

Ship-driven biopollution: how aliens transform the local ecosystem diversity in Pacific islands

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Abstract

Ships moving species across the oceans mix marine communities throughout latitudes. The introduction of new species may be changing the ecosystems even in remote islands. In tropical Pacific islands where maritime traffic is principally local, eDNA metabarcoding and barcoding revealed 75 introduced species, accounting in average for 28% of the community with a minimum of 13% in the very remote Rangiroa atoll. The majority of non-native species were primary producers –from diatoms to red algae, thus the ecosystem is being transformed from the bottom. Primary producers were more shared among sites than other exotics, confirming ship-mediated dispersal in Pacific marine ecosystems. Limited alien share and an apparent saturation of aliens (similar proportion in ports of very different size) suggests the occurrence of “alien drift” in port communities, or random retention of newly introduced aliens that reminds genetic drift of new mutations in a population.

1 Introduction

2 Tropical waters of French Polynesia shelter the best-preserved, most resilient coral
3 reefs in the world. They could be considered as temporary refuges from climate-induced
4 threats like extensive bleaching (Van Hooidek et al., 2013). Although adverse events like
5 cyclones, bleaching and predator outbreaks are leading to depletion of other coral reefs
6 (De'ath et al., 2012), in French Polynesia they seem to recover rapidly from mass mortality
7 events. This is probably due to high connectivity in the coral community network, which helps
8 larvae to replenish disturbed reefs (Vercelloni et al., 2019).

9 In addition to other threats, coral reefs suffer from biological invasions that are
10 considered as the second cause of local extinctions worldwide, just after habitat losses
11 (Clavero and Garcia-Berthou, 2005; Bellard et al., 2016). There are different gateways for the
12 introduction of exotic species into a marine habitat, the fouling of maritime vessels being
13 likely the most important (e.g. Gollasch, 2002; Godwin, 2003; Ruiz et al., 2009). International
14 shipping and intraregional transport have been reported as important mechanisms for
15 transferring marine species and alien species dissemination (Wasson et al., 2001). It is
16 important to remark the difference between exotic (alien) and invasive species. Exotic species
17 are non-native species; invasive species are non-native species that harm the recipient
18 ecosystem in different ways. Invasive species belong to diverse trophic levels, from primary
19 producers to predators, and their expansion can alter the whole ecosystem (Verges et al.,
20 2014; Bellwood and Goatley, 2017; Chandrasekaran et al., 2008; Neilson et al., 2018).

21 To date, French Polynesian coral reefs seem to resist to biological invasions better than
22 other tropical reefs like the Caribbean (Bellwood and Goatley, 2017). This tropical region and
23 every island ecosystem within are biodiversity hotspots, because although they account for
24 only 5% of the Earth's emerged land, they harbor a disproportionately high fraction of the
25 global biodiversity (Kier et al. 2009). The rich native biodiversity confers biotic resistance to
26 invasions (Stachowicz et al. 1999). Previous studies suggest that biogeographic patterns may
27 affect the success of a new invasion due to biotic and abiotic barriers. Few exotic species have
28 become naturalized within the tropics because biotic resistance can limit, even prevent, the
29 distribution, abundance and, ultimately, the establishment of non-native species (Sax, 2001).
30 This biotic resistance appears to be stronger in the tropics than in higher latitudes (Freestone
31 et al., 2013) and species richness is negatively correlated with latitude (Sax, 2001). In addition
32 to biotic resistance, French Polynesian islands are relatively isolated being away of highly
33 frequented commercial maritime routes (see Figure 1). Reduced introduction pressure also
34 contributes to protect them from major invasions. However, Polynesian reefs could be on the
35 verge of a pivotal change. The absence of major natural and anthropogenic barriers (thus
36 good connectivity reported in many studies, e.g. De'ath et al., 2012), together with local
37 maritime traffic of sails and small boats (Ardura et al., 2015), might help introduced species
38 to spread over the islands. Despite their remoteness and biogeographical pattern, surveys
39 based on conventional sampling and DNA barcoding revealed a proportion of non-indigenous
40 invertebrates higher than 15% in some locations in Moorea and Tahiti islands, especially in
41 sheltered areas (Garcia-Vazquez et al., 2020).

42 Here we have investigated the level of non-native species introduction in three French
43 Polynesian islands, using a combination of fouling and water environmental samples
44 confirmed from individual barcoding, in order to allow identification of species across all
45 Eukaryote groups. Previous studies have shown that this combination of individual DNA
46 barcoding and environmental DNA (eDNA) samples coupled with high throughput
47 sequencing methodology is a robust approach for the unambiguous inventory of communities

48 at the species level (Ardura et al., 2020). The depth of the inventory depends on reference
49 databases having enough information of all taxonomic groups (Ardura et al., 2019).

50 The final objective was to inferring the probability of invasion in each of the studied
51 islands, to ultimately determine the priority of bioinvasion control for conservation of this
52 special ecosystem of resilient coral reefs.

55 **Materials and Methods**

57 ***eDNA and biota sampling***

58 The study was developed in two islands (Moorea and Tahiti) and one atoll (Rangiroa)
59 from French Polynesia (Fig.1). Samples were collected from two locations within each island
60 during March 2018: Port Papeete and Port Phaeton in Tahiti; Avatoru (Rangiroa port) and
61 Tiputa in Rangiroa atoll; Pao-Pao and Vaia're in Moorea. Papeete has international traffic from
62 Asia, Europe and Australia (<https://www.portdepapeete.pf/fr/lignes-regulieres>), and
63 Vai'are has daily ferry connections with Port Papeete. In December 2019 random sites were
64 visited again and visible fouling biota sampled to confirm previous results with new DNA
65 barcoding. Nine sampling points were selected according to the port size: three in the
66 commercial port of Papeete, the largest one; two in the mid-size port of Vaia're in Moorea,
67 and one in each of the other small fishing ports. Three random sites separated by 100 meters
68 were sampled for water and biofouling per sampling point (Suppl.Table1). One site within
69 each location was at the entry of the port or close to it outside, to capture as much as possible
70 surrounding biota.

71 Water samples (three 3L replicates per site) and biofouling samples (three sterile
72 cotton gauzes per site, each rubbing a surface quadrat of approx. 90 cm²) were collected from
73 the nine points. An extra sample of 9L from each location was collected. Water and fouling
74 samples were taken simultaneously. Each water sample was collected in separate sterile
75 bottles and filtered immediately *in situ* with a syringe system with a Swinnex portafilters from
76 MerckMillipore® and a membrane filter with 0.2 µm pore size and 25 mm diameter. Filters
77 and gauzes were stored immediately in 100% ethanol until their processing into the
78 laboratory in the University of Oviedo (Spain).

79 To control possible contamination during the sampling, all the reusable equipment
80 and research gear were carefully cleaned with 10% bleach before and after sampling each
81 site. An extra sterilisation under UV light was done overnight during all the sampling season.
82 A closed bottle containing DI water (one per sampling point) was transported together with
83 the sampling gear, opened in the field, and processed with the rest of eDNA water samples as
84 a sampling negative control to monitor possible contaminations.

85 Immediately after taking environmental samples, samples of fouling individuals
86 morphologically recognizable, and roughly representative of all visible biota, were taken at
87 random from the same sites by the surfaces rubbed with gauzes and stored in 100% ethanol
88 for genetic barcoding. Big individuals found in the gauzes were also separated for individual
89 barcoding analysis.

91 ***DNA Barcoding***

92 DNA was extracted from a piece of tissue of fouling individuals following a standard
93 protocol (Estoup et al., 1996) employing Chelex® resin (Bio-Rad Laboratories). For algae
94 DNeasy Blood & Tissue Kits (Quiagen®) were employed following the manufacturer's
95 instructions. The tubes were stored at 4°C for immediate DNA analysis. After the analysis,
96 they were frozen at -20°C for longtime preservation.

97 A fragment of 650 base pairs (bp) within the cytochrome oxidase subunit I gene (COI)
98 was amplified by polymerase chain reaction (PCR), employing Geller *et al.* (2013) primers.
99 Additional sequencing of a fragment between 450-500 bp from the mitochondrial 16S rRNA
100 gene with Palumbi's primers (1996) was carried out to complete taxonomic identification
101 when needed. Reagent concentrations and PCR conditions are described in Suppl. Table 3.
102 PCR products were visualized in 2% agarose gels with SimplySafe™ (EURx® 20000x - 1ml).
103 Sequencing was performed in Macrogen Europe (Madrid, Spain).

104 DNA Sequences were checked and aligned with ClustalW as implemented in BioEdit
105 (Hall, 1999; Thompson et al., 1994). For species identification, the sequences were compared
106 with international references using the BLAST algorithm of GenBank against Nucleotide
107 database (<http://www.ncbi.nlm.nih.gov/>).

108 ***Environmental DNA extraction***

109 DNA was extracted from filters (one extraction per filter) with PowerWater® DNA
110 Isolation Kit (QIAGEN) according to manufacturer's instructions, under sterile conditions
111 inside a laminar flow PCR-cabinet. Filters were manipulated with previously decontaminated
112 forceps. Extraction negative controls (N = 4) and the sampling negative controls (N = 6) were
113 processed too.
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115 After removing big individuals for separate barcoding, DNA was extracted from gauzes
116 as following: gauzes were cleaned with distilled water, introduced into Stomacher® bags and
117 processed at normal speed for 120 seconds. All the liquid obtained together with the cleaning
118 water and liquid vacuumed from the gauzes were introduced into a Falcon tube and
119 centrifuged at 4000 g for 12 minutes. The pellet obtained was treated with E.Z.N.A® Soil DNA
120 kit from Omega Bio-tek for DNA extraction, following the manufacturer's instructions for 250
121 g - 1 mg samples.
122

123 ***Metabarcoding pipeline***

124 The methodological pipeline employed for metabarcoding analysis is detailed in the
125 Supporting information (SI-1)
126

127 ***Taxonomy, geographic information and invasive status***

128 Taxonomic references and geographic origin and distribution of the species assigned
129 from DNA were WoRMS (WoRMS, 2020) and AlgaeBase (Guiry and Guiry, 2020). Harmful
130 algae were checked in IOC-UNESCO list (Moestrup et al., 2020). The status of invasive species
131 was consulted in the Invasive Species Compendium (Invasive Species Compendium, 2020)
132 and IUCN Global Invasive Species Database (<http://www.iucngisd.org/gisd/>, accessed July
133 2020).
134

135 **Results**

136 The metabarcodes employed, COI and 18S genes, yielded a total of 1421467, 1069772
137 and 1113177 quality sequences from Tahiti, Moorea and Rangiroa islands respectively
138 (Suppl. Table 1). Negative controls were all DNA-free and the positive control yielded
139 sequences from all the species introduced. Rarefaction curves of molecular taxonomic units
140 (MOTUs) reached a saturation plateau in all the locations (Suppl. Fig. 1), indicating
141 representative capture of the DNA diversity present. Fouling (versus water) samples reached
142 faster the plateau and generally provided more MOTUs.
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145 Indeed, most species captured from eDNA, of all the taxa, were natives. Not only sessile
146 organisms were detected. Vagile species are also detected from eDNA such as 35 reef fish
147 (including the near-threatened black tip shark), or the native critically endangered hawksbill
148 sea turtle *Eretmochelys imbricata* (Suppl. Table 2).

149 A total of 75 exotic species were found from eDNA (28% of the 264 MOTUs identified
150 at a species level, corresponding to 18% over 415 marine MOTUs identified at genus level).
151 Individual DNA barcodes confirmed 76.5% of them. The proportion of alien species (exotic
152 plus invasive aliens) ranged from 0.37 (0.11 invasive alien species, IAS) in PaoPao to 0.13
153 (0.03 IAS) in the small port of Tiputa in Rangiroa atoll (Fig.1). Aliens were diverse in ecology
154 and origin. A few examples were the Atlantic benthonic polychaete *Syllis amica*, the
155 Mediterranean scavenger decapod *Synalpheus africanus*, the Caribbean invasive oyster
156 *Dendostrea frons*, the highly invasive Northwest Pacific *Magallana gigas* and European *Mytilus*
157 –first reports in Tahiti and Moorea-, red algae like *Solieria filiformis*; all found from Next-
158 Generation-Sequencing (NGS) metabarcodes and authenticated with individual barcodes
159 (Table 1). The Atlantic frond oyster *Dendostrea frons* found only on ship hulls in Papeete in
160 2013 (Garcia-Vazquez et al. 2020), was found attached to rocks in Papeete and Vaia're,
161 suggesting an initial colonization of natural substrates. In NGS dataset other highly invasive
162 species like the Ascidians *Styela plicata* and *Ciona intestinalis* and the algae *Chondria*
163 *coerulescens*, *Colpomenia sinuosa* and *Dasysiphonia japonica* appeared too. Alien microalgae
164 were also found, diatoms typical of the Atlantic (e.g. *Cylindrotheca closterium*, *Psammodictyon*
165 *panduriforme*, *Haslea crucifera*) and dinoflagellates, some of them known to produce algal
166 blooms and toxicity (e.g. *Alexandrium ostenfeldii*, *Margalefidinium polykrikoides*) (Table 1).

167 The composition of alien and native communities seems to be influenced by the size
168 and location of the ports. In the Neighbor Joining clustering analysis constructed from genera
169 richness in phyla, native communities grouped relatively apart from alien ones (Fig.2).
170 Moreover, they clustered by pairs, with a relative proximity of the larger ports (Papeete in
171 Tahiti and Vaia're in Moorea) to Rangiroa's (Avatoru and Tiputa). Those native communities
172 may be artificially connected from more maritime connections between larger ports and the
173 atoll than with small ones. Regarding alien phyla, the largest ports Papeete and Vai'are, were
174 clearly different, located in separate branches, expectedly because more international traffic
175 would encompass a higher diversity of aliens. Small ports were clustered by size: the two very
176 small of Rangiroa together in a cluster, then the small Phaeton (Tahiti) and PaoPao (Moorea)
177 in another cluster (Fig.2).

178 According to more ship connections, a higher proportion of aliens (25%) was shared
179 between Tahiti and Moorea than between any of those and Rangiroa (Fig.3), although a non-
180 negligible 11% of species was found in the three islands (the polychaete *Syllis amica*, three
181 diatoms *Cylindrotheca closterium*, *Navicula cryptocephala* and *Psammodictyon panduriforme*,
182 two red algae *Solieria filiformis* and *Sonderopelta capensis*, the dinoflagellate *Protoceratium*
183 *reticulatum* and the brown alga *Colpomenia sinuosa*). Many alien species were not shared
184 between islands: 36% were found only in Tahiti, 21% in Moorea and 4.5% in Rangiroa atoll,
185 remarking the importance of maritime traffic in the alien species dispersion. Only one of the
186 10 alien sponges (Porifera) was found in more than one island (*Scopalina ruetzleri* in Tahiti
187 and Moorea), while most phytoplankton species (7 out of 8) and mollusks (8 out of 12 alien
188 mollusks) were at least on two islands.

189 Regarding taxonomic and functional diversity, native and exotic species were not
190 equally distributed across taxonomic groups in these Pacific islands ($\chi_{13,3} = 53.2$, $p = 0.001$,
191 Cramer's V = 0.319). Rodophyta contained by far more aliens than any other group, followed
192 by Porifera and Mollusca (Fig.4), while Cnidaria and Ochrophyta contained less aliens. Alien

193 communities were richer in primary producers: only 30% of the native species were
194 autotrophs, while so were 46% of exotics and 41% of IAS.

195 The taxonomic profiles of the different locations were different to each other but
196 shared a common pattern: alien communities were enriched in primary producers, from
197 microscopic diatoms and dinoflagellates to macroalgae, while native community fractions
198 contained more animals (Fig.5). At genera level (N = 415 MOTUs), 55.4% and 36.5% in the
199 alien and native community fractions, respectively, were autotrophs. The distribution of
200 heterotrophic and autotrophic taxa in native communities was not different among locations
201 ($\chi^2_{2,5} = 5.5, p = .24$), nor in alien community fractions ($\chi^2_{2,5} = 1.2, p = .88$). In contrast, Wilcoxon
202 test showed that the proportion of autotrophs in native and alien fractions of these Pacific
203 communities was significantly different ($W = 21, z = 2.2, p = .027$).

204 205 206 **Discussion**

207 208 *Saturation of aliens versus natives, and "alien drift" in diverse communities*

209
210 Despite a more intense maritime traffic in Tahiti with a large international commercial
211 port in Papeete, with higher introduction pressure, and probable time lags to detection on
212 Moorea Island, similar proportion of aliens in Tahiti and Moorea islands were measured. This
213 highlights the importance of maritime traffic, both national and international, in the transfer
214 of exotic species. On the other hand, considering only mollusks with previous data, the
215 percentage of exotic species found in PaoPao, Vaia're and Papeete in 2011 (total N = 19;
216 Garcia-Vazquez et al. ,2020) was 21%; seven years later (this study) it was 29% (N = 41), not
217 significantly different ($\chi^2_{2,2} = 0.45, p = .5$) despite more exhaustive sampling and sensitive
218 identification tools. This suggests that the communities are saturated in exotic species, as
219 proposed by Sax and Gaines (2008) in islands' plant communities.

220 Could the apparent saturation of aliens in Tahiti and Moorea be an effect of aliens' easy
221 dispersal via ships, so moving freely among islands? The answer is likely not, because,
222 although the proportion of aliens over total species was similar in the two islands, most of
223 those species were found only in one of them, with only 36% shared. How would a saturation
224 mechanism operate in practice? If all the niches are occupied, even if the introduction
225 pressure persists most new arrivals will just disappear while only a few will remain in the
226 community, likely by chance if they find an empty niche (Stachowicz et al., 1999). This is
227 similar to the effect of genetic drift in the new mutations, where many new mutations will
228 appear in a population each generation but only a few will remain, if retained in the gametes
229 that will produce offspring –and a new generation.

230 Indeed, this mechanism of alien drift would operate only in absence of aggressive
231 invasion processes. If environmental conditions, or their own resistance, favor the
232 newcomers, they will stay and expand in the recipient community. Again, paralleling
233 population genetics, those aliens would remain and increase their proportion in the
234 community, like mutants subjected to positive selection do in population genomes. Ships
235 would introduce new species in French Polynesia as it is already happening around the world,
236 through ballast water, tank sediment and/or hull fouling (e.g., Gollasch, 2002; Levine and
237 D'Antonio, 2003; Ricciardi, 2016). This introduction happens as mutations introduce new
238 nucleotides in genomic pools, and those aliens will stay or remain principally by chance of
239 empty niches, and in some cases by outcompeting natives and other aliens: like genetic drift
240 and selection in genomic pools, respectively. Although in regions with intense maritime traffic
241 the rhythm of new introductions is enormous and the parallelism with mutations is far from

242 obvious, in locations like these remote and isolated islands with relatively scarce maritime
243 traffic and strong biotic and abiotic barriers (Sax, 2001), this and other hypotheses about the
244 change of communities have to be tested.

245 *Changes from the bottom of the food web*

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248 The community is changing from the bottom, suggesting that it is the most fragile niche
249 in the islands. Aliens could therefore oversee the supply of primary organic material of the
250 trophic chain in these islands, sustaining the whole community that is still rich in native
251 herbivores and carnivores. Plankton feeders have other species to eat, the same for algae
252 grazers. In a meta-analysis (Anton et al., 2019), invasive marine algae, followed by
253 crustaceans and mollusks, were the groups that mostly disturbed native taxa. Almost two
254 decades ago, Coles and Eldredge (2002) warned about the scarcity of studies of non-
255 indigenous algae in the tropical Pacific. This study resumes their concern and provides
256 evidences of algae and microalgae introductions. Attention to invasions of primary producers
257 should be especially paid, since they are able to transform the ecosystem from their very
258 bottom in different ways, compromising animal populations from sponges (De Caralt and
259 Cebrian, 2013) to reef fish (Ginther and Steele, 2018).

260 *Implications for biodiversity conservation*

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263 Increasing rate of biological invasions is one of the big challenges for biodiversity
264 conservation in the tropics, where innovative management interventions involving the local
265 society are necessary (Barlow et al., 2018). In the ports located in the middle of healthy coral
266 reefs here studied that have a fair niche occupation, a saturation of aliens around 20% of
267 species seems to occur. However, the appearance of new highly invasive bivalves like
268 *Magallana* and *Mytilus* in 2018, and the pass of *Dendostrea frons* from ships to natural rocks,
269 are serious calls of attention about a potential biosecurity risk. For the high invasive capacity
270 of these species, invasion outbreaks cannot be discarded, especially if they expand outside of
271 port areas during disturbances such as major bleaching events. This would convert the
272 process from drift-like to selection-like, following the comparison between ship-driven
273 community and population genetic evolution above. Biological invasions easily take over
274 impoverished communities with reduced diversity. This is a serious risk in depauperate coral
275 reefs with depleted diversity, where biotic resistance to invasions (Ardura et al., 2016) is no
276 longer working. Not only macroscopic exotic eukaryotes are a potential problem if niche
277 occupation decreases. Here we have detected several harmful microalgae that are a serious
278 concern for coral reefs (Bauman et al., 2010).

279 *Recommendations*

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281
282 In addition to current efforts to preserve current biodiversity and coral coverage,
283 tourists and residents can do something to prevent biological invasions in French Polynesia.
284 Measures could include, but not be limited to, cleaning scrupulously boat hulls, treating
285 carefully ballast water, taking seriously the disposal of aquarium waters in land disconnected
286 from the sea, even cleaning clothes, shoes and flip-flops before disembarking. Engagement of
287 tourists, and especially of local population, in the conservation of the amazing biodiversity of
288 tropical coral reefs, through citizen science projects (Barlow et al., 2010) or otherwise, would
289 be strongly recommended.

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Author contributions

Conceptualization, Alba Ardura, Eva Garcia-Vazquez and Serge Planes; Data curation, Eva Garcia-Vazquez; Formal analysis, Alba Ardura and Eva Garcia-Vazquez; Methodology, Alba Ardura and Eva Garcia-Vazquez; Resources, Alba Ardura, Eva Garcia-Vazquez and Anne Haguenaer; Software, Sara Fernandez Fernandez; Writing – original draft, Alba Ardura and Eva Garcia-Vazquez; Writing – review & editing, all authors.

Competing interests

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results”.

Data accessibility

Metabarcoding NGS data are in NCBI (<https://www.ncbi.nlm.nih.gov/bioproject/>) BioProject PRJNA633847, Accession SAMN14971940. Individual DNA barcodes are deposited in GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>) with the accession numbers MK934672-74, 77, 79-82, 84, 86-89, 91-94; MH197044; MT528265-74 for COI and MT487759-60 for 16S.

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Table 1. Non-native species found in this study through NGS analysis and confirmed with barcoding (in bold), and their documented native distribution sourced from WORMS database (<http://www.marinespecies.org/index.php>, accessed in March 2021).

Phylum	Species	Native distribution
Annelida	<i>Lumbrineris perkinsi</i>	Gulf of Mexico,
Annelida	<i>Perinereis anderssoni</i>	Caribbean Sea and West Atlantic
Annelida	<i>Syllis amica</i>	North Atlantic Ocean, newly cited in Mexican Pacific
Annelida	<i>Dipolydora cardalia</i>	North Pacific
Annelida	<i>Dipolydora carunculata</i>	North Pacific
Arthropoda	<i>Tigriopus californicus</i>	North Pacific
Arthropoda	<i>Lepas anserifera</i>	North Atlantic
Arthropoda	<i>Amphibalanus amphitrite</i>	Probably from West Pacific and Indian Oceans
Arthropoda	<i>Chthamalus proteus</i>	Caribbean & SW Atlantic
Arthropoda	<i>Synalpheus africanus</i>	Atlantic, Mediterranean
Arthropoda	<i>Planes minutus</i>	Widespread, putative Atlantic origin
Arthropoda	<i>Bathynomus giganteus</i>	North Pacific
Bacillariophyta	<i>Cylindrotheca closterium</i>	Atlantic Ocean
Bacillariophyta	<i>Psammodyctyon panduriforme</i>	North Atlantic
Bacillariophyta	<i>Haslea crucigera</i>	North Atlantic
Bacillariophyta	<i>Navicula cryptocephala</i>	North Atlantic
Bacillariophyta	<i>Pleurosigma strigosum</i>	North Atlantic
Bryozoa	<i>Bugula migottoi</i>	Tropical and subtropical waters, described from Brazil
Bryozoa	<i>Amathia evelinae</i>	South Atlantic ocean, described from Brazil
Bryozoa	<i>Amathia vidovici</i>	North Atlantic
Chordata	<i>Ciona intestinalis</i>	North Pacific
Chordata	<i>Perophora viridis</i>	Atlantic Ocean
Chordata	<i>Styela plicata</i>	NW Pacific
Cnidaria	<i>Gersemia rubiformis</i>	NW Atlantic
Echinodermata	<i>Diadema antillarum</i>	Caribbean
Mollusca	<i>Brachidontes mutabilis</i>	West Pacific
Mollusca	<i>Ischadium recurvum</i>	Northwest Atlantic
Mollusca	<i>Mytilus trossulus</i>	North Atlantic
Mollusca	<i>Mytilus edulis</i>	North Atlantic
Mollusca	<i>Dendostrea frons</i>	Caribbean
Mollusca	<i>Magallana gigas</i>	NW Pacific
Mollusca	<i>Saccostrea cucullata</i>	Indian Ocean
Mollusca	<i>Saccostrea glomerata</i>	New Zealand
Mollusca	<i>Paroctopus mercatoris</i>	West Central Atlantic
Mollusca	<i>Monoplex comptus</i>	North Atlantic and Indian ocean
Mollusca	<i>Discodoris confusa</i>	North Atlantic
Mollusca	<i>Phorcus sauciatu</i>	North Atlantic
Myxozoa	<i>Azadinium caudatum</i>	Atlantic Ocean
Myxozoa	<i>Triadinium polyedricum</i>	Atlantic Ocean

Myzozoa	<i>Alexandrium ostenfeldii</i>	North Atlantic HAB
Myzozoa	<i>Protoceratium reticulatum</i>	Widespread HAB, type locality NE Atlantic
Myzozoa	<i>Akashiwo sanguinea</i>	Widespread HAB, type locality NW Pacific
Myzozoa	<i>Margalefidinium polykrikoides</i>	Caribbean HAB
Ochrophyta	<i>Colpomenia sinuosa</i>	Indian Ocean
Porifera	<i>Raspailia hispida</i>	North Atlantic
Porifera	<i>Scopalina ruetzleri</i>	Atlantic Ocean
Porifera	<i>Halisarca dujardini</i>	Northeast Atlantic and Antarctic Indian Ocean
Porifera	<i>Halisarca restingaensis</i>	Caribbean
Porifera	<i>Callyspongia vaginalis</i>	West Atlantic
Porifera	<i>Xestospongia muta</i>	West Atlantic
Porifera	<i>Strongylacidon bermudae</i>	Caribbean
Porifera	<i>Mycale (Carmia) fibrexilis</i>	Northwest Atlantic
Porifera	<i>Tedania klausii</i>	Caribbean
Porifera	<i>Corticium candelabrum</i>	Atlantic Ocean
Rodophyta	<i>Ptilota serrata</i>	North Atlantic
Rodophyta	<i>Dasya caraibica</i>	Caribbean
Rodophyta	<i>Dasysiphonia japonica</i>	NW Pacific
Rodophyta	<i>Nitophyllum punctatum</i>	North Atlantic Ocean
Rodophyta	<i>Chondria coerulescens</i>	Atlantic Ocean
Rodophyta	<i>Melanothamnus collabens</i>	Atlantic Ocean
Rodophyta	<i>Melanothamnus sphaerocarpus</i>	Gulf of Mexico; invasive
Rodophyta	<i>Ptilothamnion sphaericum</i>	North Atlantic
Rodophyta	<i>Lithophyllum hibernicum</i>	North Atlantic
Rodophyta	<i>Pterocladia bartlettii</i>	Gulf of Mexico
Rodophyta	<i>Hypnea flava</i>	Brazil
Rodophyta	<i>Kallymenia feldmannii</i>	North Atlantic
Rodophyta	<i>Peyssonnelia atropurpurea</i>	Atlantic Ocean
Rodophyta	<i>Peyssonnelia boergesenii</i>	Atlantic Ocean
Rodophyta	<i>Peyssonnelia crispata</i>	Atlantic Ocean
Rodophyta	<i>Peyssonnelia inamoena</i>	Atlantic Ocean
Rodophyta	<i>Peyssonnelia luciparensis</i>	Atlantic Ocean
Rodophyta	<i>Peyssonnelia novae-hollandiae</i>	Atlantic Ocean
Rodophyta	<i>Sonderopelta capensis</i>	Atlantic Ocean
Rodophyta	<i>Solieria filiformis</i>	West Atlantic
Rodophyta	<i>Phymatolithon calcareum</i>	Atlantic Ocean

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351 **List of figures:**

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354 **Figure 1.** Map of the studied Pacific islands showing the proportion of native (green), exotic
355 (yellow) and invasive alien species (red), over the total number of species in the six locations
356 examined: Papeete-234, Phaeton-95; Vaïaré-183; Pao-Pao-104; Avatoru-127 and Tiputa 116
357 species. Invasive species as recognized in CABI (2019) Invasive Species Compendium.

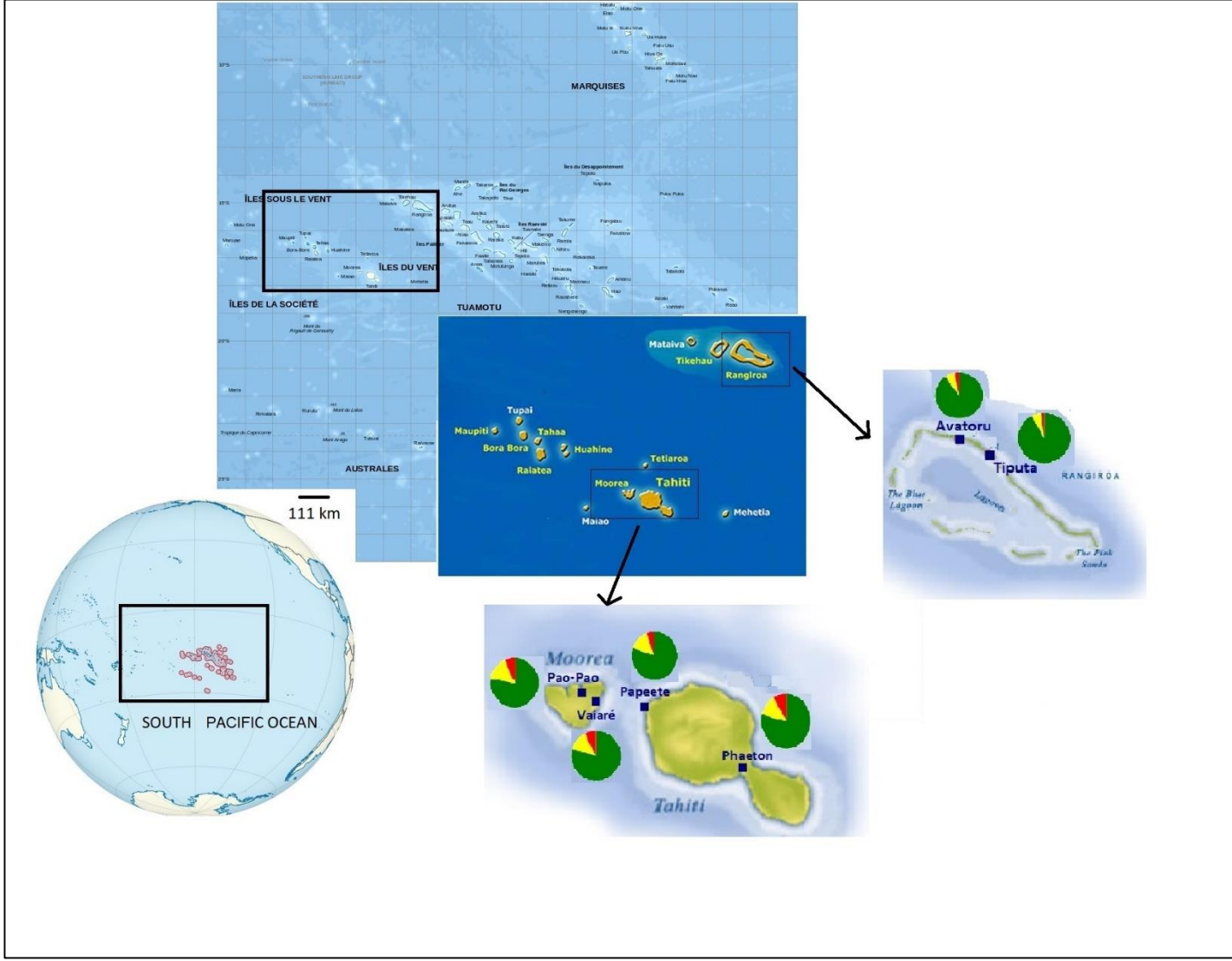
358
359 **Figure 2.** NJ clustering analysis of the native and exotic MOTU communities, where native (N)
360 and alien (A) communities cluster separately. Bootstraps: 10000, Bray-Curtis distance.

361
362 **Figure 3.** Ship connectivity with lines representing the traffic between islands (right) and
363 share of invasive species (left) in Polynesian islands as proportion of alien species in each
364 spatial fraction over the total number of aliens (N = 75). Circles are approximately
365 proportional to the maritime traffic (right) and the amount of aliens (left). Arrows represent
366 connection routes, and their width indicates roughly maritime traffic intensity; those
367 connecting Rangiroa with larger islands are dashed because there is very little traffic. Vessel
368 traffic data are sourced from the Direction Polynésienne des Affaires Maritimes, available at
369 [https://www.service-public.pf/dpam/transport-inter-insulaire/atlas-maritime-polynesien-](https://www.service-public.pf/dpam/transport-inter-insulaire/atlas-maritime-polynesien-2018/)
370 [2018/](https://www.service-public.pf/dpam/transport-inter-insulaire/atlas-maritime-polynesien-2018/) (accessed February 2021). Blue Tahiti, yellow Moorea, red Rangiroa atoll.

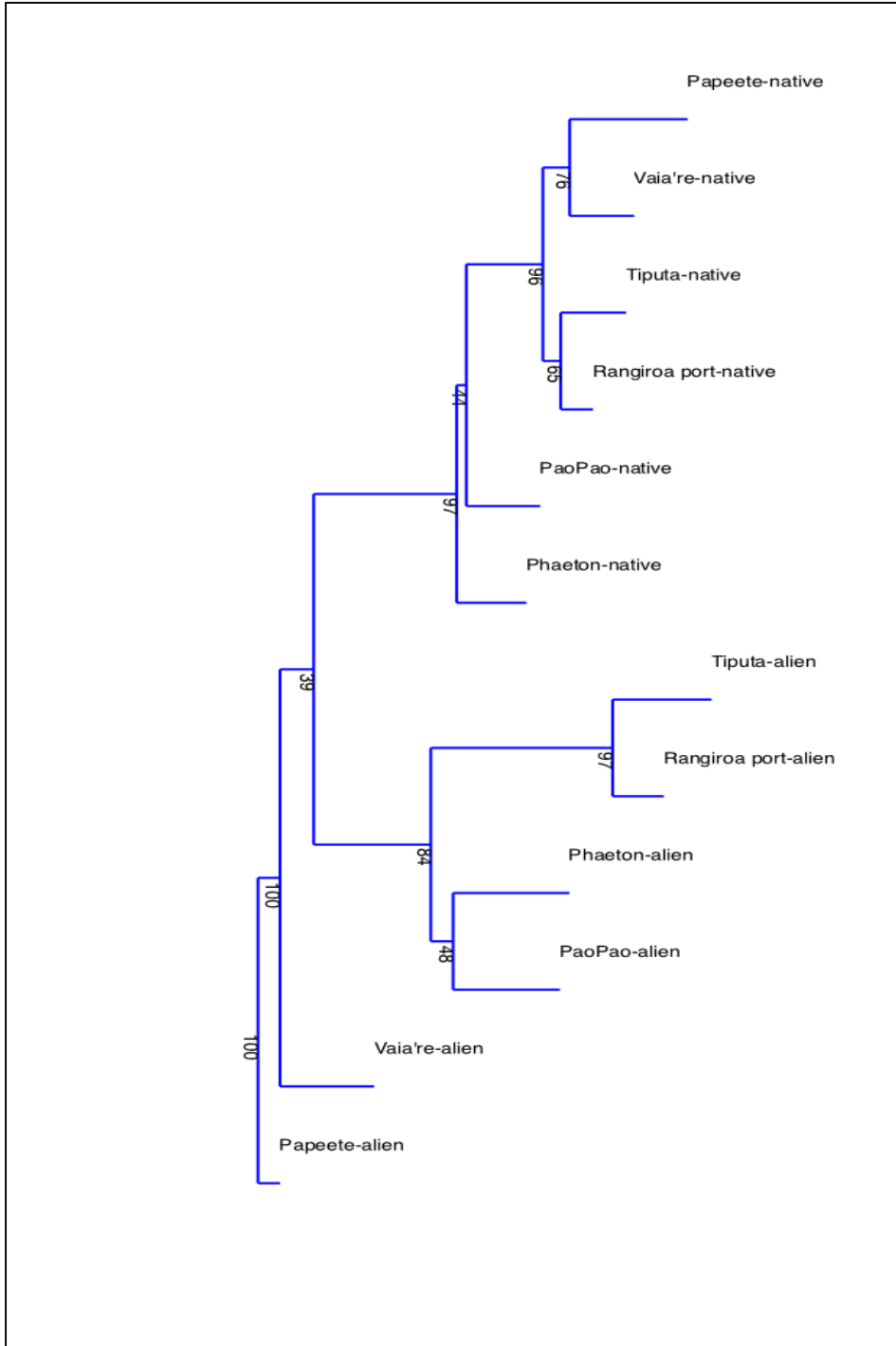
371
372 **Figure 4.** Alien and native MOTU detected in different taxa from Polynesian islands. Invasive
373 (versus non-invasive exotic) species status is recognized in CABI (2019) Invasive Species
374 Compendium. In this analysis only taxa authenticated down to a species level were
375 considered.

376
377 **Figure 5.** Community transformation in Pacific islands from the introduction of alien species
378 shown as the proportion of different taxonomic groups in alien and native fractions.
379 Autotrophs and heterotrophs in green and reddish shades, respectively. Analysis done on
380 MOTU assigned to a species level.

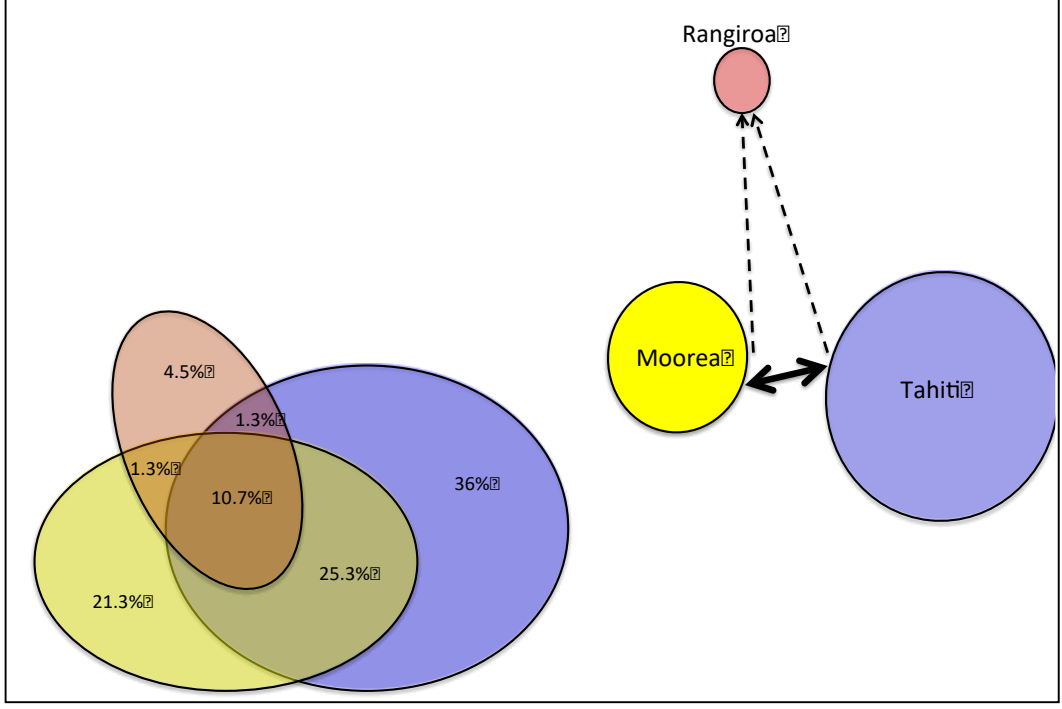
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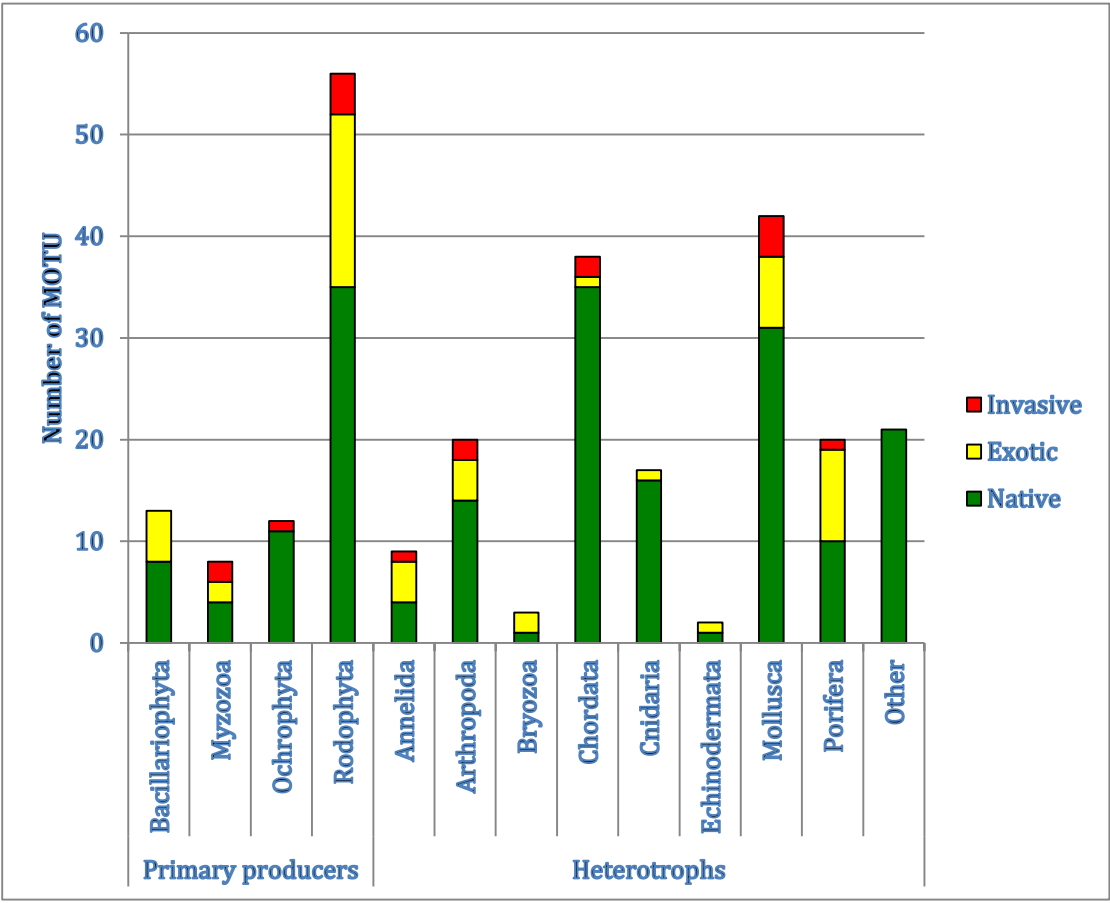
387 **Figure 2**



436 **Figure 3**
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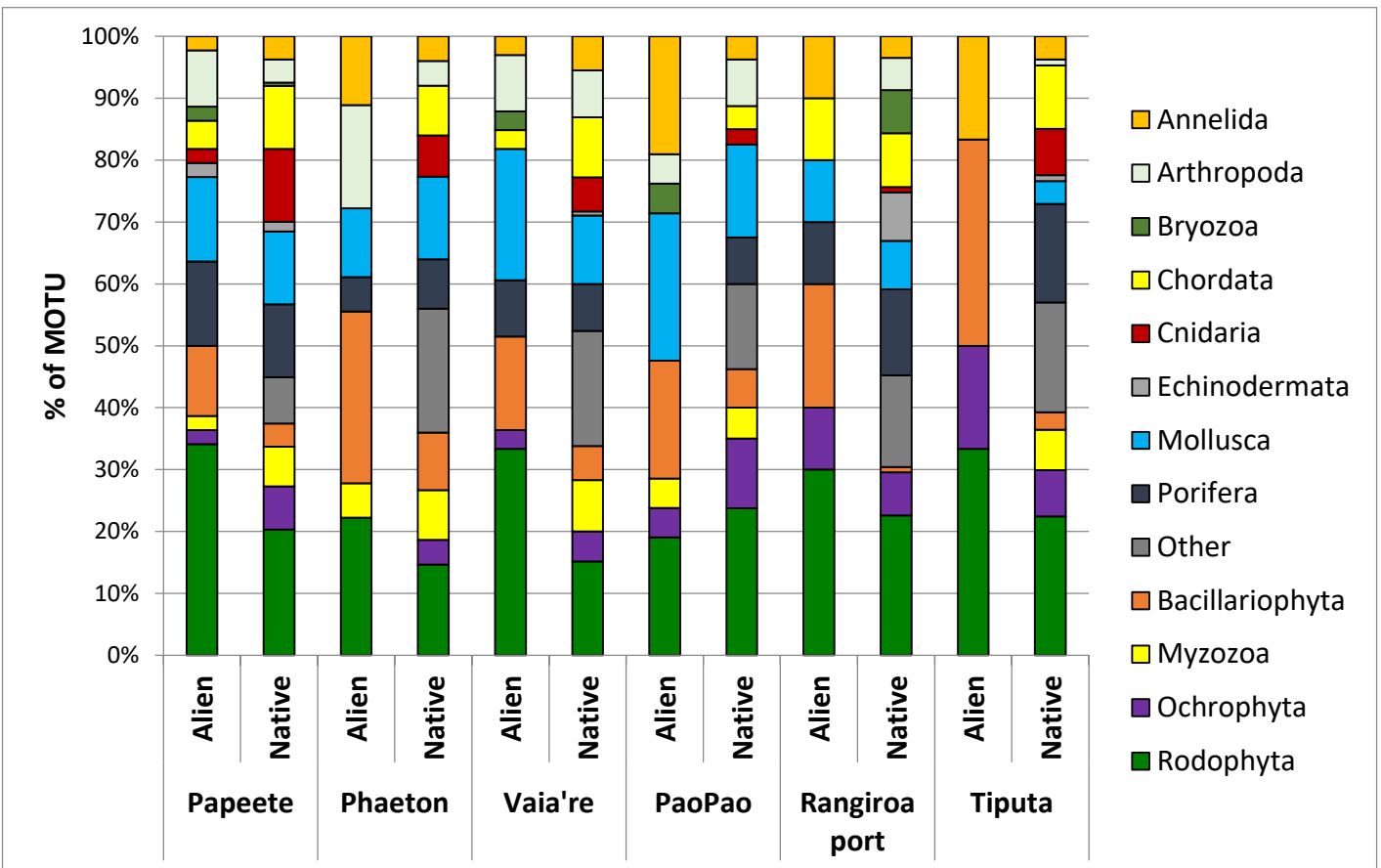


439 **Figure 4**
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Figure 5



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