent datée de près d'un siècle. Les antipathaires sont ubiquistes à Madagascar : ils sont présents tout le long de la côte ouest du pays, de l'extrême sud jusqu'aux îles touristiques de Nosy Be au nord. De l'autre côté du pays, à l'est, ils sont également présents mais beaucoup moins accessibles, la côte faisant face à l'Océan Indien étant abrupte et beaucoup plus secouée par la houle et les vents violents. Bien que les résultats obtenus lors de ces 4 années de recherche s'ancrent dans un contexte de recherche fondamentale, cette thèse comprend des informations nécessaires destinées à la protection des antipathaires. Dans ce dernier chapitre de discussion, les nouvelles informations que cette thèse a mis en évidence seront reprises et discutées par rapport à la littérature et les résultats seront croisés afin de voir comment certains résultats peuvent en expliquer ou en influencer d'autres. En guise de perspectives, les résultats permettent d'appuyer la création d'une nouvelle AMP et de dégager la meilleure option pour la protection durable des antipathaires. Dans le cadre de Madagascar, pour protéger les coraux noirs du Sud-Ouest, la priorité devrait être la mise en place d'une aire marine protégée (AMP) qui engloberait le champ d'antipathaires du Grand Récif de Tuléar ou mieux encore de faire de ce récif une AMP à part entière. La création d'une AMP est cependant un processus long, fastidieux, financièrement lourd et qui nécessite la synergie des acteurs de la protection de l'environnement tels que des membres des institutions académiques, des organisations non gouvernementales et surtout l'implication des communautés locales.

Le premier objectif de cette thèse était de caractériser la diversité d'espèces des antipathaires présents dans le Sud-Ouest et plus précisément au niveau du Grand Récif de Tuléar. Nous avons vu que dans cette région les antipathaires sont nombreux et diversifiés, en particulier au niveau de l'extrémité Nord du Grand Récif de Tuléar où leur abondance est telle qu'ils forment un champ. Auparavant, les champs d'antipathaires d'eaux peu profondes (<200 m) décrits dans le monde mentionnaient 8 espèces réparties en 6 genres et 3 familles jusqu'à 150 mètres de profondeur à Hawaii (Wagner 2015), et 16 espèces réparties en 6 genres et 3 familles jusqu'à 45 mètres de profondeur dans le parc marin de Bunaken en Indonésie (Tazioli et al. 2007). En comparaison, 18 espèces réparties en 5 genres et 2 familles furent identifiées dans le sud-ouest de Madagascar. Parmi ces 18 espèces, 6 ne correspondent pas aux descriptions originales issues de la littérature et sont soit des nouvelles espèces, soit des espèces à redécrire (car les descriptions originales sont insuffisantes pour permettre l'identification) ou encore des hybrides comme il pourrait exister au sein de la famille Myriopathidae (Lapian 2007 ; Lapian et al. 2009).

Dans cette thèse, il n'a pas été possible de nommer les 6 espèces qui ne correspondent à aucune description originale de la littérature car ceci demande une ré-étude taxonomique profonde des spécimens de musée. En effet, la description de ces probables nouvelles espèces demandait de comparer les spécimens collectés avec les holotypes et les paratypes disponibles dans les collections privées ou de musées or, pour beaucoup d'espèces d'antipathaires d'eaux peu profondes (<50 m) et notamment celles de l'Océan Indien, les types ont disparu ou les descriptions originales sont trop peu informatives. Par exemple, si on s'attarde plus en détail sur les familles des Antipathidae et des Myriopathidae, qui sont les plus représentées dans les eaux peu profondes (<50 m, avec dans une moindre mesures certaines espèces de la famille Aphanipathidae), nous voyons que sur les 117 espèces composant la première 8 espèces seulement furent décrites après 1932. De la même manière dans la seconde famille, sur 34 espèces, 9 furent décrites après 1932. Nous avons largement souligné l'importance de la microscopie électronique à balayage pour observer les caractéristiques du squelette et des épines des antipathaires, or le premier microscope de ce genre ne fut construit qu'en 1935. Il en résulte que l'état de connaissance actuel des antipathaires ne permet pas une identification complète et exhaustive de tous les spécimens rencontrés durant cette thèse, ni d'ailleurs des spécimens appartenant aux collections de musées.

Une recherche dans les bases de données en ligne montre que parmi les musées les plus proches, ceux de Bruxelles et de Londres n'abritent aucun antipathaire, tandis que celui de Leiden en possède 26 dont 25 ne sont même pas assigné à un genre. Le Smithsonian National Museum of Natural History de Washington abrite la plus grande collection d'antipathaires avec 2925 spécimens, 157 d'entre eux proviennent de l'Océan Indien toutes profondeurs confondues et 7 seulement sont originaires de Madagascar : *Antipathes pennacea* neotype (Europa Island, profondeur inconnue, collecté en 1965),

Tableau 21. Liste des spécimens originaires de Madagascar appartenant au MNHN de Paris.

Espèce	Numéro d'accession	Profondeur (m)	Expédition
Antipathes cf. *cylindrica*	MNHN-IK-2012-11221	282-333	Atimo Vatae
Antipathella sp.	MNHN-IK-2012-11222	225-282	Atimo Vatae
Antipathella sp.	MNHN-IK-2012-11223	250-300	Atimo Vatae
Stylopathes sp.	MNHN-IK-2012-10838	409-436	Atimo Vatae
Genus ?	MNHN-IK-2012-10871	225-282	Atimo Vatae
Genus ?	MNHN-IK-2012-10890	140-144	Atimo Vatae
Myriopathes sp.	MNHN-IK-2012-10908	225-282	Atimo Vatae
Genus ?	MNHN-IK-2012-10946	282-333	Atimo Vatae
Cirrhipathes sp.	MNHN-IK-2012-10989	23-25	Atimo Vatae
Cirrhipathes sp.	MNHN-IK-2012-10990	20-27	Atimo Vatae
Myriopathes sp.	MNHN-IK-2012-10991	20-22	Atimo Vatae
Cupressopathes sp.	MNHN-IK-2012-10992	13-27	Atimo Vatae
Antipathes sp.	MNHN-IK-2012-11000	13-27	Atimo Vatae
Antipathes sp.	MNHN-IK-2014-48	4	Collecté par Pichon Nosy-Be
Umbellapathes sp.	MNHN-IK-2014-205	558-592	Miriky
Stichopathes variabilis	MNHN-IK-2014-206	210-310	Miriky
Chrysopathes sp.	MNHN-IK-2014-208	435-600	Miriky
Arachnopathes sp.	MNHN-IK-2014-209	340-446	Miriky
Stichopathes cf. *papillosa*	MNHN-IK-2014-210	71-158	Miriky
Stylopathes sp.	MNHN-IK-2014-211	558-592	Miriky
Antipathes sp.	MNHN-IK-2014-212	231-237	Miriky
Antipathes sp.	MNHN-IK-2014-213	291-353	Miriky
Trissopathes tristicha	MNHN-IK-2014-214	314-353	Miriky
Lillipathes sp.	MNHN-IK-2014-215	367-369	Miriky
Stichopathes echinulata	MNHN-IK-2014-216	46-54	Miriky
Bathypathes cf. *robusta*	MNHN-IK-2014-217	331-364	Miriky
Genus ?	MNHN-IK-2014-218	220-362	Miriky
Genus ?	MNHN-IK-2014-219	244-300	Miriky
Dendrobathypathes sp.	MNHN-IK-2012-10738	71-158	Miriky
Antipathes sp.	MNHN-IK-2012-10739	71-158	Miriky
Antipathes sp.	MNHN-IK-2012-10740	71-158	Miriky
Antipathes plantagenista	MNHN-IK-2012-10785	50-107	Miriky
Stichopathes sp.	MNHN-IK-2012-10788	50-107	Miriky
Antipathes sp.	MNHN-IK-2012-10790	50-107	Miriky
Antipathes sp.	MNHN-IK-2012-10828	362-431	Miriky
Genus ?	MNHN-IK-2014-207	468-485	Miriky

Myriopathes sp. (Sakatia Island à Nosy Be, 9 m), *Stichopathes* sp. (Sakatia Island à Nosy Be, 10 m), *Cirrhipathes* cf. *anguina* (Nosy Be, profondeur inconnue), *Antipathes* cf. *ceylonensis* (Sakatia Island à Nosy Be, 20 m) et *Antipathes flabellum* (Tuléar, profondeur inconnue). Le Musée National d'Histoire Naturelle de Paris possède également une grande collection de coraux noirs avec 1347 spécimens. De ces derniers, 36 sont issus de Madagascar et sont presque entièrement déterminés par Tina Molodtsova (Tableau 21).Plusieurs conclusions peuvent être tirée de cette collection. La première concerne le grand nombre de spécimens dont l'identification est incomplète, chose habituelle pour le groupe des antipathaires. Sur les 36 spécimens malgaches, 6 ne sont pas assignés à un genre, 23 ne sont pas assignés à une espèce, 3 sont incertains en ayant la mention « confer », et seulement 4 sont complètement identifiés. Par rapport aux échantillons collectés à Tuléar durant cette thèse, la collection du MNHN de Paris présente quelques spécimens intéressants. Tout d'abord nous voyons que les échantillons malgaches de ce musée sont issus de deux expéditions. La première, appelée Miriky, avait pour objectif de prospecter une zone sous-marine peu étudiée située à des profondeurs comprises entre 100 et 1200 m. La zone s'étendait dans la partie nord du Canal du Mozambique, côté malgache, en partant de l'île de Nosy Be jusqu'au Cap d'Ambre et Majunga. Cette expédition est moins pertinente d'un point de vue bathymétrique pour la comparaison avec les espèces de cette thèse, bien que les spécimens trouvés à partir de 50 m (voir tableau 21) pourraient s'avérer intéressants à comparer. La seconde expédition, appelée Atimo-Vatae, est beaucoup plus intéressante d'un point de vue géographique et bathymétrique. Celle-ci avait pour objectif de prospecter le grand Sud en proposant une expédition composée (i) d'une prospection des alentours de Fort Dauphin, (ii) d'une prospection autour de Lavanono, avec un laboratoire de terrain et des moyens de récolte en plongée opérant dans un rayon d'une à deux heures de la base, et (iii) une mission embarquée à bord du crevettier « Nosy Be 11 » dont l'objectif était d'explorer par chalutage le plateau continental en-dessous des profondeurs plongeables. Les antipathaires issus de cette expédition sont peu nombreux comme le démontre le tableau 21, avec seulement 13 spécimens. En consultant la base de données en ligne de la campagne Atimo Vatae, il n'est pas possible de savoir si le triage des spécimens issus de la campagne est encore en cours et si ce nombre est encore susceptible d'augmenter. Sur ces 13 spécimens, 8 sont issus de profondeurs excluant toute prospection en plongée en scaphandre autonome (ici entre 140 et 436 m) comme ce fut le cas dans cette thèse. Par contre, 5 spécimens furent collectés à des profondeurs tout à fait comparables entre 13 et 27 m, le long de Fort-Dauphin (Sud-Est) et d'Itampolo (Sud-Ouest) : plusieurs *Cirrhipathes* sp., un spécimen de *Cupressopathes* sp. et un spécimen d'*Antipathes* sp. Enfin, le musée abrite également un spécimen collecté par Pichon –chercheur ayant participé à l'étude des récifs malgaches et surtout à Tuléar dans les années 70– et issu de Nosy Be, à 4 m de profondeur (*Antipathes* sp.).

Enfin, si les échantillonnages issus de ces deux expéditions ne sont pas exploitables pour comparer significativement des densités ou des diversités d'antipathaires (étant donné que les procédures n'étaient pas standardisées à cet égard), nous pouvons néanmoins faire une observation intéressante. Le plateau continental de la partie nord du Canal du Mozambique semble plus diversifié en termes de nombre de genres différents que celui du grand sud, et la quantité d'antipathaires issue de cette expédition semble également plus importante. De plus, la prospection du plateau continental du grand Sud (Fig. 71) n'a pas révélé de colonie non-branchue (du genre *Stichopathes*) comme c'est le cas au niveau du plateau continental nord du Canal du Mozambique (exemple avec *Stichopathes variabilis* ou *Stichopathes echinulata* retrouvés à cet endroit).

Figure 71. Exemples d'antipathaires remontés suite au chalutage du plateau continental pendant l'expédition Atimo Vatae. Images libres de droit et tirées du site internet du Musée National d'Histoire Naturelle de Paris.

Face à ces problèmes liés à la classification morphologique des antipathaires, le recours aux données moléculaires parait indispensable. Les gènes mitochondriaux étant peu variables chez les anthozoaires, le gène COI traditionnellement utilisé en tant que « code-barre » ne peut être utilisé. Notre choix s'est donc porté sur les régions ITS de l'ADN ribosomal à la fois de par ses caractéristiques déjà mentionnées dans le chapitre 5 mais également pour la disponibilité des séquences dans les banques de données provenant des quelques études ayant utilisé ces régions de l'ADN (Lapian et al. 2007 ; Lapian 2009 ;

Wagner et al. 2010 ; Bo et al. 2012 ; Opresko et al. 2012 ; Brugler et al. 2013 ; MacIsaac et al. 2013). Plus récemment, Bo et al. s'apprêtent à publier un article intitulé « Phylogenetic relationships of Mediterranean black corals (Cnidaria: Anthozoa: Hexacorallia) and implications for classification within the order Antipatharia », (accepté ; Bo comm. pers.). Dans chacune de ces publications (excepté la dernière non publiée dont seul l'abstract est disponible), aucun auteur ne tient compte de la variabilité intraspécifique liée aux régions ITS. L'existence d'hétérozygotie, comme nous le montrons dans le chapitre 5, est rarement mentionnée ou retrouvée dans les séquences déposées dans les banques de données. Pourtant, le séquençage des spécimens collectés durant cette thèse a révélé des cas d'hétérozygoties chez presque toutes les espèces. Sur les 40 colonies de *Stichopathes* sp. aff. *maldivensis* séquencées dans cette thèse, seules 7 séquences homozygotes furent obtenues. Après résolution des cas d'hétérozygotie, nous avons obtenus 21 haplotypes uniquement pour la région ITS-1. Bien souvent les études phylogénétiques ne se basent que sur un voire deux spécimens de la même espèce et ne permettent pas de tenir compte de cette variabilité.

Au cours de cette thèse, nous avons également tenté d'analyser la phylogénie des espèces récoltées à Madagascar sur base de données moléculaires, mais les résolutions obtenues posaient plus de questions qu'elles n'en résolvaient. Par conséquent, nous avons décidé de ne pas incorporer ce travail de phylogénie moléculaire dans l'article qui concerne la taxonomie des espèces malgaches. L'arbre phylogénétique sur la figure 72 explique la complexité du problème. Il a été réalisé suite à une analyse bayésienne des séquences hétérozygotes ITS-1 des spécimens de Madagascar en utilisant également quelques séquences disponibles dans les banques de données. Il en ressort que les genres *Cirrhipathes* et *Stichopathes* sont polyphylétique, ainsi que toutes les espèces issues de la famille Myriopathidae se regroupe en un clade avec certaines espèces de *Cirrhipathes*. Il apparait donc que (i) la région ITS-1 seule ne permet pas de discriminer certaines espèces (observé par la présence de peignes dans l'arbre), (ii) les genres *Cirrhipathes* et *Stichopathes* sont polyphylétiques, comme déjà montré pour *Stichopathes* auparavant (Lapian et al. 2007 ; Wagner et al. 2010 ; Bo et al. 2012 ; Brugler et al. 2013) et (iii) certaines espèces de la famille Antipathidae se groupent avec la famille Myriopathidae. L'utilisation de données moléculaires dans la révision taxonomique des antipathaires ajoute actuellement un niveau de difficulté supplémentaire dans un groupe où la confusion règne déjà depuis de nombreuses décennies. Pour les futures études taxonomiques concernant Madagascar et notamment des genre *Cirrhipathes* et *Cupressopathes*, il sera indispensable de découvrir de nouveaux marqueurs moléculaires afin de clarifier la situation phylogénétique des espèces classées dans ces groupes.

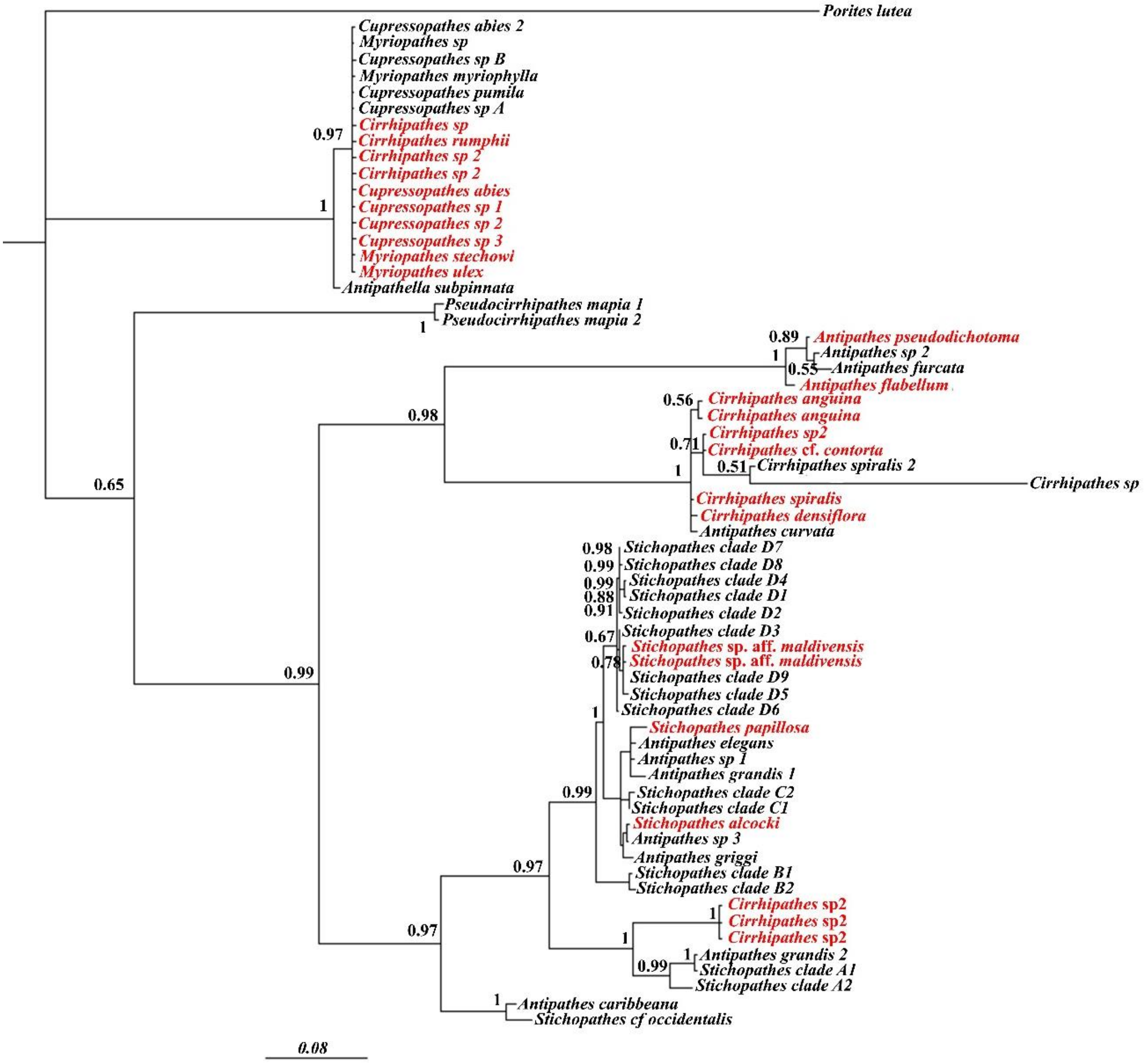


Figure 72. Exemple d'arbre obtenu par analyse bayésienne sur les séquences ITS1 des antipathaires de Tuléar. Les nombres au-dessus des nœuds représentent les probabilités postérieures associées à chaque clade.

L'étude de génétique de population que nous avons faites sur les colonies de *Stichopathes* sp. aff. *maldivensis* récoltés sur 200 km de côtes a mis en évidence 21 haplotypes de ITS-1. Les connexions analysées entre ces haplotypes suggèrent qu'ils appartiennent tous à une seule population sans sous-populations évidentes. Cette connectivité entre individus est un paramètre providentiel par rapport aux collectes illégales car le prélèvement intempestif d'individus de populations isolées génétiquement mènerait à une perte de la diversité génétique et à une fragilisation des espèces récoltées. Les résultats de notre étude diffèrent de celle de Miller (1997) qui a trouvé une différenciation génétique significative entre les sites d'échantillonnage dans les fjords de Nouvelle-Zélande. Ceci suggérant une dispersion très limitée des larves d'*Antipathes fjordensis* entre les sites, c'est-à-dire sur des distances de quelques dizaines de kilomètres. Les spécimens de *Stichopathes* sp. aff. *maldivensis* à Madagascar ne se retrouvent pas, comme dans l'étude de Miller (1997), dans un milieu confiné mais sur une côte face à la mer. Il semble pourtant y avoir une dispersion moins limitée que dans le cas étudié par Miller (1997) et cela même malgré la présence de fleuves dont les eaux qu'ils charrient auraient pu constituer des barrières naturelles contre cette dispersion. De manière générale, la connectivité génétique est en relation avec la dispersion des gamètes en période de reproduction et/ou avec la dispersion larvaire qui, toutes deux, dépendent des courants marins. Le courant des Aiguilles est le courant le plus important de l'Océan Indien. Il borde l'est de l'Afrique et transporte d'énormes masses d'eaux chaudes. C'est un courant majeur influençant le climat mondial par son rôle de transfert de chaleur et de salinité entre l'Océan Indien et l'Océan Atlantique au niveau de la pointe de l'Afrique (Fig. 73). En étudiant les isotopes stables de l'oxygène dans des colonies de *Porites* provenant d'Ifaty et de Tuléar, Zinke et al. (2014) établirent que ces coraux suivent les variations de température de surface du courant des Aiguilles depuis plusieurs siècles (plus de 300 ans), mettant ainsi en évidence l'existence d'un transfert d'eaux chaudes depuis la côte est de l'Afrique vers la côte ouest de Madagascar. Très récemment, en 2018, un nouveau courant marin fut découvert au sud-ouest de Madagascar (Ramanantsoa et al. 2018). Ce nouveau courant, nommé Southwest Madagascar Coastal Current (SMACC) est un courant dirigé vers le Sud (contrairement aux vents dominants qui vont vers le Nord) et qui s'écoule le long de la côte sud-ouest du pays (Fig. 74). Il se situe entre les latitudes 22°S et 26°S, ce qui correspond à Andavadoaka (extrémité nord de la zone d'étude de cette thèse) et à la pointe la plus au sud du pays, près de Faux-Cap. Sa longueur est d'environ 500 km et sa largeur entre 50 et 100 km. C'est un courant chaud de surface, qui s'étend depuis les 150 premiers mètres de la colonne d'eau en amont vers des profondeurs moins élevées allant jusqu'à 70 m de profondeur en aval. Les masses d'eaux portées par le SMACC ont un taux de salinité élevé et caractéristique des eaux de surface subtropicales

(Ramanantsoa et al. 2018). Sa vitesse moyenne en surface est de 20 cm·s^{-1}. Remise dans le contexte de cette thèse, cette vitesse équivaudrait à un transfert des masses d'eaux d'Andavadoaka vers Maromena en une période d'environ 11 jours, sans compter l'accélération qui apparait aux alentours de Tuléar rapportée par Ramanantsoa et al. (2018). Avec les vents dominants du sud-ouest existant dans cette région, un gyre océanique s'est installé dont le sens des courants s'effectue de façon horlogique: des eaux chaudes longent la côte sud-ouest de Madagascar du nord au sud puis s'éloignent de la côte de quelques centaines de kilomètres pour être redirigé vers le nord. Ce gyre influence probablement les upwellings du sud-ouest malgache (Ramanantsoa et al. 2018) : l'intensification des vents marins pourrait favoriser l'influence du SMACC dans les processus d'upwellings et l'intrusion des eaux chaudes provenant du Canal du Mozambique via le courant des Aiguilles dans le gyre du SMACC pourrait réduire la signature de surface des upwellings durant l'été, qui sont normalement des remontées d'eaux froides (Fig. 73, 74).

La découverte et la description de ce courant est très intéressante dans le cadre de cette thèse car elle apporte des réponses concernant l'abondance des antipathaires dans cette région et la connectivité de leurs populations. En effet, le champ d'antipathaires est soumis localement aux courants de marées dont les vitesses sont accentuées par l'effet entonnoir de la passe du récif ainsi que par les crues liées au Fiherenana qui se jette dans la mer face à lui. A plus grande échelle, le champ se situe au niveau du gyre du SMACC où justement la vitesse s'accentue dans ses environs (Ramanantsoa et al. 2018). Une des hypothèses de l'abondance des coraux noirs à cet endroit pourrait être liée à la dispersion des gamètes et des larves qui finiraient par être ramenées sur la côte par le gyre. Cependant cela impliquerait que les larves des antipathaires aient une durée de vie assez conséquente que pour pouvoir parcourir la boucle du SMACC qui remonte dans le canal du Mozambique. Nos résultats ont montré une connectivité totale entre les spécimens de *Stichopathes* sp. aff. *maldivensis* collectés d'Andavadoaka à Maromena. Cette région correspond parfaitement à la trajectoire empruntée par le SMACC. Le gyre de ce dernier empêcherait les larves des antipathaires de se disperser au-delà des côtes Sud-Ouest de Madagascar. Il est toutefois à remarquer que nous avons observé la présence d'haplotypes Indonésiens au sein de la population malgache. Les seules données relatives aux planulas des antipathaires concernent des larves asexuées formées en conditions stress et il est

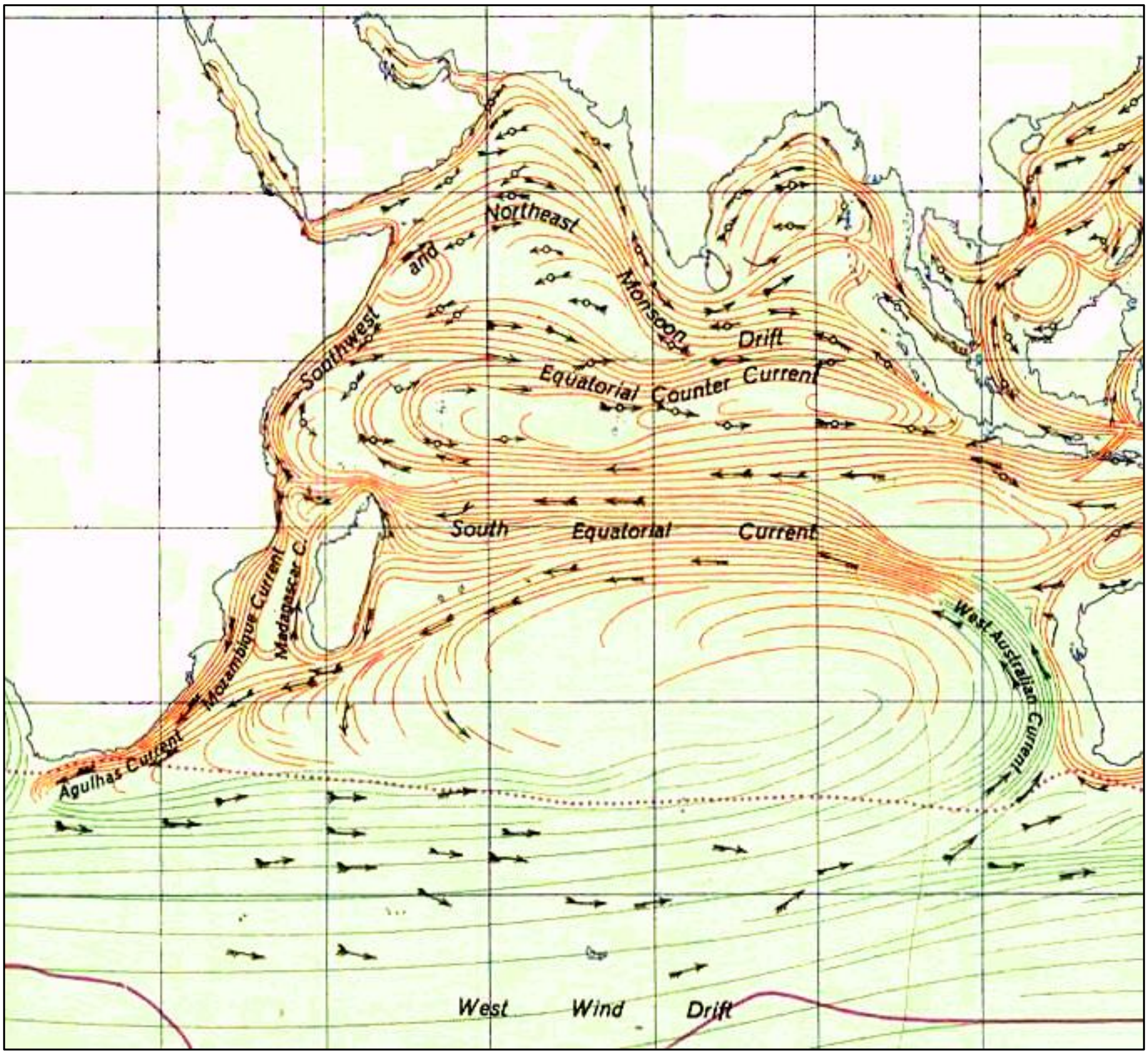


Figure 73. Carte des courants marins de l'Océan Indien. Madagascar est frappée à l'Est par le Courant Equatorial Sud en provenance de l'Asie du Sud-Est et de l'Australie. Il se divise au niveau de Madagascar et longe le Canal du Mozambique et la côte est de Madagascar. Le courant des Aiguilles quant à lui longe toute l'Afrique de l'Est transfère une partie de ses masses d'eaux chaudes au sud-ouest de Madagascar via le SMACC. Carte libre de droit.

donc impossible actuellement d'inférer la capacité de dispersion de des larves de coraux noirs en général. Un moyen de confirmer l'hypothèse de « l'emprisonnement de larves » par le gyre du SMACC serait d'étudier la structure génétique des populations entre différents endroits ciblés sur le Mozambique et sur plusieurs localités à Madagascar. Si les larves sont effectivement bloquées au sein du SMACC, nous nous attendons à trouver un isolement génétique au niveau de la côte Sud-Ouest. L'espèce idéale serait à nouveau *Stichopathes* sp. aff. *maldivensis* puisqu'elle est très abondante dans le Sud-Ouest comme l'a montré cette thèse, que des photos touristiques montrent sa présence dans le Nord-

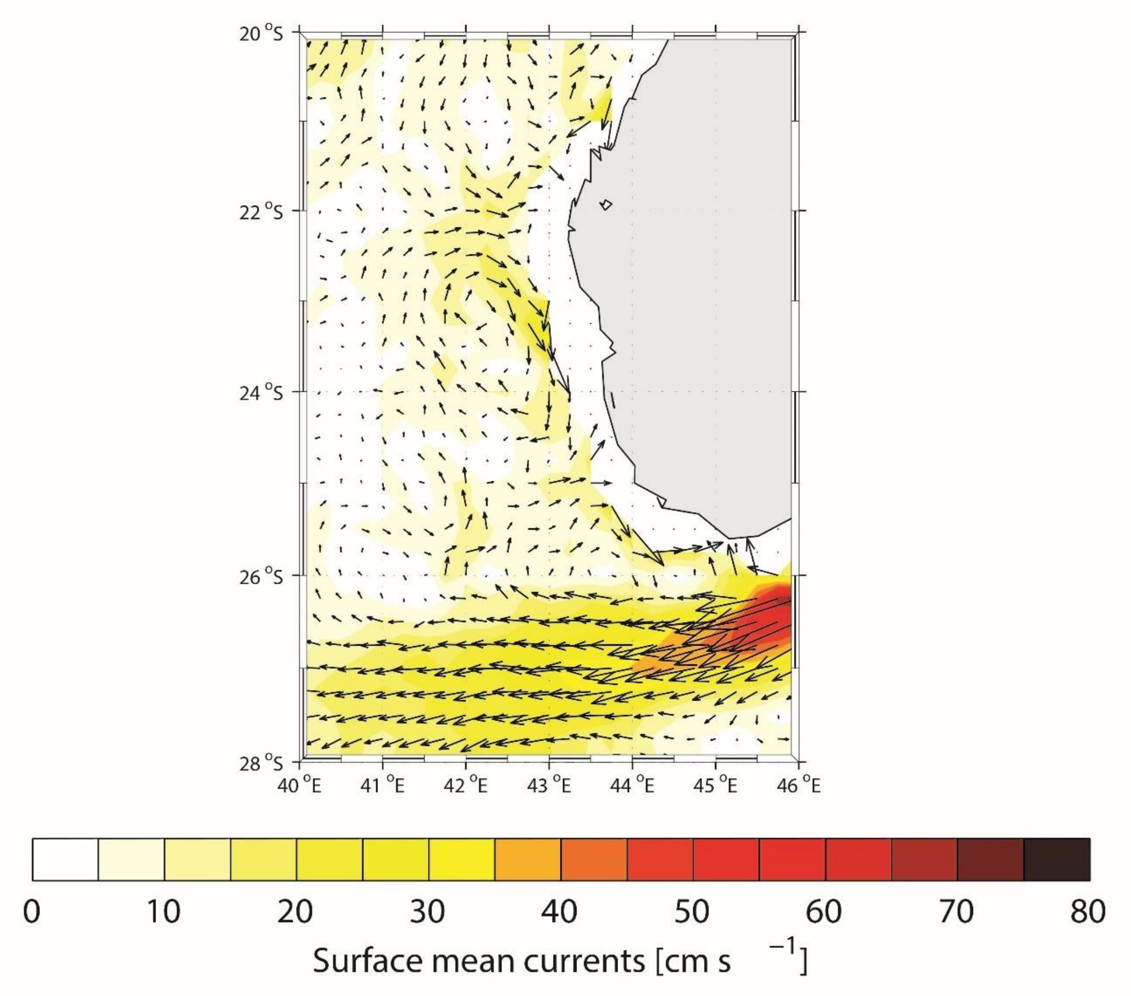


Figure 74. Direction des courants de surface au niveau du gyre du Southwest Madagascar Coastal Current. Les flèches indiquent la direction du courant tandis que l'échelle de couleur représente la vitesse en cm·s^{-1}. Illustration adaptée de Ranamantsoa et al. 2018, dans le matériel supplémentaire de l'article.

Ouest, et que sa présence est confirmée en Asie du Sud-Est par Bo et al. (2012). Seule sa présence le long du Mozambique et de Fort-Dauphin restent à déterminer. Au Mozambique, il n'existe que les travaux de Summers (1910) pour attester de la présence des antipathaires, où l'auteur décrit 4 espèces de *Stichopathes* : *S. alcocki* (identifié dans cette thèse), *S. flagellum*, *S. echinulata* et *S. gracilis*. La profondeur d'échantillonnage n'est pas précisée mais les spécimens sont issus de l'expédition du « HMS Challenger », ce qui signifie qu'ils sont probablement issus d'un dragage profond au-delà de 200 m. A Fort-Dauphin, le genre *Stichopathes* n'est pas mentionné dans la collection issue du MNHN de Paris. A long terme, l'isolement des antipathaires le long des côtes longées par le SMACC pourrait entrainer une dérive génique et une spéciation allopatrique des espèces. L'identification de marqueurs satellites semble opportun pour une telle étude : les articles de Ruiz-Ramos & Baums (2014), Ruiz-Ramos et al. (2015) et Cardona et al. (2016) concernant les microsatellites de *Leiopathes glaberrima* constituent une bonne base de travail pour la méthodologie à adapter.

La présence de ce courant SMACC est également intéressante d'un point de vue du cycle reproductif des antipathaires. Dans cette thèse nous avons étudié la reproduction sexuée de *Cirrhipathes* sp. au niveau du champ de Tuléar. Les résultats obtenus montrent qu'un pic de maturité gonadique a lieu au moment où il y a une augmentation de température de l'eau et au moment où une pluviosité accrue entraine une décharge de matière organique dans le champ de coraux noirs de Tuléar par le Fiherenana. Ce phénomène pourrait résulter dans un développement accru de phytoplancton puis de zooplancton. Cependant, l'augmentation de la turbidité entrainée par la crue du fleuve lors de la saison des pluies semble contradictoire avec le développement d'un bloom phytoplanctonique, puisque ces organismes nécessitent de la lumière afin de procéder à la photosynthèse. De plus, les résultats de l'étude des rapports isotopiques du soufre chez les coraux noirs de Tuléar contredisent également l'hypothèse d'un apport nutritif terrigène, puisque les résultats supportent une origine pélagique de la source trophique. Dès lors, un bloom de phytoplancton dans la région de Tuléar serait certainement plutôt lié aux upwellings de la région amenant des masses d'eaux froides enrichies en nutriments. Comme mentionné précédemment, ce phénomène est influencé par le SMACC. L'augmentation de chlorophylle se déroule dans la région à partir de février jusqu'avril pour atteindre son pic en mai (Ranamantsoa et al. 2018). En parallèle, les résultats obtenus dans cette thèse ont montré que la gamétogénèse s'intensifiait de décembre jusque février pour terminer par un évènement de ponte vers le mois de mars. Il se pourrait donc que l'augmentation progressive de phytoplancton (démontrée par l'augmentation de chlorophylle) entraine une augmentation progressive du zooplancton. Cette dernière pourrait expliquer l'intensification de la gamétogénèse, étant donné que le régime alimentaire des coraux noirs se base principalement sur le zooplancton. La ponte des coraux survenant un mois avant le pic de phytoplancton, on pourrait imaginer que ce décalage soit synchronisé afin que la larve nouvellement installée sur son substrat puisse bénéficier dès ses premiers jours de développement d'un maximum de ressources trophiques dans le but de stimuler sa croissance initiale et de synthétiser rapidement la plaque de base absolument nécessaire à l'ancrage de la colonie sur son substrat.

Dans le futur, certaines zones du champ de Tuléar pourraient être balisées avant la période de ponte pour recenser la présence de jeunes colonies à intervalles de temps régulier après cette dernière. De cette manière, en sachant quelles colonies et quelles tailles sont présentes dans une surface donnée, il devient possible d'observer le développement de nouvelles colonies dans la surface à intervalles de temps réguliers et ainsi déduire (i) le taux de recrutement, (ii) la vitesse de développement dès les premiers jours et (iii) la distribution spatiale voire bathymétrique des larves métamorphosées en colonies dans le champ. En outre, effectuer un tel recensement sur un minimum d'un an permettrait d'établir un intervalle de temps durant lequel les larves d'une espèce dont la période de ponte est connue peuvent survivre et être recrutées.

Si la durée de vie des larves de coraux noirs est inconnue, ce n'est pas le cas pour les colonies adultes. Dans le chapitre concerné, nous avons exposé un tableau avec les différents taux de croissance connus à l'heure actuelle, qui se basent soit sur des mesures *in situ* soit sur des expériences de datation grâce aux isotopes stables comme le carbone ou le plomb (Williams et al. 2006 ; Love et al. 2007). Nous avons présenté un taux de croissance basé à la fois sur la longueur et le diamètre du squelette de la colonie. Il est probable que la croissance d'une colonie ne soit pas constante tout au long de sa vie, d'autant plus lorsque celle-ci peut vivre pendant près d'un siècle. Pourtant une croissance continue existe chez *Leiopathes glaberrima* dont l'âge fut étudié à plusieurs reprises. Ainsi, des colonies agées de plus de 2000 ans collectés dans le Golfe du Mexique présentent des taux de croissance radiale strictement linéaires (Prouty et al. 2011). D'un autre côté, des colonies de la même espèce collectés dans les Açores présentent des taux de croissance radiale variables (Carreiro-Silva et al. 2013). Dans ces dernières, l'exemple le plus marquant est celui d'une colonie agée de 2320 ans qui possède des taux de croissance radiale de l'ordre de 4 à 5 µm/an durant les 1600 premières années de son existence, avant d'accélérer sa croissance jusqu'à 20 µm/an pendant environ 400 ans et enfin retourner au même taux de croissance initial de 4 à 5 µm/an pendant les 300 dernières années. Ces taux de croissances lents issu de la colonie agée de 2320 ans furent retrouvés en analysant des colonies plus petites en taille. Dès lors l'explication de ce taux de croissance initial lent pourrait résider dans la faible surface exposée au courant et donc limitant la quantité de nourriture disponible, tel que suggéré pour les coraux bambous

(Roark et al. 2005). Dans ce scénario, le taux de croissance augmenterait à mesure que la surface du corail s'accroîtrait mais finirait par diminuer à mesure que la colonie atteint sa taille maximale. En accroissant sa taille, la colonie pourrait paradoxalement réduire l'efficacité de sa capture de proies car les branches plus externes pourraient bloquer l'accès aux ressources des branches situées à proximité de l'axe central de la colonie. Dans le cas des antipathaires étudiés durant cette thèse, l'hypothèse du « self-shading » n'est pas envisageable étant donné que les colonies sont non branchues. La comparaison avec les données de *Leiopathes glaberrima* exposées ci-dessus est difficile car la morphologie, la bathymétrie, et l'espérance de vie sont différentes. Les colonies *Leiopathes glaberrima* vivent à des profondeurs bathyales, peuvent être âgées de plusieurs milliers d'années et sont arbustives. Dans le cas des colonies malgaches, nous avons des colonies non branchues pouvant mesurer plus de 4 m, qui vivent proches de la surface (10-30 m) et qui sont âgées d'environ 1 siècle. De plus, les conditions environnementales et l'écologie trophique des espèces sont probablement très différentes puisqu'on compare des espèces bathyales d'Atlantique avec des espèces tropicales de récifs du Canal du Mozambique. Il est donc difficile d'établir une comparaison entre ces espèces. Les taux de croissance présentés dans cette thèse sont lissés sur la durée de vie de l'antipathaire et ne tiennent pas compte d'une éventuelle variabilité temporelle. Pour savoir si le taux de croissance d'une espèce varie d'une période à l'autre, il faudrait reproduire des datations au carbone 14 en (i) délaminant les stries de croissances au KOH (4 g dans 50 ml) pendant 1 semaine afin de les isoler et de permettre un échantillonnage précis, ce traitement n'a aucune incidence sur l'analyse du carbone (Carreiro-Silva et al. 2013) ; (ii) dans le meilleur des cas échantillonner plusieurs stries adjacentes le long du rayon de l'axe squelettique afin d'avoir un maximum de précision dans la détermination de l'âge de chacune d'entre elles et ainsi inférer un taux de croissance représentatif. Le problème réside dans l'épaisseur des stries de croissances chez *Stichopathes* et *Cirrhipathes*, qui mesurent au maximum 45 µm d'épaisseur contre 100 à 200 µm chez *Leiopathes glaberrima*. Cette taille plus large a d'ailleurs permis d'isoler les stries avec des pinces après délamination chez cette dernière (Carreira-Silva et al. 2013). Techniquement, l'utilisation de la micro-fraiseuse est déjà contraignante en elle-même puisque les têtes fraiseuses en diamant mesurent 200 à 300 µm de large et entrainent par conséquent le forage de plusieurs stries d'une seule fois. L'autre aspect technique contraignant réside dans la masse nécessaire au spectromètre de masse pour obtenir une mesure. Le fraisage doit être soit profond dans la même strie soit plus étendu le long de la strie pour réunir suffisamment de squelette en poudre. Bien souvent les stries ne sont pas parfaitement concentriques mais sont irrégulières, ce qui diminue la précision de l'échantillonnage. Chez les antipathaires étudiés durant cette thèse, nous avons calculé un nombre de stries supérieur à l'âge absolu de chacune des colonies, ce qui signifie que les coraux en synthétisent plus d'une par année. Jusqu'à présent, la formation des stries de croissance était considérée comme annuelle chez les coraux noirs. La synthèse annuelle de stries de croissance est un phénomène existant chez d'autres anthozoaires tels que les scléractiniaires (Knutson et al. 1972) ou les gorgones comme par exemple *Muricea californica* (Grigg 1974) ou *Corallium rubrum* (Marschal et al. 2004). La comparaison n'est pas forcément évidente car les 3 groupes scléractiniaires, alcyonaires et antipathaires présentent chacun un squelette composé d'un matériau biochimiquement différent : de la calcite chez les premiers, de la gorgonine (substance composée de collagène) parfois associée à de la calcite chez les seconds, et de l'antipathine (substance composée de chitine) chez les derniers. Par conséquent les processus physiologiques liés à leurs synthèses sont différents et ne sont pas forcément influencés par les mêmes facteurs environnementaux. Cependant, certaines hypothèses pouvant expliquer la croissance pluri-annuelle de stries concentriques chez les antipathaires étudiés peuvent être puisées dans l'étude de Risk et al. (2002) concernant les gorgones *Primnoa resedaeformis* et *Desmophyllum cristagalli*. Chez ces espèces, les auteurs ont mis en évidence la présence de stries sub-annuelles (Risk et al. 2002) mesurant 2 à 7 µm d'épaisseur. Durant cette thèse, nous avons également montré que les stries de croissance seraient synthétisées en moyenne 2 à 3 fois par an chez les antipathaires *Stichopathes* sp. aff. *maldivensis* et *Cirrhipathes* sp. Risk et al. (2002) suggèrent que ce phénomène pourrait être dû aux marées d'équinoxes qui engendreraient un accroissement des dépôts sédimentaires sur les colonies et par conséquent un stress supplémentaire pour les coraux qui dépenseraient plus d'énergie pour lutter contre cette sédimentation et/ou qui feraient face à une diminution de leur source de nourriture. Face à ce stress, un changement métabolique se produirait et la synthèse de gorgonine laisserait place à la synthèse de calcite chez les gorgones susmentionnées. Le même phénomène pourrait se produire chez les antipathaires. En plus des marées d'équinoxes, la période de crue du fleuve Fiherenana pourrait également expliquer un accroissement de la sédimentation sur les colonies. Ceci pourrait entrainer l'alternance des couches composées de fibres de chitines et celles composée du cément protéique qui les relie (Goldberg 1991), et pourraient être liées à la disponibilité des ressources.

Les ressources trophiques utilisées par les antipathaires étudiés dans cette thèse sont de manière générale centrées sur le zooplancton, et plus particulièrement sur le mésozooplancton. La fiabilité des résultats produits par les modèles statistiques de mélanges (SIAR) dépend fortement de la variabilité des sources, qui dépend elle-même du nombre de réplicas effectués pendant l'échantillonnage, ainsi que du nombre de sources implémentées dans le modèle. C'est pourquoi les résultats obtenus vont nous permettre de nous focaliser davantage sur le zooplancton pour les futures études liées à l'écologie trophique. Dans ces nouvelles approches, l'échantillonnage réalisé avec un nombre adéquat de réplicas permettra d'évaluer s'il existe une différence entre les rapports isotopiques des sources échantillonnées le jour et la nuit, d'évaluer les rapports isotopiques de chaque fraction du zooplancton de manière plus précise, et de comparer les sources entre les saisons été/hiver, le tout rapporté aux rapports isotopiques obtenus en analysant les tissus des antipathaires.

Durant les séries de coupes histologiques réalisées dans les tissus de *Cirrhipathes* sp., il nous est apparu que cette espèce présentait des zooxanthelles (Fig. 75). Celles-ci se situent dans le gastroderme et n'apparaissent pas très abondantes, bien que nous n'ayons pas quantifié leur présence. Cette symbiose entre les coraux noirs et les zooxanthelles est déjà connue chez certaines espèces d'antipathaires d'eaux peu profondes d'Indonésie et d'Hawaii (<50 m, Bo et al. 2011 ; Wagner et al. 2011). Néanmoins il reste encore beaucoup à découvrir, notamment par rapport à la diversité de ces dinoflagellés au sein des antipathaires, à leur distribution bathymétrique, à leur rôle physiologique et à leur capacité à changer d'hôte comme c'est le cas chez les scléractiniaires. Un projet est actuellement en cours afin d'étudier les zooxanthelles des antipathaires de Tuléar. La première étape consiste à réaliser des coupes histologiques dans les tissus des 18 espèces identifiées dans le chapitre 3 afin d'établir quelles sont les espèces qui présentent des zooxanthelles et de quantifier leur abondance. Après avoir ciblé les antipathaires, des analyses génétiques basées dans un premier temps sur ITS avec des primers spécifiques aux zooxanthelles seront effectuées afin d'établir la diversité génique de ces dernières et les clades auxquels elles appartiennent. Enfin, le troisième objectif sera de collecter des tissus provenant des espèces cibles sur le terrain à différentes profondeurs et de les fixer de façon adéquate, ceci afin de caractériser plus finement l'association d'un point de vue morphologique via des techniques de microscopie électronique, et de déterminer si la profondeur influence la densité des symbiontes. Dans le futur, afin d'étudier la physiologie de l'association et de comprendre s'il existe un transfert de carbohydrates entre la zooxanthelle et son hôte, il faudrait idéalement détecter la présence de tels transferts via des composés marqués en isotopes ^{13}C ou ^{15}N mais le challenge réside dans l'isolement en quantité suffisante de ces symbiontes. Vu leur abondance, il semblerait peu probable que l'association soit obligatoire pour la survie des antipathaires.

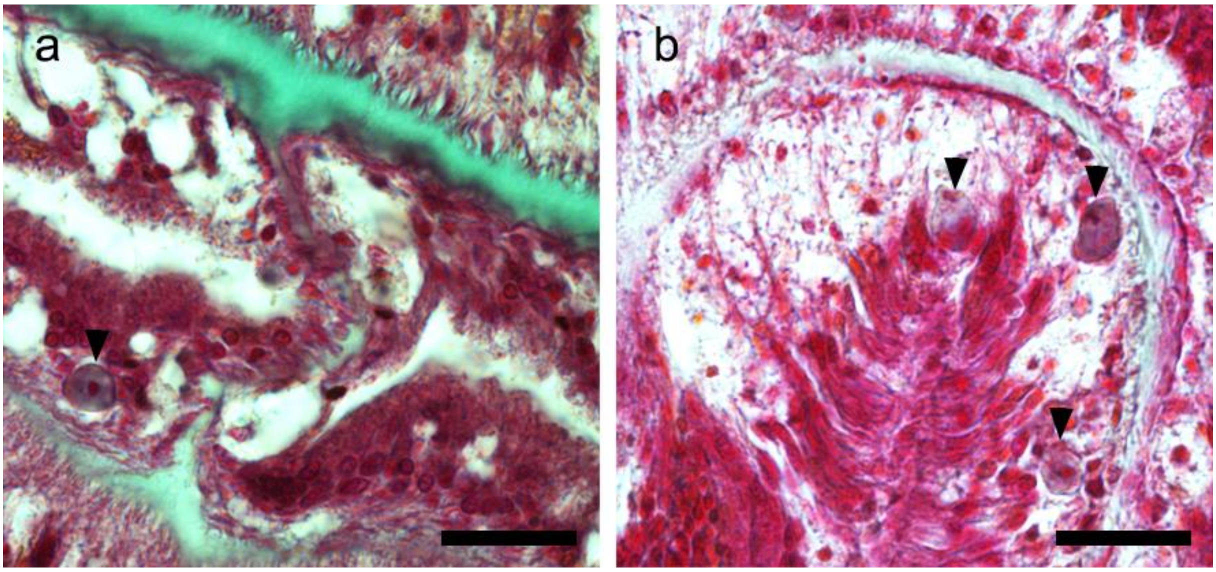


Figure 75. (a) et (b) Illustrations personnelles des zooxanthelles aperçues dans les coupes histologiques de *Cirrhipathes* sp. lors de l'étude de son cycle reproductif, pointées par les flèches noires. Elles sont caractérisées par leur taille, leur forme sphérique et leur coloration plus foncée au sein du gastroderme. Echelles : 20 µm.

Pour conclure, ce travail constitue une base solide pour de futures études plus détaillées dans chacun des domaines explorés dans cette thèse, de la taxonomie à la génétique des populations en passant par l'écologie trophique et l'étude des symbioses. Un aperçu des recherches réalisables à court terme fut donné tout le long de la discussion, ces perspectives s'ancrant toutes dans un objectif de recherche fondamentale. Dans ce manuscrit, nous avons largement insisté sur la vulnérabilité des antipathaires de Madagascar en appuyant notamment le fait qu'ils soient collectés de façon illégale ainsi qu'en mettant des chiffres sur ces exploitations. Afin d'aller au-delà de simples constatations pessimistes, la suite de ce texte aura pour volonté de proposer de façon concrète l'établissement d'un programme de protection des antipathaires à Tuléar.

PERSPECTIVES DE CE TRAVAIL EN BIOLOGIE DE LA CONSERVATION

Il y a déjà près d'un siècle, Madagascar voyait la création de ses premières zones protégées sur base d'un système hérité du passé colonial français, avec l'établissement de dix réserves naturelles en 1927 toutes strictement terrestres et la signature en 1933 de la *London International Convention for the protection of fauna and flora in Africa* (Virah-Sawmy & Gardner 2014) entrainant la création du premier Parc National de la Montagne d'Ambre en 1958 (Nicoll & Langrand 1989). Après son indépendance en 1960, Madagascar mis malheureusement la conservation de la Nature de côté au profit d'un développement agricultural plus intense et moins restreint par les mesures de protection de l'environnement (Kull 2014). Il fallut attendre les années 1990 pour retrouver une dynamique de protection de l'environnement et de la biodiversité avec la création de l'Association Nationale pour la Gestion des Aires Protégées (ANGAP) dédiée à la gestion et la mise en place des aires en partenariat avec d'autres organisations internationales (Virah-Sawmy & Gardner 2014). L'accent fut mis sur la protection stricte de la biodiversité ainsi que sur la génération de revenus liés au développement du tourisme. Néanmoins, cette vision des choses s'est rapidement avérée incompatible et infructueuse à long terme avec la gestion des ressources naturelles et le développement socio-économique des communautés locales.

En 2003, le congrès tenu à Durban (Afrique du Sud) par *l'International Union for The Conservation of Nature* (IUCN) mit un point d'honneur à l'importance d'impliquer les communautés locales dans la gestion des zones protégées afin d'allier conservation de la Nature et réduction de la pauvreté (Virah-Sawmy & Gardner 2014). La création de nouvelles aires ne se fit pas au hasard et nécessita des enquêtes au niveau national utilisant souvent des modélisations de distribution d'espèces afin de déterminer les endroits à protéger les plus propices avec en parallèle une priorité accordée aux zones où les communautés étaient les plus dépendantes vis-à-vis des ressources naturelles (Kremen et al. 2008 ; Rasoavahiny et al. 2008). De cette manière, l'utilisation durable des ressources naturelles par les communautés locales seraient implémentée et permise au sein des aires protégées (Freudenberger 2010). Cette conférence pour la protection de la biodiversité marqua également un tournant important pour Madagascar en permettant pour la première fois de déléguer la gérance et la gouvernance des zones protégées d'abord conduites par l'ANGAP, maintenant nommée Madagascar National Parks (MNP), à un regroupement synergique de communautés locales, d'organisations non gouvernementales et privées dans un projet appelé « Vision Durban ». Le tableau 22 reprend les différents types de réserves naturelles à Madagascar selon l'IUCN, avec l'introduction de nouveaux concepts et le type de gestion qui leur est appliquée. La Vision Durban introduit également deux nouveaux types de gestion des aires protégées et qui peuvent être appliquées à différents types de réserves et qui ne sont donc pas reprises dans le tableau : la gestion privée, où une compagnie privée gère de manière lucrative ou non une zone définie ; et la gestion communautaire, où les zones protégées sont des endroits naturels ou modifiés par l'Homme et qui possèdent une grande valeur écologique ou culturelle aux yeux d'une communauté indigène, qui la gère au travers de lois locales et de gouvernances ethniques. Enfin, plus récemment Madagascar s'est engagé à respecter la « Promesse de Sydney » en 2014 durant le VIème Congrès Mondial des Parcs en Australie au cours duquel l'accroissement des aires protégées fut décidé. La finalité sera la création d'un réseau d'espaces marins protégés dénommé « Zombandriake » délimitant des zones géographiques à partir de données écologiques, climatiques et socio-économiques.

A Madagascar, les modalités relatives à la mise en place administrative, pratique et socio-culturelle d'une telle réserve font l'objet d'un décret ministériel où on y définit les AMP ainsi que les différentes étapes de leur établissement et de leur fonctionnement (décret n° 2005- 013 « Organisant l'application de la loi n° 2001-005 du 11 février 2003 portant sur le Code de Gestion des aires protégées). Selon ce décret, une AMP est :

> « *Un territoire délimité terrestre, côtier ou marin en eaux larges saumâtres, continentales, ou aquatique, dont les composantes présentent une valeur particulière, notamment biologique, naturelle, esthétique, morphologique, historique, archéologique, culturelle ou cultuelle, et qui de ce fait, dans l'intérêt général, nécessite une préservation contre tout effet de dégradation naturelle et contre toute intervention artificielle susceptible d'en altérer l'aspect, la composition et l'évolution.* »

Selon ce décret, une AMP comprend :

- Un noyau dur, qui est une zone sanctuaire d'intérêt biologique, culturel, historique, esthétique, morphologique et archéologique et qui représente le périmètre de préservation intégrale. Toute activité et toute circulation y sont strictement réglementées ;

- Une zone tampon avec éventuellement des zones d'occupation contrôlée, des zones d'utilisation contrôlée ou des zones de services. C'est une zone jouxtant le noyau dur dans laquelle les activités sont limitées pour assurer une meilleure protection de l'aire protégée ;
- Une zone de protection, qui jouxte l'aire protégée dans laquelle sont admises les activités agricoles et pastorales, de pêche, de navigation ou d'autres types d'activités autorisées à titre exceptionnel par l'organisme chargé de la gestion du Réseau National des Aires Protégées et n'entraînant pas d'impacts néfastes sur l'aire protégée.

Il est communément établi que Madagascar est un des plus beaux et plus grands hotspot de biodiversité dans le monde, avec ses niveaux d'endémismes élevés et sa grande richesse d'écosystèmes et habitats différents (Myers et al. 2000). Avec ses 450 km de récifs frangeants, le Sud-Ouest possède des systèmes récifaux parmi les plus larges et les plus diversifiés de l'Océan Indien (Cooke et al. 2000). Au centre de ce système récifal se trouve un des plus grands récifs barrière du monde : le Grand Récif de Tuléar, qui se situe environ à 1.5km de la côte avec ses 18km de long et ses 33km² de surface (Pichon 1978). Les populations côtières du Sud-Ouest sont intimement –pour ne pas dire exclusivement– liées à ces récifs, et particulièrement au Grand Récif. Il représente à la fois une zone de haute importance biologique, socio-économique et culturelle et dont les connaissances relatives à ces trois facteurs sont relativement bien documentées. Cependant, le Grand Récif fait face à de nombreuses menaces anthropiques et écologiques : augmentation drastique de la démographie (Cripps & Gardner 2016), surexploitation des ressources halieutiques (Brenier et al. 2011), déforestation et augmentation de la sédimentation (Maina et al. 2012), augmentation de la pollution dans la baie (Andréfouët et al. 2013) et changements climatiques (Zinke et al. 2014). L'une des plus importantes publications de ces dernières années dresse un comparatif du Grand Récif entre celui observé en 2008 et 40 ans plus tôt (Harris et al. 2010). Dans ce rapport, il est sans surprise question d'une diminution drastique de la couverture corallienne au niveau du platier et des stocks de poissons, mais aucune mention n'est faite quant aux antipathaires.

Tableau 22. Types de réserves à Madagascar selon le modèle de l'IUCN et concepts introduits par la Vision Durban.

Catégorie IUCN	Nom à Madagascar	Définition	Gestion	Introduit par Vision Durban
I	Réserves Naturelles Intégrales (RNI)	Protection biodiversité, zones d'intérêt géologique, où les impacts anthropiques sont strictement contrôlés et limités	Gestion gouvernementale par MNP	Non
II	Parcs Nationaux (PN)	Grande aire naturelle établie afin de protéger des écosystèmes à grande échelle, en plus des espèces caractéristiques de l'endroit, mais qui fournit également un lieu adéquat pour la recherche scientifique, l'éducation, le tourisme et la culture locale.	Gestion gouvernementale par MNP	Non
III	Monuments Naturels (MN)	Protection d'un momument particulier tel qu'une carverne, un mont sous-marin, un réseau sous-terrain, etc… Souvent de petite taille mais à grande affluence touristique.	Co-gestion des aires protégées entre gouvernement (Ministère de l'Environnement, l'Administration Nationale des Forêts, …) et acteurs non gouvernementaux (associations villageoises, ONG, …)	Oui
IV	Réserves spéciales (RS)	La priorité est donnée à la gestion de la protection d'un habitat ou d'une espèce en particulier.	Gestion gouvernementale par MNP	Non
V	Paysages Harmonieux Protégés (PHP)	Zone où l'intéraction de l'Homme avec la Nature a créé avec le temps un caractère particulier, avec des valeurs écologiques, biologiques, culturelles et scéniques significatives	Co-gestion des aires protégées entre gouvernement (Ministère de l'Environnement, l'Administration Nationale des Forêts, …) et acteurs non gouvernementaux (associations villageoises, ONG, …)	Oui
VI	Réserves de Ressources Naturelles (RRN)	Dédié à la conservation d'écosystèmes et d'habitats en parallèle à l'utilisation durable des ressources naturelles et aux valeurs culturelles. L'utilisation non-industrielle des ressources est compatible avec la conservation de la Nature.	Co-gestion des aires protégées entre gouvernement (Ministère de l'Environnement, l'Administration Nationale des Forêts, …) et acteurs non gouvernementaux (associations villageoises, ONG, …)	Non

A l’heure actuelle, Madagascar comprend 52 aires marines protégées, qui ne représentent que 0.75% de la surface de ses eaux soit moins de 9000 km² sur plus de 1 200 000 km² (Fig. 76). Elles sont réparties tout autour du pays, excepté au niveau du Sud-Est où il n’existe aucune zone surveillée. C’est au niveau du Sud-Ouest que les aires marines protégées sont les plus nombreuses, alors que paradoxalement le Grand Récif n’en est toujours pas une. Il est d’ailleurs très surprenant de voir que les récifs qui l’entourent sont repris dans des zones protégées ou de gestion durable alors que lui-même n’est jamais pris en compte, bien qu’il soit la structure récifale la plus grande de la région (Figs. 76, 77 ; Tableau 23).

Tableau 23. Aires marines protégées du Sud-Ouest reprenant toute la zone d’étude de cette thèse, de Maromena à Andavadoaka. Les numéros se réfèrent à la figure 77.

Numéro de l’aire	Nom	Catégorie IUCN	Type	Statut	Année
1	Velondriake	V	Paysage Harmonieux Protégé	Désigné	2006
2	Manjaboaka	-	Aire Marine Gérée Localement	Désigné	2009
3	Soariake	VI	Réserve de Ressources Naturelles	Désigné	2008
4	Baie de Salary	-	Aire Marine Protégée	Proposé par WWF	2010
5	Fimihara	-	Arie Marine Gérée Localement	Désigné	2006
6	Tahosoa	-	Aire Marine Gérée Localement	Désigné	2008
7	Nosy Ve	II	Parc National	Désigné	2006
8	Littoral Sud Toliara	-	Aire de pêche en gestion collaborée	Proposé par WWF	2007
9	Beheloke	-	Aire Marine Gérée Localement	Désigné	2008
10	Barrière de corail Nosy Ve Androka	-	Site Ramsar et Parc National	Désigné	2017

Figure 76. Carte des Aires Marines Protégées à Madagascar. La plupart se trouvent au niveau du sud-ouest du pays, alors qu'aucune réserve n'existe sur la côte est. Données adaptées d'*Atlas of Marine Protection.*

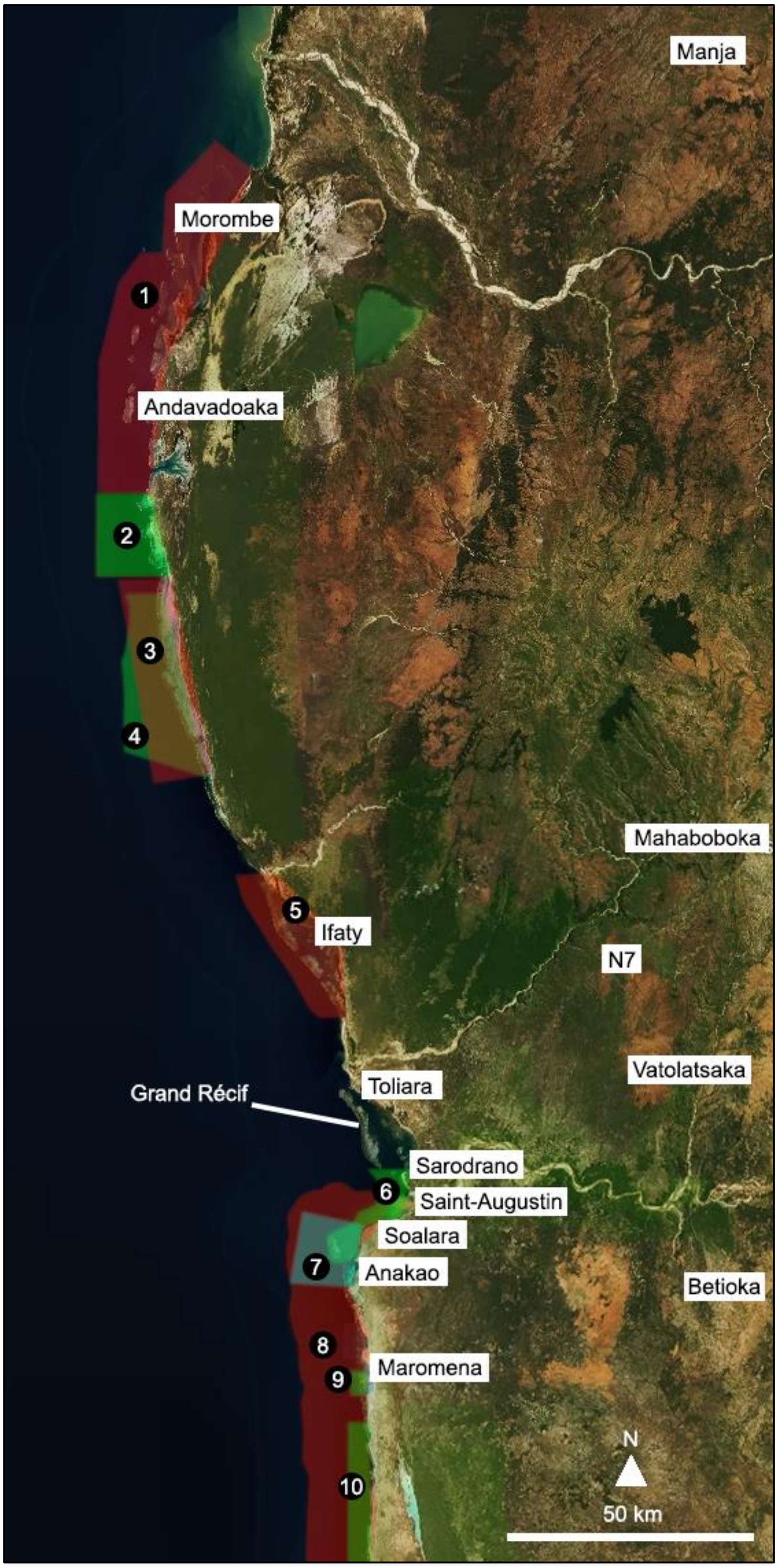


Figure 77. Détail des Aires Marines Protégées du sud-ouest de Madagascar, délimitant également la zone d'étude de cette thèse. Il apparait très clairement que les zones protégées entourent le Grand Récif sans jamais le prendre en compte. Les différentes couleurs n'ont pas de signification et permettent juste une meilleure vision des zones superposées.

Parmi les 10 aires protégées exposées ci-dessus, la Baie de Salary et le Littoral Sud Toliara ont été proposés par le WWF Madagascar mais ne bénéficient toujours pas d'un statut officiel national. La barrière de corail « Nosy Ve – Androka » possède à la fois le titre de Parc National ainsi que de site Ramsar. Ce statut fut instauré à la Convention de Ramsar (Iran) en 1971 afin de protéger les zones humides de hautes valeur biologique et écologique. Si certaines de ces zones sont directement gérées par Madagascar National Parks, nombreuses sont celles dont la gestion est déléguée aux communautés locales en collaboration avec des organisations non gouvernementales. Madagascar sert d'ailleurs d'exemple en ayant un taux de création d'aires marines gérées localement (AMGL) inégalé en Afrique de l'Est ou dans l'Océan Indien (Harris 2011). L'exemple le plus significatif de la réussite des AMGL à Madagascar est celui de l'aire de Velondriake (signifiant « vivre avec la mer ») établie conjointement entre la WCS, l'ONG Blue Ventures et l'Institut Halieutique et des Sciences Marines (IHSM) de l'Université de Toliara avec l'appui du Ministère de l'Agriculture, de l'Elevage et de la Pêche en 2006 (Harris 2007). Cette réserve de 1000 km² en fait la plus large de l'Océan Indien en plus d'être la première à déléguer la gestion aux 24 communautés locales réunies au sein de cette zone comprenant des îles, des mangroves, des lagons et des récifs coralliens (Harris 2007). Dans cette partie du pays, 85% des revenus proviennent directement de la pêche ou de la récolte sur le récif (Harris 2007). Le plan de gestion durable instaure des zones de fermetures permanentes et temporaires au cours de l'année, permettant ainsi la protection des saisons de reproduction et le maintien des stocks. Depuis, il en résulte une augmentation significative de la capture et du rendement de l'effort de pêche pour les communautés locales (Harris 2007). Cette AMGL permet également une augmentation significative des revenus pour les communautés locales, qui revendent leurs produits de la mer à des sociétés collectrices et exportatrices (Harris 2007). Grâce à ces mesures, la pêche aux poulpes dans le sud-ouest de Madagascar est reconnue comme une des premières pêches traditionnelles africaines sous la certification Marine Stewardship Council (MSC).

Les communautés locales gèrent les aires protégées par l'intermédiaire des *dina*, qui sont des lois traditionnelles établies par les villages et qui sont reconnues de manière légale par l'Etat. Les *dina* permettent notamment de réguler l'accès aux ressources, de bannir certaines pratiques de pêche destructrices, de surveiller les zones protégées et punir les infractions qui y sont commises (Andriamalala & Gardner 2010). Ces lois malgaches locales renforcent la protection des lieux et la cohésion au sein des communautés (Astuti

1995). Certaines traditions culturelles ou religieuses se transmettent également de générations en générations et portent le nom de *fady*. Ces dernières interdisent généralement l'accès ou l'exploitation de certains endroits, mais leur existence est d'avantage sacrée que liée à la sauvegarde des ressources ou de la biodiversité (Astuti 1995). Ce sont ces lois et coutumes locales qui animent le bon déroulement de la gestion des AMP, en témoignent les réunions organisées avec les chefs d'Ambatomilo (réserve de Soriake) pour obtenir les autorisations de prospecter les coraux noirs à cet endroit dans le cadre de cette thèse, ou à Andavadoaka (réserve de Velondriake) où les réunions se sont tenues avec les représentants du village et l'ONG Blue Ventures.

L'implication des communautés locales dans la gestion d'aires protégées est le seul moyen efficace et viable sur le long terme d'assurer à la fois la pérennité de la biodiversité mais également la survie et le bien-être des populations locales (White et al. 2000 ; Francis et al. 2002). Pour que ce soit efficace, trois conditions sont impératives : (i) une surveillance efficace du site à protéger, (ii) une aide continue de l'organisation qui s'occupe de l'établissement de la réserve, et (iii) la génération rapide de bénéfices pour les populations locales (Beger et al. 2004).

L'établissement d'une AMP au niveau du Grand Récif est déjà bien renseigné au travers de thèses de doctorat effectuées à l'Université de Tuléar ainsi que de publications scientifiques impliquant des partenaires étrangers (Rakotoarinivo 1994 ; Ranaivomanana 2006 ; ; Mahafina et al. 2009 ; Brenier et al. 2011 ; Mahafina 2011 ; Gardner et al. 2013 ; Ramahatratra 2014). Ratsimbazafy (2011) et Voajanahary (2011) détaillent les différentes étapes nécessaires à la mise en place d'une aire protégée gérée par les communautés locales, un processus déjà bien connu et établi dans le Sud-Ouest et dont le plan proposé ci-dessous en sera inspiré.

Dans le cas présent, la première phase consiste à informer les institutions non gouvernementales et gouvernementales des résultats obtenus durant cette thèse afin de leur exposer la nécessité de protéger le champ d'antipathaires de Tuléar. Le support scientifique de base nécessaire à la protection sera exposé par l'intermédiaire de l'IHSM, qui représenterait l'institution académique dans cette démarche participative. Durant la seconde phase du projet, ces mêmes acteurs doivent sonder et sensibiliser les villageois avec l'appui du ministère compétant. Contrairement aux autres réserves de la région, il n'est pas réaliste de promettre aux villageois une augmentation du nombre et de la diversité des ressources halieutiques conséquente et rapide grâce à la protection des coraux noirs.

Néanmoins l'écotourisme devrait être une solution viable à moyen terme et pourrait générer une source de revenu pour les populations locales. En effet, contrairement aux idées véhiculées, le Grand Récif présente de sérieux atouts pouvant attirer les amateurs de plongée. En dehors du champ de coraux noirs, d'autres sites sur le Grand Récif méritent plus de publicité de la part de l'Office du Tourisme de Tuléar (ORTU). Une gestion touristique peut être mise en place en prenant exemple sur la réserve voisine au nord, celle de FIMIHARA et qui comprend Ifaty et son site touristique, le Massif des Roses. A cet endroit, la pêche est formellement interdite et surveillée par les locaux, qui appliquent les *dina* et qui permettent le maintien des stocks de poissons pélagiques ainsi que le bon état des récifs (Belle et al. 2009). Les touristes doivent s'acquitter d'un droit d'entrée pour pouvoir plonger dans cette réserve (Belle et al. 2009). Le site bénéficie de campagnes de publicités multilingues dans les hôtels, les clubs de plongées, l'ORTU, et les livres de voyage (Belle et al. 2009). Une telle visibilité est primordiale pour l'instauration d'une activité de plongée touristique sur le Grand Récif de Tuléar.

Enfin, après un retour positif des communautés locales, la troisième phase consiste en l'établissement concret de la réserve d'un point de vue administratif et légal. Selon le modèle IUCN et le décret 2005-013 organisant l'application de la loi n°2001-005 portant Code de Gestion des Aires Protégées, le modèle de protection des antipathaires suivant est proposé :

1. Une réserve de type VI englobant l'entièreté du Grand Récif et de ses passes, c'est-à-dire une Réserve de Ressources Naturelles avec plusieurs « noyaux durs » dont un serait le champ de coraux noirs, le Grand Récif étant la zone de protection où la pêche resterait autorisée. Le noyau dur comprenant le champ d'antipathaire serait une réserve de type IV, c'est-à-dire une Réserve Spéciale où la pêche, le dragage et le mouillage seraient totalement interdits.
2. La gestion serait mise en place par des représentants des villages de l'agglomération de Tuléar. Des *dina* seraient établis afin de surveiller et punir les infractions liées à l'aire protégée et l'appui administratif, scientifique et financier proviendrait à la fois de l'état via MNP, d'institutions académiques telle que l'IHSM, et d'ONG telles que WWF et WCS.
3. Il sera nécessaire d'établir une concertation entre la délégation villageoise en charge de la réserve de la passe nord et celles des villages voisins, étant donné la

présence des antipathaires à tous ces endroits, afin de généraliser l'interdiction de la collecte de coraux noirs à toute cette partie de la côte dans un premier temps.

Dans l'AMP suggérée, le « noyau dur » de la passe nord se justifie par plusieurs raisons. Premièrement, il n'est pas possible de pratiquer une collecte durable comme c'est le cas à Hawaii étant donné l'existence d'un champ unique ne permettant pas la division en différentes zones de collectes et de fermetures et donc le rétablissement des stocks dans un intervalle de temps suffisamment large (Grigg 2001). Ensuite, l'existence de colonies presque centenaires témoignent d'un écosystème ancien dont les coraux présentent une vitesse de croissance trop faible pour être exploités durablement. Troisièmement, il fut démontré que la reproduction des antipathaires se manifeste par plusieurs événements de largage des gamètes tout au long de l'année, par conséquent une fermeture temporaire associée à une période de pêche n'est pas envisageable pour la pérennité des populations. Enfin, beaucoup d'organismes dépendent des antipathaires pour leur survie et leur reproduction, et de nouvelles espèces sont encore à découvrir comme l'a démontré cette thèse.

D'un point de vue « infrastructures », la solution la plus confortable serait la mise en place de de bouées de balisage délimitant toute la zone de noyau dur comprenant les antipathaires. Ces bouées pourraient également servir de lignes de mouillage afin d'éviter de jeter l'ancre sur les antipathaires lors de plongées scientifiques ou touristiques (Fig. 78). Le problème de telles installations réside dans le balisage apparent lui-même, car il devient facilement la cible de vols commis par les pêcheurs qui n'hésitent pas à s'approprier tout matériel susceptible d'améliorer leurs filets de pêche. De plus, la passe nord est un lieu de passage obligatoire pour les navires approchant ou appareillant le port de Tuléar et la mise en place de ces balises pourrait constituer un obstacle à ce trafic maritime. Dès lors, la solution la plus réaliste reste de se fier aux bonnes connaissances des sites par les marins et les guides touristiques, ou de se référer à des points GPS pour les clubs de plongée.

Enfin, pour établir l'organisation de la gestion de l'aire protégée, le modèle protégeant le Massif des Roses à Ifaty et son association FIMIHARA est à prendre en compte. Cette association fut créée dans le but de gérer cette AMP en réunissant à la fois des populations locales, les gouvernements régionaux et locaux, les acteurs du secteur privé tels que les opérateurs touristiques, les ONGs de protection de l'environnement ainsi que les institutions académiques. Pour le noyau dur de l'AMP de Tuléar, le nom de l'association « TANGOARAKY » est proposé, celui-ci signifiant « corail noir » en malgache (Fig. 79).

L'association comprendrait de cette manière des membres de fonction diverses ainsi qu'un comité principal. Le rôle de chaque partie dans l'association TANGOARAKY est présentée dans le tableau 24 dont la structure se base sur l'organisation implémentée dans l'association FIMIHARA (Belle et al. 2009). En conclusion, l'association aboutirait sur le long terme à (i) la protection du champ d'antipathaires et de sa biodiversité associée, (ii) au développement du secteur touristique, (iii) à la création d'emplois pour les populations locales par l'intermédiaire de gardiens ou de guides touristiques, et (iv) à la sensibilisation du grand public quant à la protection de la Nature.

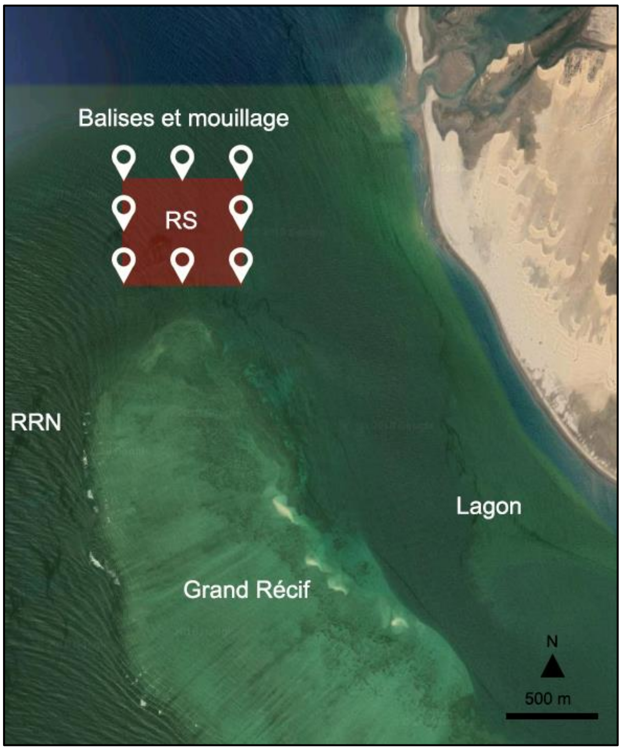


Figure 78. AMP de Tangoaraky permettant la protection du champ d'antipathaires de la passe nord du Grand Récif de Tuléar. Cette zone sera totalement fermée, c'est-à-dire que le mouillage où le chalutage/dragage sont interdits dans la zone. Les pirogues peuvent y circuler librement et y pratiquer la pêche pour autant que les filets soient en surface. Pour délimiter la zone, des bouées de balisage seront placées et seront clairement identifiables en surface par les pêcheurs ou les marins. Aussi bien les marins amenant des touristes que les bateaux de recherche scientifique de l'IHSM pourront mouiller sans risque d'endommager les antipathaires grâce à ces balises. Les gardiens veilleront à ce qu'il n'y ait aucune infraction commise dans la zone balisée.

Tableau 24. Organisation et structure de l'association Tangoaraky basées sur Belle et al. (2009).

Partie concernée	Rôle dans la réserve de coraux noirs
Communautés locales	
Pêcheurs	Respect et application des dina locales
Gardiens	Désigné par le comité TANGOARAKY, leur tâche principale est la surveillance du site
Comité de gestion locale	Composé de représentants de différents quartiers et villages de Tuléar en bord de mer, ils élaborent les dina et les amendes, désignent les gardiens et veillent au respect de la protection de l'aire, et représentent les villageois au comité TANGOARAKY
Organisations non-gouvernementales	
ONG telle que WWF ou WCS	Recherche, éducation et initiatives de conservation dans la réserve, notamment par des campagnes de sensibilisation, acteurs principaux dans la gestion de la réserve, membres et conseillers au sein de TANGOARAKY
Institution académique	
IHSM (et partenaire belge UMONS)	Apporte le support scientifique en recherche et développement nécessaire à la gestion des aires protégées, mais possèdent également un rôle d'éducation et de sensibilisation, membres et conseillers scientifiques principaux au sein de TANGOARAKY
Secteur privé	
Hôtels et clubs de plongés représentés à l'Office Régional du Tourisme de Tuéar	Vendent les tickets nécessaires à l'entrée dans la réserve, assurent la promotion publicitaire du site, sensibilisent le public à la protection de l'environnement, commentent les projets de dina via un représentant au sein du comité TANGOARAKY
Propriétaires de pirogues	Promotion du site auprès des touristes, assurent les visites et leur bon déroulement, membres de TANGOARAKY
Touristes	Paiement du droit d'entrée dans la réserve, sensibilisation relative à la conservation du site et la protection de la Nature via les campagnes de TANGOARAKY
Gouvernements	
Maire de la commune de Toliara	Approuve et signe les dina locales, membre de TANGOARAKY
Fokontany (administration de quartiers)	Consulte les propositions de dina, approuve et signe les dina, représente l'adiminstration villageoise dans le comité TANGOARAKY
Direction Régionale du Développement Rural de Toliara et Ministère de l'Agriculture, de l'Elevage et de la Pêche	Consulte, approuve et signe les dina, membres de TANGOARAKY
Chef de district et chef de police de Toliara, Ministère des Eaux et Forêts	Signe les lois locales et légalise le statut de l'association TANGOARAKY

Figure 79. Hypothétique future Aire Marine Protégée de Tangoaraky gérée par l'association portant le même nom et proposition de logo. La photo prise à -20 m illustre une scène que les touristes plongeurs pourront y admirer.

BIBLIOGRAPHIE – DISCUSSION GENERALE

Andriamalala, G., & Gardner, C. J. (2010). L'utilisation du dina comme outil de gouvernance des ressources naturelles: leçons tirées de Velondriake, sud-ouest de Madagascar. Tropical Conservation Science, 3(4), 447-472.

Andrefouet, S., Guillaume, M. M., Delval, A., Rasoamanendrika, F. M. A., Blanchot, J., & Bruggemann, J. H. (2013). Fifty years of changes in reef flat habitats of the Grand Récif of Toliara (SW Madagascar) and the impact of gleaning. Coral Reefs, 32(3), 757-768.

Astuti, R. (1995). People of the Sea: Identity and Descent among the Vezo of Madagascar (No. 95). Cambridge University Press.

Beger, M., Harborne, A. R., Dacles, T. P., Solandt, J. L., & Ledesma, G. L. (2004). A framework of lessons learned from community-based marine reserves and its effectiveness in guiding a new coastal management initiative in the Philippines. Environmental management, 34(6), 786-801.

Belle, E. M., Stewart, G. W., De Ridder, B., Komeno, R. J., Ramahatratra, F., Remy-Zephir, B., & Stein-Rostaing, R. D. (2009). Establishment of a community managed marine reserve in the Bay of Ranobe, southwest Madagascar. Madagascar Conservation & Development, 4(1).

Bo M., Baker A.C., Gaino E., Wirshing H.H., Scoccia F., Bavestrello G. (2011). First description of algal mutualistic endosymbiosis in a black coral (Anthozoa: Antipatharia). Marine Ecology Progress Series, 435: 1–11.

Bo M., Bavestrello G., Barucca M., Makapedua D.M., Poliseno A., Forconi M., Olmo E., Canapa A. (2012). Morphological and molecular characterization of the problematic whip black coral genus *Stichopathes* (Hexacorallia: Antipatharia) from Indonesia (North Sulawesi, Celebes Sea). Zoological Journal of the Linnean Society, 166: 1–13.

Brenier, A., Ferraris, J., & Mahafina, J. (2011). Participatory assessment of the Toliara Bay reef fishery, southwest Madagascar. Madagascar Conservation & Development, 6(2), 60-67.

Brugler M.R., Opresko D.M., France S.C. (2013) The evolutionary history of the order Antipatharia (Cnidaria: Anthozoa: Hexacorallia) as inferred from mitochondrial and nuclear DNA: implications for black coral taxonomy and systematics. Zoological Journal of the Linnean Society, 169(2), 312-361.

Cardona Y., Ruiz-Ramos D.V., Baums I.B., Bracco A. (2016). Potential connectivity of coldwater black coral communities in the northern Gulf of Mexico. PloS one, 11(5): e0156257.

Carreiro-Silva M., Andrews A.H., Braga-Henriques A., De Matos V., Porteiro F.M., Santos R.S. (2013). Variability in growth rates of long-lived black coral *Leiopathes* sp. from the Azores. Marine Ecology Progress Series, 473: 189–199.

Cooke, A., Ratomahenina, O., Ranaivoson, E., & Razafindrainibe, H. (2000). Madagascar. Seas at the Millennium, C. Sheppard (ed.), 1.

Cripps, G., & Gardner, C. J. (2016). Human migration and marine protected areas: Insights from Vezo fishers in Madagascar. Geoforum, 74, 49-62.

Gardner, C. J., Nicoll, M. E., Mbohoahy, T., Oleson, K. L., Ratsifandrihamanana, A. N., Ratsirarson, J., de Roland L-A. R., Virah-Sawmy, M., Zfindrasilivonona B. & Davies, Z.

G. (2013). Protected areas for conservation and poverty alleviation: experiences from Madagascar. Journal of Applied Ecology, 50(6), 1289-1294.

Grigg, R. W. (2001). Black coral: History of a sustainable fishery in Hawai'i. Pacific Science, 55(3), 291-299.

Flot J.F., Couloux A., Tillier S. (2010). Haplowebs as a graphical tool for delimiting species: a revival of Doyle’s “field for recombination” approach and its application to the coral genus *Pocillopora* in Clipperton. BMC Evolutionary Biology, 10: 372.

Flot J.F., Blanchot J., Charpy L., Cruaud C., Licuanan W.Y., Nakano Y., Payri C., Tillier S. (2011). Incongruence between morphotypes and genetically delimited species in the coral genus *Stylophora*: phenotypic plasticity, morphological convergence, morphological stasis or interspecific hybridization? BMC Ecology, 11:22.

Francis, J., Nilsson, A., & Waruinge, D. (2002). Marine protected areas in the Eastern African region: how successful are they? A Journal of the Human Environment, 31(7), 503-511.

Freudenberger, K. (2010). Paradise lost? Lessons Learnt from 25 Years of USAID Environment Programs in Madagascar, a United States Agency for International Development (USAID) Commissioned Report, Washington, United-States.

Goldberg W.M. (1991). Chemistry and structure of skeletal growth rings in the black coral *Antipathes fiordensis* (Cnidaria, Antipatharia). In Coelenterate Biology: Recent Research on Cnidaria and Ctenophora, pp. 403–409).

Grigg R.W. (1974). Distribution and abundance of precious corals in Hawaii. In Proc. Second International Symposium on Coral Reefs, Great Barrier Reef, Australia, Vol. 2, pp. 233–240.

Harris, A. R. (2007). “To live with the Sea”: Development of the Velondriake Communitny-Managed Protected Area Network, Southwest Madagascar. Madagascar Conservation & Development, 2(1).

Harris, A. R. (2011). Out of sight but no longer out of mind: a climate of change for marine conservation in Madagascar. Madagascar Conservation & Development, 6(1).

Harris, A. R., Manahira, G., Sheppard, A., Gouch, C., & Sheppard, C. (2010). Demise of Madagascar's once great barrier reef: changes in coral reef conditions over 40 years. Atoll Research Bulletin, 574, 1-18.

Knutson D.W., Buddemeier R.W., Smith S.V. (1972). Coral chronometers: seasonal growth bands in reef corals. Science, 177(4045): 270–272.

Kremen, C., Cameron, A., Moilanen, A., Phillips, S. J., Thomas, C. D., Beentje, H., Dransfield, J., Fisher B.L., Glaw, F., Good, T.C., Harper, G.J., Hijmans, R.J., Lees, D.C., Louis, E. Jr., Nussbaum, R.A., Raxworthy, C.J., Razafimpahanana, A., Schatz, G.E., Vences, M., Vieites, D.R., Wright, P.C. and Zjhra, M.L. (2008). Aligning conservation priorities across taxa in Madagascar with high-resolution planning tools. Science, 320, 222-226.

Kull, C. A. (2014). The roots, persistence, and character of Madagascar's conservation boom. In : Conservation and environmental management in Madagascar, pp. 170-195.

Lapian H.F.N. (2009) Biodiversity study of black coral (order: Antipatharia) collected from Manado, Indonesia based on rDNA internal transcribed spacer (ITS) sequences analysis. Biodiversitas, 10(1): 1-5.

Lapian H.F.N., Barucca M., Bavestrello G., Biscotti M.A., Bo M., Canapa A., Tazioli S., Olmo E. (2007) A systematic study of some Black Coral species (Antipatharia, Hexacorallia) based on rDNA internal transcribed spacers sequences. Marine Biology 151: 785–792.

Lopez-Gonzalez P. & Cunha M.R. (2010) Two new species of *Dendrobrachia* Brook, 1889 (Cnidaria: Octocorallia: Dendrobrachiidae) from the north-eastern Atlantic and western Mediterranean. Scientia Marina 74(3): 423–434.

Love M.S., Yoklavich M.M., Black B.A., Andrews A.H. (2007). Age of black coral (Antipathes dendrochristos) colonies, with notes on associated invertebrate species. Bulletin of Marine Science, 80(2): 391–399.

MacIsaac K.G., Best M., Brugler M.R., Kenchington E.L.R., Anstey L.J., Jordan T. (2013). *Telopathes magna* gen. nov. spec. nov. (Cnidaria: Anthozoa: Antipatharia: Schizopathidae) from deep waters off Atlantic Canada and the first molecular phylogeny of the deep-sea family Schizopathidae. Zootaxa, 3700(2): 237-258.

Maina, J., de Moel, H., Vermaat, J. E., Bruggemann, J. H., Guillaume, M. M., Grove, C. A., Madin, J.S., Mertz-Kraus, R. & Zinke, J. (2012). Linking coral river runoff proxies with climate variability, hydrology and land-use in Madagascar catchments. Marine Pollution Bulletin, 64(10), 2047-2059.

Mahafina, J., Lecaillon, G., Chabanet, P., Ralijaona, C., Ranaivomanana, L., Bosc, P., & Ferraris, J. (2009). Capture and culture of post-larval fish: a possible alternative to overexploitation of artisanal fisheries in Toliara (Madagascar). In: Book of Abstracts, 6th Western Indian Ocean Marine Science Association Scientific Symposium, Saint Denis, La Réunion (p. 145).

Mahafina, J. (2011). Perception et comportement des pêcheurs pour une gestion durable de la biodiversité et de la pêcherie récifale : application au niveau des réserves marines temporaires du Sud-Ouest de Madagascar. Thèse de doctorat, Université de Toliara (IH.SM), Madagascar.

Marschal C., Garrabou J., Harmelin J.G., Pichon M. (2004). A new method for measuring growth and age in the precious red coral *Corallium rubrum* (L.). Coral reefs, 23(3) : 423–432.

Molodtsova TN, Budaeva N (2007). Modifications of corallum morphology in black corals as an effect of associated fauna. B Mar Sci 81:469–479.

Myers, N., Mittermeier, R. A., Mittermeier, C. G., Da Fonseca, G. A., & Kent, J. (2000). Biodiversity hotspots for conservation priorities. Nature, 403(6772), 853.

Nicoll, M.E., & Langrand, O. (1989). Madagascar : Revue de la Conservation et des Aires Protégées. World Wide Fund for Nature, Gland, Switzerland.

Opresko D.M., Wagner D., Montgomery A.D., Brugler M.R. (2012). Discovery of *Aphanipathes verticillata* (Cnidaria: Anthozoa: Antipatharia) in the Hawaiian Islands. Zootaxa, 3348(1): 24–39.

Pichon, M. (1978). Recherches sur les peuplements à base de Scléractiniaires dans les récifs de la Baie de Tuléar (Sud-Ouest de Madagascar). Atoll Research Bulletin, 222, 1-447.

Prouty N.G., Roark E.B., Buster N.A., Ross S.W. (2011). Growth rate and age distribution of deep-sea black corals in the Gulf of Mexico. Marine Ecology Progress Series, 423 : 101–115.

Rakotoarinivo, A. W. (1994). Pêches expérimentales sur les premiers dispositifs de concentration de poissons dans la région de Toliara (sud-ouest de Madagascar). Thèse de doctorat, Université de Toliara (IH.SM), Madagascar.

Ramahatratra, F. (2014). Etude de la capacité de résilience du Grand Récif de Toliara et de sa gestion durable. Facteur : poissons herbivores. Thèse de doctorat, Université de Toliara (IH.SM), Madagascar.

Ramanantsoa, J. D., Penven, P., Krug, M., Gula, J., & Rouault, M. (2018). Uncovering a new current: The Southwest MAdagascar coastal current. Geophysical Research Letters, 45(4), 1930-1938.

Ranaivomanana, L.N.J. (2006). Identification des conditions d'appropriation de la gestion durable des ressources naturelles et des écosystèmes : cas du Grand Récif de Toliara. Thèse de doctorat, Université de Toliara (IH.SM), Madagascar.

Rasoavahiny, L., Andrianarisata, M., Razafimpahanana, A. and Ratsifandrihamanana, A.N. (2008). Conducting an ecological gap analysis for the new Madagascar protected area system. Parks, 17, 12-21.

Ratsimbazafy, R. A. (2011). L'océan au coeur de la Grande Île: Les aires marines protégées, un outil de développement durable pour Madagascar. Madagascar Conservation & Development, 6(1).

Risk M.J., Heikoop J.M., Snow M.G., Beukens R. (2002). Lifespans and growth patterns of two deep-sea corals: *Primnoa resedaeformis* and *Desmophyllum cristagalli*. Hydrobiologia, 471(1-3): 125–131.

Roark E.B., Guilderson T.P., Flood-Page S., Dunbar R.B., Ingram B.L., Fallon S.J., McCulloch M. (2005). Radiocarbon-based ages and growth rates of bamboo corals from the Gulf of Alaska. Geophysical Research Letters, 32(4).

Ruiz-Ramos D.V. & Baums I.B. (2014). Microsatellite abundance across the Anthozoa and Hydrozoa in the phylum Cnidaria. BMC genomics, 15(1): 939.

Ruiz-Ramos D.V., Saunders M., Fisher C.R., Baums I.B. (2015). Home bodies and wanderers: Sympatric lineages of the deep-sea black coral *Leiopathes glaberrima*. PloS one, 10(10): e0138989.

Summers S.L. (1910). VIII.—Antipatharians from the Indian Ocean. Journal of the Royal Microscopical Society, 30(3): 273-281.

Tazioli S, Bo M, Boyer M, Rotinsulu H, and Bavestrello G. (2007) Ecological observations of some common antipatharian corals in the marine park of Bunaken (North Sulawesi, Indonesia). Zoological Studies 46: 227–241.

Virah-Sawmy, M., & Gardner, C. J. (2014). The Durban Vision in practice: Experiences in the participatory governance of Madagascar's new protected areas. In: Conservation and environmental management in Madagascar, pp. 240-276.

Voajanahary, T.K. (2011). Nouveau zonage des réserves marines et plan de gestion des ressources naturelles dans le village Tahosoa, Saint-Augustion, Toliara. Mémoire de fin d'études, Université de Toliara (IHSM), Madagascar.

Wagner D. (2015) The spatial distribution of shallow-water (< 150 m) black corals (Cnidaria: Antipatharia) in the Hawaiian Archipelago. Marine Biodiversity Records, 8.

Wagner D., Brugler M.R., Opresko D.M., France S.C., Montgomery A.D., Toonen R.J. (2010). Using morphometrics, in situ observations and genetic characters to distinguish among commercially valuable Hawaiian black coral species; a redescription of *Antipathes grandis* Verrill, 1928 (Antipatharia: Antipathidae). Invertebrate Systematics 24: 271–290.

Wagner D., Pochon X., Irwin L., Toonen R.J., Gates R.D. (2011). Azooxanthellate? Most Hawaiian black corals contain Symbiodinium. Proceedings of the Royal Society of London B: Biological Sciences, rspb20101681.

White, A. T., Vogt, H. P., & Arin, T. (2000). Philippine coral reefs under threat: the economic losses caused by reef destruction. Marine Pollution Bulletin, 40(7), 598-605.

Williams B., Risk M.J., Ross S.W., Sulak K.J. (2006). Deep-water antipatharians: proxies of environmental change. Geology, 34(9): 773–776.

Zinke, J., Loveday, B. R., Reason, C. J. C., Dullo, W. C., & Kroon, D. (2014). Madagascar corals track sea surface temperature variability in the Agulhas Current core region over the past 334 years. Scientific reports, 4, 4393.

Annexes

FIRST RECORDS OF ILLEGAL HARVESTING AND TRADING OF BLACK CORALS (ANTIPATHARIA) IN MADAGASCAR.

TODINANAHARY G.*, TERRANA L.*, LAVITRA T., EECKHAUT I. (2016). Journal of Madagascar Conservation & Development 11(1), 6–11.
** Co-premiers auteurs.*

Introduction

Antipatharians, called black corals, are marine organisms that have attracted people for a long time. Historically, they were used by religious people and for medicinal purposes (Bruckner et al. 2008). During the Paleolithic, some populations used them as money to trade (Tescione 1968) and from antiquity to the present, they have been used worldwide mainly for jewellery. Small black coral fisheries have always existed all around the world, but it increased with the development of scuba diving equipment and techniques until it became an important source of income in several regions and countries, such as Cayman Islands, Cuba, Mexico, Taiwan, the Philippines and the Dominican Republic (Castorena and Metaca 1979). The latter three were responsible for the trade and export of 72 metric tons and 7,400,000 pieces of black coral between 1982 and 1998. In 1996, 473,000 black coral pieces imported into the United States were reported to be worth $447,000 (Guitart et al. 1997).

For now, these fisheries are best known, managed and controlled in the Hawaiian Islands. Grigg (1993) explains in detail the precious coral fisheries in the region. The black coral meadows located around them were discovered in 1958 at between 30 and 75 meters depth and 14 species were found within it, with 3 being harvested and exploited: *Antipathes griggi*, *Antipathes grandis*, and *Myriopathes ulex*. The others are found at over 100 meters depth and their diameter is not sufficient to be profitable to fishermen (Grigg and Opresko 1977). In 1969, black coral fisheries and precious coral exports to Asia were worth $2 million in Hawaii. Quickly, population and stock management problems appeared, and academic studies started in 1970 led to the development of a selective harvesting system utilizing a manned submersible (Grigg et al. 1973), allowing the development of the sustainable management of these resources. These selective fisheries and coral management are based on coral growth rates and reproductive cycles, which are supported and approved by the Western Pacific Regional Fisheries Management Council (Grigg 1976). Fishermen also need a license and an authorization to collect black corals around the Hawaiian Islands (Bruckner et al. 2008). The Hawaiian Islands make $50 million with the precious coral fisheries, with $33 million for black corals (Grigg 2010). These fishery industries employ about 1,000 people: the current wholesale value of unworked black coral being about $US 35 per pound, and the retail prices for manufactured black coral jewellery range from around $US 35 to $300 for earrings to over $US 3,000 for more ornate necklaces and bracelets (Grigg 1993, Kahng and Grigg 2005).

Elsewhere in the world, very little is known about black coral fisheries and management. At this time, the United States is the main importer, followed by Japan, but the USA is importing less than 1,000 pieces per year, which is a very small quantity of corals. The biggest provider of black corals is Taïwan, with more than 90 percent of the total of black coral legally sold, while the most harvested region is the Philippines. There is no official report of black coral trade in Africa nor in Madagascar, but it is notorious that the illegal trade occurs without exportation control and management. In Madagascar this illegal trade involves fishermen, small collectors in villages, foreign scuba divers and exporters in big cities where a kilogram of black coral is bought for €50 from fishermen.

The global conservation status of black coral has not been evaluated, but they are protected by international treaties restricting their exploitation and exportation/importation. According to the CITES (Convention on International Trade in Endangered Species of wild fauna and flora), the main threats are harvesting and invasive alien species such as the octocoral *Carijoa riise*i (Kahng and Grigg 2005). Black coral exploitation generally occurs in different steps: seabed exploration, discovery of black coral meadows, exploitation and depletion (Grigg 1989). Their stocks gradually run out, and fishermen continually search for new meadows to maintain sales. Overexploitation of black coral quickly leads to local population extinctions causing a great loss of biodiversity without a proper management plan. Conservation plans for black coral are, however, difficult to establish because these organisms are characterized by a slow growth, a delayed first reproduction (after about 10 years), a long life, an annual release of gametes, a high colony fecundity, a low recruitment of larvae and a slow rate of recovery when individuals are damaged (Parker et al. 1997). Mortality often results from sediment covering and substrate erosion (Grigg 1993). For the moment, there is no integrative biological data on shallow-water black coral populations from the Indian Ocean and it is consequently not possible to properly manage their populations in this region.

Black corals: unknown resources in Madagascar

Research on coral reefs began in Madagascar in 1961 with the establishment of the first marine station of Toliara. French researchers from the marine station of Endoume in France studied the southwestern region of Madagascar, including the Great Reef of Toliara (GRT) (E043° 20’, S23° 30’), and the coral reefs of the Bay of Ranobe (E042° 58', S23° 18'). The first published results describe the location and the physiography of these reefs (Clausade et al. 1971, Thomassin 1971, Battistini et al. 1975). Since this time, several research programs have been carried out on the coral reefs of the region (Pichon 1978, Laroche and Ramananarivo 1995, Salimo 1997, Vasseur 1997, Laroche et al. 1997, Gabrié et al. 2000, Bruggemann et al. 2012, Andréfouët et al. 2013), but none of these included black corals.

No scientific study on black corals has been made in Madagascar until now: their communities remain completely unknown. Moreover, there is no data available from the main fishery and marine science research centers of Madagascar (Fishery and Marine Science Institute-IH.SM and the National Centre for Oceanographic Research-CNRO), or from the National Environmental Research Centre (CNRE). This publication is the first to exclusively talk about antipatharians from Madagascar and the problems related to their illegal exploitation, with the lack of management.

The first official seizure of black corals

In 2014, a total of 178 kg of illegally-harvested black coral was seized by the Fisheries Control Centre (Centre de Surveillance des Pêches - CSP, based in the capital Antananarivo), in the southern regions of Anosy and Androy (Fig. 80, 81). Samples of the seizure were sent to the authors. The diameter of the base of the samples and their length were recorded with their origin (Table 25). Most of the black coral samples were fragments with a branch diameter higher than 35 mm (Fig. 80). The harvested corals seized in Tolagnaro were first branched corals with a bush-like shape before being cut into fragments. Our investigations have confirmed that 100 kg of black coral was made in

Andranobory, in the municipality of Analapatsy (Anosy region), bringing the number of official arrests in the Malagasy territory to four.

Table 25. Description of the black corals seized by the authorities in southern Madagascar. (*given as mean ± SD, ** not sampled given that the seizure happened after the sampling date).

Date of seizure	Samples	Weight (kg)	Basal Ø (mm)*	Length (mm)*	Region
March 24 2014	10	5	19.25 ± 5.67	199.4±71.65	Androy
May 15 2014	10	163	35.7 ± 6.63	395.5 ±97.22	Anosy
May 15 2014	10	10	12.05 ± 2.61	247.5 ± 72.61	Anosy
May 31 2015	NA**	100	NA	NA	Anosy

Our investigations have confirmed that 100 kg of black coral was made in Andranobory, in the municipality of Analapatsy (Anosy region), bringing the number of official arrests in the Malagasy territory to four. Dry tissues from these samples were also collected and fixed in 100% ethanol for DNA analysis. The primers LCOant forward and HCOant reverse were used to flank the CO-1 barcoding region (see Wagner et al. 2013 for the PCR conditions) and the same primers were used for DNA sequencing. Sequence analyses showed a high similarity with *Myriopathes* sp. and *Tanacetipathes* sp., two branched species belonging to the Myriopathidae family (Figure 85, unpublished data).

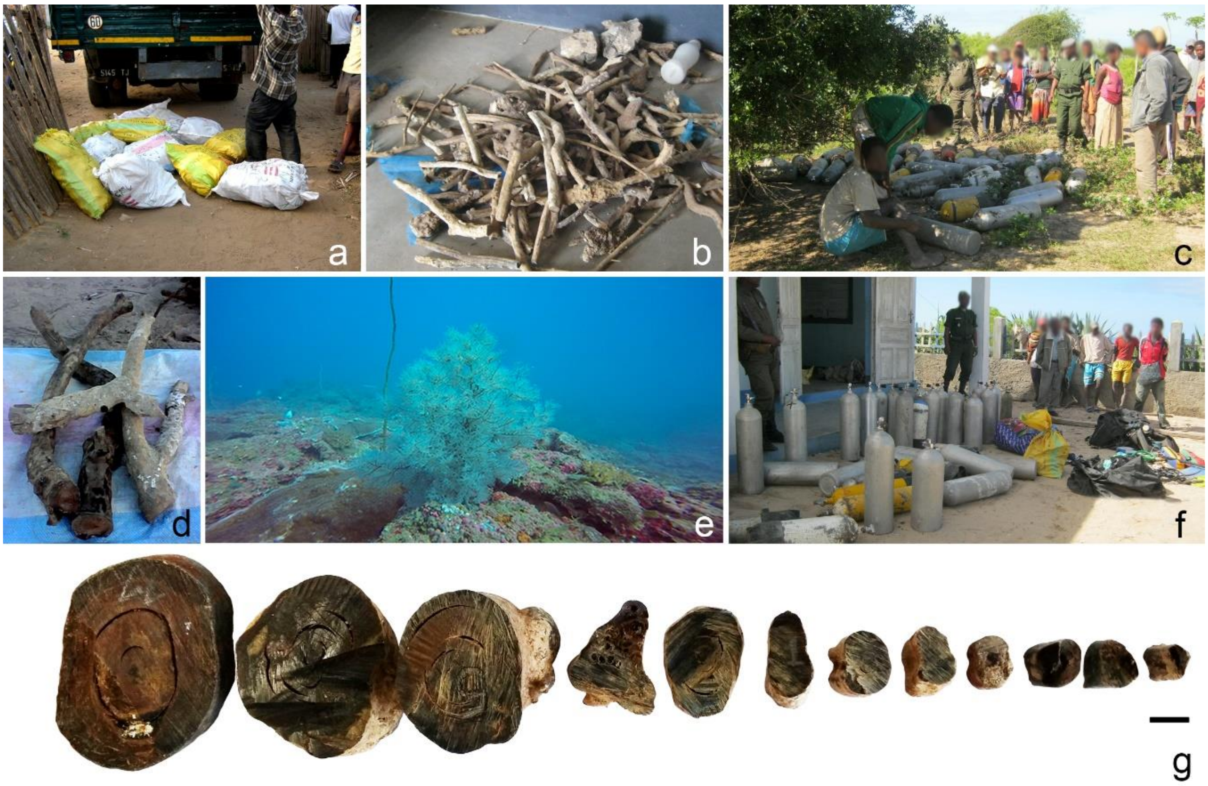
Black coral harvesting: an illegal but lucrative business

From the start of the operations in 2010 to today, it is known that antipatharians are collected from two regions of Madagascar: Anosy and Androy. These neighboring regions are located in the deep south of the country. They are famous for the aridity of their climate (especially in the Androy region) and the important richness of their marine resources (Bemiasa 2009). However, it is likely that harvesting of these marine resources occurs in other regions of the country. For example, in March 2012, a private operator officially requested an authorization to the Malagasy Ministry of Fishery and Aquatic Resources to collect black corals in four regions of Madagascar. The region of Androy was not targeted. Information from the CSP investigations were analyzed and cross-checked with interviews of local people (boat drivers, fishermen, fishing agent services and NGO staff) liable to fishing activities. Data obtained (Table 25) were used to estimate the average amount collected by each diver, each local fish trader (at the village level) and each seller (at the national level). According to all the people questioned, poachers come mainly from China with a lot of means, vehicles and diving materials (Supplementary Material, Table S1). It is estimated that a collection of 4-5 months could lead to the production of more than 5000 kilograms of black coral.

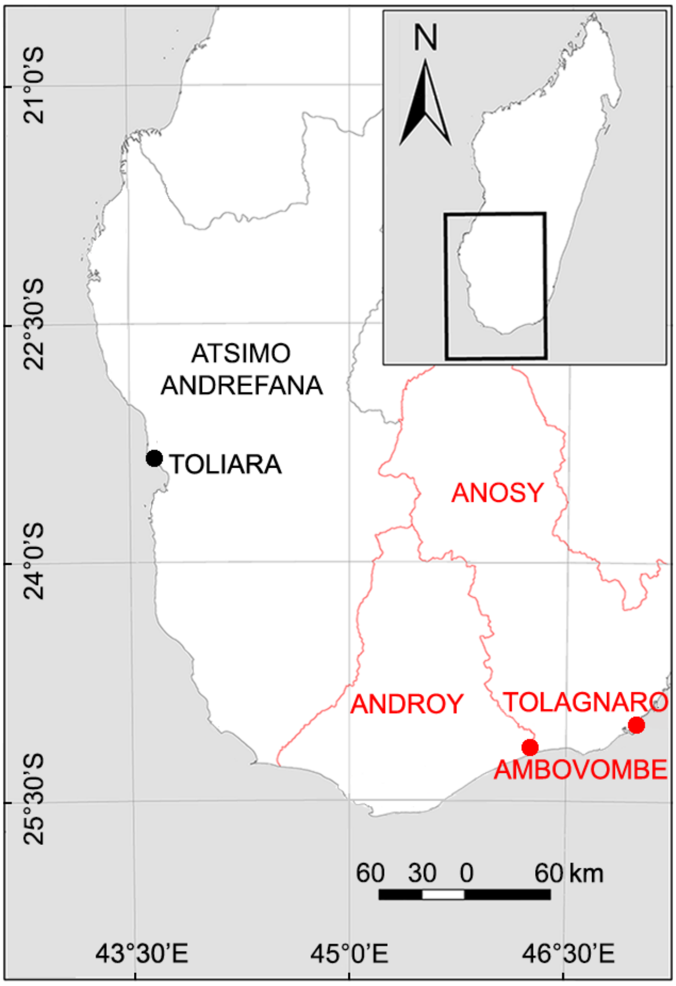
At the same time, a few farmers and managers from different regions of Madagascar contacted universities and local authorities to obtain either authorization or license to legally collect black corals (Supplementary Material, Table S2). In Madagascar, black corals are so lucrative that local people have named them the "rosewoods of the sea". Fishermen claim that the value of unworked black coral sold from the villages is between 10,000 to 15,000 Ariary per kilogram from scuba divers/fishermen to local collectors and 15,000 to 20,000 Ariary from local collectors to national collectors. The price per kilo from the capital Antananarivo for international exportation, mainly headed to Asia, ranges between 500,000 to 700,000 Ariary. In a country where a worker earns an average of 120,000 Ariary per month, black coral harvesting represents an assured money supply. According to Table 25, a scuba-diver harvesting black corals can earn 750,000 Ariary per day, his monthly salary reaching more or less 12 million Ariary. This is higher than the salary of a government official even when all costs related to the collection of corals are deducted. This income is a thousand times higher than the monthly revenue received by a traditional fisherman in Madagascar.

Table 26. Estimations of the amount of black corals collected and sold by people involved in these fisheries. (*scuba divers are professionals external to the villages).

Source of information	Scuba divers*	Fishermen	Local fishery service	NGO people
Role in the harvesting	Dive, collect, sale	Dive, collect, boat driver, sale	Should control trade	Interactions with people
Estimation of corals collected per day (kg)	50 ± 10	50 to > 300	> 100	> 40
Working day per week	4 ± 2	6 ± 1	5 ± 1	NA
Duration of the harvest season	26 weeks	84 weeks	72 weeks	NA
Estimation of total black coral collected (kg)	5.200	NA	NA	NA
Region	Anosy	Androy	Anosy	Anosy



▲ Figure 80. (a) Large bags containing dried black coral skeletons in Ambovombe, Androy. (b,d) Details of black coral skeletons collected. (c,f) Pictures of diving tanks used by poachers seized by the authorities in Kotoala, in the Ambazoa municipality of Ambovombe. (e) A branching black coral belonging to the Myriopathidae family photographed off the southwestern coast of Madagascar at approximatively 20 meters deep. This coral has the same shape and belongs to the same family as the corals harvested in Tolagnaro. (g) Pieces of black corals collected in the Anosy region showing the scale of corals that are harvested. (scale bar: 1 cm).



◄ Figure 81. Southern coast of Madagascar. The two main regions concerned by the illegal harvesting of black corals, Androy and Anosy, are highlighted in red. Their capitals are respectively Ambovombe and Tolagnaro.

Alerts, petitions and lobbying

People in Madagascar have become more and more interested in the harvest and sale of black corals, which are very lucrative. Fishermen and collectors from different regions of the country have tried to gather information about the geographical distribution and the available stock of black corals. As soon as researchers and students became aware of the fisher interest, alerts and lobbying have been launched with local authorities to inform them of the illegality of these banned fisheries.

A few months later, an alert about the illegal trade of black corals in Madagascar was launched by some students who took the initiative to start a petition. The purpose of this petition was to raise awareness and put pressure on government officials to promote the rational and sustainable management of fisheries resources. The petition, which was held in two stages, first invited the authorities to sign and publish laws prohibiting the exploitation of these resources. The second stage, which was launched after the release of a Ministerial Order, invited all administrative officials involved in the exploitation of these resources, including local authorities, the regional Director of fisheries, regions, gendarmerie and the national police, customs authorities and justice, to respect and apply the laws. The petition did not collect many signatures in three months, but the actions represented students, researchers and teachers from around the world (Madagascar, France, Norway, Belgium, United States, United Kingdom and Australia). The petition has become the forerunner of a wave of advocacy and lobbying conducted by environmental civil society organizations, such as FAMARI (Ala Fatidràn'ny Maike sy ny Riake), AVG (Alliance Voahary Gasy) and the Regional Platform of Civil Society Organizations of Atsimo Andrefana, which includes more than 250 associations and NGOs as members. These organizations are among the largest and most influential in Madagascar. All these actions have together led to the signature and publication of regional and national bylaws preventing and prohibiting any form of black coral exploitation.

Regional and national bylaws

Thanks to the willingness of leaders and managers of fisheries and marine resources service of the Region of Anosy, a regional bylaw suspending the collection of black corals in the region (Région Anosy. 2013. Arrêté n°336 REG /ANOSY du 12/12/2013 portant suspension de collecte de corail noir ou TANGOARAKY dans la Région Anosy) was enacted. This attitude of the Anosy regional authorities was an example for all the coastal areas authorities, for the responsible management of the marine resources of Madagascar. Indeed, a few weeks after the publication of the bylaw, the presence of about 10 scuba divers and black coral collectors was confirmed by local NGOs in the Androy Region. Immediately, a regional bylaw was enacted to ban black coral harvesting in the whole region (Région Androy. 2014. Arrêté n°021/14/REG/ANDROY/CR/Tangoharake du 5 Mars 2014 portant interdiction de collecte de corail noir ou “TANGOHARAKE” dans le littoral de la Région Androy). However, these bylaws did not stop the illegal traffic in either regions. Scuba divers continued quietly to collect corals and civil society, including fishermen associations and NGOs, reported anonymously the arrival of professional divers in the area, with their diving materials. Nevertheless, the use of scuba is prohibited by the Article 10 of Ordinance No. 93-022 of 4 May 1993 on the regulation of fisheries and aquaculture in Madagascar. In June 2014, a ministerial decree prohibiting the exploitation of black coral at the national level was published by the ministry of aquatic resources and fisheries (bylaw No. 21816/2014 of 12 June 2014). This decree stipulates that all forms of exploitation including the extraction, collection, storage, transport, purchase and sale of black coral (Order: Antipatharia) is strictly prohibited in the whole country. The CSP and the competent fisheries authorities are empowered to note and pursue infringements.

Application of the laws: fragility and constraints

The laws preventing the harvest of black coral in the natural environment constitute a tool for the government to better manage these resources. They allow local authorities and police to directly question any people whatever their role in the traffic: diver, collector, transporter or exporter. However, the traffic has not yet been affected by the publication of these laws. Indeed, anonymous informants from the Anosy and Androy regions were reporting the presence of black coral poachers. Every interviewed person refused to provide information on poachers for fear of being blackmailed or even condemned instead of them. These people included traditional fishermen, farmers, boat drivers, police, heads of regional or municipal services and researchers. Since the beginning of the traffic until June 2015, only four arrests and seizures were performed by the CSP. The most important seizure included 49 dive tanks, additional scuba equipment and an outboard engine for a speedboat (Table 26). The CSP also seized camp equipment, several saw blades used to cut the branches of corals and a weighing machine. Officially, no speedboat has been seized. However, unofficial sources report that the arrest that led to the largest seizure also allowed the capture of two speedboats.

The future of black corals in Madagascar

The efficient management of black coral is based on (1) the effectiveness of laws and rules that control the exploitation, (2) the protection of living stocks by the development of marine reserves, (3) the research on methods of transplantation in protected areas and reproduction in aquaria and (4) the awareness of populations especially via the education of children. The situation in the country since the beginning of the political and economic crisis in 2009 does not facilitate proper management. Black corals, among many other resources under CITES protection, have become indisputable objects of illegal harvesting and trade. At this time, the government of Madagascar cannot ensure the protection of these resources. The laws are not respected; and police and justice efforts are inefficient. The return of Madagascar to a constitutional political order in January 2013 constituted a new hope for the effective management of these natural resources. However, the situation has not improved. Indeed, several local sources, from the government, from NGOs or fishermen claim to be aware of several cases of law violation including the harvest, collection, sale and exportation of the resources mentioned above. Information about coral harvesting is growing over time. Corruption is one of the sources of the non-resolution of these problems of natural resource looting. Protection of black corals depends on the stability, independence and transparency of each concerned government entity, either directly or indirectly by the management of these natural resources, which are more and more in danger.

In Madagascar, the Decree No. 97-1455 of 18 December 1997, establishing general organization of maritime fishing, defines and clarifies the principles and guidelines set by Ordinance No. 93-022 of 4 May 1993 on the General regulation of fisheries and aquaculture. Figure 87 illustrates all the parts involved in the exploitation of the – unknown – natural stocks of natural resources, including black corals in Madagascar. Collection activities include purchase within a collection area, processing, storage, conditioning and/or transportation of fishery products, in order to sell them on the market, but they do not include fishing or direct capture in any form; or collection of animals from the aquatic environment. Any collecting authorization entitles the holder to the issuing of up to five collecting permits and is valid only in one zone as mentioned in the authorization. Furthermore, fishing activities are subject to the prior approval of the Ministry of Fisheries and Fish Resources. An industrial investment project (mining, fishing, production factory or else) that directly or indirectly affects the environment (including living natural resources) is subject to well defined rules. An environmental impact study is required for a large project, while for a small one, an environmental commitment program is enough. In any case, the decree of "compatibility of investments with the environment" (MECIE / Decree No. 2004-167 of 3 February 2004) governs all legal clauses that an operator must respect. At this time, not any conservation program for black coral can be established. A better knowledge of the biology and ecology of black corals is essential to ensure an effective management of these resources. The implementation of a conservation and management program is subject to major restrictions without a basis of reliable and updated scientific data.

Acknowledgements

We thank all the officials from the Ministry of Fisheries and Fishery Resources of Madagascar, and especially from the CSP, who provided data and information about black corals harvesting and material seizure and allowed us to analyse seized black corals. We thank all the civil society actors in Madagascar who helped by sharing information about black corals harvesting in the country. The authors are thankful to the fishermen, divers and boat pilots who accepted to be interviewed, anonymously, about their former black corals harvesting activities. Thanks to Paubert Manhatante Tsiamanaorate and Sylvain Mahazotahy for their help and the pictures they allowed to be shared in this paper. The authors are thankful to the anonymous reviewers and the editors.

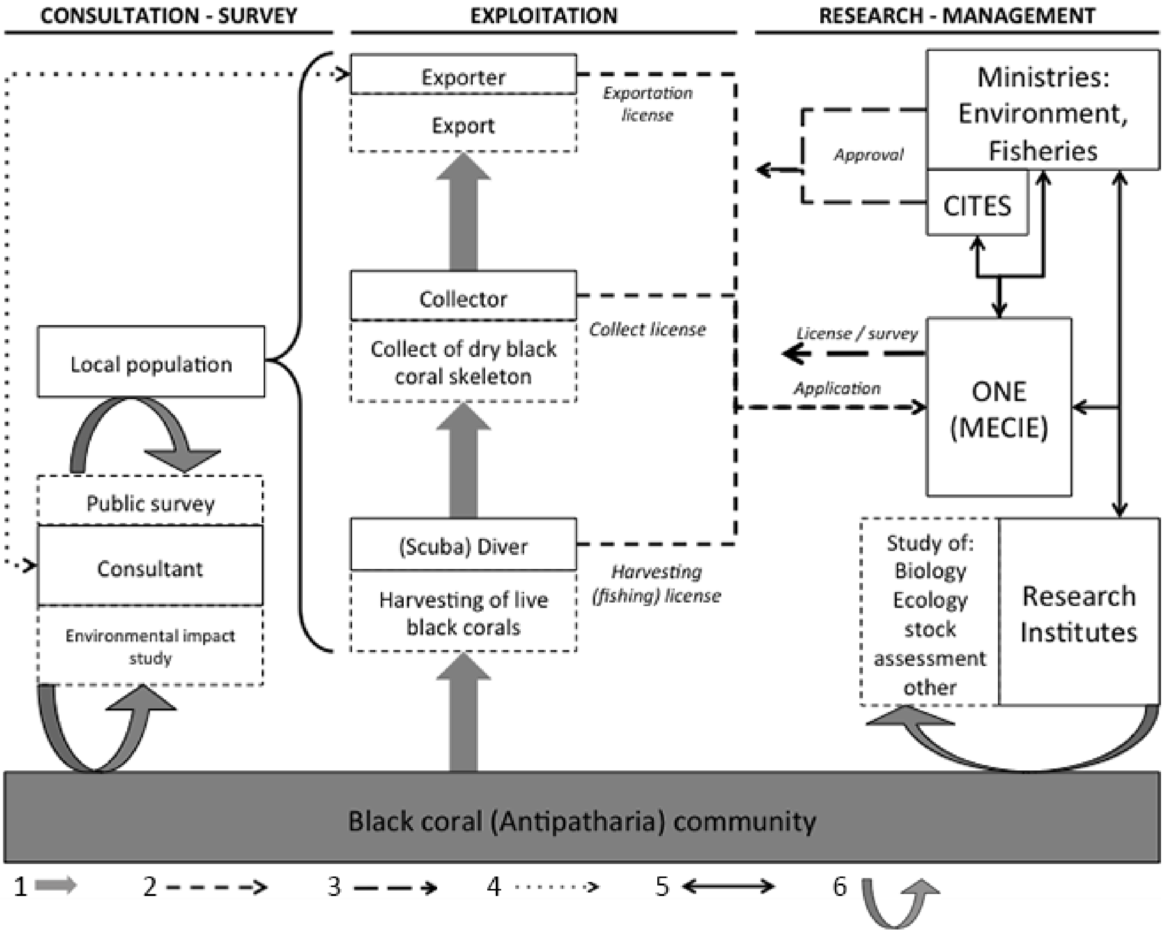


Figure 82. Diagram showing the normal procedures for black coral exploitation. The natural stock of antipatharians should be controlled through three clearly defined parts: research and management performed and ensured essentially by public centres and the government; consultation and surveys of exploitation impacts to the environment and local human societies; and exploitation itself, including harvesting, collect, transformation and exportation. Furthermore, the exploitation percentages of each part are not known yet for black corals. Meaning of the different arrows: 1- Black coral destination; 2- Application for license; 3- Approval, licensing and survey; 4- Consultancy for surveys; 5- Interaction and data exchange; 6- Research and/or survey consultation

SUPPLEMENTARY MATERIAL

Table S1. Description of the material seized by the authorities that was used to collect black corals.

Material	Number	Region of origin
Dive Tanks	49	Androy
Dive Tanks	27	Anosy
Diving Vest (Jacket)	5	Androy
Wetsuit	>5	Anosy
Wetsuit	1	Anosy
Snorkeling materials	7	Androy
Outboard motor (55cc)	2	Anosy
Hacksaw	19	Androy
Underwater shotgun	1	Androy
Water pipe	2	Androy
Suspension balance	2	Androy
Tent	2	Androy

Table S2. Collection permits delivered by the ministry of fishery and aquatic resources in 2012.

Permit n°	District	Region
512 0972	Maroantsetra	Analanjirofo
512 0409	Maroantsetra	Analanjirofo
614 0975	Taolagnaro	Anosy
614 0408	Taolagnaro	Anosy
207 0973	Nosy Be	Diana
619 0974	Morondava	Menabe
619 0410	Morondava	Menabe

Literature

Andrefouët S., Guillaume M.M.M., Delval A., Rasoamanendrika F.M.A., Blanchot J., Bruggemann J.H. (2013) Fifty years of changes in reef flat habitats of the Grand Récif of Toliara (SW Madagascar) and the impact of gleaning. Coral Reefs 32, 3: 757–768.

Battistini R., Bourrouilh F., Chevalier J.P., Coudray J., Denizot M., Faure G., Fisher J.C., Guilcher A., Harmelin-Vivien M., Jaubert J., Laborel J., Montaggioni L., Masse J.P., Mauge L.A., Peyrot-Clausade M., Pichon M., Plante R., Plaziat J.C., Plessis Y.B., Richard G., Salvat B., Thomassin B.A., Vasseur P., Weydert P. (1975) Eléments de terminologie récifale indopacifique. Téthys 7, 1: 1–111.

Bemiasa J. (2009) Dynamique des Pêcheries Traditionnelles d'Anchois, de Calmars et de Poulpes du Sud-Ouest de Madagascar : Utilisation d'Outils Océanographiques pour la Gestion des Ressources. Unpubl. Ph.D Thesis, University of Toliara, Madagascar.

Bruckner A., de Angelis P., Montgomery T. (2008) Case Study for Black Coral from Hawaii. International Expert Workshop on CITES Non-Detriment Findings, Cancun.

Bruggemann J.H., Rodier M., Guillaume M.M.M., Andrefoue¨t S., Arfi R., Cinner J.E., Pichon M., Ramahatratra F., Rasoamanendrika F., Zinke J., McClanahan T.R. (2012) Wicked social–ecological problems forcing unprecedented change on the latitudinal margins of coral reefs: the case of southwest Madagascar. Ecology and Society, 17: 4–47.

Castorena V. & Metaca M. (1979) El coral negro, una riqueza en peligro. Tecnica Pesquera 139: 22–27.

Clausade M., Gravier N., Picard J., Pichon M., Roman M.L., Thomassin B., Vasseur P., Vivien M., Vivien P. (1971) Morphologie des récifs coralliens de la région de Tuléar (Madagascar) : éléments de terminologie récifale. Téthys 2 (Suppl.) : 1–74.

Gabrié C., Vasseur P., Randriamiarana H., Maharavo J., Mara E. (2000) The coral reefs of Madagascar. In: Coral Reefs of the Indian Ocean. Their Ecology and Conservation. T. R. McClanahan, C. R. C. Sheppard and D. O. Obura (eds.), pp 411–444. Oxford University Press, Oxford.

Grigg R.W. (1976) Fishery Management of Precious and Stony Corals in Hawaii. University of Hawaii, Honolulu.

Grigg R.W. (1989) Precious coral fisheries of the Pacific and Mediterranean. In: Marine Invertebrate Fisheries: Their Assessment and Management. J. F. Caddy (ed.), pp 637–645. John Wiley and Sons, New York.

Grigg R.W. (1993) Precious coral fisheries of Hawaii and the US Pacific Islands. Marine Fisheries Review, 55(2): 50-60.

Grigg R.W. (2010) The Precious Corals Fishery Management Plan of the Western Pacific Regional Fishery Management Council. Western Pacific Regional Fishery Management Council, Honolulu.

Grigg R.W. & Opresko D.M. (1977) Order Antipatharia, black corals. In "Reef and Shore Fauna of Hawaii. Section 1: Protozoa through Ctenophora". (D. M. Devaney and L. G. Eldredge, eds), pp. 242–261. Bishop MuseumPress, Honolulu.

Grigg R.W., Bartko B., Brancart C. (1973) A new system for the commercial harvest of precious coral. University of Hawaii, Honolulu.

Guitart B., Gonzalez E., Garcia M. (1997) Evaluation of the Black Coral Populations in the Cuban West. Congreso De Ciencias Del Mar, Cuba 4: 74.

Kahng S.E. & Grigg R.W. (2005) Impact of an alien octocoral, *Carijoa riisei*, on black corals in Hawai'i. Coral Reefs 24: 556–562.

Laroche J. & Ramananarivo N. (1995) A preliminary survey of the artisanal fishery on coral reefs of the Tuleár Region (southwest Madagascar). Coral Reefs 14: 193–200.

Laroche J., Razanoelisoa J., Fauroux E., Rabenavanana M.W. (1997) The reef fisheries surrounding the south- west coastal cities of Madagascar. Fisheries Management and Ecology 4: 285–299.

Parker N.R., Mladenov P.V., Grange K.R. (1997) Reproductive biology of the antipatharian black coral *Antipathes fiordensis* in Doubtful Sound, Fiordland, New Zealand. Marine Biology, 130: 11–22.

Pichon M. (1978) Recherches sur les peuplements a`dominance d'anthozoaires dans les rećifs coralliens de Tuleár (Madagascar). Atoll Research Bulletin 222: 1–447.

Salimo R. (1997) Et́ude de la Peˆche – Collecte a P̀ied sur les Platiers du Grand Rećif de Tuleár (Sud-Ouest de Madgascar). Unpubl. DEA thesis, University of Toliara, Toliara.

Tescione G. (1968) The Italians and their Coral Fishing. Fausto Fiorentino, Napoli.

Thomassin B. (1971) Les faciès d'épifaune et d'épiflore des biotopes sédimentaires des formations coralliennes dans la région de Tuléar (Sud-Ouest de Madagascar). In: Regional Variation in Indian Ocean Coral Reefs. D. R. Stoddart and M. Yonge (eds.), pp 371–376. Symposia of the Zoological Society of London, 28. Academic Press, London and New York.

Vasseur P. (1997) Ecosystèmes côtiers en danger dans la région de Tuléar: analyse des agressions humaines et problèmes de gestion. In: Milieux et Sociétés dans le Sud-ouest de Madagascar. J.M. Lebigre (ed.), pp 97–120. Presses Universitaires de Bordeaux, Bordeaux.

Taxonomic description and 3D modelling of a new species of myzostomid (Annelida, Myzostomida) associated with black corals from Madagascar

TERRANA L. & EECKHAUT I. (2017) Zootaxa 4244(2) : 277–295.

ABSTRACT

Eenymeenymyzostoma nigrocorallium n. sp. is the first species of myzostomid worm associated with black corals to be described. Endoparasitic specimens of *E. nigrocorallium* were found associated with three species of antipatharians on the Great Reef of Toliara. Individuals inhabit the gastrovascular ducts of their hosts and evidence of infestation is, most of the time, not visible externally. Phylogenetic analyses based on 18S rDNA, 16S rDNA and COI data indicate a close relation to *Eenymeenymyzostoma cirripedium*, the only other species of the genus. The morphology of *E. nigrocorallium* is very unusual compared to that of the more conventional *E. cirripedium*. The new species has five pairs of extremely reduced parapodia located on the body margin and no introvert, cirri or lateral organs. Individuals are hermaphroditic, with the male and female gonads both being located dorsally in the trunk. It also has a highly developed parenchymo-muscular layer on the ventral side, and the digestive system lies in the middle part of the trunk. A three-dimensional digital model of this worm’s body plan has been constructed whereby the external morphology and *in toto* views of the observed organ systems (nervous, digestive and reproductive) can be viewed on-screen: http://doi.org/10.13140/RG.2.2.17911.21923.

INTRODUCTION

Myzostomida Graff, 1877 is a group of marine worm-like organisms composed of about 170 species found throughout the world and occurring in the intertidal zone to over 3,000 m depth (Grygier 2000; Eeckhaut & Lanterbecq 2005, 2014; Summers *et al.* 2014). Their phylogenetic status in relation to other animals was much discussed in the past, but recent molecular data suggest that they are members of the Annelida radiation (Bleidorn *et al.* 2007, 2009a, 2009b, 2014; Eeckhaut & Lanterbecq 2014). So far, all the described myzostomids have been obligate symbionts of echinoderms, mainly crinoids, with a few species that live in association with asteroids or ophiuroids. However, there are a few notable exceptions. Four unnamed specimens were reported from the surface of a hexactinellid sponge (Okada 1920; Grygier 2000; Eeckhaut & Lanterbecq 2014). Two unnamed species were discovered infesting anthipatharian corals, one in Puerto Rico (Goenaga 1977) and the other on the Bunaken coral reef in Indonesia (Bo *et al.* 2013). The first of these two reports is a dissertation, in which Goenaga (1977) introduced two new species of black coral. In the second, Bo *et al.* (2013) found an endoparasitic myzostomid in the whip black coral *Cirrhipathes* cf. *rumphii* (Bo *et al.* 2013). They did not name the species but gave a brief description of it. These worms were found in the host's gastric cavity and had a round, flattened body with 10 short parapodia (Bo *et al.* 2013). They were recorded only in the apical polyps all year round both in female and male colonies, and the hosting polyps were never fertile even when the neighboring zooids contained mature gametes (Bo *et al.* 2013). The myzostomids were all fertile females (Bo *et al.* 2013).

The Myzostomida is divided into 12 genera, with about 140 species placed in the genus *Myzostoma* Leuckaert, 1827, which includes mainly ectocommensal worms. *Hypomyzostoma* Perrier, 1927 and *Eenymeenymyzostoma* Summers & Rouse, 2015 are crinoid ectocommensals, the first genus erected because of the unusual morphology of the type species and the second due to the position of the type species in trees resulting from phylogenetic analysis of the myzostomids (Summers & Rouse 2014, 2015). *Notopharyngoides and Pulvinomyzostomum* species live in the digestive tube of crinoids or in cysts close to the host crinoid's mouth. *Endomyzostoma* (formerly *Cystimyzostomum*), *Contramyzostoma* and *Mycomyzostoma* are gallicolous or cysticolous species infesting the crinoid body wall. *Mesomyzostoma* and *Protomyzostomum* infest the gonads of crinoids and ophiuroids, respectively. *Asteromyzostomum and Asteriomyzostomum* species are found, respectively, on the surface and in the digestive system of asteroids.

Myzostomids are particularly interesting from the standpoint of understanding coevolution between associated metazoans and their hosts. There are reports of suggested myzostomidan infestation on Ordovician to Carboniferous crinoid stems in which one or several columnals are heavily swollen into gall-like structures provided with one large opening, but the most recent consensus is that signs of suspected infestations date from the early Jurassic, possibly to the Silurian (Hess 2010, Eeckhaut & Lanterbecq 2014). Cyst-like, bulbous forms attributed to myzostomid infestations have also been observed on Carboniferous crinoids of the Moscow region (Yakovlev 1939). Lanterbecq *et al.* (2010) first, and Summers & Rouse (2014) later, showed that during their common history, cospeciation occurred between myzostomids and their crinoid hosts (in both stalked crinoids and comatulid crinoids), with host switches and duplications events (see Lanterbecq *et al.* 2010 for more explanations).

In the present paper, we describe for the first time a new species of endoparasitic myzostomid associated with black corals, *Eenymeenymyzostoma nigrocorallium* n. sp., which was found in various anthipatharians off the southwestern coast of Madagascar. We here report on the infestation, describe the external morphology of the species studied using optical and scanning electon microscopy, analyse the internal morphology through normal and semi-thin sections and ascertain the phylogenetic position of the species based on 18S rDNA, 16S rDNA and COI data. We also reconstruct the body plan of the species based on all of these analyses and present the first 3D reconstruction of a myzostomid, including its internal structure (see supplementary material).

MATERIAL AND METHODS

Sampling

To avoid killing entire black coral colonies, fragments were collected by scuba diving at two locations within the Great Reef of Toliara off the southwestern coast of Madagascar (23°21.028'S, 43°36.883'E and 23°21.105'S, 43°36.853'E). Sampling took place during November and December 2015, at depths of 15-35 m. In the laboratory, the length of each coral fragment was measured with a tape measure to the nearest centimetre and any myzostomids associated with it were counted. When looking at the coral surface, it was not possible to see any evidence of the worm presence except for some samples with slightly protruding coral tissue (Fig. 83). Consequently, the coral tissue was removed from the entire skeleton of each fragment in small petri dishes to expose the worms. Length and width of 36 myzostomids were measured using ImageJ software (Rasband 1997) before fixing the worms in different solutions.

All the specimens of the new species have been deposited in the Museum of Natural History in Brussels (Belgium) under the following IG numbers: holotype in formol 10% buffered with sea water number INV.142000, and 3 paratypes in formol 10% buffered with sea water number INV.142001, 3 paratypes in Bouin's fluid number INV.142002 and 3 paratypes in glutaraldehyde number INV.142003.

Specimens collected by Goenaga (1977) inside *Stichopathes sp*. were obtained from the Smithsonian National Museum of Natural History for comparison with the present species (catalogue number USNM 1421641). The original label was "Puerto Rico: Edge of Shelf S. of La Parguera. 30 m. Oct-Nov. 1976 Carlos Goenaga, coll Acc. 301700 (rec. May 1977). Collected on the inside of *Stichopathes* n. sp." before being labelled as "Myzsotoma sp. 113" by Mark Grygier in 1988.

Microscopy

For histological sections, myzostomids were fixed in 3% glutaraldehyde in 0.1M cacodylate buffer for 4 h, postfixed with osmium tetroxide (1% osmium tetroxide, 0.1M sodium cacodylate, 0.27M sodium chloride; pH 7.8) and embedded in Spurr resin. 1 µm thick semi-thin sections were cut using a Reichert Austria U2 ultramicrotome equipped with a glass knife. They were then stained with a 1:1 mixture of 1% aqueous solution of azure II and 1% aqueous solution of methylene blue in 1% sodium tetraborate for 45 sec and observed with an Axioscope A1 (Zein) light microscope. Samples of black corals in which a worm was expected to be were entirely fixed in Bouin's fluid for 24h and rinsed several times with 70% ethanol. They were then dehydrated, embedded in paraffin and cut in 7µm thick sections with a Zeiss Microm HM 340E microtome. Sections were stained with a trichromatic Masson-Golden light green staining and observed to see how the parasite was positioned inside the host. Some that were stored in 70% ethanol were dissolved in 15% bleach ($NaClO_4$) solution to observe their parapodial hook apparatus with a light microscope.

For scanning electron microscopy, worm samples were fixed in Bouin's fluid for 24 h before being dehydrated in graded concentrations of ethanol and critical-point-dried using CO_2 as the transition fluid. They were then mounted on aluminium stubs before being coated with gold in a JEOL JFC-1100E sputter coater and observed with a JEOL JSM-6100 scanning electron microscope.

Molecular analyses

Sequences of the nuclear small ribosomal subunit (18S rDNA), the mitochondrial large ribosomal subunit (16S rDNA) and the mithochondrial cytochrome c oxidase subunit I (CO1) of the new species were used to ascertain its phylogenetic position. Genomic DNA was extracted using the Invitek Spin Tissue Mini Kit (Invisorb). Genes were amplified by PCR using Ready-To-Go PCR beads (GE Healthcare). Each reaction was performed in a final volume of 25 µl with 1 µl of genomic DNA. PCR profiles were as follows: 5 min at 95°C, 35 cycles of 30 s at 95°C, 30 s at 50°C (16S) or 55°C (18S and CO1), 70 s at 72°C and a final elongation of 5 min at 72°C. The 18S rDNA was amplified in 3 overlapping fragments of about 600 nucleotides each using primers 1N/8WRC, 2N/12WRC and 11W/3NRC from Eeckhaut *et al.* (2000). The primers LCO1490 and HCO2198 (Folmer *et al.* 1994) were used to amplify the first part of CO1 (600 bp). The 16S mitochondrial gene was amplified using the universal primers 16Sar and 16Sbr from Palumbi *et al.* (1997). Sequencing was performed using the same primers as the PCR reactions. Sequences were successfully obtained for *E. nigrocorallium* n. sp., edited with CodonCode Aligner (CodonCode) and deposited in Genbank under accession numbers KY449458, KY449459 and KY449460.

To assess the phylogenetic position of the new species, the sequences were aligned in ClustalX (Larkin *et al.* 2007) with the corresponding gene sequences of 41 myzostomids that constitute a representative sample of all the sequenced genera to date. The ingroup included *Endomyzostoma* (8 specimens), *Pulvinomyzostomum* (3 specimens), *Protomyzostomum* (1 specimen), *Asteromyzostomum* (1 specimen), *Eenymeenymyzostoma* (2 specimens), *Myzostoma* (12 specimens), *Hypomyzostoma* (4 specimens), *Notopharyngoides* (1 specimen), *Contramyzostoma* (1 specimen) and *Mesomyzostoma* (8 specimens). The outgroup consisted of the same 7 annelid species that had been used as outgroup taxa by Summers and Rouse (2014).

The final DNA matrix included 3339 characters that were analysed in MetaPIGA 2.1.3 (Helaers & Milinkovitch 2010). Invariable characters were detected in MetaPIGA and trimmed using the Gappyout Trimming Option (Capella-Gutiérrez *et al.* 2009). Divergence amongst sequences did not show any sign of saturation even when genes were tested separately. The resulting matrix that was subsequently analysed included 2605 characters. MP analyses were performed with PAUP*4.0b4a (Swofford 1998) using an heuristic search (SeqAdd and TBR branch-swapping). Clade supports were estimated by bootstrapping (Felsenstein 1985) (Simple SeqAdd and TBR branchswapping; 1,000 replicates). Bayesian analyses were performed using MrBayes v3.0b4 (Ronquist & Huelsenbeck 2003), using the model (GTR+I+G) selected by MrModelTest 1.0b (Nylander 2002) based on Hierarchical Likelihood Ratio Tests (the same model was also chosen by the AIC test). Four Markov chains were run simultaneously for 5 x 10^5 generations, and trees were sampled every 100 cycles for a total of 5,000 trees. The first 1,000 trees with preasymptotic likelihood scores, i.e. the first 100,000 generations, were discarded as 'burn-in'. The remaining trees were used to compute Bayesian posterior probabilities. ML analyses were performed using the Metapopulation Genetic Algorithm (MetaGA) using the software MetaPIGA 2.1.3 (Helaers & Milinkovitch 2010). The alignment was considered in the analyses either as a whole or partitioned according to the genes (six partitions). We used probability consensus pruning among four populations of four individuals each and selected the best-fitting ML nucleotide substitution model on the basis of the Akaike Information Criterion implemented in MetaPIGA. The best models that sorted from the analyses were the GTR model with rate heterogeneity without invariant. To generate an estimate of the posterior probability distribution of possible trees, we performed replicated MetaPIGA searches and stopped automatically when a series of mean relative error values among ten consecutive consensus trees remained below 5%, with a minimum of 100 replicates (Helaers & Milinkovitch 2010).

Rooting greatly influences the topology of the myzostomid trees. For estimating the phylogeny of this group, Lanterbecq *et al.* (2006) chose members of Rotifera, Platyhelminthes and Annelida to root their trees. The molecular phylogeny they recovered found two major clades, one composed of gall-, cyst- and mouth-dwelling taxa associated with crinoids (*Endomyzostoma* and *Pulvinomyzostomum*) and the other including mostly free-living taxa (*Myzostoma, Hypomyzostoma)* along with cysticolous, mouth-dwelling and internal forms (*Notopharyngoides, Contramyzostoma* and *Mesomyzostoma*). More recently, Summers and Rouse (2014) reassessed this phylogeny with a broader sample of myzostomids. By that time, multiple studies had begun to confirm that the Myzostomida are members of Annelida (Hartman *et al.* 2012, Helm *et al.* 2012) and these authors used only polychaetes as outgroups: Phyllodocida with a representative of Amphinomida, and the amphinomid *Chloeia flava*, respectively. The molecular phylogeny they obtained also comprised two clades but was different from that of Lanterbecq *et al.* (2006). One clade was composed of gallicolous and cysticolous taxa associated with crinoids (*Endomyzostoma*), and the other included all the other species with, at the base of the clade, the grouping (*Protomyzostomum* + *Asteromyzostomum)*, then *Eenymeenymyzostoma,* and then *Pulvinomyzostomum* as the sister group of the last clade, which included *Myzostoma, Hypomyzostoma, Notopharyngoides, Contramyzostoma* and *Mesomyzostoma.* In the present work, to test the influence of the outgroup on the topology of the ingroup and especially the position of the new species, we performed 127 ML analyses using the software MetaPIGA 2.1.3 (Helaers & Milinkovitch 2010) to analyse the effect of all possible single, double, triple, etc. combinations of the seven chosen outgroup species.

RESULTS

Family Eenymeenymyzostomatidae Summers & Rouse, 2015

Genus *Eenymeenymyzostoma* Summers & Rouse, 2015

Eenymeenymyzostoma nigrocorallium n. sp. Terrana & Eeckhaut (Figs 1-3)

Holotype. Great Reef of Toliara, Madagascar, 20 meters deep, 25 November 2015

Paratypes. Same data as for holotype.

Etymology. The species name is a combination of the Latin adjective '*nigrum'* and the noun '*corallium*' referring to the antipatharian host called black coral.

Material examined

A total of 162 specimens were found on the Great Reef of Toliara in Madagascar, infesting three different antipatharian species: *Stichopathes* sp. (142 specimens), *Cirrhipathes* sp. aff. *anguina* (14 specimens) and *Cirrhipathes* sp. (6 specimens) (Fig. 83). Of the 13 sampled colonies of *Stichopathes* sp. (Fig. 83A,B,C), 9 hosted myzostomids (69%) with a maximum of 39 specimens on 139 cm of coral and a maximum density of 1 individual every 0.79 cm. Single colonies of *Cirrhipathes* sp. aff. *anguina* (Fig. 83D) and *Cirrhipathes* sp. (Fig. 83E) were sampled (8 cm and 10 cm fragments respectively), and each hosted myzostomids.

Specimens "Myzostoma sp.113" collected by Goenaga in 1977 were observed with a stereomicroscope and a scanning electron microscope. The fixative used to store the specimens did not allow any molecular analyses or histological sections.

Diagnosis

Small myzostomid found in lumen of gastrovascular tracts of black corals. Body with ovoid trunk without introvert. Five pairs of small, cone-shaped parapodia located at very margin of trunk. No marginal cirrus or parapodial cirri. Cloacal opening on margin of trunk, at sagittal midline. No visible lateral organs. Body wall with cuticle-lined columnar epidermis and underlying parenchymo-muscular layer, latter especially well developed ventrally. Digestive system with pharynx, stomach and intestine, all of equal length, and two pairs of digestive diverticula. Nervous system with compact ventral nerve chain. Simultaneous hermaphrodites. Two pairs of dorsal ovaries located in parenchyma. Female genital ducts with large sagittal uterus and two pairs of dichotomized uterine branches. Uterus and intestine merging before cloacal opening. Four pairs of dorsal testicles located in parenchyma between ovaries. Male genital ducts with two pairs of deferent ducts and two pairs of seminal vesicles opening through one pair of male gonopores located dorsally to third pair of parapodia.

Description

Host ecology and morphology

The three black coral species *Stichopathes* sp., *Cirrhipathes* sp. aff. *anguina* and *Cirrhipathes* sp. hosting *E. nigrocorallium* were found in the channel between the north end of the Great Reef of Toliara and the coast at depths of 15-35 m (Fig. 83). Currents are often strong there, often with a lot of suspended organic matter from two nearby rivers, the Fiheranana and Onilahy. The three species consist of single, unbranched stems. *Stichopathes* sp. is brownish and can reach up to 5 m in length (Fig. 83C). It has a single row of polyps along the stem, which extend their tentacles at night. Both species of *Cirrhipathes* have several rows of polyps around the stem and reach up to 2m in length, sometimes forming wires (Fig. 83D,E). *Cirrhipathes* sp. aff. *anguina* is yellow (Fig. 83D) while *Cirrhipathes* sp. is dark green with orange polyps (Fig. 83E).

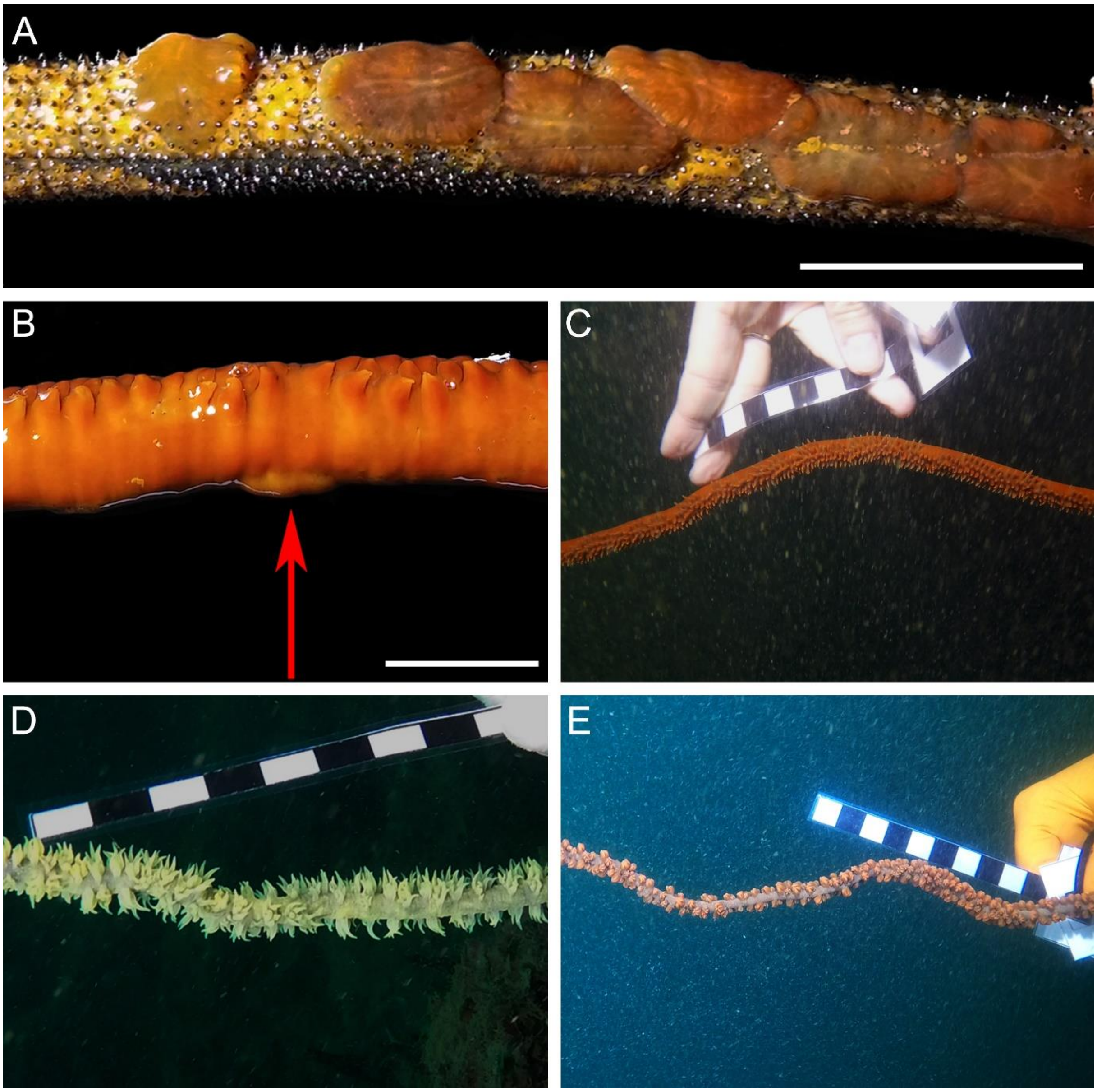


Figure 83. *Eenymeenymyzostoma nigrocorallium* n. sp. and its antipatharian hosts. (A) View of a cluster of six reddish living specimens revealed by removing overlying coral tissue; myzostomids situated within the coral tissue are often invisible. (B) In places where there are no coral polyps, the presence of myzostomids can sometimes be detected as a slight swelling of the coral surface (red arrow). (C-E) Antipatharian species recorded herein as hosts of *E. nigrocorallium*: (C) *Stichopathes* sp.; (D) *Cirrhipathes* sp. aff. *anguina;* (E) *Cirrhipathes* sp. Scales: A, B: 1mm; C, D, E, F, G: 1 cm/square.

Host-parasite relations

Eenymeenymyzostoma nigrocorallium is found in the gastrovascular ducts of these black corals. *In situ* coral infestation by myzostomids is almost impossible to detect except in *Stichopathes* sp., in which the polyps are arranged along a single face of the colony (Fig. 83B,C). The presence of these worms can then sometimes be detected on the side opposite to the polyps, with the worms appearing as very small humps on the coral surface (Fig. 83B). In the two other corals, it is impossible to detect the worms by eye because of the presence of polyps around the entire stem. Random dissections of the coral fragments confirmed the presence of myzostomids. Figure 88A shows six individuals after the removal of the upper layer of the coral soft tissue. The black skeleton (axis) of the coral is visible, covered by the thin, yellow lower layer of the soft tissue. The myzostomids sit close together. The black coral host shows no sign of any damage caused by the myzostomid presence. The skeleton spines or the different tissues are not modified at the location where the myzostomid has been found.

Worm description

Individual worms are orange to dark brown and the sagittal pharynx, stomach and intestine are visible along the sagittal midline (Fig. 83A). The white extremities of the dichotomized digestive diverticula are observable in the trunk margin, as well as two seminal vesicles with spermatophores (not observable in the picture). When separated from their hosts, the myzostomids move very slowly by folding their trunk upward and downward. They live about three days in 5-µm filtered sea water out of contact with their host. After a few hours, they lay batches of hundreds of fertilized eggs through their cloacal opening. The eggs undergo spiral divisions during the next few hours.

Sections of myzostomids inside black corals indicate that the worms are located in the gastrovascular cavities of the polyps but also in the gastrovascular ducts that connect the polyps. Figures 90A and 90B illustrate a section of an individual in a black coral. The axis of the host is bordered first by the coral epidermis, then by the thin mesoglea and the epithelium of the gastrovascular cavity of the polyp. The ventral side of the myzostomid faces the hosts's axis (Figs 90A, B). The epithelium bordering the gastrovascular cavity does not appear affected by the presence of the worm.

The myzostomids measure 1.95-7.14 mm long and 1.73-4.36 mm wide. There are no marginal cirri and the trunk margin looks smooth under a binocular microscope, but

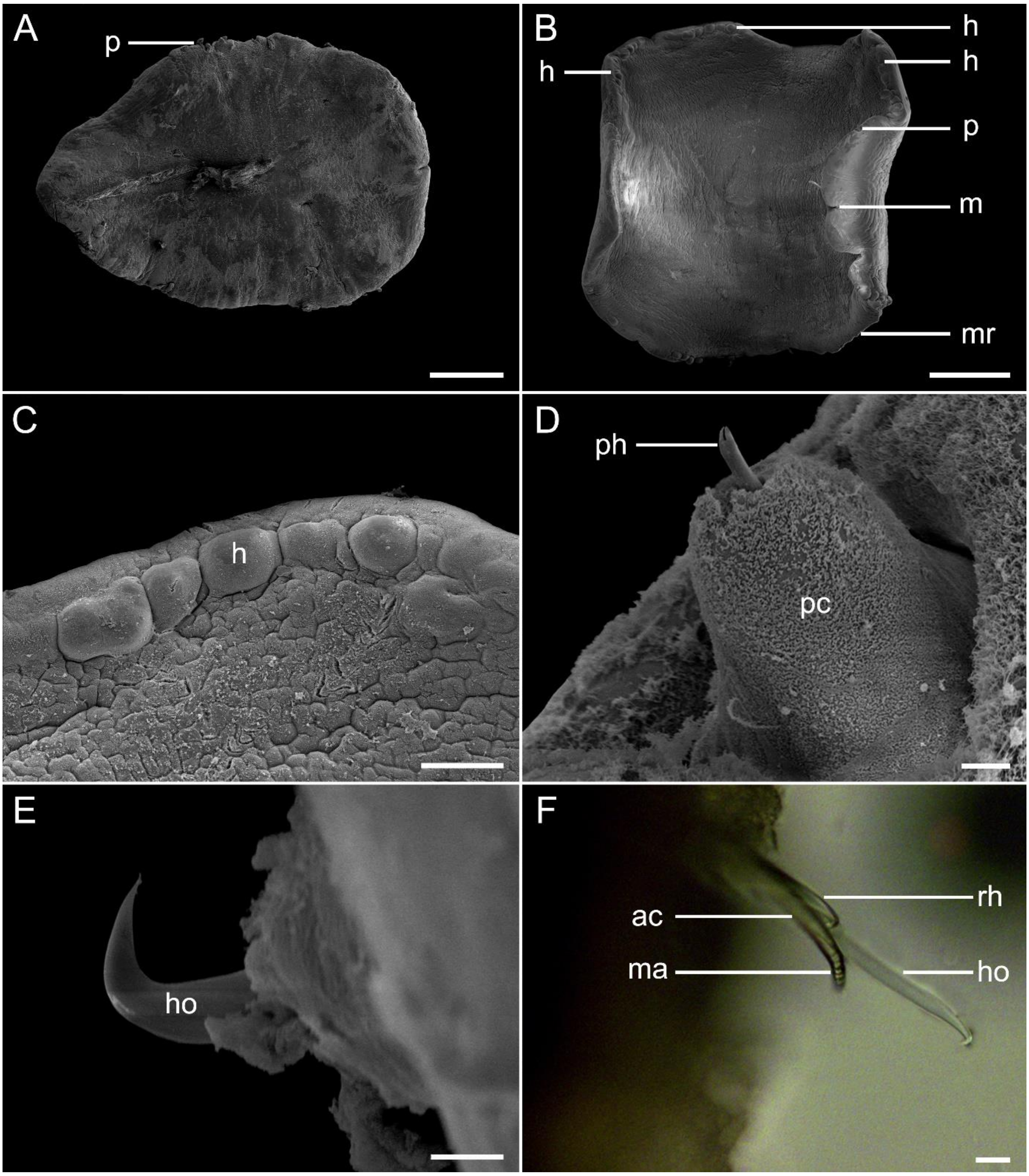


Figure 84. *Eenymeenymyzostoma nigrocorallium* n. sp. (A) SEM photo, dorsal view. (B) SEM photo, ventral view of partially folded specimen. (C) Detail of humps along margin in (B); three sets of about 10 humps each are present on each side. (D, E) Very small parapodia (not always visible), each with curved hook protruding from tip. (F) Light micrograph of parapodium digested in bleach to show entire hook apparatus. Scales: A, B: 1mm; C: 100µm; D, E: 10µm; F: 50µm. Abbreviations: ac, acicula; h, humps; ho, hook; m, mouth; ma, manubrium; mr, lateral margin; p, parapodium; pc, parapodial cone, ph, parapodial hook; rh, replacement hook.

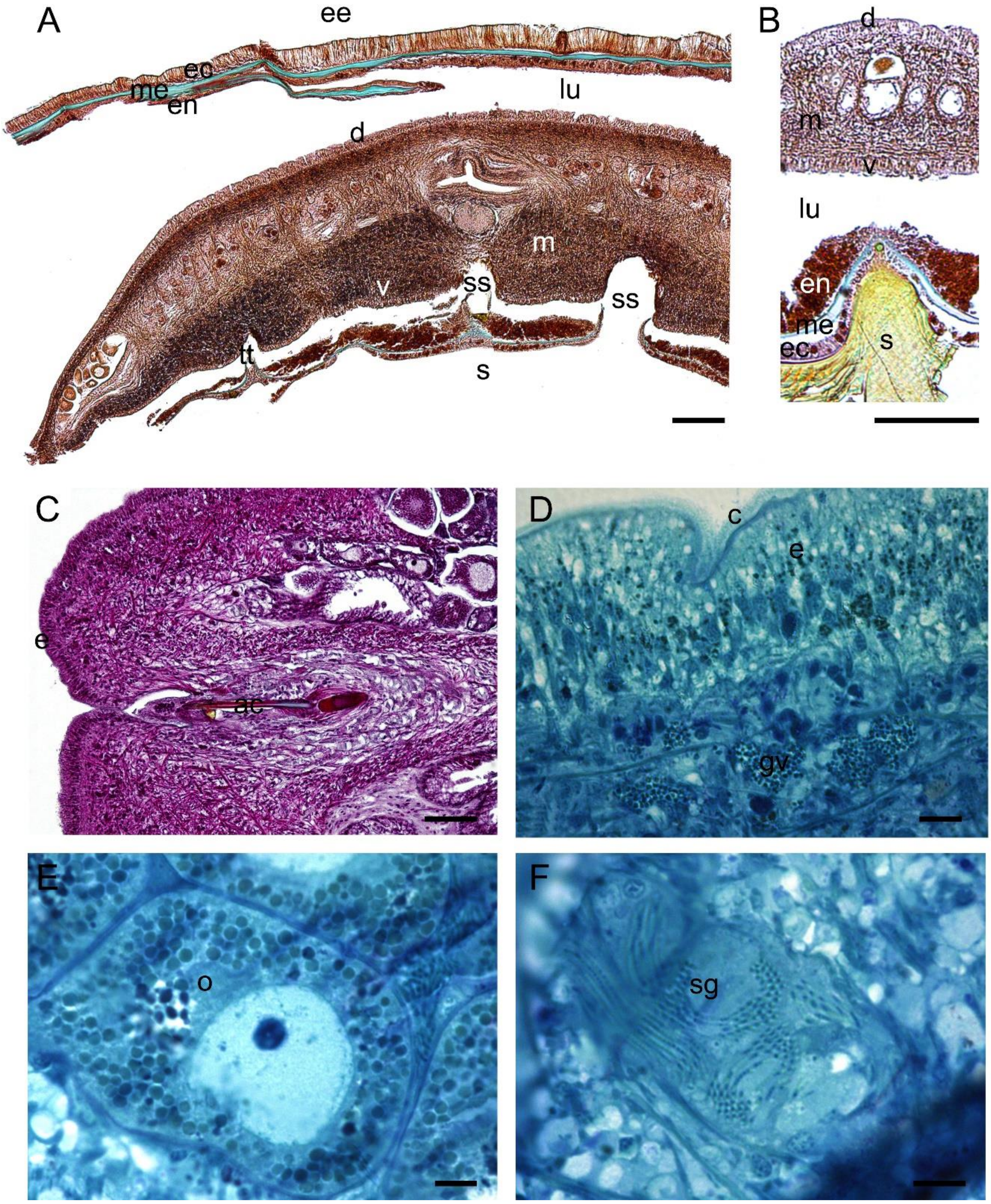


Figure 85. *Eenymeenymyzostoma nigrocorallium* n. sp., internal structure. (A) Transverse section through a black coral infested by a myzostomid lying in the lumen of a digestive duct of the coral host. (B) Detail of the host epithelial structure under the myzostomid. (C) Parapodium fully retracted into epithelial folds, thus invisible externally. (D, E, F) Hermaphroditic gonads extending out to the body margins, with spermatozoids ready to fertilize oocytes inside the gonadal ducts. Scales: A, B: 200µm; C: 60µm; D: 100µm; E, F: 10µm. Abbreviations: ac, acicula; c, cuticle; d, dorsal side of myzostomid; e, epidermis; ec, ectoderma; ee, external environment, en: endoderma; gv, granular vesicles; lu, lumen of digestive tract; m, myzostomid; mc, myoepithelial cells; me, mesoglea; o, oocyte; s, coral skeleton; sg, spermatogonia; ss, skeletal spine; v, ventral side of myzostomid.

viewed with SEM, rows of 30-µm humps are visible on the trunk margin (Fig. 84B,C). The presence of these humps may depend on the state of contraction of the individuals. Five pairs of extremely small parapodia of 60 µm in length are located on the trunk margins. They are very difficult to see under a binocular microscope and were fully revealed with SEM (Figs. 84D, E; 85C). A 12 µm long parapodial hook protrudes from each parapodial cone and has a rounded tip (Fig. 84E,F). When individuals are digested in bleach, the complete hooks are approximately 170 µm long, about twice as long as the acicula (Fig. 84F). There is a 70 µm long replacement hook at the base, and the manubrium at the tip of the acicula is rounded (Fig. 84F). Lateral organs are absent or not observed with SEM. The body wall of *E. nigrocorallum* consists of a 4 µm thick cuticle, a columnar epidermis 44 µm high and a parenchymo-muscular layer that extends between the internal organs (Fig. 85C,D). Four types of cells are commonly observed in the epidermis: covering columnar cells of 44 µm in height and 5 to 10 µm wide, ciliated cells of similar shape, glandular cells and myoepithelial cells (Fig. 85D). The glandular cells are goblet-shaped with their nucleus located in the parenchyma and sending cellular processes out to the inner surface of the cuticula (Fig. 85D). The myoepithelial cells are located below the covering cells and the ciliated cells, close to the basal lamina (Fig. 85D). The parenchymo-muscular layer is particularly well developed on the ventral side of the body (Fig. 85A).

Internally, the trunk consists of a ventral part in which the main parts of the nervous system are surrounded by a hyper-developed parenchymo-muscular layer, a mid part with the digestive system and a dorsal part with the female and the male genital systems (Figs. 90, 91). The excretory system has not been observed but is probably present; the analytical methods used here were not sufficient to reveal the presence of very small protonephridia.

The hyper-developed ventral parenchymo-muscular layer is about one third of the 80 µm total thickness of the trunk (Fig. 85A). It is probably so developed because the worm's ventral side faces the coral skeleton, which is not smooth but presents small spines; in some sections, the myzostomid's ventral body wall fits the shape of the coral spines (Fig. 85B).

The nervous system principally consists of a compact ventral nerve chain that is 430 µm long and 230 µm wide in individuals about 3.3mm long (Fig. 86B,C,D). The compact nerve chain is composed of a central part in which the neuronal bodies lie and two lateral

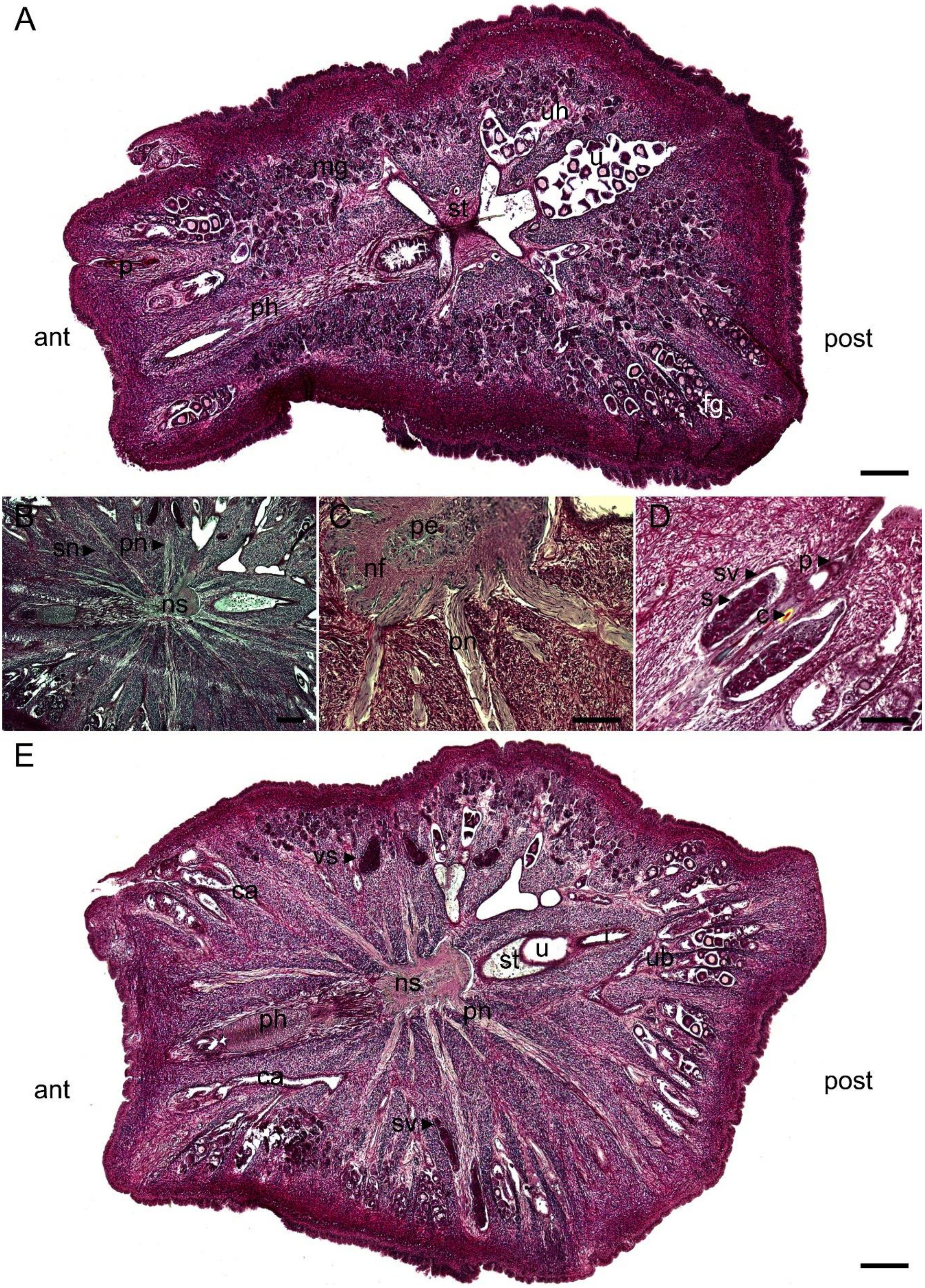


Figure 86. *Eenymeenymyzostoma nigrocorallium* n. sp., internal structure. (A, E) Upper (A) and lower (E) sections of body showing entire digestive tract, reproductive apparatus and nervous system. (B, C) Details of nervous system. (E) Two seminal vesicles associated with third parapodium, both containing spermatophores. Scales: A, E: 200µm; B: 100µm;

C, D: 50μm. Abbreviations: c, chaeta; ca, digestive caeca; fg, female gametes; i, intestine; mg, male gametes; nf, nervous fibers; ns, nervous system; p, parapodia; pe, perikarya; ph, pharynx; pn, parapodial nerve; s, spermatophore; sn, side nerves; st, stomach; sv, seminal vesicles; u, uterus; ub, uterine branches; uh, uterine horns, branches.

cords formed of the neuronal processes (Fig. 86C). These two lateral cords are connected anteriorly and posteriorly by two large nerve commissures and in between by small commissures. Five parapodial nerves, five side nerves and two pharyngeal nerves extend from each lateral cord (Fig. 86B,C,E, see also the 3D reconstruction in supplementary file). Each parapodial nerve splits in two smaller branches at the base of the respective parapodium (Fig. 86E and supplementary file). Small side nerves are located between the parapodial nerves and extend out to the body margins (Fig. 86E and supplementary file). These side nerves dichotomize into several small nerves before reaching the body margin.

The digestive system includes a tripartite pharynx, a stomach from which start two pairs of digestive diverticula and an intestine (Fig. 86 and supplementary file). The mouth appears as a slit at the trunk margin in the sagittal plane. The pharynx, the stomach and the intestine each occupies one third of the length of the body. The pharynx is divided into three parts, each of the same length. The mid part comprises the muscular bulbus typically observed in other myzostomids (Fig. 86E). It is 370 μm long and 150 μm thick (in 3 mm long individuals) and consists of a muscular layer made of radial and circular muscle fibres that assist in the swallowing of particles. In the anteriormost part, the pharyngeal epithelium is not surrounded by a muscular layer, while in the posteriormost part, the pharyngeal lumen is surrounded by longitudinal muscle fibers connected to the muscular bulbus and to the stomach (Fig. 86E). The stomach is a pouch of 300 μm in diameter (Fig. 86A). Two pairs of digestive diverticula start from its lateral sides and dichotomize to finally reach the trunk margin (Fig. 86A and supplementary file). The digestive diverticula can barely be seen through the translucent body of the worms, and the intervening branching is obscure. The intestine is about 100 μm in diameter and fuses with the uterus at the very end of the trunk to open to the exterior by a cloacal pore (see supplementary file).

All the sectioned individuals are simultaneous hermaphrodites. Both genital systems are located in the upper part of the trunk (Fig. 86A). The female genital system consists of four ovaries, a uterus and many uterine branches (Supplementary file). It lies above the digestive system in such a way that a section through the dorsal part of a worm shows the epidermis, then the parenchymo-muscular layer with developing female germinal cells, the genital duct and finally the digestive system. Oogonia, previtellogenic oocytes and vitellogenic oocytes develop in the four ovaries, each situated in one quarter of the trunk (Figs. 90E, 91A, 91E). These germinal cells grow in the parenchyma above the uterine branches, themselves located above the digestive diverticula. Mature oocytes are found in the uterine branches and in the sagittal uterus as well (Fig. 86A,E). The uterus lies above the stomach and the intestine (see supplementary file). It is about 250-300 µm in diameter in sectioned individuals at its beginning and becomes narrower up to the cloacal pore.

The male genital system comprises four testes, four deferent ducts and four seminal vesicles. The testes are diffuse with spermatocysts developing into the parenchyma between the branches of the ovaries (Fig. 85 and supplementary file). Spermatocysts of different stages, including spermatogonia, spermatids and spermatozoa, are found close together. They measure from 30 to 50 µm in diameter and are conducted by short deferent ducts into four seminal vesicles. The seminal vesicles are two pairs of sacs, each being 250 µm long and 70 µm wide (in individuals of 3.3 mm in length). The vesicles of each pair flank the third pair of parapodia (Fig. 86D). Their extremities converge and fuse into a male gonopore, the two gonopores being located above these parapodia. The spermatophores observed inside the seminal vesicles are V-shaped structures consisting of the germinal products coming from each pair of seminal vesicles. The branches of the "V" are 200 µm long and composed of many spermatocyts of about 30 µm in diameter full of spermatozoa (Fig. 86D). The point of contact of the two branches includes degenerated spermiocysts similarly to the situation in *M. cirriferum*'s spermatophore (Eeckhaut & Jangoux 1991).

Observation of museum specimens

The two specimens obtained from the Smithsonian's NMNH (catalogue number USNM 1421641) are ovoid, about 1.3-1.4 mm long and 0.8-0.9 mm wide, flattened but centrally depressed dorsally and convex ventrally (Fig. 87A,B). There are only four pairs of parapodia visible under scanning electron microscope on one side of the myzostomid, while on the other side two of them are less visible (Fig. 87A,B). The cone-shaped parapodia are located ventrally at about 100 µm of the trunk margin (Fig. 87A,B,C). They are also visible with a x50 stereomicroscope if they are not contracted, measuring 70-90µm in height including the hook (Fig. 87C). A small, round opening at one end and a slit vertical just below the edge at the other end are visible under SEM, suggesting the presence of the mouth without any introvert visible (Fig. 87B). No parapodial cirrus, no marginal cirrus and no lateral organ visible with a x50 stereomicroscope or a SEM. Male genital pores at the basis of the third pair of parapodia (Fig. 87B). The myzostomid is dorsally densely ciliated (Fig. 87D).

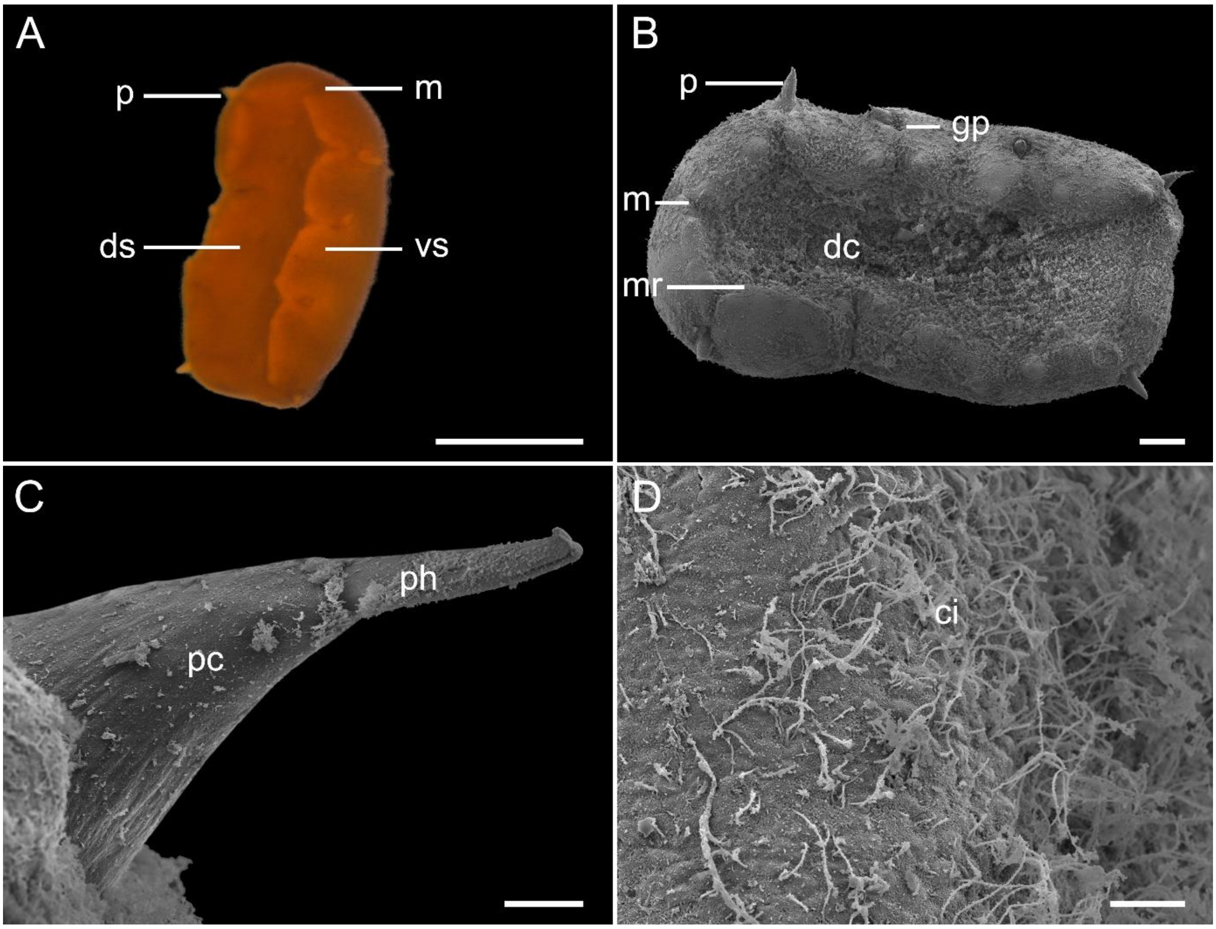


Figure 87. Undescribed specimen collected by Goenaga in 1977 on the inside of the black coral *Stichopathes* sp. (**A**) Dorsal view showing barely any structure except the parapodia. (**B**) Dorsal view under SEM showing the myzostomid ventrally convex. (**C**) View of a parapodium. (**D**) Dense cilia found on the dorsal side of the myzostomid. Scales: **A**: 500µm; **B**: 100µm; **C**,**D**: 10µm. Abbreviations: ci, cilia; dc, dorsal cilia; ds, dorsal side; gp, male genital pore; m, mouth; mr, lateral margin; p, parapodia; pc, parapodial cone; ph, parapodial hook; vs, ventral side.

Phylogenetic position

In all analyses (MP, ML and Bayesian analysis) made either on separate genes (16S rDNA, 18S rDNA and COI) or on the three genes together, the new species always grouped with *Eenymeenymyzostoma cirripedium* with probabilities superior to 98% (Figs.

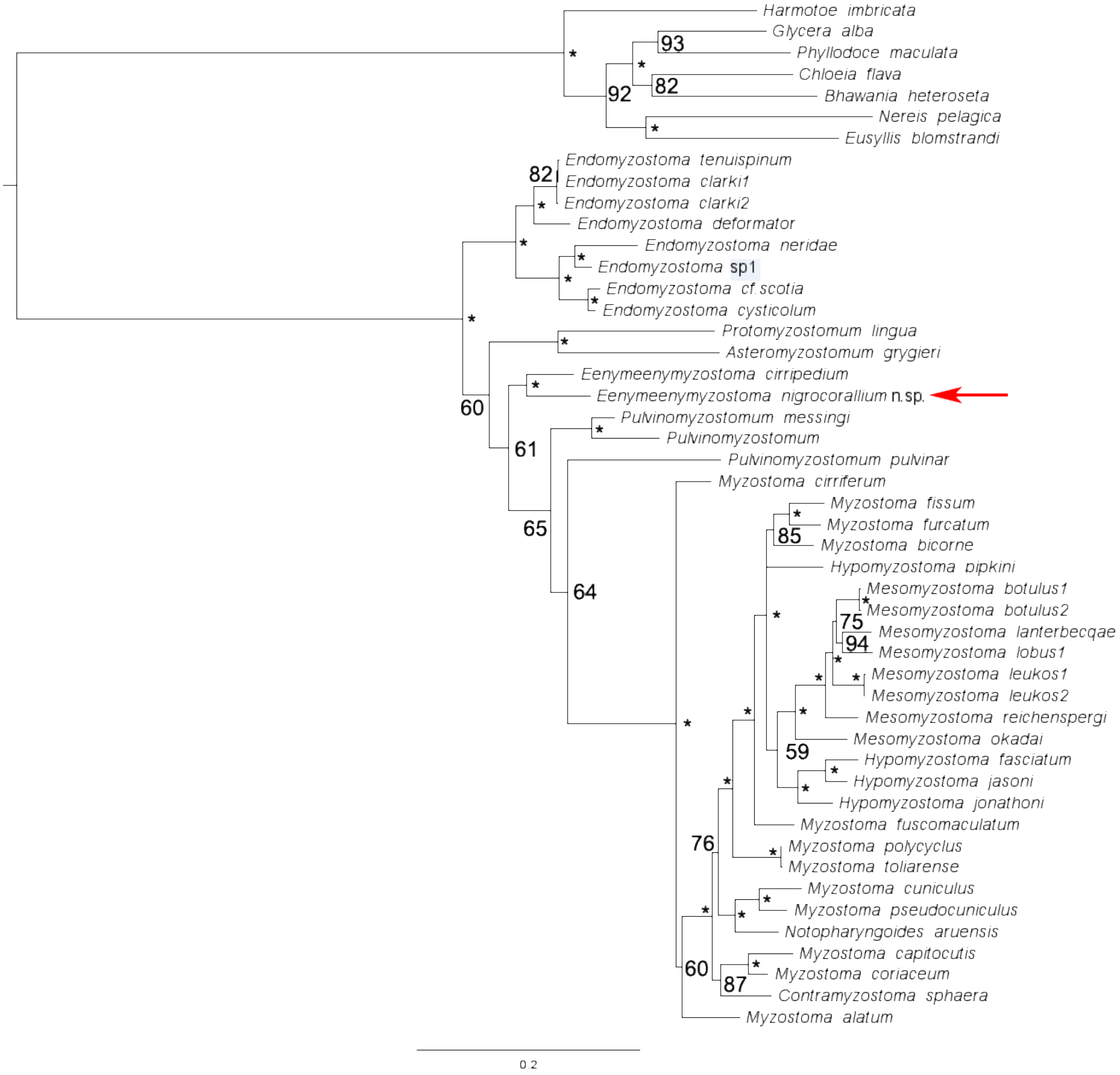


Figure 88. Phylogenetic position of *Eenymeenymyzsotoma nigrocorallium* n. sp. in a tree computed with Bayesian analysis using 16S-18S-CO1 alignment. Numbers above branches indicate posterior probabilities; a star means a posterior probability superior to 95. Red arrow points to the position of the new species.

88, 89). With an outgroup including all 7 polychaete species, two different topologies were obtained depending on the phyogenetic inference method employed (ML, MP or Bayesian). In the Baysian tree, the *Endomyzostoma* clade is sister to all the other myzostomids (Fig. 78). In the ML and MP trees, *Endomyzostoma* is in a clade together with *Protomyzostomum, Asteromyzostomum, Eenymeenymyzostoma* and *Pulvinomyzostomum* and its sister clade comprises *Myzostoma, Contramyzostoma, Notopharyngoides, Hypomyzostoma* and *Mesomyzostoma* (Fig. 88). If these 5 genera are always grouped together, regardless of the analysis or the outgroup, the phylogenetic relationships within the group are not clearly defined as shown by the low support values.

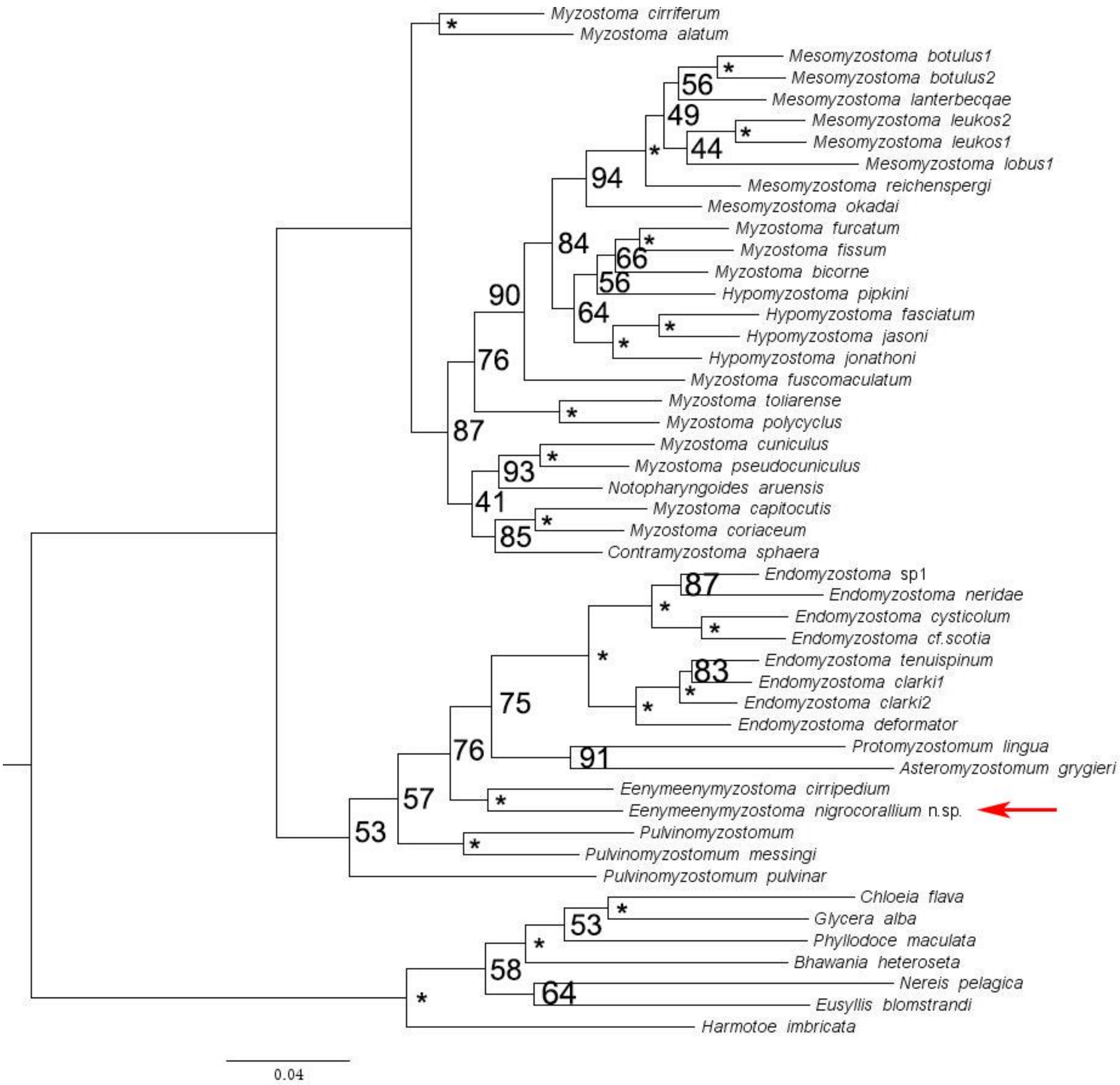


Figure 89. Phylogenetic position of *Eenymeenymyzsotoma nigrocorallium* n. sp. in a tree obtained using the maximum likelihood model (MetaPIGA). Numbers above branches indicate posterior probabilities; a star means a posterior probability superior to 95. Red arrow points to the position of the new species.

When only *Phyllodoce maculata* was taken as outgroup in Bayesian analyses (see Appendix), the pattern changed and a clade consisting of *Endomyzostoma, Protomyzostomum, Asteromyzostomum* and *Eenymeenymyzostoma* (without *Pulvinomyzostomum*) appeared as the sister group of the other myzostomids. With varying numbers of polychaete species in the outgroup, i.e. from 1 to 6, the 127 resulting ML analyses always showed the same pattern as the ML tree obtained with 7 species (Fig. 89).

DISCUSSION

Even if black corals are known to host a lot of different symbionts, most of them are ectosymbiotic (Grange 1991; Buhl-Mortensen & Mortensen 2004; Molodtsova & Budaeva 2007; Mi-Kyung & Il-Hoi 2010; Van Syoc & Dekelboum 2011). Endoparasites have been found in antipatharians (see a crustacean endoparasite for example, Grygier 1990) but reports on their presence remain scarce. *Eenymeenymyzostoma nigrocorallium* is the first myzostomid species living in association with black corals to be described. In literature, such symbioses have been reported twice (Goenaga 1977; Bo *et al.* 2013). In his dissertation at the University of Puerto Rico, Goenaga (1977) described a dark green to brown myzostomid of about 1.5 mm in length and 0.8 mm in width infesting the digestive lumen of *Stichopathes* sp. These specimens are morphologically different from *E. nigrocorallium* and they appear to be a new undescribed species. Unfortunately, the fixative used to store the specimens did not allow any molecular or histological analysis, and new sampling is needed to describe this species. Mark J. Grygier (*personal communication*), another myzostomid expert, went to Smithsonian's NMNH and studied the undescribed specimens. He considered *M. radiatum* (which is probably a synonym of *M. aureolatum*) to be the most similar species (allowing for the fact that Graff described it with the axes turned 90 degrees). Bo *et al.* (2013) found myzostomids inside the digestive lumen of *Cirrhipathes* cf. *rumphii* in Indonesia. These worms had a rounded and flattened, yellowish body of 1.0 to 1.8 mm in diameter. This minimal description, when coupled with the photos presented by Bo *et al.* (2013), is sufficient to show that *E. nigrocorallium* is a different species: the Indonesian specimens have 5 pairs of better developed parapodia and they have four white dorsal dots considered to be dorsal sense organs.

The myzostomids are observed in the gastrovascular cavity of the polyps but also in endodermal ducts between the polyps. They did not appear to be harmed by the host's digestive enzymes, a characteristic also found in other myzostomid endosymbionts of the genera *Notopharyngoides*, *Pulvinomyzostomum* and *Asteriomyzostomum* that inhabit asteroid and crinoid digestive systems. Some species of the two first genera have been studied under SEM and their surface is generally covered by cilia that are suggested to somehow protect the worm's body from enzyme digestion (Eeckhaut & Lanterbecq 2014). The trunk of *E. nigrocorallium* has some cilia (Fig. 2A,B), which could give the same kind of protection, and so has the myzostomid collected by Goenaga in 1977. The epidermis appears extremely glandular and the secretions of the glandular cells may also protect the myzostomid's body (Fig. 3A,B). Bo *et al.* (2013) found myzostomids in 30% of the colonies they sampled, and *E. nigrocorallium* infested 73% of the antipatharian colonies observed in our study. It is likely that the incidence of infestation is actually even higher, as we did not inspect the whole length of colonies but just fragments. Bo *et al.* (2013) suggested that parasitic castration of the coral polyps probably occurs.

The anatomy of *E. nigrocorallium* is unusual amongst myzostomids in having no lateral organs, no introvert and a male genital system that is located dorsally (see Grygier 2000; Eeckhaut & Lanterbecq 2005; Eeckhaut & Lanterbecq 2014). Non-observation of lateral organs is not strange as the disappearance of lateral organs has seemingly occurred at least twice in endosymbiotic myzostomids, in *Mesomyzostoma* and in many *Endomyzostoma,* according to the character state reconstruction of Lanterbecq *et al.* (2009). Jägersten (1940a) divided the Myzostomida into two orders, the Pharyngidea and the Proboscidea, on the basis of differences in the ontogenesis of the anterior body part. In the ontogeny of *Myzostoma* species (Proboscidea), the blastopore (which becomes the mouth) is located at the apex of the introvert while in the other genera (although Jägersten seems to have observed the ontogeny of the pharynx only in *Pulvinomyzostomum pulvinar*), the blastopore forms the ventral opening through which the pharynx is everted. Jägersten's (1940) classification is out of date but the absence of an introvert takes *E. nigrocorallium* away from the genus *Myzostoma*.

Individuals of *E. nigrocorallium* are simultaneous hermaphrodites with the male and female gonads located dorsally to the digestive tract. In general in myzostomids, the male gonads are located ventrally and their dorsal position in *E. nigrocorallium* is probably related to their black coral hosts. Instead of housing male gonads, the ventral parenchyma is unusually well developed, probably as a protective adaptation against the many small spines that are characteristic of black coral skeletons. The male and female genital ducts have the usual myzostomidan morphology. Female germinal cells grow in the parenchyma and fall into the uterine branches, where they are fertilized and moved to the uterus (Grygier 2000; Lanterbecq & Eeckhaut 2005; Eeckhaut & Lanterbecq 2014). Male germinal cells also grow in the parenchyma and are moved to the seminal vesicles, where spermatophores are formed. The presence of spermatophores in the seminal vesicles and fertilized eggs in the uterus in *E. nigrocorallium* indicate that they reproduce by intradermic sperm penetration: spermatophores are probably deposited on any part of the receiving individual's body surface, whereupon spermatozoa penetrate the epidermis to reach mature oocytes as it is the case in many myzostomids (Grygier 2000; Eeckhaut & Lanterbecq 2005; Eeckhaut & Lanterbecq 2014). Sexual reproduction probably occurs when they gather in small groups and touch each other. Fertilized eggs are most likely emitted into the gastrodermal cavities of polyps and then ejected to the exterior to develop into larvae before reaching other hosts.

Although the position of *E. nigrocorallium* as sister to *E. cirripedium* is unquestionable in our trees, the topology in these trees is clearly dependant on the number of species included in the outgroup and on the phylogenetic inference method employed. A consequence of this unstable tree topology is that we cannot yet be sure of the overall phylogenetic history of the myzostomids, and questions about their evolution that could otherwise be addressed by methods like character mapping remain open.

In Madagascar, the two comatulids *Comanthus* sp. aff. *wahlbergii* and *Stephanometra indica* are hosts of the myzostomids *Notopharyngoides aruensis*, *Myzostoma polycyclus*, *Myzostoma pseudocuniculus* and *Myzostoma toliarense* (Lanterbecq & Eeckhaut 2003). All of them belong to the paraphyletic family Myzostomatidae. They are morphologically very different from *E. nigrocorallium*. *Myzostoma polycyclus* and *M. pseudocuniculus* are ectocommensals, *M. toliarense* live in cysts and *N. aruensis* are in the digestive system of their hosts. The last species is a large worm with well-developed parapodia and lateral organs. Its morphology is very distinctive and the species cannot be confounded with *E. nigrocorallium*. In addition, the present phylogenetic analysis show at least that these four species, which are the only ones recorded from Madagascar so far, do not form an inclusive clade with *E. nigrocorallium*, nor with the only South African species described to date (Lanterbecq & Eeckhaut 2003, Lanterbecq *et al.* 2009) .

All of our molecular phylogenetic analyses demonstrate that the present myzostomid that infests black corals is closely related to *Eenymeenymyzostoma cirripedium* (Graff, 1885), which was formerly known as *Myzostoma cirripedium* (Summers & Rouse 2014, 2015) and is associated with stalked crinoids of the genus *Metacrinus*. Sequences for this species were first reported as "*Endomyzostoma* sp. n. 2" in Lanterbecq *et al.* (2006), and according to Summers & Rouse (2014), *Myzostoma metacrini* McClendon, 1906 is a junior synonym of *E. cirripedium*. *Eenymeenymyzostoma* was erected as a monotypic new genus to accommodate *M. cirripedium,* which was recovered by Summer & Rouse (2014) as the sister species to the well supported (unnamed) clade comprised of Pulvinomyzostomidae and Myzostomatidae. The morphology of *E. nigrocorallium* is totally different from that of *E. cirripedium*, the later having an ellipsoïdal trunk with 20 marginal cirri, five pairs of ventral, well developed parapodia with elongate parapodial cirri and four pairs of lateral organs alternating with the parapodia (Graff 1885; Summers & Rouse 2015).

The close relationship between the two species coupled with the global phylogeny of myzostomids indicates that in the evolutionary history of this group some myzostomids have shifted from stalked crinoid hosts to black coral hosts. It is not easy to detect myzostomids infesting antipatharian black corals, but we suppose that, not only on coral reefs but also in the deep sea, antipatharians may be common hosts for endosymbiotic myzostomids.

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LITERATURE

Bleidorn, C., Eeckhaut, I., Podsiadlowski, L., Schult, D., McHugh, K., Halanych, M., Milinkovitch, M., Tiedemann, R. (2007) Mitochondrial genome and nuclear sequence data support Myzostomida as part of the annelid radiation. *Molecular Biology and Evolution*, 24(8), 1690–1701. http://dx.doi.org/10.1093/molbev/msm086

Bleidorn, C., Podsiadlowski, L., Zhong, M., Eeckhaut, I., Hartmann, S., Halanych, K.M., Tiedemann, R. (2009a) On the phylogenetic position of Myzostomida: can 77 genes get it wrong? *BMC Evolutionary Biology*, 9(1), 1–11. http://dx.doi.org/10.1186/1471-2148-9-150

Bleidorn, C., Lanterbecq, D., Eeckhaut, I., Tiedemann, R. (2009b) A PCR survey of Hox genes in the myzostomid *Myzostoma cirriferum*. *Development Genes and Evolution*, 219(4), 211–216. http://dx.doi.org/10.1093/molbev/msm086

Bleidorn, C., Helm, C., Weigert, A., Eeckhaut, I., Lanterbecq, D., Struck, R., Hartmann, S., Tiedemann, R. (2014) From morphology to phylogenomics: placing the enigmatic Myzostomida in the tree of life. *In:* Wägele, J.W., Bartholomaeus, T. (Eds.), *Deep metazoan phylogeny: the backbone of the tree of life.* De Gruyter, Berlin 161-172.

Bo, M., Rouse, G.W., Martin, D., Bavestrello, G. (2013) A myzostomid endoparasitic in black corals. *Coral Reefs*, 33, 273–273.

Buhl-Mortensen, L.E.N.E., Mortensen, P.B. (2004) Symbiosis in deep-water corals. *Symbiosis*, 37(1), 33-61.

Capella-Gutiérrez, S., Silla-Martínez, J.M., Gabaldón, T. (2009) trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics*, 25(15), 1972–1973. http://dx.doi.org/10.1093/bioinformatics/btp348

Eeckhaut, I., Jangoux, M. (1991) Fine structure of the spermatophore and intradermic penetration of sperm cells in *Myzostoma cirriferum* (Annelida, Myzostomida). *Zoomorphology*, 111(1), 49-58.

Eeckhaut, I., McHugh, D., Mardulyn, P., Tiedemann, R., Monteyne, D., Jangoux, M., Milinkovitch, M. (2000) Myzostomida: a link between trochozoans and flatworms? *Proceedings of the Royal Society of London B*, 267, 1383–1392.

http://dx.doi.org/10.1098/rspb.2000.1154

Eeckhaut, I., Lanterbecq, D. (2005) Myzostomida: a review of their ultrastructure and phylogeny. *In:* Bartholomaeus, T., Purschke, G. (Eds.), *Morphology, Molecules and Evolution of the Polychaeta and Related Taxa.* Springer Netherlands, 253–275.

Eeckhaut, I., Lanterbecq, D. (2014) Myzostomida. *In:* Schmidt-Rhaesa, A.(Eds.), *Handbook of Zoology Online*. De Gruyter, Berlin.

Felsenstein, J. (1985) Phylogenies and the comparative method. *The American Naturalist*, 125, 1–15.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Biology and Biotechnology*, 3, 294–299.

Goenaga, C. (1977) Two new species of *Stichopathes* (Zoantharia; Antipatharia) with observations on aspects of their biology. Unpublished MS Thesis, University of Puerto Rico.

Grygier, M.J. (1990) A crustacean endoparasite (Ascothoracida: Synagogidae) of an antipatharian from Guam. *Micronesica*, 23(1), 15-25.

Grygier, M.J. (2000) Class Myzostomida. *In:* Beesley, P.L., Ross, G.J.B., Glasby, (Eds.), *Polychaetes and Allies: The Southern Synthesis, Fauna of Australia, Vol. 4A. Polychaeta, Myzostomida, Pogonophora, Echiura, Sipuncula*. CSIRO Publishing, Melbourne 297–330.

Graff, L. (1877) Das Genus *Myzostoma* (F.S. Leuckart). Leipzig: Verlag von Wilhelm Engelmann.

Graff, L. (1885) Description of a new species *Myzostoma*. Transactions of the Linnean Society of London, 2nd Series, Zoology. 2, 444–446. available online at http://biodiversitylibrary.org/page/8600265

Grange, K.R. (1991) Mutualism between the antipatharian *Antipathes fiordensis* and the ophiuroid *Astrobrachion constrictum* in New Zealand fjords. *Hydrobiologia*, 216/217, 297-303.

Hartmann, S., Helm, C., Nickel, B., Meyer, M., Struck, T., Tiedemann, R., Selbig, J., Bleidorn, C. (2012) Exploiting gene families for phylogenomic analysis of myzostomid transcriptome data. *PLOS ONE.* 7(1), e29843.

http://dx.doi.org/10.1371/journal.pone.0029843

Helaers, R., Milinkovitch, M. (2010) MetaPIGA v3.1: maximum likelihood large phylogeny estimation using the metapopulation genetic algorithm and other stochastic heuristics. *BMC Bioinformatics,* 11, 379. http://dx.doi.org/10.1186/1471-2105-11-379

Helm, C., Weigert, A., Mayer, G., Bleidorn, C. (2012) Myoanatomy of *Myzostoma cirriferum* (Annelida, Myzostomida): implications for the evolution of the myzostomid body plan. *Journal of Morphology,* 274, 456–466. http://dx.doi.org/10.1002/jmor.20107

Hess, H. (2010) Myzostome deformation on arms of the Early Jurassic crinoid *Balanocrinus gracilis* (Charlesworth). *Journal of Paleontology*, 84(6), 1031-1034.

Jägersten, G. (1940) Zur Kenntnis der Morphologie, Entwicklung und Taxonomie der Myzostomida. Almqvist & Wiksells.

Lanterbecq, D., Eeckhaut, I. (2003) Myzostomida from Madagascar, with the description of two new species. *Hydrobiologia* 496, 115-123. http://dx.doi.org/10.1023/A:1026180428299

Lanterbecq, D., Rouse, G.W., Milinkovitch, M., Eeckhaut, I. (2006) Molecular phylogenetic analyses indicate multiple independent emergences of parasitism in Myzostomida (Protostomia). *Systematic Biology,* 55(2), 208–227.

http://dx.doi.org/10.1080/10635150500481317

Lanterbecq, D., Hempson, T., Griffiths, C., Eeckhaut, I. (2009) *Myzostoma fuscomaculatum* (Myzostomida), a new species of myzostome from False Bay, South Africa. *Hydrobiologia*, 619(1), 145–154. http://dx.doi.org/ 10.1007/s10750-008-9606-7

Lanterbecq, D., Rouse, G.W., Eeckhaut, I. (2010) Evidence for cospeciation events in the hostsymbiont system involving crinoids (Echinodermata) and their obligate associates, the myzostomids (Myzostomida, Annelida). *Molecular Phylogenetics and Evolution*, 54, 357–371. http://dx.doi.org/10.1016/j.ympev.2009.08.011

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G. (2007) Clustal W and Clustal X version 2.0. *Bioinformatics*, 23, 2947–2948. http://dx.doi.org/10.1093/bioinformatics/btm404

McClendon, J.F. (1906) The myzostomes of the "Albatross" Expedition to Japan. *Bulletin of the American Museum of Natural History,* 23, 119–130.

Mi-Kyung, C., Il-Hoy, K. (2010) *Paramolgus orientalis* n. sp. (Copepoda: Cyclopoida: Rhynchomolgidae) Associated with an Antipatharian Coral from Korea. *Animal Systematics, Evolution and Diversity*, 26(1), 49-54.

Molodtsova, T., Budaeva, N. (2007) Modifications of corallum morphology in black corals as an effect of associated fauna. *Bulletin of Marine Science*, 81(3), 469-480.

Nylander, J.A.A. (2002) MrModeltest, version 1.1b. Available from the authors at: http://www.ebc.uu.se/systzoo/staff/nylander.html.

Okada, Y. (1920) Notes on a species of *Myzostoma* attached to a sponge. *Zoological Magazine,* Japan. 32, 324–325.

Palumbi, S.R. (1997) Nucleic acids II: The polymerase chain reaction. *In:* Hillis, D.M., Moritz, C., Mable, B.K. (Eds.). *Molecular systematics, 2nd edition* (pp. 205-247). Sinauer Associates, Sunderland, Massachusetts.

Rasband, W.S. (1997) ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA.

Ronquist, F., Huelsenbeck, J.P. (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics,* 19, 1572–1574. http://dx.doi.org/ 10.1093/bioinformatics/btg180

Summers, M.M., Al-Hakim, I., Rouse, G.W. (2014) Turbotaxonomy: 21 new species of Myzostomida (Annelida). *Zootaxa*. 3873, 301–344.

http://dx.doi.org/10.11646/zootaxa.3873.4.1

Summers, M.M., Rouse, G.W. (2014) Phylogeny of Myzostomida (Annelida) and their relationships with echinoderm hosts. *BMC Evolutionnary Biology*, 14(1), 170. http://dx.doi.org/ 10.1186/s12862-014-0170-7

Summers, M.M., Rouse, G.W. (2015) Erratum: Phylogeny of Myzostomida (Annelida) and their relationships with echinoderm hosts. *BMC Evolutionnary Biology*, 15, 33. http://dx.doi.org/ 10.1186/s12862-015-0305-5

Swofford, D. (1998) PAUP*: phylogenetic analysis using parsimony (*and other methods). Beta version 4.0.b1, Sinauer, Sunderland.

Van Syoc, R. J., Dekelboum, A.M. (2011) Oxynaspididae (Crustacea, Cirripedia) : phylogenetic and evolutionary ecology, with descriptions of three new genera and six new species. *Zootaxa*, 3103, 1-32.

Yakovlev, N. (1939) Nouveaux genres de coraux Tabulata du Permien inférieur de l'Oural et du bassin du Donetz. *Comptes Rendus de l'Académie des Sciences de l'URSS*, 24(6), 629–632.

SUPPLEMENTARY MATERIAL

Appendix I. Consensus tree of Myzostomida obtained with Bayesian analysis and with *Phyllodoce maculata* as the sole outgroup. Numbers above branches indicate posterior probabilities.

SUPPLEMENTARY MATERIAL

Appendix II. Examples of snapshots taken in the tridimensional model of the myzostomid. http://doi.org/10.13140/RG.2.2.17911.21923