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Diversity of the Pterasteridae (Asteroidea) in the Southern Ocean: a molecular and morphological approach

QUENTIN JOSSART[1*], MARC KOCHZIUS[1], BRUNO DANIS[2], THOMAS SAUCÈDE[3] and CAMILLE V. E. MOREAU[2,3]

[1]*Marine Biology, Ecology and Biodiversity, Vrije Universiteit Brussel (VUB), Brussels, Belgium*
[2]*Laboratoire de Biologie Marine, Université Libre de Bruxelles (ULB), Brussels, Belgium*
[3]*Biogéosciences, Université Bourgogne Franche Comté (UBFC), Dijon, France*

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An integrative approach is crucial in discrimination of species, especially for taxa that are difficult to identify based on morphological characters. In this study, we combine genetics and morphology to assess the diversity of Pterasteridae, a sea star family diversified in deep-sea and polar environments. Because of their derived anatomy and the frequent loss of characters during preservation, Pterasteridae are a suitable case for an integrative study. The molecular identification of 191 specimens (mostly from the Southern Ocean) suggests 26–33 species in three genera (*Diplopteraster*, *Hymenaster* and *Pteraster*), which match the morphological identification in 54–62% of cases. The mismatches are either different molecular units that are morphologically indistinguishable (e.g. *Pteraster stellifer* units 2 and 4) or, conversely, nominal species that are genetically identical (e.g. *Hymenaster coccinatus*/*densus*/*praecoquis*). Several species are shared between the Northern and Southern Hemispheres (e.g. *Pteraster jordani*/*affinis*). In conclusion, the taxonomic status of some groups is confirmed, but for others we find the need to re-evaluate the taxonomy at both genus and species levels. This work significantly increases the DNA barcode library of the Southern Ocean species and merges taxonomic information into an identification key that could become a baseline for future studies (pterasteridae-so.identificationkey.org).

ADDITIONAL KEYWORDS: Antarctica– *COI* mitochondrial DNA – cryptic species – deep sea – echinoderms – identification key – morphological systematics – phylogenetics – taxonomy.

INTRODUCTION

Taxonomy has a pivotal role in biology (Costello *et al.*, 2013). Inaccurate identifications and naming lead to misunderstandings and spurious interpretations of biological processes in various domains of the life sciences (Dayrat, 2005; Pante *et al.*, 2015). Fifteen years ago, integrative taxonomy was introduced as a promising approach to complement the traditional, morphology-based taxonomy, using new data and methods (Dayrat, 2005). Among these, molecular markers were highlighted, considering the simultaneous leaps achieved by new genetic methodologies, such as DNA barcoding (Hebert *et al.*, 2003; Stoeckle, 2003; Hebert & Gregory, 2005; Ratnasingham & Hebert, 2007; Fujita *et al.*, 2012). The number of barcoded species is currently still low in comparison to the total number of recognized species, with less than a quarter of nominal species having been barcoded in most phyla (Gong *et al.*, 2018). Nevertheless, a plethora of studies have shown the importance of using genetics and morphology alongside for discrimination at all taxonomic ranks (e.g. Richter *et al.*, 2008; Laakmann *et al.*, 2012; Pante *et al.*, 2015; Christiansen *et al.*, 2018; Peck *et al.*, 2018; Jossart *et al.*, 2019). Based on the data from the World Register of Marine Species (WoRMS), Appeltans *et al.* (2012) showed that molecular methods are significantly increasing our knowledge of marine biodiversity by helping in the detection of cryptic species and the establishment of synonymies. This is of particular importance for under-investigated taxa,

*Corresponding author. E-mail: qjossart@gmail.com

such as those including numerous species that remain unrevised since their original descriptions.

Asteroidea (i.e. sea stars or starfish) is the second-most diversified class of echinoderms, with ~1900 described species assigned to 38 families (Mah & Blake, 2012; Mah *et al.*, 2015). They show various ecological traits and are present in a broad variety of ecosystems (Mah & Blake, 2012; Lawrence *et al.*, 2013; Moreau *et al.*, 2017). For the last 20 years, considerable efforts have been made to re-evaluate the sea star phylogeny using molecular data (Knott & Wray, 2000; Janies *et al.*, 2011; Mah & Foltz, 2011a, b; Linchangco *et al.*, 2017; Moreau *et al.*, 2019). However, there is a sharp contrast in our knowledge of sea star diversity amongst families from different biogeographical regions (Feuda & Smith, 2015). This is the case for Pterasteridae Perrier, 1875, the most diverse family in the order Velatida, which includes ~130 nominal species and eight genera: *Amembranaster* Golotsvan, 1998, *Benthaster* Sladen, 1882, *Calyptraster* Sladen, 1882, *Diplopteraster* Verrill, 1880, *Euretaster* Fisher, 1940, *Hymenaster* Wyville Thomson, 1873, *Hymenasterides* Fisher, 1911, and *Pteraster* Müller & Troschel, 1842 (Mah, 2020). One unique feature of Pterasteridae is the presence of an additional (supra)dorsal membrane that produces abundant quantities of mucus (Mah & Blake, 2012; Gale, 2018). Between the dorsal and the supradorsal membranes lies a nidamental cavity, where incubation of juveniles takes place in some species (Janies, 1995). The taxonomy of the group is complicated for the following three reasons.

1. Morphologies are highly derived, and only few diagnostic characters are recognized. Most characters commonly used for species identification in other sea stars are not applicable to the family (Gale, 2018).
2. The few available characters are often indiscernible, because specimens are particularly fragile and are damaged by sampling and preservation protocols (Villier *et al.*, 2004).
3. Several species are known only from their original description based on few (poorly preserved) specimens (Villier *et al.*, 2004).

Consequently, the Pterasteridae family could benefit from an integrative taxonomic approach.

The family mainly occurs in deep cold waters, including the Arctic and Southern Oceans (Mah & Blake, 2012). Although genetic sequences are available for specimens from the Northern Hemisphere, no public data have been published for Southern Ocean species (source: www.boldsystems.org). International initiatives of the Census of Antarctic Marine Life (CAML) and of the International Polar Year (IPY) have promoted sampling efforts in the Southern Ocean (Schiaparelli *et al.*, 2013), and this momentum was at the origin of many biological campaigns. This has significantly enhanced the taxonomic and spatial coverage of the Southern Ocean biodiversity inventory, including the collection of deep-sea representatives of Pterasteridae. These new and well-preserved specimens have offered the opportunity to re-investigate the taxonomy of the family.

Based on these new samples, we have combined morphological and molecular approaches to verify whether their joint use could better assess the diversity within Pterasteridae. After an initial morphological investigation, we used a mitochondrial gene [cytochrome *c* oxidase subunit I (*COI*)] to verify how it confirms or complements the morphological identification. Subsequently, we re-investigated (*a posteriori*) specimens using a morphological approach in order to identify new characters that might differentiate species. Finally, we synthesized, for the first time, the revised taxonomy of the family and have made it available to all potential users in an open-access identification key that includes all Southern Ocean species (pterasteridae-so.identificationkey.org).

MATERIAL AND METHODS

Sampling

Specimens were collected during 16 international campaigns at sea from 1998 to 2017 (ACE, ANDEEP-3, ANDEEP-SYSTCO, ARGOS, CEAMARC 2007–2008, JR144, JR179, JR262, JR275, JR15005, MD208, MUSORSTOM 10, Poker 2, PS77, PS81 and PS96). The available specimens cover a wide distribution within the Southern Ocean (Fig. 1), including the Patagonian shelf, the South Sandwich Islands, South Georgia, South Orkney, the Shag Rocks, Kerguelen, Crozet and the Antarctic continental shelf (Adélie Land, Amundsen Sea, Antarctic Peninsula and Weddell Sea). A total of 171 specimens from these locations were included in the analysis. In order to increase the taxonomic and geographical scope, in addition to the phylogenetic resolution, 20 additional genetic sequences (see Genetic Data below) from specimens outside the Southern Ocean were added to the dataset (i.e. Fiji, South Africa, Pacific and Atlantic coasts of North America, Norway and Russia). Metadata documenting all the 191 samples can be found in the Supporting Information (Supplementary Material 1).

Morphological identification

A total of 124 Southern Ocean individuals (preserved in ethanol or frozen) were identified morphologically by the authors using original descriptions (e.g. Sladen, 1882; Koehler, 1908), identification books (Clark, 1962; Clark & Downey, 1992; McKnight, 2006) and

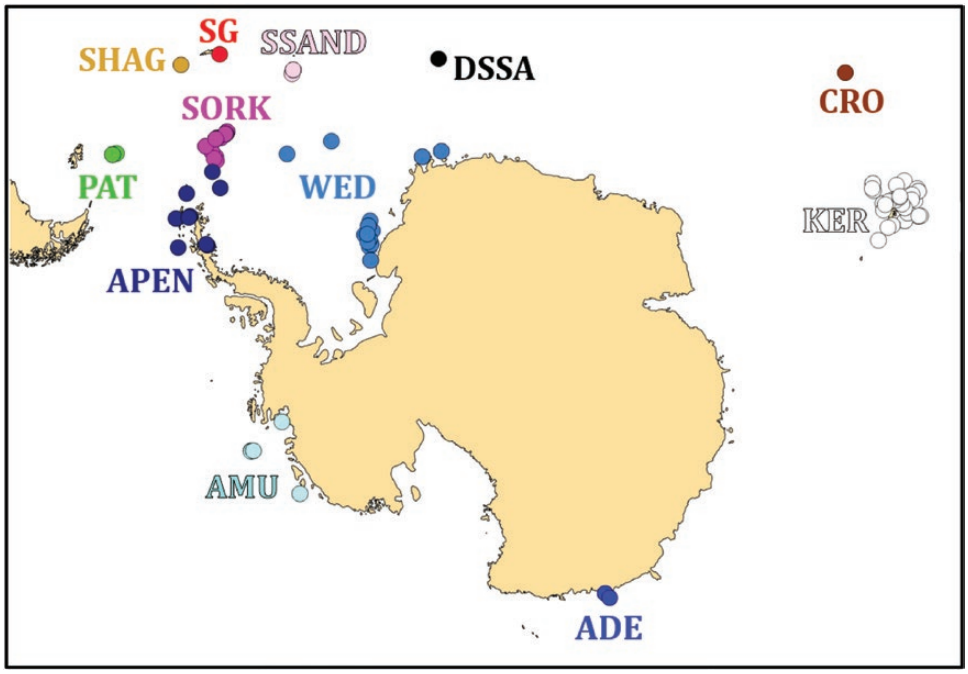

Figure 1. Sampling locations of the Pterasteridae specimens from the Southern Ocean. Abbreviations: ADE, Adelie Land; AMU, Amundsen Sea; APEN, Antarctic Peninsula; CRO, Crozet; DSSA, Deep-Sea South Atlantic; KER, Kerguelen; PAT, Patagonian shelf; SHAG, Shag Rocks; SG, South Georgia; SORK, South Orkney; SSAND, South Sandwich; WED, Weddell Sea.

contemporary scientific literature (Villier *et al.*, 2004; Gale, 2018). Subsequent to genetic analyses (see Genetic Data below), an *a posteriori* morphological investigation was carried out to look for new characters to differentiate species when new species delineations and synonymies were suggested by genetic data. Finally, the taxonomy of the family was synthesized and made available online, by building an interactive identification key through the Xper^3 portal (Fig. 2). Xper^3 is a Web portal with an easy-to-use interface that allows multiple access points (the key can be started using any character; Vignes-Lebbe *et al.*, 2016). Specimens and characters were also illustrated by drawings and macro-pictures (photographed using a camera with a macro lens, two flashes and accessories to diffuse or reflect the light; Figs 2, 3).

Genetic data

A fragment of the mitochondrial gene *COI* was sequenced (601 bp) for the 191 individuals. These genetic sequences were obtained through laboratory work in our institutes (80 individuals; see protocol below), through our private Barcode of Life Data System project (BOLD; 94 individuals) or mined from public BOLD projects (17 individuals).

DNA extractions were performed on one tube foot (podium) per sample and were based on the salting-out protocol of Sunnucks & Hales (1996). The amplification step was performed using the forward primers 'F-COI-PTE-28' (5′-GCTGGAATGATTGGAACTGC-3′) or 'LCOech1aF1' (5′-TTTTTTCTACTAAACACAAG GATATTGG-3′) and the reverse universal primer 'R-HCO2198' (5′-TAAACTTCAGGGTGACCAAAAAA TCA-3′; Folmer *et al.*, 1994). Each polymerase chain reaction (PCR) mix (25 µL) included 12.5 µL of a VWR Mastermix (2.5 units of VWR Taq polymerase, 0.4 mM of each dNTP and 1.5 mM of $MgCl_2$), 10.5 µL of molecular water, 0.5 µL each primer (10 µM) and 1 µL of the DNA extract. The PCR conditions consisted of 35 cycles for each of the three temperature steps [30 s at 95 °C (denaturation), 30 s at 48 °C (annealing) and 30 s at 72 °C (elongation)]. These cycles were preceded by 2 min at 95 °C and followed by 10 min at 72 °C. EXOSAP purification (incubation at 37 °C for 15 min followed by another at 80 °C for 15 min) was done before sending the PCR products to the MACROGEN sequencing service. Sequence editing and alignment were done using the software Geneious (Kearse *et al.*, 2012). The absence of a stop codon in the sequence was checked in the same software in order to exclude the presence of nuclear pseudo-genes.

PartitionFinder 2 (Bayesian information criterion; Lanfear *et al.*, 2016) was used within the CIPRES portal (Miller *et al.*, 2010) to select the most suitable substitution models (i.e. TRNEF+I+G for codon position 1, HKY+I+X

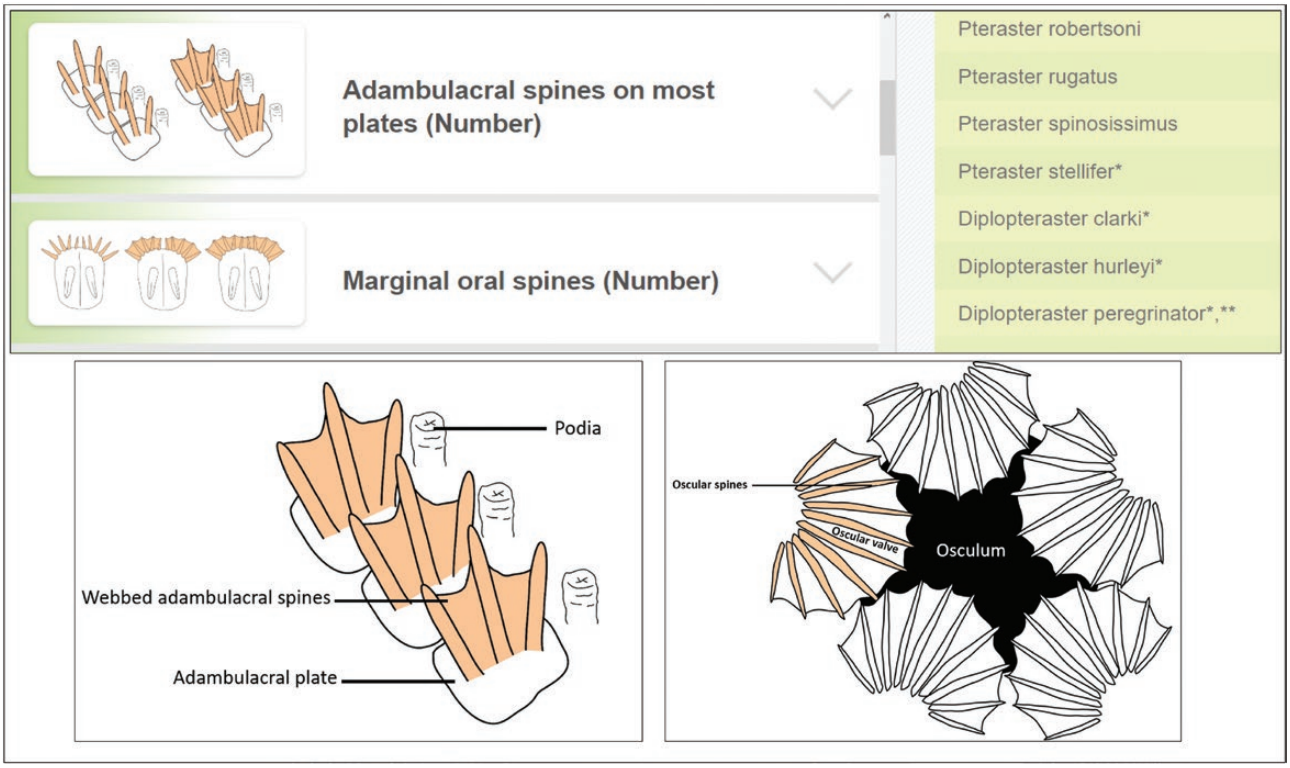

Figure 2. Interface of the Xper[3] identification key (top panel) and two examples of integrated drawings illustrating diagnostic characters (bottom left, adambulacral spines; bottom right, oscular spines).

for codon position 2 and TRN+I+G+X for codon position 3). A Bayesian phylogeny was subsequently produced using BEAST v.1.8.4 (Drummond & Rambaut, 2007) within the CIPRES portal. Based on a previous phylogeny using multiple genes, *Remaster gourdoni* Koehler, 1912 was used as the outgroup (Linchangco *et al.*, 2017). Parameters of the analysis were as follows: partitioned dataset, Yule process tree prior, Markov chain Monte Carlo of 100 × 10^6 generations sampled every 10 000 trees. TRACER v.1.6 was used to ensure an appropriate effective sampling size (ESS all > 200). TREEANNOTATOR v.1.8.4 was used to find the most likely tree, which was visualized in FIGTREE v.1.4.3 (tree.bio.ed.ac.uk/software/figtree). Node support was assessed through posterior probability (PP), with values < 0.75 not being retained and collapsed into polytomies (Huelsenbeck & Rannala, 2004). Moreover, the software DENSITREE v.2.2 was used to verify the potentiality of competing topologies among the set of trees (Bouckaert, 2010).

Three different methods of species delimitation were used to propose primary species hypotheses: one distance based [automatic barcode gap discovery (ABGD); Puillandre *et al.*, 2012] and two tree based [Bayesian Poisson tree process (bPTP; Zhang *et al.*, 2013) and generalized mixed Yule coalescent (GMYC; Fujisawa & Barraclough, 2013)]. The ABGD analysis (bioinfo.mnhn.fr/abi/public/abgd) was performed with a relative gap width of one and Kimura (K80) as the genetic distance. The bPTP analysis (species.h-its.org/ptp) was applied using 500 000 generations of Markov chain Monte Carlo, a thinning of 100 and a burn-in of 25%. Finally, the GMYC analysis (species.h-its.org/gmyc) was performed using the single threshold method. Haplotype diversity and nucleotide diversity were calculated within each species using DNASP v.6 (Rozas *et al.*, 2017).

RESULTS

INITIAL MORPHOLOGICAL IDENTIFICATION

Among the 124 morphologically investigated individuals, 91 are identified to species level. Thirty-two individuals are identified to genus level and one to family level owing to the small size of specimens (juveniles) or poor preservation that does not permit observation of diagnostic characters. Thirteen species and three genera are identified (Fig. 3). Four species of *Pteraster* are found: *Pteraster affinis* Smith, 1876, *Pteraster gibber* (Sladen, 1882), *Pteraster rugatus* Sladen, 1882 and *Pteraster stellifer* Sladen, 1882. The numbers and types of marginal oral spines are important diagnostic characters to discriminate the different *Pteraster* species, and these characters are

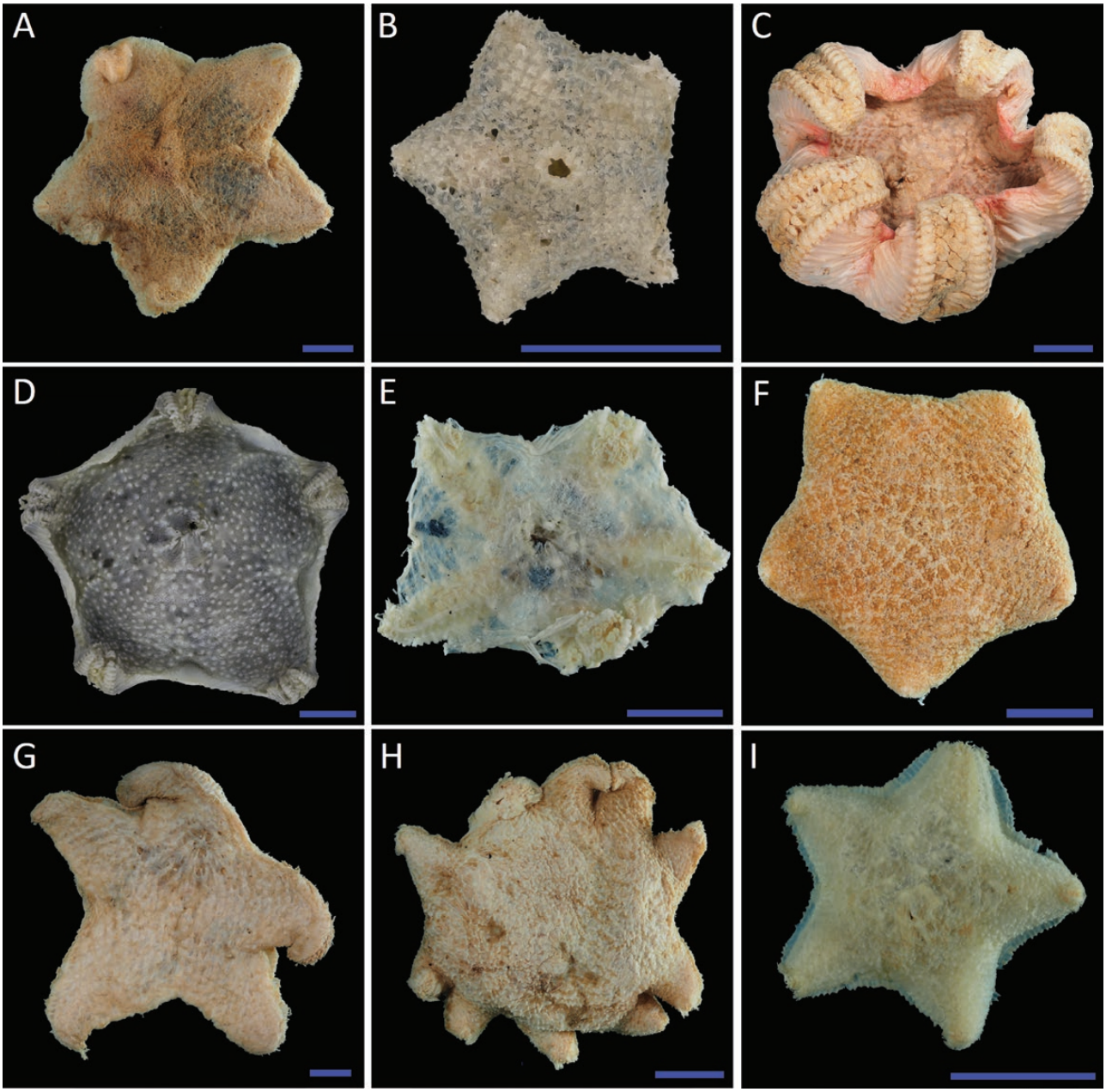

Figure 3. Aboral view of Pterasteridae species illustrating their Southern Ocean diversity. A, *Diplopteraster* sp.; B, *Hymenaster campanulatus*; C, *Hymenaster praecoquis*; D, *Hymenaster edax*; E, *Hymenaster sacculatus*; F, *Pteraster gibber*; G, *Pteraster affinis*; H, *Pteraster koelheri*; I, *Pteraster stellifer*. Scale bars: 1 cm. Photos: P. Pernet, Q. Jossart (Biologie Marine, ULB).

usually well preserved. Specimens of *Diplopteraster* are identified only up to the genus level owing to the absence of observable characters [e.g. between *Diplopteraster semireticulatus* (Sladen, 1882) and *Diplopteraster verrucosus* (Sladen, 1882); see Discussion]. Nine species of *Hymenaster* are identified: *Hymenaster campanulatus* Koehler, 1907, *Hymenaster coccinatus* Sladen, 1882, *Hymenaster densus* Koehler, 1908, *Hymenaster edax* Koehler, 1907, *Hymenaster formosus* Sladen, 1882, *Hymenaster latebrosus* Sladen, 1882, *Hymenaster perspicuus* Ludwig, 1903, *Hymenaster praecoquis* Sladen, 1882 and *Hymenaster sacculatus* Sladen, 1882. Some of these *Hymenaster* species are discriminated based on tenuous morphological differences, such as *H. densus* and *H. praecoquis*, which are differentiated based on only slight variations in the morphology of the segmental papillae and number of marginal spines (Clark, 1962).

Species delimitation (*COI*)

Species delimitation methods applied to molecular data give 26 species hypotheses for ABGD and 33 for bPTP and GMYC (Fig. 4). For each species hypothesis, the relationship between haplotype diversity and nucleotide diversity fits the expected variation for a single species, except for *Pteraster stellifer* unit 1 (Goodall-Copestake *et al.*, 2012). Morphological species identification matches molecular species delimitation in only 54% of species for ABGD and 62% for GMYC/bPTP. Within the genus *Pteraster*, species complexes are suggested within the nominal species *P. stellifer* (three to five units) and *P. affinis* (two units). Within the genus *Hymenaster*, three morphological species

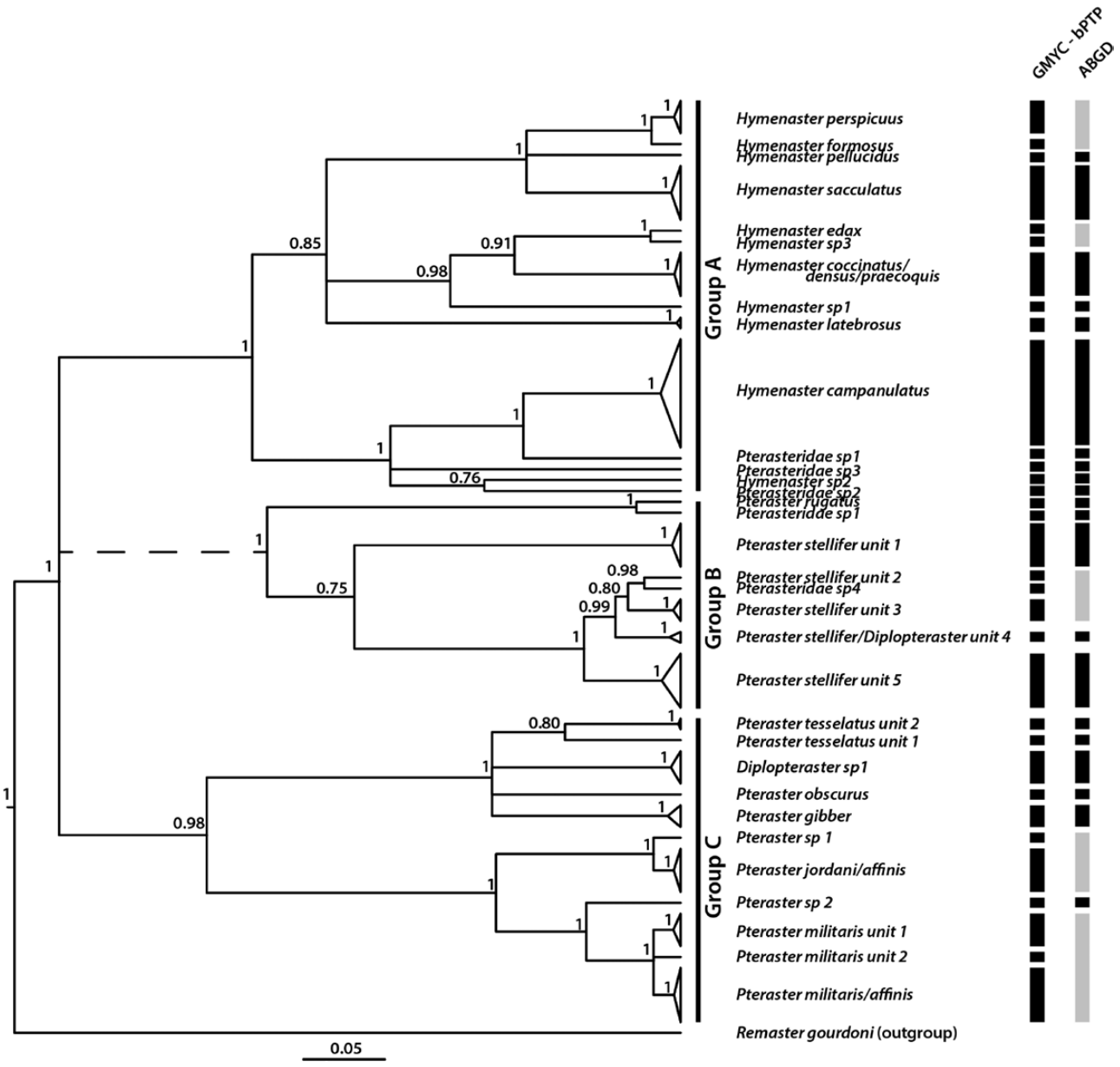

Figure 4. Bayesian phylogeny based on mitochondrial cytochrome *c* oxidase subunit I (*COI*) sequences. Values at each node are the posterior probabilities (nodes with support < 0.75 were collapsed into polytomies). The dashed line (associated with group B) indicates competing topologies from the software DensiTree. Assignments for each species delimitation method are reported as black bars [generalized mixed Yule coalescent–Bayesian Poisson tree process (GMYC–bPTP) on the left and automatic barcode gap discovery (ABGD) on the right]. Distinct GMYC–bPTP and ABGD assignments are highlighted by grey bars for the ABGD assignments.

appear to be undifferentiated genetically, suggesting that the diversity of the genus has been overestimated (*H. coccinatus*, *H. densus* and *H. praecoquis*). Moreover, four species are present in both the Southern and Northern Hemispheres: *Pteraster jordani/affinis*, *P. militaris/affinis*, *Diplopteraster* sp. 1 and *P. gibber*. In every case, Northern and Southern Hemisphere specimens are closely related within these species (proportion of distinct nucleotide sites of 0.3% for *P. jordani/affinis* and *P. militaris/affinis*, 0.7% for *Diplopteraster* sp. 1 and 1.2% for *P. gibber*).

COI PHYLOGENY

Three main groups are identified: one *Hymenaster* group (A) and two *Diplopteraster/Pteraster* groups (B and C; Fig. 4). The relationship of group B with the two other groups is unclear, as illustrated by the low posterior probability and the competing topologies from the DENSITREE output (Fig. 4; Supporting Information, Supplementary Material 2). Within group A (*Hymenaster*), *H. campanulatus* and unidentified specimens form the sister group of all other *Hymenaster* (PP = 1). Among these, *H. sacculatus* forms a subclade with *H. formosus*, *H. perspicuus* and *H. pellucidus*, and *H. coccinatus/densus/praecoquis* form another subclade with *H. edax* and *H. latebrosus* (PP = 0.85). Group B (*Diplopteraster/Pteraster*) includes *P. rugatus* and the *Diplopteraster/Pteraster stellifer* complex (PP = 1). Within group C (*Diplopteraster/Pteraster*), *P. gibber* is close to *P. obscurus*, *P. tesselatus* units 1 and 2 and *Diplopteraster* sp. 1 (PP = 1), and the other subclade includes the *P. affinis* and *P. militaris* complexes (PP = 1).

A POSTERIORI MORPHOLOGICAL RE-INVESTIGATION

After the species delimitation and phylogenetic analysis, new morphological investigations were performed to clarify the mismatch between morphological and molecular data and the status of ambiguous taxa. For unrecognized molecular units, new discriminant morphological characters were potentially identified. First, we find a different number of marginal oral spines (five vs. three) in two genetic entities initially identified under the name *P. affinis* (i.e. *P. jordani/affinis* and *P. militaris/affinis*). Second, the presence of a large, clavate suboral spine is found in several specimens of the *Diplopteraster/P. stellifer* complex, all belonging to *P. stellifer* unit 1 and *P. stellifer* unit 5. Third, the morphological re-examination of two closely related molecular units (*P. gibber* and *Diplopteraster* sp. 1) shows that a character state is shared by all specimens of these units. In fact, these specimens have a single web (for two oral plates) among marginal oral spines, whereas the other *Pteraster/Diplopteraster* specimens have free marginal oral spines or a separate web for each plate.

XPER[3] IDENTIFICATION KEY

The Xper[3] identification key includes 33 species (Figs 2, 3), i.e. all the species currently accepted in the Register of Antarctic Marine Species (RAMS; Jossart *et al.*, 2015; De Broyer *et al.*, 2020). An asterisk (and related comment) is attached to each species name for which there was a mismatch between genetic and morphological identification (i.e. *P. affinis*, *P. stellifer*, *Diplopteraster clarki* Bernasconi, 1937, *D. hurleyi*, *D. peregrinator*, *D. semireticulatus*, *D. verrucosus*, *H. coccinatus*, *H. densus* and *H. praecoquis*). Fourteen characters are selected, namely the number of arms, the type and number of paxillar spines, the type and number of adambulacral spines, the number of rows of tube feet, the type and number of suboral/marginal oral spines, the morphology of the segmental papillae, the presence of granular bodies in the supradorsal membrane, the opacity of the supradorsal membrane, the presence of muscle fibres holding the supradorsal membrane and the number of oscular spines. Several previously used descriptors are evaluated but not retained, because they are not quantifiable accurately (e.g. osculum diameter, density of spiraculae) or are undistinguishable in most specimens (e.g. body convexity). Potential new diagnostic characters that could be used in the *P. affinis* (different number of marginal oral spines) and *Diplopteraster/Pteraster stellifer* (clavate suboral spine) complexes are mentioned as comments within the key. Particular attention is devoted to make this identification key as user friendly as possible, as follows: (1) the Xper[3] platform allows the user easily to detect the remaining taxa and characters throughout the identification process (Vignes-Lebbe *et al.*, 2016); (2) any number of characters can be used in any order (multiple accesses key); and (3) numerous macro-pictures and drawings are available, illustrating whole specimens, characters and character states. This identification key is accessible at: http://pterasteridae-so.identificationkey.org/mkey.html

DISCUSSION

Our integrative approach was successful to revise species identity and phylogenetic relationships. The results call for a revision of the taxonomic status of both species and genera within the family Pterasteridae. Such a revision would not be possible without the joint use of morphological investigations and molecular analyses. We identified three of the four genera of Pterasteridae documented in the Southern

Ocean: *Diplopteraster*, *Hymenaster* and *Pteraster*, with *Calyptraster* the only genus not being encountered (Mah, 2020). Thirty-three species were identified by the bPTP and GMYC molecular approaches, which is concordant with 54–62% of the morphology-based identifications. Mismatches between morphological and genetic identifications are either attributable to different molecular units that are morphologically similar or, conversely, to morphological species being genetically identical. Several cases of species shared between high latitudes of the Northern and Southern Hemispheres were found, which could correspond to either cosmopolitanism or bipolarity (species with disjunct distribution *sensu* Darling *et al.*, 2000). After the molecular analyses, the return to morphological samples allowed the identification of potential new characters that can be used as diagnostic features to define molecular species that were previously undifferentiated based on morphology alone. Merging the available morphological and molecular results, we have synthesized the taxonomy of Pterasteridae from the Southern Ocean and built a synthetic, polytomous and open-access identification key that is intended to serve as a baseline for future taxonomic and ecological studies. Finally, this work significantly increased the DNA barcode library for the Southern Ocean pterasterid species. Before this study, no genetic sequence of Southern Ocean Pterasteridae was publicly available on the GenBank or BOLD platform. Moreover, the addition of 174 sequences extends by more than three times the public barcode library of the whole family, which also represents a 2.5% extension for the Asteroidea class and a 0.6% extension for the Echinodermata phylum (source: http://boldsystems.org).

Species delimitation

The molecular identification confirmed the species status of several species previously described on a morphological basis only (e.g. *H. campanulatus*, *H. sacculatus* and *P. rugatus*). However, discrepancies between morphological and genetic delineations were observed. These are not surprising and illustrate taxonomic uncertainties of the family already discussed in other studies (Clark & Downey, 1992; McKnight, 2006). For example, Clark & Courtman-Stock (1976) highlighted the 'ludicrous' situation in *Hymenaster*, to which > 50 nominal species have been ascribed over the last 100 years. This is well illustrated in our study by the two genetic entities gathering distinct nominal species: the unit *Hymenaster coccinatus*/*densus*/*praecoquis* and the unit *Diplopteraster peregrinator*/*semireticulatus*/*verrucosus*. Such a taxonomic issue can be related to the inadequacy of morphological characters used to discriminate species, to diagnostic characters between species instead corresponding to intraspecific variations or to the lack of taxonomic investigations of these species since they were originally described (Clark, 1962; Clark & Downey, 1992). At the other extreme, an interesting case study is provided by the species *P. stellifer*, which corresponds to a species complex (three to five species). Variations within *P. stellifer* were already noted by Clark (1962), who had proposed that two subspecies should be distinguished, '*Pteraster steliffer stellifer* Sladen, 1882' and '*Pteraster stellifer hunteri* Koehler, 1920', distinct from each other by their geographical distribution and the shape of the paxillar spinelets. Our results also suggest that *P. affinis* should encompass several distinct species that, interestingly, are closely related to species recorded in the Northern Hemisphere. Some species within these complexes correspond to unrecognized diversity, because we found (*a posteriori*) potential diagnostic characters (e.g. clavate suboral spines in *Pteraster stellifer* units 1 and 5). Some others might represent true cases of cryptic diversity, because the morphological re-investigation did not reveal any diagnostic character (e.g. among *Pteraster stellifer* units 2 and 4).

Phylogenetic relationships

As expected for a single gene phylogeny, some relationships remain partly unresolved (Gontcharov *et al.*, 2004; Sands *et al.*, 2008; Christiansen *et al.*, 2018), but most of them are supported by high posterior probabilities. Considering the uncertainty associated with the placement of group B, on the one hand, the relationship between the genus *Hymenaster* and the two other genera remains unresolved. On the other hand, both *Diplopteraster* and *Pteraster* are retrieved in groups B and C. This was also found in a previous multiple-gene phylogeny based on Northern Hemisphere species of these two genera (Mah & Foltz, 2011b). This also matches our morphological observations that diagnostic characters of the genus *Diplopteraster* are doubtful (Clark & Downey, 1992; Villier *et al.*, 2004). First, the presence of four rows of tube feet per arm might be incorrect. Close examination of all specimens at hand reveals that they possess two rows of overlapping tube feet. Second, the alternating arrangement of adambulacral plates is difficult to observe. Finally, the presence of an enlarged central paxillar spinelet cannot be observed in most specimens. Therefore, we recommend a taxonomic revision of these two genera using both genetic and morphological data. Finally, we did not find any species belonging to the genus *Calyptraster* in our collection. According to previous studies, it is the sister taxon of the genus *Hymenaster*, but only a small number of species, records and diagnostic characters

are recognized (Clark & Downey, 1992; Villier *et al.*, 2004). Moreover, there are no genetic data available (regardless of the gene) for a *Calyptraster* species. We thus recommend a thorough re-investigation of this genus in order to verify whether it should be synonymized with *Hymenaster*.

The molecular phylogeny confirmed the relationships of species already recognized as closely related, such as *H. sacculatus* and *H. perspicuus* (Clark, 1962). Moreover, the phylogeny highlighted species relationships that were previously unknown, such as the close relationships between *P. gibber* and *P. affinis*, rather than with *P. stellifer*. Another unprecedented result is the affinity of the species *P. jordani/affinis*, *P. militaris/affinis*, *Diplopteraster* sp. 1 and *P. gibber*, recorded in both the Northern and Southern Hemispheres. Depending on the species, this could correspond to either cosmopolitanism or bipolarity. This might indicate a recent migration between the two hemispheres, which would notably be facilitated by deep-sea dispersal routes (Strugnell *et al.*, 2008, 2011; Laakmann *et al.*, 2012). In fact, the Pterasteridae are known to be highly diverse and abundant in the deep sea, being one of the most represented sea star families in abyssal basins worldwide (Sibuet, 1979; Danis *et al.*, 2012).

Conclusion

Our work confirms the relevance of using molecular tools to complement morphology-based taxonomy. This is especially true for taxa that are complex to identify on a morphological basis, such as Pterasteridae. In every genus investigated, we found several species for which the taxonomy should be re-evaluated and revised. These taxonomic issues either generate unrecognized diversity or, conversely, overestimate diversity. Formal taxonomic revision of these species and genera would be premature at this stage. Further analyses are therefore needed to obtain a better picture of the diversity of the family and precise phylogenetic relationships. This implies an extensive investigation of numerous specimens, a thorough taxonomic revision and a morphological survey of holotypes. Some characters seem promising as new diagnostic features of species (e.g. oral spines), whereas other characters currently used should be abandoned (e.g. opacity of the supradorsal membrane), considering their intraspecific variability and their problematic preservation in collected specimens. The investigation of arm ossicles through X-ray photography and scanning electron microscopy also constitutes a promising prospect. Former studies of ossicle arrangement and morphology provided useful taxonomic information in different asteroid groups (Gale, 2018; Fau & Villier, 2020). In the case of Pterasteridae, primary radials (supporting the osculum) and adambulacrals seem the most pertinent ossicles to be investigated (Gale, 2018). Besides morphological studies, the use of additional nuclear genes would be interesting to turn the primary species hypotheses proposed here properly into secondary species hypotheses (Sands *et al.*, 2008; Abdelkrim *et al.*, 2018). In addition, the use of multiple genomic markers would be key to full resolution of the phylogenetic relationships within the family and to analysis of the phylogeographical patterns within species. This is, for example, a necessary condition to test different colonization scenarios between the two hemispheres for cosmopolitan or bipolar species.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Supplementary Material 1. Metadata documenting the 191 samples (Specimen ID, Expedition, Station, Vial, Year, Latitude, Longitude, Initial morphological identification, GMYC-bPTP assignments, ABGD Assignments).
Supplementary Material 2. Output from the DENSITREE program in order to verify the potentiality of competing topologies among the set of trees.