Western Indian Ocean JOURNAL OF Marine Science

Volume 17 | Issue 1 | Jan – Jun 2018 | ISSN: 0856-860X

Chief Editor José Paula

Western Indian Ocean
JOURNAL OF
Marine Science

Published biannually

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ISSN 0856-860X

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Metapenaeus dobsoni (Miers, 1878), an alien Penaeidae in Mozambican coastal waters: confirmation by mtDNA and morphology analyses

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Abstract
An alien shrimp species has been increasingly recorded in Maputo Bay, Mozambique, and its occurrence has been associated with a drastic reduction of a native and economically important Indian white shrimp, *Fenneropenaeus indicus*. Cytochrome c oxidase subunit I sequencing and body morphology analyses were used to identify this alien species. All results revealed its taxonomic identity as *Metapenaeus dobsoni* (Kadal shrimp), a native species from the eastern Indian Ocean, originally not recorded on the Mozambique coast. Maputo Bay is considered an important biodiversity hotspot and there are concerns regarding the presence of this alien species, which could weaken the biodiversity of the Bay. Moreover, the increased contribution of this small-size shrimp within the fishery is of concern because of its potential negative impacts on fisheries and economic stability in the region.

Keywords: Alien species; Molecular identification; COI; Biodiversity; *Metapenaeus dobsoni*

Introduction
Invasive species are generally associated with a number of negative impacts to native biodiversity. These impacts may affect the natural environment, the local economy and even human health (Leão *et al.*, 2011). Invasive species may start competing or become predators of native species (Hill & Lodge, 1999; Leão *et al.*, 2011; Scannella *et al.*, 2017), or even vectors of parasites and other pathogens (Torchin & Mitchell, 2004; Leão *et al.*, 2011). Shrimps and other crustaceans can host viral pathogens that are dangerous for other species inhabiting the same environment. A well-known example of dangerous viral diseases that affect shrimps is the White Spot Syndrome Virus (WSSV) (Hoa *et al.*, 2011; Bateman *et al.*, 2012). In addition, exotic species may hybridize with native species (Firmart *et al.*, 2013) and reduce local biodiversity by eliminating the natives (Dick & Platvoet, 2000). According to Shine *et al.* (2000), alien species are those occurring outside their "natural distribution", because they cannot reach this location by their own means, and some kind of human action is involved in the introduction to the new location. If an alien species becomes established in a new habitat, and is able to reproduce and generate fertile descendants with a high probability of survival, it is then regarded as an alien invasive species (Shine *et al.*, 2000; Ciruna *et al.*, 2004).

The family Penaeidae contains several important commercial species that inhabit shallow coastal waters of tropical and subtropical regions (Dall *et al.*, 1992; Chan, 1998). Nine genera belonging to this family have been identified along the southeast coast of Africa, from the Rovuma River in northern Mozambique to the Kei

River in South Africa. Most of these shrimps inhabit the continental shelf, between 5 and 70 m deep.

In Mozambique, shrimp fishery is historycally known as one of the pillars of economic growth, where *Penaeus monodon* (Giant tiger prawn), *Melicertus latisulcatus* (Western king prawn), *Metapenaeus monoceros* (Speckled shrimp), *Metapenaeus stebbingi* (Peregrine shrimp), *Marsupenaeus japonicus* (Kuruma prawn), *Fenneropenaeus indicus* (Indian white prawn), and *Penaeus semisulcatus* (Green tiger prawn) are the most frequently captured species. Among these, *F. indicus* and *M. monoceros* are the most abundant species supporting commercial fisheries. Since 2007 a new shrimp species has been recorded in Maputo Bay. This species had never been recorded in this area, or along the Mozambican coastal waters. This has created dissatisfaction among the fishing community due to a smaller body size than the native commercial species. In addition, its occurrence might be associated with a significant decrease in the abundance of *F. indicus*.

In this paper, mitochondrial DNA (mtDNA) and body morphology analyses were used to identify the species as *Metapenaeus dobsoni*, a native species from eastern Indian Ocean, and confirm that it is an alien species currently present in Maputo Bay, Mozambique. Simbine (2016) reported the presence of this species on the Mozambique coast for the first time. The present study confirms the occurrence of this species and might provide information that will be useful in its management, and protection and conservation of native species.

Materials and methods

Shrimp specimens were sampled from July 2010 to June 2011 in Maputo Bay, Mozambique (Fig. 1a, 1b). A total of 26 individuals were collected: 5 *M. monoceros*, 4 *M. stebbingi*, 5 *F. indicus*, 2 *P. monodon*, 3 *M. japonicus*, and 7 individuals of the alien species.

After collecting the samples, a piece of muscle tissue (approximately 1.5 cm^2) was extracted, avoiding damage to the morphological characteristics of the individual,

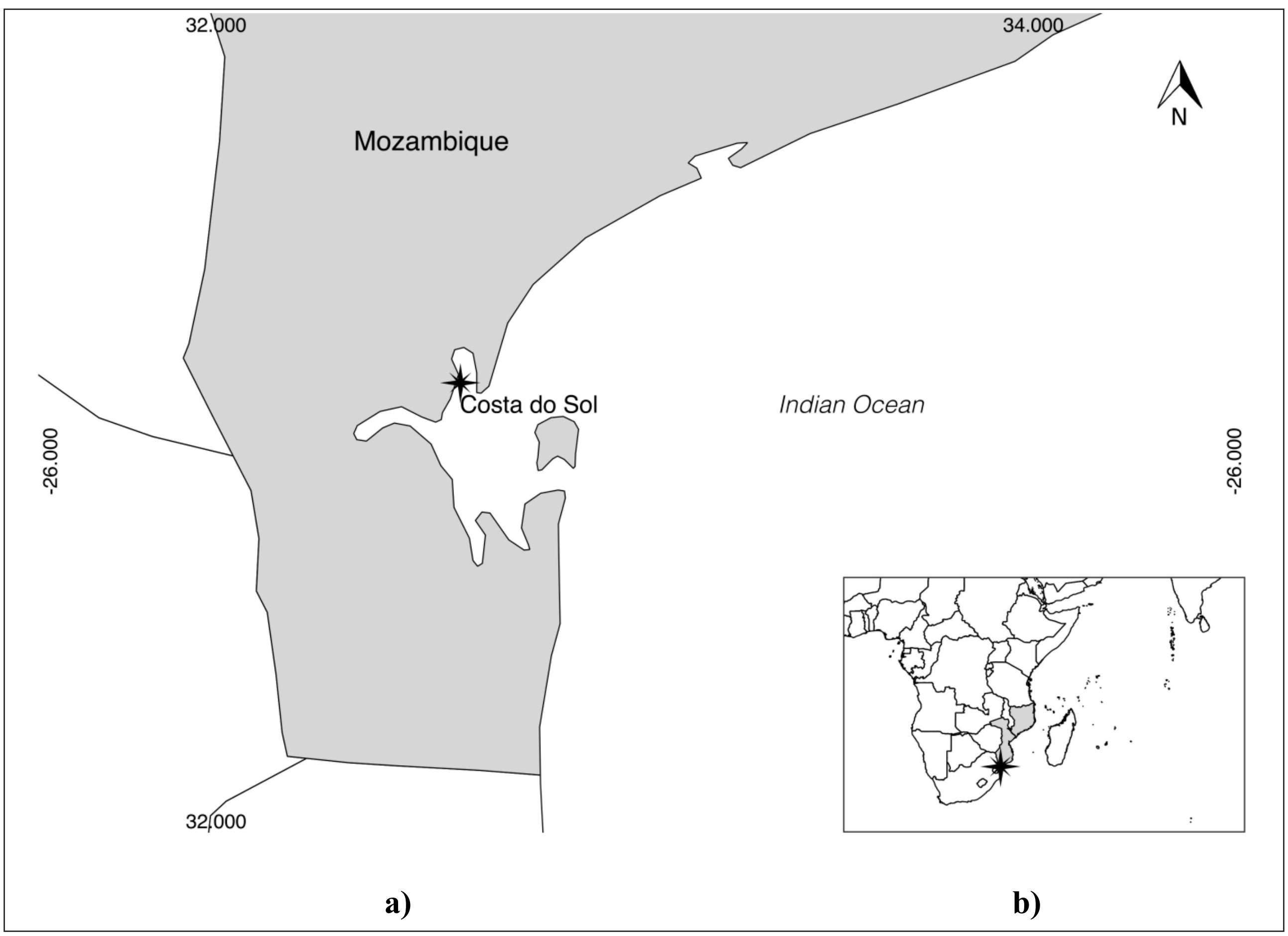

Figure 1. a) Maputo Bay showing the sampling location at 26° 11' 30"S, 32° 45' 30"E, and b) Map showing the location of Mozambique.

and immediately preserved in 95% ethanol, maintained at a temperature of 4°C until DNA extraction.

DNA extraction was carried out according the method used by Sambrook *et al.* (1989). A segment of 598 base pair (bp) of the cytochrome c oxidase subunit I (COI) mitochondrial gene was amplified using COIPenF forward and COIPenR reverse primers for penaeids. PCR was carried out in a final volume of 30 µl, containing 3.0 µl of DNA template (50ng/µl), 3µl of 10X reaction buffer, 1.5µl of 2.5mM $MgCl_2$, 2.4 µl of dNTP mixture containing 2.5mM of each dNTP, 0.9µl of each 10pmol primer (forward and reverse), and 1 U of Taq platinum (Invitrogen). The amplification protocol was carried out in a Veriti TM 96 thermocycler (Applied Biosystems) under the following conditions: 94°C for 5 min, 35 cycles of 94° C for 30 s, 51°C for 55 s and 72°C for 1 min, followed by 72°C for 30 min of final extension. Purified PCR products (Lis, 1980) were sequenced in an automatic sequencer (Korean Macrogen Inc. Service).

Nucleotide sequences were aligned using Clustal W software (Thompson *et al.*, 1994) implemented in the programme Geneious R6 (Kearse *et al.*, 2012). The genetic distance was calculated within and between species using the Mega 5.05 software (Tamura *et al.*, 2004) and Kimura 2-parameter (K2P) model. Sequences of related species were obtained from GenBank, and a genetic distance tree was obtained using the Neighbor-Joining (NJ) method (Tamura *et al.*, 2004) by bootstrapping the data with 1,500 replicates. *Macrobrachium rosenbergii*, of which sequences were obtained from GenBank, was used as an external group. Additionally, a network of haplotypes was built using Network 4.5.1.6. (Bandelt *et al.*, 1999).

Morphological analysis was performed by two groups of taxonomists from different institutions, Universidade do Estado do Rio de Janeiro (UERJ, RJ, Brazil), and Central Institute of Marine Fisheries Research (Kerala, Kochi-India). Morphology analysis (Dall *et al.*, 1992; Rao *et al.*, 2013) used five individuals of

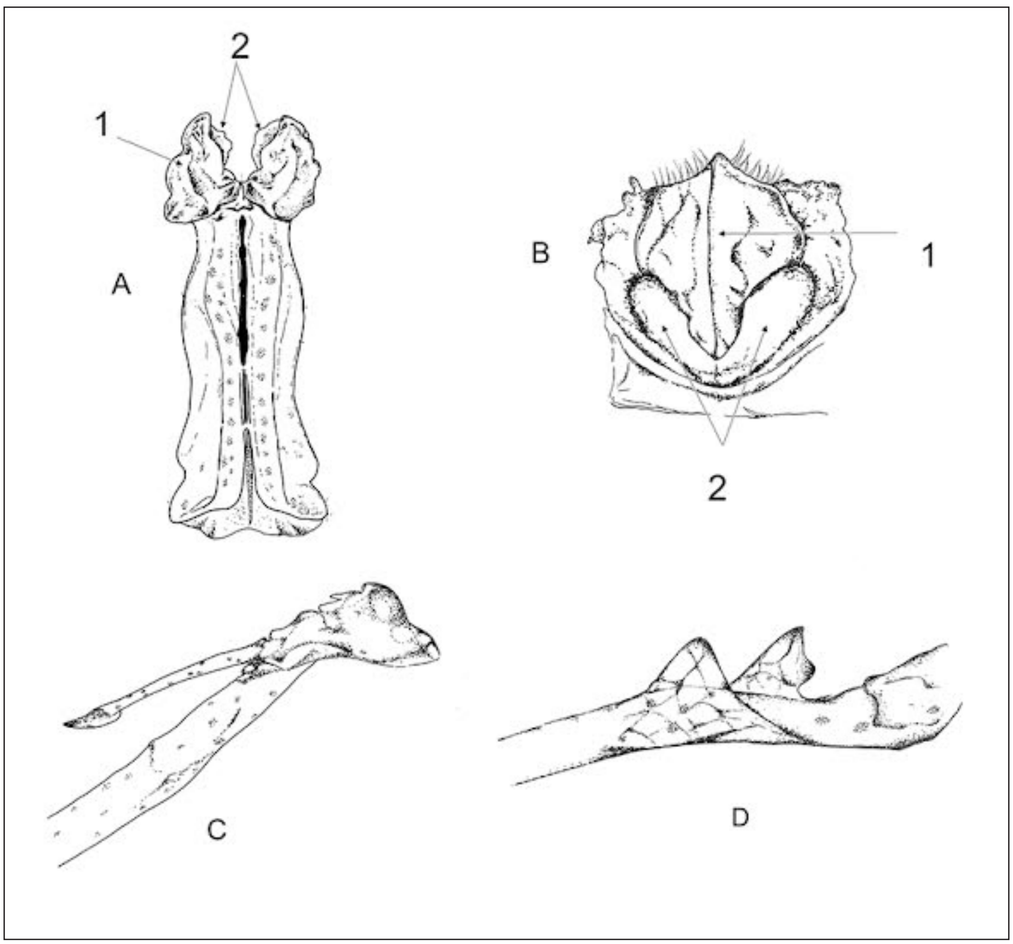

Figure 2. A(1,2); B(1,2); C and D. Female and male genitalia of *Metapenaeus dobsoni*. (A 1, 2) Petasma (ventral view) showing the disto-median and disto-lateral projection. (B 1, 2) Thelycum showing a long groove and two lateral plates. (C, D) Basial spine of the third leg (male). Drawn by Fernando Peron Magrini.

each species, comparing both Mozambican and Indian shrimp groups. Vouchers were deposited in the crustacean collection of Laboratório de Genética Pesqueira e da Conservação (LGPESC, UERJ, RJ, Brazil).

Shrimp fishery records were obtained from Instituto Nacional de Investigação Pesqueira (IIP), Mozambique, which is responsible for scientific fisheries monitoring in the country, especially focusing on shrimps. Monitoring activities include recording the type of fishing gears used (artisanal otter trawl - ARB; beach-seine - ARP; bottom gill-net – EMF; and semi-industrial bottom trawling - SEMI), the size of landed specimens, and landings. The catch data presented in this paper are from Maputo Bay.

Results

Morphological description

Traditional morphological taxonomic analysis concluded that the alien species was *Metapenaeus dobsoni*, an Indo-Pacific species originally distributed along the coasts of India, Sri Lanka, Malaysia, Indonesia, the Philippines and New Guinea (Rao *et al.*, 2013). It is characterized by the presence of a long rostrum armed with 8-9 spines on the dorsal side, with a well-marked double curve, the exopodite absent on the fifth leg of the thorax, the branchio-cardiac groove reaching almost to the middle of the carapace, the pleurobranch on seventh thoracic somite, the telson armed only with spinules, and the absence of robust ischial setae on the pereiopod. Adult males are characterized by basial robust setae of the third pereiopod with 1 or 2 large triangular spines, each disto-median projection of the petasma with a short filament on the ventral surface and another filament on the dorsal surface (Fig. 2 A, C and D). The fifth pereiopod in females is often reduced to a coxa and basis. The thelycum has a long groove dorsally unsheathed in a horseshoe-like process formed by lateral plates (Fig. 2 B).

According to the description of Rao *et al.* (2013), the body colour is transparent with red or greenish specks, the distal part of the therostrum is darker, antennules and antennae are dotted red, pereiopods and pleopods are white to pinkish, the uropods are red, darker distally with external parts of exopods red, and a double row of reddish spots on the telson with a greenish margin.

DNA sequencing

Partial sequences (598 bp) of the COI gene of this species and of the native Mozambican species *F. indicus*, *M. monoceros, M. stebbingi, M. japonicus and P. monodon* were successfully amplified. All sequences were deposited at GenBank (Accession numbers KM508829 - KM508847). Thereafter, the sequences of *M. dobsoni, Metapenaeus brevicornis, M. japonicus, Litopenaeus vannamei, P. monodon, Farfantepenaeus californiensis* and *Fenneropenaeus chinensis*, obtained from GenBank, were compared (Table 1).

The genetic distance tree with well-supported bootstrap values linked the sequences of the exotic species to previous GenBank *M. dobsoni* sequences in a unique branch. In addition, a *Metapenaeus* clade was recovered (Fig. 3), reinforcing the results of the morphological analysis. The haplotype network of *M. dobsoni* showed two distinct groups, one with samples in green from India and the other with the samples in blue from Mozambique (Fig.4).

The sequence variation between species was high. The pairwise COI divergence between *M. dobsoni* and *M. monoceros, M. brevicornis*, and *M. stebbingi* were 22.6, 24.8 and 29.9%, respectively. On the other hand, highest values (43.53% to 48.71 %) were found between *M. dobsoni* and the remaining species that did not belong to the *Metapenaeus genus* (Table 2). In contrast, the level of intraspecific variability was much lower, ranging from 0.0% (*M. monoceros, M. stebbingi and F. californiensis*) to 5.7% (*P. monodon*). *M. dobsoni* showed an intermediate value of 2.8% (Table 3).

Catch records of *F. indicus*, *M. monoceros*, *M. stebbingi, M. japonicus* and *P. monodon* species, as well as of the alien species identified herein as *M. dobsoni*, indicate a steady increase in contribution of this latter species to the total shrimp catch since the year 2007 (Table 4; Fig. 5). A concomitant decrease in catches of the main species, *F. indicus*, has also been observed in all artisanal fishing gears ($R^2>0.5$), being especially evident for the semi-industrial fleet ($R^2=0.9$), which shows a steady trend of fishing effort.

Discussion

The accurate identification of species is an important step to efficiently manage and monitor any population, especially those of great economic value (Ward, 2000; Beerkircher *et al.*, 2009). In addition to morphological taxonomy, molecular markers have successfully been used for species identification (Hebert *et al.*, 2003). Such an approach has increasingly been used for accurate identification of both terrestrial and aquatic alien species (França *et al.*, 2007; Wu *et al.*, 2011; Duggan *et al.*, 2012; Oosterhout *et al.*, 2013; Sabour *et al.*, 2013; Cruscanti *et al.*, 2015).

Table 1. Sequence access number and origin of all species obtained in GenBank.

GenBank number	Species	Origin
gi|549445155|	*Metapenaeus dobsoni*	Tamil-Nadu-India
gi|530759033	*Metapenaeus dobsoni*	Kochi, Kerala- India
gi|530759030|	*Metapenaeus dobsoni*	Kochi, Kerala- India
gi|530759027|	*Metapenaeus dobsoni*	Kochi, Kerala- India
gi|530759039|	*Metapenaeus dobsoni*	Kochi, Kerala- India
gi|530759024|	*Metapenaeus dobsoni*	Kochi, Kerala- India
gi|530759021|	*Metapenaeus dobsoni*	Kochi, Kerala- India
gi|530759018|	*Metapenaeus dobsoni*	Kochi, Kerala- India
gi|530759015|	*Metapenaeus dobsoni*	Kochi, Kerala- India
gi|530759012|	*Metapenaeus dobsoni*	Kochi, Kerala- India
gi|530759009|	*Metapenaeus dobsoni*	Kochi, Kerala- India
gi|530759006|	*Metapenaeus dobsoni*	Kochi, Kerala- India
gi|530759003|	*Metapenaeus dobsoni*	Kochi, Kerala- India
gi|530759000|	*Metapenaeus dobsoni*	Kochi, Kerala- India
gi|530758997|	*Metapenaeus dobsoni*	Kochi, Kerala- India
gi|530758994|	*Metapenaeus dobsoni*	Kochi, Kerala- India
gi|530758991|	*Metapenaeus dobsoni*	Kochi, Kerala- India
gi|530758988|	*Metapenaeus dobsoni*	Kochi, Kerala- India
gi|530758985|	*Metapenaeus dobsoni*	Kochi, Kerala- India
gi|*549445155*|	*Metapenaeus brevicornis*	Tamil Nadu- India
gi|*549445154*|	*Metapenaeus brevicornis*	Tamil Nadu- India
gi|*7374113*|	*Penaeus monodon*	Tokyo-Japan
gi|*7243610*|	*Penaeus monodon*	Australia
gi|63003723|	*Marsupenaeus japonicus*	Tokyo-Japan
gi:66276044|	*Marsupenaeus japonicus*	Tokyo-japan
gi|150375635|	*Litopenaeus vannamei*	Mexico
gi|109692170|	*Litopenaeus vannamei*	China
gi|148532179|	*Litopenaeus vannamei*	Mexico
gi|153125267|	*Fenneropenaeus chinensis*	China
gi|110287619|	*Fenneropenaeus chinensis*	China
gi|282167291|	*Macrobrachium rosenbergii*	China

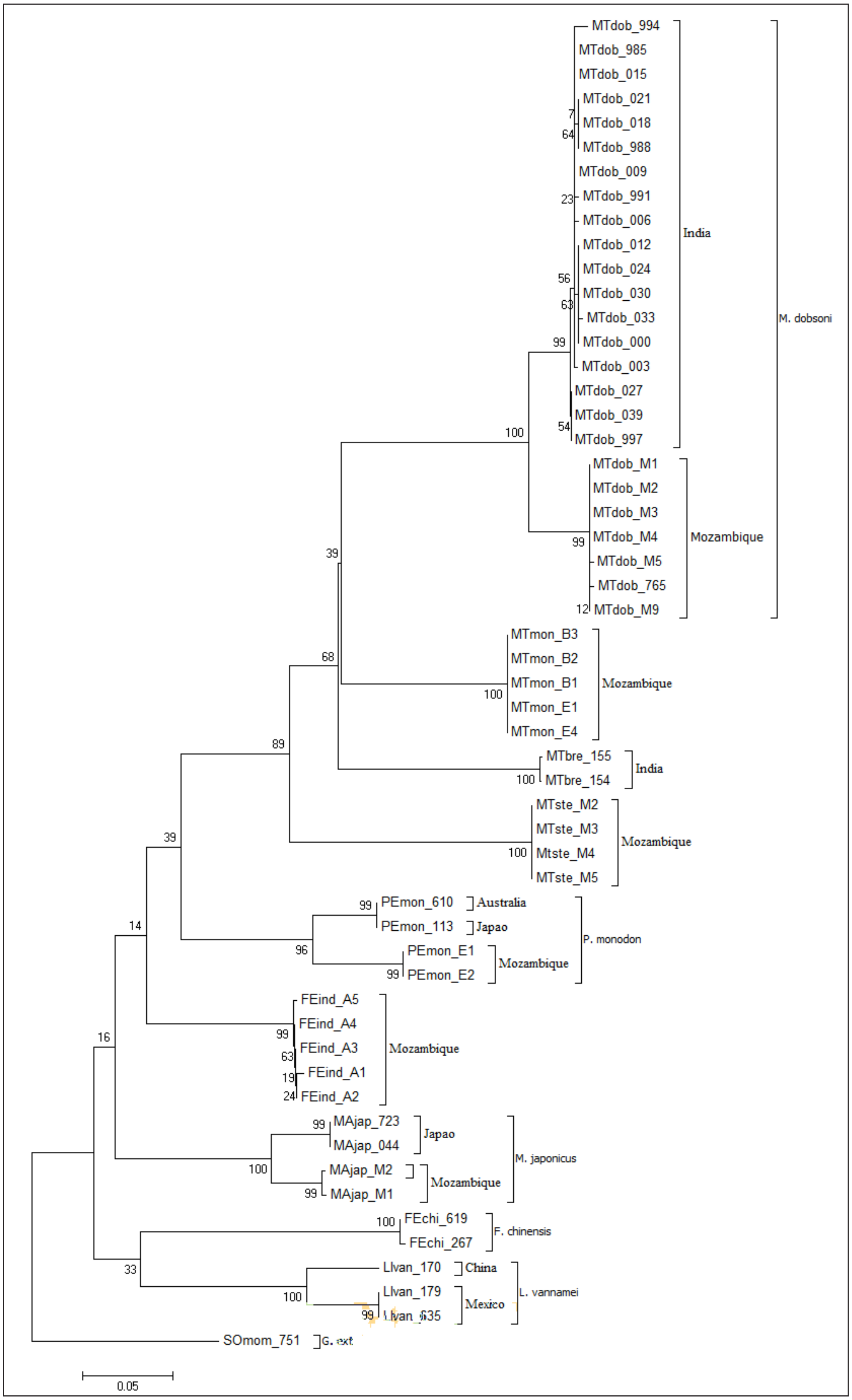

Figure 3. COI gene genetic distance tree. *Metapenaeus dobsoni* (MTdob), *Metapenaeus brevicornis* (MTbre), *Metapenaeus stebbingi* (MTste), *Metapenaeus monoceros* (MTmon), *Penaeus monodon* (PEmon), *Marsupenaeus japonicus* (MAjap), *Litopenaeus vannamei* (LIvan), *Fenneropenaeus chinensis* (FEchi), *Fenneropenaeus indicus (FEind),* and the external group *Macrobrachium rosenbergii* (SOmom).

The combination of morphology and molecular mtDNA COI analyses clearly showed that *Metapenaeus dobsoni* (Miers, 1878) is the alien species currently fished along the Mozambican coastal waters. This conclusion is grounded by the genetic distance analyses. Low genetic distance values found between *M. dobsoni* and the unknown alien species suggest that they are the same species (Table 2). This finding supports the body morphological analysis, which identified the alien species as *M. dobsoni*.

The distance genetic tree recovered two clades in the *M. dobsoni* group, suggesting a genetic population structuring within this shrimp. In this clade, a branch joined individuals from India (sequences from GenBank), while the other branch joined the studied individuals from Mozambique, suggesting that the alien individuals herein detected in Mozambique have probably derived from another area (not India) of *M. dobsoni* natural distribution. The haplotype network showed a similar result, separately grouping all samples from India (green) and from Mozambique (blue).

A recent study of exotic crustaceans inhabiting the Turkish coast indicated that *M. japonicus* has become established, replacing a native species of economic importance, *Melicertus kerathurus* (Ates *et al.,* 2013), whilst *Erugosquilla massavensis* has replaced the native *Squilla mantis* (Özcan *et al.*, 2008). Turkish waters and Mediterranean waters in general, seem to have been invaded by crustaceans through the Suez Canal resulting in settlement of Indo-Pacific migrants (Rodriguez & Suarez, 2001). However, fish and shellfish farms are abundant in lagoons and bays, possibly provided a source of exotic species. The status of invasive species in Turkey was recently assessed and presented a wide range of crustaceans, many of them of economic importance (Kapiris *et al.*, 2012; Scannella *et al.*, 2017).

In Africa, several cases of invasive species have been reported. For instance, the presence of the Japanese seaweed, *Sargassum muticum*, has recently been reported in Morocco (Sabour *et al.,* 2013). A total of 104 introduced or unknown originally alien species are listed in the Western Indian Ocean (ASCLME/SWIOFP, 2012a). Some of these species have already

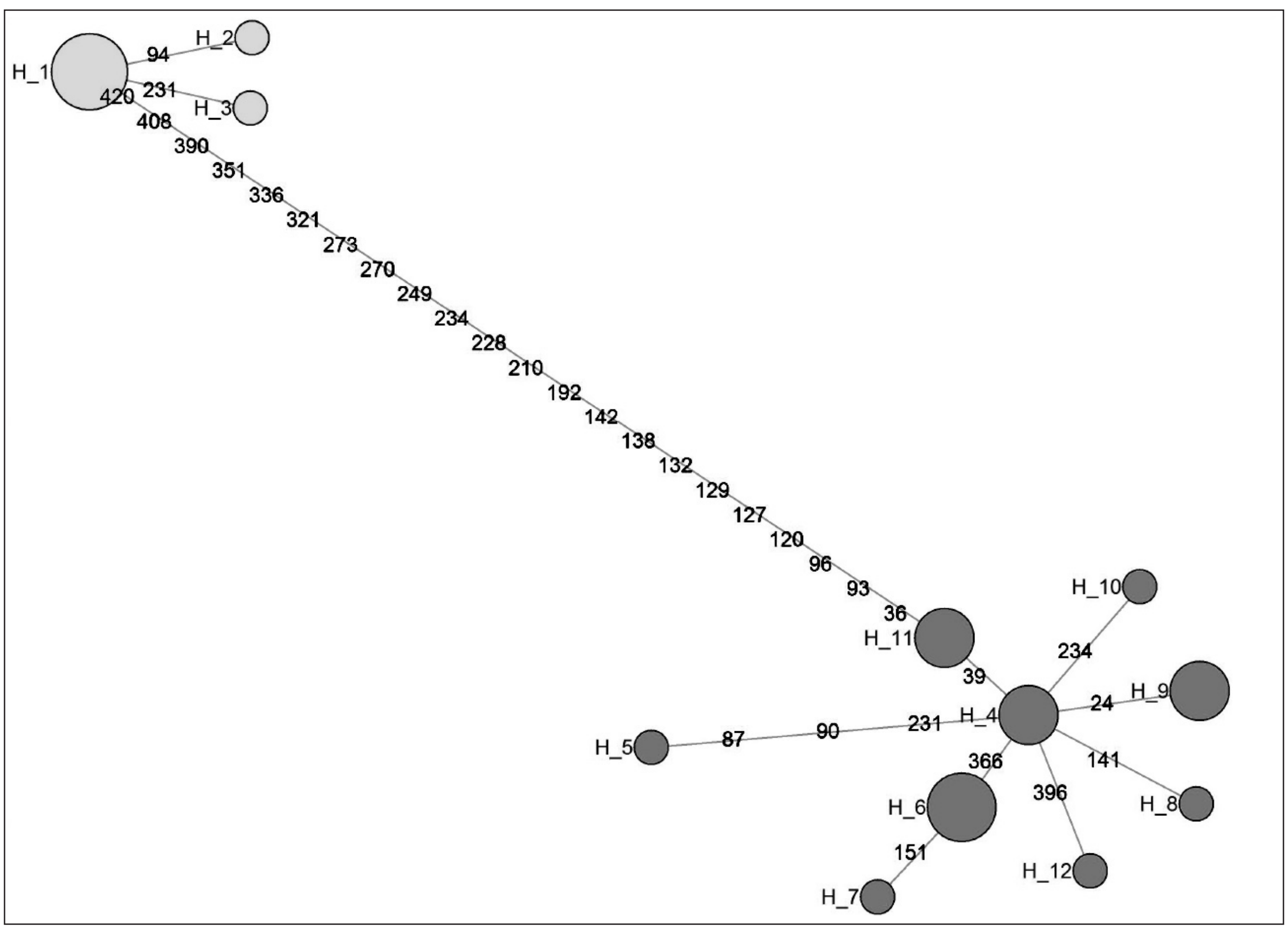

Figure 4. Haplotypes network of *M. dobsoni* showing two distinct groups, Mozambique (blue) and India (green).

been recognized as invasive species. It is therefore imperative that all the species that are on this list are considered to be potentially harmful or disturbing for biodiversity and natural ecosystems (ASCLME/SWIOFP, 2012a).

Several introduced species have been reported in Mozambique in both terrestrial and aquatic ecosystems, reducing biodiversity through hybridization, or even resulting in the extinction of native species (ASCLME/SWIOFP, 2012b). Some alien species such as *Euchornia crassipes*, *Pistia stratiotes*, *Salvinia molesta*, *Azolla filiculoides*, *Myriophyllom aquaticum* and *Lantana camara* are currently widely distributed throughout the country and have caused major negative impacts (MICOA, 2007). Corallivorous crown-of-thorns (*Acanthaster planci*) starfish has also been found on the Mozambican coast, and during 1995-1996 this species had a devastating effect on the coral reefs of Bazaruto Island (80%), and Inhambane (95% - 98%) (ASCLME/SWIOFP, 2012b). Although the data from the present study shows a large decline in the quantities of *F. indicus*, it is still not possible to conclude that *M. dobsoni* is an invasive species.

The present study identified an increase in the occurrence of *M. dobsoni* in fishery catches in Maputo Bay, probably competing with native species. If *M. dobsoni* became an invasive species it would cause great loss to local biodiversity. In addition, considering its smaller

Table 2. Genetic distance matrix between species for the COI gene. Standard errors are shown in the upper diagonal, and values of genetic distance between species in the lower diagonal.

Species	Distance									
M. dobsoni		0,3016	0,2441	0,2448	0,3430	0,1838	0,2132	0,2356	0,1775	0,3987
L. vannamei	0,4352		0,2061	0,2307	0,2101	0,2934	0,2333	0,1798	0,2737	0,3329
P. monodon	0,3375	0,2876		0,2211	0,1623	0,2637	0,2211	0,1679	0,2161	0,3799
M. japonicus	0,3671	0,3095	0,2885		0,2288	0,2867	0,2783	0,1695	0,2697	0,3543
F. chininses	0,4871	0,2805	0,2196	0,3049		0,2469	0,2042	0,1287	0,2151	0,3573
M. brevicornis	0,2484	0,4032	0,3704	0,4008	0,3085		0,1959	0,2284	0,1462	0,3095
M. stebbingi	0,2988	0,3233	0,3170	0,3952	0,2809	0,2528		0,2197	0,1867	0,4724
F. indicus	0,3249	0,2646	0,2188	0,2323	0,1919	0,3034	0,3178		0,2010	0,3113
M. monoceros	0,2263	0,3926	0,3056	0,3809	0,3050	0,2048	0,2548	0,2931		0,3097

Table 3. Kimura two-parameter average genetic distance within species for the COI gene.

Species	Distance
M. dobsoni	0,028
F. chinensis	0,002
L. vannamei	0,053
P. monodon	0,057
M. japonicus	0,042
M. brevicornis	0,002
F. californiensis	0
M. stebbingi	0
F. indicus	0,002
M. monoceros	0

Table 4. Fisheries landings in metric tons of *F. indicus, M. monoceros, M. stebbingi, M. dobsoni* and *M. japonicus* in Maputo Bay during the period from 2007 to 2016.

Fishing gear	Period	F. indicus	M. monoceros	M. japonicus	P. monodon	M. stebbingi	M. dobsoni
ARB	2007	79,0	5,1	0,1	3,6	0,1	0,0
	2008	94,6	1,7	2,7	0,0	0,0	0,0
	2009	76,7	13,8	0,3	4,5	1,2	3,5
	2010	83,5	13,6	0,0	2,8	0,0	0,0
	2011	91,9	1,5	0,0	1,8	0,0	4,7
	2012	78,4	0,0	11,8	2,0	0,0	0,0
	2013	66,0	15,0	0,0	1,0	1,0	2,0
	2014	40,0	39,0		4,0	3,0	6,0
	2015	38,0	40,0	0,0	5,0	3,0	6,0
	2016	61,0	34,0	0,0	3,0	1,0	1,0
ARP	2007	48,4	7,6	9,3	16,9	0,5	0,0
	2008	68,2	3,7	3,7	9,7	1,1	0,0
	2009	58,6	6,7	2,3	14,5	0,8	17,0
	2010	38,8	7,2	1,6	15,0	30,4	6,9
	2011	60,2	14,2	1,3	10,7	0,6	13,0
	2012	31,4	1,2	15,1	29,4	13,2	0,0
	2013	41,0	16,0	4,0	16,0	2,0	1,0
	2014	41,4	1,2	0,1	29,5	0,1	0,7
	2015	41,0	1,0	0,0	30,0	0,0	1,0
	2016	32,0	3,0	9,0	7,0	1,0	0,0
EMF	2007	94,6	0,0	0,0	5,4	0,0	0,0
	2008	94,8	2,1	0,0	0,9	0,4	0,0
	2009	92,3	4,2	0,0	1,1	0,2	2,0
	2010	85,3	1,3	0,0	10,7	0,1	2,7
	2011	90,3	3,0	0,1	2,7	0,3	0,8
	2012	91,6	2,2	0,0	8,4	0,2	0,0
	2013	92,0	3,0	1,0	2,0	0,0	0,0
	2014	86,0	4,0	2,0	4,0	0,0	0,0
	2015	85,0	4,0	2,0	4,0	0,0	0,0
	2016	87,0	3,0	0,0	3,0	0,0	0,0
SEMI	2007	22,5	28,2	2,3	3,1	9,6	20,8
	2008	21,9	4,4	4,6	5,1	8,1	41,6
	2009	18,0	12,0	1,0	1,0	6,0	54,0
	2010	53,0	20,0	0,0	1,0	2,0	21,0
	2011	43,5	21,0	0,0	0,5	2,9	30,1
	2012	34,0	22,0	0,0	0,0	3,8	39,2
	2013	21,0	6,9	0,1	1,3	4,9	59,4
	2014	14,9	13,2	0,0	0,6	1,0	66,6
	2015	13,2	13,8	0,0	1,2	5,1	57,6
	2016	14,2	17,5	0,1	1,2	6,1	51,3

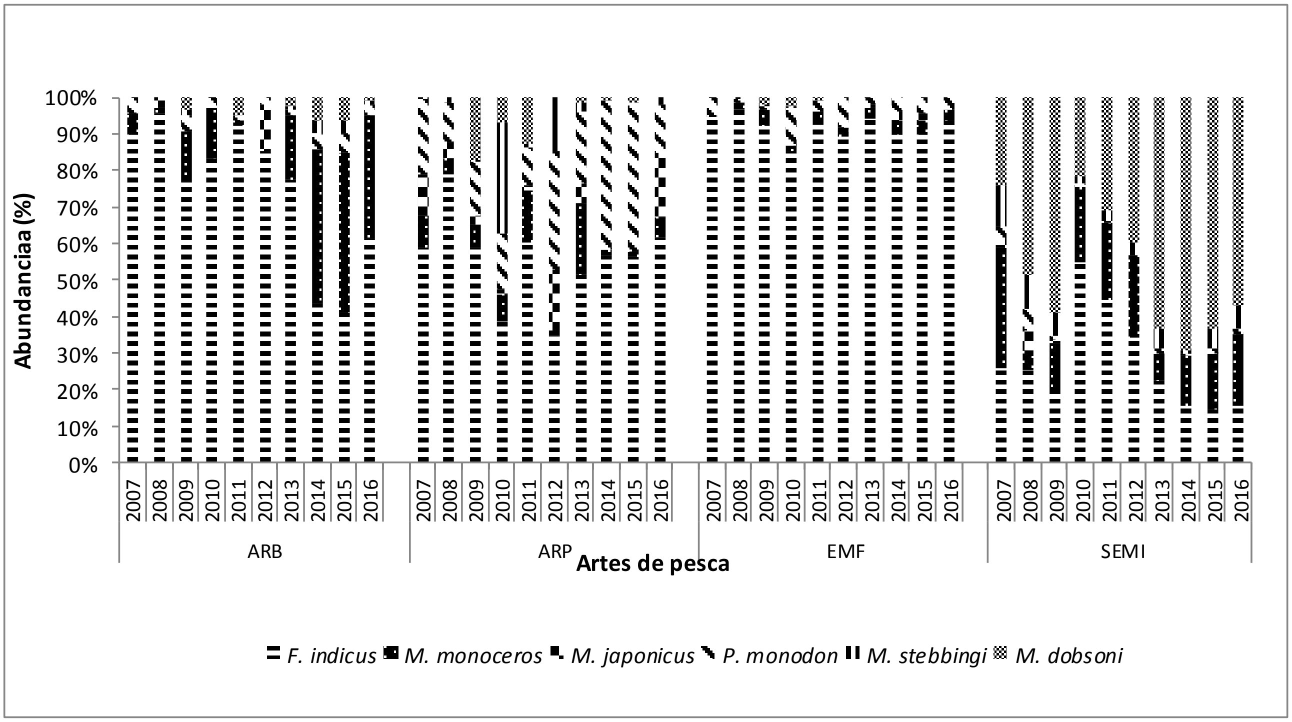

Figure 5. Shrimp landings of Maputo Bay expressed as proportion of *F. indicus*, *M. monoceros*, *M. stebbingi*, *M. japonicus*, *Penaeus monodon* and *M. dobsoni* for the years 2007 to 2016.

body size and its reduced economic value, it could result in drastic consequences for the local economy. The current status of *M. dobsoni* is of concern mainly because it may occupy similar ecological niches as native species (Rao *et al*., 2013) that are of vital importance to the economy of the country. An alien species that becomes established in a particular locality alters native biological diversity. Initially, the presence of these species may increase the local number of species, but over time it will reduce (in number and relative abundance) local biodiversity, particularly if native species are not numerous or are replaced in their natural habitat (Shine *et al.*, 2000).

In addition to the identification of the alien shrimp species, COI sequences of all penaeid shrimp occurring on the Mozambique coast are reported for the first time in this paper, enlarging the molecular knowledge of family Penaeidae populations. Similar to that observed for the *M. dobsoni* group, the distance genetic tree also revealed population structuring in *M. japonicus* and *P. monodon*, or even recent divergence of cryptic species, which could be resolved in further population studies. In both these species, the population naturally occurring on the Mozambique coast appeared in exclusive clades (African clade) compared with individuals from other areas (Japan for *M. japonicus*, and Australia for *P. monodon*), suggesting that the Mozambique coast maintains an important portion of the overall genetic variation of these shrimp. The potential negative effects that *M. dobsoni* might bring to Mozambique are quite predictable, ranging from a loss of biodiversity to economic losses. Where fishery activities involve this species, it appears that it is also accompanied by an almost total absence of the native white shrimp *F. indicus*, particularly in semi industrial fishing gear. The present study was able to confirm the identity of this alien species and provides a warning of the potential negative impacts for the local biodiversity and economy.

Acknowledgements

The authors thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), for its financial support. We are grateful to Instituto Nacional de Investigação Pesqueira (IIP) for the financial support for the publication of this paper. We also thank "Ministério de Ciências e Tecnologia de Moçambique", for granting funds to support this project, and Dr. Atanásio Brito, for his help with the first English version. We are also grateful to the team from Central Marine Fisheries Research Institute, in Cochin-Kerala, India, for the help with the taxonomic analyses.

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