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Inventory

Biodiversity of macroalgae-associated invertebrates in the marine protected area of Alcatrazes archipelago, southeastern Brazil

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Abstract: This study describes and illustrates the biodiversity of macroinvertebrates associated to *Sargassum* and *Dictyota* seaweed habitats in the Alcatrazes archipelago, the largest Marine Protected Area (MPA) in the state of São Paulo, southeast Brazil. Assemblages were sampled during the summers of 2018, 2019 and 2020 and winter of 2018 at two sites on the main island. Macroalgae containing the associated fauna were collected at approximately 10 meters deep, with six samples at each site and in each sampling campaign. *Sargassum* fronds prevailed during summer collections, whilst were absent in the winter campaigns, when *Dictyota* was the most frequent seaweed. Among invertebrates, 32 species were exclusively found on summer months, associated to *Sargassum* beds, while 12 species were only registered on winter collection, in association with *Dictyota*. In total, 91 species were identified, belonging to 60 families, 19 orders, 7 classes and 3 phyla, including Arthropoda (Malacostraca and Pycnogonida), Mollusca (Gastropoda, Bivalvia and Polyplacophora), and Echinodermata (Ophiuroidea and Echinoidea). Among the 91 species found, 73 species are new records for the Alcatrazes archipelago marine area, thus revealing the expressive invertebrate biodiversity living in association with macroalgae beds in that area, which has still been little explored. No significant difference in species diversity was found between the two sites of Alcatrazes. In addition, few specimens of two invasive species were found: *Perna perna* (Mollusca: Bivalvia) and *Ophiothela mirabilis* (Echinodermata: Ophiuroidea), which underscores the importance of monitoring different habitats within MPAs to check for possible changes in the fauna over the years. As far as our knowledge, this is the first illustrated inventory of the seaweed-associated macroinvertebrate fauna within the Alcatrazes Archipelago, one of the largest MPAs in the Brazilian coast; besides unravelling its notorious biodiversity, this can act as a reference for future monitoring of local coastal diversity.

Keywords: *Inventory; Crustacea; Pycnogonida; Mollusca; Echinodermata; Seaweed; Sargassum; Dictyota; Conservation.*

Biodiversidade de invertebrados associados a macroalgas na área marinha protegida do Arquipélago de Alcatrazes, sudeste do Brasil

Resumo: Este estudo descreve e ilustra a biodiversidade de macroinvertebrados associados a habitats de macroalgas pardas marinhas dos gêneros *Sargassum* e *Dictyota* no Arquipélago de Alcatrazes, a maior Área Marinha Protegida (AMP) do estado de São Paulo, sudeste do Brasil. As assembleias foram amostradas durante os verões de 2018, 2019 e 2020 e no inverno de 2018 em dois locais na ilha principal. As macroalgas contendo a fauna associada foram coletadas a aproximadamente 10 metros de profundidade, com seis amostras em cada local e em cada campanha amostral. Frondes de *Sargassum* predominaram nas coletas de verão, enquanto estiveram ausentes nas campanhas de inverno, quando *Dictyota* foram predominantes. Entre os invertebrados, 32 espécies foram

encontradas exclusivamente nos meses de verão, associadas aos bancos de *Sargassum*, enquanto 12 espécies foram registradas apenas na coleta de inverno, em associação com *Dictyota*. No total, foram identificadas 91 espécies, pertencentes a 60 famílias, 19 ordens, 7 classes e 3 filos, incluindo Arthropoda (Malacostraca e Pycnogonida), Mollusca (Gastropoda, Bivalvia e Polyplacophora) e Echinodermata (Ophiuroidea e Echinoidea). Das 91 espécies encontradas, 73 espécies são novos registos para a área marinha do arquipélago de Alcatrazes, revelando assim a expressiva biodiversidade de invertebrados que vivem em associação com bancos de macroalgas naquela área ainda pouco explorada. Nenhuma diferença significativa de diversidade de espécies foi encontrada entre os dois locais de Alcatrazes. Além disso, foram encontrados poucos exemplares de duas espécies invasoras: *Perna perna* (Mollusca: Bivalvia) e *Ophiothela mirabilis* (Echinodermata: Ophiuroidea), o que ressalta a importância do monitoramento de diferentes habitats dentro das AMPs para verificar possíveis alterações na fauna ao longo dos anos. Até onde sabemos, este é o primeiro inventário ilustrado da fauna de macroinvertebrados associados a algas marinhas no Arquipélago de Alcatrazes, uma das maiores AMPs da costa brasileira; além de desvendar a sua notória biodiversidade, pode servir de referência para futuro monitoramento da diversidade costeira local.
Palavras-chave: *Inventário; Crustacea; Pycnogonida; Mollusca; Echinodermata; Algas Marinhas; Sargassum; Dictyota; Conservação.*

Introduction

Brazil has an extensive coastline, encompassing over 364 million hectares of marine area. About 25% of this is designated as Marine Protected Areas (MPAs), totaling 92 million hectares (MMA 2010). Among these MPAs is the Alcatrazes archipelago, located off the northern coast of São Paulo state, within the municipality of São Sebastião. This archipelago comprises 13 islands, islets, and rocky outcrops, which are safeguarded under two types of conservation units: (1) the Tupinambás Ecological Station (ESEC Tupinambás), which was created in 1987, managed by the Instituto Chico Mendes de Biodiversidade (ICMBio), comprising an area of 2,463 hectares where public visitation is prohibited (Plano de Manejo 2017, Francini and Ramos 2014); and (2) the Alcatrazes Archipelago Wildlife Refuge (or Alcatrazes Refuge), that allows scientific research and has restricted public visitation since 2016. Alcatrazes Refuge comprises the entire Alcatrazes archipelago (except for Sapata Island and other areas already protected by ESEC Tupinambás) in addition to the surrounding marine region (Plano de Manejo 2017). It has a total of 67,479 hectares, making it the largest Conservation Unit in southeast and south Brazil and the second largest in the country overall (Plano de Manejo 2017).

The Alcatrazes archipelago is located in a relevant economic area, with the presence of the Port of São Sebastião, Petrobras units that carry out exploration and production of oil and natural gas, fishing, aquaculture, and tourism activities (Plano de Manejo 2017). Given the increase in coastal urbanization in recent decades (Marandolla et al. 2013) and the anthropogenic activities that are the main causes of marine pollution (Clark, 2001), the creation of ESEC Tupinambás and the Alcatrazes Archipelago Wildlife Refuge aimed to protect natural environments, ensuring conditions for the existence and reproduction of species or communities of local flora and resident or migratory fauna (Plano de Manejo 2017).

Macroalgae habitats are a key feature of the benthic seascape in the Alcatrazes archipelago (Aued et al. 2018; Motta et al. 2021). In marine ecosystems, macroalgae play a crucial role as primary producers in coastal food webs, which is essential for maintaining the balance of the carbon cycle and sequestering carbon from the atmosphere. This contributes to the regulation of pH in the marine environment (Hurd et al. 2009; Moraes et al. 2013). Brown macroalgae form extensive beds that can cover up to 80% of coastal areas, which form large extensions in the meso- and infralittoral rocky shores in Brazil (Paula and Oliveira-Filho 1980, Jacobucci et al. 2006). Along the São Paulo coast, brown macroalgae from the genera *Sargassum* (Phaeophyta–Fucales) and *Dictyota* (Dictyotales, Phaeophyta) are among the most prevalent types of macroalgae (Cunha et al. 2013).

These macroalgae act as habitat for a diverse associated invertebrate fauna, offering them food, protection from wave action and predators, and breeding sites (Duffy and Hay, 2000, Jacobucci et al. 2006, Christie et al. 2009). Key groups of macrofauna that inhabit these seaweed ecosystems include crustaceans, mollusks, and echinoderms (Tararam and Wakabara 1981, Leite et al. 2000, Jacobucci and Leite 2002, Tanaka and Leite 2003, Jacobucci et al. 2006), which act as links between primary producers and the other trophic levels within marine trophic webs. These organisms are also recognized as potential bioindicators of environmental quality due to their sensitivity to marine pollutants, making them important for monitoring and conservation efforts (Thomas 1993, Oehlmann and Schulte-Oehlmann 2003, Jacobucci et al. 2006).

Epifaunal invertebrates living on macroalgae meadows provide key ecological roles and ecosystem services is coastal areas, exerting major influence on fish stocks and in the abundance and diversity of invertivores species (Tano et al. 2016; Wenger et al. 2018). Rolim et al. (2019) showed that the abundance of mobile invertebrate feeder fish was increased with protection status of the area and distance to shore, with higher levels reported in Alcatrazes. During the recent expansion process of Alcatrazes Wildlife Refuge, an increase in the abundance of invertivores/herbivorous fish species, that feed on seaweed beds, has been reported in the first years after the expansion (Rolim et al. 2024). However, little is known about the macroalgae-associated invertebrate assemblages before and after this event.

Important contributions for the knowledge of benthic marine invertebrate fauna in Alcatrazes have been made over the last decades, including studies on benthic reef communities (e.g., Rocha and Bonnet 2009; Banha et al. 2019; Aued et al. 2018, Motta et al. 2021, Santana et al. 2023), sandy bottom marine invertebrates (Plano de Manejo 2017), and other populational, systematic, and management studies of particular invertebrate species (e.g., Kitahara et al. 2020; Vaga et al. 2020; Savio et al. 2021; Rezende-Gois et al. 2023). However, there is a noticeable gap in detailed surveys focused on the invertebrate biodiversity associated with marine seaweeds in the area. To address this, the present study conducted the first species-level and illustrated inventory of the biodiversity of vagile macroinvertebrates associated with *Sargassum* and *Dictyota* algae within the Alcatrazes archipelago's Marine Protected Area (MPA).

Materials and Methods

1. Study area

This study was conducted at two different sites within the Alcatrazes Island, the main island of the marine protected area (MPA) of Alcatrazes archipelago (Figure 1a–f). The first site ("Baía do 17") is part of the Tupinambás Ecological Station (ESEC Tupinambás) (24°06.357'S; 45°42.103'W), established on July 20, 1987, under Federal Decree-Law No. 94.656. The second site ("Baba de Boi") lies within the Alcatrazes Wildlife Refuge category (24°05.838'S; 45°41.291'W), created on August 2, 2016, by a separate Federal Decree-Law (unnumbered) (Figure 1e–f). Both sites feature rocky shores with similarly fragmented profiles, and samples were collected from zones dominated by fleshy brown macroalgae, but co-existing with other benthic organisms such as corals, turf algae, tunicates and poriferans.

In both localities, the predominant brown macroalgae were identified as *Sargassum furcatum* Kützing 1843 and *Dictyota menstrualis* (Hoyt) Schnetter, Hörnig & Weber-Peukert 1987. The former had been previously recorded for the area, and the later was registered for other insular areas of São Paulo (Rocha-Jorge 2015; Moraes 2018; Siqueira et al. 2024). However, both genera contain species with high intraspecific morphological variability, leading to a complex taxonomy (Mattio & Payri 2011; Bogaert et al. 2020). Therefore, we here choose to refer to them in the genus level throughout this paper.

2. Sampling and processing

Samples were collected from both Baía do 17 and Baba de Boi during four sampling campaigns: summer 2018, winter 2018, summer 2019, and summer 2020. In each campaign, the most abundant macroalgal species was selected (*Sargassum* in the summer campaigns and *Dictyota* in the winter campaign). Six random samples of algae were collected during each campaign by autonomous diving at a depth of approximately 10 meters, where the algal beds were located. Underwater, each algal thallus was individually enclosed in a bag with a 0.2 mm mesh size to prevent the loss of macrofauna. This ensured that only the fauna associated with the macroalgal thalli were collected, excluding those residing in the interspaces among the algae fronds. Once collected, the algae were brought to the surface, where they were kept in insulated coolers with seawater and ice until transported to the Center of Marine Biology at the University of São Paulo (CEBIMar/USP, in São Sebastião).

In the laboratory, each sample was successively washed three times in fresh water to remove associated fauna, which was then fixed in 70% ethanol. With a stereomicroscope and an optical microscope, specimens from the groups Mollusca (Gastropoda, Bivalvia, and Polyplacophora), Arthropoda (Malacostraca and Pycnogonida), and Echinodermata (Ophiuroidea and Echinoidea) were identified to the lowest possible taxonomic level with the aid of reference material, specific literature (e.g., Moreira and Pires 1977, Masunari and Sieg 1980, Rios 1985, LeCroy 2000, 2002, 2004, 2007, 2011, Borges et al. 2002, Amaral, Rizzo and Arruda 2005, Loyola and Silva 2006, Lacerda et al. 2011, Lacerda and Masunari 2011, Marochi and Masunari 2011, Lacerda and Masunari 2014, Longo et al. 2014, Mauro and Serejo 2015, Pires 2015), and expert taxonomists for each group. The invasion status of each species was indicated according to published guidelines (MMA 2009, Hendler et al. 2012). The best-preserved specimens from each species were photographed in the laboratory using a camera coupled to a Zeiss "Discovery V8" stereomicroscope. Image editing was done using GIMP version 2 (The GIMP development Team 2019). The material was separated in lots which were deposited in the zoological collection of the Museum of Biological Diversity of the State University of Campinas (MDBio-Unicamp), São Paulo, Brazil (ZUEC CRU 5030 – 6091; ZUEC CRU 5030 – 6091). Some specimens are still to be deposited.

3. Data analyses

To assess sampling efficiency, we created sample-based completeness curves for each location (Chao et al. 2020). Species diversity among sites was compared using a combination of sample-based interpolation and extrapolation rarefaction (Colwell et al. 2012). This comparison was based on the first three Hill numbers, representing species richness ($q = 0$), Shannon diversity ($q = 1$), and Simpson diversity ($q = 2$) (Chao et al. 2014). Rarefactions were performed with 400 bootstrap resampling iterations. Results were considered statistically significant at the $\alpha = 0.05$ level when 95% confidence intervals did not overlap. The analyses were conducted using the 'iNEXT' package (Hsieh et al., 2016) in R software version 4.3.1 (R Core Team 2023).

Results

A total of 27,456 individuals were sampled in this study, identified into 91 invertebrate species, belonging to 60 families, 19 orders, 7 classes and 3 phyla. Out of these, 59 species were found in both sampling locations, while 21 species were unique to Baba de Boi, and 11 species were unique to Baía do 17. Among all identified species, 32 were observed exclusively during summer campaigns in *Sargassum* beds, while twelve species were found solely in the single winter campaign, when only *Dictyota* beds were present.

Arthropoda was the most abundant group recorded, with 43 species of Crustacea (from the Orders Amphipoda, Isopoda, Tanaidacea, and Decapoda) and three species of Chelicerata (from the Class Pycnogonida). This was followed by Mollusca, comprising 31 Gastropoda species, seven Bivalvia species, and one single Polyplacophora. In the Echinodermata group, five species of Ophiuroidea and one of Echinoidea were identified (Figure 2). Among these taxa, 73 species are new records for the Alcatrazes archipelago (Table 1). Additionally, other less represented groups such as annelids (Polychaeta) ($n = 24$) and crustaceans from the infraorders Caridea ($n = 14$) and Anomura ($n = 38$) were also found, though most of these specimens were poorly preserved or in juvenile forms, preventing accurate identification; therefore, they were excluded from this inventory.

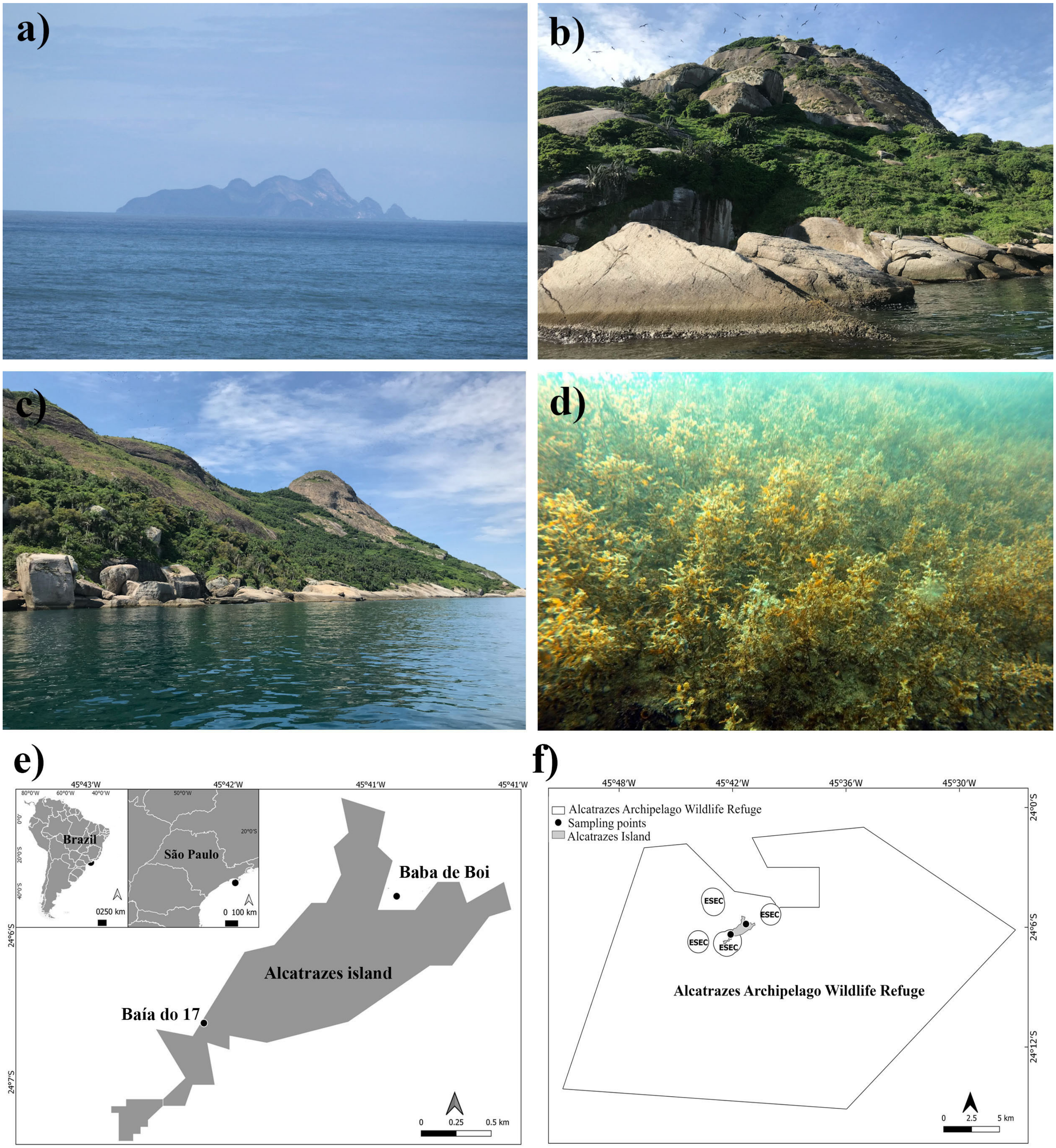

Figure 1. Characterization of the sampling area. (a) Photo of the Alcatrazes archipelago seen from the coast of São Sebastião. (b) and (c) The rocky shore. (d) Underwater photo of the *Sargassum* beds on the main island. (e) Map of the main island, in the municipality of São Sebastião, on the north coast of the São Paulo state, with the two collecting sites, Baía do 17 (ESEC Alcatrazes) and Baba de Boi (Wildlife Refuge). (f) Map of the boundaries of the two categories of protected areas (ESEC and Refuge), indicating the position of the two sampling sites.

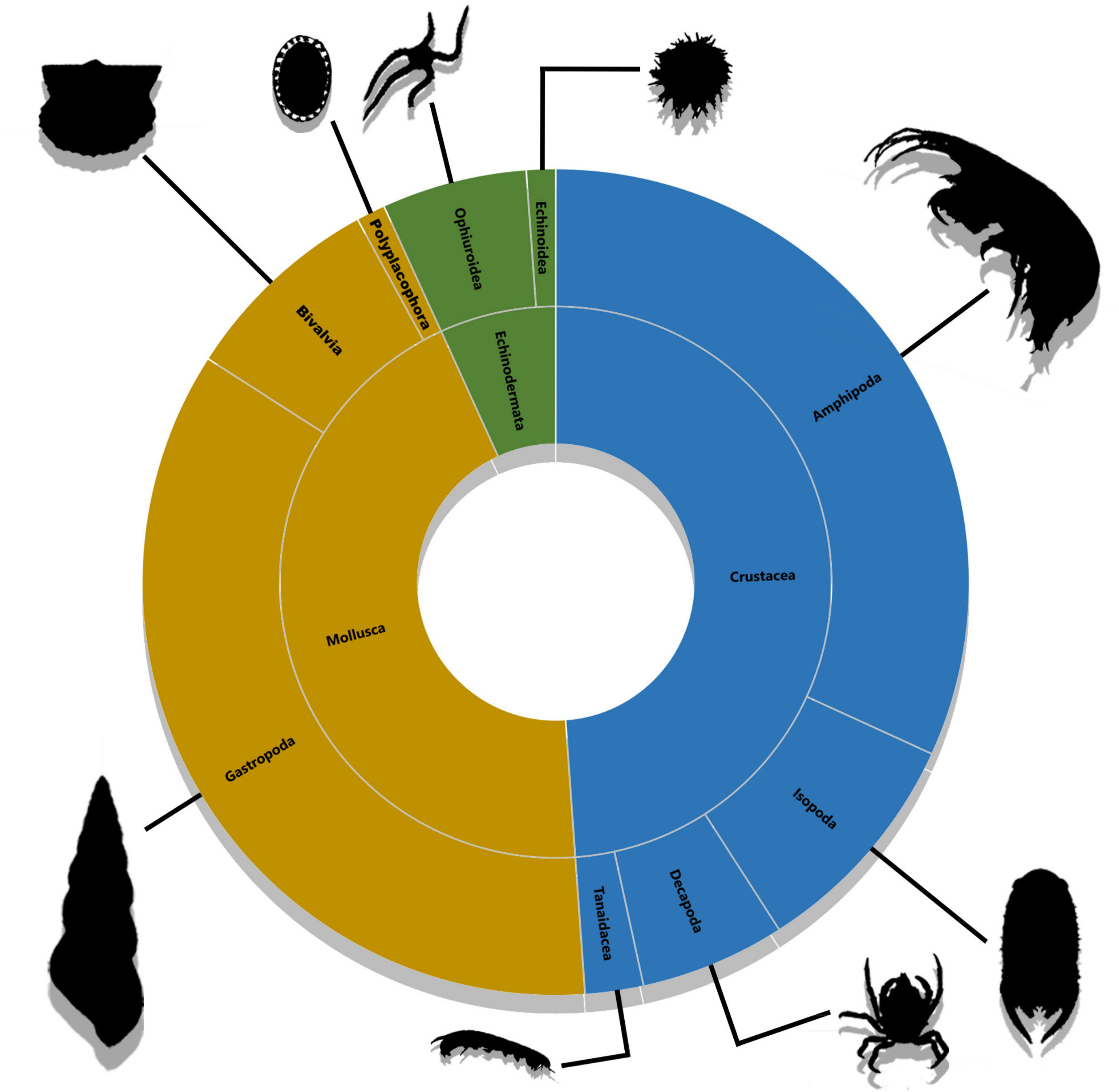

Figure 2. Total number of species in each group: Mollusca (Gastropoda, Bivalvia and Polyplacophora), Crustacea (Amphipoda, Isopoda, Decapoda and Tanaidacea) Pycnogonida and Echinodermata (Ophiuroidea and Echinoidea).

The most prominent order within the Crustacea subphylum (totaling 25,788 individuals) was Amphipoda (22,620 individuals) (Figure 3), with *Ericthonius brasiliensis* (Dana, 1853) being the most abundant species (n = 6,122) (Figure 3m). Isopoda (n = 1,700) (Figure 4a–h) was the second most abundant order within Crustacea, with *Janaira gracilis* Moreira & Pires, 1977 (n = 798) as the most prevalent species (Figure 4b). The Tanaidacea order (n = 1,410) (Figure 4i-j) had *Chondrochelia dubia* (Krøyer, 1842) (n = 1401) (Figure 5i) as the most abundant species. Brachyura, the least abundant order within Crustacea, comprised 58 individuals (Figure 5k–o), with *Mithraculus forceps* A. Milne-Edwards, 1875 (40 individuals) being the most common species (Figure 4).

The class Pycnogonida was the least represented in terms of the number of individuals, with only four sampled. Despite the low count, there was considerable diversity with three distinct species identified (Figure 5). The only species with more than one individual was *Anoplodactylus evelinae* Marcus, 1940, with two specimens (Figure 5e).

Table 1. Abundance of species associated with *Sargassum* and *Dictyota* found in the two collecting sites (Baba de Boi and Baía do 17). The collections were carried out in the winter of 2018 and in the summers of 2018, 2019 and 2020. "–" represents the absence of data; "S" represents the summer and "W" represents the winter. Species in bold were already registered in the Alcatrazes Archipelago area.

Phylum/class	Order	Species	Refuge	ESEC	Season	*Sargassum*	*Dictyota*	Abundance
MOLLUSCA								
Gastropoda	**Aplysiida**	*Phyllaplysia engeli* Er. Marcus, 1955	X	X	S	X	–	10
	Caenogastropoda	***Alaba incerta* (d'Orbigny, 1841)**	X	X	S-W	X	X	268
		Bittiolum varium (Pfeiffer, 1840)	X	X	S-W	X	X	102
		Cerithiopsis gemmulosa (C. B. Adams, 1850)	X	X	S-W	X	X	21
		Nototriphora decorata (C. B. Adams, 1850)	–	X	W	–	X	1
	Littorinimorpha	*Alvania auberiana* (d'Orbigny, 1842)	X	X	S-W	X	X	60
		Barleeia cf. *rubrooperculata* (Castellanos & D. E. Fernández, 1972)	X	X	S-W	X	X	54
		Caecum brasilicum de Folin, 1874	X	–	S-W	X	X	6
		Caecum ryssotitum de Folin, 1867	X	–	W	–	X	1
		Melanella eburnea (Megerle von Mühlfeld, 1824)	X	–	S	X	–	2
		Melanella sp.	X	–	S	X	–	1
		Amphithalamus glabrus Simone, 1996	X	X	S-W	X	X	12
		Natica sp.	–	X	W	–	X	1
	Neogastropoda	*Anachis fenneli* Radwin, 1968	X	X	S-W	X	X	96
		***Astyris lunata* (Say, 1826)**	X	X	S-W	X	X	71
		***Costoanachis sparsa* (Reeve, 1859)**	X	X	W	–	X	6
		Costoanachis sertulariarum (d'Orbigny, 1839)	–	X	S	X	–	1
		***Cerithium atratum* (Born, 1778)**	X	X	W	–	X	5
		***Claremontiella nodulosa* (C. B. Adams, 1845)**	X	X	S	X	–	6
		Stramonita brasiliensis Claremont & D. Reid, 2011	–	X	S	X	–	1
		Muricidae sp.	X	–	W	–	X	1
		***Engina turbinella* (Kiener, 1836)**	X	X	S	X	–	2
		Volvarina sp.	X	X	S-W	X	X	2
	Cephalaspidea	*Bulla occidentalis* A. Adams, 1850	X	–	S	X	–	1
		Bulla sp.	–	X	S	X	–	1
	Trochida	***Calliostoma* sp.**	X	–	S	X	–	3
		Eulithidium affine (C. B. Adams, 1850)	X	X	S	X	–	2
		Eulimastoma didymum (Verrill & Bush, 1900)	X	–	S	X	–	2
	Lepetellida	*Fisurella rosea* (Gmelin, 1791)	X	X	S	X	–	19
		Lottia subrugosa (d'Orbigny, 1846)	X	–	S	X	–	1
		Rissoela ornata Simone, 1995	X	X	S-W	X	X	326
		Ischnochiton striolatus	X	–	S	X	–	2
	Arcida	***Barbatia domingensis* (Lamarck, 1819)**	X	–	W	–	X	1
	Pectinida	***Leptopecten bavayi* (Dautzenberg, 1900)**	X	–	S	X	–	1

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Phylum/class	Order	Species	Refuge	ESEC	Season	*Sargassum*	*Dictyota*	Abundance
	Mytilida	***Modiolus carvalhoi* Klappenbach, 1966**	X	X	S-W	X	X	13
		***Musculus lateralis* (Say, 1822)**	X	X	S-W	X	X	36
		***Perna perna* (Linnaeus, 1758)**	X	X	S	X	–	4
	Ostreida	***Pinctada imbricata* Röding, 1798**	X	X	S-W	X	X	416
	Myida	*Sphenia* sp.	X	–	S	X	–	3
ARTHROPODA								
Malacostraca	**Amphipoda**	*Aora spinicornis* Afonso, 1976	X	X	S-W	X	X	1382
		Bemlos sp.	–	X	S	X	–	1
		Ampithoe marcuzzii Ruffo, 1954	X	X	S-W	X	X	16
		Ampithoe ramondi Audouin, 1826	X	X	S-W	X	X	1526
		Ampithoe sp.	X	X	S	X	–	18
		Cymadusa filosa Savigny, 1816	X	X	S-W	X	X	959
		Sunamphitoe pelagica (H. Milne Edwards, 1830)	X	X	S	X	–	5
		Hourstonius wakabarae	–	X	W	–	X	1
		Batea catharinensis Müller, 1865	X	X	S	X	–	50
		Quadrimaera quadrimana (Dana, 1852)	X	–	W	–	X	3
		Elasmopus longipropodus Senna & Souza-Filho, 2011	X	–	S	X	–	1
		Elasmopus pectenicrus (Spence Bate, 1862)	X	–	S	X	–	1
		Ericthonius brasiliensis (Dana, 1853)	X	X	S-W	X	X	6122
		Jassa slatteryi Conlan, 1990	X	X	S-W	X	X	56
		Microjassa sp.	X	X	S-W	X	X	41
		Hyale niger (Haswell, 1879)	X	X	S-W	X	X	4870
		Photis sarae Souza-Filho & Serejo, 2010	X	X	S-W	X	X	732
		Gammaropsis palmata (Stebbing & Robertson, 1891)	X	–	S-W	X	X	10
		Podocerus fissipes Serejo, 1996	X	X	S	X	–	453
		Stenothoe sp.	X	X	S-W	X	X	3145
		Lysianassa temimino Senna & Souza-Filho, 2010	X	X	S-W	X	X	12
		Leucothoe spinicarpa (Abildgaard, 1789)	X	X	S-W	X	X	6
		Caprella scaura Templeton, 1836	X	X	S-W	X	X	2688
		Caprella equilibra Say, 1818	X	X	S-W	X	X	125
		Caprella danilevskii Czerniavski, 1868	X	–	W	–	X	10
		Pseudaeginella montoucheti (Quitete, 1971)	X	X	S-W	X	X	354
		Paracaprella dubiaski Lacerda & Masunari, 2014	X	X	S-W	X	X	6
		Phtisica verae Quitete, 1979	X	X	S-W	X	X	27
	Isopoda	*Carpias minutus* (Richardson, 1902)	X	X	S-W	X	X	764
		Janaira gracilis Moreira & Pires, 1977	X	X	S-W	X	X	798

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Phylum/class	Order	Species	Refuge	ESEC	Season	*Sargassum*	*Dictyota*	Abundance
		Paracerceis sculpta (Holmes, 1904)	X	X	S-W	X	X	82
		Cymodoce brasiliensis Richardson, 1906	X	X	S-W	X	X	16
		Paranthura urochroma Pires, 1981	X	X	S	X	–	7
		Joeropsis dubia Menzies, 1951	X	X	S-W	X	X	24
		Uromunna peterseni Pires, 1985	X	X	S-W	X	X	7
		Astacilla sawayae (Moreira, 1973)	–	X	S	X	–	2
	Tanaidacea	*Chondrochelia dubia* (Krøyer, 1842)	X	X	S-W	X	X	1401
		Zeuxo coralensis Sieg, 1980	X	X	S-W	X	X	9
	Decapoda	*Acanthonyx petiverii* H. Milne Edwards, 1834	X	X	S	X	-	4
		Epialtus bituberculatus H. Milne Edwards, 1834	–	X	S-W	X	X	3
		Omalacantha bicornuta (Latreille, 1825)	X	X	S	X	–	8
		Mithraculus forceps A. Milne-Edwards, 1875	X	X	S-W	X	X	40
		***Pachygrapsus transversus* (Gibbes, 1850)**	X	X	S-W	X	X	3
Pycnogonida	**Pantopoda**	*Anoplodactylus evelinae* Marcus, 1940	X	X	W	–	X	2
		Achelia sawayai Marcus, 1940	–	X	W	–	X	1
		Callipallene gabriellae Correa, 1948	X	–	S	X	–	1
ECHINODERMATA								
Ophiuroidea	**Amphilepidida**	*Amphipholis squamata* (Delle Chiaje, 1828)	X	–	S-W	X	X	10
		Ophiactis lymani Ljungman, 1872	X	X	S-W	X	X	43
		Ophiactis savignyi (Müller & Troschel, 1842)	X	X	S	X	–	3
		***Ophioplocus januarii* (Lütken, 1856)**	X	X	S-W	X	X	40
		***Ophiothela mirabilis* Verrill, 1867**	X	–	S-W	X	X	3
Echinoidea	**Camarodonta**	***Lytechinus variegatus* (Lamarck, 1816)**	–	X	S	X	–	4

Among Mollusca (n = 1,561), Gastropoda (Figure 6a–a.e) was the most abundant class (n = 1,085), with *Rissoella ornata* Simone, 1995 (Figure 6n) being the most prevalent species (n = 326 individuals). In the Bivalvia class (n = 474) (Figure 6a.f–a.l) *Pinctada imbricata* Röding, 1798 (n = 416) (Figure 6a.h) was the most common species. As for Polyplacophora, only 2 individuals of *Ischnochiton striolatus* (Gray, 1828) (Figure 6a.m) were recorded.

Regarding the Echinodermata (103 individuals) (Figure 7), nearly all specimens were from the Ophiuroidea class (99 individuals), with *Ophioplocus januarii* (Lütken, 1856) being the most common species, totaling 40 individuals (Figure 7a). Echinoidea had just one species, *Lytechinus variegatus* (Lamarck, 1816), with four juveniles (Figure 7f).

A total of 73 species, representing 80% of the identified fauna, were newly recorded in the Alcatrazes Archipelago. The few species previously known from the archipelago include: *Astyris lunata* (Say, 1826), *Costoanachis sparsa* (Reeve, 1859), *Cerithium atratum* (Born, 1778), *Claremontiella nodulosa* (C. B. Adams, 1845), *Engina turbinella* (Kiener, 1836), *Calliostoma* sp., *Ischnochiton striolatus*, *Barbatia domingensis* (Lamarck, 1819), *Leptopecten bavayi* (Dautzenberg, 1900), *Modiolus carvalhoi* Klappenbach, 1966, *Musculus lateralis* (Say, 1822), *Perna perna* (Linnaeus, 1758), *Pinctada imbricata* Röding, 1798, *Pachygrapsus transversus* (Gibbes, 1850), *Ophioplocus januarii* (Lütken, 1856) *Ophiothela mirabilis* Verrill, 1867, and *Lytechinus variegatus* (Lamarck, 1816). Among the new records, we highlight the occurrence of the amphipod *Microjassa* sp. (Amphipoda: Ischyroceridae) (Figure 3v), as this represents only the second record of the genus in Brazil (the first was in Wakabara et al. 1991, with vague information about its occurrence).

Out of all the species examined, only two are known to be invasive: the bivalve *Perna perna* (Linnaeus, 1758) (Figure 6a.l) and the ophiuroid *Ophiothela mirabilis* Verrill, 1867 (Figure 7c). The total number of specimens was low for both species, with four records for *P. perna* and three for *O. mirabilis*.

The sample completeness curves for both sites nearly reached an asymptote, achieving approximately 95% sample coverage. This suggests that the sampling efforts in this study were adequate to represent

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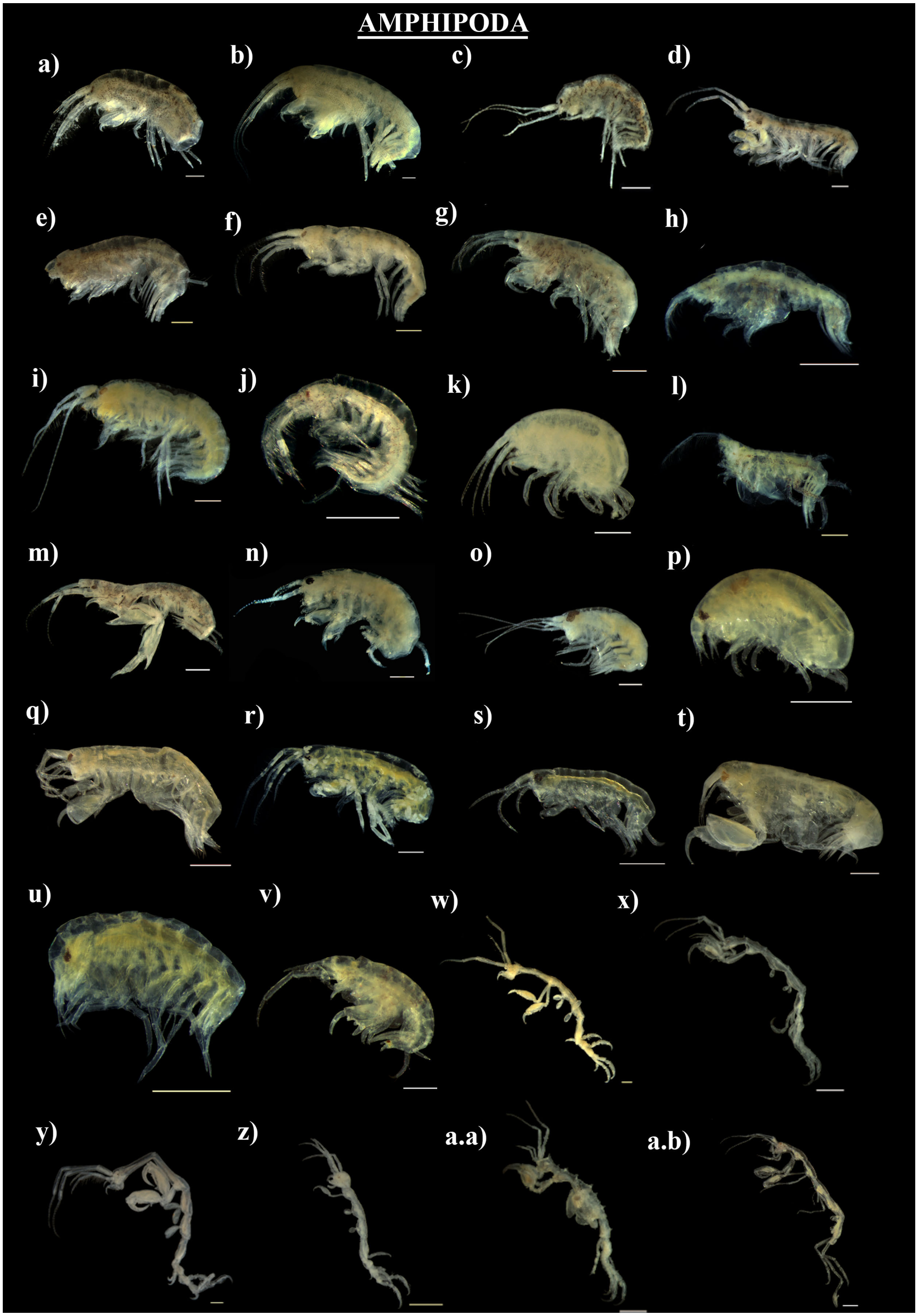

Figure 3. Amphipods collected in *Sargassum* and *Dictyota* in the Alcatrazes Archipelago. (a) *Ampithoe ramondi*, (b) *Cymadusa filosa*, (c) *Aora spinicornis*, (d) *Bemlos* sp., (e) *Ampithoe marcuzzii*, (f) *Ampithoe* sp., (g) *Photis sarae*, (h) *Gammaropsis palmata*, (i) *Sunamphitoe pelagica*, (j) *Jassa slatteryi*, (k) *Stenothoe* sp., (l) *Podocerus fissipes*, (m) *Ericthonius brasiliensis*, (n) *Hyale niger*, (o) *Batea catharinensis*, (p) *Lysianassa temimino,* (q) *Quadrimaera quadrimana*, (r) *Elasmopus longipropodus*, (s) *Elasmopus pectenicrus*, (t) *Leucothoe spinicarpa*, (u) *Hourstonius wakabarae,* (v) *Microjassa* sp., (w) *Caprella scaura*, (x) *Paracaprella dubiaski*, (y) *Caprella equilibra*, (z) *Caprella danilevskii*, (a.a) *Pseudaenginella montoucheti*, (a.b) *Phtisica verae*. Scale bars of 1 mm.

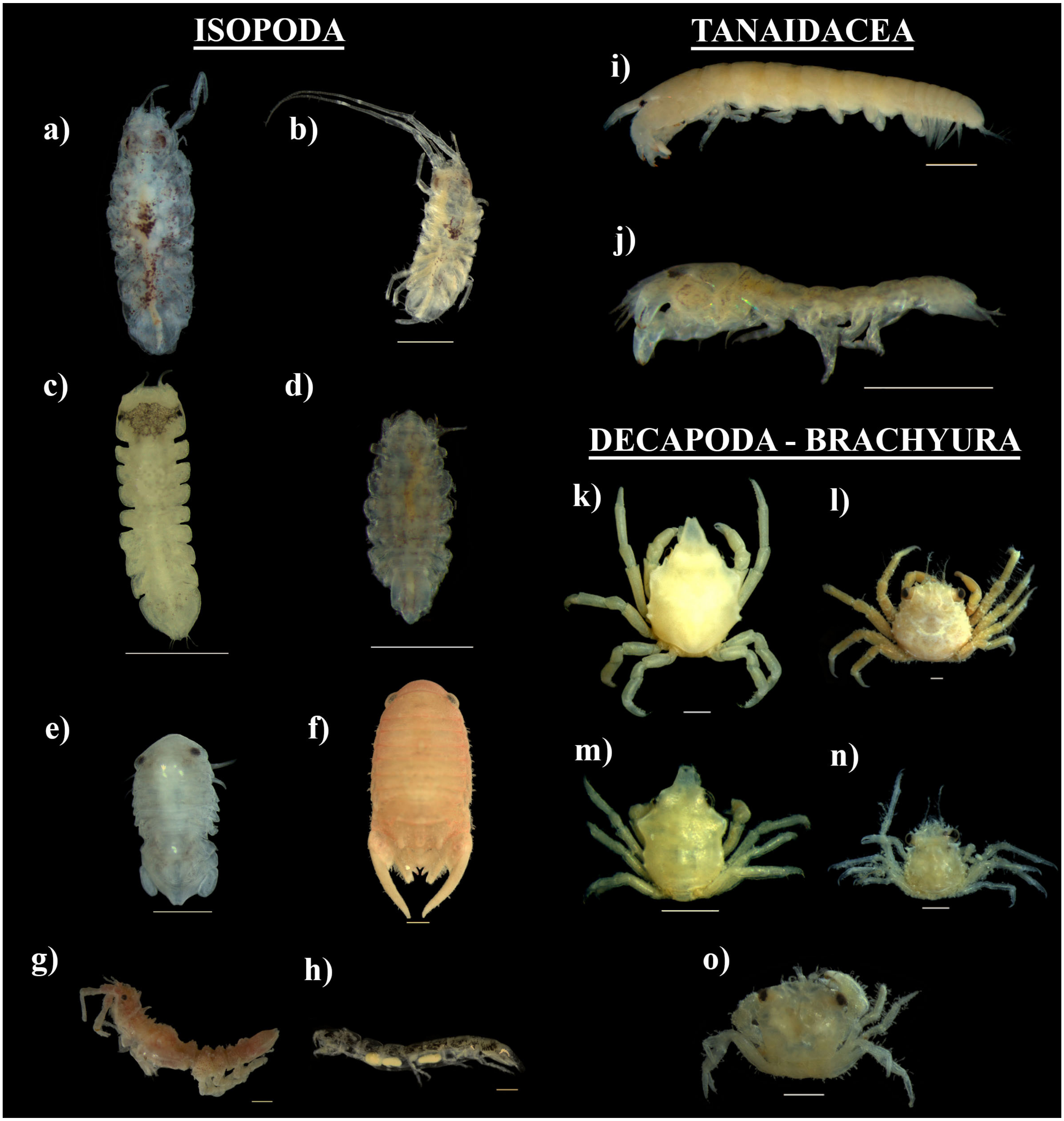

Figure 4. Isopods, tanaidaceans and brachyurans collected in *Sargassum* and *Dictyota* in the Alcatrazes Archipelago. (a) *Carpias minutus*, (b) *Janaira gracilis*, (c) *Joeropsis dubia*, (d) *Uromunna peterseni*, (e) *Cymodoce brasiliensis*, (f) *Paracerceis sculpta*, (g) *Astacilla sawayae* and (h) *Paranthura urochroma*, (i) *Chondrochelia dúbia*, (j) *Zeuxo coralensis,* (k) *Epialtus bituberculatus*, (l) *Mithraculus forceps*, (m) *Acanthonyx petiverii* (n) *Omalacantha bicornuta* and (o) *Pachygrapsus transversus.* Scale bars of 1 mm.

the macroalgae-associated macroinvertebrate species in Alcatrazes (Figure 8a). The sample-based rarefaction curves indicated that there were no significant differences in species diversity between the sampling locations within Alcatrazes (Figure 8b).

Discussion

Given the significance of the Alcatrazes Archipelago for local biodiversity and surrounding areas and considering the partial opening of

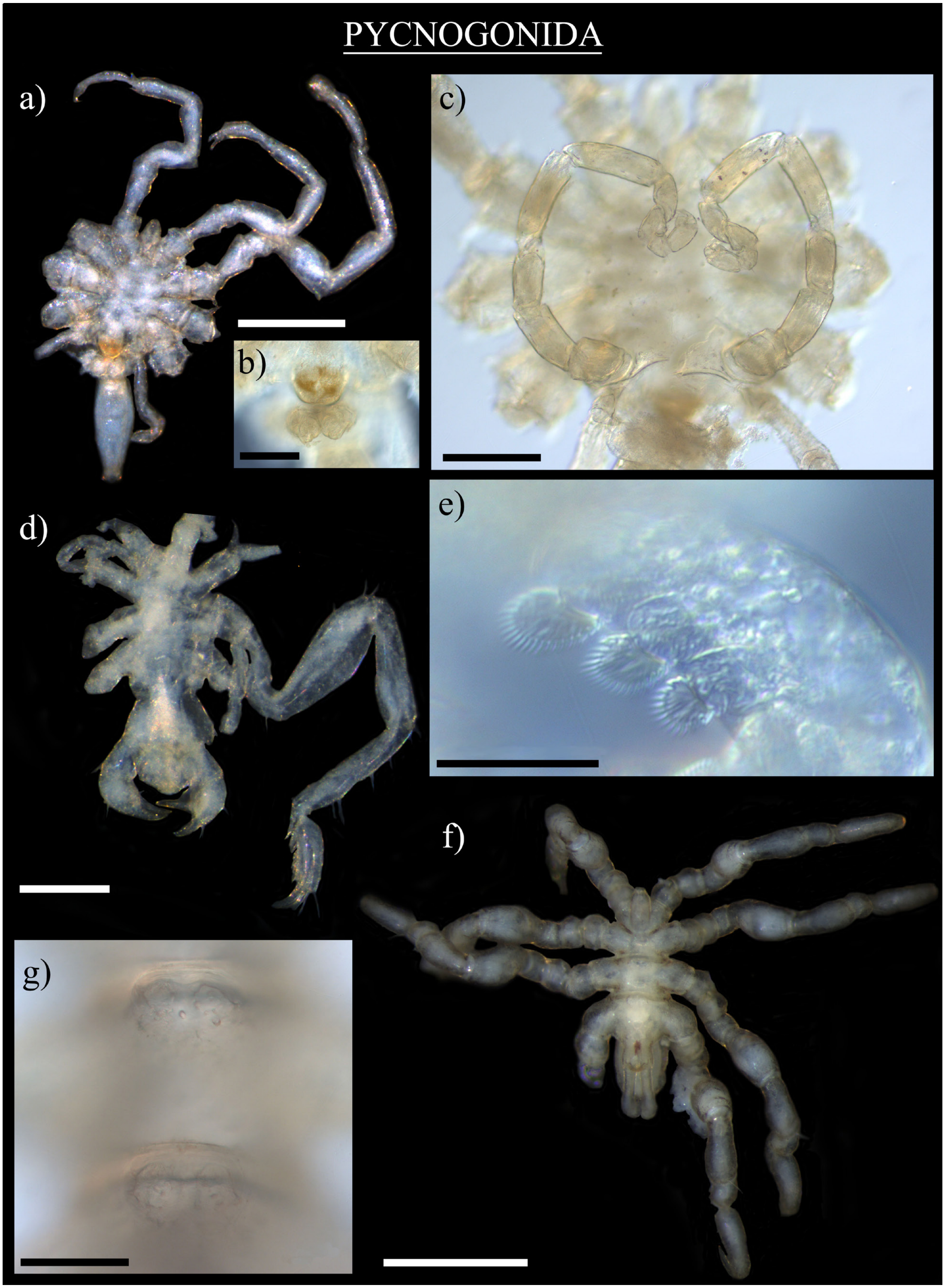

Figure 5. Pycnogonids collected in *Sargassum* and *Dictyota* in the Alcatrazes Archipelago. (a) *Achelia sawayai*, with (b) atrophied chelae and (c) ovigers; (d) *Callipallene gabriellae*, with (e) squamiform setae; (f) *Anoplodactylus evelinae*, with (g) bifid tubercles near margin of segments 1 and 2 of the trunk. Scale bars of 10 µm (e), 50 µm (b), 60 µm (g), 100 µm (c), 200 µm (a and d), and 1000 µm (f).

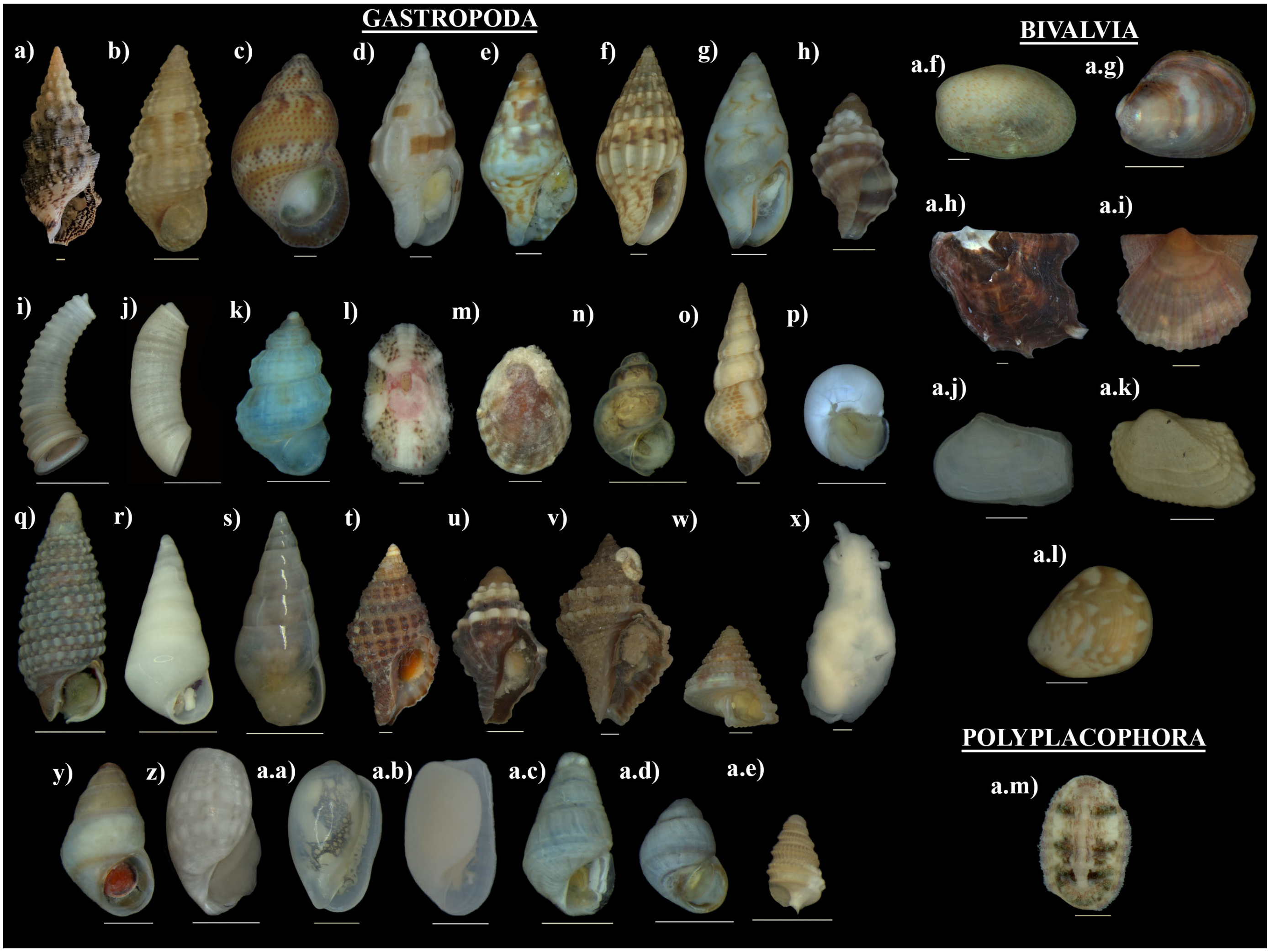

Figure 6. Mollusks collected in *Sargassum* and *Dictyota* in the Alcatrazes archipelago. (a) *Cerithium atratum* (b) *Bittiolum varium*, (c) *Elithidium affine*, (d) *Anachis fenneli*, (e) *Costoanachis sertulariarum*, (f) *Costoanachis sparsa*, (g) *Astyris lunata*, (h) *Muricidae* sp., (i) *Caecum brasilicum*, (j) *Caecum ryssotitum*, (k) *Alvania auberiana*, (l) *Fissurella rosea*, (m) *Lottia subrugosa*, (n) *Rissoella ornata*, (o) *Alaba incerta*, (p) *Natica* sp., (q) *Cerithiopsis gemmulosa*, (r) *Melanella eburnea*, (s) *Melanella* sp., (t) *Claremontiella nodulosa*, (u) *Engina turbinella*, (v) *Stramonita brasiliensis*, (w) *Calliostoma* sp., (x) *Phyllaplysia engeli*, (y) *Barleeia* cf. *rubrooperculata* (c) *Bulla occidentalis*, (a.a) *Volvarina* sp., (a.b) *Bulla* sp., (a.c) *Eulimastoma didymum*, (a.d) *Amphithalamus glabrus*, (a.e) *Nototriphora decorata,* (a.f) *Musculus lateralis*, (a.g) *Modiolus carvalhoi*, (a.h) *Pinctada imbricata*, (a.i) *Leptopecten bavayi*, (a.j) *Sphenia* sp., (a.k) *Barbatia dominguensis* and (a.l) *Perna perna* and (a.m) *Ischnochiton striolatus*. Scale bars of 1 mm in A–P.

the Wildlife Refuge for public visitation, monitoring the area has become essential for the conservation of its biodiversity and coastal habitats (Plano de Manejo 2017). With this in mind, we proposed this effort to survey and illustrate the biodiversity of marine macroinvertebrates associated with macroalgae in the Alcatrazes archipelago. Our study identified a total of 91 species, with 73 being newly recorded in the region, including at least one potentially new species.

Macroalgae form habitats for associated fauna, providing shelter, food, and breeding sites for several species of marine invertebrates (Jacobucci and Leite 2002, Jacobucci et al. 2006, Leite et al. 2009). In Brazil, one of the most significant and widespread macroalgae is *Sargassum* spp., especially along the rocky shores of Rio de Janeiro and São Paulo, where it forms extensive banks in the shallow sublittoral zone (Paula 1988, Széchy and Sá 2008). *Sargassum* spp. are perennial algae, however, at certain times of the year it drastically reduces its biomass due to environmental changes, such as temperature, nutrient levels, water movement, and transparency. These fluctuations often follow a seasonal pattern, with reproductive peaks during warmer months and almost no growth during winter in some regions (Paula and Oliveira-Filho 1980, Széchy and Paula 2000, Jacobucci and Leite 2002, Leite and Turra 2003). On the other hand, brown algae from the genus *Dictyota*, often found growing as epiphytes, can increase in abundance when *Sargassum* spp. decline, taking advantage of their seasonal absence (Paula and Oliveira-Filho 1980). This shift in dominance between *Sargassum* and *Dictyota* contributed to the greater temporal variation in the composition of the associated fauna, with 10 taxa found exclusively with *Dictyota*, thus enriching the total records of phytal macrofauna.

Amphipods were the most prevalent taxon in this study, consistent with their usual dominance in seaweed-associated invertebrate assemblages. The composition of amphipods found in the Alcatrazes Archipelago was similar to the species profiles observed in other studies

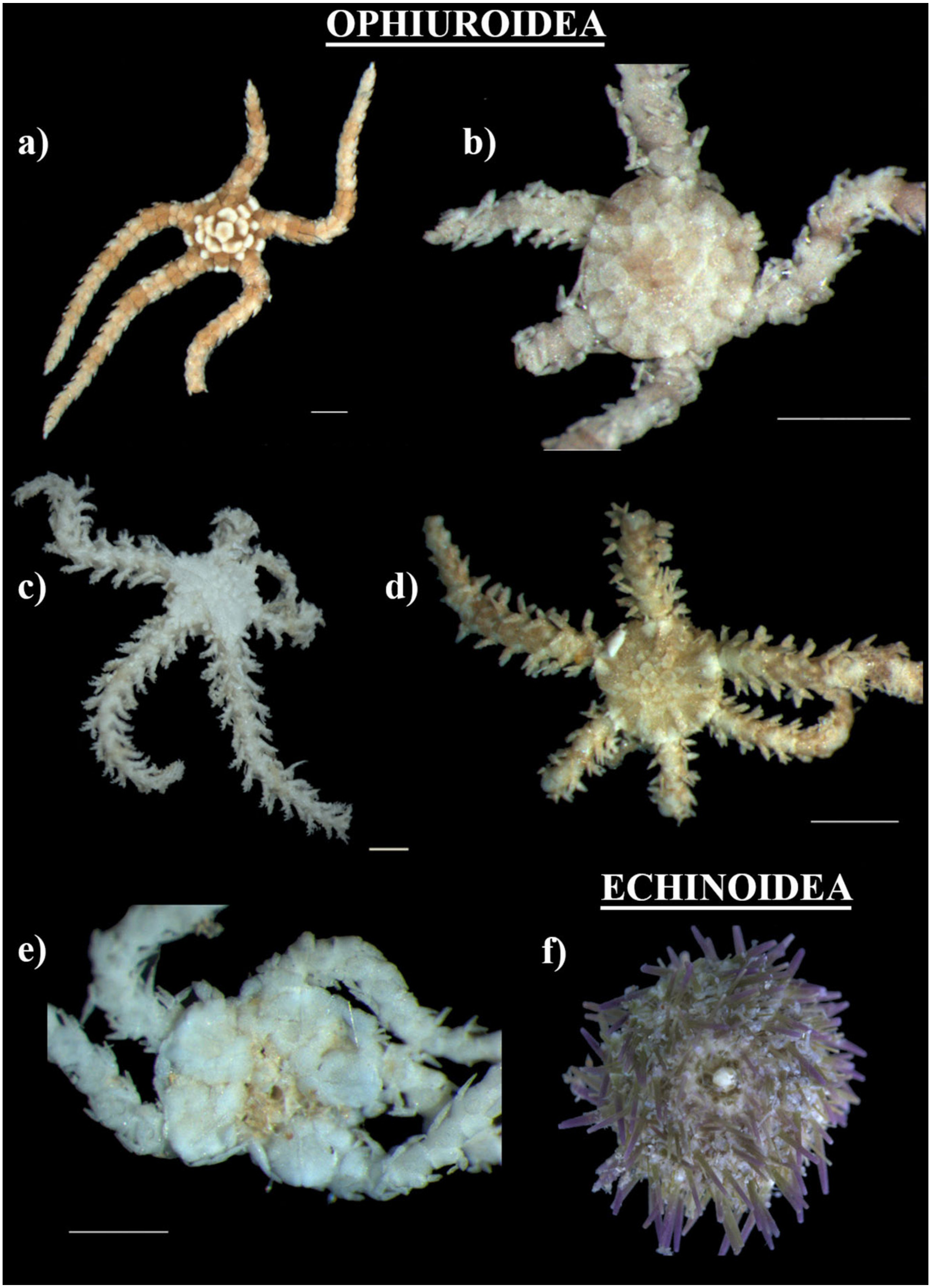

Figure 7. Echinoderms collected in *Sargassum* and *Dictyota* in the Alcatrazes Archipelago. (a) *Ophioplocus januarii*, (b) *Ophiactis lymani*, (c) *Ophiothela mirabilis*, (d) *Ophiactis savignyi*, (e) *Amphipholis squamata* and (f) *Lytechinus variegatus*. Scale bars of 1 mm in A–F.

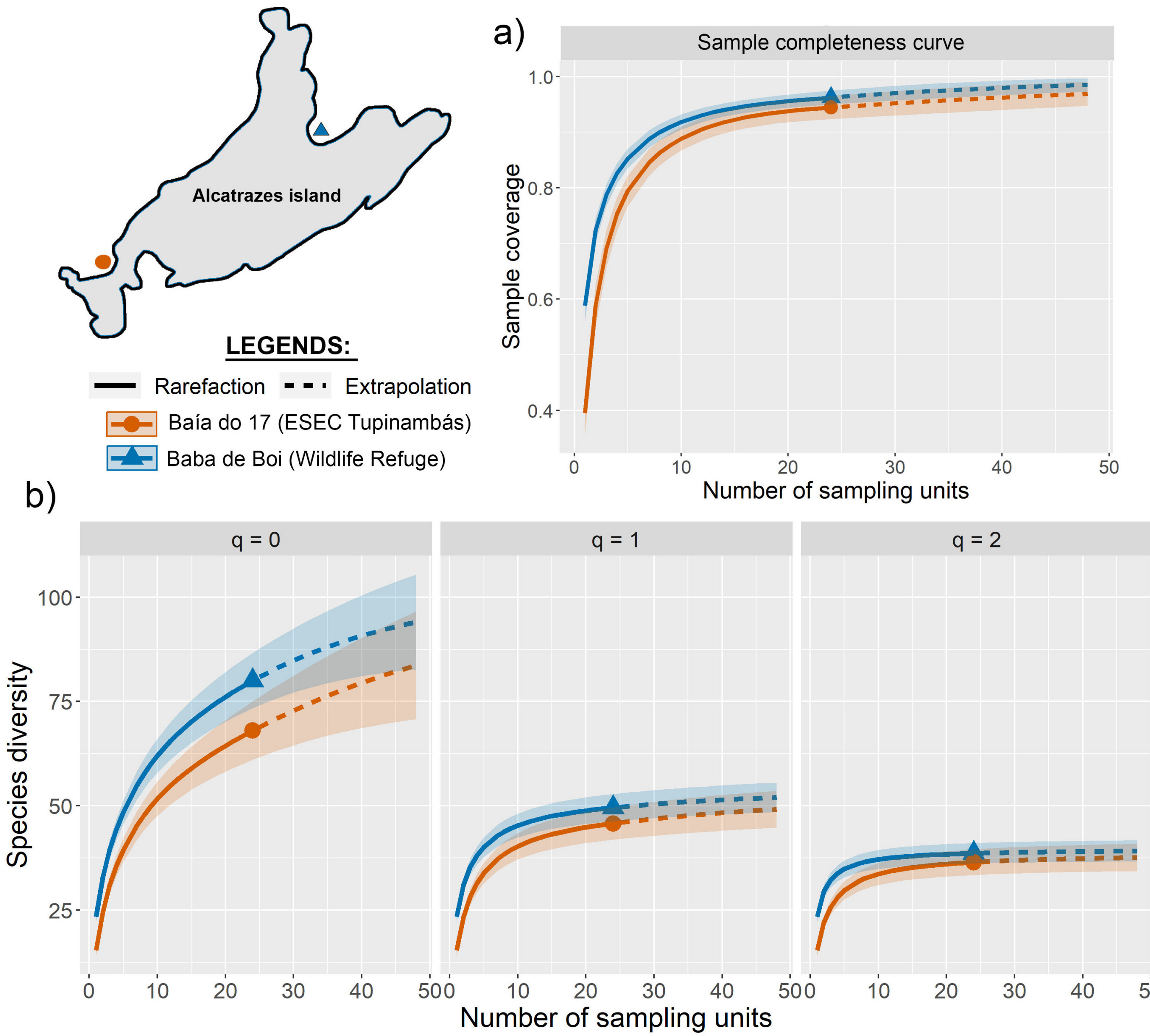

Figure 8. Sample coverage and species diversity of seaweed-associated invertebrates. (a) Sample completeness curve for Alcatrazes localities; (b) Sample-based rarefaction (solid lines) and extrapolation (dashed lines) of invertebrate diversity based on Hill numbers (left: q = 0; middle: q = 1; right: q = 2).

of both insular and continental areas in São Paulo state (Wakabara et al. 1983, Jacobucci et al. 2002, Jacobucci et al. 2006, Jacobucci and Leite 2014). For instance, in studies conducted on São Sebastião Island (e.g., Pavani 2009) and the São Sebastião coast (e.g., Leite et al. 2021), locations closer to Alcatrazes archipelago, also reported a predominance of *Hyale niger* (Haswell, 1879), *Stenothoe* sp., and *Ericthonius brasiliensis*—species that were likewise the most abundant in this study.

However, the amphipod fauna in Alcatrazes also showed unique features, most notably the discovery of a new occurrence of the genus *Microjassa* Stebbing, 1899 (Amphipoda: Ischyroceridae), which had been recorded only once on the Brazilian coast (Wakabara et al. 1991). This genus is characteristic for its small size, at most 4 mm, and for the typical "thumb" in the males' gnathopod 2 propod (Conlan 1995). Currently, there are nine known species in the genus *Microjassa*, with four from the North Atlantic (South Carolina, Florida, Gulf of Mexico, Bahamas, and the United Kingdom) and five from the North Pacific (ranging from Southeast Alaska to California and including the Mediterranean coasts of France and Italy) (Conlan 1988, 1995). The only previous record of this genus in Brazil was reported by Wakabara et al. (1991) for the species *Microjassa macrocoxa* Shoemaker, 1942, which is originally from the North Atlantic. However, the authors did not specify the location of this record or provide further details, only mentioning that it was found in an intertidal zone and in association with animal species. The new record from Alcatrazes presented in this study is the first to offer comprehensive details and reference material for the genus in Brazil. It is likely that this record represents a new species of *Microjassa*, which is currently in the process of being described.

The species composition of the other crustacean groups evaluated in this study (i.e., isopods, tanaidaceans, and brachyuran decapods) were also very similar to those of other assemblages along the São Paulo coast (e.g., Pires-Vanin 1980, Pires 1981, Mantelatto and Corrêa 1996, Jacobucci et al. 2006, Moraes 2018). Although less abundant than amphipods, isopods were also highly representative in our samples, with *Janaira gracilis* Moreira and Pires, 1977 being the most common and abundant species, similar to other coastal and insular areas of the Brazilian coast (Cunha et al. 2013, Pires 2015, Moraes 2018). For Tanaidacea, the species composition matched that found by Moraes (2018) on São Sebastião Island, with a predominance of species from the genera *Zeuxo* and *Chondrochelia*. This was consistent with Pires (1980), which found a great predominance of *Zeuxo coralensis* Sieg, 1980 in Ubatuba. Brachyuran crabs usually form a smaller group among seaweed crustaceans (Mantelatto and Correa, 1996), and their species composition in this study is aligned with that found in other regions along São Paulo's northern coast (e.g., Mantelatto and Correa 1996, Mantelatto et al. 2003 Cobo 2005, Barros-Alves et al. 2013).

The richness and abundance of Pycnogonida found in Alcatrazes was low, representing the less prevalent taxon in this study. Indeed, pycnogonids occur at low population density (Arango 2003), and although macroalgae are an important substrate for pycnogonids assemblages (Arnaud & Bamber, 1988), they represent a small portion of the associated fauna (Mukai, 1971; Tararam and Wakabara, 1981; Masunari, 1982). Despite the low number of individuals combined with poor preservation (i.e., specimens with damaged or missing appendages), the species-level identification was possible. The species identified in this study have previously been recorded as part of phytal fauna (e.g., Varoli, 1996), with *Achelia sawayai* Marcus, 1940 also emerging as the most abundant in one study (Masunari, 1982). Our results constitute the first record of these pycnogonids in Alcatrazes archipelago, providing a noteworthy contribution to the understanding of this understudied group in Brazil.

Gastropods were the second most species-rich group in this study. Regarding species composition, the most frequently encountered families in association with macroalgae along the Brazilian coast, such as Cerithiidae, Phasianellidae, and Collumbellidae (Jacobucci et al. 2006; Leite et al. 2009; Longo et al. 2019), were also common in Alcatrazes. However, the most abundant gastropod species were different from those found in other studies carried out in coastal areas of São Paulo, such as the São Sebastião Channel and Ubatuba (Longo et al. 2014, Longo et al. 2019), and from other localities in Brazil (e.g., Duarte et al. 2015; Barbosa et al. 2019). Longo et al. (2014) studied gastropod species on the rocky shores of the São Sebastião Channel, along São Paulo's northern coast, and found *Bittiolum varium* (L. Pfeiffer, 1840) and *Eulithidium affine* (C. B. Adams, 1850) to be the most abundant species. *Bittiolum varium* is cosmopolitan and often dominates in coastal areas with great anthropogenic influence, such as boating and construction activities (Longo et al. 2019). Therefore, in regions with human impact, this opportunistic species tends to prevail, leading to reduced local diversity (Longo et al. 2019, Longo et al. 2021). The lower presence of *B. varium* in Alcatrazes, compared to other studies, may be because the archipelago experiences less anthropogenic pressure than coastal areas. Meanwhile, the most abundant species in Alcatrazes were *Rissoella ornata* Simone, 1995 and *Alaba incerta* (d'Orbigny, 1841), species that are not abundant in coastal areas (although see Duarte et al. 2020, where *A. incerta* was the third most abundant gastropod on red seaweed beds in an estuarine coastal area of northeast Brazil). Rissoellids were also the most abundant gastropods in *Dictyota* spp. beds on the Abrolhos Archipelago, another insular area of Brazil (Cunha et al. 2013).

Also noteworthy in this study was the presence of 54 specimens of a *Barleeia* species (Gastropoda: Barleeiidae) that resembles *Barleeia rubrooperculata* (Castellanos and D. E. Fernández, 1972). This species has been rarely recorded along the Brazilian coast, typically found in soft-bottom areas off Rio de Janeiro and Espírito Santo states, usually at very low densities and/or in deeper waters (Nahas 1992; Nalesso et al. 2005; Macedo 2011). Therefore, this would be the first record of this species for São Paulo, and the first for Brazilian coast in association with seaweed beds (with an expressive number to be considered only an occasional occurrence). Hence, the gastropod assemblages in Alcatrazes displayed some unique characteristics when compared to previous studies along the Brazilian coast.

As for bivalve mollusks, the dominant species was *Pinctada imbricata*, followed by the mytillids *Modiolus carvalhoi* Klappenbach, 1966 and *Musculus lateralis* (Say, 1822), which aligns with findings from other studies in different regions of Brazil (Montouchet 1979, Jacobucci et al. 2006, Longo et al. 2023). We also highlight the very low abundance of *Perna perna* (Linnaeus, 1758) and the absence of *Isognomon bicolor* (C. B. Adams, 1845) in our samples. Widely distributed along the coast of Brazil, *P*. *perna* is probably a native species from the African continent that arrived in Brazil during the colonial period, trapped in the hulls of slave vessels (Oliveira et al. 2017, Silva et al. 2018). Its range is continuously expanding, often linked to human activities such as trade (Jacobucci et al. 2006). The Brazilian Ministry of the Environment (MMA 2010) classifies *P. perna* as an established invasive species, but there is ongoing debate about its invasive status, with studies focusing on its molecular biology, archaeology, and ecology (Moura-Neto 2003, Souza et al. 2003, Pierri et al. 2016, Lima and Passos 2021). This species had already been recorded in the Alcatrazes archipelago on soft bottom (Plano de Manejo 2017). As for *I. bicolor*, it was introduced into Brazilian waters by either oil platforms brought from other localities or by ballast water (Lima and Passos 2021) and its presence may have a negative impact on native species (Queiroz et al. 2023). Although it is widely distributed along São Paulo's coast, including in insular areas and in association with macroalgae beds (Domaneschi and Martins 2002; Jacobucci et al. 2006; Longo et al. 2023), we did not record it in the macroalgae beds of Alcatrazes in this study. Overall, these findings suggest a low incidence of these invasive molluscan species in the area.

The ophiuroid composition in the Alcatrazes archipelago was consistent with previous studies, with the occurrence of characteristic species associated with algae, such as *Amphipholis squamata* (Delle Chiaje, 1828), *Ophiactis lymani* Ljungman, 1872, and *Ophiactis savignyi* (Müller & Troschel, 1842) (Chao and Tsai 1995, Boffi 1972, Borges 2006, Jacobucci et al. 2006) which are also common on sandy bottoms of adjacent coastal areas (Alitto et al. 2016). However, the most abundant species in our study was *Ophioplocus januarii*

(Lütken, 1856), a benthic species that inhabits regions from the intertidal zone to depths between 30 and 100 meters (Monteiro et al. 1992). *O. januarii* consists of relatively large adults, measuring between 14 and 18 mm, and is rarely found associated with algae (Monteiro et al. 1992). This leads to the hypothesis that the macroalgae may serve as a nursery for this species, a theory supported by the significant number of juvenile individuals with reduced disk size observed in Alcatrazes (V.S.V., personal observation).

It's also noteworthy that the invasive ophiuroid species *Ophiothela mirabilis* Verrill, 1867 was observed in this study. This species is native to the Pacific and was first recorded in Rio de Janeiro in 2002 (Hendler et al. 2012). It has a distinctive yellow-orange coloration, and its ability to reproduce asexually through fission facilitates its spread (Hendler et al. 2012). The low host specificity of *O. mirabilis* makes it a generalist and opportunistic species, with reports showing its presence on more than 29 different hosts, though it is rarely found on algae and is more commonly associated with gorgonians, corals, and sponges (Granja-Fernández et al. 2014; Mantelatto 2016). Currently, *O. mirabilis* is found along the entire Brazilian coast, suggesting its spread may be facilitated by ballast water from ships (Hendler et al. 2012; Mantelatto 2016). The species had already been recorded in Alcatrazes, mainly in association with gorgonians (Plano de Manejo 2017). In this study, we also report its presence in seaweed beds, although in very low abundance.

Therefore, in both sampling areas of this study, there were unique faunal characteristics compared to other localities along the Brazilian coast, including several new occurrences and one potential new species. Notably, the incidence of known exotic species was low, which is crucial as these species could pose a threat to native biodiversity if their numbers were to increase significantly (Pimentel et al. 2005; Mantelatto et al. 2016). This highlights the importance of continuous monitoring in the area to prevent the proliferation of exotic species, thereby helping to preserve local biodiversity.

Ultimately, considering the whole invertebrate assemblages studied herein, species diversity was the same in both Alcatrazes localities. This finding suggests that despite Baía do 17 having a longer protection period as a Marine Protected Area (MPA) since 1987, while Baba de Boi gained MPA status with the establishment of the Alcatrazes Wildlife Refuge in 2016, this shorter protection period in the latter was still effective in maintaining comparable high levels of seaweed-associated invertebrate biodiversity. Rolim et al. (2024) has reported a decrease in the beta diversity (i.e., a higher faunistic stability) of fish species between areas with an older and earlier status of no-take zone within Alcatrazes arquipelago (belonging to ESEC Tupinambás and Wildlife Refuge, respectively). A similar pattern emerged here for the seaweed-associated invertebrate assemblages during the same period in Alcatrazes. Thus, increasing networks of MAPs and protection status may have positive effects on the diversity stability, for both fish and invertebrates (Chen 2021; Pettersen et al. 2022).

Furthermore, we hypothesize that the high and spatially stable invertebrate diversity in Alcatrazes' seaweed beds may have contributed to the observed increase in Labridae/Scarinae fish species, which are primarily mobile invertebrate feeders, during the initial years after the no-take zone was expanded in Alcatrazes (Rolim et al. 2024). This would emphasize the ecological role of invertebrates as critical food sources for larger marine species in coastal areas (Tano et al. 2016; Donadi et al. 2017). Therefore, monitoring and documenting seaweed-associated invertebrates can be a valuable metric for MPAs to evaluate the potential ecosystem services derived from their conservation initiatives.

This is the first comprehensive inventory of seaweed-associated macroinvertebrates in the Alcatrazes archipelago. The illustrated survey not only provides valuable records for the region but also promotes ongoing monitoring efforts in the archipelago. The information gathered here serves as a reference for future studies, helping to track natural and human-induced disturbances, the emergence of invasive species, shifts in species composition, and changes in macroalgae habitats. Monitoring macroalgae beds is crucial, as they offer food and shelter for invertebrate fauna and larger, more mobile species like fish (Donadi et al. 2017). With the recent opening of the Refuge area to public visitors, this study plays a key role in documenting the area's biodiversity during this transitional period and underscores the need for sustained, long-term monitoring.

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Associate Editor

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Pedro Augusto dos Santos Longo: Writing – original draft; review & editing; Conceptualization; Methodology; Investigation; Formal analysis; Data curation; Funding acquisition.

Karine Ferreira Ribeiro Mansur: Writing – review & editing; Conceptualization; Methodology; Investigation; Formal analysis; Data curation; Funding acquisition.

Helena Serrano: Writing – review & editing; Investigation; Data curation.

Alan Rodrigo Batistão: Writing – review & editing; Investigation; Data curation.

Fosca Pedini Pereira Leite: Writing – review & editing; Conceptualization; Methodology; Investigation; Data curation; Funding acquisition; Supervision.

Conflicts of Interest

The author(s) declare(s) that they have no conflict of interest related to the publication of this manuscript.

Ethics

This study did not involve human subjects or clinical trials which require authorization by an Institutional Committee.

Data Availability

Raw data used in this study are available in <https://doi.org/10.48331/scielodata.RDM5L0>.

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