

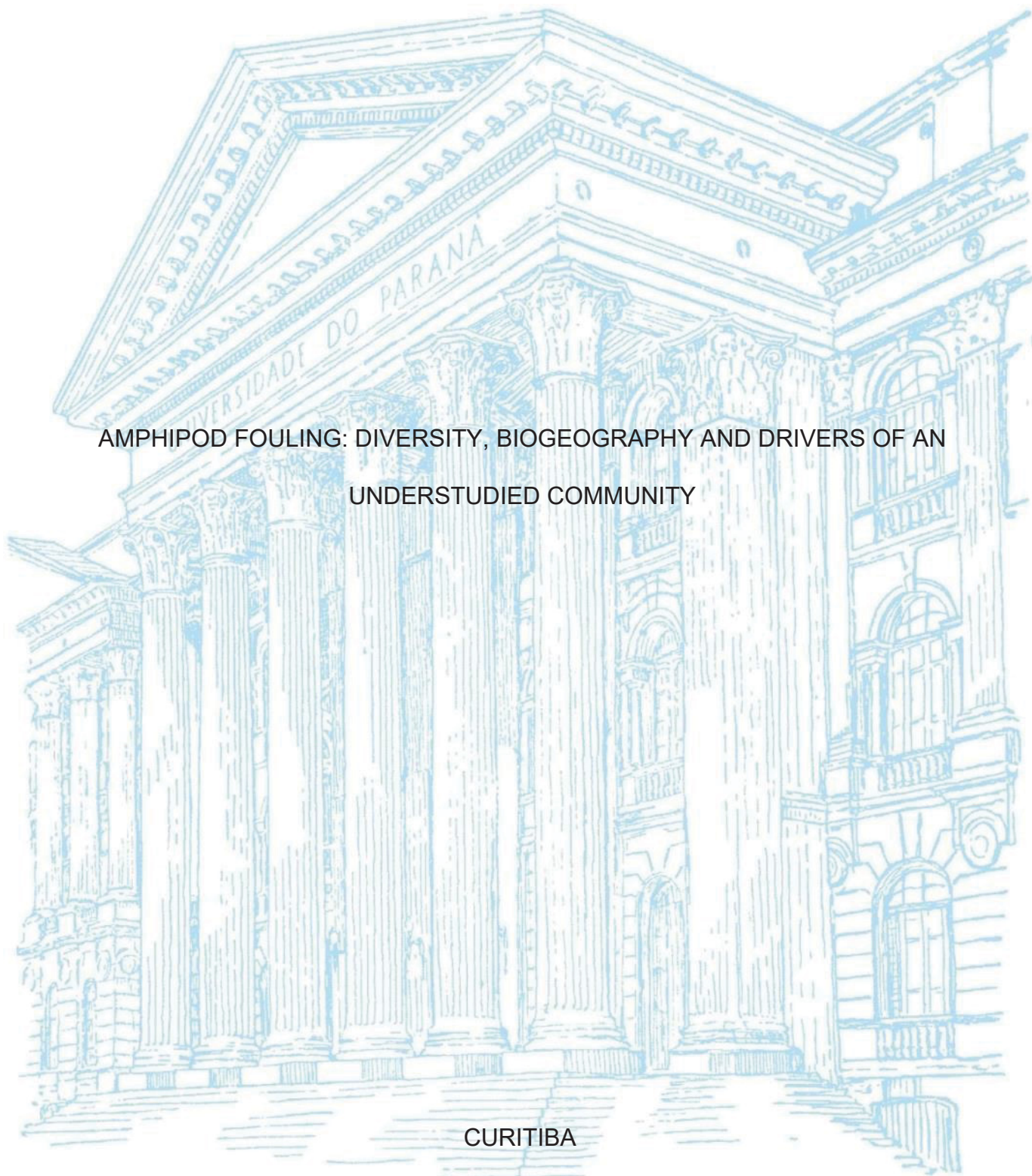
UNIVERSIDADE FEDERAL DO PARANÁ

ANDREA DESIDERATO

AMPHIPOD FOULING: DIVERSITY, BIOGEOGRAPHY AND DRIVERS OF AN
UNDERSTUDIED COMMUNITY

CURITIBA

2020



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UNDERSTUDIED COMMUNITY

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RESUMO

Substratos artificiais imersos em ambientes marinhos e estuarinos são, em geral, rapidamente colonizados por uma comunidade incrustante (*fouling*) que é conhecida por diminuir a flutuabilidade das bóias, obstruir dutos e afetar a hidrodinâmica dos barcos. No entanto, a fauna vágil do *fouling* ainda é comparativamente pouco estudada. As comunidades do *fouling* foram reconhecidas como *stepping-stones* para as espécies introduzidas não nativas (NIS), que, ao chegarem nos portos, também podem se espalhar para habitats naturais próximos. A principal hipótese desta tese - de que a proximidade de portos afeta negativamente as comunidades do *fouling* em sua composição (mais NIS perto dos portos), estrutura (proteção de barreiras ambientais entre estuários) e funcionamento (ou seja, funcionalidade de homogeneização) - é abordada em três capítulos diferentes. O primeiro capítulo testou o efeito do transporte marítimo internacional sobre a composição da assembleia de anfípodes ao longo da costa brasileira, usando códigos de barras de DNA, e explorou a possível falta de dados moleculares sobre esses crustáceos por meio de uma *gap analysis*. No estudo de caso do segundo capítulo, a propagação e as origens nativas putativas de dois habitantes comuns de comunidades do *fouling* ao redor do mundo - os anfípodes *Jassa marmorata* e *Jassa slatteryi* - foram avaliadas por meio da análise de DNA *barcoding* mitocondrial. No terceiro capítulo, as assembleias de anfípodes associadas ao *fouling*, em quatro estuários do Sul e Sudeste do Brasil, foram analisadas em diferentes aspectos de sua diversidade (taxonômica, filogenética e funcional). Para tanto, análises *network*, índices de estrutura de comunidades (diversidade alfa) e medidas de diversidade beta foram aplicadas para detectar possíveis efeitos de sazonalidade e potenciais impactos antrópicos. Além disso, uma nova espécie de anfípode e uma nova associação entre diatomáceas e anfípodes foram descritas no decorrer do desenvolvimento desta tese (publicações anexadas). Esta tese destaca que o *fouling* vágil, exemplificado aqui por crustáceos anfípodes, pode ser usada como ferramenta eficiente para detectar impactos humanos. No entanto, considerando as informações moleculares e taxonômicas, ainda há uma grande lacuna de conhecimento sobre esta comunidade artificial que precisa ser abordada para se adequar ao gerenciamento de possíveis novos impactos aos habitats naturais.

Palavras-chave: Fouling. Amphipoda. Bioinvasão. Biodiversidade. Transporte marítimo internacional. DNA barcode.

ABSTRACT

Artificial substrates immersed in marine and estuarine environments are usually rapidly colonised by a fouling community, which is known to decrease floatability of buoys, to obstruct pipelines and to affect the hydrodynamics of boats. Nevertheless, vagile fouling fauna is still comparably understudied. Fouling communities have been recognised as stepping-stones for non-native introduced species (NIS), that once arrived in ports, may also spread to neighbouring natural habitats. The main hypothesis of this thesis - that the vicinity to ports affects negatively communities of fouling in their composition (i.e. more NIS closer to ports), structure (i.e. buffering environmental barriers between estuaries) and functioning (i.e. homogenising functionality) - is addressed in three different chapters. The first chapter tested the effect of international shipping on the composition of the amphipod assemblage along Brazilian coasts using DNA barcodes and explored the possible lack of molecular data regarding these crustaceans through a gap analysis. In the case study of the second chapter, the spread and putative native origins of two common inhabitants of fouling communities around the world - the amphipods *Jassa marmorata* and *Jassa slatteryi* - were assessed through the analysis of mitochondrial DNA barcoding. In the third chapter, amphipod assemblages associated with fouling in four estuaries of South and South-East Brazil were analysed in different aspects of their diversity (i.e. taxonomic, phylogenetic and functional). For this purpose, network analyses, community structure indices (i.e. alfa diversity) and beta diversity measures were applied to detect possible effects of seasonality and potential anthropogenic impacts. Furthermore, a new amphipod species and a new association between diatoms and amphipods were described in the course of this thesis and are annexed. This thesis highlights that vagile fouling, exemplified here by amphipod crustaceans, can be used as an efficient tool to detect human impacts. Nevertheless, there is still a huge gap of knowledge considering both molecular and taxonomic information, that needs to be addressed for this artificial community, to properly manage possible further impacts on natural habitats.

Key-words: Fouling. Amphipoda. Bioinvasion. Biodiversity. Global shipping. DNA barcoding.

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PREFACE

Fouling communities have mainly been recognized for their negative effects on the hydrodynamics of ships, sinking buoys, plug piping or simply covering every substrate immersed in water (Rajagopal et al. 1991, Hunsucker et al. 2016, Want et al. 2017). Their establishment can be divided into four main steps: i) establishment of a layer of dissolved organic molecules on the surface of the substrate newly immersed; ii) bacterial colonization; iii) mainly diatoms and fungi colonization; iv) settlement and development of invertebrate larvae and algal spores (Abarzua & Jakubowski 1995). Fouling community composition have been proved to depend on various environmental conditions (e.g. water turbulence, temperature or salinity) but also the complexity or colour of substrates (Pech et al. 2002, Canning-Clode & Wahl 2010, Satheesh & Wesley 2010).

Shipping activities are recognised as one of the major vectors of non-native introduced species (NIS) in marine ecosystems (Minchin & Gollasch 2003, Seebens et al. 2013, Shenkar & Rosen 2018). If not properly treated, ships' hulls (and ballast waters) can host a rich fouling and thereby act as a global disperser of NIS that may reach international ports that are geographically too remote by natural dispersal mechanisms. Moreover, the further expansion of introduced species is aided by local boating (e.g. tourism, fishing), making the urban areas with commercial ports the hotspot and donor of NIS diversity (Clarke Murray et al. 2011, Lacoursière-Roussel et al. 2012, Ashton et al. 2014, Ferrario et al. 2017).

Fouling studies, however, seldom focused on the vagile portion of this community, which can host a considerably high diversity. From an *ad hoc* literature search on Web of Science (TS = (fouling AND communit*)), using the extended database of the last 30 years (i.e. timespan: "1990 - 2020") until July 2020 (accessed July 25th 2020) a total of 2074 publications were retrieved. Words recalling the vagile portion (e.g. motil*, mobil* or vagil*) appeared less than 100 times in title or abstract, while only the word sessil* more than 200. Also, the word amphipod was the only related to the vagile community with more than 100 mentions (179 corresponding to ~9% of papers considered), which compared with the ~20% of mentions of sessile taxa like ascidians or barnacles (~400 both), gives the extent of the taxon-bias that studies on this community are subjected to (i.e. in favour of sessile fauna). Furthermore, words like membrane*, microbi*, bacteria* or biofilm, appeared more than 500 times (i.e. 2883, 866, 586 and 901 respectively) showing an even bigger interest for the

microbial community (Fig. 1, Supplementary Tab. S1). This happens because the human efforts for preventing the fouling establishment, are focused on the first stages, which are mainly based on these taxa.

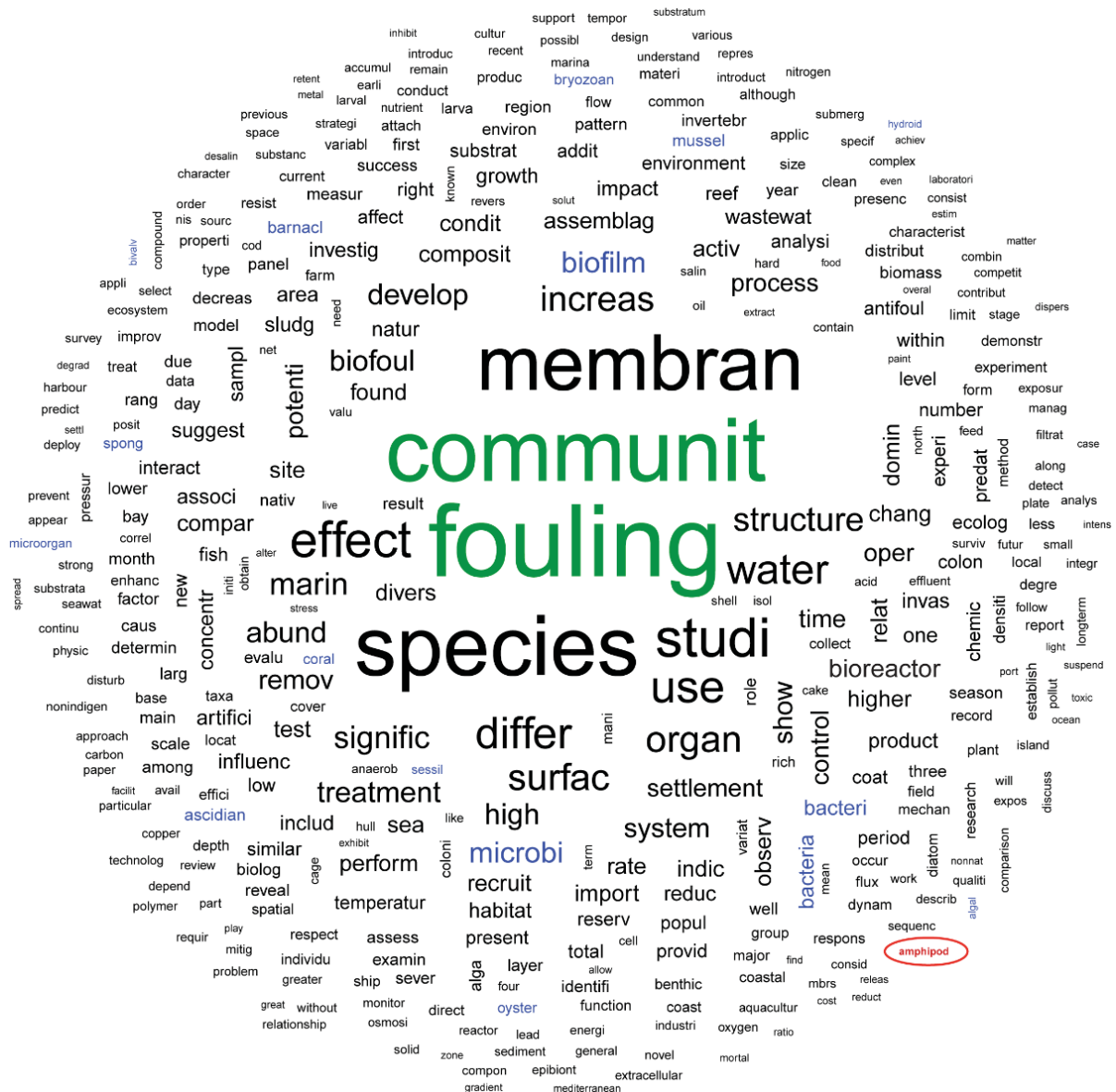


Fig. 1. Word cloud of all the words mentioned at least 100 times in title and abstract of the publications found searching for “fouling AND communit*” in the Web of Science (pps.webofknowledge.com). Green: words used for the search; blue: words relative to sessile or microorganisms; red circled: amphipod. Picture generate with the function *wordcloud* of the homonymous package of the R software R core Team 2020; www.r-project.org; <https://CRAN.R-project.org/package=wordcloud>).

The mobile fouling community is usually mainly composed of polychaetes, gastropods and crustaceans. Although species of other groups occur as well, they usually display lower diversity and abundances (Gollasch 2002, Schwindt et al. 2014, Fernandez-Gonzalez & Sanchez-Jerez 2017, Sedano et al. 2020, Meloni et al. 2020, Ros et al. 2020).

Amphipod crustaceans, for instance, can dominate fouling communities in terms of diversity, abundance, and sometimes even in biomass reaching densities of hundreds of thousands of individuals per square meter (e.g. Lee & Trott 1973, Franz 1989, Ramadan et al. 2006, Greene & Grizzle 2007, Beermann 2014). In the fouling (as in other communities), amphipods are important habitat formers because many families are able to construct tubes using a “silk” produced from the third and fourth pereopods and are known to be effective colonisers (Myers & Lowry 2003). Indeed, feeding on detritus or the bacterial and the diatom films, they are among the first macrofaunal animals able to settle on artificial substrates, providing also additional substrate (i.e. tubes) for other species (even sessile). Non-tubicolous species are specialised in clinging to substrates or other organisms (e.g. macroalgae and hydroids) or even establish symbiotic relationships with some sessile animals (e.g. cnidarians and ascidians), like the stenothoid *Stenothoe brevicornis* Sars, 1883, an obligate symbiont of the sea anemone *Actinostola callosa* (Verrill) (Vader & Krapp-Schickel 1996, Krapp-Schickel & Vader 2015). Moreover, some amphipods are also sensitive to pollutants and have been used in different bioassays accordingly (see Podlesińska & Dąbrowska 2019 for a review).

In the beginning of the 21st century, a new approach for species identification and discovery has been proposed to species discovery and diversity assessment has started. Thanks to the advent of DNA-based tools the speed at which species are being described has increased considerably easily point at the morphological inconsistencies with molecular data (i.e. species complexes). The DNA barcode, for instance, is a standard region of a gene that, once associated with a specific species, permits an easy and rapid identification (Hebert et al. 2003a). Therefore, the accuracy of the identification is correlated to the amount and quality of data (i.e. sequences) present in the public-access databases (i.e. BOLD and GenBank; Ratnasingham & Hebert 2007, Benson et al. 2013) and it improves as the coverage of taxa (i.e. quality and the number of different taxa in the libraries) increases (Weigand et al. 2019). For metazoans, a partial segment of the mitochondrial DNA of the cytochrome c oxidase 1 (COI) with approximately 650 base pairs has been established as the formal DNA barcode region. This region is recognised to have a sufficient variability to allow the diagnosis of most of the species, except only a few groups like cnidarians (Hebert et al. 2003b, Hajibabaei et al. 2007). Moreover, the use of barcodes has been proven to assist not only the species identification but also, through the combination with high-throughput sequencing (DNA metabarcoding) and analysis of environmental DNA (eDNA),

the rapid detection of NIS, which can be critical for a more efficient management of possible invasions (Hupalo et al. 2020, Duarte et al. 2021).

In attempts to measure biodiversity, scientists have developed many indices trying to characterise the different processes shaping the communities. Historically, the unit of these calculations was represented by the species identity (i.e. taxonomy). Modern approaches recognize three facets (i.e. components) of biodiversity, differentiating between taxonomic, phylogenetic and functional diversity. As already explained above, taxonomic diversity takes into consideration which species are present and, if necessary, their proportional abundance in a community. Phylogenetic diversity also includes the evolutionary distance between every species, providing additional information on the historical processes that took place, such as vicariance or local extinction (Miller et al. 2018). Functional diversity takes into consideration the ecology of every species and, through the analysis of their different combinations (in every species and between them), depicts the functions and possible ecosystem services (e.g. nutrients cycling, sediment irrigation) that are taking place in the communities (Ashford et al. 2018, Wrede et al. 2018). The study of the three facets, and the relationship between them, should provide a more comprehensive overview of the biodiversity at both a spatial and temporal level. In addition, biodiversity variability at large scale (spatial, environmental or temporal; i.e. other than single communities), is assessed through the beta diversity (Whittaker 1960, Legendre 2019). This measure can be partitioned into two additional sources of dissimilarity: the turnover (or replacement) and the richness differences (Fig. 2) (Carvalho et al. 2012). Knowledge on how multiple facets of biodiversity vary along environmental and anthropogenic disturbance gradients may help to discriminate the role of historical and present processes (e.g. current environmental changes) from the effects of anthropogenic transformation.

The vagile fouling in southern Brazil is poorly known and its amphipod diversity is even less studied. For instance, in the Paranaguá Estuarine Complex (PEC) only a couple of studies addressed it as community (i.e. not only some specific taxa), never recording more than 10 amphipod species (Correia & Loyola E Silva 1990, Neves & Rocha 2008). Furthermore, considering a molecular-data baseline, there are only few sequences of two species belonging to the Caprellidae family (i.e. *Caprella dilatata* Krøyer, 1843 and *Paracaprella pusilla* Mayer, 1890; Cabezas et al. 2013, Cabezas et al. 2019) from PEC available in the public databases, underlining the inconsistency between the already poor list of species and the related molecular data (i.e. knowledge gap).

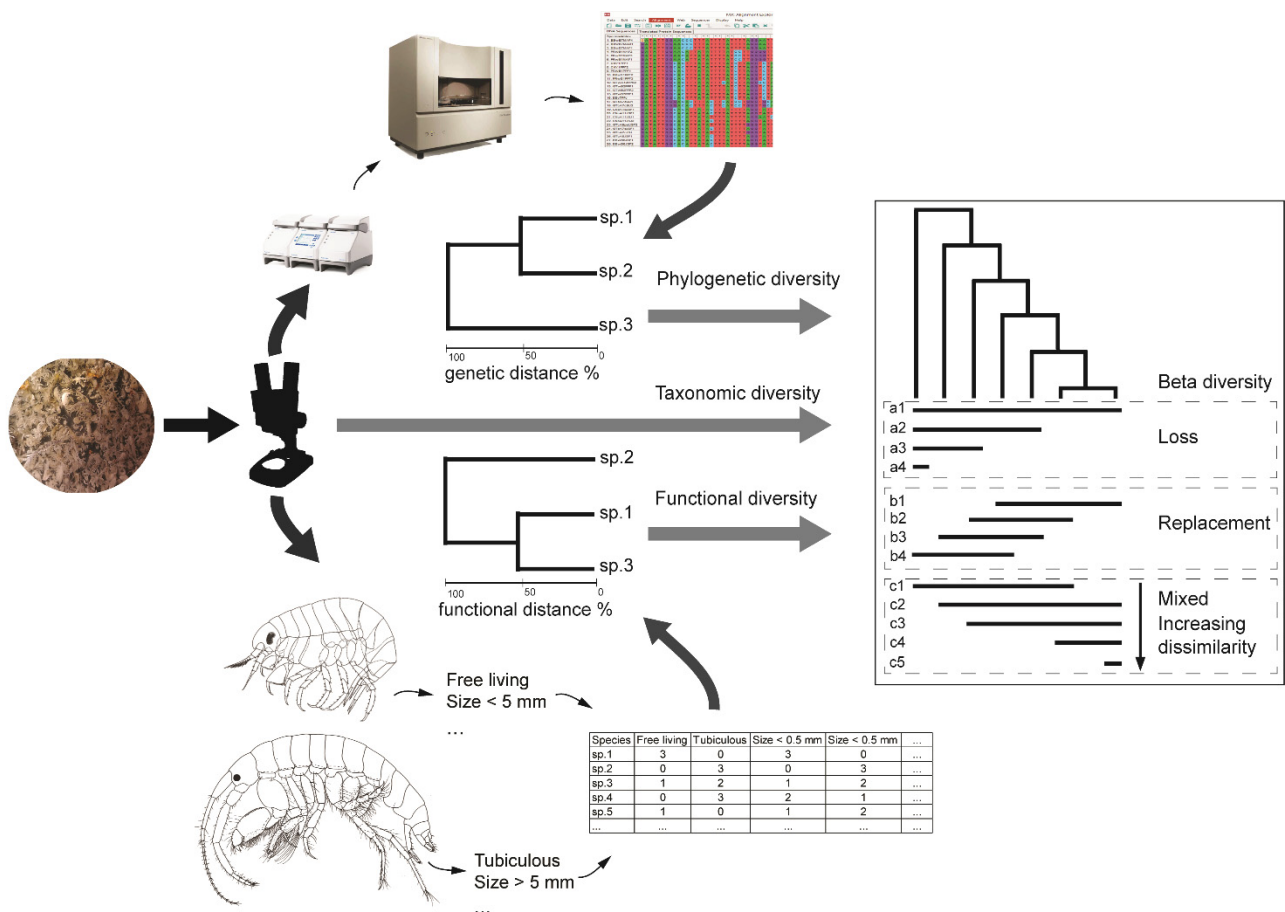


Fig. 2. Diagram of steps to calculate beta diversity for all the biodiversity facets (i.e. taxonomic, phylogenetic and functional). Taxonomic: sorting of assemblage, identification (common for every fact) and count of each species. Phylogenetic (upper part): DNA extraction, amplification and sequencing of COI for every species, reconstruction of a phylogenetic tree. Functional (lower part): compilation of a matrix of functional traits (e.g. life habitus, divided into free-living and tubicolous, and size, larger or smaller than 0.5 mm) through bibliographic research and expert's knowledge (when no-information can be found) for every species, make a cluster using Gower's distances. To underline that, the relative position of every species in each cluster/ tree (i.e. the distance between species) may be different between the clusters. For the beta diversity, taking a phylogeny/cluster of a theoretical community, three examples of possible scenarios are proposed. Referring to the first community (i.e. line) of each scenario, there is respectively: loss in richness (taxonomic, phylogenetic or functional), replacement (turnover), and a mixed scenario where only in the first two communities there is a replacement (i.e. c1 - c2) but, therefore a gradual loss of richness, and a proportional increase in dissimilarity. Two pictures of amphipods depicting *Hourstonius wakabarae* Leite & Siqueira 2013 and *Propejanice lagamarensis* Myers & Desiderato 2019, modified from Leite & Siqueira 2013, Myers & Desiderato 2019 respectively.

The current PhD project investigates the diversity of amphipods of fouling communities in relation to bioinvasion and anthropogenic impacts, using an experiment with artificial plates along four different estuaries of southern Brazil.

The thesis is organised into three chapters:

- In **Chapter I**, “*Mind the gap: missing a baseline of Brazilian fouling amphipods for proper biodiversity management*” through a gap analysis, DNA barcoding and the analysis of the international shipping of the South and South-East of Brazil, the representation of the fouling amphipods in public databases is assessed and first insights into the biodiversity baseline and bioinvasions are provided.
- In **Chapter II**, “*Ancient globetrotters - connectivity and putative native ranges of two cosmopolitan biofouling amphipods*”, the global phylogeographic structure of two common fouling species of the genus *Jassa* is explored. Using COI barcodes, the cosmopolitan distribution of both species is questioned and their putative native range evaluated.
- In **Chapter III**, “*Hitchhikers’ community: Taxonomic, phylogenetic and functional diversity among ports and estuaries*”, the biodiversity of fouling amphipods, including all its facets (i.e. taxonomic, phylogenetic and functional), is studied using four southern Brazilian estuaries as a case study. Using diversity indices, network analysis and beta diversity dissimilarity models, the effects of environmental variables, seasonality and anthropogenic impact are tested.

In the annexes, two complementary works (one published and one submitted) which were produced along with this thesis are provided:

- **Annex I**, “*A new genus and species of Aoridae (Amphipoda, Senticaudata), Propejanice lagamarensis gen. nov. sp. nov. from Brazil*”, is the description of a new species and genus of amphipod found in the LAGAMAR region and named after it: *Propejanice lagamarensis* Myers & Desiderato 2019.
- **Annex II**, “*Hidden in plain sight: co-introduction of epibiotic diatoms with amphipod crustaceans?*”, reports the presence of diatom epibionts on amphipods. With the use of scanning electronic microscopy (SEM), the coating of diatoms was described on several specimens of amphipods belonging to different species. This association is briefly presented, and possible effects are suggested along with the first record of a diatom species that is spreading around the world, indicating a possible co-introduction.

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CHAPTER I

MIND THE GAP: MISSING A BASELINE OF BRAZILIAN FOULING AMPHIPODS FOR PROPER BIODIVERSITY MANAGEMENT

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Mind the gap: missing a baseline of Brazilian fouling amphipods for proper biodiversity management

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Abstract

DNA barcodes became increasingly important over the last years as a tool for species identification and conservation management. Researchers around the world try to cover barcode libraries with as many sequences as possible, assigning sequences to described and undescribed species. In some regions and for some taxa, however, baseline inventories are still missing, blocking efforts for biodiversity management. After a gap analysis of the Brazilian amphipods' public sequences, we started a new library of fouling amphipods that increased the number of public sequences by more than 2.5-fold. Further, four new occurrence records for Brazil, between 8 to 12 genetically cryptic species and six possible new morphospecies were found. The North Western Atlantic coast, was the region with higher number of species sharing very similar haplotypes with Brazil, suggesting a natural connection. Nevertheless, a correlation with data on marine shipping activities suggested that other regions should also be involved in continuous species exchange. As the lack of baseline inventories could be even more pronounced in other developing regions of the world, both morphological and molecular studies need to be prioritized to fill an already too large gap of knowledge.

Key words: barcoding, bioinvasion, gap-analysis, global shipping, vagile

1. Introduction

The raise of DNA-based tools such as DNA barcoding and DNA metabarcoding significantly improved biodiversity studies by providing a tool for fast species identification^{1,2}. However, these tools are dependent on the availability of known DNA barcodes (reference libraries) for the target taxa^{3,4}. To more comprehensively know biodiversity and complete the reference libraries, many international projects around the world (e.g. BIOSCAN) are producing barcodes of as many species as possible⁵. Regardless of the efforts, aquatic environments are usually subject to biased focus on conspicuous taxa such as fishes, vertebrates in general or a small number of invertebrates taxa (e.g., some molluscs)^{6–8}. Of course, this is not a rule, and an increasing number of studies address different communities with the help of metabarcoding or other comprehensive techniques^{9,10}, providing important data on organisms at multiple scales (e.g., micro/macro).

DNA barcoding and DNA metabarcoding are not merely rapid species identification tools. They also play an important role in the rapid assessment of non-indigenous species (NIS), of which their early detection can be crucial for countermeasures against their spread^{11,12}. For this reason, a complete reference library of DNA barcodes of NIS species of the world would be desirable, although many NIS or cosmopolitan species are missing from the public databases, leaving an open gap that can negatively affect diversity assessments^{3,13}.

The fouling is an incrusting community which develops on artificial substrates immersed in water. It is dominant in habitats with a heavy anthropogenic impact, such as ports with their piers, pontoons and buoys. The sessile epifauna in fouling communities has been studied comparably well (e.g. barnacles, bryozoans, bivalves, cnidarians and macroalgae), which can affect the floatability and hydrodynamics of buoys and ships^{14,15}. The vagile portion of fouling comprises several species from different phyla (e.g., Annelida, Mollusca, Arthropoda)¹⁶. Amphipod crustaceans are among the most frequent taxa, often dominating fouling communities in terms of abundance, diversity and sometimes even biomass¹⁷. The ecological importance of the amphipods, exhibited for example on their role in habitat engineering through tube-building species, or their application in bioassays, is largely recognised, although sometimes disregarded^{3,18,19}. Moreover, a high cryptic diversity in amphipods has been acknowledged, where numerous molecular taxonomic units

(MOTUs; i.e., putative cryptic species) inside single morphospecies are common ^{20–23}. Nevertheless, many amphipods are known to have a widespread and near-global distribution ²⁴. Some of them are recognised neocosmopolitans (i.e. having attained a global distribution by human-dispersion) taking advantage of the global shipping to disperse among international ports and using these as stepping stones for their spread. ^{25,26}

In a conservation context, gap analysis is a method to identify biodiversity (e.g. species, ecosystems and ecological processes) not adequately represented in a protection network of a given region ²⁷. Through a proper gap analysis, countries may avoid losing natural resources, even before their discovery ²⁸. Recently, this approach has been used to evaluate also the mismatch between the species inventories and their respective molecular information in public database, pointing at taxa that need to be properly managed ^{3,4}. However, also to understand ecological processes such as bioinvasions, reliable baseline inventories are necessary ^{13,29}.

Using gap-analysis and cytochrome c oxidase 1 fragment (COI) barcoding, this study aimed to fill the gaps of knowledge and data related to the amphipods inhabiting the fouling community along the Brazilian coasts.

2. Results

2.1 Gap analysis

The application of the selection criteria to the catalogue of amphipod species of Brazil resulted in a final list of 197 currently known species in benthic marine habitats, belonging to 40 families. From those 197 species, only 33 (~16%) were detected with public COI sequences (Fig. 1; Supplementary S1), 13 species with public 18S sequences. 28S and 16S sequences were only available for two (28S) and three species (16S). It has to be underlined that 28S and 16S sequences were only available in GenBank (Supplementary Tab. S1). Many families completely lacked any public sequence and only one (Synopiidae) had more than 50% (three out of four) of the species with a sequence. Of the two most speciose families, Caprellidae and Maeridae with 21 species each, the first showed coverage of 43%, having the highest number of species represented (i.e. 9) while, the latter had no sequences (Fig. 1; Supplementary Tab. S1). The mean number of BINs was almost double compared to the morphospecies (~1.90) showing generally high intraspecific genetic diversity (Supplementary Tab. S2). Moreover, of the 1456 sequences found, only 58 were of specimens from Brazilian waters, belonging to five species: *Apohyale media* (Dana, 1853);

Caprella dilatata Krøyer, 1843; *Caprella scaura* Templeton, 1836, *Cymadusa filosa* Savigny, 1816; *Paracaprella pusilla* Mayer, 1890 (Supplementary Tab. S2).

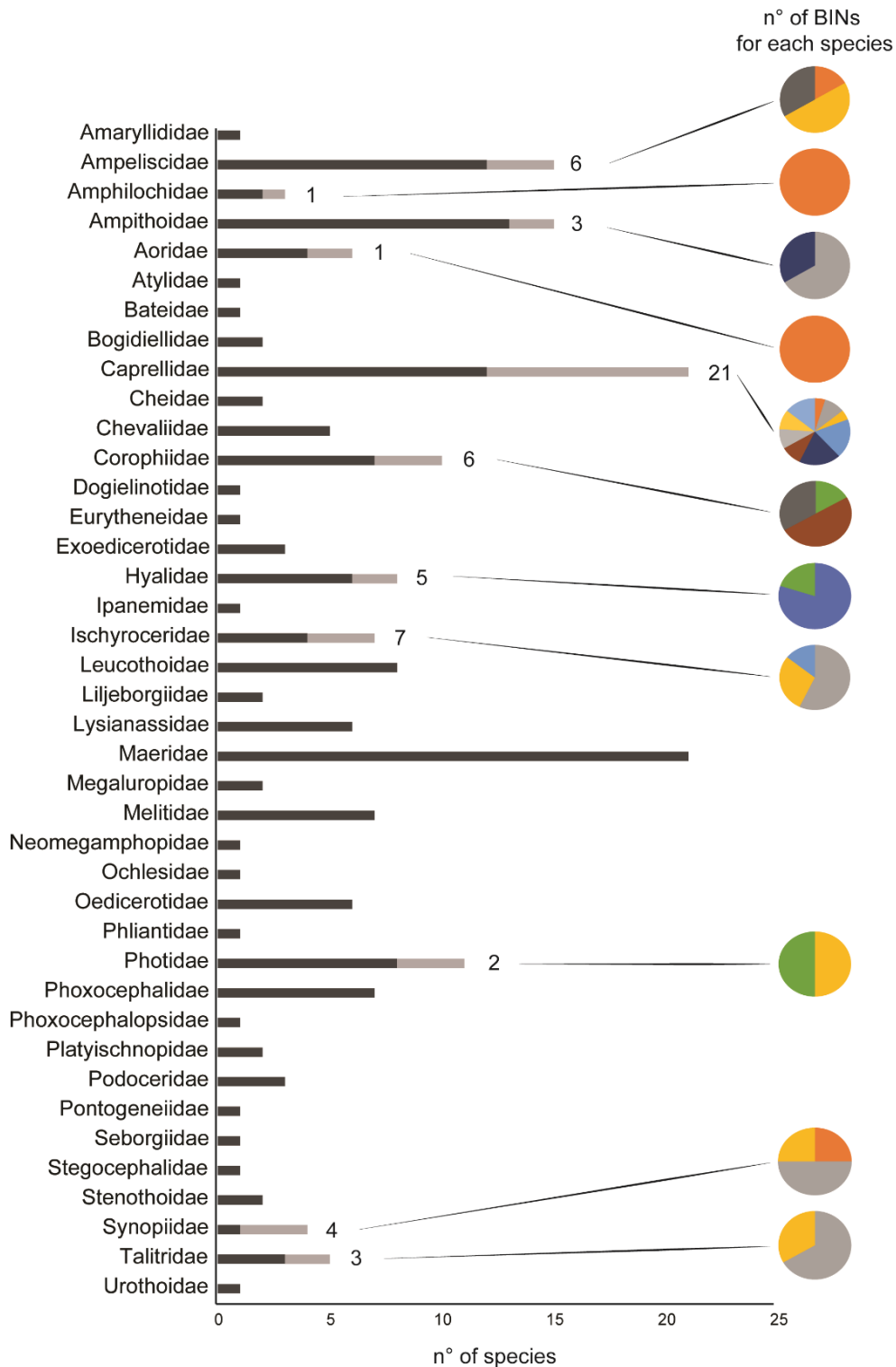


Figure 1. Number of species without (dark grey) and with (light grey) public sequences and number of BINs for each species. Size and colour of each slice proportional to the number of BINs for each species inside the family. Numbers referring to the total number of public BINs belonging to each family. Data for each species in Supplementary Table S1.

2.2 The fouling amphipods of the subtropical Brazilian coast

A total of 100 specimens, belonging to 27 fouling morphospecies, were sequenced (Fig. 2). The whole dataset was composed by 102 sequences with an average intraspecific distance of 1.89 (0.12 SE) and the highest for *Elasmopus* sp., *Erichthonius brasiliensis* (Dana, 1853) and *Stenothoe gallensis* Walker, 1904 (23.94%, 18.64% and 12.8% respectively) (Supplementary Tab. S3). The results of the BAGS analysis showed a high number of C-grade species (11), meaning that multiple BINs were present. There were also a high number of D-grade species (8), reflecting lack of data/sequences (i.e. below 3) for many species (Supplementary Fig. S1).

In the phylogeny, each morphospecies always formed a highly supported monophyletic cluster (>95% support) comprising all the different BINs; (Fig. 2). Despite the general topology reflected the one recently proposed using a more comprehensive coverage of genes ³⁰, inferences about the evolutionary relationships of amphipods are beyond the scope of this work.

Four species were recorded for Brazilian coasts for the first time: *Ampithoe valida* Smith, 1873, *Apocorophium acutum* (Chevreux, 1908), *Cerapus benthophilus* Thomas & Heard, 1979 and *Stenothoe gallensis* Walker, 1904. Two species were firstly recorded after their first description *Stenothoe georgiana* Bynum & Fox, 1977 (for Brazilian waters), *Jassa valida* (Dana, 1853) (for the World). The species *Erichthonius punctatus* (Spence Bate, 1857), could have been a first record too, although it presents some morphological incongruences with the accepted description ³¹. *Laticorophium baconi* (Shoemaker, 1934) was firstly recorded in Brazil in 2009 in the state of São Paulo ³² and afterwards in Rio de Janeiro ³³, being this the first record for states of Paraná and Santa Catarina. Finally, four species were possibly new to science: *Hourstonius* sp., *Elasmopus* sp. (belonging to the *Elasmopus pecteniscus* (Spence Bate, 1862) complex), *Cheiriphotis* sp. and *Lembos* sp.

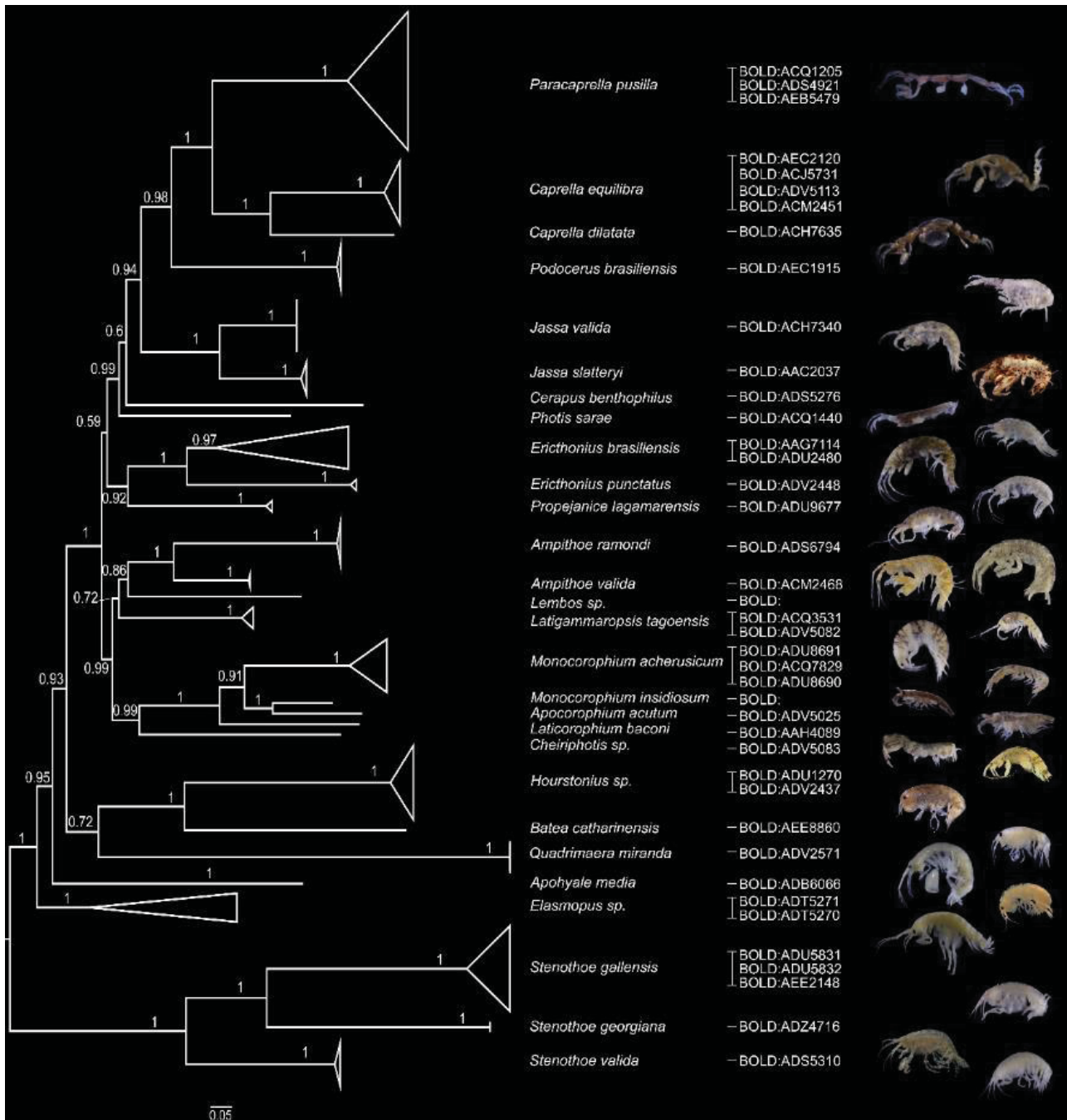


Figure 2. Midpoint-rooted maximum-likelihood phylogeny (GTR +G+I substitution model applied) based on the COI gene and BINs belonging to each morphospecies found in this work. Support higher than 70% shown above or below branches. Photographs of the amphipod morphospecies, made by AD and SS, from top: *P. pusilla* ♂, *Caprella equilibra* Say, 1818 ♀, *C. dilatata* ♀, *Podocerus brasiliensis* (Dana, 1853) ♀, *J. valida* ♀, *Jassa slatteryi* Conlan, 1990 ♂, *C. benthophilus* ♀, *Photis sarae* Souza-Filho & Serejo, 2010 ♀, *E. brasiliensis* ♀, *E. punctatus* ♀, *P. lagamarensis* ♂, *Ampithoe ramondi* Audouin, 1826 ♀, *A. valida* ♀, *Lembos* sp. ♀, *Latigammaropsis tagoensis* (Schellenberg, 1925) ♀, *M. acherusicum* ♂, *M. insidiosum* ♀, *A. acutum*, *L. baconi* ♂, *Cheiriphotis* sp. ♀, *Hourstonius* sp. ♀, *Batea catharinensis* Müller, 1865 ♀, *Quadrimaera miranda* (Ruffo, Krapp-Schickel & Gable, 2000) ♀, *S. gallensis* ♀, *S. georgiana* ♂, *Stenothoe valida* Dana, 1852 ♀. The phylogeny was generated using PhyML (<http://www.atgc-montpellier.fr/phyml/>).

2.3 Species delimitation

In general, ABGD and mPTP were more conservative within their delimitation method (i.e. by distance or topology), being the latter most conservative and showed always a lower number of MOTUs. Nonetheless, mPTP also failed to split some recognised species such as in the amphiloichid phylogeny, where two species of *Amphiloichus* and the cluster of *Hourstonius* sp. (one to two MOTUs in the other analyses) were put together while, the other three methods, recognised at least four MOTUs (Fig. 3a). For the corophiids, *Monocorophium acherusicum* (Costa, 1851) showed between two to five different MOTUs, displaying a high possibility of being a species complex. The identities of *Laticorophium baconi* and *Monocorophium insidiosum* (Crawford, 1937) were confirmed (i.e. grouped together with public sequences of these species), while the other two species were singletons without matching sequences in BOLD. In *M. insidiosum* only the cluster analysis of BOLD (BIN) showed two possible MOTUs but these were not supported by the other analyses (Fig. 3b). In the ischyrocerid, the genus *Ericthonius* displayed high variability with multiple MOTUs for both species. The sequences of *E. brasiliensis* from this study split in two clusters (one of which had also other sequences) that were supported by all the delimitation methods. Moreover, there were other two MOTUs highly supported, suggesting the presence of four cryptic species in the morphospecies *E. brasiliensis* (Fig. 3c).

Likely, *E. punctatus* showed two consistent MOTUs and one probable misidentification in BOLD (cluster with *Ericthonius difformis* H. Milne Edwards, 1830). *Cerapus benthophilus* represented the first sequence for this genus while the two *Jassa* species (*J. slatteryi* and *J. valida*) showed one clear MOTU each (Fig. 3c). In the maerids, again the mPTP was the only analysis to merge in one MOTU sequences from *Elasmopus rapax*, *E. antennatus* and *Elasmopus* sp., whereas the other three analyses recognised four different MOTUs, two of which in *Elasmopus* sp. (Fig. 3d).

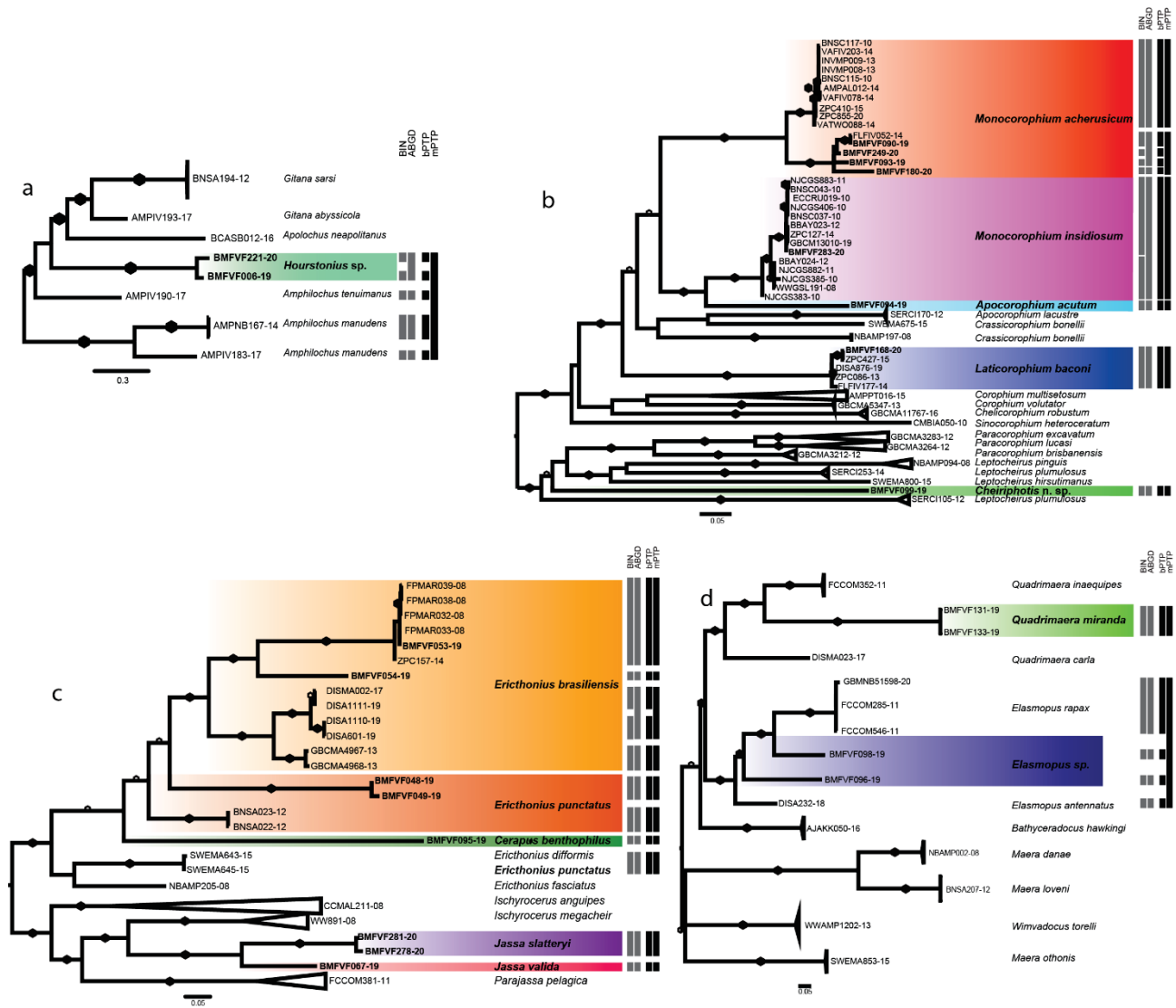


Figure 3. Maximum likelihood phylogenies of the families Amphilochidae (a), Corophiidae (b), Ischyroceridae (c) and Maeridae (d), based on the COI gene with species delimitation results. Grey and black bars representing respectively the distance-based and the results of the topology-based method of the MOTUs delimitation. ●: support >99%; ○: 85% < support < 99%. Labels referring to process-id of one sequence per haplotype, in bold the ones of this study. Species names in bold identifying the morphospecies delimited. Coloured boxes showing the cluster where the sequences of this study are located. Trees were generated using PhyML (<http://www.atgc-montpellier.fr/phyml/>).

The two species of *Ampithoe* had opposite results, confirming *A. valida* but showing a putative cryptic species supported by all the analyses for *A. ramondi* (Fig. 4a). For the stenothoids, *S. gallensis* showed between one to four MOTUs all of them from the sequences of this work with a high probability of at least three putative cryptic species (Fig. 4b).

The two species of the genus *Caprella*, like *Ampithoe*, gave opposite results, with one clear MOTU in *C. dilatata*, but multiple ones in *C. equilibra*. In the latter, one MOTU most likely belongs to the species complex of *Caprella laeviuscula* Mayer, 1903 which will not be

further discussed here, while the other MOTUs identified by the BINs were not supported by the other analyses (Fig.4c). In *Paracaprella pusilla* ABGD retrieved only two MOTUs compared to other methods (i.e. four MOTUs), identifying only the haplotype referred to BMFVF218-20 as a putative cryptic species, which was supported by all the other analyses (Fig.4d). In photids, only the BINs retrieved two MOTUs for *L. tangoensis*, although this was not supported by all the other analyses (Fig.4e).

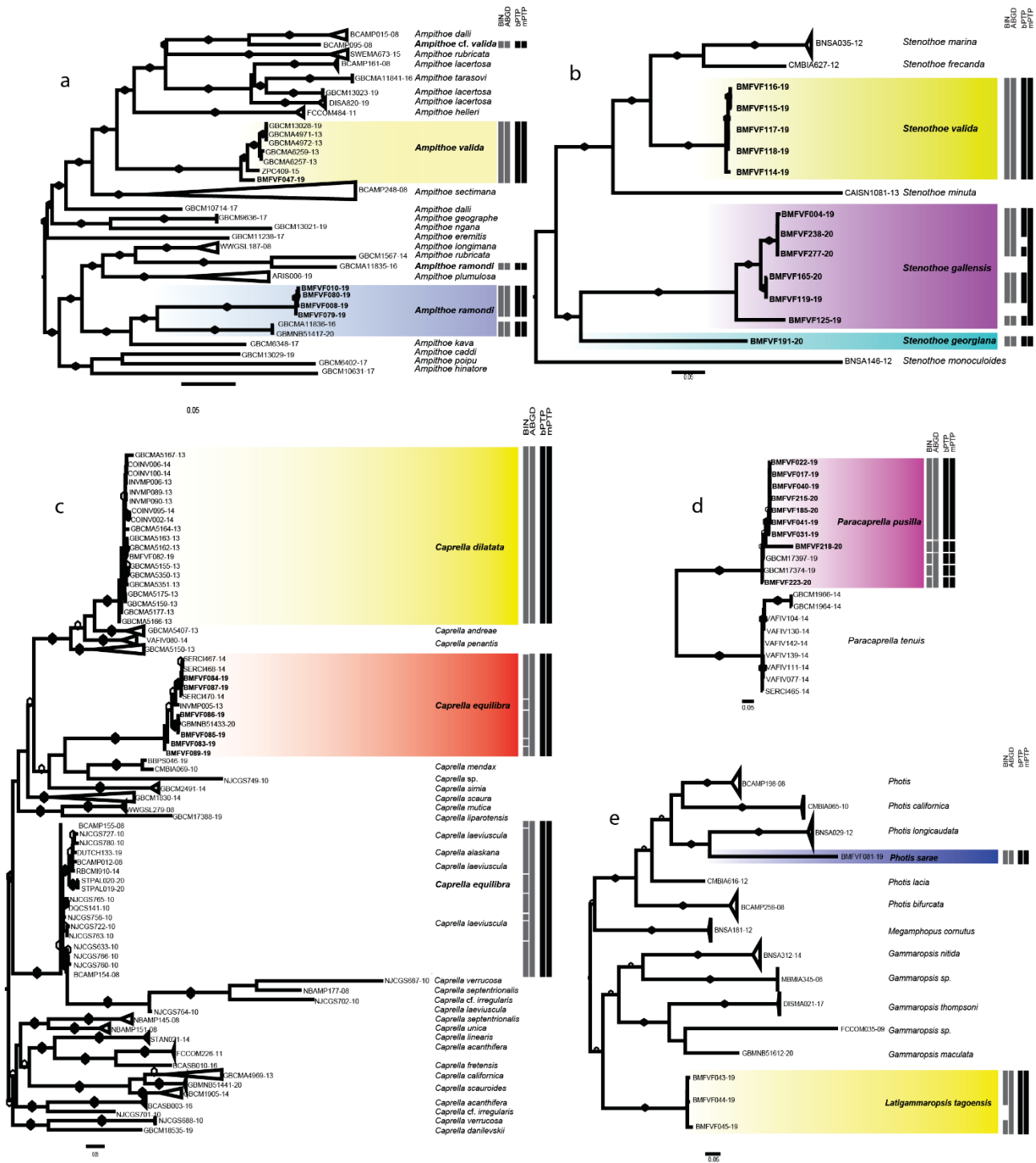


Figure 4. Maximum likelihood phylogenies of the families Ampithoidae (a) and Photidae (e) and genera Stenothoe (b) Caprella (c) and Paracaprella (d), based on the COI gene with species delimitation results. Grey and black bars representing respectively the distance-based and the results of the topology-based method of the MOTUs delimitation. Shaded grey bar (ABGD in d) referring to continuous MOTU. ●: support >99%; ○: 85%<support>99%. Labels referring to process-id of one sequence per haplotype, in bold the ones of this study. Species names in bold identifying the morphospecies delimited. Coloured boxes showing the cluster where the sequences of this study are located. Trees were generated using PhyML (<http://www.atgc-montpellier.fr/phyml/>).

2.4 Shipping and geographic connectivity

Along the four years (2015 to 2018) considered, the Southern and South-Eastern Brazilian regions exchanged more than 1,750,000,000 tons of goods via marine shipping, spanning more than half of all countries of the world. China was by far the country with most total shipping (~30% of the total), mostly exportation to it (~94%: ~540,000,000 tons) although, the remaining ~6% was enough to be second only to the USA for importation from them (USA: ~80,000,000 tons; China: ~35,000,000 tons) (Fig. 5; Supplementary Tab. S4).

Twelve of the species had COI barcode matching in at least 99.5% with those from the public databases (Fig. 5; Supplementary Tab. S5). The specimens matched with regions across all continents, Antarctica excluded. All species, except *J. slatteryi*, had at least one match with specimens from North America. *Paracaprella pusilla* was the species with more hits in different countries (7) followed by *M. insidiosum* (5).

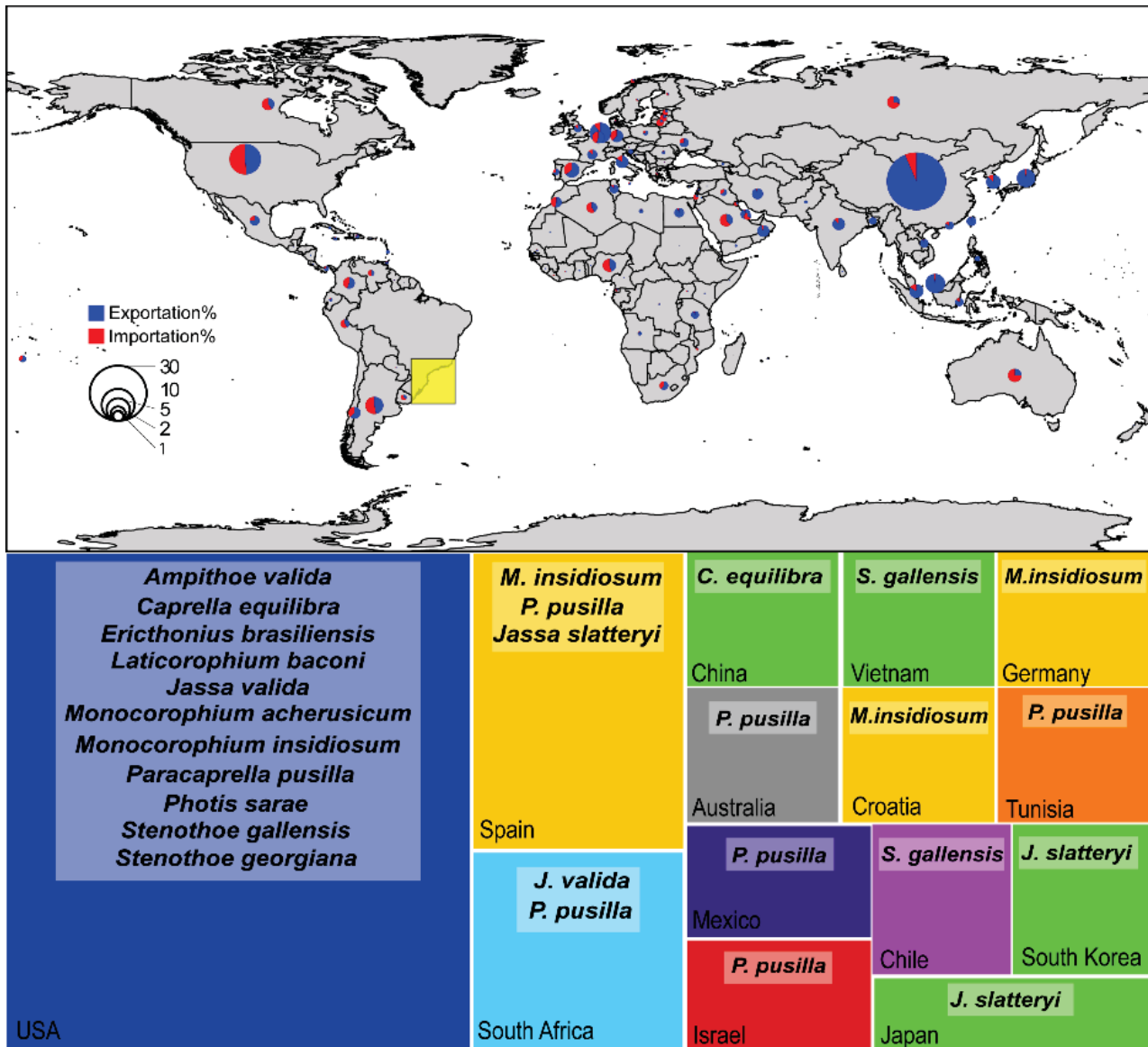


Figure 5. Above, the map of the Southern and South-Eastern Brazilian (yellow box) proportional volumes of total shipping (i.e. the percentage of the total of each country), divided into importation (red) and exportation (blue). Below, treemap of the species with a match (>99.5% of identity) with other sequences of public databases from countries other than Brazil (Supplementary Tab. S6). Colors in the treemap identify countries or regions: blue – USA; yellow – Europe; light blue – South Africa; green – Asia; grey – Australia; dark violet – Mexico; red – East Mediterranean Sea; purple – Chile; orange – South Mediterranean Sea. Map created with Qgis 3.12.3 (<https://www.qgis.org/en/site/>).

3. Discussion

The current study clearly highlighted the underrepresentation of molecular data for amphipods, an ecologically important group, in Brazilian coastal systems. The presence of a DNA sequence for less than a quarter of the species recorded in Brazil is a big gap that urges to be filled in the current scenario of species extinction, but also globalization ^{34,35}.

The high number of putative cryptic species at global scale coupled with the low number of local specimens sequenced could result in an underestimation of the real diversity present along the Brazilian coasts. Our results are emblematic of this, where multiple new records accompany multiple MOTUs.

In the last years, an increasing number of papers have been pointing out the lack of data, either taxonomic or molecular, for different taxa and regions, underlining the need to draw a clear baseline to understand what is going to be lost and what is still possible to be saved ^{4,36}. Despite our choice of taking a reference list of the 2018 ³³ was to be conservative on the calculations, the Brazilian coast resulted to be considerably understudied. In fact, numerous species were described since the catalogue was published but, most of them, without a reference sequence or barcode ^{37–40}. This knowledge further expands the gap, drawing a baseline which is far from being reached. If the lack of sequences is then coupled with the large size of the territory, the number of unexplored areas (e.g., sampling biased through accessible places or close to big universities) and the difficult taxonomy of some groups of amphipods, it is clear how “unreachable” the simple-task of drawing a baseline can be.

Our work shows how a frequently studied community, such as the fouling, can hide different new species and records in its understudied vagile portion. The presence of many cosmopolitan species and a small number of dominant ones can sometimes conceal a high diversity. While our results confirmed a global distribution of some species (e.g. *Jassa slatteryi*, *Stenothoe gallensis*, *Monocorophium insidiosum*, *Caprella equilibra*) (Fig. 5), there were many putative cryptic species that opposed the concept of cosmopolitanism. For instance, *Monocorophium acherusicum* was thought to be cosmopolitan or a NIS (in this region) in the literature ⁴¹, however our sequences were considerably different (recognized as different MOTUs in all the analyses; Fig. 3b) from the ones where the species was described in Europe (BOLD:AAU1637 in Supplementary Tab. S5). Historically, *M. acherusicum* has always been present, abundant and widespread along the Brazilian coasts, to the point that it has been described as cryptogenic ⁴². Recently, the identity of the “European” *M. acherusicum* was confirmed molecularly from Argentina ⁴³. On contrary, the specimens found in the present work shared different haplotypes with populations from North America, from South Carolina to Texas. Furthermore, in the southern region of North America, the “European” MOTU seems to be rare, as most of the *M. acherusicum* found on the databases surveyed were in the same BINs of our sequences. This suggests not only a possible connection between the two regions but also a geographic/climatic segregation

between the two cryptic species (i.e. one of more temperate waters and one from warmer ones). A different result was obtained in *Erichthonius punctatus* in which the new delimited MOTU was confirmed with morphological analyses as a new species. It presents a typical posterior projection on the base of the fifth pereopod similar to the *Erichthonius* sp.A, partially described by LeCroy in 2007. A similar situation occurred with *Jassa valida* which was never found after its description in 1853 based on specimens from Rio de Janeiro, but was abundant in our samples and there were shared haplotypes with the *Jassa* sp. of Pilgrim and Darling ⁴⁴ from South Carolina. LeCroy also partially described, in the same volume of *Erichthonius* sp. A, a *Jassa* sp. A, which resulted to be *J. valida* (Conlan, Desiderato & Beermann *in prep*). Completely different are the results we found for the species complex of *Stenothoe gallensis* where two of the identified MOTUs had two different matches with the databases. The first (haplotypes BMFVF238-20, BMFVF277-20 and BMFVF004-19 in Figure 4) matches with sequences from Chile (accession number: MT317092) and the USA (process-id: MGOTX952-19). While, the second (haplotypes BMFVF119-19 and BMFVF165-20 in Figure 4), showed 100% of identity with a unique sequence of *Stenothoe irinae* Marin & Sinelnikov, 2018, which was short (277 base pairs, accession number: MH128325), described from an aquaculture cage from Vietnam waters. Morphologically was not possible to distinguish the two MOTUs also following the description provided by the authors ⁴⁵. Furthermore, individuals of both MOTUs, matched the characters described by the authors of *S. irinae*. These results need a further investigation because it could either be a case of synonymy, where the recently described *S. irinae* is an example of the morphological plasticity of *S. gallensis*, confirming one cosmopolitan species or, that there is more than one species of this complex spread around the world.

In general, the shipping data were in agreement with our molecular results. For instance, the USA is the second country (out of 156) for volumes exchanged via-water with Brazil. On the other hand, the number of hits (i.e. shared haplotypes) with China should have been higher (only 1) as it is responsible for more than the double of goods exchanged when compared with the USA, opposing the molecular results. This is an evident artefact of our methodology based on the assumption that there is an equal number of sequences/effort (number of sequences positively correlated to the amount of effort and funds invested in barcoding) in every country, which is not true. Of the 15 countries with a hit, only three have more than 1,000 public sequences of amphipods and five have less than 100 (data accessed 08-10-2020). South Africa, for example, has only 120 sequences but two hits (Fig. 5), which is proportionally higher than the USA that has 2,161 sequences (respectively 1.67% and

0.51%). Notwithstanding, some species are known to be cosmopolitan and were recently subject of biogeographic studies at global scale ^{25,46} underlining also the importance of these type of works. So, the strong evidence of recent and/or continuous exchange of specimens could be stronger if a wider taxonomic and geographic coverage could be attained. The high number of Brazilian populations genetically close with the conspecifics from North West Atlantic, may be a result of not only historical shipping routes, but also the environmental affinity. Previous studies on other taxa (i.e. reef fishes) already underlined the taxonomic and biogeographic affinity between these regions ⁴⁷ and, recent work at a global scale on benthic amphipods recognised a biogeographic region from North Atlantic USA down to South Brazil ²⁴. Finally, the distance between areas must be taken into account as a long journey can limit the survival of hitchhikers ⁴⁸ although, it is also true that the amphipods inhabiting the fouling can have highly plastic biology, being capable of surviving in dire conditions such as changes in temperature or high salinity differences ⁴⁹.

From our results, it appears clear that extensive work must be done in order to properly address the diversity of marine amphipods, not only of the fouling community but of every habitat. Additional efforts from taxonomy experts have to be accompanied by continuous improvements in molecular data. As demonstrated by works on continental-scale in Europe ²¹, the possibility to uncover new diversity hot-spot will increase with a higher spatial and quality coverage of the libraries, and should be done as fast as possible. The possibility to discover and properly manage the current status of the major routes of species invasion will be also greatly increased if regional-focused studies will keep updating the public databases of new genetic information on the local biodiversity.

4. Materials and methods

4.1 Gap analysis

To assess possible mismatches between the current checklists of occurrence of amphipod species along Brazilian coasts and the sequences already available in public databases, a gap analysis (i.e. assess which species have publicly available DNA sequences) was performed. The catalogue of the Amphipod species occurring in Brazil was recently compiled by Serejo and Siqueira ³³. Based on this list, we selected only the marine species, present until 50 m deep, and discarded cave-dwellers and parasitic species. The taxonomic classification and name validation of the Amphipoda compiled in the lists were

made through the World Register of Marine Species (WoRMS) database (www.marinespecies.org)

The total number of public sequences for each species was retrieved from the public databases Barcode of Life Data System (BOLD) ⁵⁰ and GenBank (www.ncbi.nlm.nih.gov/Genbank) using the software R 3.6.0 (R core Team 2019; www.r-project.org) with the packages “bold” ⁵¹ (for BOLD) and “rentrez” ⁵² (for Genbank). The following genes were searched: COI, 18S, 16S and 28S. The terms used to filter the sequences from BOLD were: for COI – “COI-5P”; for 18S – “18S” or “18Sa”, for 16S – “16S” and for 28S – “28S”. In GenBank, the terms used for the search were (suggested by GenBank for the studied loci): for COI - “COI[Gene] OR CO1[Gene] OR COXI[Gene] OR COX1[Gene] OR complete genome[All Fields] OR mitochondrial genome[All Fields]”; for 18S: “18S ribosomal rna[Title] OR 18S rRNA[Title] OR 18S small subunit ribosomal RNA[Title] OR 18S ribosomal rna[Gene] OR 18S rRNA[Gene] OR 18S small subunit ribosomal RNA[Gene]”; for 16S: “16s[Title] OR 16S rRNA[Title] OR 16S[Gene] OR 16S rRNA[Gene] OR 16S mitochondrial” for 28S: “28S ribosomal rna[Title] OR 28S rRNA[Title] OR 28S small subunit ribosomal RNA[Title] OR 28S ribosomal rna[Gene] OR 28S rRNA[Gene] OR 28S large subunit ribosomal RNA[Gene]”. Only sequences with more than 500 base pairs were considered for COI since this is the minimum length required for a sequence to meet the Barcode Compliance standards ⁵⁰. Because there is no standard minimum size for the other three markers, and their fragment size varies, we chose 300 base pairs for 18S, 16S and 28S, since this is the standard minimum size of the fragments used in DNA metabarcoding studies.

The number of Barcode Index Numbers (BINs; proxy of Molecular operational taxonomic units – MOTUs) ⁵³, for each species, was retrieved from BOLD, based on the COI marker. Records co-occurring in both databases were detected through the presence of the tag “Mined from GenBank, NCBI” in BOLD’s records or availability of a GenBank’s accession number, which indicates that those BOLD records were mined from or deposited in GenBank. All the sequences mined were added to the public dataset DS-BRAMPH in BOLD.

The details of the bioinformatic pipeline, such as the scripts used for each taxonomic group and the markers searched, can be consulted at https://github.com/pedroemmanuelvieira/gap_brazil.

4.2 Specimens collection

During the years 2017 and 2018, artificial plates (made of polyethylene or of a nylon rope wrapped around an iron frame) were deployed along the subtropical Brazilian coast (i.e. in the states of Sergipe, São Paulo, Paraná, Santa Catarina), during three, six and twelve months and kept at an average depth of 1.5 meters. After recovery, the artificial plates, along with the associated fouling, were directly placed in 96% alcohol, which was changed after one day. The animals were sieved through a 500 µm mesh, the amphipods were separated and identified at the lowest possible taxonomic rank using specialized literature^{31,38,54–65}. After identification, some individuals of each species were separated and kept in 96% alcohol at a temperature not higher than 8°C.

4.3 DNA extraction, amplification and sequencing

When available, three specimens of each morphospecies (prioritizing ovigerous females) were used for molecular analysis. Depending on the animal's size, only three pleopods or with the addition of other appendices (i.e., one to three among the pereopods 5-7, one antenna, one gnathopod 2) of each specimen were used for DNA isolation. DNA extractions were performed using the QIAmp DNA Mini Kit (Qiagen) according to the manufacturer's protocols. Polymerase chain reaction (PCR) amplifications at the COI fragment were carried out using two different pairs of universal primers and following thermal cycling conditions illustrated in Table 1. The 25 µl reaction mix consisted of 0.2 mM dNTPs, 0.05 M Betain, 0.5 µM forward and reverse primers, 10 µM PCR buffer, 0.02U/µl Hotmaster Taq (5Prime GmbH), 3 µl template DNA, and molecular biology grade water to bring the mix to the final volume of 25 µl. The PCR product quality was assessed on a 2% agarose gel, sequencing was performed at a contracting sequencing facility (EUROFINS, Germany). All the sequences and metadata (e.g., coordinates) regarding the specimens produced in this study are available in the dataset DS-FOULAMP in BOLD.

Table 1. Reference of primers and cycling conditions used for the PCR amplifications of the COI barcodes

Reference	Primer	Primer Direction (5' – 3')	PCR thermal cycling conditions
Folmer et al. 1994 (65)	LCO1490	(F) GGTCAACAAATCATAAAGATATTGG	1) 94°C (2 min); 2) 36 cycles: 94°C (20 s), 42°C (20 s), 65°C (1 min); 3) 65°C (15 min).
	HCO2198	(R) TAAACTTCAGGGTGACCAAAAAATCA	
Lobo et al., 2013 (66)	LoboF1	(F) KBTCHACAAAYCAYAARGAYATHGG	1) 94°C (2 min); 2) 5 cycles: 94°C (20 s), 45°C (20 s), 65°C (1 min); 3) 35 cycles: 94°C (20 s), 54°C (20 s), 65°C (1 min); 4) 65°C (15 min).
	LoboR1	(R) TAAACYTCWGGRTGWCCRAARAAYCA	

4.4 Alignments, phylogenetic and species delimitation analyses

To check electropherograms for ambiguous bases, remove primers, and to check for stop codons and align the sequences produced in this work, MEGA X ⁶⁶ was used. Sequences of the recently described species *Propejanice lagamarensis* ⁶⁷, which was discovered in the same study, were added to the alignment and dataset. The Barcode Gap Analysis was performed with default settings in BOLD in order to assess possible overlap between intraspecific and interspecific variability. BAGS (i.e. Barcode, Audit & Grade System) ⁶⁸ was used to evaluate if the records for each morphospecies present in BOLD and this study were assigned to an exclusive BIN (grade A, B), with multiple BINs (grade C), insufficient data (grade D) or discordant (grade E). The Maximum likelihood phylogeny was inferred with PhyML ⁶⁹ using an approximate likelihood-ratio test chi squared-based (aLRT chi2-based) for the branch support as it provides results comparable to the bootstrap, but decreasing the computing times ⁷⁰. The best substitution model was tested with the SMS routine in PhyML using BIC as optimality criteria ⁷¹.

The BLAST tool of the National Center for Biotechnology Information website (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) and the Identification engine of BOLD ⁵⁰ were used for each sequence to retrieve similar sequences (i.e. the same BIN or >97% of identity) and information on the geographic distribution of similar haplotypes. For the latter, a more conservative threshold (>99.5% of identity) was chosen to increase the likelihood of a recent (<20,000 years) specimen exchange ⁷². When more than one BIN was found for one morphospecies, sequences belonging to the same species and taxonomically close BINs

(i.e. of the same family when no congeneric species were present), with at least one specimen identified at the genus level, were downloaded from BOLD (accessed 30-09-2020). An alignment for each family or genus (i.e. *Caprella*, *Paracaprella* and *Stenothoe*) was made (Supplementary S1) and collapsed using ALTER ⁷³ to single haplotypes. A phylogeny was inferred for each family, or genus, with the same procedure used for the general one. Four methods were used for species delimitation, two based on genetic distances and two on phylogenetic topology. The first two were the Cluster Sequences Analysis implemented in BOLD, and the Automated Barcode Gap Discovery (ABGD) using simple distances (i.e. p-distances) and default parameters ⁷⁴. The last two analyses were both Poisson tree processes (PTP) ⁷⁵, namely the bayesian PTP (bPTP), run on <https://species.h-its.org/ptp>, and the multi-rate PTP (mPTP) run on <https://mptp.h-its.org> both, using the phylogenies inferred with PhyML. Both methods were run with default parameters ⁷⁶. For bPTP, three different runs were done for each alignment. The trace plots resulting were checked for convergence and the congruence of runs were confirmed.

4.5 Shipping routes

Data on the shipping of all cargo types between South/South-East Brazilian states (i.e. Espírito Santo, Rio de Janeiro, São Paulo, Paraná, Santa Catarina and Rio Grande do Sul) and other countries were obtained from the Brazilian National Waterways Transportation Agency website (www.antaq.gov.br) for the years 2015, 2016, 2017, 2018 and divided into importation and exportation. The quantity of exchanged goods (i.e. tons) were used as proxy of the amount of ships travelling between countries to have a measure of connectivity between regions. These data were then compared with the number of shared species (i.e., >99.5% of genetic identity) retrieved in the previous search (i.e. BLAST).

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CHAPTER II

ANCIENT GLOBETROTTERS - CONNECTIVITY AND PUTATIVE NATIVE RANGES OF TWO COSMOPOLITAN BIOFOULING AMPHIPODS

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Ancient globetrotters - connectivity and putative native ranges of two cosmopolitan biofouling amphipods

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Abstract

The geographic distributions of some coastal marine species have appeared as cosmopolitan ever since they were first scientifically documented. In particular, for many benthic species that are associated with anthropogenic substrata, there is much speculation as to whether or not their broad distributions can be explained by natural mechanisms of dispersal. Here, we focused on two congeneric coastal crustaceans with cosmopolitan distributions – the tube-dwelling amphipods *Jassa marmorata* and *Jassa slatteryi*. Both species are common elements of marine biofouling on nearly all kinds of artificial hard substrata in temperate to warm seas. We hypothesized that the two species' modern occurrences across the oceans are the result of human shipping activities that started centuries ago. Mitochondrial DNA sequences of the CO1 fragment of specimens from

distinct marine regions around the world were analysed, evaluating genetic structure and migration models and making inferences on putative native ranges of the two *Jassa* species. Populations of both species exhibited considerable genetic diversity with differing levels of geographic structure. For both species, at least two dominant haplotypes were shared among several geographic populations. Rapid demographic expansion and high migration rates between geographically distant regions support a scenario of ongoing dispersal all over the world. Our findings indicate that the likely former native range of *J. marmorata* is the Northwest Atlantic, whereas the likely former native range of *J. slatteryi* is the Northern Pacific region. As corroborated by the genetic connectivity between populations, shipping still appears to be the more successful vector of the two species' dispersal when compared to natural mechanisms. Historical invasion events that likely started centuries ago, along with current ongoing dispersal, confirm these species' identities as true "neocosmopolitans".

Keywords: Amphipoda, Biofouling, Biological invasion, Cosmopolitan distribution, Marine dispersal, Marine shipping

1. Introduction

Human-mediated dispersal of organisms can exceed natural mechanisms, carrying species over large distances and often across natural barriers (Ashton et al. 2007; Cohen & Carlton 1998; Galil et al. 2002; Gollasch 2002; Molnar et al. 2008). For example, for as long as vessels have navigated oceans across the globe, non-native marine species have been transported and introduced into new regions as part of the fouling communities on vessel hulls and, for the last 100± years, in ballast water (Carlton 1985; Thiel et al. 2003).

Thanks to the rise of scientific documentation and description, human-mediated introductions of marine species across the globe within the last half-century have been increasingly documented. In clear contrast, the dispersal of marine species in earlier centuries is nearly undocumented and probably a great many introductions since the 1820's have yet to be recognized. Many coastal benthic species that occur in anthropogenic habitats, especially those associated with the hulls of wooden ships (i.e. fouling and boring), had probably been distributed across the oceans by human shipping activities centuries before their geographic distributions were scientifically documented for the first time. As a consequence, modern geographic distributions of some marine species show cosmopolitan occurrences (e.g. Conlan 1990; Thiel & Gutow 2005), classified as "neocosmopolitan" (sensu Darling & Carlton 2018). There is much speculation as to the likely former native

ranges of coastal benthic species, with many still not reliably assigned native or introduced status in parts of their global range (Geller et al. 2010). In parallel, there is mounting evidence from modern taxonomy and molecular research studies which increasingly cast doubt on the existence of marine benthic "eucosmopolitan" species (sensu Darling & Carlton 2018; Havermans 2016).

Here, we focused on two congeneric coastal species that are widely distributed in the world's oceans: the tube-dwelling amphipod crustaceans *Jassa marmorata* Holmes, 1905 and *Jassa slatteryi* Conlan, 1990. Despite both species being common elements of marine fouling communities in harbors ranging from temperate to warm seas, their geographic distributions are not well characterized (Marchini & Cardeccia 2017). *Jassa* species are effective colonizers of clean hard substrata through immigration of nearby adult animals, but primarily by recruitment of drifting juveniles through the water column (Beermann 2014; Franz & Mohamed 1989; Havermans et al. 2007). By these means, *Jassa* species can dominate fouling communities particularly on artificial hard substrates such as navigational buoys, harbour walls, offshore wind farms and ship wrecks, easily reaching densities of more than 1,000,000 individuals/m² and exceeding other taxa in terms of abundance and biomass (Coolen et al. 2018; Franz 1989; Scinto et al. 2007; Zintzen et al. 2008).

The long-standing difficulties in the taxonomy of the genus *Jassa* led to misidentifications and thus, unreliable and questionable species records (Conlan 1989; Conlan 1990). Furthermore, with no historical documentation of the species' native ranges prior to scientific documentation, it was next to impossible to retrace the origin and native ranges of these species. Although the historical invasion pathways of *J. marmorata* and *J. slatteryi* are difficult to reconstruct, given their ecology, we hypothesized that the current "cosmopolitan" distribution of these species is probably the result of 'ancient' invasive events (i.e. prior to scientific documentation and description), mediated by transoceanic shipping activities that started centuries ago.

Molecular approaches have often been used to retrace species' native ranges, particularly in the case of ecologically harmful invasive invertebrate species such as woodwasps (e.g. Boissin et al. 2012) and ladybird beetles (e.g. Lombaert et al. 2011). By using mitochondrial sequence data or shorter fragments such as microsatellites or SNPs, the origins and pathways of invasion or colonization can be retrieved, even in cases of complex histories, independent introductions and subsequent admixtures (e.g. Estoup & Guillemaud 2010). To this purpose, we analysed mitochondrial DNA sequences of the CO1

fragment of the two globally distributed *Jassa* species in order to evaluate their genetic structure and infer their putative former native ranges.

2. Materials and Methods

2.1 Specimen collection and taxonomic identification

Samples were collected opportunistically from artificial substrates (e.g. harbor walls, pontoons, offshore constructions) within distinct marine regions via different sampling methods. A total of 419 specimens were collected, 291 of which were identified morphologically as *Jassa marmorata* and 128 of which were identified as *J. slatteryi*, following the original descriptions provided by Conlan (1990) (Tab. 1).

2.2 DNA isolation, amplification and sequencing

If available, at least 10 specimens (including males, females, and juveniles) from each sampling location were used for molecular analysis. Two to three pereopods (pereopods 5-7, depending on the animal's size) of each specimen were used for DNA isolation. DNA extractions were performed using the QIAmp DNA Mini Kit (Qiagen) according to the manufacturer's protocols. Polymerase chain reaction (PCR) amplifications at the cytochrome c oxidase 1 fragment were carried out using the universal primers LCO1490 (5' GGTCAACAAATCATAAAGATATTGG 3') and HCO2198 (5' TAAACTTCAGGGTGACCAAAAAATCA 3') (Folmer et al. 1994). The 25 µl reaction mix consisted of 0.2 mM dNTPs, 0.5 µM forward and reverse primers, 10 µM PCR buffer, 0.02U/µl Hotmaster Taq (5Prime GmbH), 3 µl template DNA, and mol grade water to bring the mix to the final volume of 25 µl. PCR thermal cycling conditions for amplifying CO1 sequences consisted of an initial denaturation at 94 °C for 2 min, followed by 36 cycles of 94 °C for 20 s, primer annealing at 42 °C for 20 s, extension at 65 °C for 1 min, and a final extension at 65 °C for 15 mins. The PCR product quality was assessed on a 2% agarose gel, sequencing was performed at a contracting sequencing facility (EUROFINS, Germany). Material contributed by J. Coolen was processed using the same protocol as in Luttikhuisen et al. (2019).

Table 1: Sampling by region and species.

Region	Population	Sampling location	Date	<i>J. marmorata</i>		<i>J. slatteryi</i>		Collector
				total no.	sequ. no.	total no.	sequ. no.	
Northern European	Germany	Südhafen, Helgoland	12.06.2017	7	25			J. Beermann
		Südmole, Helgoland	12.06.2017	8				
		Ponton #44, Helgoland	12.06.2017	3				
		Bollwerk 2, Helgoland	12.06.2017	2				
	North Sea	Godewind.28		28	131			R. Krone
		OWF BARD1		1				
		FINO3 structure		2				
		Riffgat wind turbine R20		54				
		Riffgat wind turbine R28		5				
	Iceland	Horns rev trubine G7		41				
		Grindavik	16.08.2018	20	20	21	12	J. Beermann
	Norway	Frei	04.08.2017	16	16			V. Fernández-Gonzalez
		Vigo	01.08.2018	21	12			P. Domingues
	Atlantic Spain/ Lusitanian	A Graña	07.05.2011					J. Guerra-García
		Gijon	03.05.2011			10		
		Puerto America, Cadez	14.02.2011			7		
South West Atlantic	Argentina					10		
		Mar del Plata Port	29.11.2016	10	10			C. Rumbold
North West Atlantic	USA							
		New Haven, Connecticut	12.07.2016	12	8			S. Jungblut
North West Pacific	Japan	Otsuchi Bay, Iwate	27.06.2016			10	10	M. Kodama
		Impo, Yeosu-si	23.06.2011			6		
	South Korea	Muchangpo	28.09.2007			6	7	Y. H. Kim
		Dejuk Island	26.06.2014	2		3		
Mediterranean Sea	Mediterranean Spain	Malaga	31.07.2014			10		V. Fernández-Gonzalez
		Agua Dulce, Almeria	05.29.2014			8		
		San Pedro del Pinatar	30.05.2017	5	19		15	
		Campello	30.05.2017	7				
		Algeciras Bay	30.01.2017	13				J. Guerra-García
		Puerto Palma Mallorca	22.01.2012	2		8		
	North Africa	Ceuta	11.10.2015			10	5	
South East Pacific	Chile	Coquimbo	13.08.2018	15	15	17	17	M. Thiel
	Peru	Melchorita, Cañete	01.04.2017	11	8	3	1	A. Jiménez Campeán

2.3 Sequence editing and alignments

The software CodonCode Aligner v.8.0 (CodonCode Corporation, Deham, MA, USA) was used to check electropherograms for ambiguous bases, remove primers, and to check for stop codons to prevent inclusion of pseudogenes in the analyses. Clustal W (Thompson et al. 1994) implemented in MEGA 7.0 (Kumar et al. 2016) was used to align the resulting sequences and the basic local alignment search tool (BLAST) provided by the National Center for biotechnology information website (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) was used as a sequence control. DNA barcode data from all genetically analyzed specimens were made publicly available in the project "JASSA" in the Barcode of Life Data System (BOLD; Ratnasingham & Hebert 2007) (dx.doi.org/10.5883/DS-JASSA). In addition to the resulting 330 newly edited CO1 sequences (199+131) for the two species obtained for this study, 128 previously published sequences of *J. marmorata* and 16 sequences of *J. slatteryi* were retrieved from GenBank and BOLD. Thus, the complete dataset consisted of 458 DNA

barcodes. Additionally, 44 haplotype sequences of *J. herdmani* (Walker, 1893) from a previous work (Luttikhuisen et al. 2019) were added as outgroup (GenBank accession numbers: MH052599 – MH052642). Detailed information on the specimens and sequences are provided in Table S1.

The full alignment of 657 base pairs (bp) was collapsed to unique haplotypes, subsequently treating gaps as missing data using ALTER (Glez-Peña et al. 2010). A shorter alignment of 589 bp which excluded sequences shorter than 500 bp was chosen for the population analyses that tend to be sensitive to missing data. Finally, an alignment for each species was produced manually, preserving rare haplotypes and deleting shorter sequences of common haplotypes.

Intra- and interspecific species distances were calculated in MEGA 7.0 based on the Kimura-two-parameter (K2P) model with pairwise deletion using the full length of 657 bp sequences; these were then used to estimate genetic divergence between taxa. The K2P model was preferred over more complex and fitting models in order to ensure comparability of the results with those of published literature and other species delimitation analyses (Del Pasqua et al. 2018).

In the program DNASP 5.10 (Librado & Rozas 2009), metrics of genetic diversity within populations of at least three sequences per region and species were estimated by computing indices such as number of haplotypes (H), haplotype diversity (H_d), and nucleotide diversity (π).

To test for departure from neutrality, Fu's F_s (Fu 1997), Tajima's D (Tajima 1989) and mismatch distribution were computed in ARLEQUIN 3.5 (Excoffier & Lischer 2010) for three different spatial scales (population, region and species). The mismatch distribution was tested against the predicted outcome of the models under demographic and spatial expansion in order to give further support to the neutrality tests. Departure from neutrality as well as demographic and spatial expansion would support the hypothesis of neocosmopolitanism whereas stability would support an eucosmopolitan scenario. Fixation indices were used to analyze genetic differentiation between populations (F_{st}), among regions of populations (F_{ct}), and among populations within regions (F_{sc} ; Wright 1949). The F_{st} values were visualized in non-metric multidimensional scaling (nMDS) plots for each species in PRIMER 6 (v. 1.0.3). The significance of uncorrected pairwise F_{st} values were tested by performing 10,100 permutations in ARLEQUIN with the null hypothesis of no differentiation.

In order to test for a possible differentiation among regions or populations, one-way and two-way analyses of molecular variance (AMOVA; Excoffier et al. 1992) were performed in ARLEQUIN with 10,100 permutations for each species. A high degree of differentiation would suggest low connectivity and low genetic flow between populations, disproving the hypothesis of a neocosmopolitan distribution.

As possible differences between populations can be related to the remoteness of some locations, the relationship between geographic and genetic distances was tested with a Mantel test implemented in ALLELES IN SPACE (AIS; Miller 2005) with 10,000 permutations. Geographic distances were calculated between coordinates regardless of geographic barriers (such as continents, currents). This was set to provide good approximation and strength to the other tests and to exclude simple spatial correlation.

In addition to a putative poor differentiation, a high degree of migration between populations can provide further information on the present connectivity between populations, supporting a neocosmopolitan scenario. Migration rate among regions was assessed using MIGRATE-N 3.6.11 (Beerli 2006) on the CIPRES Science Gateway (Miller et al. 2010). By using a coalescent approach and Bayesian Markov chain Monte Carlo (MCMC) method, MIGRATE-N quantified both population size ($\theta = 2N_e\mu$ for haploid mtDNA) and migration rate ($M=m/\mu$). The product is the number of effective migrants per generation ($2N_em$). The probabilities were estimated using 10 replicates, wherein each replicate was run for 50,000,000 generations and sampled every 1,000 steps, thereby recording a total of 50,000 steps. Each replicate included four MCMC chains with relative temperatures of 1.0, 1.5, 3.0 and 100,000. Convergence was assessed to ensure that the effective sample size (ESS) was higher than 200. Migration rates were only considered when 95% of the confidence intervals (CIs) were different from zero.

A haplotype network was computed for each species using an alignment of 372 sequences and 636 bp for *J. marmorata*, and 85 sequences and 595 bp for *J. slatteryi* with the median-joining method (Bandelt et al. 1999) in the software Popart (v. 1.7).

2.4 Phylogenetic analyses and ancestral range estimations

Phylogenetic relationships among the *Jassa* species were reconstructed using only unique haplotypes with maximum likelihood (ML) and Bayesian inference (BI) of the CO1 gene fragment. The ML was inferred with PhyML (Guindon et al. 2010) using 1,000 bootstraps for the branch support. The best substitution model (GTR+I+G) was tested with the SMS routine in PhyML using both AIC and BIC as optimality criteria (Lefort et al. 2017).

A time-calibrated BI phylogeny was reconstructed in BEAST 2.5.2 (Bouckaert et al. 2014) on XSEDE (Townes et al. 2014). The GTR model of evolution, with proportion of invariant (I) and gamma shape parameters (G), determined with bModelTest (Bouckaert & Drummond 2017), and the Yule speciation model were set for priors. An uncorrelated relaxed clock with log-normal distribution was applied following the specifications proposed by Copilaş-Ciocianu et al. (2019); i.e. rates ranged from 0.7 to 1.7% Ma⁻¹ with a starting value of 1.2%. Three runs each of 20,000,000 iterations of MCMC sampled each 1,000 iterations were performed. All runs were examined using Tracer v1.7.1 and all sampled parameters achieved sufficient sample sizes (ESS>200). Tree files were combined using LogCombiner on XSEDE (1.8.4) with 15% of burn-in. The maximum clade credibility tree was generated using TreeAnnotator on XSEDE. All the XSEDE analyses were performed on the CIPRES Science Gateway (Miller et al. 2010).

The ancestral ranges of the haplotypes were estimated with the R package BioGeoBears (Matzke 2013; R Development Core Team 2018). This package is used to perform biogeographic inferences such as the estimation of ancestral ranges of species or Operational Taxonomic Units (OTUs) under different hypotheses (e.g. dispersion, founder-event speciation). Furthermore, BioGeoBears allows for the implementation of a third parameter (J) which permits a "jump speciation" in the daughter lineage, resulting in a possible different area from the direct ancestor. This feature accounts for the biology of *Jassa* species in this study by virtually including anthropogenic dispersal. The analyses were run using the DEC and DEC+J models (Matzke 2014) and a maximum of five (*J. marmorata*) and four (*J. slatteryi*) areas of possible occurrence as this was the maximum number of regions where a single haplotype was found for each species respectively. In order to avoid overinterpretation, only the nodes with at least 70% of bootstrap support and 90% of posterior probabilities were considered. The best-fitting model was selected based on Akaike's information criterion (AIC).

3. Results

The distance between species was 15.2% $\pm$ 1.7 and the average distance within species accounted 0.17% $\pm$ 0.07 for *J. marmorata*, and 0.57% $\pm$ 0.17 for *J. slatteryi*.

3.1 Genetic diversity and population structure

The alignment consisted of CO1 sequences from 475 individuals (Tab. S1). Across the entire data set, 110 sites were identified as polymorphic, 15 were singletons, and 95 were parsimony informative (comprising 36 unique haplotypes). With respect to the overall genetic diversity (all regions combined), *Jassa marmorata* (19 haplotypes) exhibited lower haplotype diversity ($H_d = 0.492$) and lower nucleotide diversity ($\pi = 0.002$) than *J. slatteryi* (17 haplotypes; $H_d = 0.804$; $\pi = 0.006$) (Tab. 2).

Within *J. marmorata*, the haplotype diversity was variable throughout marine regions, ranging from 0.884 in the NW Atlantic region, to 0.305 in the NE Pacific region. Nucleotide diversity was low among the regions, ranging from $\pi = 0.001$ in the NE Atlantic to $\pi = 0.006$ in the NW Atlantic region.

Table 2: Statistical results for *J. marmorata* and *J. slatteryi* for each marine region and sampling location. Total number of specimens (N), number of haplotypes (H), haplotype diversity (Hd), nucleotide diversity (π), Tajima's D (D) and Fu's Fs (Fs). Significant results are highlighted in bold (D confidence level < 5%; Fs confidence level < 2%). "-" = population with less than 3 specimens.

	Region	Location	N	H	Hd	π	D	Fs
<i>Jassa marmorata</i>	All		390	19	0.492	0.002	-2.101	-11.097
	Mediterranean Sea	Spain, Mediterranean	18	3	0.523	0.002	0.642	1.974
	NE Pacific	All	109	4	0.305	0.001	-0.621	0.382
		California, USA	78	3	0.378	0.001	0.111	2.001
		Oregon, USA	31	2	0.067	0.000	-1.147	-1.211
	NE Atlantic	All	211	3	0.363	0.001	0.118	0.419
		Spain, Atlantic	8	1	0.000	0.000	0.000	0.000
		North Sea	167	2	0.340	0.001	0.976	1.685
		Norway	16	2	0.533	0.001	1.529	1.362
		Iceland	20	3	0.426	0.001	-0.440	-0.377
	NW Atlantic	All	20	9	0.884	0.006	-0.433	-1.007
		Connecticut, USA	12	7	0.864	0.003	-1.304	-3.413
		New York, USA	2	2	-	-	-	-
		Virginia, USA	4	2	0.500	0.008	-0.829	3.777
		Nova Scotia, Canada	1	1	-	-	-	-
		Maine, USA	1	1	-	-	-	-
	NW Pacific	South Korea	1	1	-	-	-	-
	SE Pacific	All	22	4	0.541	0.003	-1.504	1.751
		Chile	14	1	0.000	0.000	0.000	0.000
		Peru	8	3	0.464	0.004	-1.741	2.085
	SW Atlantic	Argentina	10	3	0.644	0.001	0.222	-0.046
<i>Jassa slatteryi</i>	All		85	17	0.804	0.006	-0.734	-3.251
	Mediterranean Sea	All	20	6	0.726	0.006	0.324	1.283
		Northern Africa	4	3	0.833	0.006	-0.817	0.961
		Spain, Mediterranean	16	4	0.617	0.004	0.836	1.751
	NE Atlantic	Spain, Atlantic	12	3	0.621	0.006	2.149	4.214
	NE Pacific	California, USA	13	5	0.731	0.005	-0.738	0.865
	NW Atlantic	New York, USA	1	1	-	-	-	-
	NW Pacific	All	17	5	0.680	0.022	-2.469	7.377
		Japan	10	2	0.356	0.001	0.015	0.417
		South Korea	7	4	0.857	0.049	-1.751	5.445
	SE Pacific	All	19	3	0.433	0.002	0.035	1.524
		Chile	18	2	0.366	0.002	0.718	3.088
		Peru	1	1	-	-	-	-
	SW Atlantic	Argentina	2	2	-	-	-	-

The haplotype diversity of *J. slatteryi* also exhibited a wide range. The highest regional value of Hd = 0.731 occurred in the NE Pacific (California) while the lowest was detected in the SE Pacific (Hd = 0.433). Nucleotide diversity ranged from π = 0.022 in the NW Pacific to π = 0.002 in the SE Pacific (Tab. 2).

Table 3: Statistical results of AMOVA tests for *J. marmorata* and *J. slatteryi*. Significant results ($p < 0.005$) are highlighted in bold.

	Source of variation	df	SS	Variance components	Variation (%)	FCT	FSC	FST
<i>Jassa marmorata</i>	One-way	Among regions	5	40.67	0.16	Va	31.32	
		Within regions	380	137.01	0.36	Vb	68.68	
		Total	385	177.67	0.52			0.3132
	Two-way	Among regions	5	40.67	0.04	Va	8.07	0.0806
		Among populations	6	27.08	0.19	Vb	35.81	0.3895
		Within population	374	109.93	0.29	Vc	56.12	0.4387
		Total	385	177.67	0.52			
<i>Jassa slatteryi</i>	One-way	Among regions	4	34.96	0.47	Va	28.16	
		Within regions	75	90.85	1.21	Vb	71.84	
		Total	79	125.81	1.69			0.2815
	Two-way	Among regions	4	34.96	-0.12	Va	-7.33	-0.073
		Among populations	2	12.95	0.74	Vb	43.91	0.409
		Within population	73	77.91	1.07	Vc	63.43	0.366
		Total	79	125.81	1.68			

3.2 Demographic analysis

Tajima's D values were negative and significant for each respective species in total; this indicates an excess of rare nucleotide site variants which suggests a departure from the Wright-Fisher neutral model (Wright, 1931; Tab. 2). The Fu's F_s test, which is based on the distribution of haplotypes, showed a significant negative value for *J. marmorata* in total, indicating an excess of rare haplotypes.

The mismatch distribution analysis was in concordance with D and F_s results, for the two species, which supports the spatial and demographic expansion scenarios for both species (Tab. S2; SSD and Raggedness index).

J. marmorata did not display departure from the null hypothesis of demographic expansion for any other region except for Peru and the Northern European Seas where no significant spatial expansion was detected (Tab. S2; SSD and Raggedness index). In contrast, *J. slatteryi* displayed a significant deviation from the null model of

demographic expansion for the Mediterranean Sea as well as the North and South East Pacific, whereas there was no significance for a spatial expansion (Tab. S2; SSD and Raggedness index).

3.3 Geographical structure

The one-way AMOVA revealed significant differences among regions for both species. However, when exploring the variation among populations in the two-way test, the within-region variation was not significant (Tab. 3). While the main contribution to molecular variance was found within populations, the variation among populations within the same regions was also significant in both species.

In *J. marmorata*, specimens from the SW Atlantic showed the highest level of differentiation within NE Pacific and Northern European seas ($F_{st} = 0.71$ and 0.81 respectively). In the comparison of populations, two main groups were distinguished within *J. marmorata*: a general group with the majority of the populations, and another with Argentina and Chile. The Virginia population showed the highest average differentiation and appeared to be an outgroup (average $F_{st} = 0.776$; Fig. 1; Tab. S3, S4).

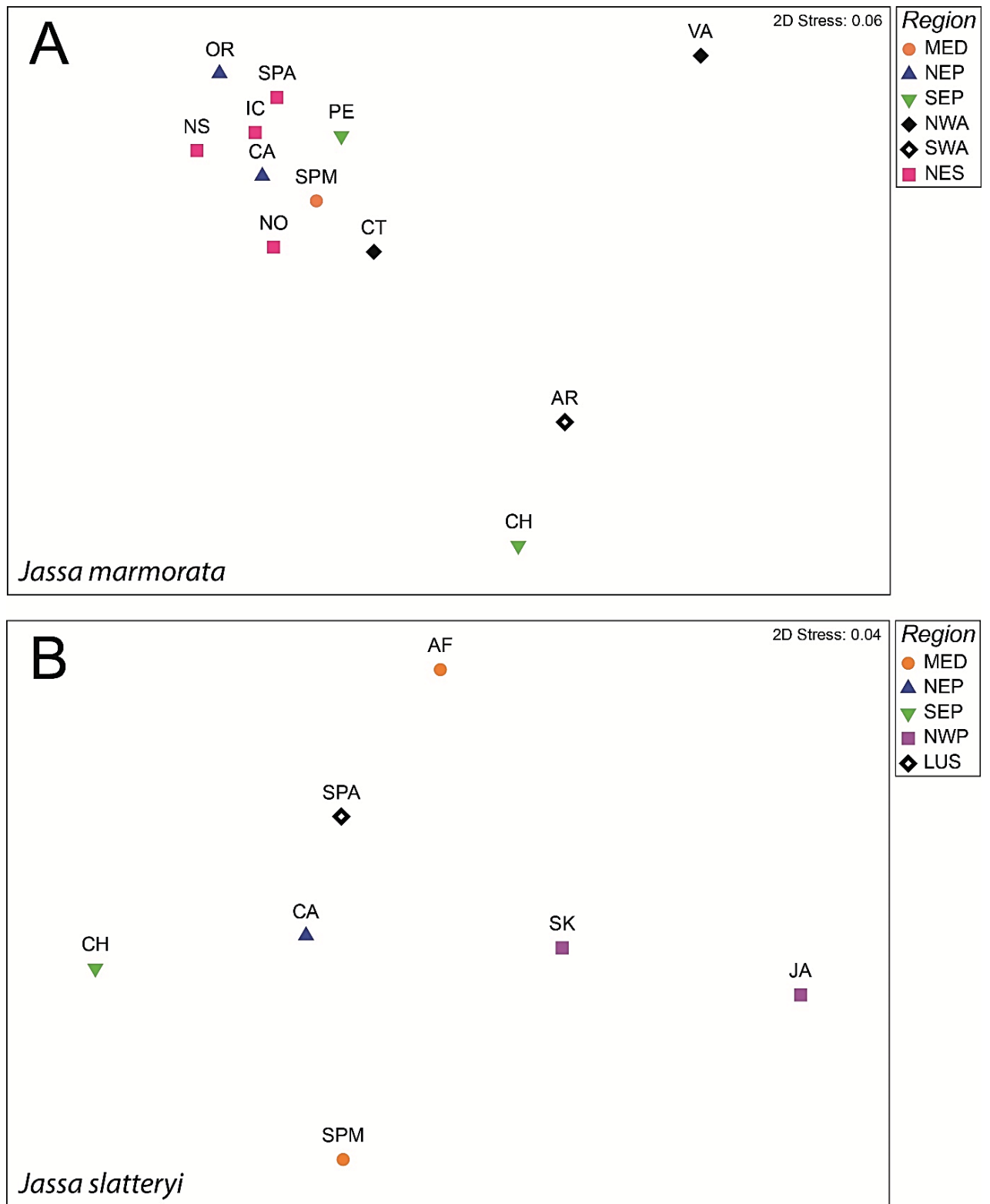


Figure 1: nMDS plots of F_{st} values between populations *J. marmorata* (A) and *J. slatteryi* (B). Abbreviations: MED = Mediterranean Sea, NEP = North East Pacific, SEP = South East Pacific, NWA = North West Atlantic, SWA = South West Atlantic, NES = Northern European seas, LUS = Iberian Peninsula.

J. slatteryi showed an overall low level of differentiation on the regional scale which was only significant for the comparison between Lusitania (Atlantic Spain) and the NE

Pacific (California) (Tab. S3, S4). Moreover, there was no obvious pattern with Japan which displayed the highest average F_{st} ($F_{st} = 0.576$; Fig. 1; Tab. S4).

For both species, only few migration rates met the assumptions ($95\% \text{ CI} > 0$; Tab. S5) and were considered in the migration model among regions. The migration between populations displayed contrasting patterns. In *J. marmorata*, no emigration was observed from Chile, but from almost every other population. In contrast, for Connecticut and Virginia there was only one population donor although multiple receivers were detected (Fig. 2; Tab. S5, S6).

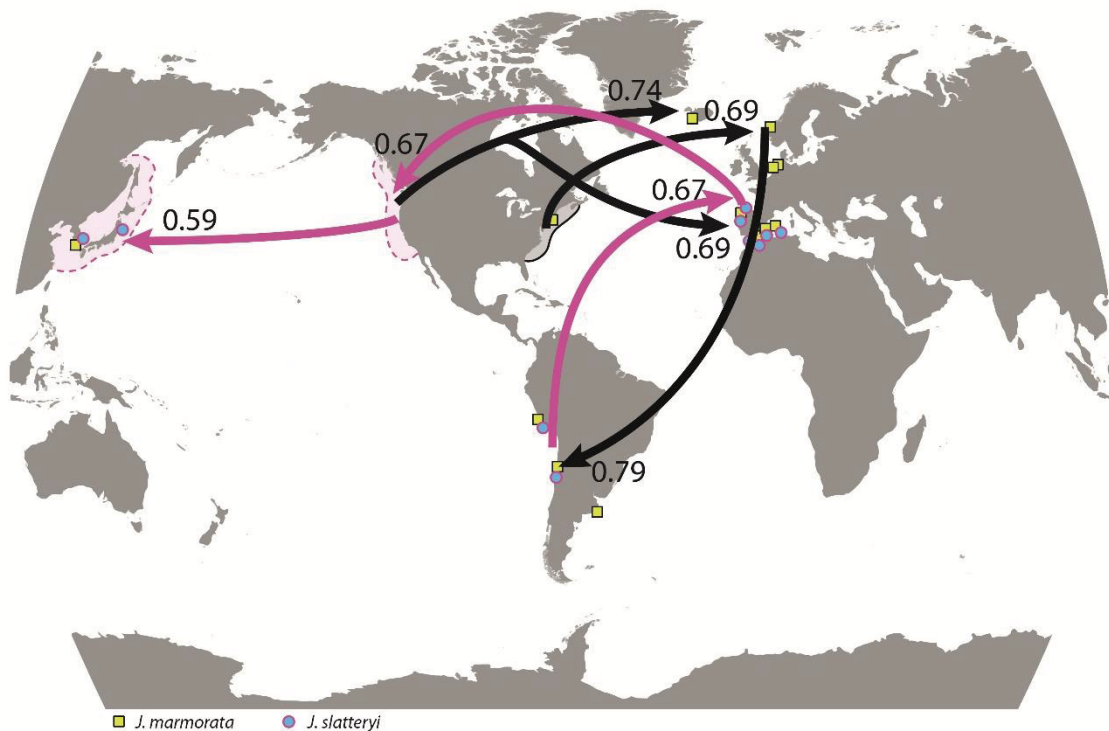


Figure 2: World map with highest significant migration rates. Rates among populations of *J. marmorata* (black) and *J. slatteryi* (purple) from non-adjacent marine regions. Symbols indicate sampling localities for each species made for this study (i.e. excluding sampling localities of previously published sequences). Patterned areas indicate the putative native ranges of *J. marmorata* (continuous black line) in the North West Atlantic and of *J. slatteryi* (dashed purple lines) in the North Pacific.

For *J. slatteryi*, neither immigration nor emigration for Mediterranean samples (Spain) and for the NW Pacific populations met the assumptions of the migration model; conversely, more emigrants were found from South Korea to Japan (Fig. 2; Tab. S5).

The haplotype network of *J. marmorata* was star-like with two main haplotypes, one of which occurred in almost every region sampled except the NW Pacific and SW Atlantic (Fig. 3). The second abundant haplotype occurred mostly in the NE Atlantic with additional records from the NW and SW Atlantic. Moreover, only three haplotypes were separated by

more than two mutations from either of the two central haplotypes (i.e. SE Pacific: 10, NW Atlantic: 8, NW Pacific: 4), while the highest distance was observed between SE Pacific and NW Atlantic haplotypes (19 steps). On the other hand, the network of *J. slatteryi* was much more dispersed in comparison and did not show any clear pattern of (geographic) segregation but included multiple missing haplotypes and three haplotypes which were present in more than two regions (Fig. 3).

There was no significant correlation between genetic and geographic distances in either *J. marmorata* or *J. slatteryi* ($p > 0.1$).

The phylogeny was poorly resolved, showing high support (posterior probabilities = 1; bootstrap support > 95) for the cluster grouping *J. marmorata* and *J. slatteryi* as well as each species (Fig. S1).

The DEC+J model significantly increased the likelihood of the DEC model in both *J. marmorata* (lnL: DEC+J = -109.76; DEC = -153.97) and *J. slatteryi* (lnL: DEC+J = -58.45; DEC = -78.23) (Tab. S7).

In *J. marmorata*, while the reconstruction of the most recent common ancestor's (MRCA of all the haplotypes) geographic range was inconclusive, it seemed to exclude the Mediterranean Sea and SW Atlantic but an ancestral divergence of a clade in the NW Atlantic was evident (Fig. S2).

Similar to *J. marmorata*, it was not possible to determine the ancestral geographic range of the MRCA of *J. slatteryi* even though in the Pacific it appeared to be comparatively more diverse. One clade was restricted to the Mediterranean Sea (Fig. S3).

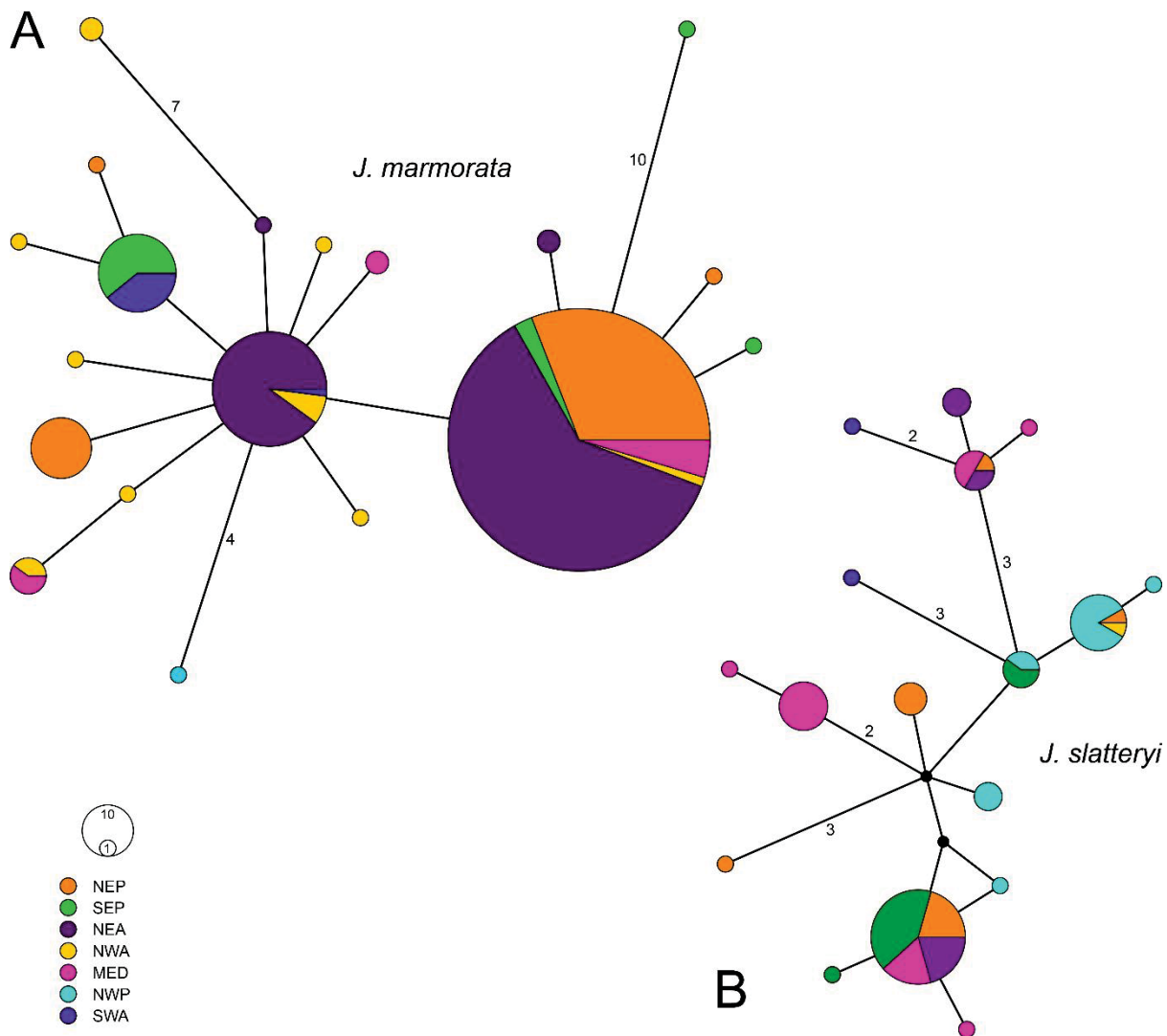


Figure 3: Median joining haplotype networks of *J. marmorata* (A) and *J. slatteryi* (B). Size of the circles are proportional to the number of specimens with relative haplotype. Black dots represent missing haplotypes, more than one mutation is reported with numbers. Abbreviations: NEP = North East Pacific, SEP = South East Pacific, NEA = North East Atlantic, NWA = North West Atlantic, MED = Mediterranean Sea, NWP = North West Pacific, SWA = South West Atlantic.

4. Discussion

Using the molecular mitochondrial marker CO1, we were able to reconstruct putative native regions (i.e. prior to their 'ancient invasion') of two cosmopolitan amphipods that are common elements of marine biofouling. The examination of the genetic variability between these two species in our analyses indicated high levels of intraspecific divergence within the *J. slatteryi* populations, as well as comparable variation within populations and among populations within regions for *J. marmorata*. A high haplotype number was detected within *J. slatteryi* when compared to *J. marmorata*, and at least two main haplotypes within each species were shared among several geographic populations.

4.1 Genetic population structure and connectivity of *Jassa marmorata*

The results indicated that the overall percentage of variation present in *J. marmorata* populations was derived almost equally from both variation within populations and variation among populations within marine regions. The highest genetic diversity (accounting for both haplotype and nucleotide diversity values) was found in the NW Atlantic region, and the lowest occurred in the NE Pacific region.

Previous research involving *J. marmorata* samples taken from assumed potential source populations along the Atlantic coast of the United States showed varying results: one population from coastal Connecticut revealed no genetic diversity at all in the population, whereas the samples analyzed from coastal South Carolina were shown to have been derived from a separate *Jassa* species-level lineage (Pilgrim & Darling 2010; JB and AD personal observation). Pacific North American coast populations of *J. marmorata* are classified as invasive and are assumed to be transfers from its native range of the Atlantic coast of North America (Chapman 2007; Conlan 1990).

In contrast to Pilgrim and Darling (2010), the results of the current study showed high genetic diversity in the presumed native range of *J. marmorata* as well as in populations from the SW Atlantic region. Nevertheless, the presence of a shared haplotype between the SW Atlantic and the SE Pacific region could signal a connection between the two. This is in accordance with the recent first record of *J. marmorata* in Argentina (Alonso de Pina 2005).

Given that introduction events transfer only a subset of native genetic diversity, invasive populations are frequently seen to exhibit lower diversity than the native source populations (Dlugosch & Parker 2008). The gene pool of introduced populations is expected to be limited due to the stochastic process of introduction mechanisms (Holland 2000). *Jassa marmorata*'s low genetic diversity in the NE Pacific is thus consistent with this expected founder effect (Pilgrim & Darling 2010). Overall haplotype and genetic diversity were lower in *J. marmorata* populations when compared to *J. slatteryi* populations despite a larger sample size. The overall genetic diversity of *J. marmorata* populations was characterized by high haplotype diversity and low nucleotide diversity, indicating only small differences between haplotypes. This is evident from the minimum spanning haplotype network which showed mostly single nucleotide differences between haplotypes in *J. marmorata*.

The obtained data for *J. marmorata* indicated differing levels of geographic structure within the sampled populations; there appeared to be substantial geographic structure within some populations (e.g. Virginia, Chile, Argentina, New York, the North Sea and Oregon)

whereas other populations were less structured (Spanish Mediterranean coast and Connecticut). These high levels of structure suggest a more limited gene flow or different selective pressures. Some *J. marmorata* populations were not panmictic throughout a marine region, but displayed high F_{st} values, indicating an absence of genetic connectivity between them. Populations from Chile-Peru as well as Connecticut-Virginia, on the other hand, could each be assigned to the same marine regions, and were characterized by high heterozygosity values. *Jassa marmorata* populations from California and Oregon exhibited genetic connectivity with populations in Norway and Iceland, which is unlikely to result from natural dispersal. Furthermore, negative Tajima's D , Fu's F_s statistics, as well as the results of the mismatch distributions for *J. marmorata* indicated a population size expansion, perhaps after a bottleneck. It can be assumed that the amount of samples was rather limited considering that the genetic diversity of these populations has probably been shaped largely by stochastic events. A lack of samples from entire marine regions remains an open issue that impedes clear inferences on the connectivity of globally distributed populations.

4.2 Genetic population structure and connectivity of *Jassa slatteryi*

The magnitude of genetic variation within *J. slatteryi* was highest within populations and lowest among marine regions. In *J. slatteryi*, there was discordance between haplotype and nucleotide diversity. Haplotype diversity was higher in the Mediterranean Sea and the NE Pacific whereas nucleotide diversity was higher in the NW Pacific. The lowest genetic diversity was found in the SE Pacific region.

Jassa slatteryi had been previously considered to be either cryptogenic or introduced to the NE Pacific (Boyd et al. 2002). Populations of *J. slatteryi* from the Pacific North American coast had been found to show similar genetic diversity indices, haplotype diversity measures, and intraspecific genetic distances as the presumed native species from that region, *J. staudei* (Chapman 2007; Pilgrim & Darling 2010). Both species exhibited diversity measures much higher than *J. marmorata* in the same area. The authors suggested that perhaps the Pacific coast of North America could represent the native range of *J. slatteryi* even though their study was geographically restricted to the continental U.S. coasts.

Our results corroborate the previous assumption that *J. slatteryi* originated from Northern Pacific regions (NE and NW). Surprisingly, there was also high genetic diversity in the populations from the Mediterranean Sea region. The highly divergent haplotypes found in the Mediterranean Sea and the private haplotypes from the SW Atlantic might be artifacts due to small sample sizes and a resulting undersampling in the putative native regions due

to the nature of our opportunistic sampling campaign. The overall genetic diversity of *J. slatteryi* populations was different from that of the *J. marmorata* populations as the overall nucleotide diversity was higher, indicating larger differences between haplotypes. This was displayed by the haplotype network where the respective haplotypes were separated by more mutations than in *J. marmorata*.

Populations of *Jassa slatteryi* also varied in their level of geographic structure, ranging from highly structured populations (Japan) to lower levels of structure (California, Spanish Atlantic coast, Chile, and South Korea) and higher gene flow between populations. In contrast to *J. marmorata* populations which showed only limited or no gene flow between populations of the same marine region, *J. slatteryi* populations did not reflect this structure between populations within the same regions. Furthermore, our findings indicate gene flow between geographically remote populations over large distances which is highly unlikely via natural dispersal (e.g., Mediterranean and Spanish Atlantic coasts with South Korea and/or Chile). The mismatch distribution for *J. slatteryi* suggested a geographic expansion but not a demographic expansion.

We identified potential connectivity and dispersal differences between *J. marmorata* and *J. slatteryi* populations in regions where both species co-occurred. *J. slatteryi* populations in Chile, California, South Korea, the Spanish Atlantic and the Mediterranean coasts were particularly closely related to each other (evident by very low F_{st} values), whereas the *J. marmorata* population from Chile showed more structure with very little gene flow between this and other populations. The F_{st} values also indicated that, for example, while there is measurable gene flow between *J. marmorata* populations from the neighboring regions of Chile and Argentina, populations of *J. slatteryi* from the same regions are not panmictic. However, both *Jassa* species showed evidence of gene flow between the Mediterranean coast of Spain and California. Both *J. marmorata* and *J. slatteryi* exhibited high haplotype diversity and low nucleotide diversity, indicating rapid demographic expansion from a small effective population size (i.e. contrasting the mismatch results in *J. slatteryi*; Avise 2000).

The scenario of ongoing dispersal and continuous input of new propagules in different marine regions all over the world is also supported by the inconclusive results of the ancestral range reconstruction of both species, confirming these species' true identities as "neocosmopolitans".

4.3 Origin and possible pathways of introduction

Maritime shipping over the past centuries has facilitated dispersal of attached fouling species on the hulls of vessels, and for the last 100± years, transported larvae and other propagules in ballast water around the world. Consequently, this facilitates gene flow between populations, particularly those with non-planktonic larvae (Carlton & Hodder 1995; Carlton 1985; Carlton 1987; Williams et al. 1988). These unintentional introductions have increased rapidly in recent decades as transit times have diminished, ship-carried trade has become globalized, and ballast-water volumes have risen (Carlton & Geller 1993; Ruiz et al. 1997; Williams & Grosholz 2008). Despite the increase in introduction of non-native species by means of maritime traffic, shipping pathways for species dispersal remains poorly understood on a global scale (Mack et al. 2000). Seebens et al. (2013) identified major shipping routes that are high-risk pathways for bioinvasions and also determined that the highest invasion risks were concentrated in a small number of ports. These invasion pathways help explain the geographic distribution of the *Jassa* populations in our study.

The ability of *Jassa* to build tubes on anthropogenic substrata and the absence of planktonic larval stages are excellent prerequisites for human-mediated transport by ships. Accordingly, specimens of both *J. marmorata* and *J. slatteryi* have been reported from hull fouling of ships and boats in different parts of the world (Castro et al. 2020; Martínez-Laiz et al. 2019; Peters et al. 2019). *Jassa marmorata* has long been recognized in the literature from worldwide locations (see Marchini & Cardeccia 2017). Although the native origin of *J. marmorata* was uncertain, the worldwide distribution of currently known *Jassa* species indicated that it could be native to the NW Atlantic region where *J. marmorata* occurs as the only species of the genus (Conlan 1990; Marchini & Cardeccia 2017). This hypothesis is supported by our findings. Although already Conlan (1990) noted a wide global distribution of *J. slatteryi*, records of this species after Conlan's original description have increasingly been reported in the past recent years - for example from Europe (Bonifazi et al. 2018; Gouillieux 2017) or South America (Rumbold et al. 2015). This is probably due to an increasing awareness of this group's taxonomy, which is corroborated by the standardized utilization of DNA barcoding for species identification. Hence, *Jassa slatteryi* had been classified as cryptogenic species with uncertain native origin (Marchini & Cardeccia 2017) but our findings indicate that the species' likely former native range is the Northern Pacific.

Jassa species were most probably transported by ship traffic between NE Atlantic ports and ports in South America (e.g. Argentina, Chile, Peru). The results of this study indicate that these shipping routes played a major role for the establishment of both *J.*

marmorata and *J. slatteryi* populations in the SE Pacific and SW Atlantic regions. The models of Seebens et al. (2013) also suggest that although the northern European Seas (NE Atlantic in our study) are recently most strongly connected to tropical and subtropical ecosystems in terms of marine traffic, with the adjacent NW Atlantic waters providing similar enough climatic conditions. Therefore, the North American Atlantic coast still dominates as the major source region of invasions into the NE Atlantic for coastal benthic species. The northern Pacific waters are also source regions for invasions to the NE Atlantic. This could explain the presence of both *J. marmorata* and *J. slatteryi* in the NE Atlantic region. Our data indicate both *Jassa* species occur along the West and East coast of the United States, which is likely due to the connection of both coasts via shipping routes through the Panama Canal. This high invasion risk canal is also passage for shipping routes to and from the NE Atlantic regions (Seebens et al. 2013). The *Jassa* populations from Argentina could have been transported there by way of the Spanish Atlantic and Mediterranean coast, as well as North Africa, and there is a high invasion probability between the NE Pacific region and the SE Pacific region (e.g. Peru and Chile).

5. Conclusions

As a result of speculative knowledge of 'ancient' invasion events and historically unsettled taxonomy, we cannot determine with certainty through which series of events these *Jassa* species achieved their neocosmopolitan distribution. It is most likely that the species' distributions are a result of human-mediated introductions which started centuries ago. Our findings indicate that the likely former native range of *J. marmorata* is the NW Atlantic region, and the likely former native range of *J. slatteryi* is the Northern Pacific (NW and NE) region. Due to the relatively fast travel speeds, extensive surface area, and independence of stochastic ocean currents (Haydar 2012), shipping still appears to be the more successful vector of *Jassa* species dispersal on a global scale as compared to natural dispersal on a local level (e.g. rafting or drifting; Thiel & Haye 2006). As with these two successful biofoulers, the occurrence of many other coastal benthic species with wide modern geographic distributions is probably the result of human shipping activities over the last centuries.

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CHAPTER III

HITCH-HIKERS' COMMUNITY: TAXONOMIC, PHYLOGENETIC AND FUNCTIONAL DIVERSITY AMONG PORTS AND ESTUARIES

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Hitch-hikers' community: taxonomic, phylogenetic and functional diversity among ports and estuaries

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Abstract

Marine fouling communities, are known to rapidly develop on artificial substrates, decreasing floatability of buoys, obstructing pipelines and modifying the hydrodynamics of ship hulls. Fouling has been recognised globally as a stepping-stone for the introduction of non-indigenous species (NIS), that once arrived in ports, may also spread to neighbouring natural habitats. In this case study, the biodiversity of fouling amphipods was studied including three facets of diversity (i.e. taxonomic, phylogenetic and functional). Following a mixed experimental design with fixed (i.e. the presence of international ports and the two seasons) and random factors (i.e. six different sites and three exposure times), settlement plates were deployed in four South Brazilian estuaries and retrieved after different periods of time. We also explored spatial and temporal modularity and diversity of respective communities along environmental and anthropogenic drivers (such as salinity, temperature, wind speed or distance from ports). Seasonality strongly affected the community structures and connectivity across the region. Possible effects of port vicinity were frequently masked by environmental and climatic variables although estuaries that harboured ports acted as connectors and hubs of species for regional fouling. Furthermore, the intensity of shipping was the major predictor of the variation in beta diversity. Finally, the lack of correlation between functional dispersion and the phylogenetic diversity for estuaries with ports (but significant for the ones without), underline how fouling communities are functionally

homogeneous despite environmental stressors, and how anthropogenic disturbance may select a specific combination of traits.

Key words: fouling community, amphipods, biodiversity, beta diversity, networks

1. Introduction

The fouling community, growing on artificial submerged substrates, is common in environments subjected to anthropic impact. It is mainly known for its sessile portion (e.g. algae, bryozoans, barnacles, benthic cnidarians, ascidians, tubicolous polychaetes, bivalves) and only marginally for its vagile organisms (e.g. planarias, gastropods, amphipods, isopods). The peculiarity making the fouling a good study community is that early colonizers, that may be substituted later by climax species in natural habitats (Connell & Slatyer 1977), never cease to prevail in this assemblage sustained by a constant anthropic disturb (e.g. introduction propagules, mechanical removal, pollutant), coupled with natural stressors (e.g. wave effects, salinity abrupt variations). Artificial substrates also harbour different, less rich and less diverse assemblages, compared to natural reefs, being commonly dominated by non-indigenous species (NIS) (Sanabria-Fernandez et al. 2018). The continuous increase in coastal modification, brought upon by the urbanization, has been greatly contributing to the global homogenization of different communities either terrestrial or aquatic (McKinney 2006, Airoidi et al. 2015, Schütz & Schulze 2015), promoting generalist species and damaging native ones. Furthermore, the either active and voluntary or the accidental human-mediated translocation of species to a new environment (i.e. introduction of NIS), otherwise non-reachable naturally (e.g. geographic and/or environmental barriers), has been contributing greatly to the disruption of native communities and consequent ecosystem functions loss (Byers 2002).

Port areas are among the most impacted in marine coastal ecosystems, being dominated by artificial substrates, dredging events, oil spill and acting as 'sink' of NIS being constantly supplied with new propagules from international shipping (Gollasch 2006, Seebens et al. 2013). Besides, ports are commonly built in estuaries where the eutrophication and the salinity variation facilitate species more tolerant instead of more sensitive (Zalmon et al.

2011). For these reasons, they play an important role in the diffusion of NIS, connecting distant environments and operating as stepping stones (Bulleri & Airoidi 2005, Kölzsch & Blasius 2011, Valdor et al. 2020).

There has been a recent drive among ecologists to understand communities not only in terms of their classical taxonomic composition (i.e. taxonomic diversity) but exploring their evolutionary structure (i.e. phylogenetic diversity) as well as the ecological functions taking place in (or rendered by) them (i.e. functional diversity) (Péru & Dolédec 2010, Flynn et al. 2011, Miller et al. 2018). Explore the phylogenetic and functional diversity allows to directly depict what are the principal drivers of a community and how they affect it (i.e. environmental filtering or niche partitioning), complementing phylogenetic relationships with specific information about traits selection taking place (Best & Stachowicz 2013, Best et al. 2013). Different indices related to functional and phylogenetic diversity have been proposed (e.g. Faith 1992, Schmera et al. 2009, Laliberté & Legendre 2010, Scheiner et al. 2017, Mammola & Cardoso 2020) and the relationship between these two facets of the biodiversity (or considered independently) have demonstrated to successfully address hypotheses regarding diverse fields such as anthropogenic impact, evolution or environmental stressors (e.g. Safi et al. 2011, Cardoso et al. 2014, Gusmao et al. 2016, Tucker et al. 2018). Furthermore, the use of beta diversity, that is, the degree of dissimilarity in the assemblage of communities along spatial and environmental gradients (Whittaker 1960, Anderson et al. 2011, see Legendre 2019 for a temporal definition of beta diversity) has been recently integrated with phylogenetic and functional diversity data (Carlo & Sabina 2008, Graham & Fine 2008, Leprieur et al. 2011, Villéger et al. 2013, Cardoso et al. 2014). Beta diversity can be decomposed in two additional sources of dissimilarity, turnover (or replacement) and richness difference (i.e. the net loss/gain of species), each reflecting specific processes acting on communities.

To understand how this “artificial community” may help predicting the cumulative degree of disturbance in estuaries, we hypothesized a strong negative effect of port vicinity on estuaries. While 'classical' diversity indices may provide an incomplete picture, this effect should be visible as a higher functional redundancy in port areas. This was tested in an integrative approach on amphipod fouling assemblages of southern Brazil estuaries. These crustaceans are important members of the fouling community, being rich in cosmopolitan and pioneer species, with functionally generalists (e.g. tubiculous and omnivore) dominating the whole community sometimes even in biomass (Beermann 2014, Ros et al. 2020). Using these crustaceans as a case study, we aimed to evaluate the supposedly high connection

of the fouling communities among estuaries assessing their spatial and temporal variability. through network analysis. Implementing a mixed experimental design, with fixed and hierarchical (i.e. random) factors, we expect to further discern the seasonal effect related to environmental fluctuations and the spatial variability of the diversity. Lastly, through the integration of all the levels of beta diversity (i.e. total, richness and replacement) on three the facets of the biodiversity (i.e. taxonomic, phylogenetic and functional), we searched for the best predictors (i.e. geographic, climatic, environmental or anthropogenic) of the regional variation of this community.

2. Material and Methods

2.1 Study area and experimental design

To test the efficiency of the fouling in testing cumulative disturbance (i.e. natural and anthropogenic), six sites into four estuaries were chosen according to their presence or absence of commercial and international ports, two without: Cananéia-Iguape estuarine complex (CN) and Guaratuba Bay (GT) and four with: Antonina (AN), Paranaguá (PR), Pontal do Sul (PN) and Babitonga Bay (BB) (Fig. 1). The sites AN, PR and PN were also chosen because they are placed on an environmental gradient in the same estuary (i.e. Paranaguá Estuarine Complex – PEC; Fig. 1B) helping further discriminating possible environmental filtering. The gradient follows the East-West axes from the high energy (high wave action and water mixing) and euhaline entrance of the estuary (PN) until the low energy and oligohaline Antonina (AN) in the inner part of it (where there is also a small international port), including Paranaguá (PR) in the midway, where the Paranaguá international port is located (Fig. 1B) (Lana et al. 2001). Also, the other three sites present comparable salinity to at least one of the sites of the PEC and so either euhaline like PN (i.e. BB) or oligo-mesohaline like AN (i.e. CN and GT).

In each of the six sites, twelve experimental sets (i.e. 72 experimental sets in total) were attached to floating objects (e.g. floating docks, fisherman's buoys, signalling buoys) in June 2017 (Fig. 1C). Each set consisted of three artificial plates (three cm from each other), made of 12x12 cm black sanded polyethylene attached to a rope ~ 1.5 m below the water surface. The individual sets were located at least 10 meters apart from each other. After 90, 180 and 360 days, a randomly chosen single plate was removed from each set. In December of 2017, after 180 days (i.e. second sampling), an additional set with only two plates was

deployed at around two meters from the first ones and one plate every 90 days randomly collected. At the end of the experiment (June 2018), there were a total of five sampling times per site. This approach was chosen to decompose the temporal variability of the communities into the two major seasons, the dry-cold (i.e. June-November) and the rainy-warm (i.e. December-May), exploring the influence of different submerging time starting in different environmental conditions (Fig. 1). Both final experimental designs (i.e. port effect and environmental gradient), presented fixed factors (i.e. port and season) and hierarchical random ones (i.e. time and site; Fig. 1D).

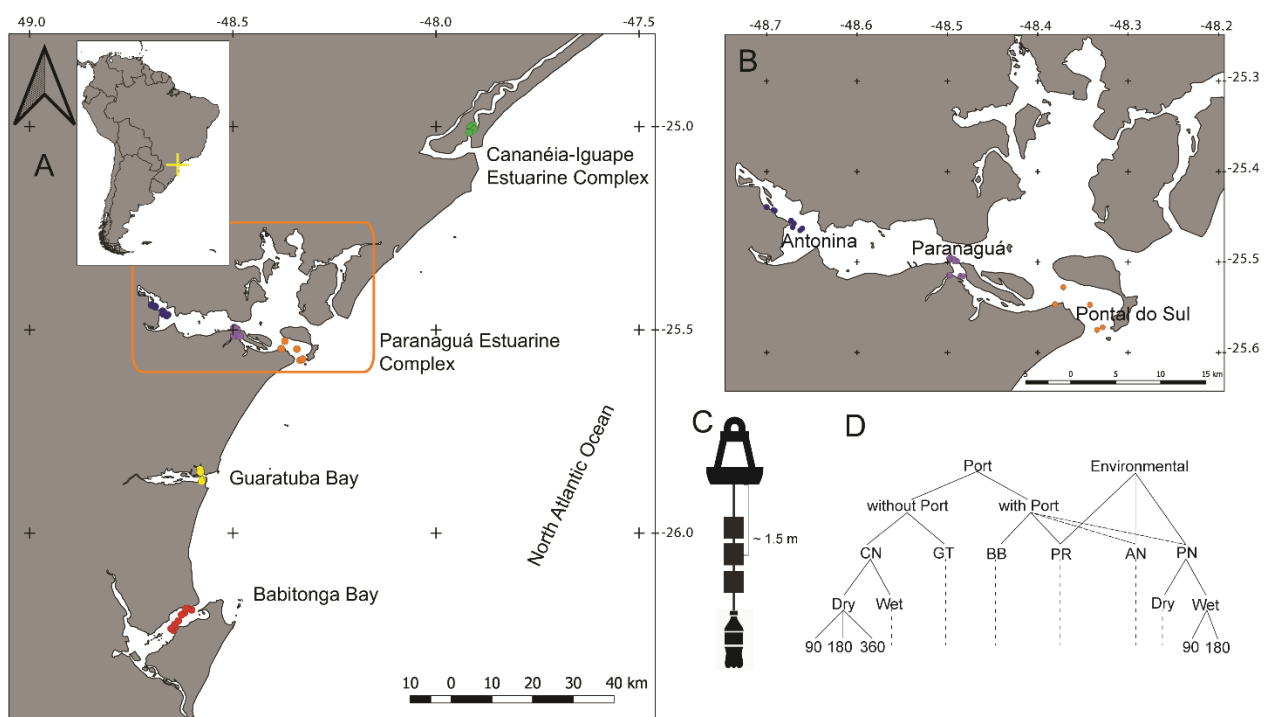


Fig. 1. Study areas and experimental set and design. A: map of the four estuaries studied. B: spatial distribution of the three sites inside the Paranaguá estuarine complex (PEC) along the environmental gradient. C: experimental set. D: experimental design distinguishing the effect of the presence of international ports (Port) and the environmental gradient (Environmental). Sites without ports: Cananéia-Iguape Estuarine Complex - Cananéia (CN, green dots) and Guaratuba Bay (GT, yellow dots). Sites with ports: Babilonga Bay (BB, red dots), Paranaguá (PR, purple dots), Antonina (AN, blue dots) and Ponta do Sul (PN, orange dots). Seasons: Dry and Wet. Days submerged: 90, 180, 360.

2.2 Sample processing and taxonomic data

Each plate, along with the associated fouling, was put in plastic bags still inside the water, to avoid escaping of mobile fauna. After recovery, the sea water was removed and replaced by 96% alcohol. After no more than one day, the vagile fauna was sieved through a 500 μ m mesh (used also during the replacement of the water) and, after a thorough search

of the plate to avoid loss of specimens, the animals were transferred into plastic pots with fresh 96% alcohol. The amphipods were then separated from the rest of the community with help of stereomicroscopes and the species identified at the lowest possible taxonomic rank through consultation of specialized literature (Bynum & Fox 1977, Myers & McGrath 1984, Conlan 1990, LeCroy 2011, Hoover & Bousfield 2001, LeCroy 2001, 2002, 2004, 2007, Valério-Berardo et al. 2007, Mauro & Serejo 2015, Krapp-Schickel 2015, Belal Hossain & Hughes 2016, Alves et al. 2018). Because several experimental sets were lost (due to storms or vandalism), when available five replicates per site and sampling time (minimum four replicates) were randomly chosen for the analyses. Then the following diversity indices and metrics were calculated for each sample using the functions *specnumber* and *diversity* of the package *vegan* in R 4.0.2 (R core Team 2020; www.r-project.org): species richness and abundance, Shannon-Weaver's H and Simpson's D as 1-D).

2.3 Phylogenetic diversity

As molecular studies on amphipods at the community level are not common, to investigate the structure and drivers of the phylogenetic diversity in the study area, a maximum likelihood phylogeny produced with the cytochrome c oxidase 1 (COI) gene fragment in a previous work (first chapter) was downloaded and pruned from the unnecessary species. Species with less than three occurrences, without a sequence in the cited work or from the study region, were excluded to more accurately represent the regional diversity. Only one sequence per species was randomly retained (Tab. S1). This choice was done to avoid over-representation of rare species but also possible cryptic species that may not have been recognised in this work, such as the ones belonging to the species complex of *Caprella penantis* Leach, 1814 (Cabezas et al. 2013). The Faith's index of phylogenetic diversity (PD), which sum the lengths of the branches from the phylogenetic tree of all species present in each community, weighting them with the relative abundances (Faith 1992), was calculated with the function *pd* of the package *picante* in R (Kembel et al. 2010).

2.4 Functional diversity

The same species retained in the phylogenetic diversity analyses were selected to allow comparability and subsequent analyses. A search in the recent literature was done to select the more appropriate functional traits to better characterise the amphipods of the fouling community (e.g. Ashford et al. 2018, Wrede et al. 2018, Liu et al. 2019, Ros et al.

2020). Common traits such as feeding habit, diet type or habitus (i.e. free living or tubiculous) were combined with ad-hoc ones (e.g. sociability, parental care) (Tab. S1). For the codification, an approach using fuzzy coding was preferred to traditional ones, to account for variability and uncertainty, given the high number of poorly studied and recently described taxa (Chevenet et al. 1994). Each functional trait was divided, for a total of 21 trait modalities, and was assigned a value of 0, 1, 2, or 3 for each species, where 0 meant no affinity and 3 complete (i.e. exclusive modality). Coding of modalities was done according to the literature on the species, field/laboratory observation (e.g. size, fecundity) or information on the closest taxon (e.g. congeneric species or from the same family) (Rand et al. 2018).

The package *ade4* was used to prepare the dataset and a matrix of Gower's distances was generated (Dray & Dufour 2007). To explore which traits were most responsible for the species ecological differentiation, a correlation between traits and relative importance were calculated with the function *kdist.cor* of the same package with R. Three functional diversity indices: functional richness (FRic: the amount of trait space occupied by species), functional evenness (FEve: the equability of abundances in trait space) and Rao's quadratic entropy (Rao's Q: is the dispersion of species in trait space weighted by their abundances) were computed with the function *dbfd* of the package *FD* in R (Laliberté & Legendre 2010).

2.5 Environmental variables

A dataset of 22 environmental variables (comprising coordinates) was compiled from different sources. Climatic predictors relative to the sea (i.e. sea surface temperature and sea surface salinity of coldest/freshest and warmest/saltiest month, average and range) were derived from the surface as 5 arcmin raster layers Bio-Oracle database (Assis et al. 2017). Monthly atmospheric data (i.e. precipitations from May 2017 to May 2018 and wind speed relative to the average monthly values from 1970 to 2000) were derived from the 2.5 arcmin raster WorldClim database (Fick & Hijmans 2017). To obtain maximum, minimum, average and range for each level of time of the experimental design (i.e. every 90 days) the data of three months were pooled together starting from the deployment of the experimental sets (i.e. June 2017). Data referring to commercial shipping (i.e. cargo and tankers) was retrieved from the 5 arcmin raster released from the European commission for the Pasta Mare Project (2017) and downloaded from Ocean Tool for Public Understanding and Science (OcToPUS; <https://octopus.zoo.ox.ac.uk/apps/ode>). Distance from international

ports was calculated in Q-gis 3.12.3 (QGIS Development Team 2012) from the raster of international ports released from National Geospatial-Intelligence Agency (2017) available in OcToPUS. Information about bathymetry was retrieved from the 30 arcsec raster available in Marspec (Sbrocco & Barber 2013). The distance from the coast was measured manually using free satellite shapefiles of the region in Qgis.

2.6 Network analyses

Network formalism was used to study the spatial association structure of amphipods to the different sampling sites. For each season (dry and wet) and for each time-step (90, 180 and 360 days) was constructed a bipartite network, by establishing links between two distinct sets of nodes (amphipod species and sampling localities/samples) representing the abundance of a given species in a sampling locality in a specific time-frame.

The spatial network structure of above-mentioned networks has been studied by using modularity (Q), a peculiar network structure arising from the appearance of densely connected groups (hereafter called modules) of nodes, with only sparser connections between groups (Newman 2006). Modularity represents a well-known and peculiar emergent property of many complex systems playing a critical role in their functionality (Newman 2006). Moreover, by providing a formal description of the pattern of aggregation between species, populations or communities, modularity can identify critical scales in specific ecological and evolutionary processes (Fletcher Jr et al., 2013).

Here, a quantitative measure of modularity (i.e. weighted modularity) was applied using a recently developed algorithm (Beckett 2016) implemented in the function *metaComputeModules* (with 999 permutations), from the package *bipartite* (Dormann et al. 2009) in R. The algorithm assigned amphipod species and sampling localities to modules to compute a modularity value (Q) that is higher when links within modules (i.e. the presence and/or abundance of amphipod species) are more prevalent and/or stronger than between modules links. Since most of the algorithms tend to be stochastic, meaning a high variability in the modularity scores and modules affiliation, the algorithm was re-run several times (n = 100) to stabilize modularity computation.

Null models were used to account for the modularity dependence on network size by using the *swap.web* algorithm (Dormann et al. 2009), which is a constrained randomization

procedure where both marginal totals (i.e. the total abundance of species *per* site and the total abundance of a species between sites) and connectance (i.e. the total number of incidences) are identical to those observed in the original matrix. To test whether or not the modular structure observed in the empirical incidence matrix was significantly non-random compared to that generated by the null model, the *z*-score was used, $z = (Q_{\text{obs}} - \mu_Q) / \sigma_Q$, where Q_{obs} is the observed modularity and μ_Q and σ_Q the mean and standard deviation of 100 randomized matrices, respectively. Values of *z* between -1.96 and 1.96 indicated a non-significant effect of a given predictor, while values greater than 1.96 and lower than -1.96 indicated a significant over and under-representation of the observed pattern.

The degree to which a network shows a significant modular structure allows to map nodes within a bi-dimensional space provided by the number of within-module (*z*-score) and among-module (*c*-score) links. This so-called ‘functional cartography’ allows assigning specific roles to nodes based on their position in the *cz*-plot. Following the modular subdivisions, the corresponding *c* and *z* values of each node were calculated by using the function ‘*czvalues*’ in the package *bipartite* of R. Also, to have an overview of the general role of the estuaries, centroids were produced with the average values of *c* and *z* of all the samples of each site. Following Guimerà and Amaral (2005), the nodes were then classified into:

1. Module hubs – nodes linked to many nodes within their own module ($z > 2.5$, $c \leq 0.62$).
2. Non-hub connectors – nodes linking several modules ($z \leq 2.5$, $c > 0.62$).
3. Network hubs – nodes acting as both connectors and module hubs ($z > 2.5$, $c > 0.62$).
4. Peripherals – nodes that have a few links within its own module and to other modules ($z \leq 2.5$, $c \leq 0.62$).

2.7 Univariate analyses

As different studies demonstrated the importance of exploring indices from different dimensions of the biodiversity (e.g. Cisneros et al. 2014), the influence of the ports and seasons with associated nested factors (sites and time respectively) were tested on the different indices and metrics of taxonomic (i.e. Richness, Abundance, *H* and *D*), functional (i.e. FRic, FEve and Rao’s *Q*) and phylogenetic diversity (i.e. PD) by generalized linear mixed models (GLMMs). GLMM is an approach for analyzing data complementing to the

fixed terms (i.e. port presence and season), random effects (Bolker et al. 2009). The best fit probability distribution family was selected for each index (between Poisson and Gaussian). The best fit was chosen with the Akaike Information Criteria (AIC), and data were checked for overdispersion (if over-dispersed data were log₁₀ transformed). For all the indices the gaussian distribution was the best fitted and therefore selected. Afterwards, the best random structure of models was selected: the full model was contrasted with models including only sites or times as random effects and also with the fixed effects model (no random effects included) for a total of four models. This procedure was also based on AIC. Lastly, the significance of fixed terms in the model was checked and non-significant terms ($\alpha > 0.05$) were dropped from the model. The final model was refitted using restricted maximum likelihood (REML) estimation and post-hoc pairwise comparisons were conducted on significant terms. Normality of residuals and homogeneity assumptions were checked using diagnostics plots according to Zuur et al. (2009). The analyses were run in R using the package *glmmTMB* and the homonymous function (Brooks, Mollie et al. 2017).

To test if the functional dissimilarity in the different communities was proportional to the higher phylogenetic distance (i.e. communities phylogenetically closer should also be functionally closer), generalized additive models (GAM) were used (Safi et al. 2011). Using gaussian distribution, the correlation between Rao's Q and PD was tested for each site (pooling together all the time-frame levels) and the whole dataset. Rao's Q was chosen over FD because, the latter, is demonstrated to be correlated to species richness (Cianciaruso et al. 2009), like PD (Safi et al. 2011, Venail et al. 2015), while the Rao's index, focusing on pairwise abundance-weighted distances between species in the trait space, is more influenced by the abundances of traits combinations rather than their presence (unlike FRic), which is desirable in a generally species-homogeneous community like the fouling. The analyses were run in R using the function *gam* of the package *mgcv* (Wood 2011).

2.8 Beta diversity partitioning

Beta diversity (i.e. the variation in community composition across space) was used to measure the compositional dissimilarity of local assemblages. The method proposed by Cardoso et al. (2014), which uses trees as a common representation for phylogenetic and functional diversity, according to which part of a global tree is shared by or unique to the compared communities, was used. Thus, the functional Gower's distance matrix was converted to cluster and then, together with the phylogeny of the amphipods, used to

quantify the taxonomic, functional and phylogenetic total beta diversity with its replacement (turnover) and richness difference components, by using the function *beta* of the *R* package *BAT* (Cardoso et al. 2015).

2.9 Generalized Dissimilarity Modelling

Generalized Dissimilarity Modelling (GDM: Ferrier et al. 2007) was used to model the variation in assemblage composition along spatial and environmental gradients using the dissimilarity matrices of beta diversity components (i.e. total, replacement and richness) for each diversity facets (i.e. taxonomic, functional and phylogenetic) as response matrices. Differently from other analyses (e.g. Mantel and partial Mantel test), GDM can accommodate nonlinearities typical in eco-geographical datasets (Ferrier et al. 2007). Nonlinearity in ecological data may occur because of the curvilinear response between increasing ecological distance and compositional dissimilarity or because of variation in the rate of compositional dissimilarity at a different position along ecogeographical gradients (Ferrier et al. 2007). After checking for multicollinearity, the environmental variables used were: geographic distances (i.e. longitude and latitude), distance from the coast, bathymetry, shipping, distance from ports, range and the average amount of precipitations, minimum and range of speed of the wind, average sea-surface temperature, sea-surface salinity of the freshest and warmest month.

GDM models and plot of the I-splines (i.e. monotone cubic spline functions) were run to assess the impact of predictor variables on the total, replacement and richness components of taxonomic, functional and phylogenetic dissimilarity matrices using the functions *gdm* and *gdm.varImp* in the package *gdm* of *R* (Fitzpatrick et al. 2020).

3. Results

3.1 Overall results

A total of 30 species of amphipods were found in this study, accounting for 441,068 individuals. The species *Podocerus brasiliensis* (Dana, 1853) and *Jassa valida* (Dana, 1853)

dominated in terms of abundance (159,844 and 123,805 specimens respectively) while different species were encountered only a few times (e.g. *Batea catharinensis* Müller, 1865, *Apohyale media* (Dana, 1853), *Cerapus benthophilus* Thomas & Heard and, with 12, 9, and 2 specimens respectively) (Fig. 2). Of the 30 species found in this study, 9 were excluded from the diversity analyses, due to lack of sequences or information on their biology (Fig. S1) to allow estimation of the functional and phylogenetic diversity.

Five functional traits were responsible for most of the differences between species (~50% of total variation): size, parental care, habitus, feeding habit and sociability (Fig. S2). On the other hand, the diet type was the trait that contributed less. There were three pairs of species that resulted functionally equal, two stenothoids (i.e. *Stenothoe valida* Dana, 1852 and *S. gallensis* Walker, 1904), two photids (i.e. *Photis sarae* Souza-Filho & Serejo, 2010 and *Latigammaropsis tagoensis* (Schellenberg, 1925)) and two introduced corophiids (i.e. *Apocorophium acutum* (Chevreux, 1908) and *Laticorophium baconi* (Shoemaker, 1934); Fig. S2). The importance of the habitus was also reflected in the cluster, dividing two main clusters with respectively free-living and tubiculous species Fig. S2.

3.2 Network analyses

The modularity was always significant when compared with the null-models and the highest values were in both times of the dry season (0.44 and 0.47 for 90 and 180 days respectively; Fig. 3A-B), while the lowest was in the 90 days of the wet season (0.23; Fig. 3C) followed by the one-year old communities (0.27; Fig. 3E). The modularity value for the total was average in comparison to the different times investigated (0.37). Excluding the whole dataset that had the highest absolute number of modules (i.e. 10; Fig. S8), the 180 days of the wet season was the time with most modules (i.e. 9; Fig. S6). On the opposite, with 5 modules, the community of one year was the one with the lowest number. The modules were heterogeneous spatially (i.e. samples from different sites frequently grouped together). AN always had at least one module shared with CN or GT (or the three together). BB was the only site that never shared a module with AN while always sharing at least one with PN or PR (or the three together; Fig. S3-4-5-6-7-8).

There were a few hubs samples and no site was completely a hub, while there was a big temporal variation in peripheral and non-hub connectors (Fig. 3). BB had a peripheral role in the dry season but a connector in the wet season (Fig. 3A-B and D-E respectively). The two sites belonging to different estuaries with opposing salinity range (i.e. AN

oligohaline and BB euhaline) had opposite topological roles between each other in both seasons but were both peripheral in the yearly communities. AN displayed the highest number of hub samples in the different times, (i.e. two inside the module and one network) as well as the highest variability in topological roles played (i.e. all of them). The network of the late community from the dry season (i.e. 180 days) was the less connected, with most peripheral samples (and sites) and fewer connectors (i.e. 25 and four respectively). In the total (i.e. whole dataset; Fig. 3F), there were two network hubs from both estuaries with and without ports (PR and CN) and four were module hubs mainly from estuaries without ports (two for AN and one for CN and GT each). Furthermore, despite AN and CN being the only peripherals sites, also these were close to the threshold used (i.e. 0.62).

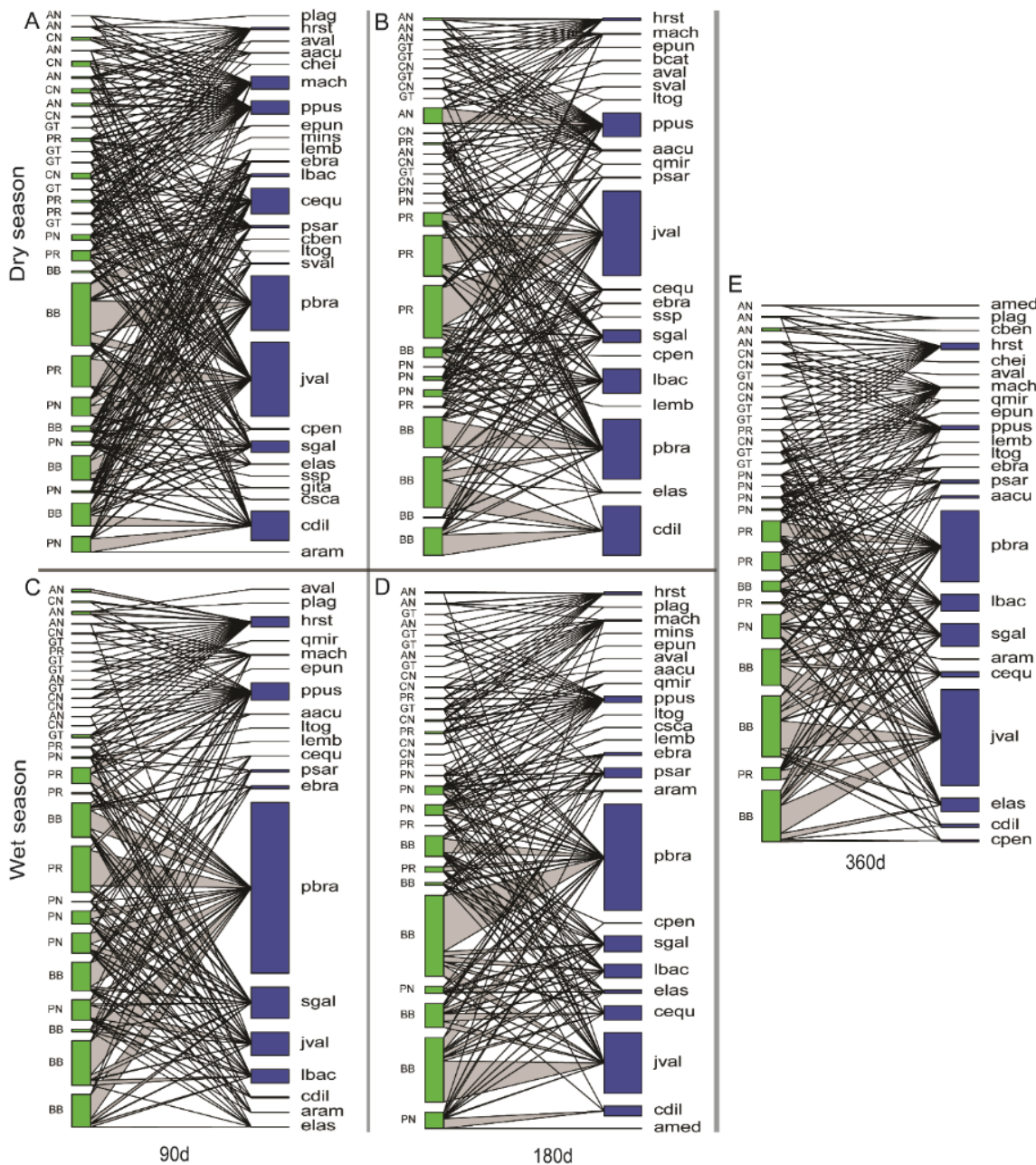


Fig. 2. Bipartite network between samples of different sites (green boxes) and species (blue boxes) for each level of time (90d and 180d) for the dry (A and B) and wet season (C and D) and the annual community (E). Black chords and grey filled triangles relative to the presence and abundance of each species (right) in each sample (left) and the proportion of each species for each community. Size of boxes proportional to species abundances. CN: Cananéia-Iguape Estuarine Complex – Cananéia; GT: Guaratuba Bay; AN: Antonina; PR: Paranaguá; PN: Pontal do Sul; BB: Babitonga Bay; hrst: *Hourstonius* sp.; plag: *Propejanice lagamarensis* Myers & Desiderato 2019; aval: *Ampithoe valida* Smith, 1873; mach: *Monocorophium acherusicum* (Costa, 1851); chei: *Cheiriphotis* sp.; sgai: *Stenothoe gallensis* Walker, 1904; lbac: *Laticorophium baconi* (Shoemaker, 1934); jval: *Jassa valida* (Dana, 1853); epun: *Erithonius punctatus* (Spence Bate, 1857); gita: *Gitanopsis* sp.; ssp: *Stenothoe* sp.; cscs: *Caprella scaura* Templeton, 1836; cdil: *Caprella dilatata* Krøyer, 1843; ltog: *Latigammaropsis tagoensis* (Schellenberg, 1925); elas: *Elasmopus* sp.; lemb: *Lembos* sp.; aram: *Ampithoe ramondi* Audouin, 1826; mins: *Monocorophium insidiosum* (Crawford, 1937); aacu: *Apocorophium acutum* (Chevreux, 1908); ppus: *Paracaprella pusilla* Mayer, 1890; sval: *Stenothoe valida* Dana, 1852; cben: *Cerapus benthophilus* Thomas & Heard, 1979; ebra: *Erithonius brasiliensis* (Dana, 1853); cpen: *Caprella penantis* Leach, 1814; cequ: *Caprella equilibra* Say, 1818; pbras: *Podocerus brasiliensis* (Dana, 1853); psar: *Photis sarae* Souza-Filho & Serejo, 2010; qmir: *Quadrimeaera miranda* (Ruffo, Krapp-Schickel & Gable, 2000); bcat: *Batea catharinensis* Müller, 1865.

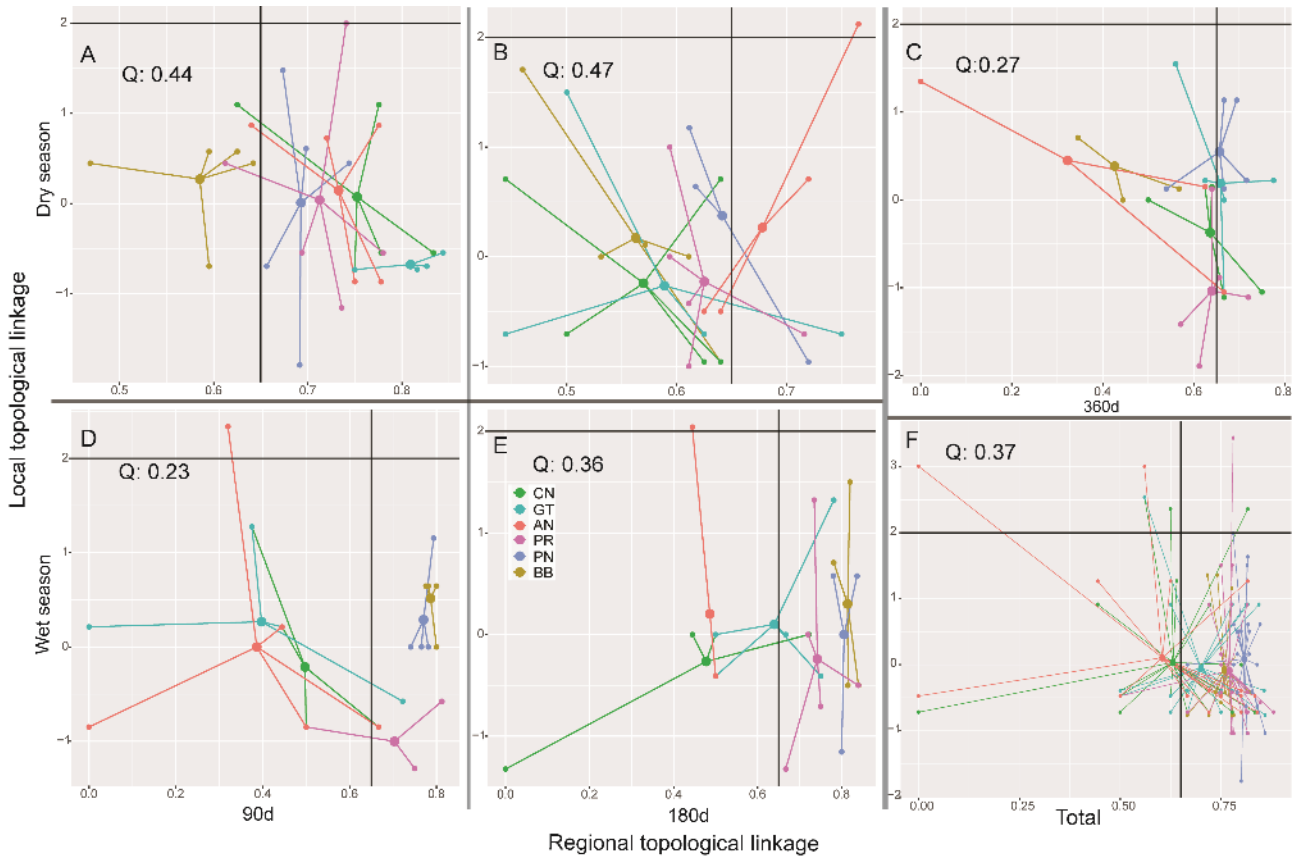


Fig. 3. Modularity (Q) and topological roles of samples and sites (small and big circles respectively) for each level of time (90d and 180d) for the dry (A and B) and wet season (D and E), for the annual community (C) and for the whole dataset together (F). Black lines in each graphic, classifying the communities into four topological roles, from the top on the left, clockwise: module hubs (numerous species of the same module and a few from others), network hubs (many species from both within and outside the module), non-hub connectors (numerous species from other modules and a few from the same) and peripherals (a few species either both within and outside the module). Colours relative to each site. CN: Cananéia-Iguape Estuarine Complex – Cananéia; GT: Guaratuba Bay; AN: Antonina; PR: Paranaguá; PN: Pontal do Sul; BB: Babetonga Bay.

3.3 Univariate analyses

A logarithmic transformation of richness and abundance was necessary to account for the overdispersion caused by some extreme values, mainly of BB (but also AN and PN for the richness). In the GLMM, the only factor that was never included in the best-fit model was the sampling time. The factor port was never significant alone but only in the interaction with season in H and D (Tab. 1e-f). Both dominance and entropy of the communities (i.e. D and H) were lower in the wet season in both levels of port but, in either season, both indices were equal between sites with and without ports. The random effect of site was included in the best-fitted model almost for every index (FRic and FEve excluded). Site was also the only factor significant for the abundance (Tab. 1b) where CN, AN and GT, showed similar and significantly lower values of the other sites, with BB showing highest abundances (regardless the season; Fig.4). Functional evenness (FEve) was lower during the wet season while nothing was significant for the FRic (Tab. 1, Fig. 5). There were no differences

between the sites CN, GT and AN in the wet season for any index, only displaying different entropy, both taxonomic and functional (H and Rao's Q respectively), as well as D, for the sites without ports in the dry season (GT < CN). A seasonally reversed result was obtained for the site PR (polyhaline) and the two euhaline sites PN and BB, where the values of every index were equal in the dry season but seldom in the wet one (Tab. 1, Fig. 4-5). PR generally showed lower values in the wet season compared with the other two sites. PN and BB were the only sites that never showed seasonality, while AN was the only one with almost always (PD excluded) lower diversity in the wet season.

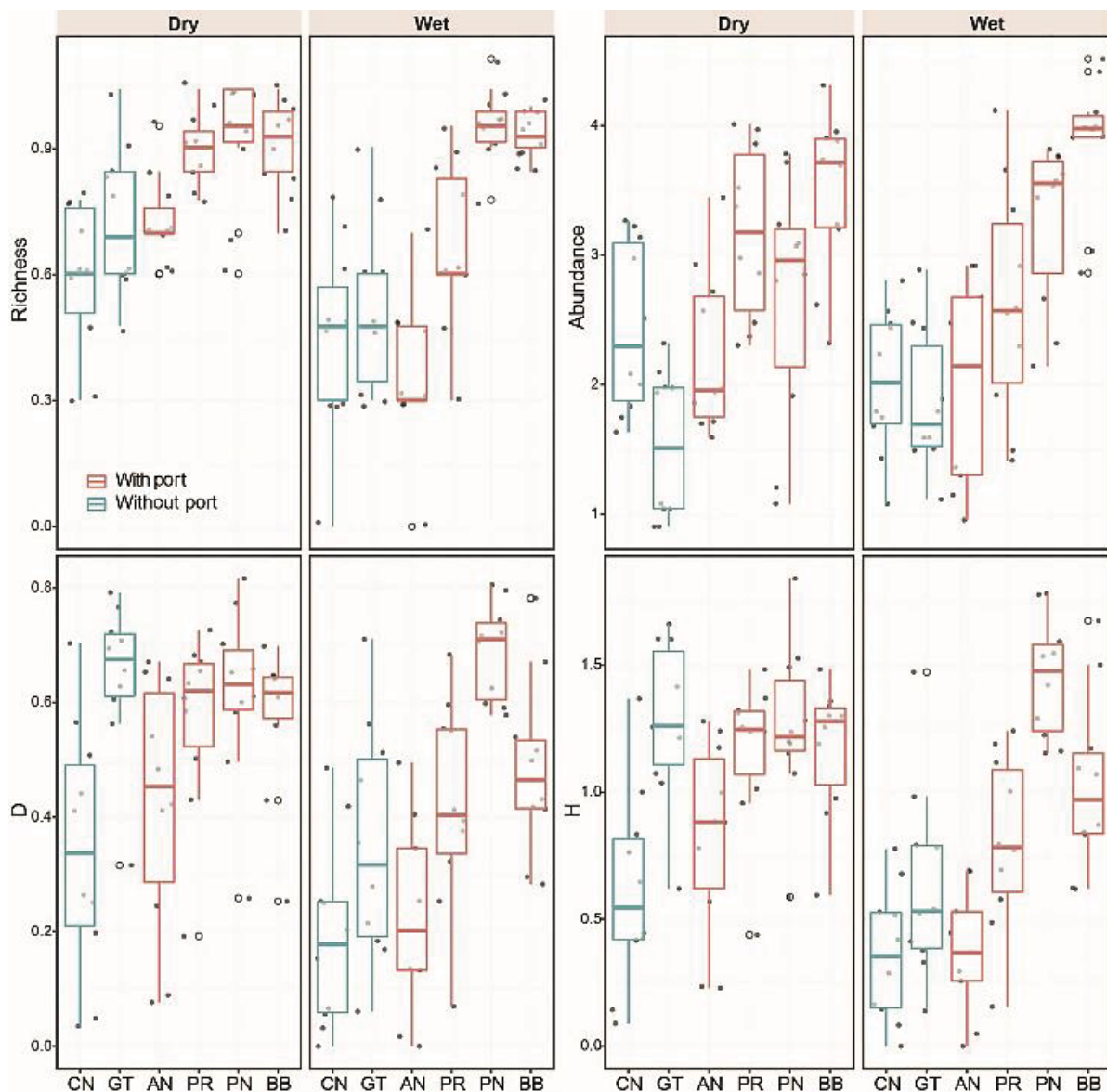


Fig. 4. Boxplots depicting the taxonomic diversity indices, namely Richness, Abundance (log10 transformed), Simpson's 1-D (D) and Shannon-Weaver's H (H) for each site and season. Open circles are referring to outliers. Colours of boxplots relative to the presence of international ports in the site (i.e. red with and blue without ports). CN: Cananéia-Iguape Estuarine Complex – Cananéia; GT: Guaratuba Bay; AN: Antonina; PR: Paranaguá; PN: Pontal do Sul; BB: Babitonga Bay.

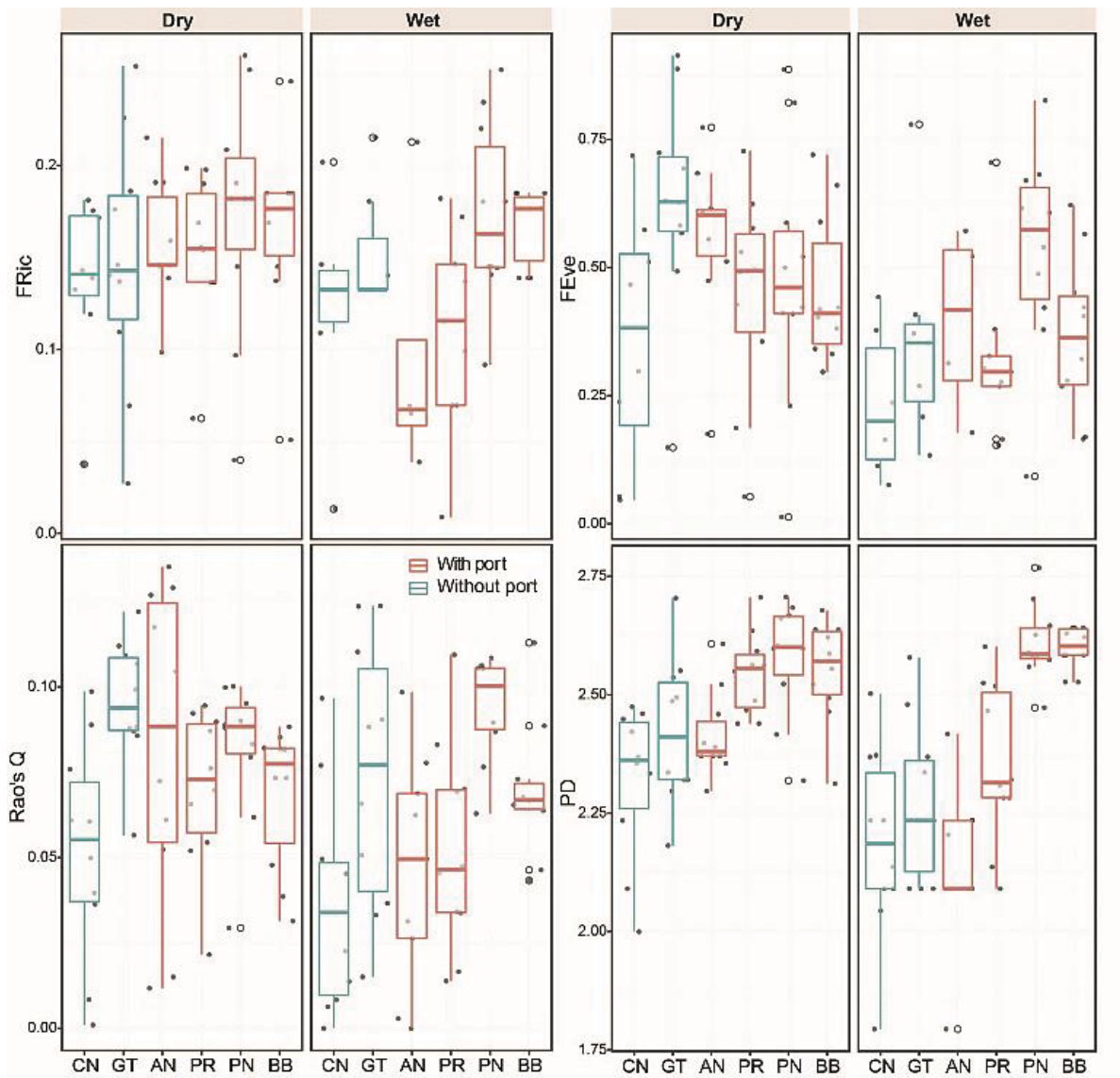


Fig. 5. Boxplots depicting the differences in the functional and phylogenetic indices (i.e. FRic, FEve, Rao's Q and PD) for each site and season. Open circles are referring to outliers. Colours of boxplots relative to the presence of international ports in the site (i.e. red with and blue without ports). CN: Cananéia-Iguape Estuarine Complex – Cananéia; GT: Guaratuba Bay; AN: Antonina; PR: Paranaguá; PN: Pontal do Sul; BB: Babitonga Bay.

Tab. 1. Analysis of deviance table and for the response of each index explored (i.e. species Richness (a) and Abundance (b), H (e), D (f), FRic (g), FEve (h), Rao's Q (c), PD (d)) to each factor of the best-fitted GLMM (in italic) and Post-hoc analyses between levels of the significant factors. Values of Chi-squared (Chisq), degrees of freedom (df) and p values (Pr(>Chisq)) are presented for each factor and significant terms ($\alpha = 0.05$) are highlighted in bold. Dr: dry season; W: wet season; CN: Cananéia-Iguape Estuarine Complex – Cananéia; GT: Guaratuba Bay; AN: Antonina; PR: Paranaguá; PN: Pontal do Sul; BB: Babitonga Bay; P: with port; nP: without port.

Factor	Chisq	df	Pr(>Chisq)	Post-hoc tests
<i>~ Port + Season + (1 Port:Site)</i>				Factor / level
(a) Richness				Dr: (CN=GT=AN);(GT=AN=PR=PN=BB);(CN#BB,PN,PR)
Port	2.76	1	0.096	W: (CN=GT=AN#BB,PN);(CN#PR);(GT=PR);(PN=BB#PR)
Season	21.47	1	0.000	Site: (CN,GT,AN,PR: Dr#W);(BB,PN: Dr=W)
(b) Abundance				Site: AN=CN=GT#PN=PR#BB
Port	3.58	1	0.059	
Season	0.03	1	0.867	
(c) Rao's Q				Dr: (CN#GT);(CN=AN=PN=PR=BB);(GT=AN=PR=PN=BB)
Port	0.18	1	0.675	W: (CN=AN=PR#PN);(CN=GT=AN=PR=BB);(GT=PN=BB)
Season	5.49	1	0.019	Site: (AN: Dr#W);(CN,GT,BB,PR,PN: Dr=W)
(d) PD				Dr: (AN=CN=GT#PN);(CN#PR,BB);(GT=PR=BB);(PR=PN=BB)
Port	2.42	1	0.120	W: (CN=GT=AN=PR#PN=BB)
Season	16.41	1	0.000	Site: (CN,GT,PR: Dr#W);(BB,AN,PN: Dr=W)
<i>~ Port * Season + (1 Port:Site)</i>				Port: Dr ≠ W always
(e) H				Season: P = nP always
Port	1.12	1	0.290	Dr: (AN=CN#GT,PN);(CN#PR,BB);(GT=PR=PN=BB)
Season	19.56	1	0.000	W: (CN=AN=GT);(CN=AN#BB,PR,PN);(GT#PN);(GT=BB=PR);(BB=PN);(PN#PR)
Port:Season	3.86	1	0.049	Site: (GT,AN,PR:Dr#W);(CN,BB,PN: Dr=W)
(f) D				Port: Dr ≠ W always
Port	0.90	1	0.342	Season: P = nP always
Season	17.69	1	0.000	Dr: (CN=AN);(CN#GT=BB=PR=PN);(AN#GT);(AN=BB=PR=PN)
Port:Season	3.90	1	0.048	W: (CN=AN=GT#PN);(CN=AN#BB);(CN#PR);(GT=AN=PR);(GT=PR=BB#PN)
				Site: (CN,GT,AN: Dr#W);(BB,PR,PN: Dr=W)
<i>~ Port + Season</i>				
(g) FRic				
Port	1.61	1	0.205	
Season	1.69	1	0.194	
(h) FEve				Season: Dr ≠ W
Port	0.52	1	0.473	
Season	8.23	1	0.004	

Despite the GAMs performed between the Rao's Q and the PD retrieved a regional significance in the correlation between the two indices, the GAMs for each site retrieved this correlation significant only for CN and GT (Fig. 6).

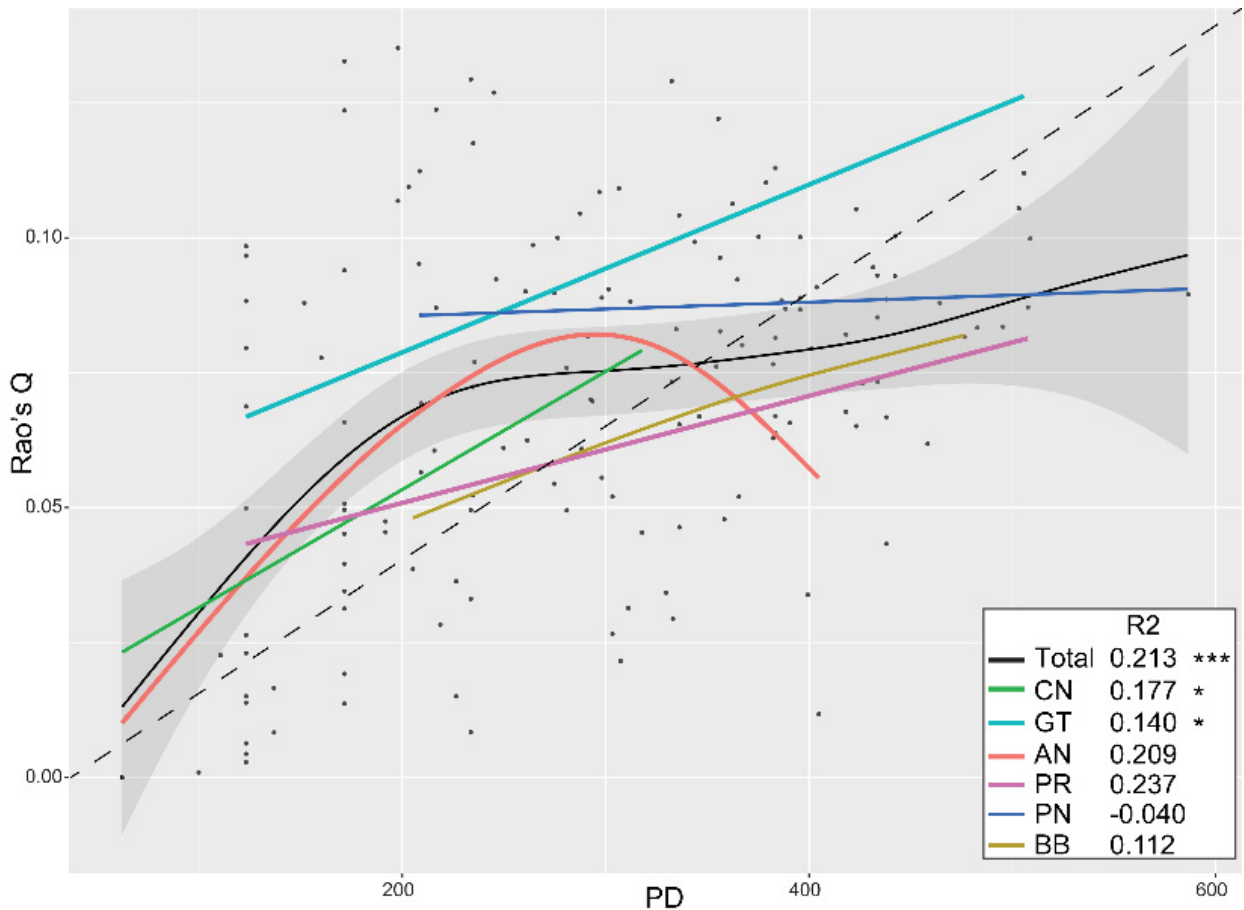


Fig. 6. Generalized additive models of the Rao's Q (i.e. functional dispersion) in function of the PD (i.e. phylogenetic diversity). Coloured splines relative to each site. Black spline with shaded area (standard error) relative to the whole dataset. Dashed line: theoretical linear correlation between phylogenetic diversity and functional dispersion. R² and significance (***: p-value < 0.001, *: p-value < 0.05) reported for each GAM. CN: Cananéia-Iguape Estuarine Complex – Cananéia; GT: Guaratuba Bay; AN: Antonina; PR: Paranaguá; PN: Pontal do Sul; BB: Babitonga Bay.

3.4 Generalized dissimilarity models

The GDMs explained more than 40% of the total dissimilarity for taxonomic and phylogenetic (~45 and ~42 respectively) while only ~27% for the functional diversity (Fig. 7A.1). The same pattern but with lower values (~27 and ~25 for taxonomic and phylogenetic respectively) was found in the replacement (i.e. higher explained percentage in taxonomic and phylogenetic than functional diversity; Fig. 7A.3) but, was inverted for the richness (Fig. 7A.2). The latter showed also the lowest explained dissimilarity proportion (~9 for taxonomic and phylogenetic and 11 for functional). For every GDM, the relationship between observed compositional dissimilarity of each pair of samples and the predictor of the regression equation was increasingly monotonic (Fig. 7B). The greatest degree of compositional change, for every level of beta and biodiversity considered, occurred along the shipping

gradient (Fig. 7C). The curves followed a logistic increment reaching a plateau at the end of the gradient for the total and the replacement (Fig. 7C.1-3) but, reached the plateau for the richness before the half of the gradient (Fig. 7C.2). The distance from the coast was the second most important predictor for total and replacement (not significant for richness) showing a rapid increment at the beginning and slowly reaching the plateau at the end (~200 meters). For the richness, the only other predictor (not for the phylogenetic diversity) was the average amount of precipitations displaying an exponential growth which starts its faster increase around the half of the gradient (Fig. 7C.2). The precipitations (average and range) were significant also in the total beta diversity showing a similar growth (Fig. 7C.1). The geographic distance was significant only for the taxonomic total diversity showing a slight increase at the highest distances (Fig. 7C.1). In the replacement only, the ecological distances showed an increment after 2.2 ms⁻¹ of the minimum wind speed (the half of the gradient) for all the biodiversity levels without reach any plateau. Only for the functional diversity, the distance from ports was significant displaying a logarithmic growth comparable with the distance from the ports.

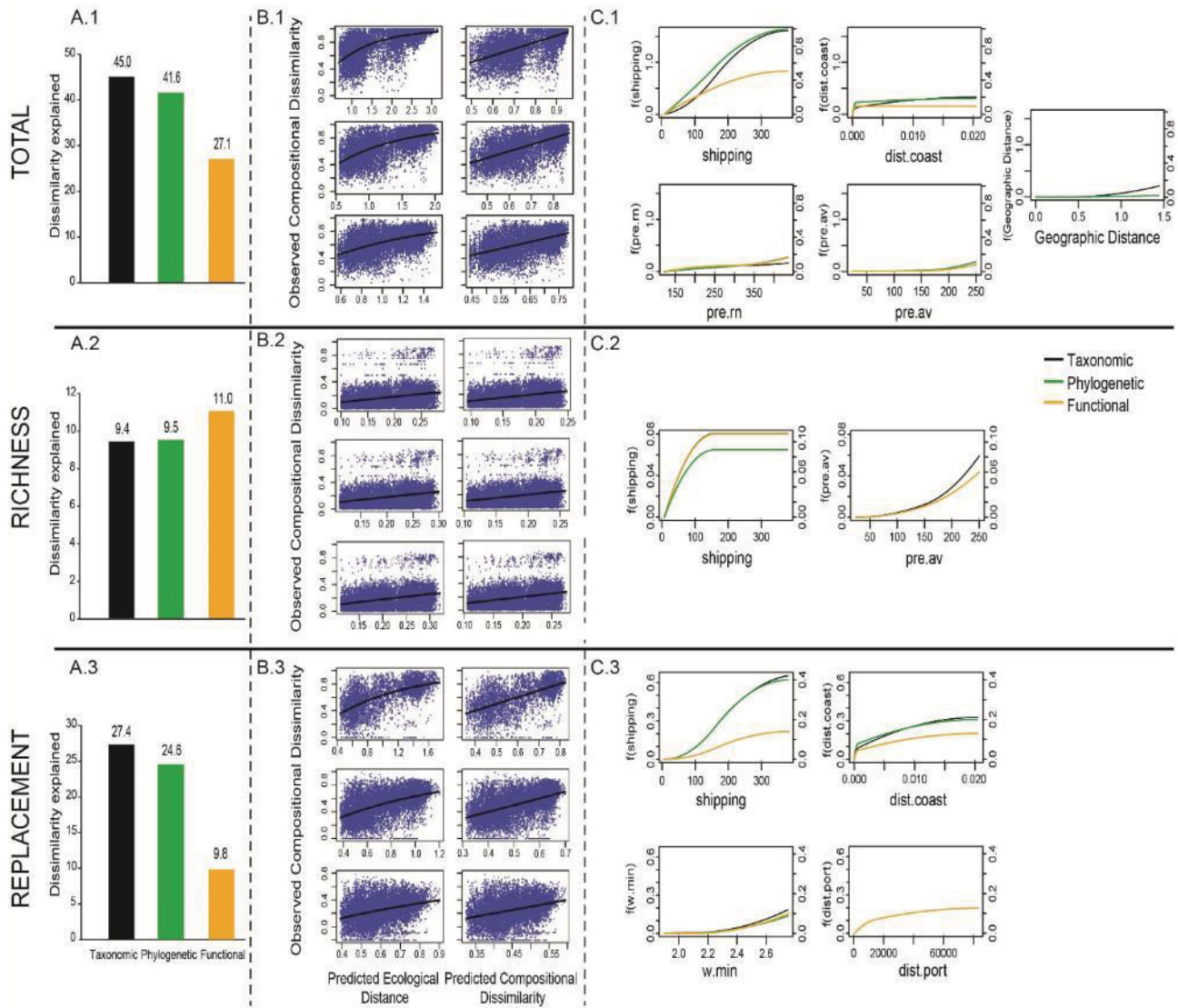


Fig. 7. Results of the GDMs. A: percentage of dissimilarity fitted by the model for the taxonomic (black), phylogenetic (green) and functional (orange) diversity for each level of beta diversity (i.e. Total, Richness and Replacement, 1-2-3 respectively). B: on the left, scatter plot of observed compositional dissimilarity of each fouling community pair and the linear predictor of the regression equation from GDM (predicted ecological distance between pairs of sampling points). On the right, scatter plot reflecting the general agreement between observed and calculated values of dissimilarity. For both, from the top results for taxonomic, phylogenetic and functional beta diversity. C: I-splines of the predicted ecological distance explained by the value of the significant variables (p -value<0.05) for each diversity (different colours). The maximum height reached by each curve indicates the total amount of compositional turnover associated with that variable and its relative importance in explaining beta diversity. Scale on the left referring to taxonomic diversity and on the right to phylogenetic and functional diversities. shipping: number of commercial ships; dist.coast: distance from coast; pre.rn: range of precipitations; pre.av: average precipitations; w.min: wind speed; dist.port: distance from the closest port.

4. Discussion

The amphipod fouling assemblages in southern Brazilian estuaries displayed marked spatial and seasonal variation visible as modularity variation in the two seasons and the relevance of climatic variables in the beta diversity. The diversity indices rejected our hypothesis on the effect of international ports affecting the fouling community, but it was the most important predictor of dissimilarity for the three components of the biodiversity and level of beta diversity displaying an increment in the differences proportionally with the number of commercial ships. The integration of different experimental designs to address human impact and environmental effects returned more complete results that made possible discern the drivers of the diversity. Moreover, our use of functional dispersion and phylogenetic diversity, and their relationship, showed how the presence of ports may homogenize the communities at local level. This may help understand which estuary is less impacted and may be an area needing protection measures, providing a useful tool for conservation management.

4.1 Taxonomic, phylogenetic and functional indices

While time of sampling seemed to have an effect in the networks, showing in the 180 days always a higher modularity when compared to the respective 90 days communities, the lack of statistical significance in all the indices suggests that the structure of the communities is mostly driven by the seasonality. In fact, most of the indices showed lower values in the wet season when compared to the dry one. This difference could be related to the high climatic variability (i.e. instability) of the subtropical summer that characterizes the whole region (Grace et al. 2008, Gusmao et al. 2016, Bettim & Haddad 2017). The conspicuous increase in the sudden rainfall during the summer, as well as being the warmest season, is coupled with the daily variations of an estuarine environment (i.e. seawater entering deeply the estuaries during high tides). As a consequence, sites closer to the entrance of estuaries tend to be more stable (at least for the salinity) compared with the inner ones (Lana et al. 2001). For our results, this can be one of the explanations of the lack of significant difference between seasons in PN and BB in almost every index studied (FEve excluded). Notwithstanding, other factors may be important in these areas such as the wave effect, which could detach overgrowing animals leaving every time an open topological space (Ros et al. 2016) and consequently a functional one.

The functional diversity of the communities was only partially explained by the predictors or at least the independent variables of this study (i.e. experimental design). The FRic was the only index that did not show any significant correlation. This suggests either that the functional space is well occupied or that this may not be an index sensitive enough to detect differences between fouling communities (known to be functionally homogeneous). Its inefficiency can be due to the assumptions using convex hulls and considering only the extreme values of functional combinations and not all the variation between them (Mouchet et al. 2010). Though, despite there is literature supporting the use of these indices to detect impact in communities (e.g. Jia et al. 2020), our results support the other works in which FRic and FEve failed to detect significant variation between impacted and reference sites also in other taxa (Gusmao et al. 2016). Similarly, Rao's Q did not show any port effect on the communities. On the other hand, it was one of the only indices in which CN and GT were not similar, at least in the dry season (i.e. H and D indices). High functional dispersion is expected in non-impacted communities, which implies that CN may be more disturbed than GT in the dry season. This could reflect the strong cold fronts coming from the south that, in the winter, enter the estuaries (Besnard 1950, Alcántara-Carrió et al. 2018).

In general, the taxonomic and phylogenetic diversity indices had a more marked resolution for the seasonal effect rather than the ports effect, displaying more evident differences in the wet season. Although, these were inverse to the expectation showing higher values in the sites with ports and higher salinity. Among the functional indices, only Rao's Q displayed also a spatial variation (i.e. between sites) although, it did not detect any effect of the ports. The poor efficacy of FRic and FEve was already found in other works (Van Der Linden et al. 2015, Dolbeth et al. 2016, Gusmao et al. 2016) and also here confirmed, suggesting that they may be excluded in future analyses of impact evaluation, but might be still considered for descriptive works. For these reasons, we suggest to take carefully into account these indices when studying the fouling community (or at least its vagile portion) as the multidimensionality of the sessile assemblage may provide almost infinite space (if the fractal area of every component is taken into consideration) for "healthful" communities even when they are overpopulated. Nevertheless, the functional redundancy of the assemblage seems to promote gregarious and generalist species, increasing the niche space and decreasing the interspecific competition.

4.2 Temporal and spatial networks

The generally low modularity and abundance of connectors in the yearly communities and the whole dataset found in our results of the topological roles in the regional network, seem to reflect the ecology of the fouling community. Our experimental set (as well as other human-made substrates, like the buoys) is suspended in the water column and may be colonized by those species that are either naturally dispersed in the water or that are stochastically moved closer. As fouling exists mainly due to the presence of human activity (e.g. artificial substrate, marinas) what would be a stochastic event of colonization for a natural community turns rather common and in urban areas, this is even higher. Moreover, this also helps species disperse against natural means (e.g. currents direction) (e.g. Clarke Murray et al. 2011, Valdor et al. 2019). Nevertheless, in our results, two main groups were somehow consistent in every time, grouping the two sites without commercial ports (i.e. CN and GT) with the oligohaline site of the PEC (AN), and the other two sites of the PEC (i.e. PR and PN) with the last site with international port (BB). This result seems to oppose our main hypothesis, showing that the effect of the presence of international ports may be buffered, or even overtaken, by environmental variables, such as salinity.

4.3 Phylogenetic constraints on functional dispersion

The results of the GAMs showed that at a regional level the fouling community seems to generally follow a pattern of a positive correlation between the phylogenetic diversity and functional traits dispersion, meaning that there is an accumulation of diverse functions along a phylogenetic gradient (Safi et al. 2011). Nevertheless, at the local level (i.e. in each site) this correlation was significant only for sites lacking an international port (i.e. CN and GT). The contrasting results (i.e. between regional and local scales) may reflect the general resistance of the system at the regional scale (Cappelatti et al. 2020). In the site AN, which displayed comparable results to the two sites without ports in the other analyses (also for Rao's Q), the correlation between these two indices was not significant which made it "more similar" to the other sites with commercial ports. This result is important also because these three sites share a closer salinity (oligo-mesohaline) and if only the indices would have been taken into consideration, the salinity might have been the variable recognized as most important. The lack of a correlation between functional dispersion and phylogenetic diversity it is an even stronger signal of functional redundancy than the already low Rao's Q values ($0 < \text{Rao's Q} < 1$; maximum value found ~ 0.125 ; Fig. 5). This seems to be in accordance with the concept of environmental sorting (or filtering), where specific parameters restrict the

suitability of a given area to be inhabited (Bueno et al. 2016, Conradi & Kollmann 2016, Shivaprakash et al. 2018). Indeed, stronger phylogenetic clustering has been reported many times as an indicator of environmental filtering in more stressful environments (Best & Stachowicz 2014, Cadotte & Tucker 2017). Although, in this case, the filtering is applied to the functionality of the fouling community and not to its taxonomic (and consequently phylogenetic) composition, therefore, decreasing functional dispersion. Furthermore, it is not a natural gradient of stress (e.g. salinity, temperature) but the result of the cumulative disturbance that takes place in estuaries with international ports and increasing urbanization.

It is interesting to see how even in the sites with the highest richness (i.e. PN and BB) and phylogenetic diversity (i.e. the same of the richness + AN) there is high functional redundancy strongly suggesting an effect of the port presence which was not detected by the GLMM of the Rao's Q alone. Our choice of a measure of the functional dispersion rather than the simple diversity/richness (e.g. FD or FRic) seems to have a higher resolution power on discriminating also more subtle patterns, otherwise overlooked. Further tests, comparing different communities or at different scales, are necessary to properly evaluate the efficiency of this relationship, but our results suggest a higher power than the single indices.

4.4 Beta diversity drivers

The high proportion of phylogenetic beta diversity explained in our results suggests a selection of specific taxa. This is partially expected as the fouling community is by definition heavily disturbed and characterized by generalist species. The phylogenetic relatedness of a community bears many different ecological meanings that may be overlooked in the choice of the traits (Best et al. 2013). Nevertheless, the reduced representativity of the total ecological variation may be voluntary (as in this work), willingly reducing the possible functions to a specific selection. Our results support the use of integrated approaches using both facets of diversity (i.e. phylogenetic and functional) as a phylogenetic signal may carry historic or more complex processes that could be better explained with a more selected set of variables (e.g. functional traits).

As the geographic distance was significant only for the total taxonomic beta diversity, our results support the presence of other drivers independent from the spatial location. This has been confirmed by the results of network analysis and implying a some-sort of connection among sites, due to natural (e.g. rafting substrates with currents) or human-mediated dispersion. For instance, in the last decades, it has been recognized the

importance of local boating for the regional dispersion of fouling species (e.g. Wasson et al. 2001, Ashton et al. 2014, Ferrario et al. 2016, Kauano et al. 2016). Notwithstanding, the geographic distance was not significant for phylogenetic and functional beta diversity, implying a major role of other filtering variables.

Indeed, the main driver of beta diversity among the fouling communities studied appears to be the number of commercial ships visiting the areas. The data used in this work are somehow old (i.e. 2010) and as there was an increase in shipping during the last years (see <http://www.antaq.gov.br>) the effect could have been of an even greater extent. The increase of beta diversity (reaching an asymptote) between sites with high shipping means that the presence of ports is boosting the local diversity (either taxonomic, phylogenetic or functional). Notwithstanding, this increase does not have to be mistaken for a positive effect. In the scenario of marine bioinvasion (such as the port environment), it has to be remembered that beta diversity increase when local widespread species are substituted by non-native species entering the community (and does not turn ubiquitous) (Socolar et al. 2016).

The effect that the distance from the coast had on the total beta diversity, but mainly in the replacement, means a gradual change in fouling community and an increase in the taxonomic, phylogenetic and functional heterogeneity between sites/communities far from the coastline. Large distances from the coast or mainland mean also a scarcer supply of propagules when the taxon considered lacks larval stage (like amphipods), meaning that colonization is more stochastic and related to sporadic events (Hachich et al. 2015, Desiderato et al. 2019, Vieira et al. 2019). In this situation, the floating docks of marinas, or artificial structures of ports in general, may provide an almost permanent (depending on the biology of the species) source of species that may contribute to the homogenization of the communities closer to them (Meyer et al. 2017, Martins et al. 2017).

The significant effect of the precipitations (both the average and the range) is given by the strong correlation with the seasons found also in our previous analyses (i.e. network and diversity indices). It is important to underline how the average precipitations were the only other predictor (excluding shipping) in the beta diversity richness of the taxonomic and functional facets. This means a higher difference in the net number of species (i.e. loss) and functional combinations in the wet season. This can be related again to the higher environmental variability of this season, characterized by sudden changes in salinity (i.e.

sudden rainfalls) and temperature (i.e. warmest subtropical season). Moreover, the minimum wind speed was one of the predictors in the replacement (for all the facets of diversity), supporting again the effect of seasonality as stronger winds blow in the winter influencing the establishment of some species, as suggested previously (Neves et al. 2007). These two opposing results may reflect a pattern of environmental/climatic driving of the studied communities. Thus, the communities may be more influenced by the precipitations, displaying beta diversity richness (i.e. net number of species) changes in the wet season, while more affected by the winds and the replacement/turnover of species (functions) in the dry season.

The lack of correlation with salinity, despite one of our experimental design addressed it, might be also an artefact of the source of our environmental data. The values for the inner portion of estuaries is usually the result of predictions based on values from oceanographic buoys situated outside of them. This can easily produce a miscalculation if the freshwater input is localized far from the entrance of the estuary (such as in the PEC). Thus, our data related to it may have scarcely represented the real difference present among the sites hiding possible correlations.

5. Conclusion

In this work, we show how the structure of the amphipod assemblages of the fouling community is strongly influenced by the seasons and their diversity components decreasing in the wet season. Also, despite displaying a strong effect of the presence of port activities on all the facets of the biodiversity (i.e. taxonomic, phylogenetic and functional) and all the levels of the beta diversity, this was not evident in the other analyses (i.e. network and diversity indices). Furthermore, the relationship between functional dispersion and phylogenetic diversity showed important insights on the functional homogenization effect of ports on this globally found community. Finally, our results show the importance of integrative approaches to anthropogenic impact evaluation as it can affect communities in a compound way that might be difficult to depict and, thus, overlooked.

6. References

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GENERAL CONCLUSIONS

In the current thesis, I highlight how a commonly overlooked taxon of the vagile fouling may provide important insights into community ecology and bioinvasion mechanisms. Further, I demonstrate that there is still a clear lack of data (either molecular or taxonomic) for amphipod fouling communities, preventing effective management measures for both bioinvasions and biodiversity.

A main hypothesis that was tested in the experiment was that the vicinity of international ports should affect the fouling communities (e.g. disrupted community structures and functioning) and estuaries with already many records of NIS were specifically selected. Fouling communities commonly host introduced or invasive species due to its constitution (i.e. artificial substrates subjected to continuous disturbance). This may already partially explain the high richness found in this region which could be a result of former (recent or historical) introductions. The first chapter demonstrates how South Brazil seems to form a bioregion extending up to the southern coasts of the North-West Atlantic Ocean (USA), where also many of the found cryptic species were located. These findings are supported by other global-scale works on amphipods and other taxa, although this still needs to be better explored. Indeed, the past connection of the two continents (i.e. North and South America) have already shown a remnant signature on terrestrial animals but future works should explore if similarities in the marine realm could be due also to contemporary drivers (i.e. human-mediated transport).

The results of every chapter underline the strong effect of shipping on vagile fouling communities. For the global dispersal of two species in the genus *Jassa* as well as for the regional dissimilarities (i.e. beta diversity) of the communities, shipping activities always appeared to be the main driving factor. Nevertheless, seasonality had a stronger effect on the similarity between South Brazilian estuaries and on the structures of local communities (i.e. alpha diversity) than port vicinity and other environmental gradients. These results were possible thanks to the integrated experimental design that I chose, including both, the port effect, and the environmental gradient. Actually, the exclusion of the latter (i.e. excluding the most oligohaline impacted site) would have resulted in a stronger effect of the ports masking the environmental changes related to the seasonality (i.e. stronger winter winds and abundant summer rainfalls). Furthermore, the functional diversity (i.e. functional dispersion) appeared to be independent from the evolutionary history of those communities that were impacted by ports, whereas functional diversity and evolutionary history were correlated in

absence of ports. This demonstrates the pressure on fouling communities for the selection of specific traits, resulting in similar (or even identical) functional profiles in fouling communities across the world, although this may still be subjected to climatic and/or environmental-driven variation, as showed in the third chapter.

Thus, this thesis highlights how global-scale phylogeographic works are necessary to unravel the current status and past origin of fouling species. However, regional-scale studies are essential for appropriate management decisions and are crucial for the prediction of global-scale patterns. Although fouling communities are widespread, they require an in-depth look on all levels of diversity using integrated and/or specific approaches to address evolutionary and ecological questions.

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ANNEX I

A NEW GENUS AND SPECIES OF AORIDAE (AMPHIPODA, SENTICAUDATA), *PROPEJANICE LAGAMARENSIS* GEN. NOV. SP. NOV. FROM BRAZIL

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A new genus and species of Aoridae (Amphipoda, Senticaudata), *Propejanice lagamarensis* gen. nov. sp. nov. from Brazil.

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Abstract

A new monotypic genus of aorid amphipod *Propejanice* **gen. nov.** is described and figured from Brazil. The single species, *P. lagamarensis* **sp. nov.** was collected from artificial plates suspended in the sea in Paraná and Sao Paulo States, Brazil. The new genus appears to be morphologically closest to the genus *Janice* Griffiths, 1973 from Moçambique. Both genera are phylogenetically close to the genus *Grandidierella* Coutière, 1904.

Key words: taxonomy, Crustacea, Amphipoda, Aoridae, Brazil, new genus, new species, *Propejanice*

1. Introduction

A number of specimens of an apparently undescribed species of aorid amphipod were collected from artificial substrates in two estuaries in southern eastern Brazil. These estuaries are in one of the last regions with Atlantic temperate forest and are part of the Lagamar Mosaic. One of them includes two international harbours, Antonina and Paranaguá, the former being adjacent to the sampling site. The material is attributed here to a new genus allied to the genus *Janice* Griffiths, 1973 that is currently known only from

Moçambique. An elongate divergent merus on the gnathopod 2 is a shared character state between these two genera. The ancestor of these genera is assumed to have occurred along the joint eastern south American – western African coast line before the formation of the south Atlantic around 100-120 My.

2. Materials and Methods

Artificial plates were submerged for three, six and twelve months and kept at an average depth of 1.5 meters. The artificial plates were either of polyethylene or made of a nylon rope wrapped around an iron frame. The plates recovered, along with fouling associated, were directly placed in 96% alcohol, which was changed after one day.

The material was dissected in 70% alcohol and the parts mounted on glass microscope slides in glycerine for examination under a Nikon Optiphot microscope with interference contrast attachment and drawn with the aid of a drawing tube. Type material is deposited in the Museu Nacional do Rio de Janeiro, Brazil.

For molecular analyses, DNA was extracted from one or two pereopods using the QIAmp DNA Mini Kit (Qiagen) according to the manufacturer's protocols. In order to provide the DNA barcode of this new genus, the Folmer fragment of the mitochondrial cytochrome oxidase subunit 1 gene (CO1) was amplified using the universal primers LCO1490/HCO2198 (Folmers et al. 1994). Each reaction consisted of 0.2 mM dNTPs, 0.5 μ M of forward and reverse primers, 10 x PCR-buffer, 0.02U/ μ l Hotmaster Taq (5Prime), 3 μ l of template DNA, and water to make the mix to the final 25 μ l. PCR thermal cycling conditions consisted of an initial denaturation at 94 °C for 2 min, followed by 36 cycles of 94 °C for 20 s, primer annealing at 42 °C for 20 s, extension at 65 °C for 1 min, and lastly a final extension at 65 °C for 15 mins. DNA barcodes were stored and made available in the Barcode of Life Data System (BOLD, Ratnasingham and Hebert, 2007).

BOLD accession numbers: BMFVF005-19, BMFVF055-1

Abbreviations used in figures: Md = mandible palp; G1, 2 = gnathopods 1, 2; P3–7 = pereopods 3–7; U1–3 = uropods 1–3; F = female; M = male.

3. Systematic section

Suborder Senticaudata Lowry & Myers, 2013

Infraorder Corophiida Leach, 1814

Parvorder Corophiidira Lowry & Myers, 2013

Superfamily Aoroidea Stebbing, 1899

Family Aoridae Stebbing, 1899

Propejanice gen. nov

Diagnosis. *Head* anteroventral margin moderately excavate; ocular lobes obtuse, only moderately produced. *Antenna 1* peduncular article 3 much shorter than peduncular article 2. *Antenna 2* subpediform. *Mandible* palp article 3 not expanded distally. *Gnathopod 1* enlarged in male, larger than gnathopod 2, carpocheate. *Gnathopod 2* in male slender, setose, merus elongate, subquadrate, divergent. *Pereopod 7* longer than pereopod 6, *Uropod 3* uniramous.

Type species. *Propejanice lagamarensis* sp. nov. by monotypy.

Etymology. From the name of its closest relative *Janice* (a name derived from the Hebrew Yochanan), with the Latin prefix 'prope' meaning close to.

Remarks. *Propejanice* gen. nov. closely resembles *Janice* Griffiths, 1973. It shares with it, the long divergent merus on gnathopod 2. It differs from it, however, in the carpocheate male gnathopod 1 (subcheate in *Janice*) in which the posterodistal margin of the carpus is drawn out into a long acute spine. *Janice spinidactyla* Griffiths is known from a single male specimen. It closely resembles the female of *Propejanice lagamarensis* sp. nov. described here. If, when further material of *Janice spinidactylus* Griffiths is available, it is shown that Griffith's specimen is in fact a female and furthermore that males of the species have a carpocheate gnathopod 1, then *Propejanice* may have to be synonymised with *Janice*. For the moment we accept that the unique specimen of *Janice spinidactyla* is, as indicated by

Griffiths (1973) a male and accordingly we erect the genus *Propejanice* **nov. gen.** for *P. lagamarensis* **sp. nov.**

Myers & Lowry (2003), placed the little known genus *Janice* in the Unciolidae because of the close resemblance of the gnathopod 2 to that of the unciolid *Paracorophium* Stebbing, 1899. It is clear now after study of material of the new genus, that *Janice* Griffiths and *Propejanice* **gen. nov.** should both be correctly placed in the Aoridae and that the peculiar gnathopod 2 is a homoplasy.

4. *Propejanice lagamarensis* sp. nov.

(Figs 1–3)

Type Material. Holotype male 5.0 mm (MNRJcarcino 029412) artificial rope plate, 1.5 m, on marina buoy, Antonina, Paraná, Brazil, 12.09.2017, A. Desiderato.

Other material. 5 male, paratypes (MNRJcarcino 029413), same data holotype; 1 female, 1 juvenile (MNRJcarcino 029414), artificial polyethylene plate, 1.5 m on signal buoy, Antonina, Paraná, Brazil, 04.06.2018, A. Desiderato; 1 juvenile (MNRJcarcino 029415), artificial polyethylene plate, 1.5 m floating dock/gasoline recharge station, Cananeia, Sao Paulo, Brazil, 06.06.2018, A. Desiderato.

Type locality. Antonina, Paraná State, Brazil.

Etymology. Named after the Lagamar Mosaic that includes both the estuaries in which the species was found.

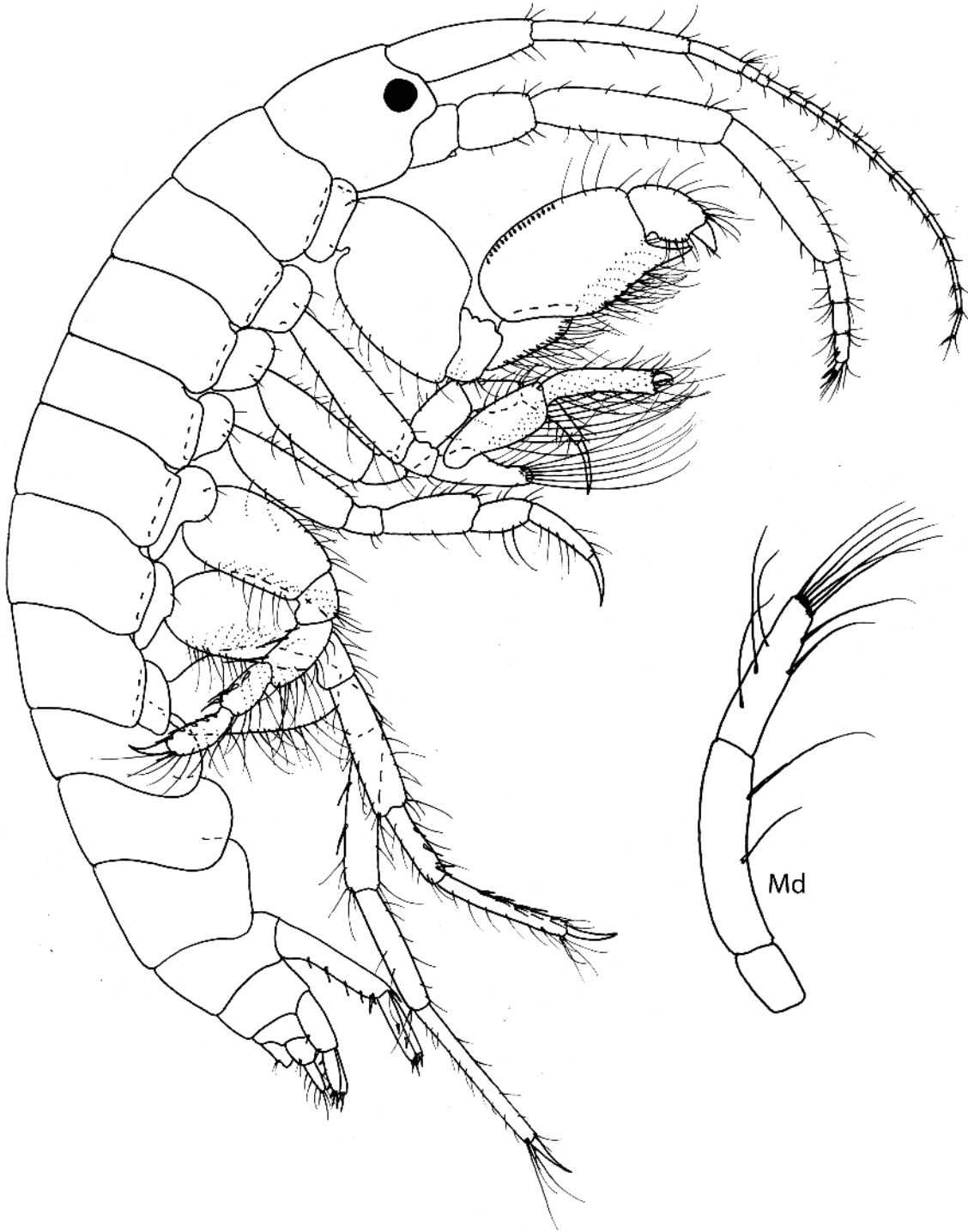


FIGURE 1. *Propejanice lagamarensis* sp. nov. male holotype, Antonina, Paraná, Brazil

Description. Head. *Ocular lobes* obtuse; eyes of moderate size, black. *Antenna 1* peduncular articles in the ratios 8:8.5:3; primary flagellum sub-equal in length with peduncle with 16 articles; accessory flagellum minute with one rudimentary fused article bearing

terminal setae. *Antenna 2* subpediform, a little shorter than antenna 1; peduncular article 5 a little shorter than 4, flagellum shorter than peduncular article 5, with 4 articles. *Mandible* palp article ratios 2:6:4.5, article 3 subrectangular, with long apical and subapical setae.

Pereon. *Gnathopod 1* coxa much longer than broad, subrectangular, anterior margin rounded, unproduced; basis almost as broad as long; carpus large, subrectangular, anterior margin proximally broadly rounded, anteroproximal to midproximal margin with a single row of stridulating ridges; posterior margin weakly concave, $1.5 \times$ as long as broad, the posterior distal margin produced into a well developed, slender spine, propodus short, less than half length of carpus, posterior margin with distinct flange; dactylus becoming short, triangular, obtuse in large adult males. *Gnathopod 2* coxa subrectangular, a little longer than broad; basis elongate, $4 \times$ as long as broad, anterior margin substraight; merus elongate, subquadrate, diverging from carpus, with numerous, long pectinate distal setae; carpus elongate, more than $2 \times$ as long as broad, anterior margin weakly setiferous, posterior margin with very long pectinate setae; propodus subequal in length with carpus, narrow, with weakly convex anterior and straight posterior margin, palm oblique; dactylus short, fitting palm, strongly falcate. *Pereopods 3–4* coxae evenly convex anteriorly; dactylus two thirds length of propodus. *Pereopods 5–7* in the length ratios 5:7:8; *pereopods 6–7* basis with long setae on the posterior margin.

Pleon. *Epimera 1–3* rounded. *Uropod 1* peduncle much longer than rami, with a stout, distal, inter-ramal spiniform process; inner ramus longer than outer with two marginal setae, outer ramus lacking marginal setae. *Uropod 2* peduncle broad, a little longer than outer ramus, lacking an inter-ramal process; inner ramus longer than outer. *Uropod 3* uniramous; peduncle subsquare, with strongly expanded flange on inner margin; ramus $2 \times$ length of peduncle with a small second article and several long marginal and distal setae. *Telson* as broad as long with nearly straight ventral margin, dorsolateral crests each with a group of 2 long setae, lateral margins each with 2 setae.

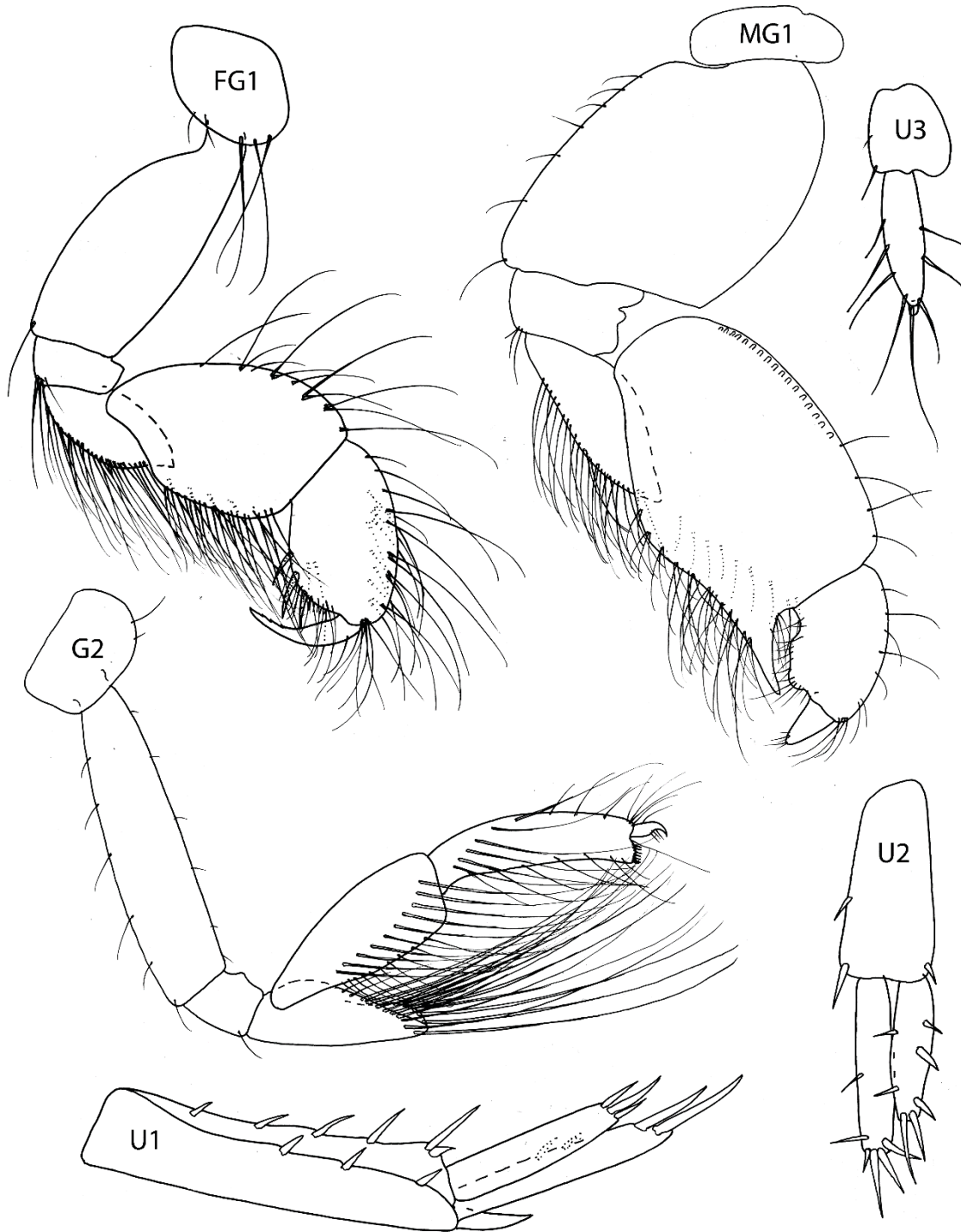


FIGURE 2. *Propejanice lagamarensis* sp. nov. male holotype, female paratype, Antonina, Paraná, Brazil.

Female 5.0 mm (sexually dimorphic characters). *Gnathopod 1* basis a little over 2 × as long as broad, anterior margin weakly convex; carpus subovoid, only a little longer than broad, anterior and posterior margins strongly setose; propodus about two thirds length of

carpus, subovoid, palm oblique, not clearly defined from posterior margin, which bears 3 strong robust setae; dactylus elongate, two thirds length of propodus.

Habitat. Brackish waters between 8 and 25 psu.

Remarks. The male gnathopod 1 of *P. lagamarensis* closely resembles that of *Grandidierella perlata* Schellenberg, 1938 from Fiji. It shares with that species the presence of stridulating ridges on the anteroproximal margin of the carpus of the male gnathopod 1. It differs from that species, however, in the characteristic elongate, divergent merus of the male gnathopod 2.

When species occur as fouling in harbours historically frequented by international vessels, there is always the possibility that some species may have been transported anthropogenically. The plausibility of this explanation will only be resolved after more extensive studies along both the Brazilian and African coasts. At the moment, *Propejanice* **gen. nov.** has never been reported from any previously studied harbours anywhere in the world. The occurrence of the sibling pair *Janice* and *Propejanice*, one in the Atlantic and one in the Indian Ocean could be explained by the occurrence of an ancestor along the contiguous coastlines of what are now South America and Africa (Gondwana), 100 – 120My. With the opening of the South Atlantic during the late Cretaceous, the two genera would have become isolated from one other. This latter hypothesis would be supported by the discovery of the genus in Brazil well away from man-made harbours together with failure to find the genus in Africa despite more extensive studies.

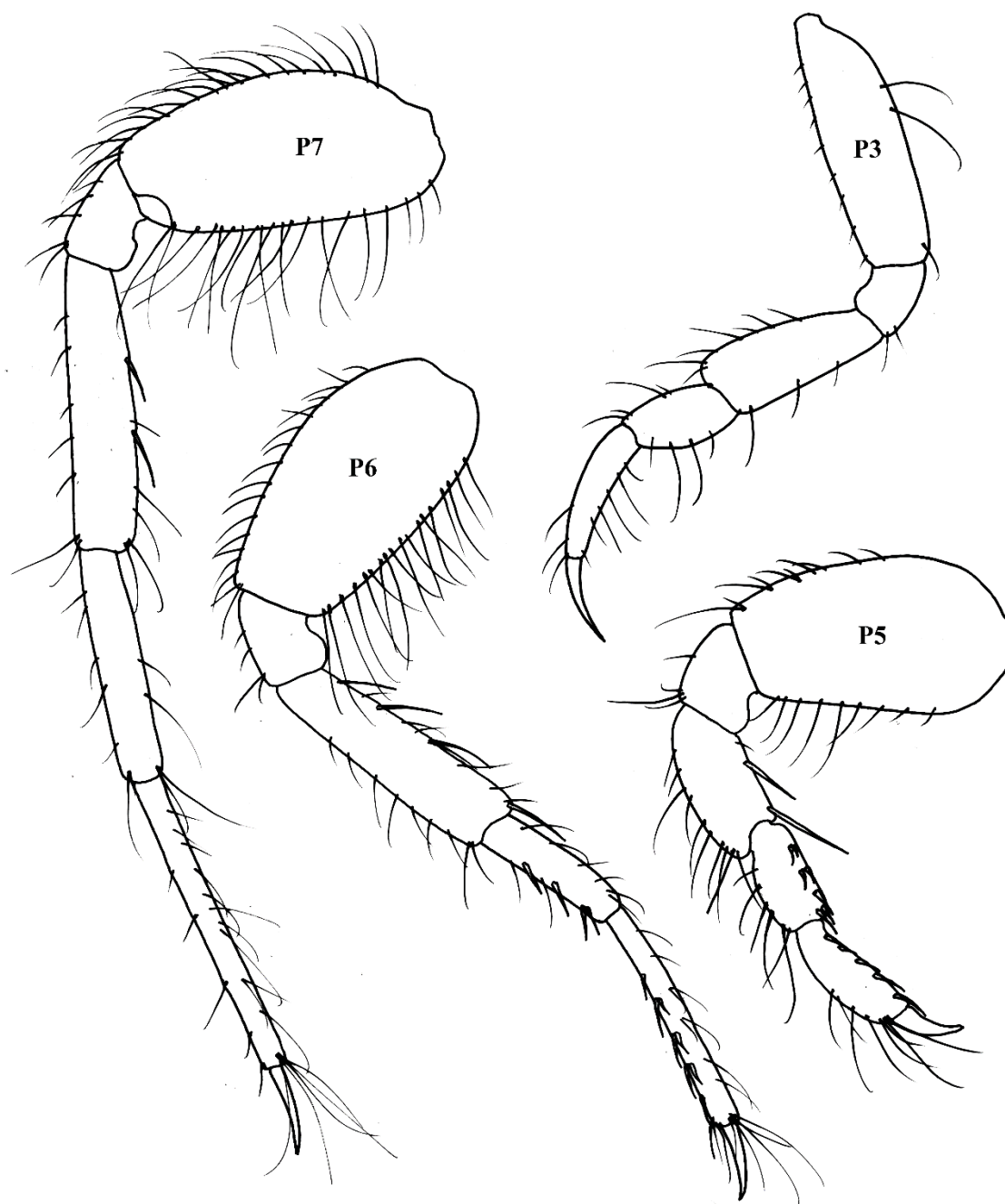


FIGURE 3. *Propejanice lagamarensis* **sp. nov.** male holotype, Antonina, Paraná, Brazil.

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ANNEX II

HIDDEN IN PLAIN SIGHT: CO-INTRODUCTION OF EPIBIOTIC DIATOMS WITH AMPHIPOD CRUSTACEANS?

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Hidden in plain sight: co-introduction of epibiotic diatoms with amphipod crustaceans?

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Abstract

In fouling communities of three estuaries in southern Brazil, individuals belonging to three different amphipod species were found harbouring a dense coverage of epibionts: the two globally widespread caprellids *Caprella equilibra* and *Paracaprella pusilla*, as well as the ischyrocerid *Jassa valida*. Further SEM analyses assigned the epibionts to 14 different species of epibiotic diatoms. This is the first record of diatoms attached to amphipods. The occurrence of the diatom species *Amphora helenensis* represents the first report for Brazilian waters and the second record for the whole SW Atlantic Ocean, respectively. As some diatoms were associated with the cryptogenic caprellids, a co-introduction of the diatoms to Brazil on amphipod specimens seems likely. The underlying transport mechanisms are still poorly understood, while any effects on the animals and possible implications for the ecosystem of this amphipod-diatom association remain to be investigated.

Keywords: Amphipoda, Bacillariophyceae, Basibiont, Biofouling, NIS

1. Introduction

Many studies have focused on human-based transport mechanisms of non-indigenous species (NIS), but processes of co-introduction through associations such as parasitism or epibiosis are still understudied (Vanhove et al. 2016; Goedknecht et al. 2016). These associations allow simultaneous multiple introductions that can consequently affect ecosystems on several levels. With mycorrhizal associations, for example, the transfer of entire host plants also leads to co-introductions and spillover of fungal species, affecting native fungal assemblages (Santolamazza-Carbone et al. 2019). Furthermore, introductions of non-indigenous plants can also result in co-invasions of forest pests such as gall-inducing insects (Csóka et al. 2017). In marine environments, the canopy-forming macroalga *Sargassum muticum* (Yendo) Fensholt, 1955 was probably introduced to North American and European shores with the Pacific oyster *Magallana gigas* (Thunberg, 1793) (formerly known as *Crassostrea gigas*), which was intentionally brought for aquaculture purposes. The invasive seaweed outcompetes native canopy-forming macroalgae, dominating tidal pools and compromising intertidal communities (Engelen et al. 2015).

Marine ecosystems face an increased risk of the spread of NIS because even geographically distant locations are virtually connected through the oceans. This is further amplified by intensive human shipping activities, increasing the dispersal of species through hull fouling, ballast water and other vessel components (e.g. Gollasch 2002; Seebens et al. 2016). In addition, ports meet most of the requirements for the successful introduction of NIS. Ports feature continuous pressure of propagules in usually eutrophic environments and are modified by artificial structures where native species can lose their potential competitive advantage (Byers 2002; Blackburn et al. 2014). Hard substrata in ports are usually characterized by fouling communities consisting of r-strategists, filter feeders and scavengers (Mayer-Pinto et al. 2018). These communities are known to be comparably rich in NIS, which frequently outcompete native species in the colonization of these substrates (Megina et al. 2016).

Amphipod crustaceans can dominate marine and estuarine fouling communities in terms of abundance and sometimes even biomass. At least 55 amphipod species are recognised as introduced or invasive in at least one marine region around the world, and more than half of them are directly associated with biofouling (Marchini and Cardeccia

2017). Accordingly, amphipods should be effective vectors for any amphipod-associated organism.

In a field experiment designed to study fouling communities related to ports, artificial plates of polyethylene and rope (nylon rope wrapped around a squared iron frame) were deployed in three estuaries of southern Brazil between June 2017 and June 2018. After three months, the plates were collected and individually preserved in 96% ethanol. On-site of the experimental setup, additional samples of the fouling were scraped from buoys and preserved in ethanol for further comparison. All amphipod individuals were sorted and identified to the species level. During this step, some amphipods displayed conspicuous levels of epibiosis and were separated for further examination using a scanning electron microscope (SEM). The specimens were mounted on aluminium stubs with a small piece of adhesive carbon tape, completely air-dried at room temperature and kept in an air desiccator jar. The stubs were then sputter-coated with gold in a Baltec metallizer and observed in a JEOL JSM 6360-LV electron microscope at an acceleration voltage of 15 Kv and a 7 mm working distance (Fig. 1A).

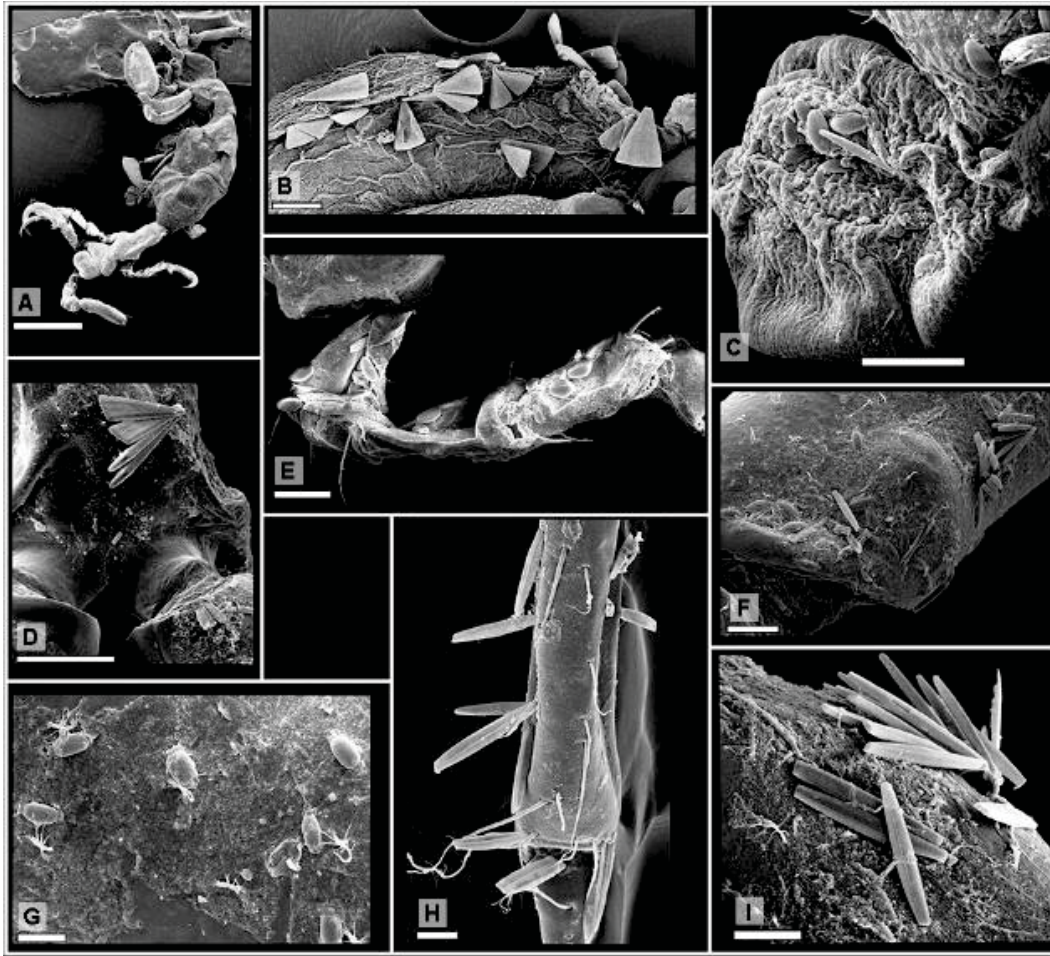


Fig. 1. Diatom distributions on amphipod body parts. A: Female specimen of *Caprella equilibra* (scale: 500 μm); B: *Licmophora abbreviata* on the dactyl of gnathopod 2 of *C. equilibra* (scale: 50 μm); C: Diatoms *Amphora* and *Tabularia* on a gill of *Paracaprella pusilla* (scale: 50 μm); D: *Licmophora ehrenbergii* on the head of *P. pusilla* (scale: 100 μm); E: *Amphora helenensis* on the pereopod 5 of *P. pusilla* (scale: 50 μm); F: Diatoms *Halamphora* and *Tabularia* on pereonite 5 of *P. pusilla* (scale: 50 μm); G: *Halamphora coffeiformis* on pereonite 4 of *P. pusilla* (scale: 20 μm); H: *Tabularia fasciculata* on pereopod 5 of *C. equilibra* (scale: 20 μm); I: *Tabularia affinis* on the head of *P. pusilla* (scale: 20 μm)

2. Results & Discussions

A total of 14 different diatom species belonging to seven families were identified. The families Catenulaceae and Naviculaceae were the most species-rich, with three species each. The diatoms found are typically benthic species, i.e. attached to substrates employing a variety of mucilage secretions. The most common structures were mucilaginous peduncles in *Tabularia* spp. and *Licmophora* spp. (Fig. 1I - 2B), along with mucilage pads as in *Amphora helenensis* M.H. Giffen, 1973, *Halamphora coffeiformis* (C. Agardh) Mereschowsky, 1903, *Cocconeis* spp. (Fig. 1E, G - 2I). Other species, such as *Navicula* spp., seemed to attach to amphipod individuals using mucilaginous secretions of the raphe (Fig. 2L, O). Although no quantitative analysis was performed, some diatom taxa were

dominant, such as *Tabularia affinis* (Kützinger) Snoeijs, 1992, *Licmophora* spp., *H. coffeiformis* and *A. helenensis*, comprising approximately 50% of the total diatom composition in the majority of amphipod individuals analysed. All the diatoms found here have been reported elsewhere from other substrates, such as macroalgae and rocks (Snoeijs 1992; Witkowski et al. 2000), indicating that these species are rather opportunistic to grow on invertebrates. Although some diatoms are known to be specialized epizoic on planktonic copepods (Fernandes and Calixto-Feres 2012), only a few works dealt with the growth of diatoms on benthic invertebrates. Usually, diatoms of the genera *Navicula*, *Amphora* and *Cocconeis* are known to occur on several species of cnidarians (Romagnoli et al. 2007), sponges (Totti et al. 2005) and gastropods (Gillan and Cadée 2000; D'Alelio et al. 2011).

The biraphid diatom *A. helenensis* found in this work represents the first record for Brazilian coasts. This species has been recorded repeatedly around the world outside of its type locality (South Africa). Recent records include the coastal waters of Australia and New Zealand (Harper et al. 2012), Europe (The Netherlands; Veen et al. 2015), China (Yellow Sea; Witkowski et al. 2016) and Mexico (Pacific Ocean; López-Fuerte et al. 2019). We found *A. helenensis* in the Paranaguá estuarine complex, an environment where many taxonomic studies have been performed since 1960 (see Procopiak et al. 2006), suggesting a likely recent introduction to South Brazil. However, it cannot be ruled out that this species had been overlooked or misidentified with other closely related species at other locations in the SW Atlantic, where it was recorded only once in Argentina (Hassan et al. 2006). Therefore, *A. helenensis* can be classified as cryptogenic.

Twelve specimens of amphipods bore epibiotic diatoms (Tab. 1). The specimens belonged to only three cryptogenic species: the two globally distributed caprellids *Caprella equilibra* Say, 1818 and *Paracaprella pusilla* Mayer, 1890 and the ischyrocerid *Jassa valida* (Dana, 1853). The highest diversity of epibionts was found on a male specimen of *P. pusilla* with seven different diatom species on its cuticula (Tab. 1). Three diatom species were each found only once (i.e. *Melosira moniliformis* C. Agardh, 1824, *Rhoicosphenia marina* (Kützinger) M. Schmidt, 1889 and *Tabularia fasciculata* (C. Agardh) D.M. Williams & Round, 1986) on caprellids. The diatoms were distributed over the whole body of the animals, but the appendages seemed to exhibit a higher coverage with epibionts than the rest of the body.

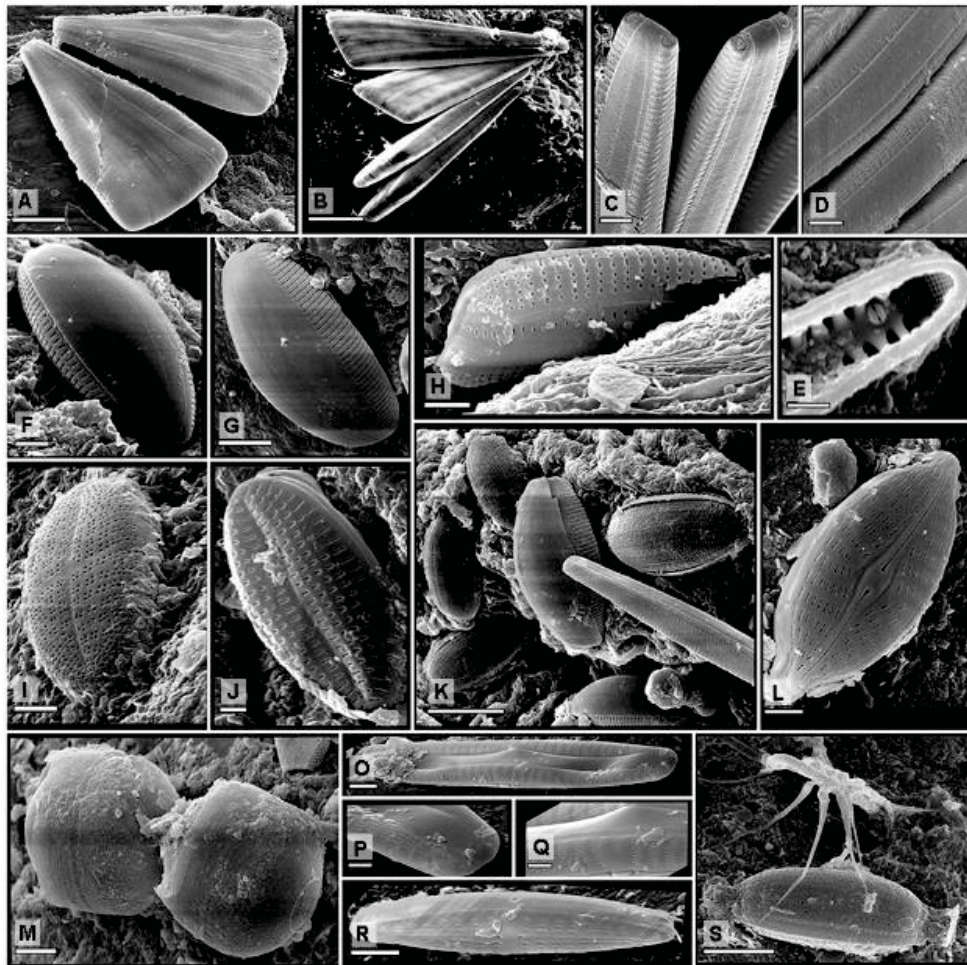


Fig. 2. Epibiotic diatom species found on fouling amphipods in south Brazilian estuaries. A: overall view of two frustules of *Licmophora abbreviata* (scale: 10 μ m); B: four cells attached by short mucilage stalks of *Licmophora ehrenbergii* (scale: 20 μ m); C-D: details of frustules showing the ocellulimbus at the apices of *Tabularia affinis* (scale: 2 μ m) and E: internal view of valve apex with rimoportula (scale: 1 μ m); F: lateral view of a frustule of *Amphora pediculus* (scale: 2 μ m); G: lateral view of a frustule of *Amphora helenensis* (scale: 5 μ m); *Rhoicosphenia marina* (scale: 2 μ m); I: Frustule attached by mucilage extruded through the raphe of *Cocconeis dirupta* (scale: 2 μ m); J: Frustule attached by mucilage extruded through the raphe of *Cocconeis* sp.1 (scale: 1 μ m); K: *Amphora*, *Halamphora* and *Tabularia* (scale: 10 μ m) on a gill of *P. pusilla*; L: Frustule in valve view, with a central raphe system of *Navicula platyventris* (scale: 2 μ m); *Melosira moniliformis* (scale: 5 μ m); O: *Navicula* sp.1 (scale: 2 μ m) with P-Q: two details of apical and central regions (scale: 1 μ m); R: *Navicula* sp.2 (scale: 2 μ m); S: Frustule in girdle view of *Halamphora coffeiformis* (scale: 5 μ m) covered by a seta of *Paracaprella pusilla*

Possible interactions of diatoms with their amphipod hosts are completely unknown. Although it could be possible that high diatom densities on fragile body parts such as the gills or sensory appendages (Fig. 1C, H) may lead to handicaps (e.g. interferences in gas exchange, reproduction or sensorial activity; Ohtsuka et al. 2011), the amphipods and the diatoms seem to display a basibiont-epibiont association.

Although to our knowledge, this work represents the first description of this association, there is most likely a previously overlooked example of a caprellid-diatom association in Krapp et al. (2006). In their work, the authors meticulously illustrated specimens of *Caprella scaura* Templeton, 1836 which show clear diatom epibionts not mentioned in the work (see figures 5, 6 in Krapp et al. 2006). Furthermore, commonly applied cleaning procedures for SEM preparation most likely prevent many epibiotic discoveries. In fact, most epibiotic diatom species are small (<50 µm) and difficult or nearly impossible to detect with light microscopy only.

Tab. 1. Overview of amphipod and diatom species recorded for each analysed sample. Substrates: PP - polyethylene plate; B - buoy; RP - rope plate.

Estuary	Substrate	Lat S	Long W	Amphipod species	Sex	<i>Amphora helenensis</i> M.H.Giffen 1973	<i>Amphora pediculus</i> (Kützing) Grunow-Heurck, 1885	<i>Halimphora coffeiformis</i> (C. A. Agardh) Levikov, 2009	<i>Cocconeis</i> sp. 1	<i>Cocconeis dirupta</i> Gregory, 1857	<i>Licmophora cf. abbreviata</i>	<i>Licmophora ehrenbergii</i> (Kützing) Grunow, 1867	<i>Melosira moniliformis</i> (O. Muller) Agardh, 1824	<i>Navicula platyventris</i> F. Meister 1935	<i>Navicula</i> sp. 1	<i>Navicula</i> sp. 2	<i>Rhicosphenia marina</i> (Kützing) M.Schmidt, 1889	<i>Tabularia affinis</i> (Kützing) P. Snoeijls, 1992	<i>Tabularia fasciculata</i> (C. Agardh) D.M.Williams &
Cananeia-Iguape Estuary Complex	PP	25.021	47.918	<i>P. pusilla</i>	Male			x	x							x			
		25.027	47.914		Juvenile												x		
Paranaguá Estuary Complex	B	25.467	48.663	<i>P. pusilla</i>	Male					x	x	x					x		
	PP	25.473	48.649	<i>C. equilibra</i>	Female					x								x	x
	B	25.497	48.491	<i>C. equilibra</i>	Male						x	x		x	x				
	RP	25.549	48.389		Juvenile	x		x										x	
				<i>J. valida</i>	Male			x											
				<i>P. pusilla</i>	Male			x							x			x	
	PP	25.549	48.388	<i>P. pusilla</i>	Female	x	x	x		x									
					Male	x	x	x	x	x				x				x	
Guaratuba Bay	PP	25.875	48.589	<i>C. equilibra</i>	Juvenile	x	x						x						
					Juvenile				x										

3. Conclusions

Our findings represent the first description of an epibiotic association between diatoms and amphipods. In the current case, an association between globally distributed amphipods and epibiotic diatoms likely led to a co-introduction of diatoms with the animals. Amphipods seem to be potent vectors for epibiotic organisms such as diatoms and ciliates (e.g. Fernandez-Leborans et al. 2016), especially in fouling communities. Along with increasing marine shipping activities around the world, stochastic events such as co-

introductions on small benthic invertebrates could increasingly promote the establishment of NIS in native communities, eventually leading to a homogenization of marine fouling communities.

4. Acknowledgements

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Supplementary material

Preface

Tab.S1 List and respective count for words appearing more than 10 times in the literature searched on Web Of Science between 1990 and 2020.

Word	Count	Word	Count	Word	Count	Word	Count
fouling	3866	test	627	substrat	443	invertebr	340
communit	3366	perform	625	within	442	benthic	335
speci	3202	higher	603	chemic	438	effici	334
membran	2883	indic	602	period	438	field	334
studi	1927	wastewat	592	number	436	type	334
effect	1775	habitat	588	similar	436	group	330
use	1613	bacteri	586	predat	430	occur	325
differ	1557	sludg	581	ecolog	427	rich	323
water	1437	product	579	biomass	426	method	321
result	1288	impact	577	pattern	425	oyster	321
surfac	1280	natur	566	barnacl	423	flux	318
organ	1219	growth	560	success	423	record	317
increas	1163	investig	559	panel	421	spong	317
structur	1086	suggest	559	model	414	form	316
marin	1030	howev	556	mussel	408	major	316
signific	960	reduc	554	year	408	variat	316
develop	939	concentr	544	day	405	function	315
biofoul	912	invas	542	season	405	base	314
biofilm	901	associ	537	environ	402	presenc	314
abund	898	one	533	determin	400	respect	314
treatment	893	influen	530	ascidian	398	common	312
high	878	area	529	distribut	398	cover	311
microbi	866	elsevi	525	decreas	391	dynam	311
system	861	bacteria	522	assess	389	characterist	310
show	811	artifici	520	measur	381	coastal	310
remov	796	sampl	518	first	380	diatom	309
time	759	includ	514	due	379	plant	309
bioreactor	719	mbr	513	month	376	respons	309
process	710	temperatur	513	larg	375	experiment	308
potenti	698	experi	512	role	373	data	307
control	695	reserv	497	bay	372	size	307
settlemen	693	right	494	factor	372	locat	306
oper	688	present	493	evalu	368	bryozoan	303
chang	682	analysi	484	rang	366	depth	302
found	666	coat	484	layer	365	mechan	300
activ	653	fish	481	main	365	research	300
domin	651	low	474	densiti	363	attach	299
relat	651	colon	472	region	361	clean	296
assembla	647	level	472	alga	360	taxa	293
divers	646	reef	469	lower	360	degre	292
composit	638	popul	463	identifi	356	establish	292
condit	638	total	457	caus	352	spatial	292
site	638	new	455	among	351	coast	288
compar	637	affect	454	nativ	350	local	287
sea	636	antifoul	454	biolog	349	vari	284
rate	634	provid	452	examin	349	larva	283
recruit	631	environment	450	scale	344	mani	283

observ	630	addit	449	sever	343	applic	282
import	629	interact	449	reveal	341	demonstr	282
limit	282	stage	224	mbrs	197	epibiont	173
individu	280	design	223	physic	197	comparison	171
produc	280	filtrat	222	greater	196	compon	171
current	278	introduct	222	oxygen	195	extracellular	171
report	275	like	221	previous	195	acid	170
variabl	275	will	220	repres	195	obtain	170
direct	274	follow	219	sourc	195	analys	169
resist	271	consid	218	depend	194	oil	168
flow	270	avail	217	describ	194	various	168
improv	265	competit	217	earli	194	discuss	167
less	265	possibl	217	mean	194	futur	167
properti	264	recent	217	net	194	hard	167
pressur	263	sediment	217	survey	194	mitig	166
treat	263	strong	216	osmosi	193	extract	165
coloni	258	detect	215	work	193	live	165
enhanc	258	introduc	215	appear	191	estim	164
collect	256	larval	215	carbon	191	find	164
materi	255	consist	214	cod	190	settl	164
ship	254	select	214	expos	190	suspend	164
conduct	253	shell	214	known	190	achiev	163
coral	253	submerg	214	technolog	190	allow	163
farm	253	seawat	212	cultur	189	bivalv	163
reactor	249	character	211	harbour	188	great	163
along	248	hull	211	lead	188	laboratori	163
energi	247	nutrient	210	prevent	188	overal	163
initi	247	valu	210	requir	187	stress	163
contribut	244	approach	209	part	185	even	162
manag	244	compound	209	continu	184	light	162
monitor	244	nonindigen	209	paper	184	mediterranean	162
complex	242	revers	209	without	184	port	162
industri	242	accumul	207	longterm	183	mortal	161
disturb	241	particular	205	nis	183	desalin	160
salin	240	exposur	204	novel	183	food	160
marina	239	general	203	isol	182	reduct	159
small	239	review	203	tempor	182	solut	159
anaerob	238	combin	202	appli	180	exhibit	158
format	237	four	202	solid	179	ocean	158
posit	237	sequenc	202	support	179	facilit	157
substanc	237	space	202	problem	178	intens	157
understan	237	cage	201	qualiti	178	substratum	157
correl	234	feed	201	integr	177	cost	156
aquacultu	233	order	201	polymer	177	dispers	156
ecosyster	233	predict	201	contain	176	paint	156
strategi	233	substrata	201	surviv	176	spread	155
cake	231	remain	199	effluent	175	zone	155
thus	231	copper	198	microorgan	175	play	154
cell	230	deploy	198	north	175	amphipod	153
sessil	227	island	198	relationship	175	case	153

plate	226	need	198	nitrogen	174	inhibit	153
specif	224	pollut	198	term	174	releas	153
retent	153	averag	136	life	123	typic	111
algal	152	host	136	reproduct	123	exot	110
alter	152	invad	136	dissolv	122	histori	110
matter	152	summer	136	film	122	maximum	110
ratio	152	transport	136	morpholog	122	set	110
gradient	151	wherea	136	near	122	australia	109
hydroid	151	wide	136	challeng	121	induc	109
metal	151	distinct	135	explain	121	western	109
nonnat	151	unit	135	strain	121	boat	108
degrad	150	contrast	134	behavior	120	demand	108
toxic	150	despit	134	origin	120	phase	108
adhes	149	state	134	shown	120	place	108
analyz	149	around	133	littl	119	rocki	108
immers	149	lake	133	offshor	119	aerat	107
paramet	149	mix	133	separ	119	epiphyt	107
therefor	149	per	133	vector	119	vertic	107
contamin	148	confirm	132	atlant	118	microscopi	106
crab	148	primari	132	polym	118	succession	106
negat	148	solubl	132	sinc	118	transfer	106
often	148	spp	132	tmp	118	estuari	105
platform	148	tropic	132	macroalga	117	harbor	105
rapid	148	commerci	131	manipul	117	juvenil	105
root	148	clear	130	srt	117	biotic	104
consequ	147	oxid	130	whether	117	heat	104
especi	147	practic	130	altern	116	larger	104
aim	146	pretreat	130	optim	116	shallow	104
intertid	146	red	130	consider	115	polysaccharic	103
key	146	secondari	130	loss	115	balanus	102
subtid	146	tunic	130	stabl	115	event	102
techniqu	146	enrich	129	five	114	infect	102
week	146	foulant	129	grow	114	orient	102
evd	145	risk	129	involv	114	permeat	102
fauna	145	knowledg	128	long	114	prefer	102
recoveri	145	south	128	reach	114	account	101
particl	144	amount	127	situ	114	aerob	101
polychaet	144	abil	126	adult	113	econom	101
focus	143	convent	126	filter	113	explor	101
power	143	mgl	126	gene	113	seagrass	101
exist	142	age	125	health	113	bottom	100
float	142	anim	125	taxonom	113	forc	100
protein	142	global	125	via	113	furthermor	100
shift	142	gulf	125	cycl	112	issu	100
southern	142	load	125	macrofoul	112	maintain	100
content	141	much	125	moreov	112	metabolit	100
highest	139	throughout	125	plastic	112	singl	100
across	138	vessel	125	protect	112	station	100
carri	138	either	124	resourc	112	alien	99
generat	138	smp	124	wast	112	close	99

declin	137	better	123	biodivers	111	final	99
mangrov	137	inform	123	frequenc	111	genus	99
might	99	mobil	92	open	83	scienc	77
molecular	99	subsequ	92	tile	83	soft	77
northern	99	suitabl	92	toward	83	trophic	77
quantifi	99	varieti	92	women	83	warm	77
still	99	coverag	91	accord	82	advanc	76
util	99	extent	91	andor	82	bulk	76
fraction	98	least	91	consumpt	82	caprellid	76
made	98	length	91	corros	82	employ	76
pacif	98	transmembrai	91	fullscal	82	given	76
probabl	98	coupl	90	green	82	numer	76
propos	98	intern	90	poor	82	spring	76
regard	98	nanoparticl	90	suppli	82	temper	76
river	98	proport	90	toler	82	attract	75
second	98	substanti	90	way	82	eduli	75
six	98	critic	89	act	81	hrt	75
weight	98	end	89	featur	81	incorpor	75
becom	97	human	89	good	81	literatur	75
dri	97	anthropogen	88	link	81	microb	75
hybrid	97	benefit	88	phosphorus	81	promot	75
led	97	buoy	88	refer	81	shape	75
ultrafiltr	97	genera	88	specimen	81	aquat	74
concern	96	interest	88	stabil	81	cluster	74
electron	96	tissu	88	trend	81	construct	74
floc	96	deposit	87	winter	81	dose	74
highlight	96	mytilus	87	california	80	intermitt	74
anmbr	95	standard	87	constant	80	regul	74
assay	95	strength	87	discharg	80	resid	74
eastern	95	surround	87	modifi	80	taken	74
gastropod	95	approxim	86	rather	80	tank	74
occurr	95	diseas	86	reject	80	target	74
point	95	elev	86	estuarin	79	upper	74
predomin	95	free	86	extend	79	block	73
profil	95	inorgan	86	filament	79	didemnum	73
publish	95	antarct	85	graze	79	extens	73
biocid	94	attribut	85	propagul	79	famili	73
tool	94	fluctuat	85	seawe	79	fuel	73
world	94	last	85	conclus	78	lack	73
bed	93	liquor	85	engin	78	percentag	73
encrust	93	multipl	85	help	78	quantit	73
epifaun	93	rna	85	mode	78	recreat	73
frequent	93	complet	84	modul	78	scan	73
mass	93	egg	84	reflect	78	wave	73
municip	93	facil	84	seem	78	wood	73
object	93	hydrodynam	84	usa	78	care	72
patient	93	plankton	84	behaviour	77	colonis	72
sustain	93	pore	84	brazil	77	competitor	72
climat	92	shore	84	chlorin	77	crustacean	72
gas	92	consum	83	column	77	hypothes	72

hypothesi	92	creat	83	concret	77	ozon	72
implic	92	hydraul	83	durat	77	peak	72
promis	72	distanc	65	late	61	cyprid	56
restrict	72	european	65	move	61	decad	56
veloc	72	exclus	65	pearl	61	height	56
aggreg	71	extern	65	pile	61	histor	56
correspon	71	freshwat	65	project	61	instal	56
yet	71	make	65	rare	61	intestinali	56
aspect	70	member	65	rock	61	magnitud	56
avoid	70	modif	65	seri	61	occupi	56
capabl	70	static	65	status	61	onto	56
defens	70	widespread	65	urchin	61	permeabl	56
document	70	arriv	64	articl	60	recommend	56
persist	70	black	64	baltic	60	serpulid	56
salt	70	certain	64	biodegrad	60	slight	56
simultan	70	conclud	64	capac	60	snail	56
solitari	70	exempl	64	deriv	60	agent	55
absenc	69	except	64	florida	60	allevi	55
damag	69	foundat	64	indirect	60	best	55
grazer	69	past	64	offer	60	cue	55
sensit	69	reus	64	steel	60	eight	55
subject	69	serv	64	worm	60	electr	55
urban	69	almost	63	adjac	59	elimin	55
belong	68	appar	63	defin	59	emerg	55
contact	68	attent	63	dock	59	independ	55
detail	68	identif	63	drink	59	index	55
drag	68	short	63	extrem	59	parallel	55
minim	68	simul	63	fed	59	pdms	55
replic	68	submers	63	influent	59	remot	55
tube	68	yield	63	later	59	step	55
upon	68	advantag	62	mexico	59	abiot	54
abl	67	ambient	62	neritina	59	appropri	54
amphitrit	67	central	62	patch	59	date	54
devic	67	clam	62	preval	59	geograph	54
domest	67	cool	62	reservoir	59	mill	54
epibenth	67	differenti	62	theori	59	must	54
epibiota	67	east	62	thick	59	network	54
expect	67	effort	62	adapt	58	proteobacteri	54
heterogen	67	exchang	62	purpos	58	respond	54
imag	67	far	62	regim	58	shear	54
relev	67	ident	62	shelter	58	thermal	54
symptom	67	insight	62	statist	58	vexillum	54
togeth	67	kelp	62	biota	57	blue	53
address	66	real	62	coagul	57	concept	53
becam	66	antibiot	61	context	57	countri	53
eelgrass	66	ballast	61	essenti	57	entir	53
epibiot	66	brown	61	isopod	57	equip	53
femal	66	bugula	61	mainten	57	herbivor	53
movemeni	66	compris	61	mollusc	57	injuri	53
smaller	66	dna	61	partial	57	insid	53

bodi	65	element	61	reason	57	longer	53
channel	65	genet	61	run	57	matur	53
pilot	53	characteris	49	zooplankton	47	multivari	44
replac	53	compos	49	agnp	46	name	44
rough	53	deep	49	ammonium	46	normal	44
transplant	53	defenc	49	attempt	46	pac	44
air	52	dreissena	49	calcul	46	perlucidum	44
bound	52	fluoresc	49	caprella	46	physiolog	44
ceram	52	mark	49	clinic	46	san	44
denitrif	52	nitrif	49	delta	46	servic	44
display	52	polyp	49	enabl	46	shade	44
hydrophot	52	receiv	49	eukaryot	46	social	44
implemen	52	recov	49	expand	46	uniqu	44
intermedi	52	reliabl	49	faster	46	autumn	43
lowest	52	versus	49	forward	46	boundari	43
methan	52	ammonia	48	heterotroph	46	configur	43
most	52	barrier	48	horizont	46	electrod	43
pool	52	cultiv	48	lagoon	46	excel	43
prey	52	microfoul	48	liquid	46	fulli	43
sens	52	outsid	48	mesocosm	46	moder	43
trait	52	pathway	48	odor	46	nich	43
violaceus	52	percent	48	phytoplanktor	46	ombr	43
white	52	pioneer	48	prepar	46	precipit	43
worldwid	52	plot	48	progress	46	prove	43
advers	51	residu	48	public	46	shipwreck	43
aombr	51	scaura	48	tidal	46	stressor	43
foulingrele	51	silicon	48	trace	46	suscept	43
gel	51	simpl	48	understood	46	usual	43
male	51	stream	48	basi	45	actual	42
opportun	51	tradi	48	basin	45	carrier	42
shellfish	51	trial	48	benefici	45	copolym	42
side	51	assembl	47	bloom	45	digest	42
wall	51	brackish	47	charg	45	efficaci	42
america	50	class	47	chemistri	45	embr	42
august	50	coeffici	47	debri	45	equal	42
batch	50	cryptogen	47	interspecif	45	fact	42
electroche	50	encount	47	interv	45	fluid	42
fragment	50	expans	47	pontoon	45	highthroughput	42
greatest	50	fix	47	procedur	45	inhabit	42
japan	50	glass	47	screen	45	now	42
matrix	50	heavi	47	styela	45	option	42
metabol	50	irrevers	47	take	45	pilotscal	42
necessari	50	latter	47	adsorpt	44	shrimp	42
periphytor	50	microscop	47	anoth	44	wet	42
prior	50	nation	47	bacteroidet	44	acceler	41
regardless	50	primarili	47	constitut	44	alway	41
restor	50	pseudomona	47	convers	44	analyt	41
subtrop	50	question	47	feasibl	44	annual	41
systemat	50	rise	47	gradual	44	broad	41
ulva	50	shortterm	47	groom	44	ciona	41

volum	50	synthet	47	hydrophil	44	compet	41
cathod	49	whole	47	infrastructur	44	difficult	41
distinguisl	41	motil	39	suppress	37	recycl	35
dramat	41	nanofiltr	39	traffic	37	sign	35
emiss	41	phylogenet	39	train	37	suspens	35
everi	41	prolifer	39	under	37	transit	35
experienc	41	pvc	39	untreat	37	zealand	35
face	41	quorum	39	action	36	angl	34
favor	41	reaction	39	anemon	36	array	34
largest	41	smbr	39	assist	36	call	34
media	41	southeastern	39	balanc	36	collector	34
microalga	41	spectroscopi	39	beyond	36	descript	34
microbionr	41	wash	39	bioga	36	diet	34
microfiltr	41	bioactiv	38	captur	36	earlier	34
min	41	coexist	38	chain	36	europ	34
nhn	41	compost	38	clog	36	fast	34
park	41	conserv	38	crustacea	36	ferment	34
pathogen	41	draw	38	dens	36	fit	34
recogn	41	electrophores	38	gain	36	fundament	34
remark	41	fabric	38	illustr	36	interpret	34
underwat	41	fatti	38	newli	36	land	34
valid	41	fisheri	38	note	36	mesh	34
wind	41	harm	38	perna	36	metamorphos	34
antibacter	40	infest	38	pose	36	notabl	34
caribbean	40	jassa	38	scientif	36	oliv	34
china	40	microhabitat	38	sem	36	reclam	34
chlorophy	40	nine	38	special	36	refug	34
elegan	40	pelag	38	web	36	repeat	34
epifauna	40	septemb	38	acidif	35	third	34
input	40	serious	38	bare	35	antibiofoul	33
program	40	seven	38	cmbr	35	april	33
random	40	turn	38	copepod	35	choic	33
silver	40	unknown	38	count	35	crucial	33
spat	40	access	37	crude	35	easili	33
star	40	bacterium	37	daili	35	exceed	33
start	40	botrylloid	37	dmf	35	fold	33
threat	40	deterior	37	drive	35	groundwat	33
west	40	diamet	37	forest	35	heavili	33
zebra	40	driven	37	framework	35	hong	33
adriat	39	enzym	37	full	35	juli	33
alreadi	39	epibiosi	37	granular	35	kong	33
connect	39	exclud	37	harvest	35	landfil	33
crassostr	39	improvisus	37	henc	35	largescal	33
diffus	39	incid	37	humic	35	medium	33
distil	39	nitrit	37	maricultur	35	methodolog	33
eutroph	39	obvious	37	mediat	35	moor	33
evolut	39	particul	37	modern	35	navicula	33
goal	39	quantiti	37	octob	35	nearbi	33
half	39	sensor	37	other	35	nitrat	33
latitud	39	sewag	37	outcom	35	nitrifi	33

mixtur	39	slow	37	phyla	35	phylum	33
molecul	39	suffici	37	raw	35	qualit	33
sand	33	item	31	visual	30	propens	28
seedl	33	limpet	31	wettabl	30	proteinlik	28
smallscal	33	macroinvertet	31	africa	29	rural	28
speed	33	macrophyt	31	canada	29	solar	28
though	33	maritim	31	conspecif	29	srts	28
tubeworm	33	mat	31	discov	29	stone	28
turbid	33	nevertheless	31	drug	29	tend	28
ulcer	33	opportunistic	31	excess	29	textur	28
wild	33	protocol	31	fail	29	watersipora	28
acet	32	purif	31	floccul	29	antarctica	27
adher	32	pyrosequenc	31	fungal	29	arctica	27
antimicrot	32	shark	31	hand	29	build	27
baselin	32	signal	31	hmbr	29	calcar	27
beach	32	soil	31	intak	29	corallin	27
bentho	32	sound	31	june	29	core	27
biopolym	32	top	31	labscal	29	cyanobacteria	27
boulder	32	unlik	31	litter	29	delay	27
categori	32	absent	30	march	29	detach	27
coal	32	amphiphil	30	meet	29	detriment	27
crystal	32	australian	30	nest	29	feeder	27
leav	32	autotroph	30	neutral	29	give	27
manmad	32	background	30	perspect	29	herbivori	27
medic	32	backwash	30	possess	29	immobil	27
minor	32	blade	30	programm	29	indian	27
old	32	boiler	30	retain	29	infrar	27
parasit	32	bryozoa	30	scallop	29	inhibitori	27
physicocf	32	cca	30	secret	29	iron	27
postsettl	32	colour	30	threshold	29	list	27
raft	32	confoc	30	whose	29	macrofauna	27
renew	32	deepsea	30	xray	29	microbiolog	27
sector	32	dgge	30	anod	28	minimum	27
spore	32	done	30	away	28	nacl	27
stimul	32	drop	30	brazilian	28	pen	27
supernat	32	forag	30	citi	28	pest	27
synthesi	32	govern	30	dimens	28	phenol	27
tbt	32	hydrozoan	30	dye	28	player	27
termin	32	linear	30	elucid	28	polychaeta	27
transform	32	mimic	30	england	28	polymorpha	27
alon	31	older	30	fossil	28	predatori	27
anox	31	osmot	30	innov	28	princip	27
biocak	31	particip	30	line	28	scenario	27
comprehe	31	phenomenon	30	lipid	28	taxon	27
dead	31	plan	30	macroalg	28	team	27
denatur	31	robust	30	methanogen	28	terrestri	27
dissimilar	31	schlosseri	30	nearshor	28	upwel	27
dog	31	subtorquata	30	onlin	28	valuabl	27
driver	31	ten	30	overgrowth	28	accept	26
fiber	31	therebi	30	pier	28	accur	26

instead	31	trajectori	30	preliminari	28	american	26
instrumen	31	view	30	preserv	28	amphipoda	26
amphora	26	bacillus	25	illumina	24	interview	23
bioinvas	26	begin	25	inocul	24	intraspecif	23
bivalvia	26	built	25	kda	24	just	23
brachiopo	26	canal	25	laboratorysca	24	kpa	23
calcium	26	cascad	25	leach	24	manufactur	23
center	26	cooccur	25	listerianum	24	massiv	23
chile	26	difficulti	25	miner	24	maxim	23
coastlin	26	dom	25	nanocomposi	24	microfluid	23
colloid	26	dreissenid	25	nanotub	24	mlss	23
compact	26	efb	25	neither	24	nois	23
cut	26	formul	25	northeast	24	none	23
deeper	26	francisco	25	polar	24	owe	23
deliveri	26	fungi	25	polymorph	24	permit	23
diagnosi	26	gammaprotec	25	porous	24	photograph	23
drill	26	interfac	25	prospect	24	prokaryot	23
fertil	26	ion	25	respir	24	quick	23
gdm	26	isotop	25	shorter	24	recircul	23
hollow	26	itali	25	stronger	24	reli	23
ice	26	kind	25	swro	24	reproduc	23
inner	26	laser	25	theoret	24	rope	23
januari	26	leachat	25	topographi	24	seawal	23
macrofaur	26	magnet	25	transmiss	24	shelf	23
nontox	26	nutrit	25	urinari	24	synthes	23
passiv	26	phosphat	25	vesiculosus	24	tendenc	23
perman	26	proxim	25	zinc	24	turf	23
pharmace	26	resili	25	alphaproteobac	23	ultim	23
polyethyle	26	sulfat	25	arabian	23	unclear	23
preferenti	26	took	25	buildup	23	veget	23
problematic	26	transect	25	burrow	23	whilst	23
puls	26	weak	25	cape	23	wound	23
pump	26	adopt	24	centuri	23	zoospor	23
regular	26	archipelago	24	cold	23	amphibalanus	22
seed	26	betaproteobac	24	cosmopolitan	23	bag	22
silica	26	biocarri	24	decemb	23	bioassay	22
situat	26	botryllus	24	definit	23	biodeposit	22
smooth	26	classifi	24	diplosoma	23	bioluminesc	22
southwest	26	clone	24	emphas	23	bore	22
stalk	26	converg	24	exhaust	23	brush	22
storag	26	cord	24	favour	23	carbohydr	22
superior	26	dispos	24	fill	23	chlorid	22
technic	26	dosag	24	finfish	23	coincid	22
thin	26	educ	24	flat	23	compart	22
tunicata	26	etc	24	gac	23	comput	22
univers	26	former	24	gap	23	cours	22
valv	26	fouler	24	graft	23	discoveri	22
verifi	26	fresh	24	grew	23	edg	22
volatil	26	fucus	24	ground	23	error	22
argentina	25	giga	24	hydrogen	23	fall	22

assum	25	gobi	24	iii	23	fine	22
author	25	hour	24	induct	23	homogen	22
hous	22	india	21	mfc	20	factori	19
impli	22	invers	21	nitzschia	20	faec	19
insitu	22	membraneba	21	olr	20	fluidiz	19
irradi	22	mollusca	21	ongo	20	friend	19
kinet	22	monsoon	21	outer	20	ftir	19
librari	22	morph	21	panicea	20	gill	19
marmorat	22	nps	21	peninsula	20	gorgonian	19
match	22	nylon	21	percept	20	grown	19
micro	22	overview	21	pig	20	home	19
microalg	22	perturb	21	porifera	20	hydrolysi	19
micropollu	22	polici	21	share	20	indopacif	19
middl	22	portion	21	shoot	20	instanc	19
mollusk	22	rhizophora	21	suit	20	lmh	19
nas	22	seafloor	21	synergist	20	lost	19
next	22	shock	21	tag	20	macrobenth	19
partit	22	sink	21	thought	20	mangl	19
pcrdgge	22	stand	21	thousand	20	mutual	19
pipe	22	stock	21	tree	20	naocl	19
prolong	22	turbul	21	trigger	20	neglig	19
pulp	22	wider	21	upstream	20	nitrospira	19
pure	22	wreck	21	urin	20	nurs	19
quit	22	afmbr	20	viabil	20	pcr	19
resolut	22	anoxicox	20	vulner	20	pcs	19
rustica	22	ascidia	20	warmer	20	pes	19
southwest	22	atom	20	zno	20	pigment	19
speciessp	22	autopsi	20	zwitterion	20	polystyren	19
startup	22	besid	20	accompani	19	quench	19
survivorsh	22	biogen	20	aegean	19	rais	19
vibrio	22	branch	20	afm	19	sargassum	19
vicin	22	brood	20	aggress	19	scleractinian	19
aeruginos	21	cancer	20	architectur	19	scrape	19
alpha	21	cellular	20	aromat	19	seab	19
anamnox	21	charact	20	asphalten	19	seen	19
assign	21	criteria	20	attack	19	sequenti	19
bight	21	cross	20	awar	19	settler	19
biogeogra	21	doc	20	believ	19	shallowwat	19
chronic	21	enigmaticus	20	biofilt	19	slower	19
cirripedia	21	evolv	20	candid	19	solvent	19
classic	21	express	20	carolina	19	spallanzanii	19
clava	21	faunal	20	concurr	19	spawn	19
cnidaria	21	fewer	20	conspicu	19	suffer	19
costeffect	21	firmicut	20	copperbas	19	sulfur	19
deal	21	hawaii	20	decay	19	suspensionfe	19
deter	21	healthi	20	detritus	19	swim	19
endem	21	hygien	20	disinfect	19	symbiont	19
errata	21	lab	20	dung	19	toc	19
galloprovir	21	left	20	ensur	19	viabl	19
hydrocarb	21	margin	20	equilibrium	19	viridi	19

immedi	21	mesograz	20	exert	19	weather	19
ind	21	meter	20	exploit	19	young	19
acorn	18	ospw	18	cryptic	17	round	17
alaska	18	otherwis	18	cylind	17	safeti	17
amongst	18	output	18	databas	17	sanmbr	17
ancmbr	18	overlap	18	deliv	17	santa	17
aris	18	pair	18	denitrifi	17	schizoporella	17
arrang	18	pco	18	electrodialysi	17	sedentari	17
arthropod	18	pellet	18	enteromorpha	17	slime	17
biofloccul	18	petroleum	18	epss	17	sodium	17
blood	18	photosynthet	18	extra	17	spirobranchus	17
bod	18	potent	18	fjord	17	sulfid	17
bring	18	powder	18	fractur	17	summar	17
bubbl	18	prevail	18	galapago	17	sydney	17
bugensi	18	prop	18	hiatella	17	symbiot	17
cation	18	quantif	18	hole	17	tannin	17
color	18	repair	18	hypoxia	17	thread	17
complic	18	salmon	18	inde	17	tio	17
compress	18	sandi	18	indigen	17	topic	17
cpt	18	section	18	inoculum	17	trade	17
depress	18	sexual	18	intersleek	17	transloc	17
dioxid	18	shed	18	lca	17	transpar	17
domain	18	sidestream	18	linza	17	travel	17
encapsul	18	smps	18	littor	17	turbin	17
episod	18	southeast	18	loos	17	ubat	17
erad	18	spacer	18	magnesium	17	video	17
geometri	18	spain	18	mfcs	17	adequ	16
giant	18	span	18	mfombr	17	aembr	16
hardbottor	18	sparg	18	millepor	17	algin	16
hinder	18	sport	18	morbid	17	annelida	16
hospit	18	terebran	18	mudflat	17	aqueous	16
householc	18	threaten	18	nematod	17	archaea	16
hydrogel	18	tract	18	neuron	17	asian	16
jet	18	tubular	18	nonoat	17	bank	16
keep	18	twophas	18	northeastern	17	basic	16
lifetim	18	unionid	18	northwest	17	broader	16
linnaeus	18	vagin	18	oral	17	chamber	16
mar	18	virginica	18	outdoor	17	children	16
marsh	18	visibl	18	overgrown	17	chilensi	16
meadow	18	wale	18	periostracum	17	chiton	16
meanwhil	18	zero	18	periwinkl	17	chosen	16
mesophil	18	ankl	17	phenolog	17	circul	16
microbiotz	18	apart	17	portug	17	codn	16
multispec	18	aquif	17	post	17	compani	16
nanoscal	18	bilg	17	postpartum	17	condens	16
never	18	biofilmform	17	probe	17	continent	16
notic	18	byssus	17	profession	17	crayfish	16
novemb	18	ccatreat	17	radiat	17	dark	16
occup	18	centr	17	rank	17	desir	16
oceanogrz	18	cleaner	17	regress	17	discrimin	16

oligotroph	18	contributor	17	respiratori	17	diverg	16
optic	18	copyright	17	retriev	17	donor	16
encourag	16	scarc	16	defend	15	surpris	15
enemi	16	search	16	expens	15	taxonomi	15
enter	16	sedimentari	16	faecal	15	thermophil	15
epoxi	16	seek	16	foraminifera	15	thorough	15
erect	16	singapor	16	game	15	transient	15
eri	16	slide	16	hydrat	15	TRUE	15
fad	16	societi	16	idh	15	twostag	15
failur	16	sootblow	16	interfer	15	uasb	15
fecund	16	spectrometri	16	intrins	15	undersid	15
feedwat	16	spill	16	inventori	15	visit	15
filterfeed	16	stainless	16	irrig	15	walk	15
flexibl	16	subsurfac	16	latissima	15	washington	15
foreign	16	sun	16	macroorgan	15	waterborn	15
fourier	16	tanzania	16	map	15	wooden	15
franc	16	throughput	16	massachuset	15	worker	15
freeliv	16	tide	16	miseq	15	yacht	15
greec	16	tubastraea	16	narrow	15	academ	14
halichondria	16	ultrasound	16	nontarget	15	accuraci	14
hold	16	undertaken	16	nonwoven	15	adjust	14
hrts	16	unfoul	16	northwestern	15	aerial	14
hydrozoa	16	vascular	16	outlin	15	affin	14
inc	16	viviparus	16	oxidas	15	aggrav	14
infaun	16	yearold	16	perceiv	15	agreement	14
infer	16	zostera	16	person	15	aid	14
inject	16	accomplish	15	phenomena	15	alcohol	14
institut	16	acidlik	15	plicata	15	aluminum	14
limeston	16	acut	15	plus	15	amplifi	14
meiofauna	16	ahl	15	postmonsoon	15	anchor	14
metagenom	16	aob	15	precis	15	archaeal	14
methanol	16	arboresc	15	predictor	15	arctic	14
moss	16	attain	15	principl	15	ash	14
naclo	16	attend	15	pronounc	15	autonom	14
night	16	attenu	15	prototyp	15	bar	14
obelia	16	augment	15	provis	15	barent	14
otus	16	azor	15	putat	15	beer	14
outbreak	16	blend	15	rdna	15	branchiomma	14
peopl	16	british	15	relax	15	cartridg	14
photocatalysi	16	brought	15	retard	15	cement	14
polydimethylsiloxan	16	cas	15	rout	15	cervic	14
popular	16	cay	15	rule	15	columbia	14
poros	16	cladophora	15	senil	15	compressor	14
premonsoon	16	closterium	15	seta	15	consortia	14
proper	16	clsm	15	shannon	15	constitu	14
proven	16	communityba	15	skeleton	15	constraint	14
reactiv	16	congen	15	smell	15	cylindrotheca	14
rear	16	consortium	15	sole	15	death	14
recognis	16	contrari	15	sought	15	decentr	14
relief	16	convert	15	stead	15	deterr	14

rotat	16	crossflow	15	stochast	15	dietari	14
sandston	16	crosssect	15	supplement	15	discret	14
displac	14	purifi	14	dive	13	pine	13
disrupt	14	pusilla	14	divid	13	profound	13
downstream	14	quinon	14	dorbigni	13	promin	13
drastic	14	radic	14	drainag	13	prone	13
eburneus	14	refineri	14	drawback	13	pseudoalteror	13
embryo	14	regener	14	elder	13	ptfe	13
emphasi	14	return	14	enclos	13	questionnair	13
empir	14	rio	14	enough	13	readili	13
empti	14	sam	14	entri	13	realtim	13
ethanol	14	scotland	14	equilibra	13	reclaim	13
explan	14	scuba	14	equival	13	redox	13
feo	14	seafood	14	evidenc	13	rest	13
fever	14	skin	14	exponenti	13	rigor	13
filler	14	spite	14	februari	13	rins	13
fingerprint	14	temporari	14	ferrous	13	safe	13
flatsheet	14	texa	14	ficopomatus	13	sav	13
flush	14	tight	14	footbal	13	sensori	13
foulsmel	14	toxin	14	front	13	sheet	13
frame	14	trflp	14	fucata	13	sock	13
gave	14	uptak	14	granul	13	spirorbid	13
hawaiian	14	viscos	14	gravitydriven	13	stem	13
ill	14	vital	14	grit	13	sympatr	13
impair	14	acryl	13	held	13	tow	13
inlet	14	avicennia	13	herein	13	twice	13
inwat	14	back	13	hypochlorit	13	uncag	13
irgarol	14	ban	13	imped	13	updat	13
king	14	bandag	13	intent	13	velig	13
latitudin	14	began	13	ireland	13	viz	13
leaf	14	beta	13	leg	13	wadden	13
macroscop	14	bind	13	legisl	13	wellknown	13
madeira	14	biocontrol	13	lesion	13	yeast	13
manner	14	bioeros	13	littorea	13	zonat	13
mathemat	14	biomed	13	manhattensi	13	acclim	12
metabarcod	14	bipolar	13	metridium	13	acquir	12
molgula	14	breed	13	microtopograj	13	affili	12
multidimension	14	brief	13	mission	13	agricultur	12
muticum	14	brine	13	mlemb	13	algorithm	12
neighbor	14	bsa	13	modular	13	alkalin	12
non	14	come	13	mouth	13	anion	12
overcom	14	complementa	13	mre	13	antibodi	12
overlook	14	compromis	13	mud	13	antiscal	12
phospholipid	14	confin	13	mutant	13	arsenic	12
piggeri	14	coordin	13	nitric	13	asexu	12
pillar	14	corridor	13	nob	13	backflush	12
placement	14	cps	13	nov	13	barcod	12
planctomycet	14	darwin	13	odour	13	basibiont	12
plata	14	debat	13	optimis	13	benzothiazol	12
presum	14	deplet	13	pain	13	bicolor	12

prioriti	14	dictyota	13	pass	13	bile	12
protozoa	14	die	13	pennat	13	biochem	12
biotechnolog	12	nauplii	12	ago	11	eros	11
bismuth	12	nonfoul	12	albumin	11	fate	11
bodega	12	odessa	12	amin	11	fep	11
break	12	onset	12	ammoniaoxid	11	fga	11
calcifi	12	optimum	12	amplicon	11	fombr	11
canopi	12	oregon	12	anticip	11	founder	11
catalyt	12	outlet	12	aquarium	11	fractal	11
chromat	12	overgrow	12	ascidiacea	11	fragili	11
coars	12	patent	12	ascidiella	11	fulvic	11
communitylevel	12	phenotyp	12	asia	11	generalist	11
concomit	12	pit	12	aspersa	11	genom	11
consecut	12	polyurethan	12	aurelia	11	germani	11
crown	12	price	12	australi	11	germin	11
crustos	12	reciproc	12	balthica	11	gmelin	11
curv	12	regist	12	bear	11	grand	11
cypri	12	robot	12	biovolum	11	hardsubstr	11
deem	12	routin	12	bond	11	hatch	11
del	12	rugos	12	border	11	hemispher	11
destroy	12	saccharina	12	candida	11	hierarch	11
digit	12	sallei	12	carapac	11	hullfoul	11
diminish	12	satur	12	caspian	11	hundr	11
dot	12	save	12	caviti	11	hydroxyl	11
dredg	12	securi	12	cbz	11	hypox	11
egypt	12	slope	12	cellulos	11	ideal	11
elsewher	12	spatiotempor	12	census	11	impress	11
epizo	12	stationari	12	cfid	11	inactiv	11
esp	12	storm	12	cfu	11	incub	11
eventu	12	strait	12	chines	11	inevit	11
flora	12	sudden	12	chromatograp	11	inhibitor	11
foulingresist	12	surfaceassoc	12	clariti	11	insect	11
get	12	translat	12	communic	11	insuffici	11
grass	12	transmit	12	conjunct	11	intend	11
guild	12	trap	12	contemporari	11	intervent	11
habit	12	tributyltin	12	correct	11	invest	11
immigr	12	twelv	12	corrobor	11	ionian	11
increment	12	ubiquit	12	crop	11	ionic	11
insert	12	uncommon	12	cryptobenth	11	janeiro	11
japanes	12	vast	12	deadend	11	jellyfish	11
know	12	vermetid	12	decapod	11	kembla	11
lie	12	versatil	12	determinist	11	kept	11
lobster	12	villag	12	disintegr	11	kill	11
lowcost	12	vitro	12	disjunct	11	lbep	11
lowstrength	12	voltag	12	district	11	lesser	11
macro	12	zeta	12	diver	11	lifehistori	11
mainland	12	absolut	11	dntd	11	lime	11
matern	12	accident	11	drift	11	log	11
mbmbr	12	acinetobact	11	droplet	11	longlin	11
migrat	12	acoust	11	dual	11	lurida	11

million	12	african	11
monom	12	agenc	11
microcosm	11	steril	11
mitochondri	11	subleth	11
mytilopsi	11	surg	11
northward	11	threedimensic	11
novosibirsk	11	timber	11
nurseri	11	titanium	11
octopod	11	toulon	11
oppos	11	track	11
ostrea	11	turnov	11
pack	11	ultraviolet	11
panama	11	umbil	11
peat	11	uniform	11
philippinarum	11	unpollut	11
phyllosoma	11	unusu	11
picophytoplankton	11	welfar	11
polymeras	11	wetland	11
polyvinyl	11	worship	11
prolaps	11	engag	11
proli	11	entrap	11
radiata	11	manual	11
rainwat	11	methacryl	11
realist	11	spot	11
resembl	11	stain	11
reson	11		
ribosom	11		
rigida	11		
ruben	11		
sabellida	11		
saccostrea	11		
saginaw	11		
schedul	11		
sclerobiont	11		
scour	11		
seastar	11		
sensu	11		
sepsi	11		
sewer	11		
shorelin	11		
sitespecif	11		
smallest	11		
smpc	11		
smz	11		
soccer	11		
socioeconom	11		
softwar	11		
solgel	11		
speciesrich	11		
spectrum	11		

SUPPLEMENTARY MATERIAL CHAPTER I

Mind the gap: missing a baseline of Brazilian fouling amphipods for proper biodiversity management

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Tab. S1 List of 197 species retained for the gap analyses and the relative number of sequences for each of the four genes searched (i.e. COI, 18S, 28S and 16S) divided into the total (TOTAL), if exclusive of GenBank or BOLD (GenBank exclusive and BOLD exclusive respectively) or if present in both (GenBank and BOLD).

[illegible]

Tab. S2 Family, genus and BINs for each species found along the Brazilian coasts.

Family	Genus	Species	BINS
Ampeliscidae	<i>Ampelisca</i>	<i>Ampelisca brevisimulata</i>	BOLD:AAO4463
Ampeliscidae	<i>Ampelisca</i>	<i>Ampelisca cristata</i>	BOLD:AAO4467
Ampeliscidae	<i>Ampelisca</i>	<i>Ampelisca cristata</i>	BOLD:AAZ2195
Ampeliscidae	<i>Ampelisca</i>	<i>Ampelisca cristata</i>	BOLD:ACH0282
Ampeliscidae	<i>Ampelisca</i>	<i>Ampelisca pseudobicarinata</i>	BOLD:AAO4466
Ampeliscidae	<i>Ampelisca</i>	<i>Ampelisca pseudobicarinata</i>	BOLD:AAU1980
Ampithoidae	<i>Ampithoe</i>	<i>Ampithoe ramondi</i>	BOLD:ADA9884
Ampithoidae	<i>Ampithoe</i>	<i>Ampithoe ramondi</i>	BOLD:ADD1828
Hyalidae	<i>Apohyale</i>	<i>Apohyale media</i>	BOLD:ACX2630
Hyalidae	<i>Apohyale</i>	<i>Apohyale media</i>	BOLD:ACX2667
Hyalidae	<i>Apohyale</i>	<i>Apohyale media</i>	BOLD:ADB6065
Hyalidae	<i>Apohyale</i>	<i>Apohyale media</i>	BOLD:ADB6066
Amphilocheidae	<i>Apolochus</i>	<i>Apolochus neapolitanus</i>	BOLD:ADD0145
Aoridae	<i>Bemlos</i>	<i>Bemlos unicornis</i>	BOLD:AAI8655
Caprellidae	<i>Caprella</i>	<i>Caprella andreae</i>	BOLD:ACH8095
Caprellidae	<i>Caprella</i>	<i>Caprella danilevskii</i>	BOLD:AAW8076
Caprellidae	<i>Caprella</i>	<i>Caprella danilevskii</i>	BOLD:AEA9737
Caprellidae	<i>Caprella</i>	<i>Caprella dilatata</i>	BOLD:ACH7635
Caprellidae	<i>Caprella</i>	<i>Caprella equilibra</i>	BOLD:ADV5113
Caprellidae	<i>Caprella</i>	<i>Caprella equilibra</i>	BOLD:ACM2451
Caprellidae	<i>Caprella</i>	<i>Caprella equilibra</i>	BOLD:ACM2452
Caprellidae	<i>Caprella</i>	<i>Caprella equilibra</i>	BOLD:AEC7312
Caprellidae	<i>Caprella</i>	<i>Caprella penantis</i>	BOLD:ACA3998
Caprellidae	<i>Caprella</i>	<i>Caprella penantis</i>	BOLD:ACH9338
Caprellidae	<i>Caprella</i>	<i>Caprella penantis</i>	BOLD:ACH9339
Caprellidae	<i>Caprella</i>	<i>Caprella penantis</i>	BOLD:ACM2623
Caprellidae	<i>Caprella</i>	<i>Caprella scaura</i>	BOLD:AAW8077
Caprellidae	<i>Caprella</i>	<i>Caprella scaura</i>	BOLD:ADC8262
Ampithoidae	<i>Cymadusa</i>	<i>Cymadusa filosa</i>	BOLD:AA4495
Ischyroceridae	<i>Erichthonius</i>	<i>Erichthonius brasiliensis</i>	BOLD:AAG7114
Ischyroceridae	<i>Erichthonius</i>	<i>Erichthonius brasiliensis</i>	BOLD:ACH6329
Ischyroceridae	<i>Erichthonius</i>	<i>Erichthonius brasiliensis</i>	BOLD:ADP3130
Ischyroceridae	<i>Erichthonius</i>	<i>Erichthonius brasiliensis</i>	BOLD:ADR2250
Ischyroceridae	<i>Erichthonius</i>	<i>Erichthonius brasiliensis</i>	BOLD:AAC2037
Photidae	<i>Gammaropsis</i>	<i>Gammaropsis thompsoni</i>	BOLD:ADP1035
Ischyroceridae	<i>Jassa</i>	<i>Jassa marmorata</i>	BOLD:AAA3311
Ischyroceridae	<i>Jassa</i>	<i>Jassa slatteryi</i>	BOLD:AAC2037
Corophiidae	<i>Laticorophium</i>	<i>Laticorophium baconi</i>	BOLD:AAH4089
Corophiidae	<i>Monocorophium</i>	<i>Monocorophium acherusicum</i>	BOLD:AAU1637
Corophiidae	<i>Monocorophium</i>	<i>Monocorophium acherusicum</i>	BOLD:ACQ7829
Corophiidae	<i>Monocorophium</i>	<i>Monocorophium acherusicum</i>	BOLD:ADU8691
Corophiidae	<i>Monocorophium</i>	<i>Monocorophium insidiosum</i>	BOLD:AAE1628
Corophiidae	<i>Monocorophium</i>	<i>Monocorophium insidiosum</i>	BOLD:AAE9749
Caprellidae	<i>Paracaprella</i>	<i>Paracaprella pusilla</i>	BOLD:ACQ1205
Caprellidae	<i>Paracaprella</i>	<i>Paracaprella pusilla</i>	BOLD:AEB5479
Caprellidae	<i>Paracaprella</i>	<i>Paracaprella tenuis</i>	BOLD:ACM2928
Caprellidae	<i>Paracaprella</i>	<i>Paracaprella tenuis</i>	BOLD:ACQ5152
Hyalidae	<i>Parhyale</i>	<i>Parhyale hawaiiensis</i>	BOLD:AAX1005
Photidae	<i>Photis</i>	<i>Photis longicaudata</i>	BOLD:ACG9506
Caprellidae	<i>Phtisica</i>	<i>Phtisica marina</i>	BOLD:AAF6487
Caprellidae	<i>Phtisica</i>	<i>Phtisica marina</i>	BOLD:ABW2045
Caprellidae	<i>Phtisica</i>	<i>Phtisica marina</i>	BOLD:ACX1183
Talitridae	<i>Platorchestia</i>	<i>Platorchestia monodi</i>	BOLD:AAB3402
Talitridae	<i>Platorchestia</i>	<i>Platorchestia monodi</i>	BOLD:ACB6373
Synopiidae	<i>Synopia</i>	<i>Synopia ultramarina</i>	BOLD:AAG7100
Synopiidae	<i>Syrrhoe</i>	<i>Syrrhoe crenulata</i>	BOLD:AAG7107
Synopiidae	<i>Syrrhoe</i>	<i>Syrrhoe crenulata</i>	BOLD:ABY3672
Talitridae	<i>Talitroides</i>	<i>Talitroides alluaudi</i>	BOLD:ACH9326
Synopiidae	<i>Tiron</i>	<i>Tiron biocellata</i>	BOLD:ACH0518

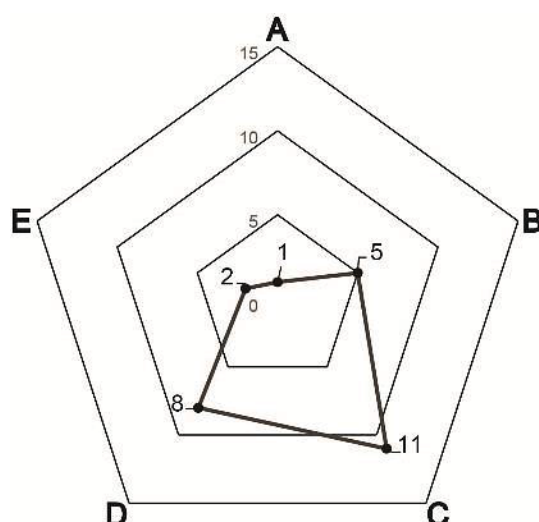


Fig. S1 Results of BAGS analysis and amount BINs for each grade. Letters referring to the grades of BAGS for each morphospecies. A: congruent, >10 specimens; B: congruent, <10 specimens; C: multiple BINs; D: insufficient data (< 3 specimens); E: discordant, no further specimen (singleton)

Tab. S3 Average and maximum genetic distances within morphospecies (Mean Intra-Sp and Max Intra-Sp respectively) with closest species (Nearest Species), relative BIN (Nearest Neighbour) and genetic distance (Distance to NN) for each morphospecies.

Family	Species	Mean Intra-Sp	Max Intra-Sp	Nearest Species	Nearest Neighbour	Distance to NN
Amphilochidae	<i>Houstonius</i> sp.	4.92	8.64	<i>Latigammaropsis tagoensis</i>	BMFVF044-19	31.35
Ampithoidae	<i>Ampithoe ramondi</i>	0.43	0.77	<i>Propejanice lagamarensis</i>	BMFVF005-19	21.02
Ampithoidae	<i>Ampithoe valida</i>	0.31	0.31	<i>Latigammaropsis tagoensis</i>	BMFVF044-19	20.83
Aoridae	<i>Propejanice lagamarensis</i>	1.08	1.08	<i>Erichthonius brasiliensis</i>	BMFVF054-19	20.01
Bateidae	<i>Batea catharinensis</i>	N/A	0	<i>Erichthonius brasiliensis</i>	BMFVF052-19	32.3
Caprellidae	<i>Caprella dilatata</i>	N/A	0	<i>Caprella equilibra</i>	BMFVF084-19	22.16
Caprellidae	<i>Caprella equilibra</i>	2.79	3.98	<i>Caprella dilatata</i>	BMFVF082-19	22.16
Caprellidae	<i>Paracaprella pusilla</i>	4.37	10.42	<i>Erichthonius brasiliensis</i>	BMFVF054-19	23.34
Corophiidae	<i>Apocorophium acutum</i>	N/A	0	<i>Monocorophium insidiosum</i>	BMFVF283-20	15.83
Corophiidae	<i>Cheiriphotis</i> sp.	N/A	0	<i>Monocorophium acherusicum</i>	BMFVF180-20	25.83
Corophiidae	<i>Laticorophium baconi</i>	N/A	0	<i>Monocorophium insidiosum</i>	BMFVF283-20	20.45
Corophiidae	<i>Monocorophium acherusicum</i>	3.62	6.72	<i>Apocorophium acutum</i>	BMFVF094-19	17.5
Corophiidae	<i>Monocorophium insidiosum</i>	N/A	0	<i>Apocorophium acutum</i>	BMFVF094-19	15.83
Hyalidae	<i>Apohyale media</i>	N/A	0	<i>Erichthonius brasiliensis</i>	BMFVF054-19	24.99
Ischyroceridae	<i>Cerapus benthophilus</i>	N/A	0	<i>Erichthonius brasiliensis</i>	BMFVF054-19	23.51
Ischyroceridae	<i>Erichthonius brasiliensis</i>	7.45	18.64	<i>Propejanice lagamarensis</i>	BMFVF005-19	20.01
Ischyroceridae	<i>Erichthonius punctatus</i>	1.57	1.57	<i>Erichthonius brasiliensis</i>	BMFVF054-19	22.85
Ischyroceridae	<i>Jassa slatteryi</i>	0.62	1.24	<i>Jassa valida</i>	BMFVF187-20	15.98
Ischyroceridae	<i>Jassa valida</i>	0	0	<i>Jassa slatteryi</i>	BMFVF279-20	15.98
Maeridae	<i>Elasmopus</i> s p.	15.96	23.94	<i>Latigammaropsis tagoensis</i>	BMFVF044-19	26.41
Maeridae	<i>Quadrinemaera miranda</i>	0.08	0.15	<i>Ampithoe ramondi</i>	BMFVF010-19	30.36
Photidae	<i>Latigammaropsis tagoensis</i>	2.09	2.35	<i>Ampithoe valida</i>	BMFVF046-19	20.83
Photidae	<i>Photis sarae</i>	N/A	0	<i>Erichthonius brasiliensis</i>	BMFVF054-19	22.54
Podoceridae	<i>Podocerus brasiliensis</i>	0.81	1.72	<i>Erichthonius brasiliensis</i>	BMFVF054-19	26.18
Stenothoidae	<i>Stenothoe gallensis</i>	4.3	12.8	<i>Stenothoe valida</i>	BMFVF117-19	29.73
Stenothoidae	<i>Stenothoe georgiana</i>	0	0	<i>Stenothoe valida</i>	BMFVF118-19	26.47
Stenothoidae	<i>Stenothoe valida</i>	0.68	1.4	<i>Stenothoe georgiana</i>	BMFVF190-20	26.47

Tab. S4 Goods exchanged via-shipping (expressed in millions of tons) by South and South-East Brazil with countries divided into imported (Import) and exported (Export) goods and their sum (Tot). Proportion of goods exchanged from 2015 to 2018 with each country compared with the total (%tot shipping). Imp% and Exp%: percentage of importation or exportation of goods in comparison with the total exchanged with the relative country

Country	Export	Import	Tot	Exp%	Imp%	%tot shipping
China	541505578	36069324	577574902	94	6	33
United States of America	78999840	82948702	161948541	49	51	9
Netherlands	66417042	7260181	73677222	90	10	4
Malaysia	61251430	1511387	62762818	98	2	4
Japan	54845495	1214241	56059736	98	2	3
Argentina	24207115	27339970	51547085	47	53	3
Spain	24666535	13750024	38416559	64	36	2
South Korea	30193441	4364485	34557926	87	13	2
Singapore	26984607	5280257	32264864	84	16	2
Australia	7550003	22189401	29739404	25	75	2
Nigeria	12669365	15563782	28233147	45	55	2
Saudi Arabia	10516408	16078924	26595332	40	60	2
Russia	7457181	18765609	26222789	28	72	1
Germany	16036463	9762795	25799258	62	38	1
Italy	21619223	3989021	25608244	84	16	1
India	22414863	2864788	25279651	89	11	1
Belgium	13509481	11229169	24738650	55	45	1
Canada	9784309	14782289	24566599	40	60	1
Chile	14366718	8969338	23336056	62	38	1
Oman	22012475	1165079	23177553	95	5	1
Colombia	12057784	9749170	21806953	55	45	1
Iran	19312610	667652	19980262	97	3	1
Morocco	10021881	8560703	18582585	54	46	1
Algeria	7633539	10745006	18378545	42	58	1
Bahrain	15076775	818756	15895531	95	5	1
France	14002333	1891244	15893576	88	12	1
Mexico	11505545	3987791	15493336	74	26	1
Egypt	14512496	959494	15471989	94	6	1
Taiwan	14288094	555932	14844026	96	4	1
United Arab Emirates	9817771	3561430	13379201	73	27	1
Turkey	11489271	1307634	12796904	90	10	1
South Africa	7618873	4802921	12421794	61	39	1
Peru	5046728	7331938	12378666	41	59	1
Vietnam	11608101	273607	11881708	98	2	1
Hong Kong S.A.R.	9273940	2088353	11362293	82	18	1
Indonesia	9713490	1477498	11190988	87	13	1
Thailand	9057819	575769	9633588	94	6	1
United Kingdom	7908204	1205941	9114145	87	13	1
Bangladesh	8618633	30186	8648819	100	0	0
Trinidad and Tobago	5093154	2981847	8075002	63	37	0
Latvia	1729930	6318338	8048268	21	79	0
Qatar	2647831	5237570	7885400	34	66	0
Lithuania	1471721	6298312	7770032	19	81	0
Iraq	4621621	2341260	6962881	66	34	0
Venezuela	3056291	3239489	6295780	49	51	0
Portugal	4211723	1735463	5947186	71	29	0
Uruguay	1822787	3498830	5321617	34	66	0
Estonia	1057145	3788593	4845738	22	78	0
Philippines	4557788	54674	4612461	99	1	0
Israel	1060118	3408632	4468749	24	76	0
Poland	2949695	998049	3947744	75	25	0
Panama	2995278	885436	3880714	77	23	0
Libya	3083381	130018	3213400	96	4	0
Slovenia	3030946	97844	3128790	97	3	0

Kuwait	991313	1634282	2625595	38	62	0
Dominican Republic	2115750	501995	2617745	81	19	0
Angola	2237167	369201	2606368	86	14	0
Norway	508136	1758282	2266418	22	78	0
Saint Lucia	2068773	14825	2083598	99	1	0
Jamaica	1752045	133417	1885462	93	7	0
Romania	1529002	264733	1793735	85	15	0
Ecuador	1362916	339675	1702590	80	20	0
Pakistan	1621764	42346	1664111	97	3	0
Tunisia	1059693	235159	1294852	82	18	0
Equatorial Guinea	432526	861656	1294182	33	67	0
Malta	891414	312556	1203970	74	26	0
Finland	329696	842644	1172340	28	72	0
Mozambique	176396	924489	1100885	16	84	0
Yemen	1000798	135	1000933	100	0	0
Georgia	936300	35107	971408	96	4	0
Greece	187010	631478	818489	23	77	0
Cuba	794596	11896	806492	99	1	0
Sweden	200366	556358	756724	26	74	0
Senegal	633040	109870	742910	85	15	0
Lebanon	494774	110915	605690	82	18	0
Denmark	345547	253834	599380	58	42	0
Democratic Republic of the Congo	371155	132700	503855	74	26	0
Ghana	492753	3229	495982	99	1	0
Ukraine	225016	230118	455135	49	51	0
Mauritania	422986	22	423007	100	0	0
Croatia	362911	6340	369251	98	2	0
Jordan	343002	15637	358638	96	4	0
Gambia	302731	1549	304280	99	1	0
Nicaragua	275688	5076	280764	98	2	0
Kenya	278183	1282	279465	100	0	0
Sri Lanka	253629	25003	278632	91	9	0
Liberia	38476	229752	268229	14	86	0
Togo	224449	24252	248701	90	10	0
Mauritius	234266	316	234582	100	0	0
New Zealand	184410	33997	218406	84	16	0
Syria	214225	1313	215538	99	1	0
Ivory Coast	122048	92844	214891	57	43	0
Costa Rica	192532	19590	212122	91	9	0
Cyprus	208398	152	208549	100	0	0
Somalia	200000	0	200000	100	0	0
Sierra Leone	183695	12662	196357	94	6	0
Benin	177582	17292	194874	91	9	0
Paraguay	120905	59644	180549	67	33	0
Iceland	164911	114	165026	100	0	0
Austria	78598	79049	157648	50	50	0
Guatemala	104987	47215	152202	69	31	0
Bulgaria	57624	92487	150111	38	62	0
United States Virgin Islands	31086	105774	136860	23	77	0
Puerto Rico	114100	5217	119317	96	4	0
Djibouti	113372	27	113399	100	0	0
Guinea	96794	8015	104808	92	8	0
Cameroon	102302	1710	104011	98	2	0
Eritrea	100924	0	100924	100	0	0
Ireland	84884	13389	98273	86	14	0
El Salvador	68633	18435	87068	79	21	0
Honduras	41664	25875	67540	62	38	0
Guyana	37847	26440	64287	59	41	0
Sudan	51020	1569	52590	97	3	0
North Korea	51837	0	51837	100	0	0

Haiti	42206	3249	45455	93	7	0
Saint Helena	16000	16000	32000	50	50	0
Swaziland	30886	0	30886	100	0	0
Central African Republic	30000	28	30028	100	0	0
Suriname	14643	12252	26894	54	46	0
Madagascar	17143	227	17371	99	1	0
Cape Verde	8211	6981	15193	54	46	0
Aruba	13706	32	13738	100	0	0
Bermuda	13020	29	13048	100	0	0
Antigua and Barbuda	12011	0	12011	100	0	0
Albania	10179	820	10998	93	7	0
Guinea Bissau	9756	626	10382	94	6	0
Cayman Islands	3815	6171	9987	38	62	0
East Timor	226	9137	9363	2	98	0
Barbados	7948	287	8236	97	3	0
Namibia	4234	1257	5491	77	23	0
Cambodia	989	3722	4710	21	79	0
Belize	4630	12	4642	100	0	0
Comoros	2908	0	2908	100	0	0
Gabon	2642	66	2708	98	2	0
Myanmar	1842	412	2254	82	18	0
Grenada	2140	0	2140	100	0	0
Montenegro	1298	501	1799	72	28	0
Republic of Congo	1585	5	1590	100	0	0
Switzerland	707	778	1485	48	52	0
Maldives	1387	0	1387	100	0	0
United Republic of Tanzania	318	269	587	54	46	0
French Polynesia	219	219	437	50	50	0
Niue	151	163	314	48	52	0
Dominica	268	34	302	89	11	0
New Caledonia	243	30	273	89	11	0
Seychelles	268	0	268	100	0	0
Uganda	33	220	253	13	87	0
Saint Kitts and Nevis	204	0	204	100	0	0
Papua New Guinea	164	0	164	100	0	0
Sao Tome and Principe	104	57	161	65	35	0
Saint Vincent and the Grenadines	136	0	136	100	0	0
Lesotho	0	108	108	0	100	0
Laos	0	54	54	0	100	0
Bosnia and Herzegovina	18	18	37	50	50	0
Curaçao	29	0	29	100	0	0
Brunei	0	5	5	0	100	0

Tab. S5 Species with matches in public databases with relative accession numbers, country of origin of the sequences (State: Country) and if they are public or private (accessibility)

Species	database	Accession number	State:Country	accessibility
<i>Ampithoe valida</i>	Genbank	KU906082	Virginia:USA	public
<i>Ampithoe valida</i>	Genbank	KP254641	Virginia:USA	public
<i>Ampithoe valida</i>	Genbank	KP254396	Virginia:USA	public
<i>Ampithoe valida</i>	Genbank	KP254345	Virginia:USA	public
<i>Ampithoe valida</i>	Genbank	GU048371	South Carolina:USA	public
<i>Ampithoe valida</i>	Genbank	GU048265	Washington:USA	public
<i>Ampithoe valida</i>	Genbank	GU048399	California:USA	public
<i>Ampithoe valida</i>	Genbank	GU048360	Connecticut:USA	public
<i>Ampithoe valida</i>	Genbank	GU048377	South Carolina:USA	public
<i>Caprella dilatata</i>	Genbank	KC146194	Brazil	public
<i>Caprella dilatata</i>	Genbank	KC146191	Brazil	public
<i>Caprella equilibra</i>	Genbank	KU905820	Virginia:USA	public
<i>Caprella equilibra</i>	Genbank	KU906051	Virginia:USA	public
<i>Caprella equilibra</i>	Genbank	KU905974	Virginia:USA	public
<i>Caprella equilibra</i>	Genbank	KU905863	Virginia:USA	public
<i>Caprella equilibra</i>	BOLD	HZPLY1083-13	China	public
<i>Caprella equilibra</i>	BOLD	MGOTX244-19	USA:Texas	private
<i>Erichthonius brasiliensis</i>	Genbank	MH825931	Florida:USA	public
<i>Erichthonius brasiliensis</i>	BOLD	DISA244-18	North Pacific	public
<i>Erichthonius brasiliensis</i>	BOLD	DISA032-18	North Pacific	public
<i>Jassa slatteryi</i>	BOLD	JASSA147-19	South Korea	public
<i>Jassa slatteryi</i>	BOLD	JASSA148-19	South Korea	public
<i>Jassa slatteryi</i>	BOLD	JASSA149-19	South Korea	public
<i>Jassa slatteryi</i>	BOLD	JASSA137-19	Japan	public
<i>Jassa slatteryi</i>	BOLD	JASSA138-19	Japan	public
<i>Jassa slatteryi</i>	BOLD	JASSA139-19	Japan	public
<i>Jassa slatteryi</i>	BOLD	JASSA140-19	Japan	public
<i>Jassa slatteryi</i>	BOLD	JASSA142-19	Japan	public
<i>Jassa slatteryi</i>	BOLD	JASSA143-19	Japan	public
<i>Jassa slatteryi</i>	BOLD	JASSA144-19	Japan	public
<i>Jassa slatteryi</i>	BOLD	JASSA145-19	Japan	public
<i>Jassa slatteryi</i>	BOLD	INVMP003-13	Argentina	public
<i>Jassa slatteryi</i>	BOLD	JASSA172-19	Spain	public
<i>Jassa slatteryi</i>	BOLD	JASSA173-19	Spain	public
<i>Jassa slatteryi</i>	BOLD	JASSA203-19	Spain	public
<i>Jassa slatteryi</i>	BOLD	JASSA202-19	Spain	public
<i>Jassa slatteryi</i>	BOLD	JASSA193-19	Spain	public
<i>Jassa slatteryi</i>	BOLD	JASSA196-19	Spain	public
<i>Jassa slatteryi</i>	BOLD	JASSA198-19	Spain	public
<i>Jassa slatteryi</i>	BOLD	JASSA174-19	Spain	public
<i>Jassa valida</i>	Genbank	HM466379	Hawaii	public
<i>Jassa valida</i>	Genbank	HM466381	Hawaii	public
<i>Jassa valida</i>	Genbank	HM466382	Hawaii	public
<i>Jassa valida</i>	Genbank	HM466385	Hawaii	public
<i>Jassa valida</i>	Genbank	HM466380	Hawaii	public
<i>Jassa valida</i>	Genbank	HM466383	Hawaii	public
<i>Jassa valida</i>	Genbank	HM466384	Hawaii	public
<i>Jassa valida</i>	Genbank	GU048146	South Carolina,USA	public
<i>Jassa valida</i>	BOLD	MGOTX373-19	Texas:USA	private

<i>Jassa valida</i>	BOLD	IZFPI055-20	Florida:USA	private
<i>Jassa valida</i>	BOLD	ISWIO027-19	South Africa	private
<i>Laticorophium baconi</i>	BOLD	MGOTX137-19	Texas:USA	private
<i>Laticorophium baconi</i>	BOLD	MGOTX407-19	Texas:USA	private
<i>Laticorophium baconi</i>	BOLD	MGOTX419-19	Texas:USA	private
<i>Monocorophium acherusicum</i>	BOLD	FLFIV052-14	Florida:USA	public
<i>Monocorophium acherusicum</i>	BOLD	FLFIV018-14	Florida:USA	public
<i>Monocorophium acherusicum</i>	BOLD	FLFIV418-18	Florida:USA	private
<i>Monocorophium acherusicum</i>	BOLD	IZFPI238-20	Florida:USA	private
<i>Monocorophium acherusicum</i>	BOLD	INIRL045-16	Florida:USA	private
<i>Monocorophium acherusicum</i>	BOLD	MGOTX928-19	Texas:USA	private
<i>Monocorophium acherusicum</i>	BOLD	MGOTX998-19	Texas:USA	private
<i>Monocorophium acherusicum</i>	BOLD	SERC12179-19	Virginia:USA	private
<i>Monocorophium insidiosum</i>	Genbank	MG313882	Canada	public
<i>Monocorophium insidiosum</i>	Genbank	MG310516	Canada	public
<i>Monocorophium insidiosum</i>	Genbank	HM900381	Canada	public
<i>Monocorophium insidiosum</i>	Genbank	MH138328	New York:USA	public
<i>Monocorophium insidiosum</i>	Genbank	MH138327	New York:USA	public
<i>Monocorophium insidiosum</i>	Genbank	MG320366	Canada	public
<i>Monocorophium insidiosum</i>	Genbank	HQ966518	Canada	public
<i>Monocorophium insidiosum</i>	Genbank	HM900380	Canada	public
<i>Monocorophium insidiosum</i>	Genbank	MH231164	New York:USA	public
<i>Monocorophium insidiosum</i>	Genbank	HQ966519	Canada	public
<i>Monocorophium insidiosum</i>	Genbank	KT209419	North Sea: German Bight	public
<i>Monocorophium insidiosum</i>	Genbank	MG936088	Canada	public
<i>Monocorophium insidiosum</i>	Genbank	KU697731	Spain	public
<i>Monocorophium insidiosum</i>	Genbank	KT209031	North Sea: German Bight	public
<i>Monocorophium insidiosum</i>	BOLD	HRB678-18	Croatia	public
<i>Paracaprella pusilla</i>	Genbank	MK764211	Tunisia	public
<i>Paracaprella pusilla</i>	Genbank	MK764210	Tunisia	public
<i>Paracaprella pusilla</i>	Genbank	KF743535	Mexico	public
<i>Paracaprella pusilla</i>	Genbank	KF743525	Mexico	public
<i>Paracaprella pusilla</i>	Genbank	MK764262	Mexico	public
<i>Paracaprella pusilla</i>	Genbank	MK764213	Israel	public
<i>Paracaprella pusilla</i>	Genbank	MK764146	Spain	public
<i>Paracaprella pusilla</i>	Genbank	MK764145	Spain	public
<i>Paracaprella pusilla</i>	Genbank	MK764166	Spain	public
<i>Paracaprella pusilla</i>	Genbank	MK764144	Spain	public
<i>Paracaprella pusilla</i>	Genbank	MK764165	Spain	public
<i>Paracaprella pusilla</i>	Genbank	MK764164	Spain	public
<i>Paracaprella pusilla</i>	Genbank	MK764163	Spain	public
<i>Paracaprella pusilla</i>	Genbank	MK764162	Spain	public
<i>Paracaprella pusilla</i>	Genbank	MK764159	Spain	public
<i>Paracaprella pusilla</i>	Genbank	MK764158	Spain	public
<i>Paracaprella pusilla</i>	Genbank	MK764157	Spain	public
<i>Paracaprella pusilla</i>	Genbank	MK764153	Spain	public
<i>Paracaprella pusilla</i>	Genbank	MK764151	Spain	public
<i>Paracaprella pusilla</i>	Genbank	MK764148	Spain	public
<i>Paracaprella pusilla</i>	Genbank	MK764118	Spain	public
<i>Paracaprella pusilla</i>	Genbank	MK764116	Spain	public

<i>Paracaprella pusilla</i>	Genbank	MK764115	Spain	public
<i>Paracaprella pusilla</i>	Genbank	MK764114	Spain	public
<i>Paracaprella pusilla</i>	Genbank	MK764110	Spain	public
<i>Paracaprella pusilla</i>	Genbank	MK764099	Spain	public
<i>Paracaprella pusilla</i>	Genbank	MK764098	Spain	public
<i>Paracaprella pusilla</i>	Genbank	MK764086	Spain	public
<i>Paracaprella pusilla</i>	Genbank	MK764081	Spain	public
<i>Paracaprella pusilla</i>	Genbank	MK764209	Spain	public
<i>Paracaprella pusilla</i>	Genbank	MK764197	Spain	public
<i>Paracaprella pusilla</i>	Genbank	MK764195	Spain	public
<i>Paracaprella pusilla</i>	Genbank	MK764191	Spain	public
<i>Paracaprella pusilla</i>	Genbank	MK764190	Spain	public
<i>Paracaprella pusilla</i>	Genbank	MK764188	Spain	public
<i>Paracaprella pusilla</i>	Genbank	MK764185	Spain	public
<i>Paracaprella pusilla</i>	Genbank	MK764184	Spain	public
<i>Paracaprella pusilla</i>	Genbank	MK764183	Spain	public
<i>Paracaprella pusilla</i>	Genbank	MK764178	Spain	public
<i>Paracaprella pusilla</i>	Genbank	MK764177	Spain	public
<i>Paracaprella pusilla</i>	Genbank	MK764168	Spain	public
<i>Paracaprella pusilla</i>	Genbank	MK764123	Spain	public
<i>Paracaprella pusilla</i>	Genbank	MK764121	Spain	public
<i>Paracaprella pusilla</i>	Genbank	KF743519	Spain	public
<i>Paracaprella pusilla</i>	Genbank	KF743516	Spain	public
<i>Paracaprella pusilla</i>	Genbank	KF743512	Spain	public
<i>Paracaprella pusilla</i>	Genbank	KF743510	Spain	public
<i>Paracaprella pusilla</i>	Genbank	KF743497	Spain	public
<i>Paracaprella pusilla</i>	Genbank	KF743496	Spain	public
<i>Paracaprella pusilla</i>	Genbank	KF743495	Spain	public
<i>Paracaprella pusilla</i>	Genbank	KF743491	Spain	public
<i>Paracaprella pusilla</i>	Genbank	KF743488	Spain	public
<i>Paracaprella pusilla</i>	Genbank	MK764219	Australia	public
<i>Paracaprella pusilla</i>	Genbank	MK764217	Australia	public
<i>Paracaprella pusilla</i>	Genbank	MK764215	Australia	public
<i>Paracaprella pusilla</i>	Genbank	MK764227	Australia	public
<i>Paracaprella pusilla</i>	Genbank	MK764225	Australia	public
<i>Paracaprella pusilla</i>	Genbank	MK764224	Australia	public
<i>Paracaprella pusilla</i>	BOLD	FLTWO875-18	Florida:USA	public
<i>Paracaprella pusilla</i>	BOLD	FLTWO871-18	Florida:USA	public
<i>Paracaprella pusilla</i>	BOLD	FLTWO956-18	Florida:USA	public
<i>Paracaprella pusilla</i>	BOLD	FLTWO955-18	Florida:USA	public
<i>Paracaprella pusilla</i>	BOLD	FLTWO561-18	Florida:USA	public
<i>Paracaprella pusilla</i>	BOLD	FLFIV400-18	Florida:USA	public
<i>Paracaprella pusilla</i>	BOLD	ISWIO025-19	South Africa	private
<i>Photis sarae</i>	BOLD	FLFIV422-18	Florida:USA	private
<i>Photis sarae</i>	BOLD	FLFIV406-18	Florida:USA	private
<i>Photis sarae</i>	BOLD	FLFIV398-18	Florida:USA	private
<i>Photis sarae</i>	BOLD	FLFIV396-18	Florida:USA	private
<i>Photis sarae</i>	BOLD	FLFIV419-18	Florida:USA	private
<i>Photis sarae</i>	BOLD	FLFIV437-18	Florida:USA	private
<i>Photis sarae</i>	BOLD	FLFIV443-18	Florida:USA	private

<i>Stenothoe gallensis</i>	Genbank	MH128325	Vietnam	public
<i>Stenothoe gallensis</i>	Genbank	MT317092	Chile	public
<i>Stenothoe gallensis</i>	Genbank	MT317093	Chile	public
<i>Stenothoe gallensis</i>	Genbank	MT317091	Chile	public
<i>Stenothoe gallensis</i>	BOLD	MGOTX952-19	Texas:USA	private
<i>Stenothoe georgiana</i>	BOLD	SERCI2483-19	Virginia:USA	private

CHAPTER II

Ancient globetrotters - connectivity and putative native ranges of two cosmopolitan biofouling amphipods

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All the following supplementary materials are available online at:

<https://peerj.com/articles/9613/>

Figure S1: Phylogenetic time tree of *J. marmorata*, *J. slatteryi* and *J. herdmani* derived with BEAST.

Posterior probabilities and bootstrap support are provided when higher than 90% and 70% respectively. Blue bars refer to the 95% highest posterior density intervals for the nodes with at least 90% posterior probabilities.

Figure S2: BioGeoBears ancestral range reconstruction under the DEC+J model of *J. marmorata*. Pie charts at the nodes and edges are proportional to the probability of each region before and after the speciation event.

Figure S3: BioGeoBears ancestral range reconstruction under the DEC+J model of *J. slatteryi*. Pie charts at the nodes and edges are proportional to the probability of each region before and after the speciation event.

Table S1: Complete overview of all sequences used for the analyses (collected in this study, and obtained from GenBank and BOLD). Information includes species identity, locality, number of specimens (n), accession numbers, and references.

Table S2: Sum S (SSD), Raggedness index and relative p-values of the mismatch distributions under the models of demographic expansion and spatial distribution for *J. marmorata* and *J. slatteryi*.

Significant p-values are highlighted in bold. Regions represented by only one population are annotated in the same line with "/". OR: Oregon; CA: California; NS: North Sea; NO: Norway; IC: Iceland; SPA: Atlantic Spain; CT: Connecticut; VA: Virginia; SPM: Mediterranean Spain; CH: Chile; PE: Peru; JA: Japan; SK: South Korea.

Table S3: Pairwise Fst indices between regions. Significant Fst values (p-value < 0.05) are highlighted in bold. JM: *Jassa marmorata*; JS: *Jassa slatteryi*. NEP: North East Pacific; NES: North european seas; NWA: North West Atlantic; MED: Mediterranean Sea; SEP: South East Pacific; NWP: North West Pacific; LUS: Iberian Peninsula.

Table S4: Pairwise Fst indices between populations. Significant Fst values (p-value < 0.05) are highlighted in bold. JM: *Jassa marmorata*; JS: *Jassa slatteryi*. SPM: Mediterranean Spain; OR: Oregon; CA: California; NS: North Sea; NO: Norway; IC: Iceland; SPA: Atlantic Spain; CT: Connecticut; VA: Virginia; MED: Mediterranean Sea; CH: Chile; PE: Peru; JA: Japan; SK: South Korea; AR: Argentine; AF: Africa.

Table S5: Migration rates between regions. The source of migration is given in the first row; consistent results are highlighted in bold (always >0). JM: *Jassa marmorata*; JS: *Jassa slatteryi*. NEP: North Eat Pacific;

NES: North european seas; NWA: North West Atlantic; MED: Mediterranean Sea; SEP: South East Pacific; NWP: North West Pacific; LUS: Iberian Peninsula.

Table S6: Migration rates between populations. The source of migration is given in the top row; consistent results are highlighted in bold (always >0). JM: *Jassa marmorata*; JS: *Jassa slatteryi*. OR: Oregon; CA: California; DE: Germany; NS: North Sea; NO: Norway; IC: Iceland; SPA: Atlantic Spain; CT: Connecticut; VA: Virginia; MED: Mediterranean Sea; CH: Chile; PE: Peru; JA: Japan; SK: South Korea.

Table S7: Summary of likelihood results and the statistics for model comparison of BioGeoBears.

CHAPTER III

Hitchhikers' community: taxonomic, phylogenetic and functional diversity among ports and estuaries

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Tab. S1. Fuzzy-coded functional traits for each peracarid species collected. Each species was scored in a range of 0-3 based on its affinity degree for each category, with 0 being no affinity to a trait category and 3 being complete affinity. Feeding habit: surface deposit feeder (f.sdf), suspension feeder (f.sf), predator (f.pr), grazer (f.gr); Diet type: omnivore (dt.o), carnivore (dt.c), herbivore (dt.h); Environmental position: epibenthic (ep.e), infaunal (ep.i), epibiotic (ep.b); Adult movement: swimmer (am.sw), crawler (am.c); Life habit: free-liver (ha.fl), tubiculous (ha.tb); Fecundity: < 20 eggs (fec.1), 20-40 eggs (fec.2), > 40 eggs (fec.3); Size (cm): 0-0.5 (si.0), 0.5-1 (si.1), >1 (si.2); Sociability: solitary (so.so), gregarious (so.g); Parental care: limited (pc.l), extended (pc.e).

Species	Feeding habit				Diet type			Environmental position			Adult movement		Life habit		Fecundity			Size			Sociability		Parental care	
	f.sdf	f.sf	f.pr	f.gr	dt.o	dt.c	dt.h	ep.e	ep.i	ep.b	am.sw	am.c	ha.fl	ha.tb	fec.1	fec.2	fec.3	si.0	si.1	si.2	so.so	so.g	pc.l	pc.e
<i>Hourstonius</i> sp.	1	0	2	0	2	2	0	2	0	2	1	2	3	0	2	1	0	2	1	0	3	0	3	0
<i>Stenothoe gallensis</i> Walker, 1904	1	0	2	1	2	2	0	1	0	2	1	2	3	0	1	2	0	1	2	0	3	0	3	0
<i>Stenothoe valida</i> Dana, 1852	1	0	2	1	2	2	0	1	0	2	1	2	3	0	1	2	0	1	2	0	3	0	3	0
<i>Monocorophium acherusicum</i> (Costa, 1853)	1	2	0	1	2	1	1	2	1	0	2	2	0	3	2	1	0	0	3	0	0	3	1	2
<i>Laticorophium baconi</i> (Shoemaker, 1934)	2	2	0	1	2	1	1	2	1	0	2	2	0	3	0	2	1	0	3	0	0	3	1	2
<i>Apocorophium acutum</i> (Chevreux, 1908)	2	2	0	1	2	1	1	2	1	0	2	2	0	3	0	2	1	0	3	0	0	3	1	2
<i>Jassa valida</i> (Dana, 1853)	1	2	1	0	2	2	0	2	0	2	2	2	1	2	0	1	2	0	3	0	1	2	2	1
<i>Enicthonius brasiliensis</i> (Dana, 1853)	2	2	0	0	3	0	0	2	0	1	1	2	0	3	0	1	2	0	3	0	2	1	2	1
<i>Erithonius punctatus</i> (Spence Bate, 1857)	2	2	0	0	3	0	0	2	0	1	1	2	0	3	3	0	0	0	3	0	2	1	2	1
<i>Caprella dilatata</i> Krøyer, 1843	2	2	1	0	3	0	0	1	0	2	0	3	3	0	0	1	2	0	1	2	0	3	0	3
<i>Caprella equilibra</i> Say, 1818	1	2	2	0	2	1	0	1	0	2	0	3	3	0	0	1	2	0	1	2	0	3	0	3
<i>Paracaprella pusilla</i> Mayer, 1890	1	1	1	1	3	0	0	1	0	2	0	3	3	0	1	2	0	0	2	1	0	3	0	3
<i>Podocerus brasiliensis</i> (Dana, 1853)	2	2	0	0	3	0	0	2	0	2	1	2	3	0	2	2	0	0	2	1	0	3	0	3
<i>Photis sarae</i> Souza-Filho & Serejo, 2010	2	1	0	0	2	1	0	2	0	2	2	2	0	3	3	0	0	0	3	0	1	2	1	2
<i>Latigammaropsis tagoensis</i> (Schellenberg, 1925)	2	1	0	0	2	1	0	2	0	2	2	2	0	3	3	0	0	0	3	0	1	2	1	2
<i>Elasmopus</i> sp.	2	0	0	1	2	1	1	3	0	0	2	2	3	0	0	1	2	0	2	1	2	1	1	2
<i>Quadrimaera miranda</i> (Ruffo, Krapp-Schickel & Gable, 2000)	2	1	0	0	2	1	1	2	1	0	2	2	3	0	3	0	0	0	2	1	3	0	1	2
<i>Lembos</i> sp.	2	1	0	0	2	1	1	2	1	0	2	2	0	3	3	0	0	0	3	0	3	0	2	1
<i>Propejanice lagamarensis</i> Myers & Desiderato, 2019	0	2	0	0	2	1	0	2	1	0	2	2	0	3	2	1	0	0	3	0	3	0	2	1
<i>Ampithoe valida</i> Smith, 1873	2	0	1	2	1	0	2	2	0	1	2	2	2	1	0	0	3	0	0	3	2	1	2	1
<i>Ampithoe ramondi</i> Audouin, 1826	2	0	1	2	2	0	1	2	0	1	2	2	2	1	0	0	3	0	0	3	2	1	2	1

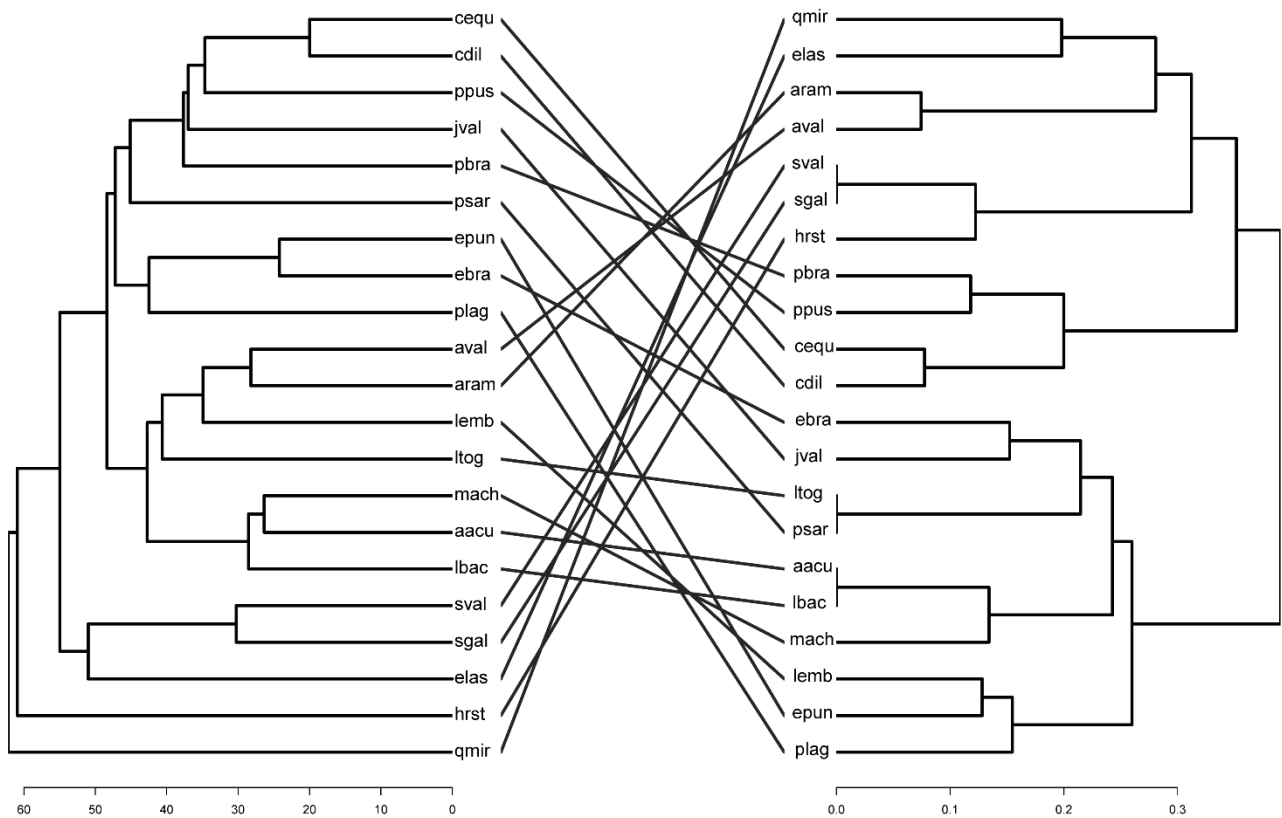


Fig. S1. Phylogeny (left) and functional cluster (right) of the 21 species studied. Black lines relative to the position change of each species in the two trees. Phylogeny of the COI adapted from Desiderato et al. *in prep* (first chapter). Cluster based on Gower's distances. hrst: *Hourstonius* sp.; plag: *Propejanice lagamarensis* Myers & Desiderato 2019; aval: *Ampithoe valida* Smith, 1873; mach: *Monocorophium acherusicum* (Costa, 1851); sgai: *Stenothoe gallensis* Walker, 1904; lbac: *Laticorophium baconi* (Shoemaker, 1934); jval: *Jassa valida* (Dana, 1853); epun: *Erithonius punctatus* (Spence Bate, 1857); cdil: *Caprella dilatata* Krøyer, 1843; ltog: *Latigammaropsis tagoensis* (Schellenberg, 1925); elas: *Elasmopus* sp.; lemb: *Lembos* sp.; aram: *Ampithoe ramondi* Audouin, 1826 aacu: *Apocorophium acutum* (Chevreux, 1908); ppus: *Paracaprella pusilla* Mayer, 1890; sval: *Stenothoe valida* Dana, 1852; cben: *Cerapus benthophilus* Thomas & Heard, 1979; ebra: *Erithonius brasiliensis* (Dana, 1853); cequ: *Caprella equilibra* Say, 1818; pbras: *Podocerus brasiliensis* (Dana, 1853); psar: *Photis sarae* Souza-Filho & Serejo, 2010; qmir: *Quadrimeaera miranda* (Ruffo, Krapp-Schickel & Gable, 2000).

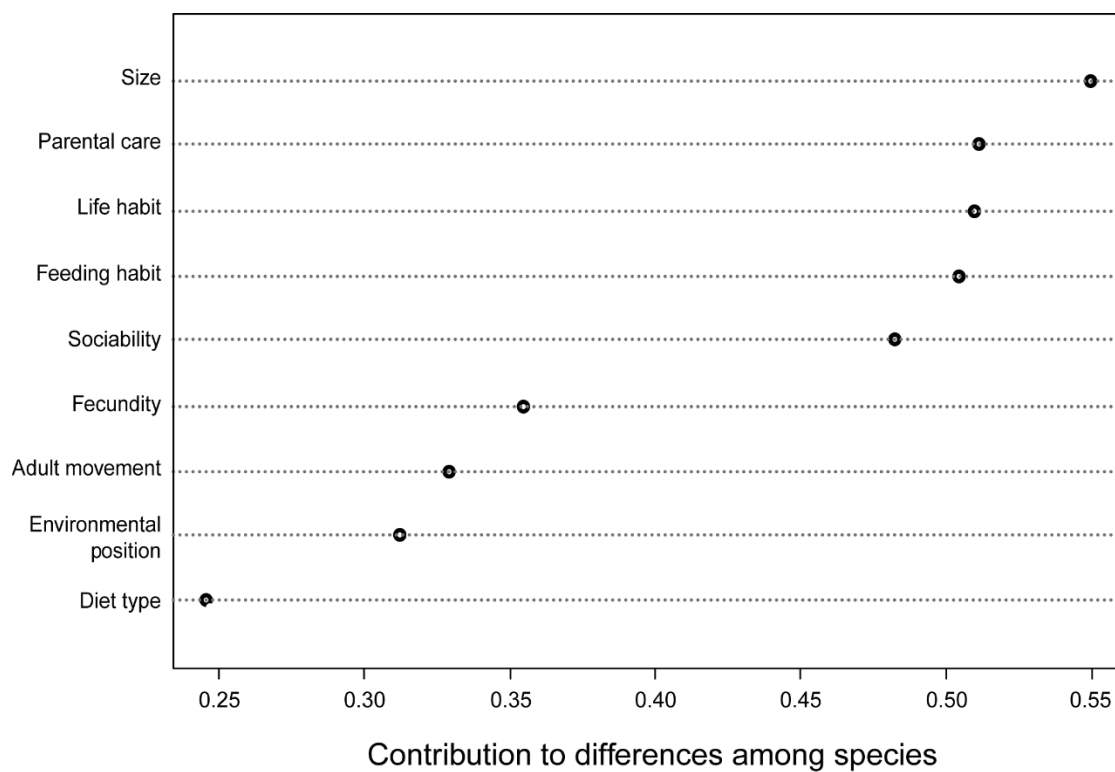


Fig. S2. Contribution of each trait (black circles) to the species differences.

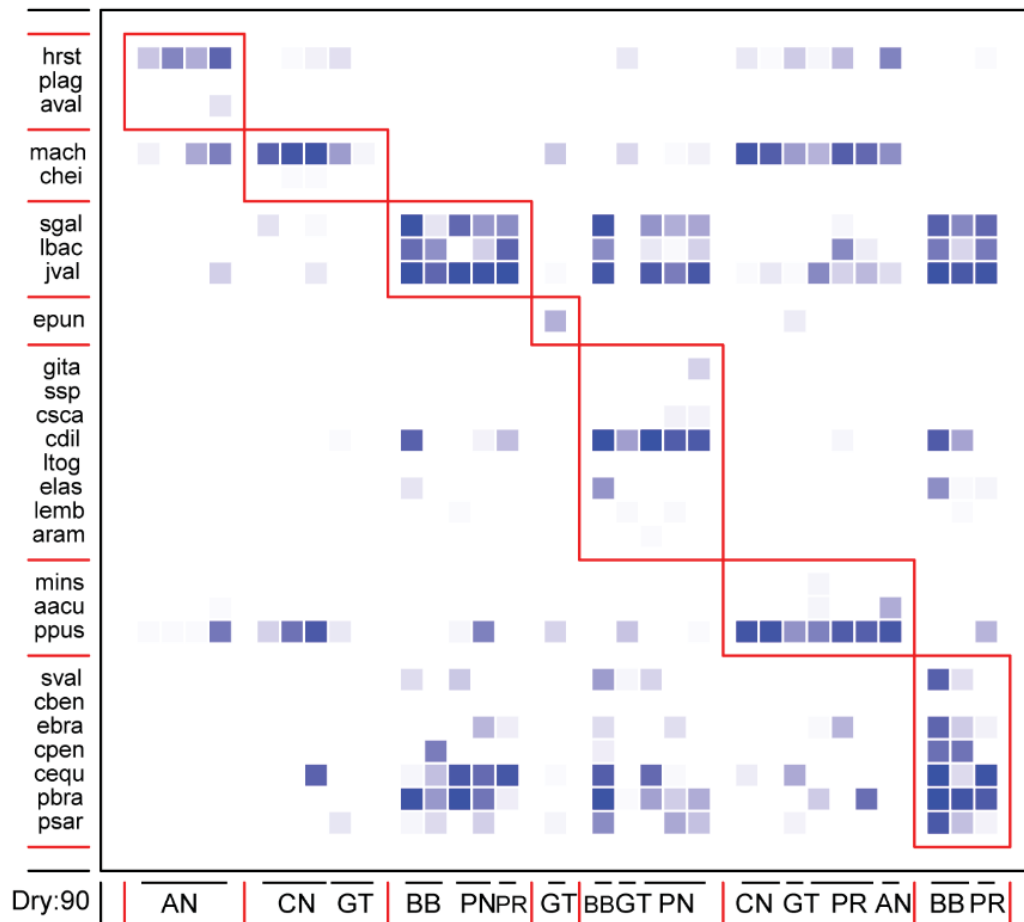


Fig. S3. Modules (red lines) of the 90 days communities of the dry season (Dry:90). Colored squares showing relative abundance of species in each sample (white = absence; dark blue = high abundance). Black lines relative to different samples of the sites. CN: Cananéia-Iguape Estuarine complex – Cananéia; GT: Guaratuba Bay; AN: Antonina; PR: Paranaguá; PN: Pontal do Sul; BB: Babitonga Bay. hrst: *Hourstonius* sp.; plag: *P. lagamarensis*; aval: *A. valida*; mach: *M. acherusicum*; chei: *Cheiriphotis* sp.; sgai: *S. gallensis*; lbac: *L. baconi*; jval: *J. valida*; epun: *E. punctatus*; gita: *Gitanopsis* sp.; ssp: *Stenothoe* sp.; csca: *Caprella scaura* Templeton, 1836; cdil: *C. dilatata*; ltog: *L. tangoensis*; elas: *Elasmopus* sp.; lemb: *Lembos* sp.; aram: *A. ramondi*; mins: *Monocorophium insidiosum* (Crawford, 1937); aacu: *A. acutum*; ppus: *P. pusilla*; sval: *S. valida*; cben: *C. benthophilus*; ebra: *E. brasiliensis*; cpen: *Caprella penantis* Leach, 1814; cequ: *C. equilibra*; pbras: *P. brasiliensis*; psar: *P. sarae*.

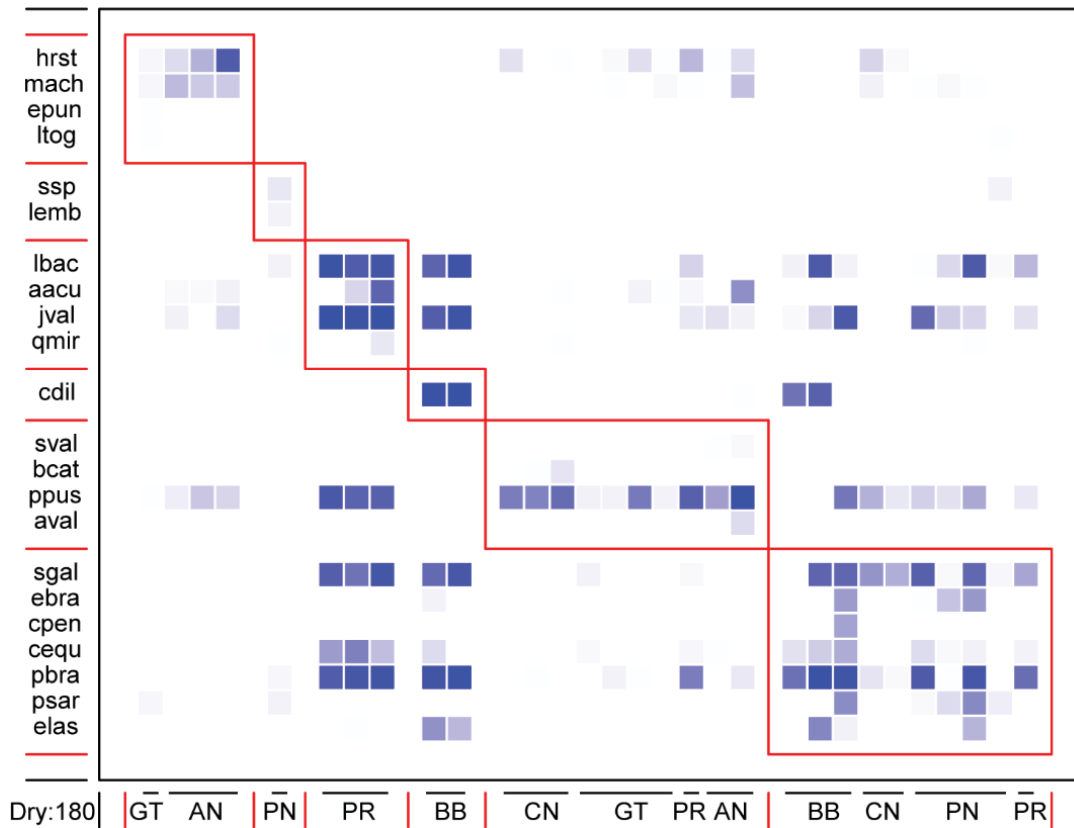


Fig. S4. Modules (red lines) of the 180 days communities of the dry season (Dry:180). Colored squares showing relative abundance of species in each sample (white = absence; dark blue = high abundance). Black lines relative to different samples of the sites. CN: Cananéia-Iguape Estuarine complex – Cananéia; GT: Guaratuba Bay; AN: Antonina; PR: Paranaguá; PN: Pontal do Sul; BB: Babitonga Bay. hrst: *Hourstonius* sp.; mach: *M. acherusicum*; epun: *E. punctatus*; ltog: *L. tagoensis*; ssp: *Stenothoe* sp.; lemb: *Lembos* sp.; lbac: *L. baconi*; aacu: *A. acutum*; jval: *J. valida*; qmir: *Q. miranda*; cdil: *C. dilatata*; sval: *S. valida*; bcat: *B. catharinensis*; ppus: *P. pusilla*; aval: *A. valida*; sgall: *S. gallensis*; ebra: *E. brasiliensis*; cpen: *C. penantis*; cequ: *C. equilibra*; pbras: *P. brasiliensis*; psar: *P. sarae*; elas: *Elasmopus* sp.



Fig. S5. Modules (red lines) of the 90 days communities of the wet season (Wet:90). Colored squares showing relative abundance of species in each sample (white = absence; dark blue = high abundance). Black lines relative to different samples of the sites. CN: Cananéia-Iguape Estuarine complex – Cananéia; GT: Guaratuba Bay; AN: Antonina; PR: Paranaguá; PN: Pontal do Sul; BB: Babitonga Bay. hrst: *Hourstonius* sp.; mach: *M. acherusicum*; qmir: *Q. miranda*; plag: *P. lagamarensis*; aval: *A. valida*; sgal: *S. gallensis*; elas: *Elasmopus* sp.; aram: *A. ramondi*; aacu: *A. acutum*; jval: *J. valida*; cdil: *C. dilatata*; ebra: *E. brasiliensis*; psar: *P. sarae*; epun: *E. punctatus*; cequ: *C. equilibra*; ppus: *P. pusilla*; ltog: *L. tagoensis*; lemb: *Lembos* sp.; lbac: *L. baconi*; pbras: *P. brasiliensis*.

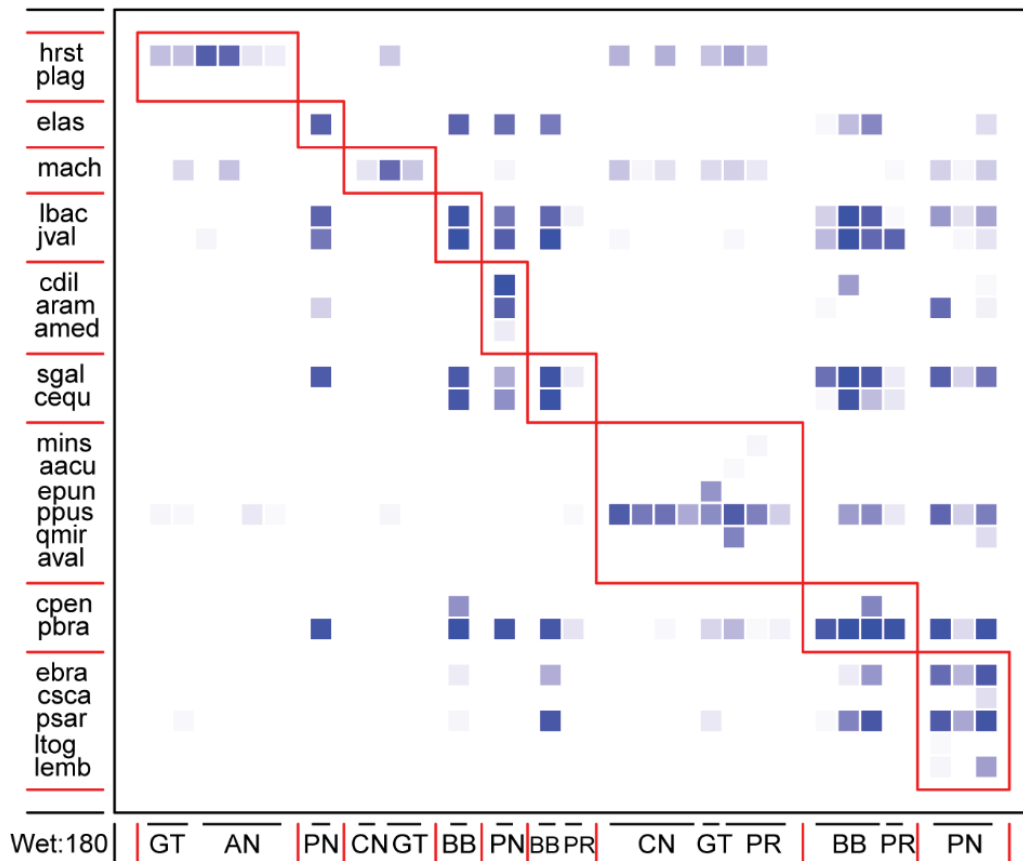


Fig. S6. Modules (red lines) of the 180 days communities of the wet season (Wet:180). Colored squares showing relative abundance of species in each sample (white = absence; dark blue = high abundance). Black lines relative to different samples of the sites. CN: Cananéia-Iguape Estuarine complex – Cananéia; GT: Guaratuba Bay; AN: Antonina; PR: Paranaguá; PN: Pontal do Sul; BB: Babitonga Bay. hrst: *Hourstonius* sp.; plag: *P. lagamarensis*; elas: *Elasmopus* sp.; mach: *M. acherusicum*; lbac: *L. baconi*; jval: *J. valida*; cdil: *C. dilatata*; aram: *A. ramondi*; amed: *A. media*; sgal: *S. gallensis*; cequ: *C. equilibra*; mins: *M. insidiosum*; aacu: *A. acutum*; epun: *E. punctatus*; ppus: *P. pusilla*; qmir: *Q. miranda*; aval: *A. valida*; cpen: *C. penantis*; pbras: *P. brasiliensis*; ebra: *E. brasiliensis*; csca: *C. scaura*; psar: *P. sarae*; ltog: *L. tagoensis*; lemb: *Lembos* sp.

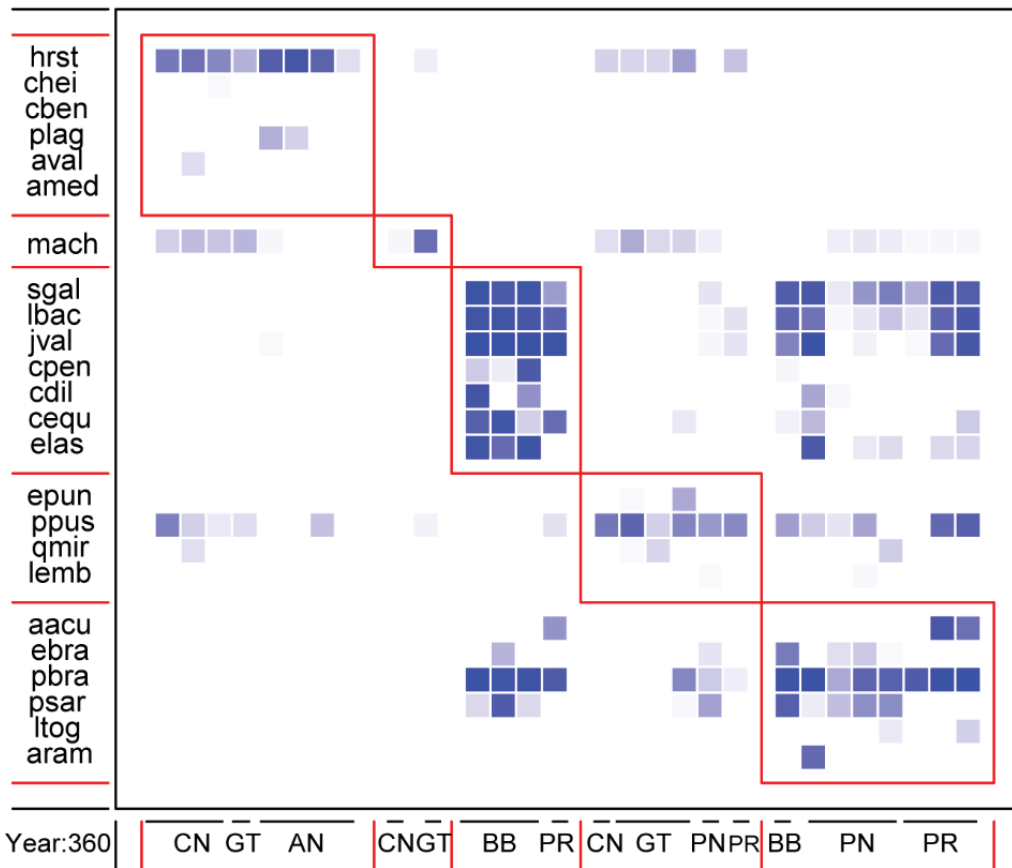


Fig. S7. Modules (red lines) of the yearly communities (Year:360). Colored squares showing relative abundance of species in each sample (white = absence; dark blue = high abundance). Black lines relative to different samples of the sites. CN: Cananéia-Iguape Estuarine complex – Cananéia; GT: Guaratuba Bay; AN: Antonina; PR: Paranaguá; PN: Pontal do Sul; BB: Babitonga Bay. hrst: *Hourstonius* sp.; chei: *Cheiriphotis* sp.; cben: *C. benthophilus*; plag: *P. lagamarensis*; aval: *A. valida*; amed: *A. media*; mach: *M. acherusicum*; sgal: *S. gallensis*; lbac: *L. baconi*; jval: *J. valida*; cpen: *C. penantis*; cdil: *C. dilatata*; cequ: *C. equilibra*; elas: *Elasmopus* sp.; epun: *E. punctatus*; ppus: *P. pusilla*; qmir: *Q. miranda*; lemb: *Lembos* sp.; aacu: *A. acutum*; ebra: *E. brasiliensis*; pbras: *P. brasiliensis*; psar: *P. sarae*; ltog: *L. tagoensis*; aram: *A. ramondi*.

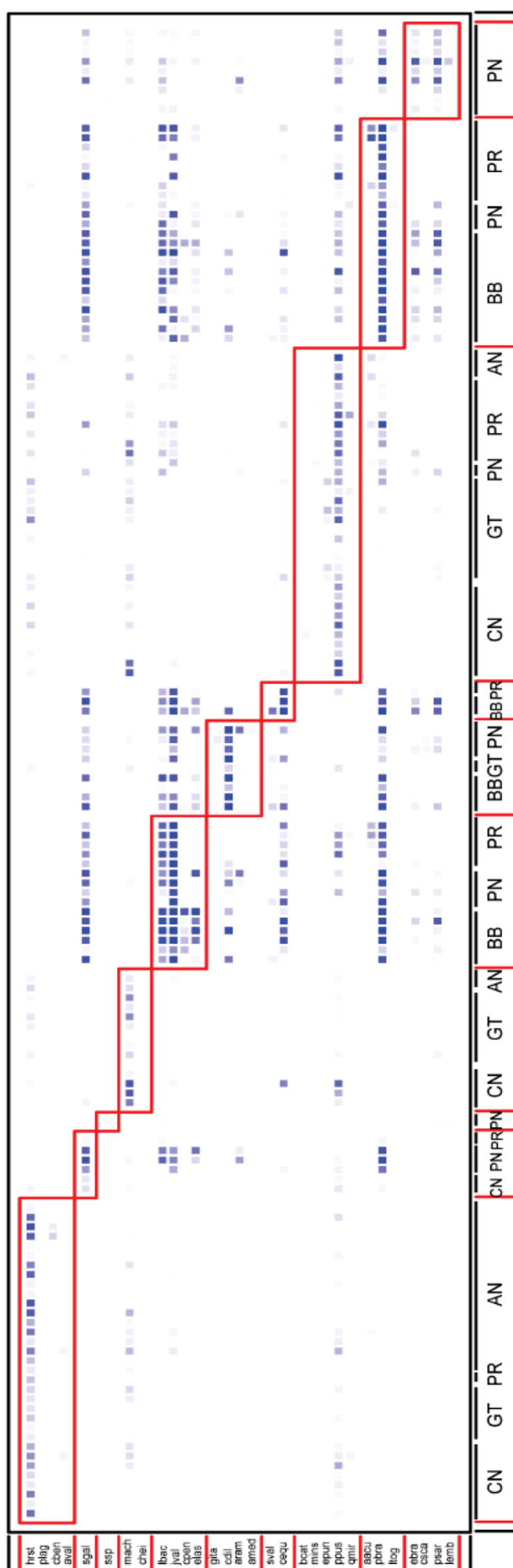


Fig. S8. Modules (red lines) of the whole dataset. Colored squares showing relative abundance of species in each sample (white = absence; dark blue = high abundance). Black lines relative to different samples of the sites. CN: Cananéia-Iguape Estuarine complex – Cananéia; GT: Guaratuba Bay; AN: Antonina; PR: Paranaguá; PN: Pontal do Sul; BB: Babitonga Bay. hrst: *Hourstonius* sp.; plag: *P. lagamarensis*; aval: *A. valida*; mach: *M. acherusicum*; sgai: *S. gallensis*; lbac: *L. baconi*; jval: *J. valida*; epun: *E. punctatus*; gita: *Gitanopsis* sp.; ssp: *Stenothoe* sp.; cdi: *C. dilatata*; ltog: *L. tagoensis*; elas: *Elasmopus* sp.; lemb: *Lembos* sp.; aram: *A. ramondi*; mins: *M. insidiosum*; aacu: *A. acutum*; ppus: *P. pusilla*; sval: *S. valida*; bcat: *B. catharinensis*; cben: *C. benthophilus*; ebra: *E. brasiliensis*; cpen: *C. penantis*; psar: *P. sarae*; chei: *Cheiriphotis* sp.; csca: *C. scaura*; amed: *A. media*.