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3 **Diversity of the Pterasteridae (Asteroidea) in the Southern Ocean:**  
4 **a molecular and morphological approach**  
5

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13

14 **ABSTRACT**

15 An integrative approach is crucial in discrimination of species, especially for taxa that  
16 are difficult to identify on a morphological basis. In this study, we combined genetics and  
17 morphology to assess the diversity of the Pterasteridae, a sea star family diversified in  
18 deep-sea and polar environments. Because of their derived anatomy and the frequent  
19 loss of characters during preservation, the Pterasteridae are a case in point for an  
20 integrative study. The molecular identification of 191 specimens (mostly from the  
21 Southern Ocean) suggested 26 to 33 species from 3 genera (*Hymenaster*, *Diplopteraster*,  
22 *Pteraster*), which matched the morphological identification in 54 to 62 % of cases. The  
23 mismatches were either different molecular units that are morphologically  
24 indistinguishable (e.g. *Pteraster stellifer* units 2&4) or, conversely, nominal species being  
25 genetically identical (e.g. *Hymenaster coccinatus/densus/praecoquis*). Several species  
26 were shared between Northern and Southern Hemispheres (e.g. *Pteraster*  
27 *jordani/affinis*). In conclusion, the taxonomic status of some groups was confirmed but  
28 we found, for others, the need to reevaluate the taxonomy at both the genus and species  
29 levels. This work significantly increases the DNA barcode library of the Southern Ocean  
30 species and merged taxonomic information into an identification key that could be a  
31 baseline for future studies ([pterasteridae-so.identificationkey.org](http://pterasteridae-so.identificationkey.org)).

## 32 INTRODUCTION

33 Taxonomy has a pivotal role in biology (Costello et al. 2013). Inaccurate identifications  
34 and naming lead to misunderstandings and spurious interpretations of biological  
35 processes in various domains of life sciences (Dayrat 2005; Pante et al. 2015). Fifteen  
36 years ago, integrative taxonomy was introduced as a promising approach to  
37 complement the traditional, morphology-based taxonomy, using new data and methods  
38 (Dayrat 2005). Among these, molecular markers were in the spotlight, considering the  
39 simultaneous leaps achieved by new genetic methodologies such as DNA barcoding  
40 (Stoeckle 2003; Hebert et al. 2003; Hebert & Gregory 2005, Ratnasingham & Hebert  
41 2007; Fujita et al. 2012). Nowadays, the number of barcoded species is still low  
42 compared to the total number of recognized species, less than a quarter of nominal  
43 species being barcoded in most phyla (Gong et al. 2018). Nevertheless, a plethora of  
44 studies have shown the importance of using genetics and morphology alongside for  
45 discrimination at all taxonomic ranks (e.g. Richter et al. 2008; Laakmann et al. 2012;  
46 Pante et al. 2015; Christiansen et al. 2018; Peck et al. 2018; Jossart et al. 2019). Based on  
47 the data from the World Register of Marine Species (WoRMS), Appeltans et al. (2012)  
48 showed that molecular methods are significantly increasing our knowledge of marine  
49 biodiversity by helping in the detection of cryptic species and the establishment of  
50 synonymies. This is of particular importance for under-investigated taxa, such as those  
51 including numerous species that remain unrevised since their original descriptions.

52 The Asteroidea (*i.e.* sea stars or starfish) is the second most diversified class of  
53 echinoderms, with around 1,900 described species assigned to 38 families (Mah & Blake  
54 2012; Mah et al. 2015). They show various ecological traits and are present in a broad  
55 variety of ecosystems (Mah & Blake 2012; Lawrence et al. 2013; Moreau et al. 2017). For

56 the last twenty years, considerable efforts have been made to reevaluate sea star  
57 phylogeny using molecular data (Knott & Wray 2000; Janies et al. 2011; Mah & Foltz  
58 2011a, 2011b; Linchangco et al. 2017; Moreau et al. 2019). However, there is a sharp  
59 contrast in our knowledge of sea star diversity, namely amongst families from different  
60 biogeographic regions (Feuda & Smith 2015). This is the case of the Pterasteridae  
61 Perrier, 1875, the most diverse family in the Order Velatida, which includes around 130  
62 nominal species and eight genera: *Amembranaster* Golotsvan, 1998, *Benthaster* Sladen,  
63 1882, *Calyptraster* Sladen, 1882, *Diplopteraster* Verrill, 1880, *Euretaster* Fisher, 1940,  
64 *Hymenaster* Wyville Thomson, 1873, *Hymenasterides* Fisher, 1911, *Pteraster* Müller &  
65 Troschel, 1842 (Mah 2020). One unique feature of the Pterasteridae is the presence of  
66 an additional (supra)dorsal membrane producing abundant quantities of mucus (Mah &  
67 Blake 2012, Gale 2018). Between the dorsal and the supradorsal membranes is a  
68 nidamental cavity where incubation of juveniles takes place in some species (Janies  
69 1995). The taxonomy of the group is complicated for three reasons: (1) morphologies  
70 are highly derived and only few diagnostic characters are recognized. Most characters  
71 commonly used for species identification in other sea stars are not applicable to the  
72 family (Gale 2018); (2) the few available characters are often indiscernible as specimens  
73 are particularly fragile and get damaged by sampling and preservation protocols (Villier  
74 et al. 2004); (3) several species are only known from their original description based on  
75 few (poorly preserved) specimens (Villier et al. 2004). Consequently, the Pterasteridae  
76 family is a case in point of a group that could benefit from an integrative taxonomic  
77 approach.

78 The family mainly occurs in cold waters from the deep sea to the Arctic and the Southern  
79 oceans (Mah & Blake 2012). Unfortunately, although genetic sequences were available

80 for specimens from the Northern Hemisphere, no public data was published for  
81 Southern Ocean species (source: boldsystems.org). International initiatives of the  
82 Census of Antarctic Marine Life (CAML) and of the International Polar Year (IPY) have  
83 promoted sampling efforts in the Southern Ocean (Schiaparelli et al. 2013) and this  
84 momentum was at the origin of many biologic campaigns until today. This significantly  
85 enhanced the taxonomic and spatial coverage of the Southern Ocean biodiversity  
86 inventory, including the collection of deep-sea representatives of the Pterasteridae.  
87 These newly and well-preserved specimens have offered the opportunity to  
88 reinvestigate the taxonomy of the family. Based on these new samples, we combined  
89 morphological and molecular approaches to verify whether their joint use could better  
90 assess the diversity within the Pterasteridae. After an initial morphological  
91 investigation, we used a mitochondrial gene (Cytochrome c Oxidase subunit I - COI) to  
92 verify how it confirms or complements the morphological identification. Subsequently,  
93 we reinvestigated (*a posteriori*) specimens using a morphological approach in order to  
94 identify new characters that may differentiate species. Finally, we synthesized, for the  
95 first time, the revised taxonomy of the family and made it available to all potential users  
96 in an open-access identification key that includes all the Southern Ocean species  
97 (pterasteridae-so.identificationkey.org).

## 98 MATERIAL & METHODS

### 99 Sampling

100 Specimens were collected during seventeen international campaigns undertaken at sea  
101 from 1998 to 2017 (ACE, ANDEEP-3, ANDEEP-SYSTCO, ARGOS, CEAMARC 2007-2008,  
102 JR144, JR179, JR262, JR275, JR15005, MD208, MUSORSTOM 10, POKER II, PS77, PS81,  
103 PS96). Available specimens cover a wide distribution within the Southern Ocean (Figure  
104 1), including the Patagonian shelf, South Sandwich Islands, South Georgia, South Orkney,  
105 Shag Rocks, Kerguelen, Crozet and the Antarctic continental shelf (Adélie Land,  
106 Amundsen Sea, Antarctic Peninsula, Weddell Sea). A total of 174 specimens from these  
107 locations were included in the analysis. Moreover, in order to increase the taxonomic  
108 and geographical scope, as well as the phylogenetic resolution, 20 additional genetic  
109 sequences (see below) from specimens outside the Southern Ocean were also added to  
110 the dataset (*i.e.* Fiji, South Africa, Pacific and Atlantic coasts of North America, Norway,  
111 Russia). Metadata documenting all the 191 samples can be found in Supplementary  
112 Material 1.

### 113 Morphological identification

114 A total of 124 Southern Ocean individuals (preserved in ethanol or frozen) were  
115 identified morphologically by the authors using both original descriptions (e.g. Sladen  
116 1882, Koehler 1908), identification books (Clark 1962, Clark & Downey 1992, McKnight  
117 2006) and contemporary scientific literature (Villier et al. 2004, Gale 2018).  
118 Subsequently to genetic analyses (see below), an “*a posteriori*” morphological  
119 investigation was carried out to look for new characters to differentiate species when  
120 new species delineations and synonymies were suggested by genetic data. Finally, the  
121 taxonomy of the family was synthesized and made available online, building an  
122 interactive identification key through the Xper<sup>3</sup> portal (Figure 2). Xper<sup>3</sup> is a web portal

123 with an easy-to-use interface allowing multiple accesses (the key can be started using  
124 any character; Vignes-Lebbe et al. 2016). Specimens and characters were also illustrated  
125 by drawings and macro pictures (photographed using a camera with a macro lens, two  
126 flashes and accessories to diffuse or reflect the light, Figure 2, Figure 3).

### 127 **Genetic data**

128 A fragment of the mitochondrial gene “Cytochrome c Oxidase subunit I (COI)” was  
129 sequenced (601 base pairs) for the 191 individuals. These genetic sequences were  
130 obtained through laboratory works in our institutes (80 individuals, see protocol  
131 below), through our private Barcode of Life Data System project (BOLD; 97 individuals)  
132 or mined from public BOLD projects (17 individuals).

133 DNA extractions were performed on one tube foot (podia) per sample and were based  
134 on the salting-out protocol of Sunnucks and Hales (1996). Amplification step was  
135 performed using the forward primers “F-COI-PTE-28” (5'-GCTGGAATGATTGGAAGTGC-  
136 3') or “LCOech1aF1” (5' TTTTTTCTACTAAACACAAGGATATTGG”) and the reverse  
137 universal primer “R-HCO2198” (5'- TAAACTTCAGGGTGACCAAAAAATCA'; Folmer et al.  
138 1994). Each PCR mix (25 µl) included 12.5 µl of a VWR Mastermix (2.5 units of VWR Taq  
139 polymerase, 0.4 mM of each dNTP and 1.5mM of MgCl<sub>2</sub>), 10.5 µl of molecular water, 0.5  
140 µl each primer (10 µM), and 1 µl of the DNA extract. PCR conditions consisted of 35  
141 cycles for each of the three temperature steps [30 s at 95 °C (denaturation), 30 s at 48 °C  
142 (annealing), and 30 s at 72 °C (elongation)]. These cycles were preceded by 2 min at 95  
143 °C and were followed by 10 min at 72 °C. EXOSAP purification (incubation at 37 °C for 15  
144 minutes followed by another at 80°C for 15 minutes) was done before the sending of  
145 PCR products to the MACROGEN sequencing service. Sequence editing and alignment  
146 were done using the software Geneious (Kearse et al. 2012). The absence of stop codon

147 in the sequence was checked in the same software in order to exclude the presence of  
148 nuclear pseudogenes.

149 PartitionFinder2 (BIC criterion, Lanfear et al. 2016) was used within the CIPRES portal  
150 (Miller et al. 2010) to select the most suitable substitution models (*i.e.* TRNEF+I+G for  
151 codon position 1, HKY+I+X for codon position 2 and TRN+I+G+X for codon position 3). A  
152 Bayesian phylogeny was subsequently produced using BEAST 1.8.4 (Drummond &  
153 Rambaut 2007) within the CIPRES portal. Based on a previous phylogeny using multiple  
154 genes, *Remaster gourdoni* Koehler, 1912 was used as the outgroup (Linchangco et al.  
155 2017). Parameters of the analysis were: partitioned dataset, Yule process tree prior,  
156 Markov chain Monte Carlo (MCMC) of  $100 \times 10^6$  generations sampled every 10,000  
157 trees. Tracer v1.6 was used to ensure an appropriate effective sampling size (ESS all  
158 above 200). TreeAnnotator v1.8.4 was used to find the most likely tree that was  
159 visualized in FigTree v1.4.3 ([tree.bio.ed.ac.uk/software/figtree](http://tree.bio.ed.ac.uk/software/figtree)). Node support was  
160 assessed through posterior probability (pp), with values lower than 0.75 not retained  
161 and collapsed into polytomies (Huelsenbeck & Rannala 2004). Moreover, the software  
162 DensiTree 2.2 was used to verify the potentiality of competing topologies among the set  
163 of trees (Bouckaert 2010).

164 Three different methods of species delimitation were used to propose primary species  
165 hypotheses: one distance-based (ABGD- Automatic Barcode Gap Discovery: Puillandre et  
166 al. 2012) and two tree-based (bPTP-Bayesian Poisson Tree Process: Zhang et al. 2013  
167 and GMYC-Generalized Mixed Yule Coalescent: Fujisawa & Barraclough 2013). The  
168 ABGD analysis ([bioinfo.mnhn.fr/abi/public/abgd](http://bioinfo.mnhn.fr/abi/public/abgd)) was performed with a relative gap  
169 width of 1 and K80 as the genetic distance. The bPTP analysis ([species.h-its.org/ptp](http://species.h-its.org/ptp))  
170 was applied using 500,000 generations of MCMC, a thinning of 100 and a burn-in of 25  
171 %. Finally, the GMYC analysis ([species.h-its.org/gmyc](http://species.h-its.org/gmyc)) was performed using the single



172 threshold method. Haplotype diversity and nucleotide diversity were calculated within  
173 each species using DnaSP 6 (Rozas et al. 2017).

## 174 RESULTS

### 175 Initial morphological identification

176 Among the 124 morphologically investigated individuals, 91 were identified to species  
177 level. Thirty-two individuals were identified to genus level and one to family level due to  
178 the small size of specimens (juveniles) or poor preservation not permitting observation  
179 of diagnostic characters. Thirteen different species and three genera were identified  
180 (Figure 3). Four species of *Pteraster* were found: *Pteraster affinis* Smith, 1876, *Pteraster*  
181 *gibber* (Sladen, 1882), *Pteraster rugatus* Sladen, 1882, and *Pteraster stellifer* Sladen,  
182 1882. The number and type of marginal oral spines were important diagnostic  
183 characters to discriminate the different *Pteraster* species and these characters are  
184 usually well preserved. Specimens of *Diplopteraster* were only identified up to the genus  
185 level due to the absence of observable characters (e.g. between *Diplopteraster verrucosus*  
186 (Sladen, 1882) and *Diplopteraster semireticulatus* (Sladen, 1882); see discussion). Nine  
187 species of *Hymenaster* were identified: *Hymenaster campanulatus* Koehler, 1907,  
188 *Hymenaster coccinatus* Sladen, 1882, *Hymenaster densus* Koehler, 1908, *Hymenaster edax*  
189 Koehler, 1907, *Hymenaster formosus* Sladen, 1882, *Hymenaster latebrosus* Sladen, 1882,  
190 *Hymenaster perspicuus* Ludwig, 1903, *Hymenaster praecoquis* Sladen, 1882 and  
191 *Hymenaster sacculatus* Sladen, 1882. Some of these *Hymenaster* species were  
192 discriminated based on tenuous morphological differences, such as *H. densus* and *H.*  
193 *praecoquis* only differentiated based on slight variations in the morphology of the  
194 segmental papillae and number of marginal spines (Clark 1962).

### 195 Species delimitation (COI)

196 Species delimitation methods applied to molecular data gave 26 species hypotheses for  
197 ABGD and 33 for bPTP and GMYC (Figure 4). For each species hypothesis, the  
198 relationship between haplotype diversity and nucleotide diversity fitted the expected

199 variation for a single species, except for *Pteraster stellifer* unit 1 (Goodall-Copestake et  
200 al. 2012). Morphological species identification only matched with molecular species  
201 delimitation in 54 % and 62 % of species for ABGD and GMYC/bPTP, respectively.  
202 Within the genus *Pteraster*, species complexes were suggested within the nominal  
203 species *P. stellifer* (3 to 5 units) and *P. affinis* (2 units). Within the genus *Hymenaster*,  
204 three morphological species appeared to be undifferentiated genetically, suggesting that  
205 the diversity of the genus was over-estimated (*H. coccinatus*, *H. densus* and *H.*  
206 *praecoquis*). Moreover, four species are present in both the Southern and Northern  
207 Hemispheres: *Pteraster jordani/affinis*, *P. militaris/affinis*, *Diplopteraster sp1* and *P.*  
208 *gibber*. In every case, Northern and Southern Hemisphere specimens were closely  
209 related within these species (p-distance of 0.3 % for *P. jordani/affinis* and *P.*  
210 *militaris/affinis*, 0.7 % for *Diplopteraster sp1* and 1.2 % for *P. gibber*).

## 211 **COI phylogeny**

212 Three main groups were identified: one *Hymenaster* group (A) and two  
213 *Diplopteraster/Pteraster* groups (B and C; Figure 4). The relationship of group B with the  
214 two other groups was unclear, as illustrated by the low posterior probability and the  
215 competing topologies from the DensiTree output (Figure 4; Supplementary material 2).  
216 Within group A (*Hymenaster*), *H. campanulatus* and unidentified specimens was the  
217 sister group of all other *Hymenaster* (pp = 1). Among these, *H. sacculatus* formed a  
218 subclade with *H. formosus*, *H. perspicuus* and *H. pellucidus*, while *H.*  
219 *coccinatus/densus/paecoquis* formed another subclade with *H. edax* and *H. latebrosus*  
220 (pp = 0.85). Group B (*Diplopteraster/Pteraster*) included *Pteraster rugatus* as well as the  
221 *Diplopteraster/Pteraster stellifer* complex (pp = 1). Within group C  
222 (*Diplopteraster/Pteraster*), *Pteraster gibber* was close to *P. obscurus*, *P. tessellatus* units

223 1&2 and *Diplopteraster sp1* (pp = 1); while the other subclade included the *Pteraster*  
224 *affinis* and *P. militaris* complexes (pp = 1).

### 225 ***A posteriori* morphological re-investigation**

226 Subsequently to species delimitation and phylogenetic analysis, new morphological  
227 investigations were performed to clarify the mismatch between morphological and  
228 molecular data and the status of ambiguous taxa. For unrecognized molecular units, new  
229 discriminant morphological characters were potentially identified. First, we found a  
230 different number of marginal oral spines (5 *versus* 3) in two genetic entities initially  
231 identified under the same species name *P. affinis* (*i.e.* *P. jordani/affinis* and *P.*  
232 *militaris/affinis*). Secondly, the presence of a large, clavate suboral spine was found in  
233 several specimens of the *Diplopteraster/P. stellifer* complex, all belonging to *P. stellifer*  
234 *unit 1* and *P. stellifer unit 5*. Thirdly, the morphological re-examination of two closely  
235 related molecular units (*Pteraster gibber* and *Diplopteraster sp1*) showed that a  
236 character state was shared by all specimens of these units. In fact, these specimens had a  
237 single web (for two oral plates) among marginal oral spines while the other  
238 *Pteraster/Diplopteraster* specimens harbored free marginal oral spines or a separate  
239 web for each plate.

### 240 **Xper<sup>3</sup> identification key**

241 The Xper3 identification key included 33 distinct species (Figures 2 and 3), namely all  
242 the species currently accepted in the Register of Antarctic Marine Species (RAMS; Jossart  
243 et al. 2015; De Broyer et al. 2020). Moreover, an asterisk (and related comment) was  
244 attached to each species name, for which there was a mismatch between genetic and  
245 morphological identification (*i.e.* *Pteraster affinis*, *P. stellifer*, *Diplopteraster clarki*, *D.*  
246 *hurleyi*, *D. peregrinator*, *D. semireticulatus*, *D. verrucosus*, *Hymenaster coccinatus*, *H.*  
247 *densus*, *H. praecoquis*). Fourteen characters were selected, namely the number of arms,

248 the type and number of paxillar spines, the type and number of adambulacral spines, the  
249 number of rows of tube feet, the type and number of suboral/marginal oral spines, the  
250 morphology of the segmental papillae, the presence of granular bodies in the  
251 supradorsal membrane, the opacity of the supradorsal membrane ,the presence of  
252 muscle fibres holding the supradorsal membrane and the number of oscular spines.  
253 Several previously used descriptors were evaluated but not retained, because they were  
254 not accurately quantifiable (e.g. osculum diameter, density of spiraculae) or  
255 undistinguishable in most specimens (e.g. body convexity). Potential new diagnostic  
256 characters that could be used in the *Pteraster affinis* (different number of marginal oral  
257 spines) and *Diplopteraster/Pteraster stellifer* (clavate suboral spine) complexes were  
258 mentioned as comments within the key. A particular attention was devoted to make this  
259 identification key as user-friendly as possible: (1) the Xper<sup>3</sup> platform allows the user to  
260 easily detect the remaining taxa and characters throughout the identification process  
261 (Vignes-Lebbe et al. 2016); (2) Any number of characters can be used in any order  
262 (multiple accesses key); (3) Numerous macro-pictures and drawings are available,  
263 illustrating both whole specimens, characters and character states. This identification  
264 key is accessible through the following link: [pterasteridae-so.identificationkey.org](http://pterasteridae-so.identificationkey.org).

265 **DISCUSSION**

266 Our integrative approach was successful to revise species identity and phylogenetic  
267 relationships. The results call for a revision of the taxonomic status of both species and  
268 genus within the family Pterasteridae. Such a revision would not be possible without a  
269 joint use of morphological investigations and molecular analyses. We identified three of  
270 the four genera of Pterasteridae documented in the Southern Ocean: *Diplopteraster*,  
271 *Hymenaster* and *Pteraster*, with *Calyptraster* the only genus not being encountered (Mah  
272 2020). Thirty-three species were identified by the bPTP and GMYC molecular  
273 approaches, which was concordant with 54 to 62 % of the morphology-based  
274 identifications. Mismatches between morphological and genetic identifications were  
275 either due to different molecular units that are morphologically similar or, conversely, to  
276 morphological species being genetically identical (see below). Several cases of species  
277 shared between high latitudes of the Northern and Southern Hemispheres were found,  
278 which could correspond either to cosmopolitanism or bipolarity (species with disjunct  
279 distribution *sensu* Darling et al. 2000). Following the molecular analyses, the return to  
280 morphological samples allowed identification of potential new characters that can be  
281 used as diagnostic features to define molecular species that were previously  
282 undifferentiated based on morphology alone. Merging available morphological and  
283 molecular results, we synthesized the taxonomy of the Pterasteridae from the Southern  
284 Ocean and built a synthetic, polytomous and open-access identification key that is  
285 intended to serve as a precious baseline for future taxonomic and ecological studies.  
286 Finally, this work significantly increases the DNA barcode library for Southern Ocean  
287 species. Prior to this study, no genetic sequence of Southern Ocean Pterasteridae was  
288 publicly available on GenBank or BOLD platforms. Moreover, the addition of 174  
289 sequences extends by more than three times the public barcode library of the whole

290 family, which also represents a 2.5 % extension for the Asteroidea class and a 0.6 %  
291 extension for the Echinodermata phylum (source: boldsystems.org).

## 292 **Species delimitation**

293 The molecular identification confirmed the species status of several species previously  
294 described on a morphological basis only (e.g. *Hymenaster campanulatus*, *H. sacculatus*,  
295 *Pteraster rugatus*). On the other hand, discrepancies between morphological and genetic  
296 delineations were observed. These are not surprising and illustrate taxonomic  
297 uncertainties of the family already discussed in other studies (Clarke & Downey 1992;  
298 McKnight 2006). For example, Clark & Courtman-Stock (1976) highlighted the  
299 'ludicrous' situation in *Hymenaster*, to which more than 50 nominal species have been  
300 ascribed over the last 100 years. This is well illustrated in our study by the two genetic  
301 entities gathering distinct nominal species: the unit *Hymenaster*  
302 *coccinatus/densus/praecoquis* and the unit *Diplopteraster*  
303 *peregrinator/semireticulatus/verrucosus*. Such a taxonomic issue can be related to the  
304 inadequacy of morphological characters used to discriminate species, to diagnostic  
305 characters between species rather corresponding to intraspecific variations or to the  
306 lack of taxonomic investigations of these species since they were originally described  
307 (Clark 1962; Clark & Downey 1992). On the other extreme, an interesting case study is  
308 provided by the species *Pteraster stellifer* that corresponds to a species complex (3 to 5  
309 species). Variations within *P. stellifer* were already noticed by Clark (1962) who had  
310 proposed to distinguish two subspecies, "*Pteraster stellifer stellifer* Sladen, 1882" and  
311 "*Pteraster stellifer hunteri* Koehler, 1920", distinct from each other by their geographic  
312 distribution and the shape of the paxillar spinelets. Our results also suggest that  
313 *Pteraster affinis* should encompass several distinct species that, interestingly, are closely  
314 related to species recorded in the Northern Hemisphere (see below). Some species

315 within these complexes correspond to unrecognized diversity as we found (*a posteriori*)  
316 potential diagnostic characters (e.g. clavate suboral spines in *Pteraster stellifer* units  
317 1&5). Some others might represent true cases of cryptic diversity as the morphological  
318 re-investigation did not reveal any diagnostic character (e.g. among *Pteraster stellifer*  
319 *unit 2 and 4*).

### 320 **Phylogenetic relationships**

321 As expected for a single gene phylogeny, some relationships remained partially  
322 unresolved (Gontcharov et al. 2004; Christiansen 2008; Sands et al. 2008), but most of  
323 them are supported by high posterior probabilities. Considering the uncertainty  
324 associated with the placement of group B, on the one hand, the relationship between the  
325 *Hymenaster* genus and the two other genera remains unresolved. On the other hand,  
326 both *Diplopteraster* and *Pteraster* were retrieved in groups B and C. This was also found  
327 in previous multiple-gene phylogeny based on Northern Hemisphere species of these  
328 two genera (Mah & Foltz 2011b). This also matches our morphological observations that  
329 diagnostic characters of the genus *Diplopteraster*, are doubtful (Clark & Downey 1992,  
330 Villier et al. 2004). First, the presence of four rows of tube feet per arm might be  
331 incorrect. The close examination of all specimens at hand reveals that they rather  
332 possess two rows of overlapping tubefeet. Secondly, the alternating arrangement of  
333 adambulacral plates is very difficult to observe. Finally, the presence of an enlarged  
334 central paxillar spinelet could not be observed on most specimens. Therefore, we  
335 recommend a taxonomic revision of these two genera using both genetic and  
336 morphological data. Finally, we did not find any species belonging to the genus  
337 *Calyptraster* in our collection. According to previous studies, it is the sister taxon of the  
338 genus *Hymenaster*, but only a small number of species, records and diagnostic characters  
339 are recognized (Clark & Downey 1992; Villier et al. 2004). Moreover, there is no genetic



340 data available (regardless of the gene) for a *Calyptroaster* species. We thus recommend a  
341 thorough re-investigation of this genus in order to verify whether it should not be  
342 synonymized with *Hymenaster*.

343 The molecular phylogeny confirmed the relationships of species already recognized as  
344 closely related species such as *Hymenaster sacculatus* and *H. perspicuus* (Clark 1962).  
345 Moreover, the phylogeny highlighted species relationships that were previously  
346 unknown, such as the close relationships between *Pteraster gibber* and *P. affinis*, rather  
347 than with *P. stellifer*. Another unprecedented result is the affinity of the species *P.*  
348 *jordani/affinis*, *P. militaris/affinis*, *Diplopteraster sp1* and *P. gibber*, recorded both in the  
349 Northern and Southern Hemispheres. Depending on the species, this could correspond  
350 either to cosmopolitanism or bipolarity. This might indicate a recent migration between  
351 the two hemispheres, which would notably be facilitated by deep-sea dispersal routes  
352 (Strugnell et al. 2008, 2011; Laakmann et al. 2012). In fact, the Pterasteridae are known  
353 to be highly diverse and abundant in the deep sea, being one of the most represented sea  
354 star families in abyssal basins worldwide (Sibuet 1979; Danis et al. 2012).

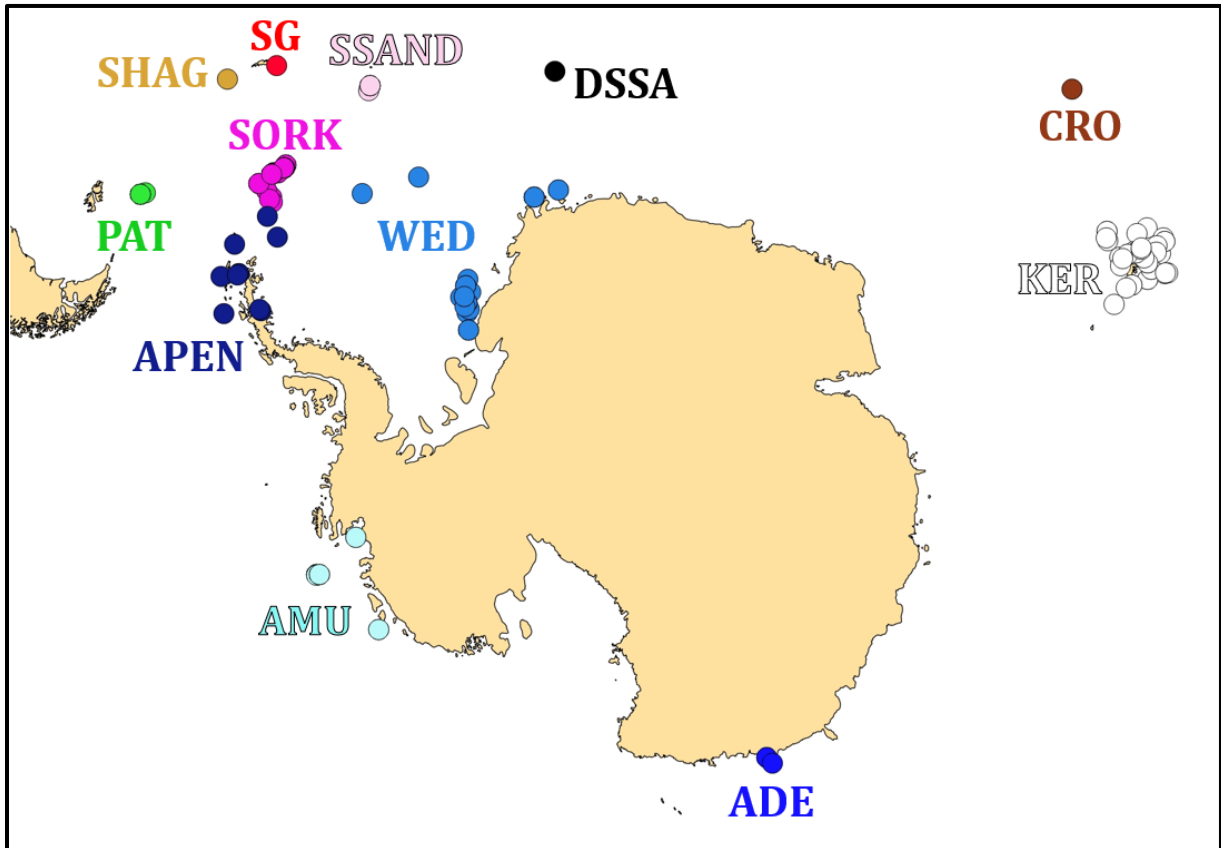
## 355 **CONCLUSION**

356 Our work has confirmed the relevance of using molecular tools to complement  
357 morphology-based taxonomy. This is especially true for taxa that are complex to identify  
358 on a morphological basis such as the Pterasteridae. In every genus investigated, we  
359 found several species for which the taxonomy should be reevaluated and revised. These  
360 taxonomic issues generate either unrecognized or conversely, overestimated diversity.  
361 Formal taxonomic revision of these species and genera would be premature at this  
362 stage. Further analyses are therefore needed to obtain a better picture of the diversity of  
363 the family and precise phylogenetic relationships. This implies an extensive  
364 investigation of numerous specimens, a thorough taxonomic revision and morphological

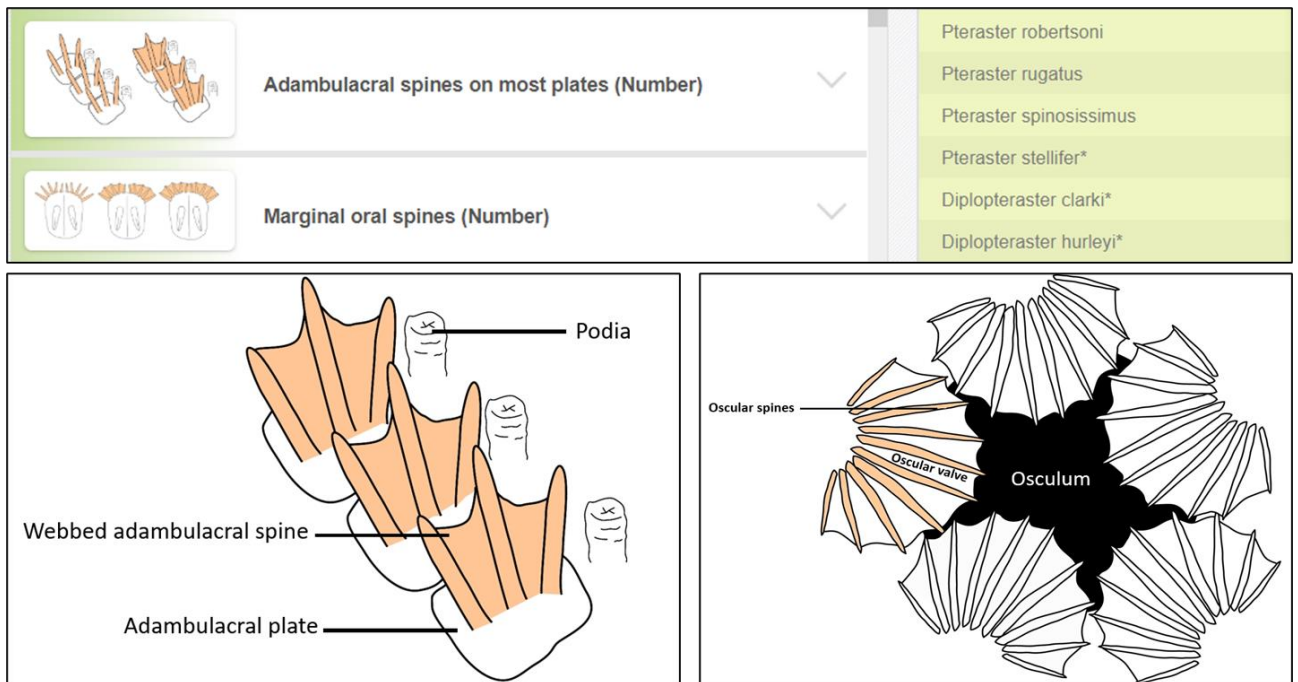
365 survey of holotypes. Some characters seem promising as new diagnostic features of  
366 species (*e.g.* oral spines), while other characters currently used should be abandoned  
367 (*e.g.* opacity of the supradorsal membrane), considering their intraspecific variability  
368 and their problematic preservation in collection specimens. The investigation of arm  
369 ossicles through X-ray photography and electron microscopy (SEM) also constitutes a  
370 promising prospect. Former studies of ossicle arrangement and morphology provided  
371 useful taxonomic information in different asteroid groups (Gale 2018, Fau & Villier  
372 2019). In the case of Pterasteridae, primary radials (supporting the osculum) and  
373 adambulacrals seem the most pertinent ossicles to be investigated (Gale 2018). Besides  
374 morphological studies, the use of additional nuclear genes would be interesting to  
375 properly turn the primary species hypotheses proposed here into secondary species  
376 hypotheses (Sands et al. 2008; Abdelkrim et al. 2018). In addition, the use of multiple  
377 genomic markers would be key to fully resolve phylogenetic relationships within the  
378 family and analyze phylogeographic patterns within species. This is, for example, a  
379 necessary condition to test different colonization scenarios between the two  
380 hemispheres for cosmopolitan or bipolar species.

381 **FIGURES**

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

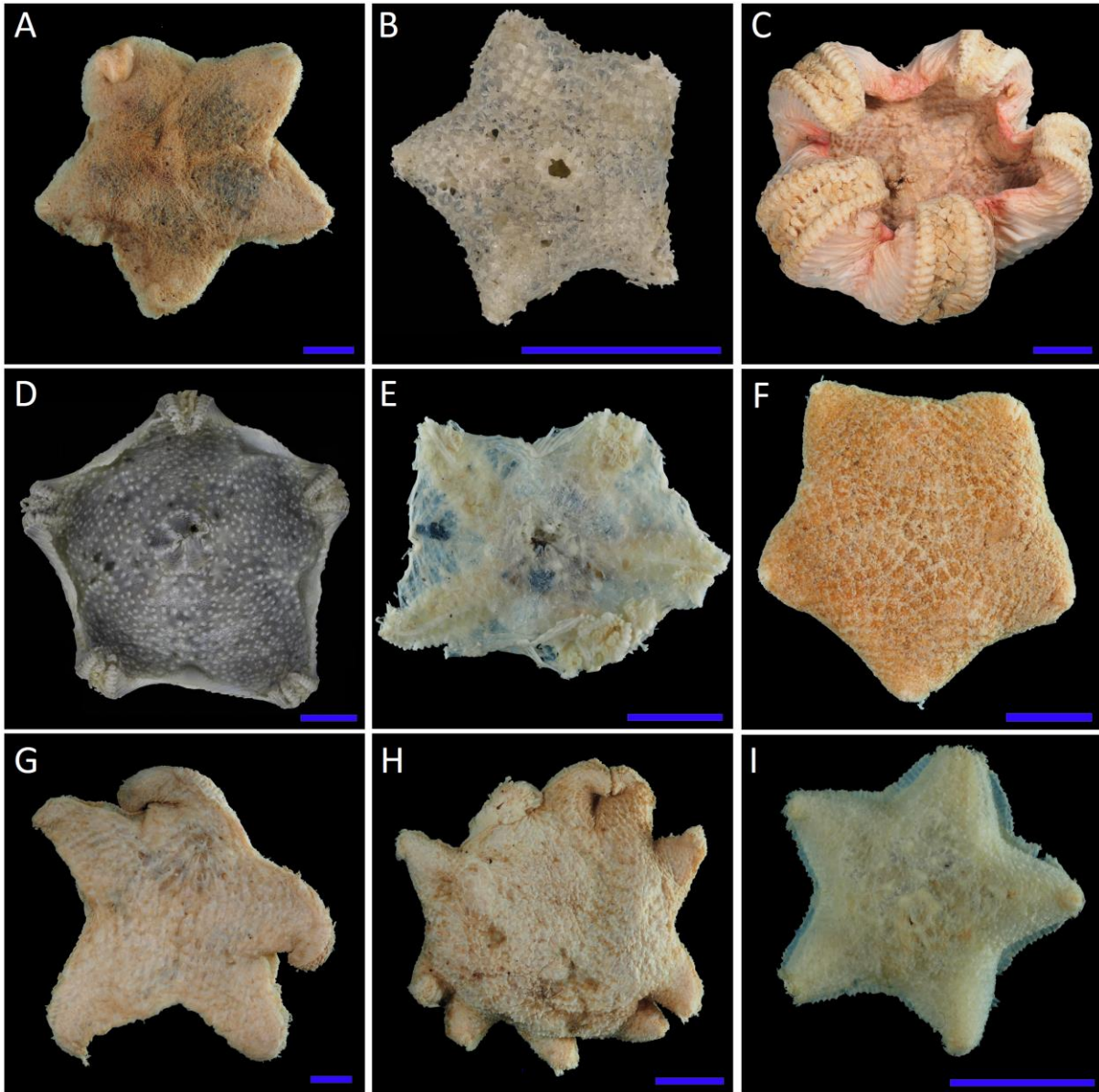


386 **FIGURE 2** - Interface of the Xper<sup>3</sup> identification key (top) and two examples of  
387 integrated drawings illustrating diagnostic characters (adambulacral spines: bottom left;  
388 oscular spines: bottom right).

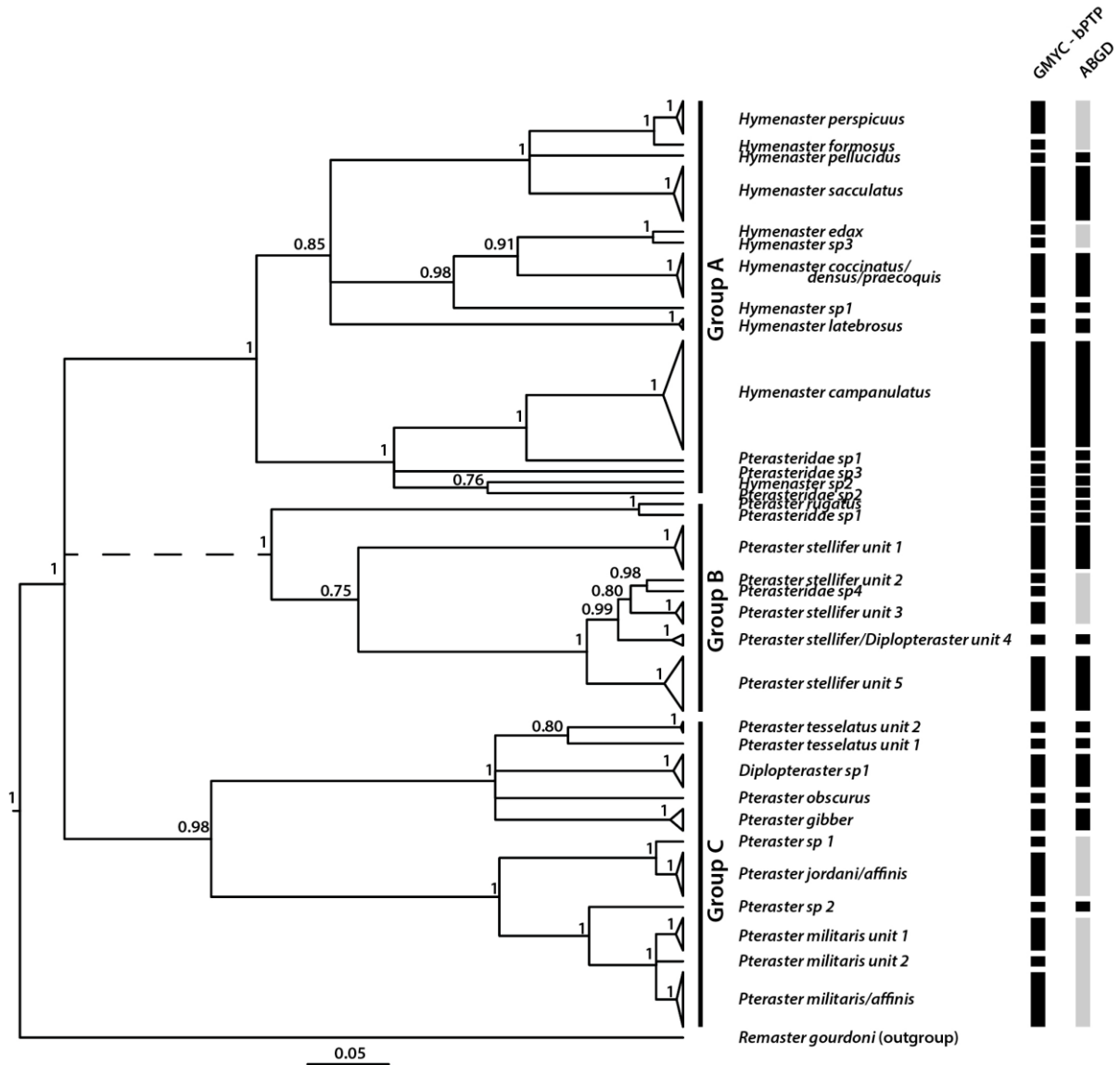


389  
390

391 **FIGURE 3** – Aboral view of Pterasteridae species illustrating their Southern Ocean  
392 diversity. A: *Diplopteraster* sp; B: *Hymenaster campanulatus*; C: *Hymenaster praecoquis*;  
393 D: *Hymenaster edax*; E: *Hymenaster sacculatus*; F: *Pteraster gibber*; G: *Pteraster affinis*; H:  
394 *Pteraster koelheri*; I: *Pteraster stellifer*. Scale bars : 1 cm.



395 **FIGURE 4** – Bayesian phylogeny based on mitochondrial Cytochrome c Oxidase subunit I  
 396 (COI) sequences. Values at each node are the posterior probabilities (nodes with support  
 397 lower than 0.75 were collapsed into polytomies). The dashed line (associated to the  
 398 group B) indicate competing topologies from the software DensiTree. Assignments for  
 399 each species delimitation method are reported as black bars (GMYP-bPTP on the left and  
 400 ABGD on the right). Distinct GMYP-bPTP and ABGD assignments are highlighted by grey  
 401 bars for the ABGD assignments.



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