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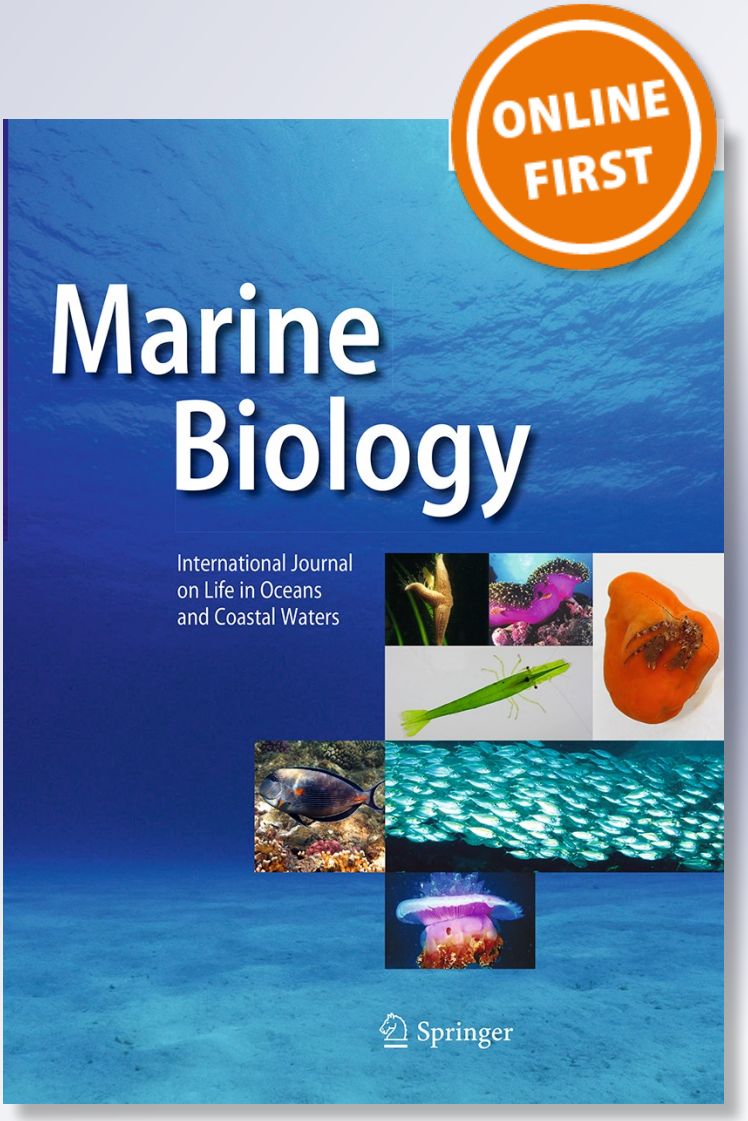

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ORIGINAL PAPER

New insight on population genetic connectivity of widespread amphidromous prawn *Macrobrachium lar* (Fabricius, 1798) (Crustacea: Decapoda: Palaemonidae)

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Abstract Due to the sparse and unstable nature of insular freshwater habitats, marine larval dispersal of amphidromous species is considered a critical element of population persistence. We assessed population genetic structure of freshwater prawn *Macrobrachium lar* across its range that encompasses two biogeographic barriers: the vast open ocean separating Western and Central Pacific regions and the Indo-Malay archipelago separating Indian and Pacific oceans. A total of 173 samples collected from 21 islands throughout the Indo-Pacific were sequenced at 16S and 28S rDNA. We observed distinct genetic isolation of populations located at the eastern and southwestern edge of the species range but no evidence of an effect of the Indo-Pacific barrier. Differentiation patterns are consistent with a stepping-stone model of dispersal. Genetic differences of Central Pacific populations may reflect founder events associated with colonization of isolated islands, or be a signature of a past bottleneck after population depletion caused by drastic climatic events.

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Introduction

In the tropical Indo-Pacific, insular freshwater systems are mainly dominated by diadromous species. Indeed, the colonization of these habitats, subject to extreme climatic and hydrologic seasonal variations, requires specific adaptations of the life cycle of the freshwater organisms (Keith 2003; McDowall 2007). Spending part of their life cycle at sea, diadromous species are able to escape drought or cyclonic flood events through the colonization of new environments via oceanic dispersal. As a consequence, dispersal abilities of diadromous organisms that allow migration between insular habitat patches are considered a critical element to population persistence and species viability, both at local and large spatial scales (Keith 2003; Keith et al. 2011; Feutry et al. 2013a).

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Amphidromy is a type of diadromy that involves several families of decapod crustaceans (e.g, Chen et al. 2009; Wowor et al. 2009), teleost fishes (e.g., Holthuis 1980; Keith et al. 1999, 2002; Marquet et al. 2003), and gastropod snails (e.g., Myers et al. 2000; Kano and Kase 2003; Cook et al. 2009; Crandall et al. 2010). Amphidromous species spawn in fresh or estuarine water and larvae drift downstream to the sea where they undergo a planktonic phase before returning to rivers to grow and reproduce (McDowall 2007; Wowor et al. 2009). Although ecologically considered freshwater organisms (Bandel and Riedel 1998; Smith et al. 2003), patterns of population connectivity in amphidromous species are expected to be more similar to that of sedentary marine species with high dispersal abilities. Indeed, pelagic larval durations estimated from laboratory cultures of larvae in some amphidromous gastropods and crustaceans, or from fish otoliths, are comparable to that found in marine species with high dispersal abilities (cf. Crandall et al. 2010 and references therein). Accordingly, several population genetic studies have shown that amphidromous organisms with long-lived larvae display low or non-existent genetic structure among populations (Chubb et al. 1998; Hodges and Allendorf 1998; Myers et al. 2000; Waters et al. 2000; Kano and Kase 2004; Berrebi et al. 2005; Page et al. 2013). However, most of these studies have been undertaken at relatively small geographical scale (i.e., island or archipelago). Indeed, for widely distributed amphidromous species, very little is known about patterns of population connectivity at large spatial scales; therefore, how larval dispersal may counteract the effects of the geographic isolation of insular freshwater habitats is unknown.

Based on a few case studies relying on widespread geographic sampling, species persistence in insular freshwater systems may partially rely on multiple colonization events, which are influenced by the species dispersal abilities, distances between habitat patches, presence–absence of favorable oceanic currents, and island biogeography (Murphy and Cowan 2007; Crandall et al. 2010; Lord et al. 2012; Feutry et al. 2013b). An important finding of these population genetic studies has been the identification of a certain degree of spatial genetic structuring among biogeographic provinces, reflecting the effectiveness of biogeographical barriers for limiting larval dispersal. The background hypothesis supporting these results is that oceans are partitioned into biogeographical provinces distinguished by habitat discontinuities (Briggs 1974; Gaither et al. 2010). Thus, large-scale physical barriers separating biogeographical provinces can sporadically shape species distributions and intraspecific genetic structure over time, mainly acting on species displaying dispersal potential through the marine environment (e.g., Planes and Fauvelot 2002; Gaither et al. 2010, 2011; Lord et al. 2012). An example of a biogeographical barrier in the Indo-Pacific is the vast expanse of open ocean separating the Western and Central Pacific islands (Gillespie et al. 2008). For most widespread Indo-Pacific marine species, the Central Pacific region that includes the Hawaiian, Cook, Society, Marquesas, Austral, Gambier, and Tuamotu archipelagos is located beyond their dispersal limits and generally constitutes the eastern end of species ranges. Therefore, Indo-Pacific species that occur up to the Central Pacific are generally thought to have colonized eastern remote islands through a stepping-stone pattern of dispersal. Accordingly, genetic studies of widely distributed marine and freshwater species have demonstrated genetic divergences between Western and Central Pacific populations associated with patterns of isolation by distances (e.g., Palumbi et al. 1997; Planes and Fauvelot 2002; Crandall et al. 2010; Gaither et al. 2010; Winters et al. 2010; Lord et al. 2012).

Another example of a major biogeographical barrier in the Indo-Pacific is the aggregation of islands in the Indo-Malay archipelago separating the Indian and Pacific oceans (the Indo-Pacific Barrier; Briggs 1974). Recent genetic studies have suggested that sea-level fluctuations of Plio-Pleistocene times influenced the partitioning of intraspecific variation in organisms with ranges that span the Indo-Pacific (e.g., Bowen et al. 2001; Horne et al. 2008; Minegishi et al. 2008; Gaither et al. 2010; Winters et al. 2010; Lohman et al. 2011). For amphidromous species that require both marine and freshwater habitats, sea-level fluctuations may have conflicting effects on the population connectivity. While lowering sea level would disrupt gene flow by decreasing marine dispersal routes among isolated freshwater systems, it would also facilitate marine dispersal by decreasing the spacing and increasing the size of freshwater habitat patches (i.e., expansion of brackish and freshwater habitats, cf. Hewitt 2000; Voris 2001). Consistent with this, patterns of genetic structure between Indian and Pacific populations vary from one taxon to another (e.g., Dodson et al. 1995; Lavery et al. 1995, 1996; de Bruyn et al. 2004a, b, 2005; de Bruyn and Mather 2007; Page et al. 2007; Crandall et al. 2008; Minegishi et al. 2008; Lord et al. 2010; Feutry et al. 2013b).

In this study, we focused on one of the most widespread amphidromous prawn species, *Macrobrachium lar* (Fabricius, 1978). This species is distributed from the Marquesas Islands in the Central Pacific to the east coast of Africa (Short 2004; Fig. 1). *M. lar* is a large-sized species (males have 61 mm maximum carapace length or 195 mm total length; Short 2004), which lives in a variety of environments, from surface freshwater streams, rivers, or lakes to estuaries and coastal ponds (Shokita 1979; Keith et al. 2006) down to underground streams in caves (Wowor 2012). *M. lar* is an opportunistic detritivorous species with

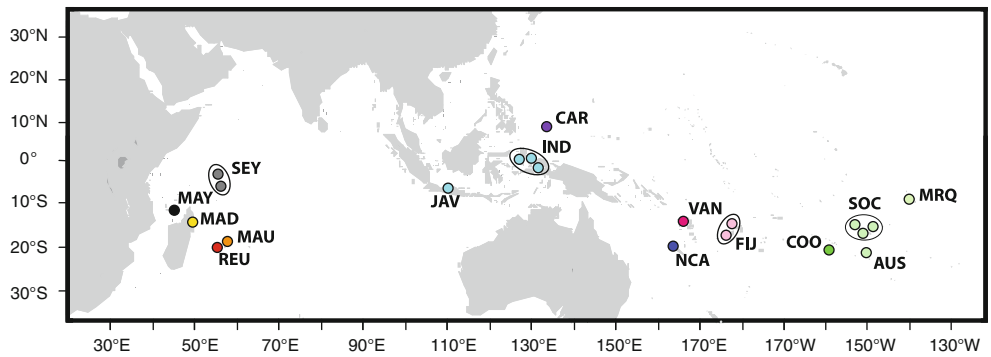

Fig. 1 Map of the Indo-Pacific showing localities where *M. lar* was sampled. *Colored circles* correspond to the 21 islands sampled. The individuals from these islands were partitioned into 15 populations indicated with circles or ellipses and three-letter population abbreviation: *SOC* Tahiti, Raiatea, and Moorea; *MRQ* Marquesas; *AUS* Austral Islands; *COO* Cook Islands; *NCA* New Caledonia; *VAN* Vanuatu; *FIJ* Fiji and Hoorn Islands; *IND* Papua, Waigeo, and Halmahera; *JAV* Java; *CAR* Caroline Islands; *SEY* Mahé and Silhouette; *MAY* Mayotte; *MAD* Madagascar; *REU* Réunion; *MAU* Mauritius (cf. Table 1)

an r-reproductive strategy in that females produce a large number of small eggs (up to 40,000 eggs, 0.7 mm in length) that are incubated for approximately 20 days. The small eggs give rise to free-swimming larvae that are euryhaline and go through 12 zoeal stages, lasting about 90 days as plankton, before they reach the juvenile stage and become bottom dwellers (Atkinson 1977; Keith et al. 2006; Wowor et al. 2009). In tropical islands, *M. lar* is an integral part of traditional diets and is usually threatened by agricultural and domestic pollution. Anecdotal reports from fishermen of the Southwest Indian Ocean (e.g., Mayotte, Réunion, and Madagascar) indicate that populations of *M. lar* have decreased substantially during the last decade. For biological conservation perspectives, it is therefore quite important to know how oceanic larval dispersal contributes to population viability across large geographical scales.

The aim of the present study is to gain an in-depth understanding of population genetic structuring in a widespread amphidromous species. Based on samples obtained from 21 islands distributed throughout the species range of *M. lar*, we analyzed mitochondrial and nuclear DNA sequence data from 173 individuals to address two main questions: (1) what are the connectivity patterns among *M. lar* populations across its geographical range?, and (2) do biogeographical barriers, such as the isolation of the Central Pacific islands and the Indo-Pacific Barrier, affect population genetic structure in *M. lar*?

Materials and methods

Sampling

One hundred and seventy-three individuals of *M. lar* were collected from 21 island locations (Fig. 1; Table 1). Geographic coverage was obtained by sampling from five biogeographic regions in the Indo-Pacific Ocean: (1) the Central Pacific Ocean, CPO (Tahiti, Raiatea, and Moorea: *Society Islands*; Ua huka: *Marquesas Islands*; Rurutu: *Austral Islands*; Rarotonga: *Cook Islands*); (2) the Southwest Pacific Ocean, SWPO (Grande Terre: *New Caledonia*; Santo: *Vanuatu*; Futuna: *Hoorn Islands*; Taveuni: *Fiji*); (3) the Northwest Pacific Ocean, NWPO (Papua, Waigeo, Halmahera, and Java: *Indonesia*; Palau: *Caroline Islands*); (4) the Northwest Indian Ocean, NWIO (Mahé and Silhouette: *Seychelles Islands*; Mayotte: *Comoros Island*); and (5) the Southwest Indian Ocean, SWIO (*Madagascar*; Réunion and Mauritius: *Mascarene Islands*) (Table 1; Fig. 1). Specimens were collected by electrofishing (Portable Dekka 3,000 electric device, Germany) during several expeditions conducted by the Muséum national d'Histoire naturelle of Paris between 2004 and 2011, by the Indonesian Institute of Sciences between 2006 and 2010, or were generously provided by colleagues (see Acknowledgements). In the field, either entire specimens were sampled or a piece of pereiopod was clipped off of live prawns that were then released back into the river. All samples were fixed in 95 % ethanol for molecular analysis.

PCR amplification and DNA sequencing

Total genomic DNA was extracted from muscle tissue using NucleoSpin^{R} 96 Tissues (Macherey-Nagel) following manufacturer's instructions. A fragment of the mitochondrial 16S rDNA gene was amplified using universal primers 16Sa-L and 16Sb-H2 (Palumbi 1996), and a fragment of the nuclear 28S rDNA gene including the D1 and D2 domains (Hassouna et al. 1984; Palumbi et al. 1991) was amplified using primers C1 and D2 (Jovelin and Justine 2001). PCR amplification was performed in 25 µl reactions, containing approximately 3 ng template DNA, 2.5 mM $MgCl_2$, 0.26 mM of each nucleotide, 0.3 µM of

Table 1 Details of *M. lar* locations sampled, including biogeographic region, countries, islands, sample sizes at each location (Ss), and abbreviated names given to the analyzed populations (Populations)

Regions	Countries	Islands	Ss	Populations	*n*
Central Pacific Ocean	*Society Islands*	Tahiti	2		
–	–	Raiatea	1		
–	–	Moorea	1	SOC	4
–	*Marquesas*	Ua huka	2	MRQ	2
–	*Austral Islands*	Rurutu	1	AUS	1
–	*Cook Islands*	Rarotonga	26	**COO**	**26**
Southwest Pacific Ocean	*New Caledonia*	Grande Terre	20	**NCA**	**20**
–	*Vanuatu*	Santo	11	**VAN**	**11**
–	*Hoorn Islands*	Futuna	1		
–	*Fiji*	Taveuni	3	FIJ	4
Northwest Pacific Ocean	*Indonesia*	Papua	12		
–	–	Waigeo	2		
–	–	Halmahera	2	**IND**	**16**
–	–	Java	1	JAV	1
–	*Caroline Islands*	Palau	19	**CAR**	**19**
Northwest Indian Ocean	*Seychelles Islands*	Mahé	1		
–	–	Silhouette	2	SEY	3
–	*Mayotte Island*	Mayotte	29	**MAY**	**29**
Southwest Indian Ocean	*Madagascar*	Madagascar	14	**MAD**	**14**
–	*Mascarene Islands*	Réunion	18	**REU**	**18**
–	–	Mauritius	5	**MAU**	**5**

Locations with only 1 or 2 individuals were pooled with nearby locations in order to increase the number of individuals within the populations used in the AMOVA (*n*). Populations used in the AMOVA and the Mantel's test are given in bold characters

each primer, 5 % DMSO, and 1.5 units of QBIOTAQ polymerase (MPBiomedicals). Amplification products were generated by an initial denaturation step of 4 min at 94 °C followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 51 °C for 16S and 56 °C for 28S for 40 s, and extension at 72 °C for 1 min. PCR products were sequenced bidirectionally at Genoscope (Evry, France) using PCR primers. Chromatograms were viewed, edited, and alignment assembled using BioEdit Sequence Alignment Editor Version 7.0.4.1 (Hall 1999).

DNA analyses

Mitochondrial DNA

The genetic diversity of the 16S gene fragment was examined using Arlequin 3.5 (Excoffier and Lischer 2010). For each geographic region, genetic diversity indices were estimated by computing the number of mitochondrial haplotypes (N_H), number of segregating sites (S; Watterson 1975), nucleotide diversity (π; Tajima 1983; Nei 1987, i.e., average number of nucleotide differences between pairs of sequences), and haplotype diversity (H_d; Nei 1987).

To visualize genealogical relationships among haplotypes and their geographic distribution, a median-joining network was built from the 16S dataset using Network 4.1.1.2 (Bandelt et al. 1999) with equal weights for variable sites. A maximum parsimony algorithm was applied to simplify the complex branching pattern and generate a network representing all of the most parsimonious intraspecific phylogenies.

The degree of genetic differentiation among populations was assessed using Arlequin 3.5 with an analysis of molecular variance (AMOVA; Excoffier et al. 1992), for which the individuals from the 21 islands sampled were partitioned into fifteen populations (Table 1). Sites with small sample size were pooled with nearby populations (i.e., Raiatea and Moorea were added to the nearby Tahiti island, SOC; Futuna was added to Fiji, FIJ; Waigeo and Halmahera were added to Papua, IND; Silhouette was added to Mahé, SEY) or left alone if geographically too isolated (i.e., Marquesas, MRQ; Austral Islands, AUS; Java, JAV). Ultimately, populations including less than five individuals were discarded from this analysis (i.e., SOC, MRQ, AUS, FIJ, JAV, SEY, Table 1). Genetic differentiation between each pair of populations was also assessed by calculating pairwise F_{ST} values (Wright 1949).

To investigate broader-scale patterns of structuring, a hierarchical AMOVA was performed using the five Indo-Pacific biogeographic regions as populations (i.e, CPO, SWPO, NWPO, NWIO, and SWIO), and oceans as regions (i.e., Indian and Pacific) in the analysis. This approach allowed increasing the number of individuals in each analyzed group by relieving sample size constraints. In fact, all individuals sequenced were included in this analysis, even those from small or isolated populations (i.e.,

SOC, MRQ, AUS, FIJ, JAV, SEY). Significance of *F*-statistics was calculated from 10,000 replicate analyses based on samples drawn randomly and an alpha value of 0.05 (Excoffier and Lischer 2010).

To test for isolation by distance, distances between populations (Table 1) were calculated using great-circle distances (i.e., orthodromic distances) based on the sampling site coordinates (the geographical center of the pooled sites was used as the location for the pooled samples) and plotted against pairwise F_{ST} using a Mantel's test based on 10,000 permutations (Mantel 1967). Mantel's test was performed using the online version of GENEPOP (http://genepop.curtin.edu.au/, Raymond and Rousset 1995) with log-transformed pairwise F_{ST} (calculated as $F_{ST}/[1 - F_{ST}]$) and the natural logarithm of the geographical distances (calculated as Ln(d)) (Rousset 1997; Rousset and Raymond 1997).

The demographic history of *M. lar* was inferred using the Fu's *Fs*-statistic (Fu 1997), implemented in Arlequin 3.5. Analyses were performed from genetically homogeneous regions. Significantly, negative values of Fu's *Fs* values indicate a genetic pattern expected under population growth.

Nuclear DNA

Patterns of genetic structuring established from mitochondrial 16S were compared to those from nuclear 28S. The genealogical history of nuclear genes is independent from that of mitochondrial genes (Nichols 2001); therefore, recovering the same structuring pattern across marker types gives strong support for observed patterns of population partitioning. The 28S dataset consisted of sequences from at least four individuals from all five separate regions described in Table 1. A median-joining network was built using the same parameters as those used for the mitochondrial dataset.

Results

Mitochondrial DNA

A total of 173 mitochondrial 16S sequences were obtained from *M. lar* and deposited in GenBank (Accession Numbers: KC506830–KC507002). Analyzed 16S sequences were 509 base pairs long and contained 16 polymorphic sites that defined 26 unique haplotypes. Haplotype diversity (Hd) was generally low, with the lowest value being 0.459 in SWIO and the highest being 0.761 in NWPO. Nucleotide diversity was relatively low ranging from 0.662 to 2.102 (Table 2).

The haplotype network showed a geographic structure with four main groups (Fig. 2a): (1) the SWIO; (2) the CPO, *plus* one haplotype from Indonesia (Papua); (3) the SWPO, *plus* two haplotypes from Indonesia (Papua, Waigeo, and Halmahera), three haplotypes from Caroline Islands (Palau), two haplotypes from Mayotte, and one haplotype from Seychelles Islands (Silhouette and Mahé); and (4) the NWPO, *plus* one haplotype from Mayotte. These four groups were connected by five central haplotypes distributed from Réunion and Madagascar in the SWIO to Ua huka in the CPO. Haplotypes from the NWIO appeared in groups (3), (4), and in the central haplotypes (Fig. 2a).

Table 2 Summary statistics describing sequence polymorphism at 16S gene for each analyzed population of *M. lar*

Populations	N_H	S	π SD	H_d SD	F_S P value
Central Pacific Ocean	10	10	1.412	0.721	**−4.605**
$N = 33$			±0.886	±0.078	**0.004**
Southwest Pacific Ocean	4	3	0.662	0.559	−0.444
$N = 35$			±522	±0.046	0.356
Northwest Pacific Ocean	9	9	1.911	0.761	−1.954
$N = 36$			±1.114	±0.050	0.157
Northwest Indian Ocean	6	6	2.102	0.702	0.569
$N = 32$			±1.203	±0.072	0.652
Southwest Indian Ocean	6	5	1.051	0.459	−1.23
$N = 37$			±0.713	±0.096	0.234

Significant *Fs* values ($\alpha \leq 0.05$) are given in bold characters

N number of sequences, N_H number of haplotypes, S number of segregating sites, π nucleotide diversity (mean number of pairwise differences), H_d haplotype diversity with standard deviation (SD), *Fs* Fu's *Fs*-statistics with associated P values

Overall, significant genetic differentiation was detected among the nine analyzed populations ($F_{ST} = 0.674$, P value $<10^{-5}$). This genetic structure was partly due to populations from the CPO and the SWIO regions, which both contained several private haplotypes (as denominated by Slatkin 1985). When removing populations from these regions, the F_{ST} estimate, although still significant (P value $= 10^{-3}$), decreased to 0.124. Pairwise F_{ST} comparisons (Table 3) indicated high level of population structure between Central Pacific and all other Western islands. Strong genetic differentiation was also found between Mauritius, Réunion, Madagascar Islands, and all other populations analyzed. Significant genetic structure was observed between Mayotte versus New Caledonia and Vanuatu. Interestingly, Mayotte showed significant but lower levels of structure when compared with Indonesia and Caroline Islands. Similarly, pairwise F_{ST} values between New Caledonia and Indonesia showed lower levels of population structure. No genetic differentiation was detected between New Caledonia and Caroline Islands, Vanuatu and Caroline Islands, and Vanuatu and Indonesia. There was no significant genetic structure between

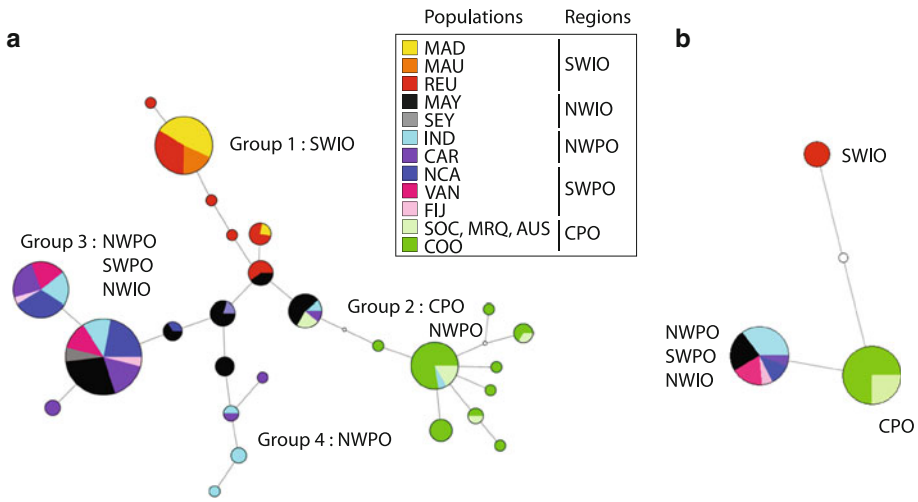

Fig. 2 Median-joining network obtained from the 16S (**a**) and 28S (**b**) genes. *Circles* are color-coded based on general location (*key box*, cf. Fig. 1) and are sized according to the relative abundance of each haplotype. Each *line* connecting a *circle* equals one mutation. Small *unfilled circles* equal missing or unsampled haplotypes

Table 3 Pairwise F_{ST} values calculated from mtDNA haplotypes of nine populations of *M. lar* (below diagonal) and associated *P* values (above diagonal)

	COO	NCA	VAN	CAR	IND	MAY	MAD	REU	MAU
COO		$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$
NCA	**0.869**		0.999	0.313	0.024	0.001	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$
VAN	**0.861**	−0.065		0.532	0.103	0.007	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$
CAR	**0.814**	0.011	−0.011		0.279	0.008	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$
IND	**0.739**	**0.116**	0.081	0.010		0.021	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$
MAY	**0.718**	**0.223**	**0.208**	**0.136**	**0.096**		$<10^{-5}$	$<10^{-5}$	$<10^{-5}$
MAD	**0.852**	**0.900**	**0.909**	**0.821**	**0.741**	**0.651**		0.058	0.999
REU	**0.756**	**0.762**	**0.740**	**0.678**	**0.593**	**0.481**	0.118		0.123
MAU	**0.846**	**0.912**	**0.930**	**0.810**	**0.704**	**0.632**	−0.097	0.119	

Significant pairwise F_{ST} values ($\alpha \leq 0.05$) are given in bold characters

COO Cook Islands, *NCA* New Caledonia, *VAN* Vanuatu, *CAR* Caroline Islands, *IND* Indonesia, *MAY* Mayotte, *MAD* Madagascar, *REU* Réunion Island, *MAU* Mauritius

Madagascar and Réunion, Indonesia and Caroline Islands, and Vanuatu and New Caledonia. When populations were grouped by biogeographic regions, a significant amount of genetic variation was apportioned among regions ($F_{ST} = 0.680$, *P* value $<10^{-5}$), with pairwise comparisons involving the CPO and the SWIO regions yielding the highest F_{ST} values (ranging from 0.642 to 0.845; Table 4). In contrast, lower levels of genetic structure were observed between SWPO and NWPO, and between NWPO and NWIO. Interestingly, pairwise F_{ST} values showed that the NWIO was less genetically differentiated from the NWPO that is about 8,820 km away, than from the SWIO that is only about 1,540 km away (Table 4). When regions were grouped by ocean basin, significant level of genetic structuring was observed between the Pacific and Indian oceans ($F_{CT} = 0.242$, *P* value $<10^{-5}$). When removing CPO and SWIO populations from these two regions (i.e, Pacific vs. Indian), the F_{CT} estimate, although still significant (*P* value $= 10^{-5}$), decreased to 0.133. Fu's *Fs* values were significantly negative only for the CPO region (Table 2).

Table 4 Pairwise F_{ST} values calculated from mtDNA haplotypes of five biogeographic regions in the Indo-Pacific (below diagonal) and associated *P* values (above diagonal)

	CPO	SWPO	NWPO	NWIO	SWIO
CPO		$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$
SWPO	**0.845**		0.012	0.001	$<10^{-5}$
NWPO	**0.731**	**0.065**		0.008	$<10^{-5}$
NWIO	**0.712**	**0.199**	**0.085**		$<10^{-5}$
SWIO	**0.781**	**0.826**	**0.710**	**0.642**	

Significant pairwise F_{ST} values ($\alpha \leq 0.05$) are given in bold characters

CPO Central Pacific Ocean, *SWPO* Southwest Pacific Ocean, *NWPO* Northwest Pacific Ocean, *NWIO* Northwest Indian Ocean, *SWIO* Southwest Indian Ocean

A Mantel test showed a significant and positive correlation between genetic differentiation and geographical distances (in kilometers) based on the study of nine populations from the Indo-Pacific ($Z = -8.573$, *P* value $= 0.008$).

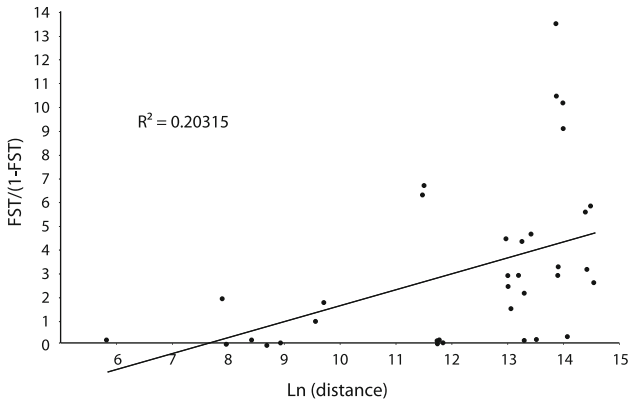

Fig. 3 Isolation by distance. Genetic distance $F_{ST}/(1 - F_{ST})$ calculated for each population pair from 16S gene plotted against geographic distance Ln(d) in kilometers. Regression line is pictured with a *black line*

However, geography explained only 20 % of the genetic variation among populations ($R^2 = 0.203$, Fig. 3).

Nuclear DNA

A total of 58 nuclear 28S sequences of 741 base pairs were obtained from *M. lar* and deposited in GenBank (Accession Numbers: KC507003–KC507060). From the 173 individuals analyzed at 16S, a subset comprised of 4 individuals from Réunion (SWIO), 8 from Mayotte (NWIO), 15 from Cook Islands, 6 from Society Islands (CPO), 6 from Vanuatu, 4 from New Caledonia, 2 from Fiji (SWPO), 12 from Papua, and 1 from Caroline Islands (NWPO) were sequenced at 28S (Fig. 2b). The 28S dataset contained only 6 polymorphic sites, all of which were parsimony informative. The haplotype network corroborated the mitochondrial partitioning, with Réunion and Cook *plus* Society populations being divergent from all other populations (i.e., NWIO, NWPO, and SWPO). In fact, individuals from NWIO, NWPO, and SWPO, all held identical sequences (Fig. 2b).

Discussion

Our study of the freshwater prawn *M. lar* revealed significant levels of population genetic structure across its geographic range. However, molecular analyses indicated that most of this genetic structure is a result of the genetic isolation of populations within the Central Pacific and the Southwest Indian Ocean regions. When these regions were removed from analyses, population structure decreased significantly, revealing a lower level of genetic structure across the West Pacific, and between Indian and Pacific Ocean basins. This pattern of weak connectivity across the Indonesian archipelagoes and strong isolation of the peripheral populations (i.e., located at the outer edge of the species range) have also recently been observed in marine reef fishes (Gaither et al. 2010, 2011; Winters et al. 2010). Accordingly, our Mantel's test revealed significant isolation by distance among the Indo-Pacific populations, indicating that dispersal should be more likely to occur between nearby populations following a stepping-stone pattern. In this context, considering the whole species range of *M. lar*, peripheral populations that are more isolated would have reduced rates of colonization and higher levels of genetic differentiation than central populations. However, the linear relationships between genetic differentiation and geographic distances explained less than 20 % of the total variance. This weak correlation suggests that gene flow is affected by more than just the simple spatial proximity of *M. lar* populations and is discussed hereafter and compared with existing genetic studies.

Isolation of peripheral geographic locations

The Central Pacific Ocean (CPO)

Genetic analyses revealed distinct populations at the eastern margin of the geographic range of *M. lar*. From the haplotype networks, the CPO populations were genetically differentiated from all other Indo-Pacific populations. This genetic differentiation was supported by significant and high pairwise F_{ST} values, in both population and regional comparisons. These results suggest that the geographic isolation of the CPO may represent a strong barrier to dispersal, separating Central and Western Pacific populations of *M. lar*. However, both the small genetic distances and the presence of shared ancestral haplotypes between these regions suggest that populations have been connected by gene flow in the past. Although quite long, the pelagic larval duration of *M. lar* (90 days) might be inadequate to cross, in a single generation, the thousands of kilometers that separate Central and Western Pacific regions (cf. Crandall et al. 2010). However, one might expect that these distant regions have possibly been connected through a stepping-stone model of dispersal. This possibility corroborates numerous studies carried out on the terrestrial fauna of the Central Pacific islands showing that most lineages have western origins and colonized the remote locations of the Central and Eastern Pacific progressively, from the less isolated western islands (references reviewed in Gillespie et al. 2008).

Moreover, the star-like pattern of the haplotype network and the neutrality statistics in the CPO region were consistent with a scenario of past colonization events followed by demographic expansions. Under this scenario, reduced genetic diversity might result from founder effects associated with colonization of isolated islands or, alternatively,

be a signature of past bottlenecks after population depletions caused by drastic climatic events. Such patterns are commonly found in highly dispersive organisms inhabiting spatially fragmented or ephemeral habitats. This has been observed in several widespread reef fish species (e.g., *Lutjanus fulvus*, Gaither et al. 2010; *Naso unicornis,* Horne et al. 2008; *Scarus psittacus,* Winters et al. 2010), but also in amphidromous species living in oceanic islands (Myers et al. 2000; Kano and Kase 2004; Lord et al. 2012).

Another major result of this study was that the CPO populations were genetically less differentiated from some haplotypes from the NWPO and NWIO regions than from haplotypes collected nearer in the SWPO region, suggesting differential probabilities of gene exchanges between these regions. Although sampling bias cannot be ruled out, connectivity patterns in *M. lar* may thus be explained by factors other than geographic distance only. Ocean currents may have a strong influence on population genetic structuring (White et al. 2010; Page et al. 2013). However, on a large geographical scale, present-day oceanic currents are usually not fully consistent with patterns of connectivity in marine species (Benzie 1998; Lessios 1998; Planes and Fauvelot 2002). Indeed, similar patterns of genetic differentiation were identified in two amphidromous species, the gastropod *Neritina canalis* (Crandall et al. 2010) and the fish *Sicyopterus lagocephalus* (Lord et al. 2012). Likewise, a sharp genetic break has been found between the Central and Western Pacific populations within the sea urchin species *Echinometra mathaei* and *E. oblonga* (Palumbi et al. 1997), the coral reef fishes *Acanthurus triostegus* (Planes and Fauvelot 2002) and *Scarus psittacus* (Winters et al. 2010), and the snapper fishes *Lutjanus kasmira* and *L. fluvus* (Gaither et al. 2010). The biogeographic genetic break has commonly been explained by a combination of parameters, such as past changes in climate and water mass circulation and random long-distance dispersal events (e.g., Palumbi et al. 1997; Planes and Fauvelot 2002; Lord et al. 2012). Thus, besides geographic isolation and present-day oceanic currents, historical factors should be taken into account to explain high divergence of the Central Pacific populations. If the barrier to gene flow has been more permeable in the past, during periods of low sea level or current reversals, then CPO populations of *M. lar* could have historically been in contact, at least sporadically, with Northwestern Pacific populations through episodic migration events.

The Southwest Indian Ocean (SWIO)

Populations of *M. lar* in the Indian Ocean do not represent a genetically homogeneous assemblage, with high divergence found between geographically close localities. Both the haplotype networks and pairwise F_{ST} comparisons revealed significant genetic structuring between the NWIO populations (Mayotte and Seychelles islands) and a group made up of the SWIO populations (Madagascar, Réunion, and Mauritius). This pattern is in agreement with what was previously observed in the reef fish *Myripristis berndti* for which genetic structure analyses indicated an important isolation of Réunion and Madagascar from Mayotte (Muths et al. 2011). The geographic isolation and the biogeographic location of Réunion Island (i.e, surrounded by deep-sea areas) and the relative rarity of the species on the neighboring island (i.e., Mauritius) have been proposed to explain the genetic isolation of Réunion populations. Directionality of the prevailing oceanic currents in the Southwest Indian Ocean may have also amplified the effect of geographic distance. The Southern Equatorial Current flows in the upper 600 m from east to west between about 10° and 15°S and splits at the east coast of Madagascar into the southward East Madagascar Current and the northward East African Coastal Current (Chapman et al. 2003). The geographic location of the NWIO and SWIO populations on either side of the current may have limited genetic exchange between the two regions. In contrast, the weak genetic differentiation observed in the amphidromous fish *S. lagocephalus* between Comoros and Réunion islands by Lord et al. (2012) suggests the existence of continuous flows between those biogeographic regions that would allow inter-regional gene flow to occur. Although *S. lagocephalus* presents a longer pelagic larval duration (averaging 130 days, Lord et al. 2010) than that of *M. lar*, the high levels of population structure we observed at a small spatial scale between the SWIO and the NWIO populations are surprising. The pelagic larval duration of *M. lar* should be sufficient to allow dispersal between these islands. This genetic differentiation could reflect selective constraints on specific larval behaviors, such as orientation and sensory abilities that prevent larva from going far at sea. Some studies have shown that amphidromous prawns, including *Macrobrachium* species, can adopt larval strategies to avoid environmental stress and predation (Read 1986; Gamba and Rodriguez 1987; Paula 1998). Depending on surrounding physical factors, such strategies can indirectly lead to larval retention and self-recruitment (Sponaugle et al. 2002). Much remains to be elucidated with regard to the basic biology, ecology, and evolution of *M. lar.*

Population connectivity between Indian and Pacific oceans

The Indo-Malay archipelago: a permissive biogeographic barrier?

Molecular analyses indicated that the Indo-Malay archipelago did not act as a strong barrier to gene flow among

the Indian and the Pacific populations of *M. lar*. Although the AMOVA revealed that the populations were differentiated from one another, the presence of shared haplotypes between the NWPO (i.e, Indonesia and Caroline Islands), the NWIO (i.e, Mayotte and Seychelles islands), and the SWPO (i.e, New Caledonia, Vanuatu, Hoorn Islands, and Fiji) in the 16S haplotype network supported the hypothesis that the larval dispersal of *M. lar* may have in the past allowed some short-distance migrations from one island to another across these biogeographical regions. Although the variability of the nuclear marker was much lower than that of the 16S mtDNA, the 28S data tended to support this hypothesis, as these regions appeared genetically homogeneous while populations located at the outer edge of the species range were slightly differentiated. The number and proximity of islands in and around the Indo-Malay archipelago may have increased the connectivity and decreased bottleneck effects in the region. Consistent with this, in the Indo-Malay archipelago, *M. lar* had high genetic diversity, lower levels of genetic structure both among populations within the region as well as between each of the NWIO and SWPO regions, and no departure from mutation-drift equilibrium. Our results may therefore support the "Pleistocene marine dispersal hypothesis" proposed by de Bruyn and Mather (2007) based on genetic data from the giant prawn *Macrobrachium rosenbergii*. Expansion of freshwater systems and reduction of geographic distances between freshwater habitat patches may have promoted marine dispersal in the Indo-Malay archipelago. Even though a certain level of population structure was observed in *M. lar* between distant regions, our results provide no evidence of a profound effect of the Indo-Pacific barrier. If present, the effects of this barrier on the connectivity of *M. lar* were no stronger than the isolating mechanisms observed at the outer edge of the species range.

Overall, our results reinforce the hypothesis that the primary factor driving connectivity in *M. lar* may be the geographic distances between habitat patches. Between the NWPO and the NWIO, coastlines bordering the northern part of the Indian Ocean display numerous freshwater systems that may represent suitable habitats for *M. lar*. In this context, even with a short larval life, NWPO and SWIO populations could be connected through a stepping-stone model of dispersal. The same hypothesis can be drawn for the NWPO and the SWPO regions that are linked by numerous insular freshwater systems.

Conclusion

Our study provides new and comprehensive insights into the genetic connectivity of the widespread amphidromous prawn *M. lar*. Three major results are highlighted. First, the Central Pacific populations, located at the eastern edge of the species range, present highly significant population structure with all other Indo-Pacific populations. The vast distances of open ocean separating the Western and Central Pacific islands might have a strong effect in the limitation of larval dispersal. Second, the Indo-Pacific Barrier did not act as a strong barrier to gene flow among Indian and Pacific populations; last, a genetic break is observed at a small spatial scale, between the Southwest and the Northwest populations of the Indian Ocean.

In the tropical Indo-Pacific, insular freshwater populations are subject to recurrent local extinctions caused by extreme climatic and hydrological seasonal variations. Therefore, population genetic structure might be increased by recurrent bottleneck events (McDowall 2007). Although sampling bias cannot be ruled out, such effects were not detected in the central regions of the species range that gather a high number of islands separated by small geographic distances. This suggests that gene exchanges might have been frequent in central regions of the species range. However, here, patterns of population genetic structure vary in accordance with the biogeographic region and the scale of the habitat patches. This suggests that *M. lar* probably has a complex pattern of connectivity within the Indo-Pacific.

Connectivity in *M. lar* may result from a stepping-stone pattern of dispersal that might have been relatively high in the past across the NWIO, NWPO, and the SWPO, but that decreased at the end of the species range where past bottleneck events are detected. Possible explanation would be that peripheral freshwater habitats, physically separated from central freshwater habitats, might substantially differ by environmental parameters. Organisms are generally not simultaneously adapted to these contrasting environmental conditions. The unusual ecological conditions of peripheral freshwater systems could strengthen geographical barriers and limit gene flow (e.g., Gaither et al. 2010). However, these hypotheses are difficult to test. Additional data, including larger number of individuals, additional nuclear polymorphic nuclear markers, and measures of ecological parameters, are required to strengthen our interpretations.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards All research presented in the manuscript was conducted in accordance with all applicable laws and rules set forth by their governments and institutions, and all necessary permits were in hand when the research was conducted.

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