Beware of oysters. Rapid advance of non-native species in tropical Pacific islands

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Abstract

Non-indigenous species can become a problem for the ecosystem health, especially when their distribution grows to the detriment of native species. In this moment, they can become invasive species. In marine ecosystems, the maritime transport is the principal gate and corridor for the movement of alien species. The genetic identification, using barcoding tools, of different oyster species in ports of the remote French Polynesia islands and atolls, showed a significant increase of exotic versus native oyster species between 2011 and 2018. This supports the spread of exotic species with the maritime traffic as the main cause. Moreover, the 11% of inaccurate identification at species level obtained in this study shows the need to complete the genetic databases.

Keywords:

French Polynesia, oysters, non-native species spreading, barcoding.

1. Introduction

Contrary to what is often thought, any ecosystem is fragile in itself. That is, if enough pressure is applied over it (Barabás et al., 2017). Coral reefs are usually robust, balanced, dynamic, self-regulating ecosystems that will have larger or smaller populations of the species of plants and animals that live there, depending on the climate conditions: water temperature, sunlight, salinity, carbonates etc. (https://www.noaa.gov/education/resource-collections/marine-life/coral-reef-ecosystems).

However, in some situations, this balance can be quickly and easily thrown out of whack: among others, it happens when new coming species arrive and disturb the host ecosystem. Most non-indigenous species (NIS) do not become invasive or cause problems in their new locations: many have great benefits to society, for example in agriculture, horticulture, forestry and aquaculture. However, the subset of NIS that do become invasive have major environmental, economic, public health or political implications for the country or countries concerned (IUCN, 2000, Global overview of the management of invasive alien species - http://www.fao.org/3/y5968e/y5968e04.htm). These NIS pose threats for marine biodiversity (Bax et al., 2003; Molnar et al., 2008), and therefore for marine resources, adding their impacts to those of overfishing, climate change, pollution, and habitat destruction. They can drastically affect structure and function of the host ecosystem. Many aquatic biological communities are impaired by the uncontrolled spread of invaders (Horgan and Mill, 1997; Bax et al., 2003), and productive activities such as aquaculture, fishing and shellfish harvesting may be severely affected (Hayes and Sliwa, 2003; Neil et al., 2006), even threatening food security in many regions (Nuñez and Pauchard, 2010)

Oysters are marine bivalve molluscs with a worldwide distribution. Some of them are key species for different ecosystems due to their ecological services as filter-feeders and reef builders (Guo et al., 2018). Besides, they are important from the economic point of view, being support for aquaculture and fisheries industries around the world, with an annual production of 5.6 million metric tons (FAO, 2018). They generally have a high tolerance to different environmental conditions, being well adapted to sessile life in estuaries, intertidal and shallow waters with highly fluctuating environmental conditions since they can tolerate prolonged air exposure and extreme variations in salinity and temperature (Galtsoff, 1964). This remarkable resilience is possible thanks to their high genomic diversity and complexity, critical for the adaptation to changing environments (Guo et al., 2015). These characteristics make many oyster species good targets for aquaculture, dominating bivalve aquaculture production in many regions (Herbert et al., 2016). Despite of their resilience, some native oysters have been on decline because of overfishing, habitat destruction, other invasive oysters, and diseases transferred among species from different origin (Beck et al., 2011), because they lack adaptive immunity, although thrive in microbe-rich environments as filter-feeders. The protection and management of oyster resources and wild population in general require a good understanding of genetic diversity and classification. Moreover, genetic diversity is also a critical resource for genetic improvement and sustainable aquaculture of oysters (Guo, 2009).

On the other hand, the same characteristics that make them good for aquaculture help oyster populations to establish in the wild, with potential to displace native species and modify habitats and ecosystems. Although ecological impacts of mollusc farming are small relative to other forms of aquaculture (Naylor et al., 2000), oyster culture is

responsible for many biological invasions. For example, the widely cultured Japanese or Pacific oyster *Magallana* (formerly *Crassostrea*) *gigas* is already considered a cosmopolitan species, established around the world (Miossec et al., 2009). Together with aquaculture, shipping is believed to be one of the most important pathways for transfer of indigenous species across marine regions (Leppäkoski et al., 2002), since more than 90% of the global trade goods are transported by ship (International Maritime Organization, 2018, https://wwwcdn.imo.org/localresources/en/KnowledgeCentre/Documents/CAB%20258%20MAY%202018.pdf). This pathway involves several potential vectors—transport of organisms in ballast water and ballast tank sediments, and fouling of hull, sea chests, anchors, and anchor chains, etc. (Hewitt et al., 2009). Ballast water (BW) is recognized as the most significant of these vectors (Molnar et al., 2008). Therefore, the place where the boats stay and interchange their ballast water will be the principal core of marine invasions: the ports (Seebens, 2013). According to Molnar et al. (2008), the molluscs fall into the most prevalent group of invasive species and can have a tremendous impact on aquatic ecosystems, being the oysters, an important group also driven by the commercialization of aquaculture species (Miralles et al., 2016; Pejovic et al., 2016).

Oysters are "ecosystem engineers" like corals – they create three-dimensional structures as they settle and grow on each other. Left undisturbed, these oyster reefs provide a habitat for an incredible biodiversity of organisms, serving as a food source, nursery ground and refuge for many species, boosting fish stocks (David, 2020). However, exotic oysters introduced via shipping may threat not only coral reefs, but also their own native biodiversity (Beck et al., 2011; zu Ermgassen et al., 2020). Moreover, invasive oysters may put native oyster aquaculture at risk outcompeting native resources and transferring diseases (Ruesink et al., 2005). Here we will focus on one of the best-preserved coral reef ecosystems in the world, French Polynesia (van Hooidonk et al., 2013; Vercelloni et al., 2019), where the culture of the native pearl oyster *Pinctada margaritifera* is a priority resource (Ky et al., 2019 and references therein). If exotic oysters arrive via port, and start settling down and expanding, both coral reefs and native oyster aquaculture may be at risk. We have analysed oysters from ports of larger Polynesia islands (Moorea and Tahiti), and from remote atolls (Rangiroa and Tikehau). Oysters are difficult to classify and identify due to their high plasticity in shell morphology and the presence cryptic species (Harry, 1985; Lam and Morton, 2006). The molecular techniques are shown as a good tool to solve this situation and they have been developed in the past two decades to oyster classification and improved the understanding of oyster species diversity and taxonomy (Bayne, 2017). For this reason, we have used DNA barcoding for oyster identification, being COI the chosen marker, due to has been the barcode of choice employed in the first marine barcoding projects and many others that have followed, with one of the most comprehensive databases to date (Hebert et al., 2003; Ward et al., 2009; Ardura et al., 2019).

2. Material and methods

2.1. Study area and sampling

We focused on port areas that are the main entry of invasive molluscs in Polynesia (e.g. Ardura et al., 2021). The international Tahitian Port of Papeete is connected by ferry to Vai´are port in Moorea, with two companies operating several times a day all the year. Molluscs were sampled from different sectors and ships long-time docked in Papeete port (September 2011, October 2018) and the small port of Phaeton (2018) in Tahiti;

and from Vai'are ferry port and marina, and the small port of Pao-Pao in Moorea (2011 and 2018). In 2018 the small ports of Rangiroa and Tikehau were also sampled (Figure 1).

Sampling was made as is described in Ardura et al. (2015). Briefly, sampling was carried out by randomly selecting specimens of molluscs from rocky areas of approximately 10 m^2 in the intertidal range, accessible from the shore - and with snorkel in inaccessible points. Representative sampling per species was carried out, this meaning that the number of samples taken from a species was proportional to the observed abundance of such species in the sampling area. Individuals were picked at random within species. In total, at least 100 mollusc samples were obtained per port and year, except for Phaeton, Rangiroa and Tikehau ports that were sampled only in 2018 (Table 1), with different number of oysters per site and year (Table 2).

2.2. Genetic analysis

Total DNA was extracted from the samples using the E.Z.N.A. Mollusc DNA kit (IOMEGA, bio-tek), following manufacturer's instructions. The tubes were stored at 4°C for immediate DNA analysis, and aliquots were frozen at −20°C for long time preservation.

DNA barcodes are a powerful tool for species detection and identification, as multiple investigations have previously shown around the world and ecosystems (e.g., Hebert et al., 2003; Ardura et al., 2010; Ardura et al., 2015*)*, and the mitochondrial cytochrome oxidase (COI) subunit I gene barcode has been used extensively for population genetic studies, phylogeography, speciation and systematics. Therefore, for this study, a fragment of the cytochrome oxidase subunit 1 (COI) gene was chosen and amplified by the polymerase chain reaction (PCR), using the primers described by Geller et al. (2013). These primers are modified from the primers designed by Folmer et al. (1994), for use as suitable tools for routine DNA barcoding, surveys of all taxa, and metazoan metabarcoding (Geller et al., 2013). The amplification reaction was performed in a total volume of 40 µl, consisting of 1× Promega (Madison, WI) buffer, 2.5 mM MgCl2, 0.25 mM dNTPs, 20 pmol of each primer, approximately 20 ng of template DNA and 1 U of DNA Taq polymerase (Promega), and the following PCR conditions: initial denaturing at 95°C for 5 minutes, 35 cycles of denaturing at 95°C for 1 minute, annealing at 48°C for 1 minute, extension at 72°C for 1 minute and a final extension at 72°C for 5 minutes.

PCR products were visualised in 2% agarose gels with 2.5µl of 10 mg/ml simply safe (EURx). Sequencing was performed at Macrogen Europe (The Netherlands).

2.3. Sequence edition and phylogenetic analysis

Sequences were visualized and edited employing the BioEdit Sequence Alignment Editor software (Hall, 1999) and aligned with the ClustalW application (Thompson et al., 1994) included in BioEdit. The sequences obtained were compared with international databases employing the software BLAST within NCBI (http://www.ncbi.nlm.nih.gov/) and BOLD system (http://www. boldsystems.org/) for identifying the species.

Sequences obtained in this work were used together with GenBank reference sequences from different genera of Ostreidae family (Table 3) for subsequent phylogenetic analysis.

The phylogenetic analysis was performed with the software MEGA 6.0 (Tamura et al., 2013). The phylogenetic tree containing GenBank reference and present COI sequences was reconstructed using Neighbor Joining (NJ) algorithm. The best suited molecular substitution model of sequence evolution and accompanying evolutionary parameter values for the data were chosen using the same software. Robustness of the NJ topology was assessed using 1000 bootstrap replicates.

2.4. Data analysis

The oyster species found in this study were classified as native or NIS according to current inventories of Moorea fauna and the native distribution of each species following WoRMS Editorial Board (2021) and World Register of Marine Species and the IUCN. Available from http://www.marinespecies.org and https://www.iucnredlist.org/, respectively. Accessed January 2021.

Temporal differences in the oyster communities of Pao-Pao, Vaiare marina and ferry station and Port Papeete between 2011 and 2018 were tested using Chi-square test, assuming equal composition if the null hypothesis is true, and confirmed with Monte Carlo (9999 permutations). Statistical analysis was performed with the free PAST software (Hammer et al., 2001).

3. Results

In total, 170 oyster samples were analysed: 38 in 2011 and 132 in 2018, with different proportion over the total bivalves sampled (Table 1). 152 of them identified at species level (Table 2), corresponding to six species. The rest were identified only at genus level and will not be considered here because the focus is invasive species, and genera with invasive species may also contain non-invasive species. Data of 2011 have been partially published in Ardura et al. (2015). One COI sequences per haplotype obtained at species level were submitted to GenBank (https://www.ncbi.nlm.nih.gov/genbank/) with accession numbers below (GB-AN).

Five native species were detected. *Pinctada maculata* was found in Port Papeete (Tahiti Island) in 2011, but not in 2018 (GB-AN-MW767827). Two *Isognomon* species were detected in 2018: *I. nucleus* in Pao-Pao (Moorea) (GB-AN-MK934687) and Tikehau atoll; and *I. perna* in Port Papeete (Tahiti) and in Tikehau, with the same haplotype (GB-AN-MK934692). Nine *Alectryonella plicatula* in Avatoru (Rangiroa atoll) in 2018, corresponding to the same haplotype (GB-AN-MT487759) (Table 2).

Two exotic oyster species were identified in this study: the Natal rock oyster *Saccostrea cucullata* – native to the Indian Ocean and the Red Sea- and the Frond oyster *Dendostrea frons* – native to Caribbean Sea. These two species are catalogued as invasive species in the Mediterranean Sea (Streftaris et al., 2005). In 2011, the Frond oyster (*D. frons*) was detected only on three ships docked in Port Papeete. In 2018 this oyster was present into the port of Papeete, already on port structures, and in Vai'are ferry station and marina, thus being in both Tahiti and Moorea islands. On the other hand, in 2011 the Natal rock oyster (*S. cucullata*) was present only in Pao-Pao (Moorea); in 2018 it was indeed in Pao-Pao too, and also in Vai'are ferry station, Port Papeete and Port Phaeton (Tahiti) (Table 2).

The three COI sequences of Frond oyster obtained from the three ships sampled in Port Papeete in the Tahiti Island in 2011, 400 nucleotides long, were best matched to *Dendostrea frons*, corresponding to three new different haplotypes found in the sampling developed in 2011 by Ardura et al. (2015), uploaded to the GenBank database and available with accession numbers MH197042-43-44 (Table 2). The fourteen COI sequences obtained from individuals sampled in 2018, corresponded to one haplotype best matched to a Caribbean haplotype with GenBank accession number KP455014 (Pagenkopp-Lohan et al., 2015), it was uploaded to the GenBank database and available with accession number MW843009. Because *D. frons* shows several genetic lineages in the database and the taxonomic designation of GenBank animals may be incorrect, only the best match obtained in this particular case was taken into account to develop the phylogenetic tree (Figure 2). The best match was determined using a nucleotide identity threshold >97% for COI barcode as indicated in Ward et al. (2005), and also taking into account the coverage, e-value and the quality of the alignment, in order to minimize potential unspecific identifications.

The nine COI sequences of Natal rock oyster obtained from Pao-Pao (Moorea) in 2011 were identified as *Saccostrea cucullata* from BLAST, corresponding to the same haplotype and uploaded to GenBank with the accession number KT149315. The 44 COI sequences obtained in 2018 best matched with the haplotype found in 2011 (GB-AN-MK934686).

The dataset containing the haplotypes detected at species level, together with reference sequences for different genera of Ostreidae family (Table 3) best fitted the mutation model of Tamura and Nei (1993), with a proportion of invariant sites of 0.04 and gamma distribution value 0.29 (TrN+G+I). Using these settings, we reconstructed a NJ phylogenetic tree with 2000 bootstrap replication. Each species clustered with the references belonging to the same genus (Figure 2). The tree shape, with the genera distributed in different branches and good bootstrap values, confirmed the genetic identification done from DNA barcoding and BLAST, and was consistent with the currently accepted molecular phylogeny of oysters (Tëmkin et al., 2010).

Comparing the proportion of the different species of oysters found in the samples of 2011 and 2018 (in the sites sampled both years i.e., Pao-Pao, Vai'are and Port Papeete), a clear increase of alien species was found in 2018 (Table 4). The two distributions of alien versus native oysters were significantly different the two years (χ^2 = 17.411, p = $3.01*10^{-5}$, d.f. = 1, Monte Carlo p = 0.0001).

4. Discussion

Despite of the difference in the strategy of sampling in both years due to the lack of samplings in the two atolls and Phaeton in 2018, all the sampled sites had a good sampling coverage, with at least 100 sampled molluscs. The results demonstrate that several islands of French Polynesia are experiencing an increase of the proportion of exotic oyster species that are invasive in other regions. This is an important, worrying discovery that calls for special attention to this type of molluscs. The gate and corridor for the movement of alien oysters between islands is likely the maritime transport. Species found in 2011 only in Tahiti are now present in Moorea, and reciprocally. *D. frons*, found only in Port Papeete in 2011, is today in the main ports of Moorea (Vai'are ferry station and marina) and Tahiti (Port Papeete); while *S. cucullata* (only in Pao-Pao in 2011) occurs in all the analysed ports of the large islands today, including the small Port Phaeton in Tahiti.

The introduction history in the French Polynesia is somewhat different in these two exotic species. While *D. frons* was detected and genetically ascertained for the first time in 2011 (Garcia-Vazquez et al., 2020), *S. cucullata* had been cultured in the Philippines (Blanco et al. 1951; Blanco, 1956), and in French Polynesia decades ago (Aquacop, 1982) although it is no longer produced therein. It was described in samples taken from natural rocks in 2009 (Tröndle and Boutet, 2009). However, none of these species appears in French Polynesia in the geographic distribution described in the World Register of Marine Species (WoRMS) in 2021 (AphiaID- 420779 and 181316 for *D. frons* and *S. cucullata* in WoRMS, respectively). On the other hand, the relatively high variability of *D. frons*, with three haplotypes detected, would suggest multiple introduction hits (Ardura et al., 2016; Miralles et al., 2016; 2018). The importance of maritime transport in the dispersal of exotic species and the impact that these species have on native communities is strongly suggested from our data. Significant differences were found in the composition of foreigners versus natives between 2011 and 2018, the proportion of non-indigenous individuals increasing significantly in only seven years to the detriment of native species. Furthermore, the presence of new oysters in remote ecosystems supports this hypothesis since it is the only possible gateway for the oysters that are not deliberately imported for cultivation. Previous studies have found the presence of alien oysters associated with ports, as is the case of *Ostrea stentina* and *Magallana gigas* in the central Cantabrian Sea (Pejovic et al., 2016) or *M.gigas* in the Marmara Sea (Turkey), between the Black and Mediterranean Sea and in where the invasion of alien species results from a combination of marine transportation, between Black and Mediterranean Sea, and aquaculture activities of non-native species (Özcan-Gökçek et al., 2020).

It is also important to highlight the presence of *A. plicatula* in the Rangiroa atoll. Although this species is considered to be resident in the Indo-Pacific Ocean (https://www.iucnredlist.org/; http://www.marinespecies.org/), it had not been recorded in previous inventories in French Polynesia (Tröndle and Boutet, 2009). Therefore, to our knowledge, and taking also into account the distribution described in the World Register of Marine Species and the IUCN Red List, this is the first record in French Polynesia.

Some of these species were also detected from French Polynesian islands through Next Generation Sequencing (NGS) on environmental DNA (Ardura et al., 2021), highlighting the utility of DNA identification tools for the early detection of new coming species. The use of DNA is especially useful in the identification of oysters with planktonic larval phases and morphologically polymorphic (cryptic species) which makes the identification by traditional methods very difficult (Bayne, 2017). However, in the present study based on individual DNA barcoding, we found that 10.5% of the samples could not be identified to the species level. As well as in metabarcoding studies, the depth of the taxonomic inventory depends on reference databases having enough information of all taxonomic groups (Ardura, 2019). Higher barcoding efforts would be recommended to expand and enrich reference databases with more haplotypes and variants from different regions, so ensuring adequate geographical coverage especially in still understudied tropical regions (Weigand et al., 2019; Garcia-Vazquez et al., 2021).

5. Conclusion

The significant differences in the composition of native versus exotic oyster species between 2011 and 2018 show the spread of invasive species between islands, with local maritime traffic being likely the most influential factor in this spread. It is recommended to control the traffic of local boats to mitigate or prevent the spread of exotic species to the detriment of the autochthonous ones.

On the other hand, our results, with around 11% of oysters identified only at a genus level, show that it is more than necessary to improve the genetic database, in order to achieve the identification of all the samples; especially when the same genus may include native and exotic species, even invasive ones.

CRediT authorship contribution statement

Alba Ardura: Conceptualization, Sampling, Barcoding work, Formal analysis, Methodology, Resources, Writing - original draft, review & editing. **Almudena Gonzalez Sanz:** Barcoding work. **Laura Clusa:** Barcoding work. **Serge Planes:** Review the final version. **Eva Garcia-Vazquez:** Conceptualization, Sampling, Data curation, Formal analysis, Methodology, Resources, Writing - review & editing.

Declaration of competing interest

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.

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Table 1. Number of mollusc individuals sampled in 2011 and 2018, by sampling site. It presents the number of molluscs analysed, classified as gastropods and bivalves, and the percentage of oysters over the total number of bivalves.

	2011				2018					
	MOOREA		TAHITI		MOOREA		TAHITI		RANGIROA	TIKEAHU
	VAI'ARÉ	PAOPAO	PAPEETE	PHAETON	VAI'ARÉ	PAOPAO	PAPEETE	PHAETON	AVATORU PORT	TIKEHAU PORT
GASTROPODS	110	92	279	-	92	79	55	55	77	30
BIVALVES	0	9	30	-	48	61	63	125	57	75
% OYSTERS OVER TOTAL BIVALVES	0	100%	100%	-	70%	72%	90%	25%	39%	100%

Table 2. Number of oyster individuals identified at species level found in different years and areas. In grey, non-native species.

		Alectryonella plicatula	*Dendostrea frons*		*Isognomon nucleus*	*Isognomon perna*	*Pinctada maculata*	*Saccostrea cucullata*		
	ORIGIN	Native	Central America		Native	Native	Native	SE Africa		
	YEAR	**2018**	**2011**	**2018**	**2018**	**2018**	**2011**	**2011**	**2018**	
MOOREA ISLAND	VAI'ARÉ	0	0	11	8	0	0	0	3	
	PAOPAO	0	0	0	1	0	0	9	15	
TAHITI ISLAND	PAPEETE	0	3	3	0	3	26	0	8	
	PHAETON	0	-	0	0	0	-	-	18	
RANGIROA ATOLL	AVATORU PORT	9	-	0	0	0	-	-	0	
TIKEHAU ATOLL	TIKEHAU PORT	0	-	0	19	16	-	-	0	
	TOTAL	**9**	**3**	**14**	**28**	**19**	**26**	**9**	**44**	**152**

Table 3. Haplotypes of each species detected in different islands and atolls from French Polynesia in 2011 and 2018, and references sequences from Ostreidae genera. GB-accession number: GenBank accession number. In grey, non-native species in studied area.

	Species	Haplotyes with GB-accession number and sampling location	Year	Reference
Oyster species detected by the authors	*Alectryonella plicatula*	MT487759-Rangiroa atoll	2018	This study
	Dendostrea frons	MH197042-Port Papeete customs Ship 1	2011	This study
	Dendostrea frons	MH197043-Port Papeete customs Ship 2	2011	This study
	Dendostrea frons	MH197044-Port Papeete customs Ship3	2011	This study
	Dendostrea frons	MW843009-Moorea and Tahiti Islands	2018	This study
	Isognomon nucleus	MK934687-Moorea Island and Tikehau atoll	2018	This study
	Isognomon perna	MK934692-Port Papeete and Tikehau atoll	2018	This study
	Pinctada maculata	MW767827-Port Papeete	2011	Ardura et al., 2015
	Saccostrea cucullata	KT149315-Moorea and Tahiti Islands	2011 - 2018	Ardura et al., 2015
Reference sequences from different genera of Ostreidae family	*Crassostrea brasiliana*	KX436142-Brazil		Moreira et al., 2017
	Dendostrea frons	KP455014-Caribbean		Pagenkopp-Lohan et al., 2015
	Magallana hongkongensis	KP976208-China		Shen et al., 2016
	Magallana gigas	KX345125-Taiwan		Unpublished
	Ostrea lurida	KT317529-Gulf of California		Raith et al., 2016
	Ostrea stentina	KY986335-China		Unpublished
	Saccostrea mordax	HQ661029-China		Liu et al., 2011
	Saccostrea palmula	KT317579-Gulf of California		Raith et al., 2016

Table 4. Native and alien oyster individual sampling (percentage) in the shared sampling point between 2011 and 2018 (Pao-Pao, Port Papeete and Vaia´re – marina and ferry).

	NATIVE	ALIEN	TOTAL
2011	26 (68,4%)	12 (31,6%)	38
2018	12 (23%)	40 (77%)	52

Figure legends:

Figure 1: Sampling points in Moorea and Tahiti Islands and Tikehau and Rangiroa atolls. Map modified from commons. wikimedia, Licensed under the Creative Commons Attribution-Share Alike 3.0 Unported license. Red line shows the daily transport by ferry between Tahiti and Moorea Island.

Figure 2. Neighbour-Joining tree based on COI gene haplotypes found in different areas and years. Bootstrap values in percent over 50%. Branches containing different genera are coloured in different colours. Three different families, Margaritidiae, Isognomoniade and Ostreidae are marked in black, pink and blue, respectively. Codification for each sample: species acronym-sample code-Ref (if taken from the GenBank) or year (if sampled in this study). Sample code-Area-Island-Year. Area codes: PP: Port Papeete (Moorea Island); TP: Tiputa Port (Tiputa atoll); PaoPao: Pao-Pao Port (Moorea Island); VF: Vaia´re Ferry (Moorea Island); VM: Vaia´re Marina (Moorea Island); Av: Avatoru (Rangiroa atoll). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Figure 1.

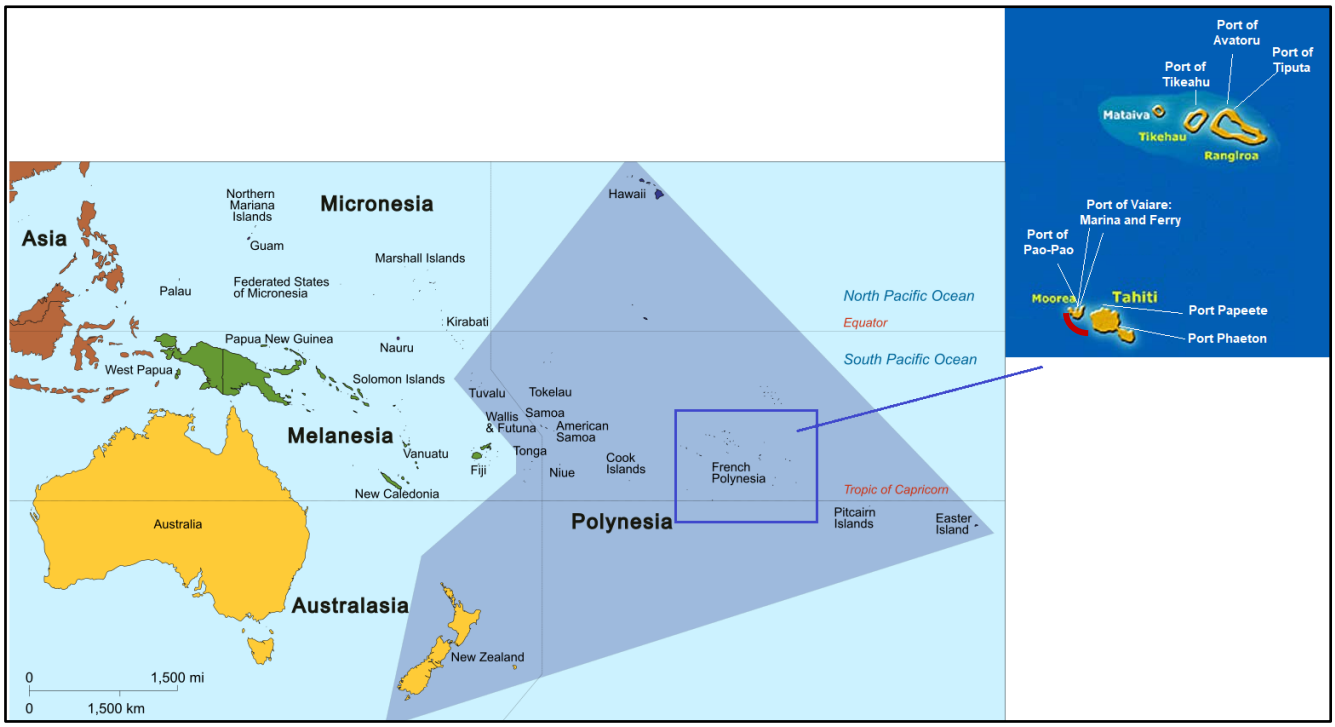
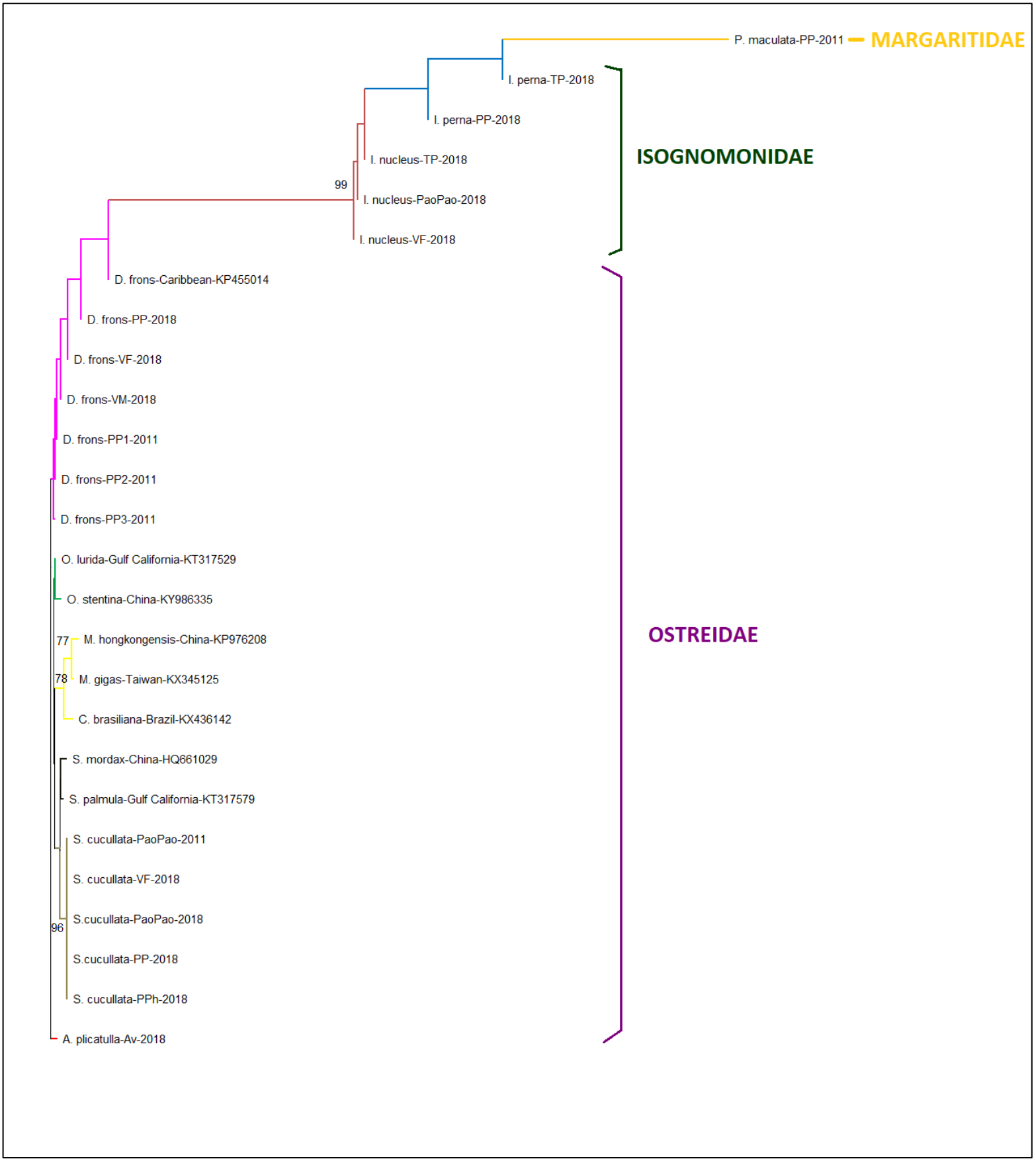

Asia
Northern Mariana Islands
Micronesia
Guam
Marshall Islands
Palau
Federated States of Micronesia
Kirabati
Papua New Guinea
Nauru
West Papua
Solomon Islands
Tuvalu
Tokelau
Wallis & Futuna
Samoa
American Samoa
Melanesia
Vanuatu
Tonga
Cook Islands
Fiji
Niue
French Polynesia
New Caledonia
Tropic of Capricorn
Australia
Polynesia
Pitcairn Islands
Easter Island
Australasia
New Zealand
Hawaii
North Pacific Ocean
Equator
South Pacific Ocean
0
1,500 mi
0
1,500 km
Port of Avatoru
Port of Tiputa
Port of Tikeahu
Mataiva
Tikehau
Rangiroa
Port of Vaiare: Marina and Ferry
Port of Pao-Pao
Moorea
Tahiti
Port Papeete
Port Phaeton

Figure 2.


P. maculata-PP-2011
MARGARITIDAE
I. perna-TP-2018
I. perna-PP-2018
I. nucleus-TP-2018
ISOGNOMONIDAE
99
I. nucleus-PaoPao-2018
I. nucleus-VF-2018
D. frons-Caribbean-KP455014
D. frons-PP-2018
D. frons-VF-2018
D. frons-VM-2018
D. frons-PP1-2011
D. frons-PP2-2011
D. frons-PP3-2011
O. lurida-Gulf California-KT317529
O. stentina-China-KY986335
77
M. hongkongensis-China-KP976208
78
M. gigas-Taiwan-KX345125
C. brasiliana-Brazil-KX436142
S. mordax-China-HQ661029
S. palmula-Gulf California-KT317579
S. cucullata-PaoPao-2011
S. cucullata-VF-2018
S.cucullata-PaoPao-2018
96
S.cucullata-PP-2018
S. cucullata-PPh-2018
A. plicatulla-Av-2018
OSTREIDAE

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