Article

Preliminary Assessment of Sea Star (Echinodermata, Asteroidea) Diversity in the Coastal Magellanic Region (South Chile) and Their Geographical Distribution

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Abstract: Sea stars are a diverse and important component of the Southern Ocean benthos. However, scarce information is available regarding their diversity, distribution, evolution, and taxonomic uncertainties persist for multiple taxa. The Magellanic Region (south of Chile) remains under-sampled despite its pivotal location for species distribution and diversity, being located at the crossroad of three ocean basins. In this study, we assessed the biodiversity of coastal Magellanic sea stars and their affinities with other oceanic bioregions. An integrative approach combining morphological identification with DNA barcoding was implemented to highlight taxonomic discrepancies such as suspected synonymy and unrecognised diversity. Firstly, we identified a total of 15 species from the coastal Magellanic Region and reported the occurrence of Cycethra frigida Koehler, 1917 for the first time in this region. The distribution of these 15 species ranged from only in South America to circumpolar, bipolar, or possibly cosmopolitan. Secondly, we highlighted possible synonymy in two species pairs within the genera Anasterias and Odontaster. This preliminary biodiversity assessment forms an important baseline for monitoring and conservation purposes, especially in the face of distribution shifts as a response to climate change and the increased presence of invasive species. Developmental mode has previously been suggested to be important in shaping biogeographical patterns. However, developmental mode was insufficient to explain the observed patterns, and other factors (e.g., physiological constraints, competition, bathymetrical range, and the possibility of passively rafting on kelp) are suggested to be at least equally important. Finally, an increase in barcoding effort is needed to better capture phylogeographic patterns for each species, both by increasing the number of specimens investigated and by covering a broader geographical range.

Keywords: Southern Ocean; sub-Antarctic; DNA barcoding; integrative taxonomy; starfish

1. Introduction

The Magellanic Region, located at the southernmost tip of Chile, is an under-sampled region in the Southern Ocean [1,2] (Figure 1). This region encompasses the official Chilean region Magallanes: from Isla Aldea in the north to the most southern point of Chile (Antarctic Peninsula excluded). It is located in the sub-Antarctic between the Antarctic Polar Front and the sub-Tropical Front (Figure 1). The Magellanic Region is located at a pivotal position in the World Ocean, at a crossroad between three ocean basins (the Pacific, Atlantic, and Southern Ocean). Such a central geographic position suggests a rich and complex species composition originating from these three distinct basins [3].
Sea stars (class Asteroidea) are a major component of the Southern Ocean’s ecosystems with 294 accepted species belonging to seven different orders and 38 families [4]. Their ecological importance lies in their relatively large body size, their diverse diets, their presence in nearly all marine environments, and the fact that they commonly occupy high trophic levels [5–7].

Despite being a diversified and essential part of the Southern Ocean benthos, only scarce information is available regarding sea stars’ diversity, evolution, and ecological roles [8]. Recent efforts have been made to increase sea star biodiversity knowledge in the Southern Ocean (e.g., [9–12]), including the Magellanic Region [8,13–15]. However, the resulting estimations are inconsistent and often cover different geographical areas and depth ranges. Biodiversity assessments, including species lists, form an important baseline for monitoring and conservation purposes, especially in the face of distribution shifts associated with climate change and the increased presence of invasive species [1].

To unravel the possibly overlooked sea star diversity and untangle taxonomic discrepancies such as the presence of synonymous species (e.g., the sea stars Glabraster antarctica [15,16] and Marthasterias [17]) or cryptic species (e.g., Henricia [18,19]), an integrative approach combining genetic and morphological data is increasingly more common (e.g., [11,17–24]).

The widely used fragment of the Cytochrome c Oxidase subunit I (COI) gene (i.e., genetic barcode) has been demonstrated to be an effective marker in species identification (i.e., DNA barcoding) of echinoderms [17,18,23,25,26]. By using a clustering algorithm, these barcodes, and their associated individuals, are assigned to different BINs (Barcode Index Numbers) in the internationally recognised platform “Barcode of Life” [27]. Each BIN corresponds to one putative species. However, the number of barcoded marine species remains low [11,28]. For example, only 582 sea star BINs are publicly available in BOLD compared to the almost 1950 accepted asteroid species in the World Asteroidea Database [29]. These DNA barcode libraries do not only aid in species identification but are also implemented in other fields such as population genetics, phylogenetics, and community-based studies [30,31]. The need for a solid DNA barcode library covering a wide range of taxonomic groups is therefore fundamental.

Marine faunal affinities between the Magellanic Region and other regions of the Southern Ocean have been highlighted for decades (e.g., [32]) and are still studied in detail, both with Antarctica (e.g., [33–35]) and the sub-Antarctic areas [8,36–39]. Generalisations, however, are complicated as connectivity is taxon-dependent and largely related to bathymetrical ranges and life history traits such as developmental modes [38,40]. As for many benthic invertebrates, sea stars exhibit different types of development modes, either with (broadcasting) or without (e.g., brooding or oviposition) a pelagic larval stage. These strategies are expected to significantly shape species’ biogeographical patterns, with broadcasting taxa being expected to show less spatial structure [2]. Dispersal ability deduced from developmental mode alone is, however, not sufficient, and eco-physiological constraints, other life history traits, and biological interactions should also be taken into account [41,42].

The objectives of this study were to first assess sea star diversity in the under-sampled coastal Magellanic Region using an integrative approach combining morphological and molecular tools. This represents a significant addition to the DNA barcode reference library (BOLD). Additionally, the faunal affinities (phylogeographic patterns) of the Magellanic Region with other Southern Ocean bioregions were assessed and related to the developmental modes of the species investigated.
2. Materials and Methods

2.1. Sample Collection and Morphological Identification

A total of 26 locations (Figure 2, Table S2) were sampled in the Magellanic Region between 2016 and 2023 using various methods (SCUBA diving, snorkelling, and intertidal sampling). Specimens were identified morphologically using the scientific literature (e.g., [11,13,43–48]) and were preserved frozen at −20 °C or in 96% ethanol and stored at the Laboratorio de Ecosistemas Marinos Antárticos y Subantártico (LeMAS) at the University of Magellan in Punta Arenas, Chile.

Figure 1. Overview of the major islands in the Southern Ocean and the position of the major fronts: Polar Front variations (blue lines, PF) and the sub-Tropical Front (black line, STF). The sub-Antarctic is indicated in the grey hashed area. Abbreviations: Fal = Falkland Islands, SG = South Georgia, SOrk = South-Orkney Islands, SSan = South-Sandwich Islands, Cro = Crozet Islands, Ker = Kerguelen, Hrd = Heard Island.

Figure 2. Map of sampling locations in the Magellanic Region (pale blue), Chile. Because several
stations are close together in the Magellanic strait and on Isla Navarino, a zoom-in is also shown. Abbreviations: A = Isla Alta, BA = Bahía Acashuauia, BM = Bahía Mejillones, BQ = Buque Quemado, CH = Copihue Channel, E = Eleuterio Channel, FB = Fuerte Bulnes, FI = Faro San Isidro, GA = García Island, (I)PM = (Isla) Paso Mackinlay, MO = Canal de las Montañas, PB = Paradise Bay, PC = Punta Carrera, PD = Punta Delia, PM = Pia Fjord Moraine, PN = Puerto Navarino, PR = Parker Island, PW = Puerto Williams, R = Diego Ramírez Island, RB = Rinconada Bulnes, ROB = Bahía Robalo, SO = Otway Sound, SP = South of Punta Arenas, SU = Summer Island, TBV = Top Bahía Virginia.

2.2. COI Amplification

Genomic DNA extractions were performed on podia tissue using the DNeasy Blood & Tissue kit by Qiagen. For COI amplification, we used a PCR mix of 6.25 µL Accustart Toughmix (QuantaBio), 4.75 µL ultrapure water, and 0.25 µL (10 µM) of each primer. A volume of 1.5 µL of template DNA was then added for the reaction. The amplification of COI was performed using the forward primer LCOech1aF1 (5′-TTTTTTCTACTAAACAAGGATATTGGG-3′) and the reverse primer jgHCO2198 (5′-TAIACYTCGGRTGICCCRAARYCA-3′). All primers were tailed with an M13 tail (forward: 3′-CAGGAAACAGCTATGAC-5′, reverse: 3′-TGTAAAACGACGGCCAGT-5′) to ease sequencing. The PCR protocol consisted of an initial denaturation step at 95 °C for 5 min followed by 40 cycles of denaturation at 95 °C for 45 s, primer annealing at 45 °C for 45 s, and elongation at 72 °C for 45 s ending with a final elongation at 72 °C for 3 min. PCR products were then purified with 3 µL of VWR ExoCleanUp FAST added to 7 µL of PCR product following the provided instructions for temperature cycles. Sequencing was performed by Macrogen Europe. Obtained sequences were edited by removing primer sequences, reassigning low-quality base pairs, and checking for the absence of stop codon using the software Codoncode Aligner v10.0.2. Sequences were then aligned using the Muscle algorithm [50] implemented in Codoncode Aligner.

2.3. DNA Barcoding and Database Compilation

Sequences were compared to BOLD references to assign each specimen to a BIN. Supplementary DNA sequences were added for the same identified species from the entire Southern Ocean to study global species distributions. These sequences were obtained in two ways: (1) additional samples from other regions (curated at host institutions) were processed following the protocol mentioned above and (2) publicly available sequences within our species’ BINs were mined from BOLD. All specimens belonging to each BIN (=single putative species) were included, even if referenced under different species names (e.g., Anasterias rupicola and Odontaster meridionalis included as part of the Anasterias antarctica and Odontaster penicillatus BINs).

2.4. Bioinformatics

Genetic diversity indices (number of haplotypes, nucleotide diversity π, and haplotype diversity Hd) were calculated for each putative species using the software DnaSP v6.12.03 [51]. Genetic variability was inferred by calculating mean intraspecific p-distance in MEGA v10.2.0 [52]. These indices were only calculated for species with three or more sequences.

To further assess the credibility of the species identification and confirm the results obtained using BIN delineation, we ran all sequences together using the online species delineation program ASAP (Assemble Species through Automatic Partitioning: https://bioinfo.mnhn.fr/abi/public/asap/ (accessed on 8 June 2023)). Default settings using the Kimura 2P model were selected. In addition, the relationship between nucleotide diversity and haplotype diversity was compared to the results from Goodall-Copestake et al. (2012) to verify whether this relationship fits the one expected for a single species [53].
Occurrences accessible from GBIF [54], the Global Biodiversity Information Facility, were obtained for the species that could be identified at the species level. These were mapped in QGIS v3.10.10 [55] together with distributions of barcoded specimens within the same BIN on BOLD.

Haplotype networks were generated using the TCS method in Popart (http://popart.otago.ac.nz/ (accessed on 23 June 2023)) [56] to visualise the frequency and geographic distribution of haplotypes within each species.

3. Results

3.1. Sea Star Diversity in the Magellanic Region

A total of 383 specimens were collected from the Magellanic Region belonging to 15 different species within 13 genera, 10 families, and four orders (Figure 3 and Table S1). Genetic COI sequences from 262 specimens (fragment of 658 bp) were newly obtained (Table 1). No stop codon was detected. We were able to assign 12 of the 15 species to a unique BIN in BOLD (Figure 3, Table 1). The seven specimens of Henricia sp. and five specimens of Perknaster sp. did not result in a match in BOLD indicating the absence of these two species in online genetic databases. The species delimitation provided by ASAP resulted in the same 15 species as in BOLD (Figure 3). Within the BIN of Anasterias antarctica, the specimens from Kerguelen identified as Anasterias rupicola were present. The same situation was found for specimens from Kerguelen identified as Odontaster meridionalis within the BIN of Odontaster penicillatus.

The final dataset, including 475 additional sequences mined from BOLD, consisted of 737 sequences. Most sequences belonged to the species Glabraster antarctica (377) while only two were available for Lophaster stellans (Table 1). The genetic variability within each putative species ranged from 0.17% to 1.71%. Glabraster antarctica displayed the highest genetic diversity for all four indices (N haplotypes = 146, H dred = 0.980, π = 0.0171, and genetic variability = 1.71%) (Table 1). Cycethra frigida had the lowest genetic variability (0.17%) and nucleotide diversity (π = 0.0017), Lophaster stellans had the lowest number of haplotypes (2), and Pteraster gibber had the lowest haplotype diversity (Hd = 0.533). Genetic variability was more than twice as high in G. antarctica (1.71%) compared with the majority of the other species (maximum 0.58% in Odontaster penicillatus). Only C. verrucosa (0.95%) also showed higher variability. Glabraster antarctica was also the only one where the relationship between nucleotide diversity and haplotype diversity was higher than the expected variation within a species according to results from Goodall-Copestake et al. (2012) [53].

Morphological identifications were mostly in line with the genetic approach. However, the two Cycethra species were initially morphologically seen as one species due to the smaller C. frigida being hardly distinguishable superficially from juveniles of C. verrucosa. Henricia sp. and Perknaster sp. specimens were not identified at the species level. These genera have proven very challenging to identify, and a complete taxonomic and phylogenetic re-evaluation would be necessary [8,19,43,57,58].
Table 1. Taxonomic table of Magellanic sea star species indicating the number of new sequences, number of extra sequences mined from BOLD, total number of sequences, their associated genetic diversity indices, the species’ BIN in BOLD, and their developmental mode. Abbreviations: $\pi$ = nucleotide diversity, N haplotypes = number of haplotypes, Hd = haplotype diversity.  

<table>
<thead>
<tr>
<th>Order</th>
<th>Family</th>
<th>Genus</th>
<th>Species</th>
<th>N New Sequences</th>
<th>N Sequences BOLD</th>
<th>N Total</th>
<th>$\pi$</th>
<th>N Haplotypes</th>
<th>Hd</th>
<th>Genetic Variability (%)</th>
<th>BOLD BIN</th>
<th>Developmental Mode</th>
</tr>
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<td>Forcipulatida</td>
<td>Asteriidae</td>
<td>Anasterias</td>
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<td>19</td>
<td>112</td>
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<td>14</td>
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<td>Labidiasterida</td>
<td>Labidiaster</td>
<td>Labidiaster radiossus Lütken, 1871 [62]</td>
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<td>2</td>
<td>19</td>
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<td>21</td>
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<td>0.51</td>
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<td>5</td>
<td>18</td>
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<td>0.699</td>
<td>0.23</td>
<td>BOLD:ACI1273</td>
<td>Brooder 1</td>
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<tr>
<td></td>
<td>Cyctherida</td>
<td>Cycthera</td>
<td>Cycthera frigida Koehler, 1917 [66]</td>
<td>3</td>
<td>12</td>
<td>15</td>
<td>0.0017</td>
<td>5</td>
<td>0.695</td>
<td>0.17</td>
<td>BOLD:ADG2622</td>
<td>Broadcaster 2</td>
</tr>
<tr>
<td></td>
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<td>Peknaster sp.</td>
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<td>5</td>
<td>0</td>
<td>5</td>
<td>0.0021</td>
<td>4</td>
<td>0.900</td>
<td>0.31</td>
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<td>Cycthera verrucosa Philippi, 1857 [65]</td>
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<td>0</td>
<td>33</td>
<td>0.0106</td>
<td>14</td>
<td>0.884</td>
<td>0.95</td>
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<tr>
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<td>3</td>
<td>12</td>
<td>15</td>
<td>0.0017</td>
<td>5</td>
<td>0.695</td>
<td>0.17</td>
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<td>Broadcaster 2</td>
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<tr>
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<td>Odontasterida</td>
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<td>Diplodontias singularis Muller and Troschel, 1843 [67]</td>
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<td>0</td>
<td>11</td>
<td>0.0023</td>
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<td>0.23</td>
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<td></td>
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<td>64</td>
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<td>27</td>
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<td>0.58</td>
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<td>Broadcaster 1,3</td>
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<td>357</td>
<td>377</td>
<td>0.0171</td>
<td>146</td>
<td>0.980</td>
<td>1.71</td>
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<td>20</td>
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<td>Lopaster stellans Sladen, 1889 [70]</td>
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<td>1</td>
<td>2</td>
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<td>NA</td>
<td>NA</td>
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<tr>
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<td>Pterasterida</td>
<td>Pteraster</td>
<td>Pteraster affinis * Smith, 1876 [69]</td>
<td>3</td>
<td>20</td>
<td>23</td>
<td>0.0018</td>
<td>10</td>
<td>0.707</td>
<td>0.18</td>
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<td>Brooder and broadcaster 1,4</td>
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<tr>
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<td></td>
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<td>Pteraster gibber Sladen, 1882 [71]</td>
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<td>9</td>
<td>10</td>
<td>0.0038</td>
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<td>0.37</td>
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<td>737</td>
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</table>

* Corresponds to the P. militaris/affinis clade in [11].
Figure 3. Visualisation of the 15 species present in the Magellanic Region. Red lines indicate species delineation using ASAP while blue lines indicate the separation into different BINs in BOLD. The size of each line is proportional to the number of sequences obtained for each species.

3.2. Biogeography and Faunal Affinities

3.2.1. Occurrences vs. Barcoded Occurrences

Specimens belonging to the genus Henricia and Perknaster could not be identified at the species level. In consequence, no occurrences could be retrieved from GBIF for this analysis. Five species (Henricia sp., Perknaster sp., Cycethra verrucosa, Cosmasterias lurida, and Diplodontias singularis) had no publicly available DNA barcodes prior to our work. Only two species (Glabraster antarctica and Asterina fimbriata) had enough barcoded specimens to confirm their distribution indicated by GBIF (Figure 4, red *). The other 11 species need additional COI barcoding effort in certain geographical areas to confirm their known distribution (Figure 4). This is the case for most of the specimens occurring on the Antarctic continental shelf, for which COI barcodes remain very scarce.
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Figure 4. Known species distributions from GBIF (yellow circles) compared to occurrences with COI barcodes in BOLD (red diamonds). * = species for which COI barcodes cover the entire known distribution. (A) South America-only distribution, (B) sub-Antarctic distribution (C) sub-Antarctic and Antarctic Peninsula distribution, (D) True circum-Antarctic distribution. (See Figure S3 for bigger maps per species).

3.2.2. Estimating Species Distribution

Most species showed a circumpolar distribution in GBIF in contrast to a more restricted distribution in BOLD. Based on these barcoded specimens, four biogeographical patterns were found:

1. South America-only

Four species (i.e., Asterina fimбриata, Cosmasterias lurida, Cycethra verrucosa, and Diplodon-tias singularis) were only present in South America (Figure 4A). This restricted distribution was only confirmed by GBIF for A. fimбриata. Cosmasterias lurida also occurred on the Antarctic Peninsula, D. singularis was additionally found on the Antarctic Peninsula and Tristan Da Cunha, and C. verrucosa occurred all over the Antarctic continent.

2. Sub-Antarctic

A second biogeographical pattern contained species occurring in the sub-Antarctic only, north of the Polar Front (Figure 4B). Six species fitted this pattern based on barcoded specimens. In contrast, GBIF contributed a wider distribution range including either all of Antarctica (Anasterias antarctica, Lophaster stellans, Odontaster penicillatus, and Solaster regularis) or at least the Antarctic Peninsula (Pteraster gibber). Cycethra frigida had very few records, making it difficult to determine its distribution reliably, and its occurrence in the Macquarie Islands could not be confirmed by barcodes. In this project, we could, however, extend its known distribution to the Magellanic Region.

3. Sub-Antarctic and Antarctic Peninsula

A third pattern (Figure 4C) included two species with a distribution mainly in the sub-Antarctic, but each had few sequences originating from either the Antarctic Peninsula (Labidaster radiosus) or the Scotia Arc (Pteraster affinis). GBIF occurrences indicated, however, a circumpolar distribution for them. Pteraster affinis also had the particularity to include one barcoded specimen from the Northern Hemisphere.
4. True circum-Antarctic

The last species (*Glabraster antarctica*) showed a true circum-Antarctic distribution with both barcodes and GBIF occurrences being spread over the entire Southern Ocean (Figure 4D).

The three species known for their brooding behaviour (*Anasterias antarctica, Asterina fimbriata, and Pteraster affinis*) did not display a more geographically limited distribution than the broadcasting species (Table 1).

4. Discussion

4.1. Sea Star Diversity in the Magellanic Region

4.1.1. Species Richness

In the present study, we recorded 15 sea star species in the coastal waters of the Magellanic Region. We obtained congruent results using both the ASAP and BIN methods to delineate species based on COI barcodes. Previous studies reported a higher species richness with up to 22 species shallower than 40 m [5,13,33,46]. Combined, those studies reported 34 different species. Differences between this study and previous works are most likely due to different geographical ranges (the Magellanic Region, only Tierra del Fuego, or the whole of Chile), different sampling sites, the fact that our sampling was restricted to 40 m, and observer bias. Eleven of our thirteen species identified at the species level were also reported in Argentina [72]. The latter study, however, focused on deep-sea sea star occurrences, and it would be interesting to also investigate shallow neighbouring regions like Argentina and the Falkland Islands. We reported *Cycethra frigida* for the first time in the Magellanic Region. Based on the open resource database GBIF and scientific literature, this species was previously recorded in Kerguelen and Macquarie Island only [45,73]. *Henricia* sp. could not be identified at the species level morphologically or molecularly. *Henricia* species are known for their high degree of intraspecific and even intrapopulation morphological variation and are capable of interbreeding [19,44]. This may have led to taxonomic oversplitting into the almost 100 species that are accepted in this genus, which makes identifying specimens morphologically challenging [19,44]. The sequences could also not identify our specimens as they did not result in a match in BOLD, indicating the absence of this species in the online genetic database. We added seven more sequences for a *Henricia* species in the reference database in the hope that the taxonomic issues in this genus will be resolved in the future.

Our five specimens of *Perknaster* could also not be identified at the species level. This genus also requires taxonomic revisions. Moreau (2019) already indicated possible cryptic species and a challenging morphological identification for certain species, especially in small specimens [9,44]. Three species have been recorded from the Magellanic region, namely, *P. aurantiacus, P. densus* [54], and *P. sladeni* [74]. These species should, however, be easily morphologically distinguished from the rest of the species within the genus, which is not the case in our specimens [43]. We suspect our specimens to belong to either *P. fuscus, P. antarcticus, P. aurorae*, or *P. charcoti*. The sequences could also not identify our specimens because of the absence of the corresponding species in the BOLD genetic database.

The registered intraspecific variation values (0.17–1.71%) are in line with Ward et al. (2008), who reported intraspecific variation within 37 species of asteroids ranging from 0% to 1.85% [25]. However, the observed relation between nucleotide diversity and haplotype diversity was higher than expected in *Glabraster antarctica* [53]. This contrasts with ASAP and BIN species delineations indicating one species. Earlier studies already indicated *Glabraster antarctica* to be one single species morphologically [16] and molecularly albeit showing complex genetic structuring based on geography explaining the high molecular variation in this species [15].

4.1.2. Taxonomic Discrepancies Highlighting the Importance of an Integrative Approach

Genetic identifications with DNA barcoding were mostly in line with morphological identifications. However, the two *Cycethra* species were difficult to differentiate morpho-
logically due to adults of *Cycethra frigida* resembling juveniles of *Cycethra verrucosa* also present in the same sampling locations. However, a closer re-examination allowed us to differentiate them morphologically by the number of adambulacral and furrow spines (Figure S2) [44,45]. The Magellanic Region is so far the only region where both species are reported together.

Taxonomic discrepancies can also lead to overestimations of biodiversity (e.g., [11,15,17]). Specimens within this *Anasterias* genus found in the intertidal of Kerguelen are named *A. rupicola* (Figure 5A, yellow), but they are named *A. antarctica* when found in South America (Figure 5B purple). Genetics in this study pointed out that they are likely to be a single species occurring in both regions and its status should be revised with scrutiny. Previous authors already pointed out the complexity of species delimitation within the *Anasterias* genus, notably within the Magellanic–Falkland area [44]. Additionally, *A. rupicola* was called an intermediate morphotype by Clark (1962) [43].

A similar observation was made for *Odontaster meridionalis* (Kerguelen, Figure 5B yellow) and *O. penicillatus* (South America, Figure 5B purple) forming a similar genetic entity. Janosik and Halanych (2013) indicated the morphological distinctness of *O. meridionalis* [48]. However, as Moreau et al. (2021) pointed out, there seems to be a misidentification of the associated specimens, which was confirmed in our analysis (the sequences belong to *Asterina fimbriata* [4]. Additionally, two other *Odontaster* species, *Odontaster roseus* and *Odontaster pearsei*, have been repeatedly misidentified as *O. meridionalis* [12]. Altogether, this questions the existence of *O. meridionalis* as a different species [12].

These examples illustrate the importance of combining morphology with molecular approaches to avoid under- or overestimating biodiversity, as has already been highlighted in previous studies by Janosik et al. (2011), Layton et al. (2016), and Knott et al. (2018) as well [9,18,19].

![Figure 5](image.png)

**Figure 5.** Haplotype networks built with the TCS method using COI sequences (658 bp). Colours depict the geographic origin of the specimen (see legend) with black dots being missing haplotypes. The size of the circles indicates the haplotype frequency (Legend: outer circle: 10 samples, inner circle: 1 sample). (A) The 112 COI sequences from individuals within the BIN ‘BOLD:AAA8344’ identified as *Anasterias rupicola* (all originating from Kerguelen, in yellow) and *Anasterias antarctica* (originating from various locations). (B) The 64 COI sequences within the BIN ‘BOLD:ABW1983’ identified as *Odontaster meridionalis* (all originating from Kerguelen, in yellow) and *Odontaster penicillatus* (originating from various locations). Haplotype networks for the other species can be found in Supplementary Figure S1.

Despite the small number of species recorded, several taxonomic discrepancies were found (e.g., the presence of *Cycethra frigida* almost went unrecognised, and the possibility
of two synonymous species pairs). This suggests that numerous taxonomic discrepancies such as these remain to be unveiled.

4.2. Geographical Patterns of Magellanic Sea Stars in the Southern Ocean

As suggested in this work, confirming species identifications using genetic tools can be crucial to determining species distributions more reliably. However, no barcode data were available prior to this study for *Henricia* sp., *Perknaster* sp., *Cosmasterias lurida*, *Cycethra verrucosa*, and *Diplodontias singularis*, and only two species had barcoded specimens covering their known distribution. GBIF indicated a circumpolar distribution for most species compared with a more limited distribution based on barcoded specimens. The biggest discrepancy was found in *Cycethra verrucosa*, where the circumpolar distribution shown by GBIF only has barcoded specimens from the Magellanic Region despite the relatively extensive barcoding effort available for Southern Ocean sea stars. Either GBIF shows an overestimation due to misidentifications or BOLD shows an underestimation due to the lack of DNA barcodes covering the full distributional range of a species. Whether a species’ true distribution is closer to the one obtained from GBIF occurrences or BOLD can be resolved by geographically expanding the barcoding effort and/or verifying all GBIF occurrences (e.g., completed in [72] for Argentine deep-sea sea stars). Barcodes are especially scarce for individuals originating from the Antarctic continental shelf. Other than taxonomical errors, georeferencing without mentioning the coordinate accuracy of occurrences can form a big hurdle towards accurate species distribution patterns [75,76].

Despite these uncertainties, Magellanic sea star distribution varies among species, ranging from a South America-only distribution, even in GBIF (e.g., *Asterina fimbriata*), to a possible sub-Antarctic-only distribution (e.g., *Anasterias antarctica*), and a circumpolar distribution, even in BOLD (e.g., *Glabraster antarctica*). Two species had a distribution pattern mainly within the sub-Antarctic but with occurrences on the Antarctic Peninsula and the Scotia Arc. These affinities for some species support the hypothesis of a stepping-stone process along the Scotia Arc to connect the Magellanic region and the Antarctic Peninsula in both directions. This also highlights the pivotal position of South America in biogeography within the Southern Ocean [33,35].

Factors Influencing Species Distribution

The developmental mode has been suggested to be an important factor in shaping biogeographical patterns in sea stars [2 and references therein]. In our study, the brooders did not show a more restricted distribution compared to the broadcasters. In the case of the brooder *Pteraster affinis*, our genetic data indicate that the Magellanic specimen belongs to the *Pteraster affinis/militaris* clade from Jossart et al. (2021), which includes one specimen from Canada (identified as *P. militaris*) [11]. Therefore, it appears that the clade “*Pteraster affinis/militaris*” has a very broad distribution range with a presence in both the Southern and Northern Hemispheres. Sampling outside of the Southern Ocean is required to determine whether this species has a true bipolar distribution [11] or a cosmopolitan distribution with occurrences outside the polar regions that are not yet sampled.

Even though one brooder, *Asterina fimbriata*, was restricted to the Patagonian shelf, the same restricted distribution also occurs in one broadcasting species: *Cosmasterias lurida*.

In the case of the brooder *Anasterias antarctica*, its wide distribution could be linked to possible dispersal through passive rafting on floating kelp rafts [42,76–78], as species within this genus have already been observed on floating kelps [78,79]. Many species on these rafts are brooders, allowing them to remain on a raft for multiple generations [80,81]. Smiths (2002) estimated that, at any time, 70 million floating kelp rafts are drifting in the Southern Ocean north of the Polar Front [82]. Fraser et al. (2018) revealed that large numbers of these kelp rafts are able to cross the Polar Front and that climate change will enhance the frequency and importance of these kelp rafting events in the future [81]. Despite the uncertainty in hitting land soon enough for the species to survive and washing ashore in
a suitable habitat for the species to settle, the large number of rafts suggests that at least some will be successful each year, facilitating colonisation by its epifauna.

Dispersal ability alone is not sufficient in explaining species’ distribution range [41,42]. Environmental factors combined with species’ physiological constraints as well as other life history traits and biological interactions should also be considered [3,83].

Physiological constraints play a major role in species distribution, especially temperature tolerance when crossing oceanic fronts [84]. Crossing these fronts coincides with a sudden drop or increase in temperature. Antarctic species generally have a lower thermal limit, but their sensitivity to the rapidness of temperature changes is taxon-specific [85]. Echinoderms might be more sensitive to chronic temperature changes but better at resisting an acute and temporary temperature change associated with the crossing of an oceanfront, for example [85]. Other environmental factors like lower salinity in the Magellanic fjords might also explain the absence of some species [86–88].

Finally, the two species with the broadest distribution also had the largest depth range, with Glabraster antarctica being present down to 3200 m [59] and Pteraster affinis down to 1800 m [11]. This enlarges their possible distribution range, and deep-sea migration makes connection possible among populations from South America and Kerguelen, Antarctica, and other sub-Antarctic islands [11,36].

5. Conclusions

In this preliminary biodiversity assessment, we recorded a sea star diversity of 15 species from the coastal Magellanic Region. We recorded Cycethra frigida for the first time in this region. This is only the beginning of unravelling the sea star biodiversity in this region, as more sampling sites and less accessible locations could still host an undiscovered diversity. We also emphasised the need to combine morphology and genetics in species identification to gain the most accurate estimate of biodiversity. We indicated the possible synonymy of two species pairs within the Anasterias and Odontaster genera. In order to determine reliable species distributional ranges, the barcoding effort needs to be expanded to cover a wider geographical range, starting by verifying GBIF occurrences and increasing the scarce availability of barcodes for individuals originating from the Antarctic continental shelf. Despite this, Magellanic sea stars with a narrow distributional range (South America-only) and circum-polar to bipolar or cosmopolitan ranges were found. Although developmental mode has been suggested to determine species distribution, relying on this alone is insufficient, and other explaining factors should be considered.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15111129/s1, Figure S1: All haplotype networks of species with more than three sequences; Figure S2: Diagnostic morphological characters of the two Cycethra species; Figure S3: Species distributions; Table S1: All metadata; Table S2: Sampling site information including coordinates and maximum depth.

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