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A synopsis of Tunicata biodiversity in Brazil

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ABSTRACT. The Tunicata, despite the relatively low species diversity among the invertebrates, has always received attention not only due to their ecological importance, especially in fouling communities, but also for several species that are studied as models for genetics and the evolution of development, as well as being a prolific source of natural products. In Brazil, research during the last 60 years has considerably increased our knowledge of benthic and planktonic tunicates, resulting from the work of several research teams. In this review, we provide information on the biodiversity of coastal Brazil along with an analysis of geographic distribution, sampling effort, the locations and status of taxonomic collections, and research specialists working on this group.

KEY WORDS. Ascidians, larvaceans, southwestern Atlantic, thaliaceans, Urochordata.

INTRODUCTION

Tunicata is the sister group of the Vertebrata within the phylum Chordata as proposed by molecular data and studies of nervous system development (Manni et al. 2004, Delsuc et al. 2006). It currently comprises 3145 valid species (Shenkar et al. 2023) distributed in two classes, Ascidiacea with 3068 species, most of them with benthic adult forms, and Appendicularia with 77 holoplanktonic species. Although most textbooks and the World Register of Marine Species (https://www.marinespecies.org/) still have Thaliacea as a tunicate Class, more than 20 years of genetic studies and more recently phylogenomics show that the pelagic thaliaceans have evolved within the Ascidiacea (Swalla et al. 2000, Delsuc et al. 2018, Kocot et al. 2018). Tunicates are covered by the tunic, a complex tissue formed by cellulosic fibers and living cells (Kimura et al. 2001, Hirose 2009) that offers protection, attachment to the substrate, camouflage, and a sophisticated self-recognition system (Franchi and Ballarin 2017). They are filter feeders that rely on a mucous net produced by the endostyle inside the pharynx to capture or concentrate food (Henschke et al. 2016, Conley et al. 2018). Their circulatory system has a heart with two pacemakers that alternate control in a bidirectional blood flow (Cain et al. 2020). Most tunicates are hermaphrodites and while the larval phase is absent or very reduced in the planktonic forms, the benthic adult ascidians present a planktonic stage in which the larva resembles a tadpole, with an ovate trunk and an elongated tail, responsible for dispersal (Lambert C 2005).

Among Ascidiacea, all holoplanktonic forms are in the taxon Thaliacea (previously Class Thaliacea) and comprise three orders, Salpida, Doliolida, and Pyrosomatida (Esnal and Daponte 1999a, 1999b, 1999c). Thaliaceans are characterized by the presence of oral (inhalant siphon) and atrial (exhalant siphon) openings at opposite ends of the body (Esnal 1981). They are transparent organisms, approximately cylindrical in shape. The life cycle of most species presents an alternation of generations, with individuals performing either asexual or sexual reproduction. Currently, there are 83 valid species (Shenkar et al. 2023).

The benthic forms within Ascidiacea are known as ascidians or sea squirts. Ascidians are sessile marine animals that are most often found on hard substrates, from the intertidal region to great depths. Very few species tolerate water with low salinity, such as in estuaries, and thus there are no fresh-water or terrestrial species (Lambert G 2005). The greatest diversity of ascidians is found in the Indo-Pacific region, but species can be found at all latitudes from the tropics to polar regions (Shenkar and Swalla 2011). Adult ascidians may be solitary or colonial, distributed in three Orders: Aplousobranchia, Phlebobranchia, and Stolidobranchia (Shenkar and Swalla 2011). Aplousobranchia became colonial early in the evolution of the group. This Order evolved a variety of forms of budding that have resulted in great species diversification, which today includes around half of all known ascidian species. Most species in Phlebobranchia are solitary and have a relatively short life cycle, but colonial forms are also present. Families in this Order have a variety of body plans, which may indicate that the group is polyphyletic, and thus further study is required to resolve this possibility. Stolidobranchia has long-lived solitary species with resistant, and often leathery, tunics. But, within this group, coloniality arose more than once and gave rise to a variety of colonial genera in one of its families (Styelidae).

Ascidians are important for a variety of reasons. Ecologically, they are a major component of the sessile community and often dominate on artificial substrates (Bullard et al. 2004). In addition to competing with other sessile organisms for substrate space, they are often included in the diet of invertebrates and fish (Lambert G 2005). People also eat some species, such as *Pyura chilensis* Molina, 1782 in Chile, *Halocynthia roretzi* (Drasche, 1884) in Japan, and *Styela clava* Herdman, 1881 in South Korea (Lambert et al. 2016). Ascidians are often transported by boats, from recreational boats to cargo ships, and as a consequence, some species become important invasives and cause problems in shellfish farms (McKindsey et al. 2007). Also, ascidians may harbor interesting chemical compounds that are currently used in cancer treatment, while many other biological properties are also investigated for a large array of species (Jimenez et al. 2020, Wilke et al. 2021).

Appendicularians are exclusively marine organisms, whose name is due to the presence of a tail as a locomotory appendix. They are also known as Larvacea because of their resemblance to the larvae of ascidians (Gorsky and Palazzoli 1989). They occur in all oceans, both inshore and offshore (Esnal 1999, Carvalho and Bonecker 2016). The appendicularians have been classified as jellyfish (Chamisso 1821), mollusks (Mertens 1830), zoophytes (Quoy and Gaimard 1833), and ascidian larvae (Müller 1847). The group was only recognized as a Class within the Subphylum Tunicata by Lohmann (1933). Currently, about 82 species of Appendicularia are known belonging to the families Oikopleuridae, Fritillariidae, and Kowalevskiidae. Oikopleuridae has the largest number of genera and species described (Esnal and Castro 1977, Esnal 1999, Fenaux et al. 1998). The diagnostic characteristics at the species level are the shape of the trunk, endostyle, spiracles, wall of the stomach, gonad, tail, amphi- and subcordal cells.

Tunicate research in Brazil has been growing since the 1960s with a focus on the record and description of new species (Dias et al. 2013) which provided the basis for the development of more recent studies of phylogeny, biogeography, ecology, and developmental evolution. Here, we expand upon prior reviews (Rodrigues et al. 1999, Rocha et al. 2011) with a brief history of taxonomic research on the different tunicate taxons, and update our knowledge of biodiversity, geographical distributions, taxonomic collections, and specialists in Brazil.

MATERIAL AND METHODS

The historical review of Tunicate taxonomic research in Brazil was based on published literature, while the information on collections and research groups was obtained through interviews with collection curators and group leaders.

The Brazilian Fauna Taxonomic Catalogue (CTFB – http://fauna.jbrj.gov.br/) furnished the species list, while the classification at Orders and Family levels followed the World Register of Marine Species (Shenkar et al. 2023). We used QGIS software version 3.28.4 LTR to create the distribution maps of the Appendicularia and Ascidiacea, with data obtained through databases from the scientific collections: Federal University of Paraná (UFPR), Oceanographic

Institute of the University of São Paulo (IOUSP), and State University of Rio de Janeiro (UERJ), and literature review of the species listed in CTFB to find all reports and their geographical coordinates. In the case of planktonic samples, many coordinates refer to oceanographic samples and thus more than one species could be represented.

RESULTS

History of the study of the benthic Ascidiacea in Brazil

The earliest reports of ascidians along the Brazilian coast were a consequence of the global oceanographic expeditions at the end of the 19th century and the few species reported were contributions by a variety of authors, including Herdman (1880, 1886), Traustedt (1882, 1893a), Michaelsen (1907, 1923) and Hartmeyer (1912). In shallow coastal waters, the first reports were by Luederwaldt (1929) at São Sebastião Island (Ilha de São Sebastião), in the state of São Paulo, who sent the samples to the National Museum of the United States and the American Museum of Natural History. Subsequently, Van Name (1945) included this material in his monograph "The North and South American Ascidians", which is considered an obligatory reference for any systematic study of the Ascidiacea from this region.

The first account on tunicates published by Brazilian authors was made by Moure et al. (1954), recording species from Paranaguá Bay. Subsequently, the expeditions of the vessels Calypso, Atlantis II, and Chain in the 1960s added descriptions of additional species (Monniot C 1970, Monniot F 1971) along with several censuses carried out in the states of Rio de Janeiro and São Paulo from the 1950s to the 1980s by Brazilians and others (review in Rocha et al. 2011, Dias et al. 2013). By 1977, new species from coastal Brazil had been described by eight taxonomists, of whom only one was Brazilian: Dr. Sérgio de Almeida Rodrigues (Rodrigues 1962, 1966, 1977). At that time, the type locality of 30 species was in Brazil. In the following 50 years, another 27 species were described by 17 taxonomists, only two of which were not Brazilians (Fig. 1).

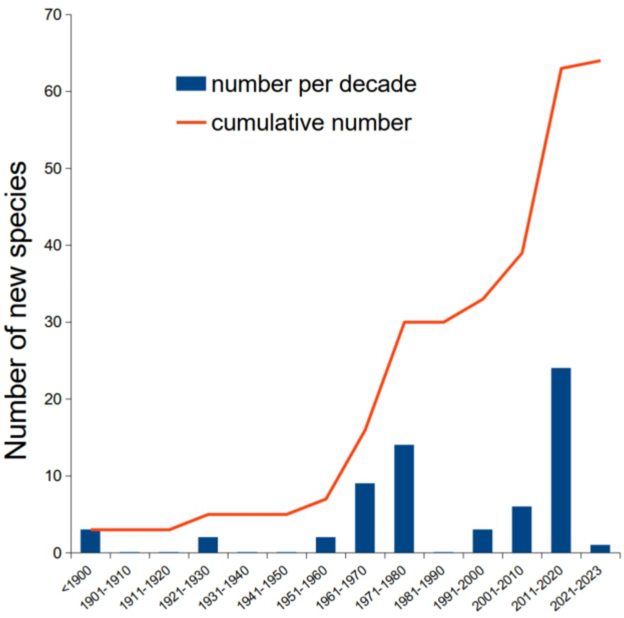

Figure 1. Cumulative number of new ascidian species descriptions from the coast of Brazil. Of the 136 species known from Brazil, almost half have the type locality in the country, and half of those have been described in the last 40 years.

Professor Sérgio de Almeida Rodrigues was responsible for training the first ascidiologists in the country, where he and his students focused on the region around the São Sebastião Canal, and the Marine Biology Center (Centro de Biologia Marinha, CEBIMar) of the University of São Paulo (USP) for logistical support. Under Professor de Almeida's guidance, students carried out both systematic and ecological studies of Brazilian ascidians, and consequently, a reasonable number of studies were published in the 1990s (Rodrigues and Rocha 1993, Rocha and Monniot 1995, Rodrigues et al. 1998). At Rio de Janeiro, Professor Sérgio Henrique Gonçalves da Silva, from the Marine Biology Department at the Federal University of Rio de Janeiro investigated marine fouling communities and performed some ecological studies (Silva et al. 1989), including the role of predators on one invasive species, *Ciona robusta* Hoshino & Tokioka, 1967 (reported as *C. intestinalis*). However, only much later research from that lab focused on ascidian biodiversity (Marins et al. 2009, Marins et al. 2010).

Beginning in the 1980s, the dissemination of SCUBA diving granted access to research in deeper waters (up to 30 m deep) and *in situ* photography and preservation of samples in much better condition, as well as a better understanding of their autecology. At that time, biodiversity studies also began in the southern states of Paraná and Santa Catarina (e.g., Rocha and Nasser 1998, Rocha et al. 2005). Just before the turn of the century, the richness of 70 species previously estimated for the state of São Paulo (Rodrigues et al. 1999) was confirmed. Subsequently (2000–2010), biodiversity studies began in the northeast, focused on the states of Ceará (e.g., Cascon and Lotufo 2006) and Bahia (e.g., Rocha et al. 2012a), which found a wide diversity of species, including some new species. More recently, the states of Espírito Santo, Paraíba and Pernambuco, and oceanic islands such as Rocas Atoll, have been added to the list (e.g., Rocha et al. 2012b, Oliveira et al. 2014, Paiva et al. 2015). The paucity of information on ascidian diversity, including the still better-known south and southeast, justifies increased efforts in discovering and understanding the ascidian fauna of Brazil, faced with an increasing rate of degradation of coastal habitats.

History of the study of planktonic Ascidiacea in Brazil

The first records of the Thaliacea from the Brazilian coast were made by the great oceanographic expeditions in the South Atlantic (Traustedt 1893b, Apstein 1894, Borgert 1894, Garstang 1933, Krüger 1939). The first study by a Brazilian scientist was carried out by Tavares (1967) who recorded three species off Santos and Cananéia, in the State of São Paulo. Almost two decades later, Bonecker started his studies at Rio de Janeiro, about the distribution of six species (Bonecker 1983) and the ecology of large aggregations of the salpa *Thalia democratica* (Forskål, 1775) (Bonecker et al. 1995). At the end of the 1990s, research expanded towards the coastal and oceanic regions of Ceará and Pernambuco, in northeastern Brazil (Neumann-Leitão et al. 1998) while Amaral et al. (1997) and Esnal and Daponte (1999a, 1999b, 1999c) recorded 35 species of thaliaceans in Brazilian south waters. In the 2000s, the REVIZEE project (Evaluation of the potential sustainability of living resources within the exclusive economic zone, in Portuguese) increased sampling efforts of pelagic organisms along the Brazilian continental shelf expanding the knowledge about the distribution of many species (Bonecker and Quintas 2006a, 2006b). Carvalho and Bonecker (2008) recorded the first occurrence of the pyrosome *Pyrosomella verticillata* (Neumann, 1909) in the western South Atlantic region. In the same year, Diaz et al. (2008) recorded for the first time the salpa *Thalia cicar* van Soest, 1973 in the Equatorial Atlantic region. The oil exploration in the deep sea by the Brazilian company Petrobras opened the first opportunity to study deeper water organisms including 10 species of Thaliacea found at different depths of the Campos Basin (Bonecker et al. 2014). A few time-series studies were also performed revealing temporal and spatial patterns of species distribution (Nogueira-Jr 2012, Dias et al. 2018). All this research expanded the knowledge of species distribution in Brazil, but no new species were found and described.

History of the study of Appendicularia in Brazil

One of the first surveys of Appendicularia in Brazil was carried out by Björnberg and Forneris (1956) who recorded nine species in Fernando de Noronha archipelago, in northeastern Brazil. Another pioneering study showed the influence of seasonality and water masses' environmental characteristics on the distribution of these organisms, from Rio de Janeiro to Rio Grande do Sul (Forneris 1965, Tundisi 1970, Esnal and Castro 1977). The report of Appendicularia continued to be very scarce and focused on the southeast and southern regions (Valentin 1984, Dadon and Esnal 1995, Eskinazi-Sant'Anna and Björnberg 2006, Vega-Pérez et al. 2011) with the exception of a few studies in the northeastern region (Neumann-Leitão et al. 1998, Ramos 2007, Larrazábal et al. 2009, Bonecker and Carvalho 2006, Dias et al. 2020) and ocean islands (Díaz 2007). Given that new species were never found in Brazil, the review by Esnal (1999) listing 35 species in Brazil is still one of the most important for the group in the region.

Long-time series studies have been important to reveal temporal patterns of species distribution (Dias and Bonecker 2008, Carvalho and Bonecker 2010, Dias et al. 2018). Other ecologically-oriented research tried to determine species that are indicators of water quality in estuarine environments (Carvalho et al. 2016) or open water (Carvalho and Bonecker 2016, Tosetto et al. 2022) and which species are associated with deep water masses (Bonecker et al. 2014). The importance of Appendicularia species in the carbon flux and ocean productivity has also been the object of research (Miyashita 2010, Miyashita and Lopes 2011).

Biodiversity of Tunicata in Brazil

To date, records of Ascidiacea in Brazil comprise 136 species in 15 families (Appendix 1). The most speciose families are Didemnidae (38 spp.), Styelidae (28 spp.), and Polyclinidae (12 spp.) and these families are also among the most speciose worldwide (Shenkar and Swalla 2011). The most species-rich genera are *Didemnum* (19 species, Didemnidae), *Ascidia* (10, Ascidiidae), *Aplidium* (8, Polyclinidae), and Eudistoma (8, Polycitoridae). Most records are in the order Aplousobranchia (945; 516 in N + NE, 419 in SE + S), followed by Stolidobranchia (510; 178 in N + NE, 328 in SE + S) and Phlebobranchia (188; 70 in the N + NE, 118 in SE + S) (Fig. 2).

Thaliaceans found on the Brazilian coast comprise 36 species, seven of which belong to the order Doliolida, two Pyrosomatida and 27 Salpida (Appendix 1). The vast majority of species were recorded in the study by Esnal and Daponte (1999a, 1999b, 1999c). The only new record for the class on the Brazilian coast was for the pyrosome *P. verticillata* in the Campos Basin region, a study that expanded the distribution

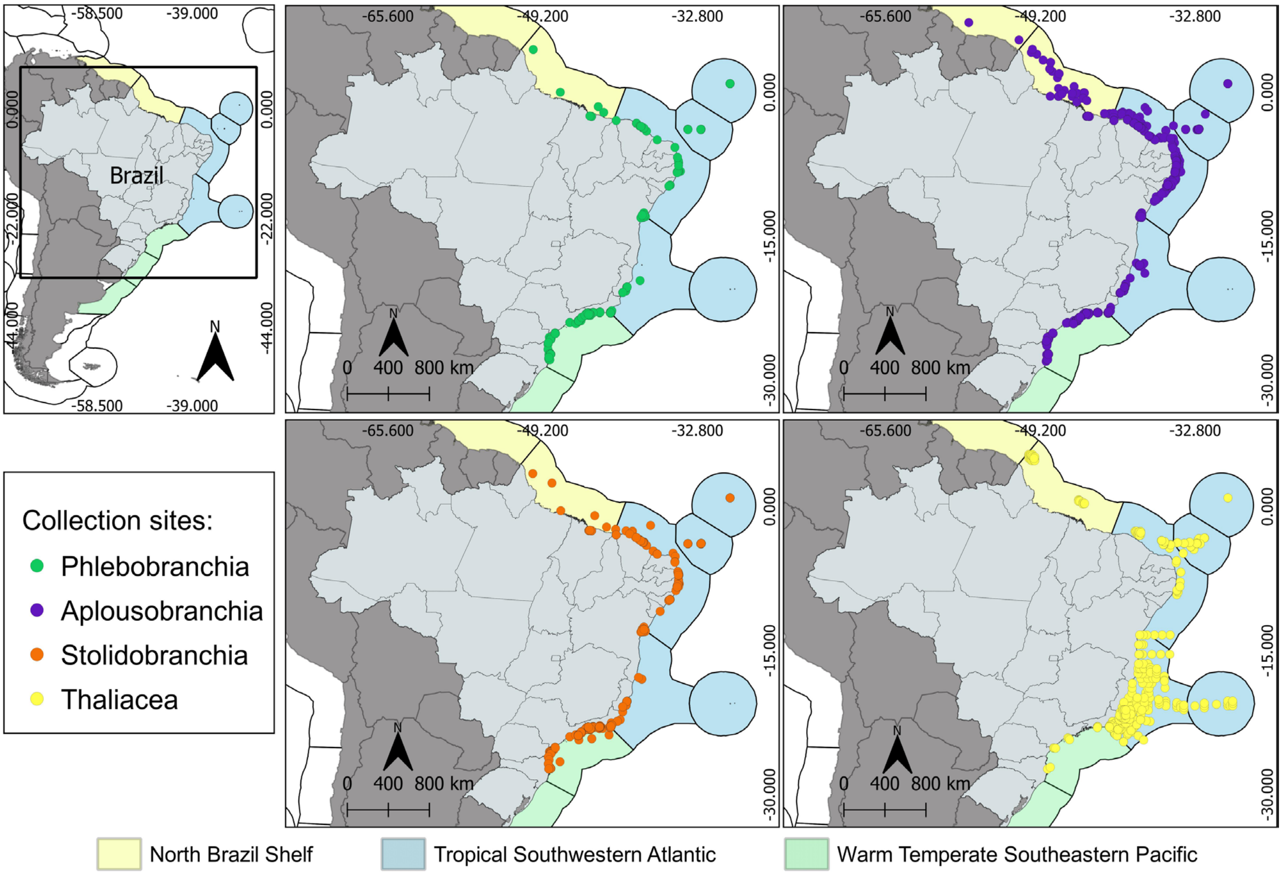

Figure 2. Distribution of Ascidiacea records along the coast of Brazil by Order, based on published research and vouchers in the scientific collections of the Federal University of Paraná (UFPR), Oceanographic Institute of the University of São Paulo (IOUSP), and State University of Rio de Janeiro (UERJ). The subdivisions in the map refer to the ecoregions proposed by Spalding et al. (2007), grouped in provinces by color. Each dot represents at least one record, and the abundance of records in the same site is not represented.

of this species not only to Brazil but also to the Western South Atlantic (Carvalho and Bonecker 2008).

The Appendicularia in Brazil comprise the 35 species recorded by Esnal (1999) and belong to the order Copelata. Among these species, 18 belong to the Fritillaridae, 16 to the Oikopleuridae, and one to the Kowalevskiidae (Fig. 3, Appendix 1). Despite being more than 20 years old, the study by Esnal (1999) fully records the number of species found on the Brazilian coast, and no new occurrence has been observed for the region since then.

Research groups

Currently, all the research groups that are active in studying ascidian biodiversity in Brazil are concentrated in three states in the southeastern and southern regions, which suggests that more attention should be directed to the northeastern and northern regions. The oldest is led by Rosana M. Rocha, of the Federal University of Paraná, where systematic and ecological studies are underway. Another group leader is Tito M.C. Lotufo, who worked for 12 years at the Federal University of Ceará and has more recently (2014) moved to the Oceanographic Institute of the University of São Paulo (IO-USP), where systematics and ecology are also the main fields of research. Also in São Paulo, Gustavo Muniz Dias, of the Federal University of ABC (UFABC), leads a group that is focusing on the ecology of ascidians, and Federico D. Brown A., of the University of São Paulo (IB-USP), leads a group working on genetics of development and the evolution of asexual reproductive system in ascidians. Luis Felipe Skinner, of the State University of Rio de Janeiro (UERJ), works on

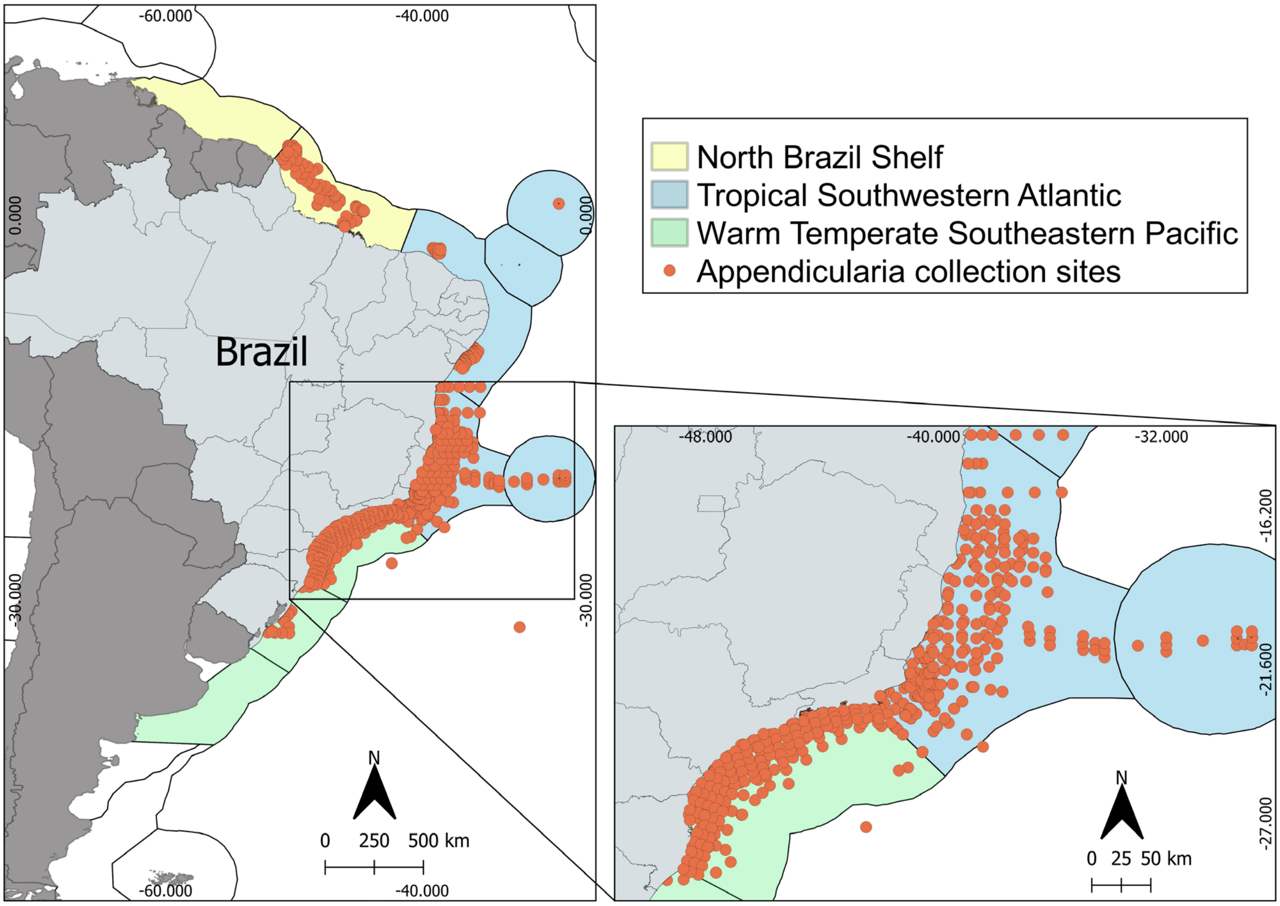

Figure 3. Distribution of Appendicularia records along the coast of Brazil, based on published research. The subdivisions in the map refer to ecoregions proposed by Spalding et al. (2007), grouped in provinces by color. Each dot represents at least one record, and the abundance of records in the same site is not represented.

ascidian ecology, including exotic species, and more recently is surveying the species found along the coast of the state of Rio de Janeiro. The most recently established research group is led by M. Tarciana Vieira Fortaleza, of the Federal Institute of Education, Science and Technology of Pará (IFPA), working on ascidian systematics.

In addition to these groups, other researchers include ascidians as biological models in their research. Andrea Junqueira (Federal University of Rio de Janeiro – UFRJ) works on the ecology of rocky shores and invasive species. Roberto G.S. Berlinck (University of São Paulo - São Carlos), Letícia V. Costa-Lotufo (University of São Paulo - São Paulo), Paula Christine Jimenez (Federal University of São Paulo – UNIFESP, Santos) and Diego Veras Wilke (Federal University of Ceará - UFC) focus on the biochemistry of natural products and their pharmaceutical effects. Mauro Sérgio Gonçalves Pavão (Federal University of Rio de Janeiro – UFRJ) works on the biochemistry of *Styela plicata* (Lesueur, 1823) with a focus on medical applications. Also in Rio de Janeiro, Cintia Monteiro de Barros, of the Federal University of Rio de Janeiro (NUPEM – UFRJ), focuses on neuroscience, immunology, and biochemistry of macromolecules of ascidians.

Research groups working on Appendicularia and Thaliacea in Brazil, are even fewer and most of them carry out studies on the ecology of zooplankton, contributing to the record of species and the knowledge of their spatial and temporal patterns. Among the oldest are the ones led by Luz Amelia Vegas-Pérez, of the Oceanographic Institute of the University of São Paulo (IO-USP), and by Sergio Luiz Costa Bonecker and Pedro Freitas de Carvalho at the Federal University of Rio de Janeiro (UFRJ). It is worth highlighting two very active research groups in northeastern Brazil, one led by Sigrid Neumann Leitão from the Oceanographic Department of the Federal University of Pernambuco (UFPE) and a more recent one, led by Miodeli Nogueira Júnior, from the Federal University of Paraíba (UFPB), who works with the taxonomy of gelatinous organisms.

Scientific Collections

Five principal collections of ascidians exist in Brazil. The collection in the Zoology Department of the Federal University of Paraná has the largest number of samples (~ 4000 identified as species and ~2000 samples identified as genus or family). Most samples are from Brazil, but many other countries are also represented. Information on the Brazilian data of this collection is available on the Portal of Brazilian Biodiversity (https://sibbr.gov.br/). The next largest collection is the Prof. Edmundo Nonato Biological Collection (ColBIO) associated with the laboratory of the previously mentioned Professor Tito Lotufo of the Oceanographic Institute of USP. This collection has over 4,000 samples, 70% of which are identified at least to genus. Most samples are from northeastern Brazil and Brazilian oceanic islands, and a vast number of specimens from Antarctica and some from the Gulf of Mexico, French Polynesia, New Caledonia, and Oman. The third collection is held in the Zoology Museum of the USP in which most material was collected by Prof. Sergio Rodrigues in the coastal region of the state of São Paulo and contains most of the type material of species described from Brazil. Two more recent collections are in the Department of Sciences at the State University of Rio de Janeiro (UERJ/FFP) and in the Department of Zoology at the Federal University of Paraiba (UFPB- LIPY). The first one has approximately 2,000 properly identified specimens and a significant number of samples identified at genus or family from Rio de Janeiro and the second has ~600 vouchers from Bahia to Maranhão, and around half of the samples are identified at species level. Due to the fact that until the 1960s most studies were carried out by foreign researchers, collections of Brazilian ascidians can be found in a variety of international institutions, including The National Museum of Natural History (Smithsonian) in Washington, D.C., the American Museum of Natural History in New York, The Natural History Museum in London, and the Muséum Nationale d'Histoire Naturelle in Paris.

Plankton collections including Appendicularia and Thaliacea are more scarce. The Zooplankton and Ichthyoplankton Laboratory Collection, at the Department of Zoology of the Federal University of Rio de Janeiro (LIZI – UFRJ), has a total of 9,076 vouchers of Appendicularia, and 4,484 of Thaliacea. ColBIO in the University of São Paulo also holds a large number of zooplankton samples with pelagic tunicates, mainly collected off the southeastern and southern Brazilian coast.

DISCUSSION

What we do know and do not about Brazilian tunicate diversity

Most marine taxa in the Western Atlantic have their diversity concentrated in the tropical region, with maximum numbers at intermediary latitudes, between southern Bahia and northern Espirito Santo. For ascidians, the state of Bahia also has the largest number, with 70 species recorded, followed by São Paulo (68 spp.), Espírito Santo (50 spp.), Ceará (49 spp.) and Rio de Janeiro (47 spp.). These numbers, however, certainly are biased towards regions with more specialists and more collection efforts. Tropical States having fewer studies include Amapá, Pará, Maranhão, Piauí, and Sergipe, with three to 12 species reported. The central coastal region, including Espírito Santo and Bahia, seems to be quite diverse, given that current results come from studies that have been concentrated in a few places (Salvador in Bahia and Guarapari in Espírito Santo) with a large gap in records between these two sites, with exception of a few records in the south of Bahia (Fig. 2). Another important gap occurs along the coast of Rio Grande do Sul, formed mainly by sandy beaches. However, the inner shelf also contains reefs formed by paleo beach rocks with a diverse fauna of Bryozoa (Ramalho and Calliari 2015), which suggests that ascidians could also be present. Even in São Paulo, with a large number of records, the majority of the samples were gathered in the region of the São Sebastião Canal, while both the northern and southern coasts have rarely been studied (Dias et al. 2013). Islands in Brazil have also received little attention, including both near shores as well as oceanic islands, where we can expect to find more species. For example, recent studies of the islands near Ilha Grande Bay and Cabo Frio in Rio de Janeiro have found new species (Oliveira et al. 2019a, 2019b), and a few others are in the process of being described. Another recent study at the Rocas Atoll found 12 species, five of which are new which indicates a surprising level of endemism (Paiva et al. 2015).

Few species of ascidians have been found in deeper waters of the continental shelf and slope despite increased efforts in the last 50 years (Monniot F 1971, Rocha 2004, Tâmega et al. 2013). This may reflect true low biodiversity in these deeper waters, or difficulty in recognizing animals while analyzing samples considering the tendency of deep water species to be very small (less than 5 mm), often with incrustations from sediments on the tunic.

Brazilian knowledge about thaliaceans and appendicularians is still very limited compared to benthic metazoans, or even other zooplanktonic groups. Records of Thaliacea are concentrated between São Paulo and Bahia, while records of Appendicularia extend a little further south until Rio Grande do Sul, and also in the mouth of the Amazon River, between Pará and Amapá (Figs 2, 3) in the north region. The sampling of plankton depends on oceanographic cruises and this could explain important advances in planktonic tunicates reports after the enforcement of the REVIZEE program in 1994–1998, that combined efforts of scientists and the Brazilian Navy. Also, regions with regular traffic of Navy ships, such as the Trindade - Martim Vaz archipelago on the coast of Espírito Santo, have been well surveyed, but there are still large areas of the Brazilian coast that have not been sampled. Further, the mesh size of plankton nets and the timing of sampling (diurnal or nocturnal) have a great influence on the richness and abundance of tunicates collected (Tosetto et al. 2022). It is interesting to note that there is a good cover of Appendicularia records in the mouth of the Amazon River system, but we cannot say the same about Thaliacea records, suggesting an ecological difference between those two groups.

Exotic species

More recently, the recognition that several species can be, and have been, transported by ocean-going vessels and smaller boats of coastal waters, or even rafting on flotsam, has generated interest in studies of the detection of exotic and potentially invasive species (Rocha and Kremer 2005, Skinner et al. 2016), environmental conditions that may facilitate that process (Marins et al. 2010) and their impact on fouling communities (Rocha et al. 2009, Kremer et al. 2009, Oricchio et al. 2019). About 25% of the species reported in Brazil are considered introduced in one or more regions of the country. Species introductions have become more frequent in the past decades, including even previously undescribed species (Kremer et al. 2011). As most inventories were conducted in the last 50 years, it is difficult to determine if disjunct distributions result from recent introduction events. For instance, the Brazilian subtropical coast shares many species with the Caribbean region that are absent in the tropical region (Dias et al. 2013). In the past decades of continuous assessments especially in the southeastern and southern coasts of Brazil, some invasions could be detected, including from *Ciona robusta*, *Clavelina oblonga* Herdman, 1880, *Cnemidocarpa irene* (Hartmeyer, 1906), *Didemnum perlucidum* Monniot, 1983, *Eusynstyela* sp., *Polycarpa tumida* Heller, 1878, *Pyura beta* Skinner, Rocha & Counts, 2019, *Pyura gangelion* (Savigny, 1816), *Rhodosoma turcicum* (Savigny, 1816), and *Styela plicata* just to mention the more relevant (Rocha et al. 2012c, Skinner et al. 2019, Barboza and Skinner 2021). However, the number of cryptogenic species is quite large and suggests that as these species receive more study and their origins are determined, the number of introduced species will possibly rise.

Gene sequencing and population genetic studies have been central to the definition of the connectivity between Brazilian and other populations, and to reveal possible routes of species spread (Barros et al. 2009, Rocha et al. 2012c, 2019, 2021, Barros and Rocha 2021). Few introduced species are originally from Europe or the Pacific Ocean, while many introductions are Caribbean species that were found in the southeast or south, without records for the intervening northeast coast. Niche modeling is another tool that has been useful to access regions with adequate environments to receive exotic species, and to predict species invasion given the presence of transport vectors (Lins et al. 2018, Lins and Rocha 2023).

The definition of exotic species for planktonic animals is still more challenging, given the lack of physical barriers to the dispersal of species and the wide geographical distribution of many species. We are not aware of any exotic planktonic ascidian or Appendicularia in Brazil.

New avenues for tunicate research in Brazil

The increase in taxonomic surveys in the last fifty years opened the opportunity for research in a variety of other topics having ascidians as models, including ecology (Rocha 1991, Dias and Delboni 2008, Dias et al. 2008, Hiebert et al. 2019), biogeography (Moreno et al. 2014), life cycles (Rocha et al. 1999), natural products (Berlinck et al. 2004, Jimenez et al. 2020), genetics and phylogeny (Dias et al. 2006, Moreno and Rocha 2008, Oliveira et al. 2017), developmental biology (Jiménez-Merino et al. 2019, Alié et al. 2021, Hiebert et al. 2021), neuro-immunological regulation and regeneration (Souza et al. 2020, Correa et al. 2023).

For the planktonic tunicates, biodiversity knowledge opened the opportunity toward ecological-oriented research, for example, trying to determine species that are indicators of water quality in estuarine environments (Carvalho et al. 2016) or open water (Carvalho and Bonecker 2016) and which species are associated with deep water masses (Bonecker et al. 2014). The importance of Appendicularia species in the carbon flux and ocean productivity has also been the object of research (Miyashita 2010, Miyashita and Lopes 2011). The vertical migration of the population of *T. democratica* was another topic of research (Resgalla-Jr et al. 2004).

A good knowledge of the specific composition of the Brazilian tunicate fauna is essential for the further development of many relevant initiatives. From nature conservancy to drug discovery, this information is the basis for scientific advancement on many fronts. Continuous support for training new generations of taxonomists and also for the creation and maintenance of good biological collections including curated barcodes for all samples must be kept in the scientific funding agendas.

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Appendix 1. List of species in the subphylum Tunicata registered in the Taxonomic Catalog of the Brazilian Fauna (TCBF). The number in parentheses indicates the number of species in the family.

Class Ascidiacea
- Cionidae (1)
  - *Ciona robusta* Hoshino & Tokioka, 1967
- Ascidiidae (12)
  - *Ascidia curvata* (Traustedt, 1882)
  - *Ascidia interrupta* Heller, 1878
  - *Ascidia cf multitentaculata* (Hartmeyer, 1912)
  - *Ascidia nordestina* Bonnet & Rocha, 2011
  - *Ascidia papillata* Bonnet & Rocha, 2011
  - *Ascidia santosi* Millar, 1958
  - *Ascidia scalariforme* Bonnet & Rocha, 2011
  - *Ascidia sydneiensis* Stimpson, 1855
  - *Ascidia tenue* Monniot, 1983
  - *Ascidia viridina* Paiva, Oliveira-Filho & Lotufo, 2015
  - *Phallusia nigra* Savigny, 1816
  - *Phallusia recifensis* (Millar, 1977)
- Agnezidae (1)
  - *Agnezia celtica* Moniot & Monniot, 1974
- Corellidae (1)
  - *Rhodosoma turcicum* (Savigny, 1816)
- Perophoridae (8)
  - *Ecteinascidia conklini* Berrill, 1932
  - *Ecteinascidia minuta* Berrill, 1932
  - *Ecteinascidia styeloides* (Traustedt, 1882)
  - *Ecteinascidia turbinata* Herdman, 1880
  - *Perophora bermudensis* Berrill, 1932
  - *Perophora multiclathrata* (Sluiter, 1904)

Perophora regina Goodbody & Cole, 1987
Perophora viridis Verrill, 1871
Diazonidae (1)
Rhopalaea sp. (Sluiter, 1898)
Clavelinidae (2)
Clavelina oblonga Herdman, 1880
Clavelina brasiliensis (Millar, 1977)
Stomozoidae (1)
Stomozoa gigantea (Van Name, 1921)
Polycitoridae (9)
Cystodytes dellechiajei (Della Valle, 1877)
Eudistoma alvearium Rocha & Oliveira, 2014
Eudistoma carolinense Van Name, 1945
Eudistoma clavatum Rocha & Bonnet, 2009
Eudistoma recifense Millar, 1977
Eudistoma saldanhai Millar, 1977
Eudistoma spiculiferum, Millar, 1977
Eudistoma vannamei Millar, 1977
Eudistoma versicolor Rocha & Oliveira, 2014
Euherdmanidae (2)
Euherdmania fasciculata F. Monniot, 1983
Euherdmania vitrea Millar, 1961
Polyclinidae (12)
Aplidium accarense (Millar, 1953)
Aplidium antillense (Gravier, 1955)
Aplidium elongatum Rocha, Gamba & Zanata, 2012
Aplidium lobatum Savigny, 1816
Aplidium multisulcatum Millar, 1977
Aplidium pentatrema (Monniot, 1972)
Aplidium selenium Rocha, Gamba & Zanata, 2012
Aplidium traustedti Millar, 1977
Polyclinum aurantium Milne-Edwards, 1841
Polyclinum constellatum Savigny, 1816
Polyclinum molle Rocha & Costa, 2005
Sidneioides peregrinus Kremer, Metri & Rocha, 2011
Holozoidae (2)
Distaplia bermudensis Van Name, 1902
Distaplia stylifera (Kowalewsky, 1874)
Didemnidae (38)
Didemnum ahu Monniot & Monniot, 1987
Didemnum apersum Tokioka, 1953
Didemnum aurantium Rocha & Neves, 2015
Didemnum cineraceum Sluiter, 1898
Didemnum digestum Sluiter, 1909
Didemnum flammacolor Rocha & Neves, 2015
Didemnum galacteum Lotufo & Dias, 2007
Didemnum granulatum Tokioka, 1954
Didemnum halimedae Monniot, 1983
Didemnum lambertae Rocha & Neves, 2015
Didemnum ligulum Monniot, 1983
Didemnum longigaster Rocha & Neves, 2015
Didemnum perlucidum Monniot, 1983
Didemnum psammatodes (Sluiter, 1895)
Didemnum rochai Paiva, Oliveira-Filho & Lotufo, 2015
Didemnum rodriguesi Rocha & Monniot, 1993
Didemnum speciosum (Herdman, 1886)
Didemnum tetrahedrum Dias & Rodrigues, 2004
Didemnum vanderhorsti Van Name, 1924
Diplosoma citrinum Rocha & Gamba, 2015
Diplosoma listerianum (Milne-Edwards, 1841)
Diplosoma cf. spongiforme (Giard, 1872)
Leptoclinides brasiliensis Michaelsen, 1923
Leptoclinides coronatus Oliveira, Carvalho & Rocha, 2019
Leptoclinides crocotulus Paiva, Oliveira-Filho & Lotufo, 2015
Leptoclinides latus Monniot, 1983
Leptoclinides lotufoi Oliveira, Carvalho & Rocha, 2019
Leptoclinides torosus Monniot F., 1983
Lissoclinum fragile (Van Name, 1902)
Lissoclinum perforatum (Giard, 1872)
Lissoclinum verrilli (Van Name, 1902)
Polysyncraton amethysteum (Van Name, 1902)
Polysyncraton cabofriense Oliveira & Rocha, 2019
Polysyncraton maurizeliae Paiva, Oliveira-Filho & Lotufo, 2015
Trididemnum maragogi Rocha, 2002
Trididemnum orbiculatum (Van Name, 1902)
Trididemnum rocasensis Paiva, Oliveira-Filho & Lotufo, 2015
Trididemnum solidum (Van Name, 1902)
Styelidae (28)
Botrylloides giganteus (Pérès, 1949)
Botrylloides niger Herdman, 1886
Botryllus humilis (Herdman, 1891)
Botryllus planus (Van Name, 1902)
Botryllus schlosseri (Pallas, 1766)
Botryllus tabori Rodrigues, 1962
Botryllus tuberatus Ritter & Forsyth, 1917
Cnemidocarpa irene (Hartmeyer, 1906)
Eusynstyela tincta (Van Name, 1902)
Monandrocarpa stolonifera Monniot, 1970
Polyandrocarpa anguinea (Sluiter, 1898)
Polyandrocarpa pilella (Herdman, 1881)
Polyandrocarpa zorritensis (Van Name, 1931)
Polycarpa arnoldi (Michaelsen, 1914)
Polycarpa foresti Monniot, 1970
Polycarpa itapoa Rocha & Moreno, 2000
Polycarpa nivosa (Sluiter, 1998)
Polycarpa cf. reviviscens Monniot & Monniot, 2001

Polycarpa spongiabilis Traustedt, 1883
Polycarpa tumida Heller, 1878
Styela canopus (Savigny, 1816)
Styela cearense Oliveira-Filho & Lotufo, 2015
Styela eurygaster Millar, 1977
Styela glans Herdman, 1881
Styela multicarpa Barros & Rocha, 2021
Styela plicata (Lesueur, 1823)
Symplegma brakenhielmi (Michaelsen, 1904)
Symplegma rubra Monniot, 1972
Pyuridae (9)
Herdmania pallida (Heller, 1878)
Microcosmus anchylodeirus Traustedt, 1883
Microcosmus exasperatus Heller, 1878
Microcosmus helleri Herdman, 1881
Pyura beta Skinner, Rocha & Counts, 2019
Pyura gangelion (Savigny, 1816)
Pyura mariscata Rodrigues, 1966
Pyura millari Rodrigues, 1966
Pyura vittata (Stimpson, 1852)
Molgulidae (9)
Bostrichobranchus digonas Abbott, 1951
Gamaster guillei Monniot C., 1994
Molgula braziliensis Millar, 1958
Molgula eugyroides Traustedt, 1883
Molgula fortuita Monniot & Monniot, 1984
Molgula phytophila Monniot, 1970
Molgula pyriformis Herdman, 1881
Molgula salvadori Monniot, 1970
Paraeugyrioides vanname (Monniot, 1970)
Class Thaliacea
Doliolidae (7)
Dolioletta gegenbauri (Uljanin, 1884)
Doliolina (Doliolinetta) intermedia (Neumann, 1906)
Doliolina (Doliolina) muelleri (Krohn, 1852)
Dolioloides rarum (Grobben, 1882)
Doliolum denticulatum Quoy & Gaimard, 1834
Doliolum nationalis Borgert, 1893
Doliopsoides meteori Krüger, 1939
Pyrosomatidae (2)
Pyrosoma atlanticum Péron, 1804
Pyrosomella verticillata (Neumann, 1909)
Salpidae (27)
Cyclosalpa polae Sigl, 1912
Brooksia rostrata (Traustedt, 1893)
Cyclosalpa affinis (Chamisso, 1819)
Cyclosalpa bakeri Ritter, 1905
Cyclosalpa danae van Soest, 1975
Cyclosalpa floridana (Apstein, 1894)
Cyclosalpa pinnata (Forskål, 1775)
Helicosalpa virgula (Vogt, 1854)
Soestia zonaria (Pallas, 1774)
Ihlea magalhanica (Apstein, 1894)
Ihlea punctata (Forskål, 1775)
Pegea bicaudata (Quoy & Gaimard, 1826)
Pegea confoederata (Forskål, 1775)
Ritteriella amboinensis (Apstein, 1904)
Ritteriella retracta (Ritter, 1906)
Salpa aspera Chamisso, 1819
Salpa fusiformis Cuvier, 1804
Salpa maxima Forskål, 1775
Salpa thompsoni Foxton, 1961
Salpa younti van Soest, 1973
Thalia cicar van Soest, 1973
Thalia democratica (Forskål, 1775)
Thalia longicauda (Quoy & Gaimard, 1824)
Thalia orientalis Tokioka, 1937
Thetys vagina Tilesius, 1803
Traustedtia multitentaculata (Quoy & Gaimard, 1834)
Iasis cylindrica (Cuvier, 1804)
Class Appendicularia
Fritillaridae (18)
Appendicularia sicula Fol, 1874
Fritillaria aberrans Lohmann, 1896
Fritillaria aequatorialis Lohmann, 1896
Fritillaria antarctica Lohmann, 1905
Fritillaria borealis Lohmann, 1896
Fritillaria drygalskii Lohmann & Bückmann, 1923
Fritillaria formica Fol, 1872
Fritillaria fraudax Lohmann, 1896
Fritillaria gracilis Lohmann, 1896
Fritillaria haplostoma Fol, 1872
Fritillaria helenae Buckmann, 1923
Fritillaria megachile Fol, 1872
Fritillaria messanensis Lohmann & Bückmann, 1924
Fritillaria pellucida (Busch, 1851)
Fritillaria borealis sargassi Lohmann, 1896
Fritillaria tenella Lohmann, 1896
Fritillaria venusta Lohmann, 1896
Tectillaria fertilis (Lohmann, 1896)
Kowalevskiidae (1)
Kowalevskia tenuis Fol, 1872
Oikopleuridae (16)
Althoffia tumida Lohmann, 1892
Folia gracilis Lohmann, 1892
Folia mediterranea (Lohmann, 1899)

Megalocercus abyssorum Chun, 1887
Oikopleura (Vexillaria) albicans (Leuckart, 1853)
Oikopleura (Vexillaria) cophocerca (Gegenbaur, 1855)
*Oikopleura (Coecaria) fusiformis cornutogastr*a Aida, 1907
Oikopleura (Vexillaria) dioica Fol, 1872
Oikopleura (Coecaria) fusiformis Fol, 1872
Oikopleura (Coecaria) gracilis Lohmann, 1896
Oikopleura (Coecaria) intermedia Lohmann, 1896
Oikopleura (Coecaria) longicauda (Vogt, 1854)
Oikopleura (Vexillaria) parva Lohmann, 1896
Oikopleura (Vexillaria) rufescens Fol, 1872
Pelagopleura oppressa (Lohmann, 1914)
Stegosoma magnum (Langerhans, 1880)