Molecular ecology of the freshwater shrimp Caridina natalensis and comparative analysis with other amphidromous species (Decapoda, Teleostei, and Gastropoda)

Valentin de Mazancourt, Ahmed Abdou, Magalie Castelin, Céline Ellien, Clara Lord, Marion Mennesson, Clémentine Renneville, Gérard Marquet, Philippe Keith

To cite this version:

Valentin de Mazancourt, Ahmed Abdou, Magalie Castelin, Céline Ellien, Clara Lord, et al.. Molecular ecology of the freshwater shrimp Caridina natalensis and comparative analysis with other amphidromous species (Decapoda, Teleostei, and Gastropoda). Hydrobiologia, 2023, 10.1007/s10750-023-05283-7 . hal-04142539

HAL Id: hal-04142539
https://hal.sorbonne-universite.fr/hal-04142539

Submitted on 27 Jun 2023

ACCEPTED MANUSCRIPT

Molecular ecology of the freshwater shrimp Caridina natalensis and comparative analysis with other amphidromous species (Decapoda, Teleostei, and Gastropoda)

This Accepted Manuscript (AM) is a PDF file of the manuscript accepted for publication after peer review, when applicable, but does not reflect post-acceptance improvements, or any corrections. Use of this AM is subject to the publisher's embargo period and AM terms of use. Under no circumstances may this AM be shared or distributed under a Creative Commons or other form of open access license, nor may it be reformatted or enhanced, whether by the Author or third parties. By using this AM (for example, by accessing or downloading) you agree to abide by Springer Nature's terms of use for AM versions of subscription articles: https://www.springernature.com/gp/open-research/policies/accepted-manuscript-terms

The Version of Record (VOR) of this article, as published and maintained by the publisher, is available online at: https://doi.org/10.1007/s10750-023-05283-7. The VOR is the version of the article after copy-editing and typesetting, and connected to open research data, open protocols, and open code where available. Any supplementary information can be found on the journal website, connected to the VOR.

For research integrity purposes it is best practice to cite the published Version of Record (VOR), where available (for example, see ICMJE's guidelines on overlapping publications). Where users do not have access to the VOR, any citation must clearly indicate that the reference is to an Accepted Manuscript (AM) version.

Accepted manuscript

Molecular ecology of the freshwater shrimp *Caridina natalensis* and comparative analysis with other amphidromous species (Decapoda, Teleostei, and Gastropoda)

VALENTIN DE MAZANCOURT[1,2*], AHMED ABDOU[1], MAGALIE CASTELIN[3], CÉLINE ELLIEN[1], CLARA LORD[1], MARION MENNESSON[1], CLÉMENTINE RENNEVILLE[1], GÉRARD MARQUET[4], AND PHILIPPE KEITH[1]

[1] *Unité Biologie des Organismes et Écosystèmes Aquatiques (UMR BOREA 8067), Muséum national d'Histoire naturelle – CNRS – Université de Caen Normandie – Sorbonne Université – IRD – Université des Antilles; CP26, 57 rue Cuvier, 75005 Paris, France.*

[2] *Museum für Naturkunde, Leibniz Institute for Evolution and Biodiversity Science, Invalidenstraße 43, 10115 Berlin, Germany*

[3] *Institut de Systématique, Évolution, Biodiversité (UMR ISYEB 7205), Muséum national d'Histoire naturelle – CNRS – Sorbonne Université – EPHE – Université des Antilles; CP50, 57 rue Cuvier, 75005 Paris, France.*

[4] *96 rue de Richelieu, 75002 Paris, France.*

[*] *Corresponding author. E-mail: valentin.seizilles_de_mazancourt@sorbonne-universite.fr*

Abstract

Due to their life cycle shared between rivers and oceans, amphidromous organisms serve as intriguing models for studying biogeography. To investigate the implications of their unique life history, we examined the population structure of the amphidromous shrimp Caridina natalensis across its known range in the South Western Indian Ocean. A total of 118 specimens were collected from 7 islands (Mayotte, Mohéli, Mahé, Praslin, Silhouette, Mauritius and Madagascar) and the African mainland (South Africa), and their 16S rRNA and Cox1 mitochondrial genes were sequenced. Our findings reveal significant regional structure among archipelagos, suggesting complex patterns of dispersal involving successive events of extinction-recolonization. By conducting a comparative analysis with six other amphidromous species from the South Western Indian Ocean, based on literature sources, we were able to draw conclusions regarding the amphidromous biogeography of the area. Furthermore, we propose a novel classification of amphidromous species, considering their population structure and life history traits. We defined four categories of increasing dispersal abilities and decreasing genetic population structure: 1. Land-locked species; 2. Species with reduced or facultative amphidromy; 3. Species with common amphidromy; and 4. Super-amphidromous species. Lastly, we identified the Comoros Islands (namely Mayotte and Mohéli) as a critical area for the dispersal of amphidromous species, emphasizing the need for prioritizing conservation efforts in this region.

Keywords: Amphidromy, Biogeography, Conservation, Population structure, Haplotype networks, mitochondrial markers

Competing interests: The authors declare no financial or non-financial interests that are directly or indirectly related to the present work.

Acknowledgements

Comoros archipelago: thanks to Ibrahim Yahaya, Abdallah Ahmed Soilihi and Halidi Ahmed Ben Ali. We are also grateful to Conservation International (CI) and particularly to F. Hawkins and CI Madagascar.

Mayotte: Thanks to DAF (Direction de l'agriculture et de la forêt) and ARDA (Association Réunionaise de Développement de l'Aquaculture).

Seychelles: We thank H. Grondin of ARDA (Association Réunionaise de Développement de l'Aquaculture) in Reunion Island for giving us specimens from this archipelago.

Magagascar: We thank the DIAMSOI team (The IH-SM from Tuléar with Dr Man-Wai Rabenevanana and the MNHN from Paris with E. Feunteun, P. Keith and T. Robinet) and the ARDA team (P. Bosc, H. Grondin and P. Valade). We are grateful to Joe Aride from Madagascar National Parcs (MNP-ANGAP) and to the manager of Masoala National Parc at Maroantsetra. Lastly, we want to thank all the Responsible Chiefs of the areas concerned for their kind permission, and the villages and communities who have always heartily received us and helped us in our prospecting of rivers. This research was financed by the Institut Français de la Biodiversité (IFB) for the DIAMSOI program (2007-2010).

South Africa: We are grateful to Patrick Kubheka and Helen James for facilitating collecting permits for us with provincial conservation urgencies in KwaZulu-Natal and Eastern Cape (permit no. CRO69/17CR), respectively. We thank Nick and Tim McClurg, Patrick Kubheka, Lazola Maliwa and Pascal Tiberghien for their help in the field and Albert Chakona (SAIAB) for lending us his electric fishing gear.

Thanks to D. Christopher Rogers for providing us specimens from Mauritius.

Thanks to the Service de Systématique Moléculaire of the MNHN.

Introduction

Insular freshwater systems of the tropical Indo-Pacific are mainly dominated by diadromous species (*i.e.* organisms that migrate between freshwater and marine environments during their life cycle, see Myers, 1949). This particular life trait represents a very important adaptation for the colonization of these distinctive habitats, which are oligotrophic environments subject to extreme climatic and hydrologic seasonal variations (Keith, 2003; McDowall, 2007). Spending part of their life cycle at sea, diadromous species are able to escape drought or cyclonic flood events in the rivers, and to colonize new and sometimes distant environments through their oceanic dispersal phase. Amphidromy, a particular case of diadromy, is well represented in insular freshwater macrofaunal biodiversity (Keith, 2003, McDowall, 2007, Keith and Lord, 2011b). In amphidromous species, the adults grow, feed and reproduce in rivers. After hatching, larvae use the river flow to drift to the sea where they undergo larval development (Shen and Tzeng, 2002, 2008; Crandall et al., 2009; Lord et al., 2010; Ellien et al., 2020). After larval development in coastal or open ocean environments, the competent larvae (*i.e.* post-larvae) must find and enter the mouth of a coastal river or stream. They actively swim towards the river mouth, probably attracted by the freshwater signal, which they can perceive from the open sea (Murphy and Cowan, 2007). In contact with desalinated water at the river mouth, post-larvae metamorphose into juveniles and migrate up to the adult freshwater habitat, sometimes at considerable distances from the sea.

The larvae of amphidromous organisms are planktonic during the marine stage and as such, their dispersal patterns are influenced by the currents, which they cannot resist, as well as by biotic factors such as their breeding season, potential diel vertical migration, food availability, specific behaviour which varies between species, more or less lengthy larval duration, etc. Even if PLD (Pelagic Larval Duration) is not a proxy of larval dispersal capacities, the longer an individual stays at sea, the further it may go from its hatching site, but also the higher the probability it dies before encountering a suitable habitat to pursue its life cycle (Heim-Ballew et al., 2020). This context makes the marine dispersal phase difficult to study, and no larva of amphidromous species has ever been caught at sea. Besides, amphidromy may show variations across taxa depending on the physiology and/or behaviour of the larvae. The study of population structure through haplotype networks can enlighten us on the dispersal capacities of amphidromous species and in the Indian Ocean, hypotheses on larval dispersal vary according to the taxa considered (e.g. Wood et al., 2019). Some species are widespread throughout the Indo-West Pacific area and show no population structure in the Indian Ocean as there is a strong connectivity between the different areas studied; these species probably have good dispersal abilities (see Cook et al., 2009). Species with a more structured population pattern with clear geographical isolation can indicate limited dispersal abilities or different larval behaviour and/or ecology.

When no data is available about the biology of a species, studying its population structure can be a good proxy to predict its resilience against threats, in our case, the capacity to colonize new habitats or recolonize habitats after catastrophic events (natural or caused by anthropogenic activities), but also to inform about connectivity between isolated landmasses.

Amphidromous species are known in many taxa, namely decapod crustaceans in the families Atyidae and Palaemonidae (Dennenmoser et al., 2010; Bauer, 2013), gastropods in the family Neritidae (Crandall et al., 2009; Abdou et al., 2015) or fish in the families Eleotridae, Gobiidae and Kuhliidae (Chubb et al., 1998; Keith and Lord, 2011a, 2011b; Feutry et al., 2013; Mennesson et al., 2015).

Caridina natalensis De Man, 1908 is an amphidromous shrimp of the family Atyidae commonly found in the lower reaches of rivers flowing to the South-West Indian Ocean (SWIO). It has a very complex taxonomic history, being formerly known as *C. longirostris* H. Milne Edwards, 1837 in Mayotte (see Hoareau and Valade, 2017 or Lagarde et al., 2020 for example), in Reunion Island (see Keith et al., 1999), in Seychelles (Keith et al., 2006) and in Madagascar (Holthuis, 1965) or as *C. brachydactyla* De Man, 1908 in South Africa (Richard and Clark, 2010), but it was recently revised and is now considered a valid species (de Mazancourt et al., 2019), a taxonomic stabilisation which facilitates the understanding of its distribution area.

The aim of this paper is to study the genetic structure of *Caridina natalensis* populations occurring on Comoros (Mayotte and Mohéli), Seychelles (Mahé, Praslin and Silhouette), Mascarenes (Mauritius), Madagascar and South Africa and to infer possible dispersal trajectories of larvae. By conducting this analysis, it will be possible to assess the level of genetic isolation among these populations, thereby identifying key areas crucial for the dispersal of the species, which will help implementing appropriate measures to manage and protect the habitats.

Using numerous specimens collected by teams from the Muséum national d'Histoire naturelle (MNHN, Paris) over the last 20 years in rivers of South Africa, North-East Madagascar, Comoros, Mascarenes and Seychelles, we explored the population genetic structure of *C. natalensis*. In order to investigate the existence of shared patterns of connectivity among species with this life cycle, we compared its genetic structure to that of other amphidromous organisms found in the Western Indian Ocean region, such as the decapod crustacean *Macrobrachium lar* (Fabricius, 1798) (see Castelin et al., 2013), the gastropod *Septaria* *borbonica* (Bory de Saint-Vincent, 1804) (see Abdou, 2021), *Neritina stumpffi* Boettger, 1890 (see Abdou et al., 2019) and three fish, *Eleotris klunzingerii* Pfeffer, 1893 (Mennesson et al., 2018), *Kuhlia rupestris* (Lacepède, 1802) (see Feutry et al., 2013) and *Sicyopterus lagocephalus* (Pallas, 1770) (see Lord et al., 2012).

Material and Methods

Field data collection

A total of 118 individuals of *Caridina natalensis* were sampled from Seychelles Islands (Praslin, Mahé and Silhouette), Comoros Islands (Moheli and Mayotte), Mauritius Island, Madagascar and South Africa (Supp. Tab. 1). All specimens were collected by electrofishing (portable Dekka 3,000 electric device, Germany) and by hand nets (for South African localities). In the field, caught specimens were fixed in 95% ethanol then preserved in 75% ethanol. All material has been deposited in the collections of the MNHN in Paris (France).

DNA extraction, amplification and sequencing

DNA was isolated from abdominal tissues using the semi-automatic Eppendorf ep-Motion 5075 robot following the NucleoSpin 96 Tissue Core (Macherey-Nagel) protocol. Fragments of the two mitochondrial markers *16S* rRNA (~ 510 bp) and *Cytochrome c oxydase subunit 1* (*Cox1*) (~ 650 bp) gene were amplified using respectively the pairs of primers 16Sar-Lmod (TACTTCTGCCTGTTTATCAAAAA) / 16Sbmod (GGTCTGAACTCAAATCATGTAAA) (de Mazancourt et al. 2019) and LCOI1490 (GGTCAACAAATCATAAAGATATTGG) / HCO2198 (TAAACTTCAGGGTGACCAAAAAATCA) (Folmer et al., 1994). DNA amplification was performed in 20µl PCR reactions, containing approximately 3 ng of template DNA, 2.5 mM MgCl2, 0.26 mM of each nucleotide, 0.3 µM of each primer, 5% DMSO, 1 ng of BSA and 1.5 units of QBIOTAQ polymerase (MP Biomedicals). Amplification products were generated by an initial denaturation step of 4 min at 94°C followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 52°C (*16S*) or 48°C (*Cox1*) for 40 sec, extension at 72°C for 60 sec and a final extension step at 72°C for 7 min.

PCR products were sequenced using the same primers and in both directions to insure the accuracy of base calls. Chromatograms were viewed and edited using Geneious v.8 software (http://www.geneious.com/ (Kearse et al. 2012)). All sequences were deposited in GenBank (Numbers XXXXXX to XXXXXX for *16S* and XXXXXX to XXXXXX for *Cox1*, see Supp. Tab. 1).

Molecular analyses

DNA sequenced for the study and two sequences retrieved from GenBank as outgroups (*Caridina gracilipes* and *C. typus*) were aligned using MEGA X software (Kumar et al., 2018) with Muscle algorithm (Edgar, 2004) and concatenated using Geneious. Using Bayesian information criterion in jModelTest (Darriba et al., 2012; Guindon and Gascuel, 2003) the GTR + G + I model was retained for both concatenated markers (*16S* rRNA and *Cox1*). Best-scoring Maximum Likelihood (ML) trees were estimated using RAxML HPC2 v.8.2.10 (Stamatakis, 2014) and best-scoring Bayesian Inference (BI) trees were estimated using MrBayes v.3.2.6 (Ronquist and Huelsenbeck, 2003), both methods implemented in the Cyber Infrastructure for Phylogenetic Research (CIPRES) portal v.3.1. Miller et al., 2010) (https://www.phylo.org/) with the previously determined models, running for 10,000,000 generations, a sampling frequency of 2,000 and a burn in of 25%. Support for nodes was determined using posterior probabilities calculated by MrBayes implemented in CIPRES. One hundred independent searches, each starting from distinct random trees, were conducted. Robustness of the nodes was assessed using non-parametric bootstrapping (Felsenstein 1985) with 1,000 bootstrap replicates.

Genetic diversity of *16S* and *Cox1* markers was examined using DNAsp v.6 (Rozas et al., 2017) and Arlequin v.3.5 (Excoffier and Lischer, 2010) first separately and then concatenated. For each population, 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) (Supp. Tab. 2).

To visualize the relationships among haplotypes and their geographical distribution, median-joining networks (MJN) were constructed using the program NETWORK 10.2 (Bandelt et al., 1999) with equal weights for variable sites (epsilon = 0) and connection cost calculation method. To delete superfluous median vectors and links that are not contained in the shortest tree, a post-processing maximum parsimony calculation was run.

The degree of genetic differentiation among populations was assessed using Arlequin v.3.5 with an analysis of molecular variance (AMOVA; Excoffier et al., 1992), for which the individuals were partitioned into five geographical populations according to the locality they were sampled from (Table 1). Genetic differentiation between each pair of populations was also assessed by calculating pairwise F_{ST} values (Wright, 1949). The demographic history of the various populations of *C. natalensis* was inferred using Fu's F_S statistics (Fu, 1997) implemented in the same program. To test for isolation by distance, distances between sampled populations were plotted against pairwise F_{ST} using a Mantel's test based on 10,000 permutations (Mantel, 1967) implemented in XLSTAT program (© Addinsoft) with log-transformed pairwise F_{ST} (calculated as $F_{ST}/(1 - F_{ST})$) and geographic distances in km (Rousset, 1997; Rousset and Raymond, 1997).

Results

Molecular results

A total of 104 *16S* sequences of 511 bp and 109 *Cox1* sequences of 651 bp were obtained with 95 specimens having both genes sequenced (Supp. Tab. 1) The concatenated dataset only included specimens that had information for the 16S at least.

Phylogenetic analyses.

The topologies of trees obtained separately from each marker were congruent, allowing us to concatenate the datasets. Topologies derived from both the ML and BI analyses were congruent (Figs. S1, S2, S3 and S4). Each concatenated tree obtained (Fig. 1) supports the monophyly of our specimens (PP = 1; B = 100), thus confirming they were correctly identified as *C. natalensis*. Within that clade, the deep nodes are poorly supported in both methods. Shallower clades are better supported, especially in BI, with PP ranging from 0.75 to 1 and appear to be geographically structured, with one clade of some specimens from Madagascar (PP = 0.89; B = 71), one with Mayotte and Moheli specimens (PP = 0.98; B = 62), one containing all specimens from the Seychelles and Mauritius (PP = 0.99; B = 48) and one clade (PP = 0.9; B = 72) containing two subclades, one with the remaining Malagasy specimens (PP = 0.99; B = 69) and the other with South African specimens (PP = 0.75; B = 42). Additionally, 4 South African specimens have an unresolved position within the *C. natalensis* clade.

(Figure 1)

Haplotypes analyses.

16S sequences contained 27 polymorphic sites defining 23 unique haplotypes, whereas *Cox1* sequences contained 43 polymorphic sites defining 33 unique haplotypes. Overall haplotype diversity (H_d) was quite high, with 0.892 ± 0.012 for *16S* and 0.918 ± 0.012 for *Cox1*. The concatenated dataset contained 71 polymorphic sites defining 43 unique haplotypes, with a haplotype diversity of 0.944 ± 0.014. When analysing by locality, H_d was generally low, with the lowest value being 0.471 in Madagascar (24 indiv.), and the highest being 0.792 in South

Africa (22 indiv.). These numbers exclude Mauritius for which the number of haplotypes was too low to provide accurate statistics (Supp. Tab. 3).

The median joining network (MNJ) of the concatenated dataset revealed three clear clusters: (dotted circle 1 in Fig. 2) Comoros Islands (Mayotte and Mohéli islands), Seychelles (Silhouette, Praslin, Mahé) and Mauritius with shared haplotypes and Madagascar with South Africa; well separated from (dotted circle 2) Madagascar alone; and (dotted circle 3) South Africa alone (Fig. 2). The network of the more variable *Cox1* showed a stronger geographic structure with five main genetic clusters: (1) Mayotte and Mohéli islands; (2) Silhouette, Praslin, Mahé (Seychelles) together with Mauritius islands; (3) Madagascar with South Africa; (4) Madagascar alone; and (5) South Africa alone (Fig. S5). Overall, significant genetic differentiation was detected among the eight analysed sampling localities in both *16S* (F_{ST} = 0.691, p-value < 10^{-5}) and *Cox1* (F_{ST} = 0.733, P value < 10^{-5}) (Supp. Tab. 4). Pairwise F_{ST} comparisons indicated a significantly strong level of population structure between most analysed sampling localities, except between Seychelles and Mauritius, for which no significant genetic differentiation was detected despite the great geographical distance between these two areas (Supp. Tab. 5). Based on the network and on the F_{st} results there seems to be 3 to 4 distinct populations of *C. natalensis* in the Indian Ocean: Comoros (COM), Seychelles + Mauritius (SEY+MAU), South Africa (SAF) and Madagascar (MAD). The Fst value between SAF and MAD is 0.566 and is statistically significant, showing the presence of a genetic structure, but these two localities also share haplotypes. The SAF+MAD population seem to have intermediate haplotypes between COM and SEY+MAU.

Fu's F_S value was significantly negative only for the Comoros Islands (Supp. Tab. 2), indicating a genetic pattern expected under population growth. A Mantel test showed no significant correlation (p-value = 0.139) between genetic differentiation and geographical distances (in kilometres) based on the study of the *16S* of the five geographical areas sampled. Geography explained only about 10% of the genetic variation among populations (r = 0.100, Fig. 3).

(Figure 2)
(Figure 3)

Comparison with other amphidromous species from SWIO with published haplotype networks

Through a bibliographic search, we were able to gather and compile data on six species of amphidromous organisms for which genetic population structure has been studied in the SWIO: the decapod crustacean *Macrobrachium lar* (Fabricius, 1798) (see Castelin et al, 2013), the gastropods *Septaria borbonica* (Bory de Saint-Vincent, 1804) (see Abdou, 2021) and *Neritina stumpffi* Boettger, 1890 (see Abdou et al., 2019) and three fish *Eleotris klunzingerii* Pfeffer, 1893 (see Mennesson et al., 2018), *Kuhlia rupestris* (Lacépède, 1802) (see Feutry et al., 2013) and *Sicyopterus lagocephalus* (Pallas, 1770) (see Lord et al., 2012) (Table 1, Fig. 4).

(Table 1)
(Figure 4)

Discussion

Population structure of Caridina natalensis

Analyses of the population structure of eight locations of *C. natalensis* throughout its distribution range in the South-West Indian Ocean region enable the proposal of hypotheses regarding patterns of larval dispersal. Haplotype networks show strong genetic divergences among Northeast Madagascar, South Africa, Comoros, and Seychelles. Interestingly, haplotypes from the Seychelles have been found in Mauritius. This genetic differentiation is supported by AMOVAs suggesting an important and significant regional structure. The absence of significant structure between Seychelles and Mauritius populations suggests that larval exchanges are or have been possible between these regions separated by 1,700 km. These results mostly show a consistency between geographic distribution and genetic structure of the populations and highlight the existence of potential barriers or pathways for gene flows between the surveyed regions (Barber et al, 2002). These barriers that seem particularly important between Seychelles and the other islands studied (especially when analysing the *Cox1*) could be explained by the distance between these localities. Indeed, Seychelles are distant by 1,420 km from Madagascar and the Comoros whereas the populations sampled in Mayotte and Madagascar are only separated by 570 km. However, Mauritius being 1,700 km away from Seychelles and 900 km from Madagascar harbours a *16S* haplotype shared with Seychelles but none with Madagascar. The study of specimens from Reunion Island would have been useful to confirm the pattern observed in specimens from Mauritius, but unfortunately *C. natalensis* has not been collected there despite several surveys conducted in the recent years and it is likely extirpated. Connectivity between Seychelles and Mauritius has been shown in parrotfishes but was explained by the Mascarene plateau serving as a stepping stone for migration rather than a current system connecting the two regions (Visram et al., 2010); that hypothesis does not hold for *C. natalensis*. The genetic distance thus cannot be entirely explained by geographic distance, which is confirmed by the results of the Mantel's test (Fig. 3). Since this species has a marine larval phase, the oceanic currents are likely to play a major role in the dispersal of the larvae, shaping the population structure (Roberts, 1997). It is however important to note that the observed genetic structure might be due to sampling bias, and increasing the number of individuals at each locality or including new localities would maybe have decreased the observed degrees of genetic structuration.

The distribution of genetic diversity of *C. natalensis* at the scale of its distribution range results from the geographic isolation of the freshwater habitats, but also from its complex way of dispersal. Indeed, one of the surprising results of this study is the coexistence of two distinct genetic clusters in Madagascar and in South Africa (with shared haplotypes between both localities seemingly intermediate between Seychelles/Mauritius and Comoros Islands). Considering the environmental characteristics of the islands (i.e. climatic and hydrological instability) and biological characteristics of the lifecycle of amphidromous organisms (i.e. oceanic larval dispersal), coexistence of these genetically distinct groups strongly suggests successive events of extinction/recolonization. In a fragmented habitat, spatio-temporal heterogeneity of different habitat patches can lead to local extinctions of populations (Fahrig, 2002). It is probably currently the case in Reunion Island. Extinction can however be counterbalanced by dispersal to more favourable habitat. The structure and functioning of these populations result in a dynamic system of meta-populations (Hanski, 1999) where populations are connected to their neighbours by stochastic mechanisms of dispersal, *i.e.* migration between populations. According to this hypothesis, populations in Madagascar and South Africa could for example be constituted of migrants through allo-recruitment processes (i.e. recruitment of post-larvae from distant populations) and local genotypes through auto-recruitment processes (i.e. post-larvae from local populations). Persistence of migrants in these areas could be facilitated by continuous in-flow of migrants coming from distant source populations with more favourable conditions (Hanski, 1999; Kritzer and Sale, 2004). It seems that the Comoros population is in expansion, displaying a distinct star-shaped structure (Fig. 2). The central geographic position of the archipelago in the SWIO would indeed provide good dispersal opportunities for the larvae to all the other suitable habitats of the area. However, the oceanic circulation of the Indian Ocean and in particular the strong southward Agulhas current (Fig. 5) prevents the circulation of southern haplotypes towards Seychelles in the North. The shape of the haplotype network and our analyses do not provide sufficient evidence to draw any definite conclusion about the area of origin of *C. natalensis*, but one can hypothesize that its ancestors arrived from the East carried by the dominant South Equatorial current (as shown for Atyidae by Bernardes et al., 2021) and settled in Comoros or Seychelles from where they colonized the other surrounding localities.

The population structure observed in *C. natalensis* appears to be intermediate between those observed in other amphidromous species of *Caridina*. For *C. typus* and *C. leucosticta*, occurring in Japan, the population was structured with few haplotypes at high frequencies, resulting in a star-shaped network, whereas *C. multidentata* exhibited a high number of haplotypes at low frequencies resulting in a scattered network (Fujita et al., 2016; Bernardes et al., 2017; Wood et al., 2019). In the case of our study, *C. natalensis* has a comparatively lower number of haplotypes with low frequencies. Furthermore, no clear geographical pattern could be discerned in the population structure of the previously cited species, whereas *C. natalensis* exhibits strong differentiation according to geography. On the other hand, some landlocked (*i.e.* with an abbreviated larval phase taking place entirely in freshwater) species like *C. indistincta* in Australia (Page and Hughes, 2007) or *C. africana* in South Africa (Wood et al., 2019) can show the opposite with extreme geographic structure, having haplotypes restricted to single watersheds. This would indicate that *C. natalensis* has lower dispersal abilities than amphidromous species like *C. typus*, *C. leucosticta* or *C. multidentata*, but higher than landlocked species. This could be due to a shorter larval duration (fewer zoeal stages), lower salinity tolerance or different behaviour of the larvae (Fujita et al., 2016).

Comparison with other amphidromous organisms and discussion on SWIO biogeography

The seven amphidromous species considered in this study all have in overall high haplotype diversity (e.g. Lord et al., 2012; Castelin et al., 2013), suggesting reduced connectivity between populations. In the case of widespread species, *i.e.* with Indo-Pacific distributions, Indian Ocean populations almost always show some degree of isolation compared to Pacific populations. Indeed, usually, the West Pacific populations (roughly from Indonesia to Samoa) show no structure but are isolated from French Polynesian populations (e.g. Abdou et al., 2019; Castelin et al., 2013). Moreover, judging from haplotype diversity geographical patterns, these widespread species all seem to have originated in the West Pacific and later dispersed to the Indian Ocean, never the other way around, which is congruent with the dominant oceanic currents flowing westward from Indonesia to the African coasts (Bernardes et al., 2021). Another similarity shared by all these amphidromous species is that the Comoros Islands (Mayotte + Comoros) seem to be a haplotype diversity hotspot. Its central position in the SWIO and at a crossroads of oceanic currents (Fig. 5) could indeed lead to an increased haplotype diversity as larvae originating from various locations could recruit there.

Studied amphidromous species show differences in their population structure. Firstly, three of them are restricted to the Indian Ocean (the shrimp *C. natalensis*, the snail *S. borbonica* and the fish *E. klunzingerii*) whereas the others are found on both sides of the Indo-Pacific barrier and have a wider distribution in the Indo-Pacific region. Within the SWIO, most species show a low regional structure, with haplotypes being shared by several populations. This contrasts with the results of the present study on *C. natalensis* where there is significant population structure according to geography. This difference could suggest that *C. natalensis* has lower dispersal abilities compared to the other amphidromous species considered here. Almost all species have a high haplotype diversity across the SWIO, with the exception of the goby *Sicyopterus lagocephalus* that shows a low haplotype diversity at the scale of the Indian Ocean which could be explained by a recent colonisation of the region by individuals originating from the Pacific (Lord et al., 2012) or better dispersal abilities in the area due to differences in the life history traits such as an extended marine larval dispersal which can .range from 3 to 6 months (Lord *et al.*, 2012) or different behaviour.

Freshwater species could be classified into four categories according to their population structure (Table 2):

- Land-locked species: having a very strong regional structure and high haplotype diversity due to their very low dispersal ability (i.e. direct development entirely in freshwater, no migration).
- Species with reduced amphidromy: showing some regional structure and/or high haplotype diversity due to limited dispersal abilities (i.e. short planktonic larval phase, development in estuarine conditions).
- Common amphidromous species: having a weak regional structure and high haplotype diversity due to their good dispersal abilities (i.e. long planktonic larval phase).
- Super-amphidromous species: having no regional structure and a lower haplotype diversity due to their good dispersal abilities (i.e. very long planktonic larval phase in fully marine conditions).

This classification enables the inference of life history traits of species from their population structure and to predict their distribution, both aspects being critical when assessing the conservation status of a species.

Our results are consistent with studies investigating pelagic larvae dispersal using models of SWIO oceanic surface currents. Gamoyo et al. (2019) studied the dispersal pathways of coral larvae testing four different PLD (from 5 to 60 days) and found that the Comoros archipelago was central in the connectivity network of the region for PLDs longer than 5 days. They explained this pattern by the oceanic circulation in the area, characterized by whirling currents all year round in the Mozambique Channel (Fig. 5). Furthermore, the authors identified geographic clusters that agree with our population structure for PLDs shorter than 30 days (with Seychelles separated from Comoros, which are both separated from Madagascar for instance) which would support a hypothesis of a shorter PLD for *C. natalensis* in contrast to common amphidromous species in the same genus like *C. typus* with a PLD ranging from 18 to 48 days (Nakahara et al., 2005).

Conservation implications

Haplotype networks can indeed be used as a tool for conservation biology of amphidromous species as a proxy for life history traits (Cook et al., 2009; Fujita et al., 2016; Wood et al., 2019), to assess if and which populations are increasing, decreasing or stable (Chubb et al., 1998) and to identify areas of conservation priority. In the case of *C. natalensis*, our study showed that the populations seem to be increasing in most surveyed areas, and that the species is likely to belong to the reduced-amphidromous type (Table 5). According to our bibliographic search, the Comoros Islands (Mayotte + Comoros) seem to be a critical area for amphidromous species as all the species considered show their highest haplotype diversity there (Table 4) and should be given priority for conservation efforts.

Amphidromous species require particular attention when implementing conservation efforts due to the fact that they follow the same rules as marine species for their larval life but are limited to freshwater habitats in adulthood. As a result, they should not be treated in the same way as primary freshwater species (*sensu* Myers, 1938) nor as marine species. The colonisation of habitats of amphidromous species throughout their current distribution area relies on a stepping-stone process using island streams (Han et al., 2022). Unfortunately, insular freshwater habitats are heavily threatened due to pollution like in Mauritius (pesticides from sugarcane monoculture) or Reunion Island (bleach poisoning for commercial fishing) or in the Comoros, where rivers are used as running water for domestic purposes (washing clothes, dishes, etc.). This pollution can lead to eutrophication of the watercourses, often associated with deoxygenation of the water, incompatible with the recruitment of certain amphidromous species (C. Ellien, pers. comm.). Then, some of these stepping-stones have already disappeared, like in

Rodrigues Island where no permanent rivers remain due to deforestation (see de Mazancourt et al., 2021). Two freshwater shrimps have already been impacted, as *C. natalensis* seems to have been extirpated from Reunion Island and *Macrobrachium hirtimanus*, endemic to the Mascarenes (assessed by the IUCN as Endangered in 2013) has not been reported since the 1980's (Keith et al., 2006). Amphidromous species rely on the sea and on the entire river courses to fulfil their life cycle as they undertake two migrations: downstream migration at hatching and upstream migration as they recruit back to rivers. Continous ocean-river corridors should be maintained and river systems from lower to upper courses should remain free of any impassable barrier. These conditions are absolutely necessary for the maintenance of amphidromous species.

Conclusions

Our study has given new insights into the population structure of the amphidromous shrimp *C. natalensis*. By comparing it with other amphidromous species from the South Western Indian Ocean, we were able to draw conclusions about the local biogeography, highlighting the importance of the Comoros Islands as a central hub for the dispersal of amphidromous species. Furthermore, a new classification of amphidromous species was proposed based on population structure and life history traits. Further research is required to enhance our understanding of the biology of these species, as there are still many unknown aspects regarding their larval development (such as larval stage duration, depth, salinity, etc.). These factors could influence our understanding of their dispersal abilities and facilitate the implementation of a more informed conservation strategy..

References

Abdou, A. (2021). New taxonomic and phylogeographic data on three nominal species of the genus *Septaria* Férussac, 1807 (Gastropoda: Cycloneritida: Neritidae). *Zootaxa*, *4915*(1), 28–40. https://doi.org/https://doi.org/10.11646/zootaxa.4915.1.2

Abdou, A., Keith, P., & Galzin, R. (2015). Freshwater neritids (Mollusca: Gastropoda) of tropical islands, amphidromy as a life cycle, a review. *Revue d'écologie (Terre et Vie)*, *70*(4), 387–397.

Abdou, A., Lord, C., Keith, P., & Galzin, R. (2019). Phylogéographie de *Neritina stumpffi* Boettger, 1890 et *Neritina canalis* Sowerby, 1825 (Gastropoda, Cycloneritida, Neritidae). *Zoosystema*, *41*(12), 237–248. https://doi.org/10.5252/zoosystema2019v41a12

Atkinson, J. M. (1977). Larval Development of a Freshwater Prawn, *Macrobrachium lar* (Decapoda, Palaemonidae), Reared in the Laboratory. *Crustaceana*, *33*(2), 119–132. https://doi.org/10.1163/156854077x00025

Bandelt, H. J., P. Forster, and A. Rohl. (1999). "Median-Joining Networks for Inferring Intraspecific Phylogenies." *Molecular Biology and Evolution* 16 (1): 37–48. https://doi.org/10.1093/oxfordjournals.molbev.a026036

Barber, P. H., Palumbi, S. R., Erdmann, M. V, & Moosa, M. K. (2002). Sharp genetic breaks among populations of *Haptosquilla pulchella* (Stomatopoda) indicate limits to larval transport: patterns, causes, and consequences. *Molecular Ecology*, *11*, 659–674.

Bauer, Raymond T. (2013). "Amphidromy in Shrimps: A Life Cycle between Rivers and the Sea." *Latin American Journal of Aquatic Research* 41 (4): 633–50. https://doi.org/10.3856/vol41-issue4-fulltext-2

Bebler MH, Foltz DW (2004). Genetic Diversity in Hawaiian Stream Macroinvertebrates. *Micronesica* 37(1): 119-128.

Bernardes, Samuel C., Almir R. Pepato, Thomas von Rintelen, Kristina von Rintelen, Timothy J. Page, Hendrik Freitag, and Mark De Bruyn. (2017). "The Complex Evolutionary History and Phylogeography of *Caridina typus* (Crustacea: Decapoda): Long-Distance Dispersal and Cryptic Allopatric Species." *Scientific Reports* 7 (1). https://doi.org/10.1038/s41598-017-08494-w

Bernardes, S. C., von Rintelen, K., von Rintelen, T., Pepato, A. R., Page, T. J., & de Bruyn, M. (2021). Ecological changes have driven biotic exchanges across the Indian Ocean. Scientific Reports, 11(1), 1–10. https://doi.org/10.1038/s41598-021-02799-7

Carini, G., & Hughes, J. M. (2004). Population structure of *Macrobrachium australiense* (Decapoda: Palaemonidae) in Western Queensland, Australia: the role of contemporary and historical processes. *Heredity 2004 93:4*, *93*(4), 350–363. https://doi.org/10.1038/sj.hdy.6800510

Castelin, M., P. Feutry, M. Hautecoeur, G. Marquet, D. Wowor, G. Zimmermann, and P. Keith. (2013). "New Insight on Population Genetic Connectivity of Widespread Amphidromous Prawn *Macrobrachium Lar* (Fabricius, 1798) (Crustacea: Decapoda: Palaemonidae)." *Marine Biology* 160 (6): 1395–1406. https://doi.org/10.1007/s00227-013-2191-y

Chubb A.L., Zink R.M., Fitzsimons J.M. (1998). Patterns of mtDNA Variation in Hawaiian Freshwater Fishes: The phylogeographic Consequences of Amphidromy. *The Journal of Heredity* 89(1): 8-16.

Cook D.B., Bernays S., Pringle C.M., Hughes J.M. (2009). Marine dispersal determines the genetic population structure of migratory stream fauna of Puerto Rico: evidence from island-scale population recovery process. Journal of the North American Benthological Society 28: 709-718. https://doi.org/10.1899/09-008.1

Crandall, E. D., Taffel, J. R. and Barber, P. H. (2009). "High Gene Flow Due to Pelagic Larval Dispersal among South Pacific Archipelagos in Two Amphidromous Gastropods (Neritomorpha: Neritidae)." *Heredity* 104 (6): 563–72. https://doi.org/10.1038/hdy.2009.138

Darriba, D., Taboada, G. L., Doallo, R., and Posada, D. (2012). "JModelTest 2: More Models, New Heuristics and Parallel Computing." *Nature Methods* 9 (8): 772–772. https://doi.org/10.1038/nmeth.2109

Dennenmoser S., Thel M., Schubart C. D. (2010). High genetic variability with no apparent geographic structuring in the mtDNA of the amphidromous river shrimp *Cryphiops caementarius* (Decapoda: Palemonidae) in northern-central Chile. *Journal of Crustacean Biology*, 30: 762-765. https://doi.org/10.1651/09-3273.1

Edgar, R. C. (2004). "MUSCLE: Multiple Sequence Alignment with High Accuracy and High Throughput." *Nucleic Acids Research* 32 (5): 1792–97. https://doi.org/10.1093/nar/gkh340

Excoffier, L., Smouse, P. E., & Quattro, J. M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics*, *131*(2), 479–491. https://doi.org/10.5962/bhl.title.86657

Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, *10*(3), 564–567.

Fahrig, L. (2002). Effect of habitat fragmentation on the extinction threshold: A synthesis. *Ecological Applications*, *12*(2), 346–353. https://doi.org/10.1890/1051-0761(2002)012[0346:eohfot]2.0.co;2

Felsenstein, J. (1985). “Confidence Limits on Phylogenies: An Approach Using the Bootstrap.” *Evolution* 39 (4): 783–91.

Feutry, P., Valade, P., Ovenden, J. R., Lopez, P. J., Keith, P., Feutry, P., Valade, P., Ovenden, J. R., Lopez, P. J., & Keith, P. (2012). Pelagic larval duration of two diadromous species of Kuhliidae (Teleostei: Percoidei) from Indo-Pacific insular systems. *Marine and Freshwater Research*, *63*(5), 397–402. https://doi.org/10.1071/MF11243

Feutry P, Vergnes A, Broderick D, Lambourdiere J Keith P, Ovenden JR (2013). Stretched to the limit: can a short pelagic larval duration connect adult populations of an Indo-Pacific diadromous fish (*Kuhlia rupestris*)?. *Molecular ecology* 2013 22(6): 1518-1530.

Fu, Y. X. (1997). Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, *147*(2), 915–925. https://doi.org/genetics.org//147/2/915

Fujita, Junta, Kei Zenimoto, Akira Iguchi, Yoshiaki Kai, Masahiro Ueno, and Yoh Yamashita. (2016). “Comparative Phylogeography to Test for Predictions of Marine Larval Dispersal in Three Amphidromous Shrimps.” *Marine Ecology Progress Series* 560: 105–20. https://doi.org/10.3354/meps11911

Gamoyo, M., Obura, D., & Reason, C. J. C. (2019). Estimating Connectivity Through Larval Dispersal in the Western Indian Ocean. *Journal of Geophysical Research: Biogeosciences*, *124*(8), 2446–2459. https://doi.org/10.1029/2019JG005128

García-Velazco, H., Maeda-Martínez, A. M., Obregón-Barboza, H., Rodríguez-Almaraz, G., Villalobos-Hiriart, J. L., & Murugan, G. (2014). Evidence of Oceanic Dispersal of a Disjunctly Distributed Amphidromous Shrimp in Western North America: First Record of *Macrobrachium occidentale* from the Baja California Peninsula. *Journal of Crustacean Biology*, *34*(2), 199–215. https://doi.org/10.1163/1937240X-00002217

García-Velazco, H., Maeda-Martínez, A. M., Obregón-Barboza, H., Campos-Torres, O., & Murugan, G. (2017). The systematics of the Mexican populations of *Macrobrachium digueti* (Bouvier, 1895) (Decapoda: Caridea: Palaemonidae). *Journal of Crustacean Biology*, *37*(2), 168–186. https://doi.org/10.1093/JCBIOL/RUX008

Guindon, S, and Gascuel, O. (2003). “A Simple, Fast, and Accurate Algorithm to Estimate Large Phylogenies by Maximum Likelihood.” *Systematic Biology* 52: 696–704.

Han, C., Lai, C., Huang, C., Wang, I.-C., Lin, H., & Wang, W. (2022). Phylogeographic Structuring of the Kuroshio-Type Prawn Macrobrachium japonicum (Decapoda: Palaemonidae) in Taiwan and Ryukyu Islands. *Diversity*, *14*(8), 617. https://doi.org/10.3390/d14080617

Hanski, I. (1999). *Metapopulation ecology*. Oxford University Press.

Heim-Ballew, H., Moody, K. N., Blum, M. J., McIntyre, P. B., & Hogan, J. D. (2020). Migratory flexibility in native Hawai’ian amphidromous fishes. *Journal of Fish Biology*, *96*(2), 456–468. https://doi.org/10.1111/jfb.14224

Hoarau, P. E., & Valade, P. B. (2017). Note sur la fécondité de *Caridina longirostris* H . Milne Edwards , 1837 et de son évolution au cours de l’incubation sur une petite rivière du Nord de Mayotte , archipel des Comores , Sud-Ouest de l’océan Indien (Decapoda : Atyidae). *Cahiers Scientifiques de l’océan Indien Occidental*, *8*, 5–12.

Holthuis, L. B. (1965). The Atyidae of Madagascar. *Mémoires Du Muséum National d'Histoire Naturelle, Série A, Zoologie, 33*(1), 1–48.

Hughes, J. M., Bunn, S. E., Hurwood, D. A., Choy, S., & Pearson, R. G. (1996). Genetic differentiation among populations of Caridina zebra (Decapoda: Atyidae) in tropical rainforest streams, northern Australia. *Freshwater Biology*, *36*, 289–296.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., & Drummond, A. (2012). Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, *28*(12), 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Keith, P., Vigneux, E., & Bosc, P. (1999). *Atlas des poissons et des crustacés d'eau douce de la Réunion*. Paris: Muséum national d'Histoire naturelle.

Keith, P. (2003). "Biology and Ecology of Amphidromous Gobiidae of the Indo-Pacific and the Caribbean Regions." *Journal of Fish Biology* 63 (4): 831–47. https://doi.org/10.1046/j.1095-8649.2003.00197.x

Keith, P., Marquet, G., Valade, P., Bosc, P., & Vigneux, E. (2006). *Atlas des poissons et des crustacés d'eau douces des Comores, Mascareignes et Seychelles*. Paris: Muséum national d'Histoire naturelle.

Keith, P., Lord C. (2011a). Biology and Ecology of amphidromous Gobiidae in the Indo-pacific and the Carrbean regions. *Journal of Fish Biology* 63:831-847.

Keith, P., Lord C. (2011b). Tropical freshwater gobiies: amphidromy as a life cycle. In: Patzner RA, Van Tessel JL, Kovacic M, Kapoor BG (eds). *The Biology of gobies*. Science Publishers, St Helier: 119-128.

Kritzer, J. P., & Sale, P. F. (2004). Metapopulation ecology in the sea: from Levins' model to marine ecology and fisheries science. *Fish and Fisheries*, *5*(2), 131–140. https://doi.org/10.1111/J.1467-2979.2004.00131.X

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, *35*(6), 1547–1549. https://doi.org/10.1093/molbev/msy096

Lagarde, R., Teichert, N., Valade, P., & Ponton, D. (2020). Structure of small tropical island freshwater fish and crustacean communities: A niche-or dispersal-based process? *Biotropica*, 1–12. https://doi.org/10.1111/btp.12865

Levins, R. (1969). Some Demographic and Genetic Consequences of Environmental Heterogeneity for Biological Control. *Bulletin of the Entomological Society of America*, *15*(3), 237–240. https://doi.org/10.1093/besa/15.3.237

Liao, T. Y., Lu, P. L., Yu, Y. H., Huang, W. C., Shiao, J. C., Lin, H. Du, Jhuang, W. C., Chou, T. K., & Li, F. (2021). Amphidromous but endemic: Population connectivity of *Rhinogobius gigas* (Teleostei: Gobioidei). *PLOS ONE*, *16*(2), e0246406. https://doi.org/10.1371/JOURNAL.PONE.0246406

Lord C, Brun C, Hautecoeur M, Keith P (2010). Comparaison of the duration of the marine larval phase estimated by otolith microstructures analysis of three amphidromous *Sicyopterus* species (Gobiidae: Sicydiinae) from Vanuatu and New Caledonia insights an endemism. *Ecology of Freshwater Fish* 19: 26-38.

Lord, C., Lorion, J., Dettai, A., Watanabe, S., Tsukamoto, K., Cruaud, C., & Keith, P. (2012). From endemism to widespread distribution: Phylogeography of three amphidromous *Sicyopterus* species (Teleostei: Gobioidei: Sicydiinae). *Marine Ecology Progress Series*, *455*, 269–285. https://doi.org/10.3354/meps09617

Lord, C., Maeda, K., Keith, P., & Watanabe, S. (2015). Population structure of the asian amphidromous Sicydiinae goby, *Stiphodon percnopterygionus*, inferred from mitochondrial COI sequences, with comments on larval dispersal in the northwest pacific ocean. *Vie et Milieu*, *65*(2), 63–71.

Mantel, N. (1967). The Detection of Disease Clustering and a Generalized Regression Approach. *Cancer Research*, *27*(2 Part 1).

Maruyama, A., Yamada, Y., Yuma, M., & Rusuwa, B. (2001). Stable nitrogen and carbon isotope ratios as migration tracers of a landlocked goby, *Rhinogobius sp.* (the orange form), in the Lake Biwa water system. *Ecological Research*, *16*, 697–703.

de Mazancourt, V., Castelin, M., Renneville, C., Mlambo, M. C., Marquet, G., & Keith, P. (2019). Revalidation of *Caridina natalensis* De Man, 1908 (Crustacea: Decapoda: Atyidae) in the South western Indian Ocean. *Zootaxa*, *4543*(3), 375–387. https://doi.org/10.11646/zootaxa.4543.3.3

de Mazancourt, V., Klotz, W., Marquet, G., Mos, B., Rogers, D. C., & Keith, P. (2021). New Insights on Biodiversity and Conservation of Amphidromous Shrimps of the Indo-Pacific islands (Decapoda: Atyidae: *Caridina*). In T. Kawai & D. C. Rogers (Eds.), *Recent Advances in Freshwater Crustacean Biodiversity and Conservation* (pp. 381–404). CRC Press. https://doi.org/10.1201/9781003139560-12

McDowall, R M. (2007). “On Amphidromy, a Distinct Form of Diadromy in Aquatic Organisms.” *Fish and Fisheries* 8 (1): 1–13. https://doi.org/10.1111/j.1467-2979.2007.00232.x

Mennesson, M., Tabouret, H., Pécheyran, C., Feunteun, E., & Keith, P. (2015). Amphidromous life cycle of *Eleotris fusca* (Gobioidei: Eleotridae), a widespread species from the Indo-Pacific studied by otolith analyses. *Cybium*, *39*(4), 249–260. https://doi.org/10.26028/cybium/2015-394-002

Mennesson, M., Bonillo, C., Feunteun, E., & Keith, P. (2018). Phylogeography of *Eleotris fusca* (Teleostei: Gobioidei: Eleotridae) in the Indo-Pacific area reveals a cryptic species in the Indian Ocean. *Conservation Genetics*, *19*, 1025–1038.

Miller, Mark A., Wayne Pfeiffer, and Terri Schwartz. (2010). “Creating the CIPRES Science Gateway for Inference of Large Phylogenetic Trees.” In *Proceedings of the Gateway Computing Environments Workshop (GCE)*, 1–8. New Orleans.

Murphy, C. A., & Cowan, J. H. (2007). Production, Marine Larval Retention or Dispersal, and Recruitment of Amphidromous Hawaiian Gobioids: Issues and Implications. *Bishop Museum Bulletin in Cultural and Environmental Studies, 3,* 63–74.

Myers, G. S. (1938). *Fresh water fishes and west Indian zoogeography* (Vol. 3465). US Government Printing Office.

Myers, G. S. (1949). Usage of Anadromous, Catadromous and Allied Terms for Migratory Fishes. *Copeia*, *1949*(2), 80–97. https://doi.org/10.2307/1438482

Myers, M. J., Meyer, C. P., & Resh, V. H. (2000). Neritid and thiarid gastropods from French Polynesian streams: how reproduction (sexual, parthenogenetic) and dispersal (active, passive) affect population structure. *Freshwater Biology*, *44*(3), 535–545. https://doi.org/10.1046/J.1365-2427.2000.00599.X

Nakahara, Y., Hagiwara, A., Miya, Y., & Hirayama, K. (2005). Larval Rearing of Three Amphidromous Shrimp Species (Atyidae) under Different Feeding and Salinity Conditions. *Aquaculture Science*, *53*(3), 305–310.

Nei, M. (1987). *Molecular Evolutionary Genetics*. https://doi.org/10.7312/nei-92038

Oliveira, C. M. C. A., Terossi, M., Mantelatto, F. L., Oliveira, C. M. C. A., Terossi, M., & Mantelatto, F. L. (2019). Phylogeographic structuring of the amphidromous shrimp *Atya scabra* (Crustacea, Decapoda, Atyidae) unveiled by range-wide mitochondrial DNA sampling. *Marine and Freshwater Research*, *70*(8), 1078–1093. https://doi.org/10.1071/MF18272

Page, T. J., & Hughes, J. M. (2007). Radically different scales of phylogeographic structuring within cryptic species of freshwater shrimp (Atyidae: *Caridina*). *Limnology and Oceanography*, *52*(3), 1055–1066. https://doi.org/https://doi.org/10.4319/lo.2007.52.3.1055

Richard, J., & Clark, P. F. (2010). *Caridina* H. Milne Edwards, 1837 (Crustacea: Decapoda: Caridea: Atyoidea: Atyidae) - Freshwater shrimps from eastern and Southern Africa. *Zootaxa*, *337*(2372), 305–337.

Roberts, C. M. (1997). Connectivity and management of Carribean coral reefs. *Science, 278*(5342), 1454–1457. https://doi.org/10.1126/science.278.5342.1454

Ronquist, F, and Huelsenbeck, J. P. (2003). “MrBayes 3: Bayesian Phylogenetic Inference under Mixed Models.” *Bioinformatics* 19: 1572–74. https://doi.org/10.1093/bioinformatics/btg180

Rossi, N., & Mantelatto, F. L. (2013). Molecular Analysis of the Freshwater Prawn *Macrobrachium olfersii* (Decapoda, Palaemonidae) Supports the Existence of a Single Species throughout Its Distribution. *PLOS ONE*, *8*(1), e54698. https://doi.org/10.1371/JOURNAL.PONE.0054698

Rousset, F. (1997). Genetic Differentiation and Estimation of Gene Flow from F-Statistics Under Isolation by Distance. *Genetics*, *145*, 1219–1228.

Rousset, F., & Raymond, M. (1997). Statistical analyses of population genetic data: new tools, old concepts. *Trends in Ecology & Evolution*, *12*(8), 313–317. https://doi.org/10.1016/S0169-5347(97)01104-X

Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J. C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S. E., & Sánchez-Gracia, A. (2017). DnaSP 6: DNA Sequence Polymorphism Analysis of Large Data Sets. *Molecular Biology and Evolution, 34*(12), 3299–3302. https://doi.org/10.1093/molbev/msx248

Stamatakis, A. (2014). “RAxML Version 8: A Tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies.” *Bioinformatics* 30 (9): 1312–13. https://doi.org/10.1093/bioinformatics/btu033

Shen, K. N. and Tzeng, W. N. (2002a). Formation of a metamorphosis check in otoliths of the amphidromous goby *Sicyopterus japonicus*. *Marine Ecology Progress series* 228:205-211.

Shen, K. N. and Tzeng, W. N. (2002b). Reproductive strategy and recruitment dynamics of amphidromous goby *Sicyopterus japonicus* as revealed by otolith microstructures. *Journal fish* *biology* 73.2497-2512.

Taillebois, L., Castelin, M., Ovenden, J. R., Bonillo, C., & Keith, P. (2013). Contrasting Genetic Structure among Populations of Two Amphidromous Fish Species (Sicydiinae) in the Central West Pacific. *PLOS ONE*, *8*(10), e75465. https://doi.org/10.1371/JOURNAL.PONE.0075465

Tajima, F. (1983). Evolutionary relationship of DNA sequences in finite populations. *Genetics*, *105*(2), 437–460. https://doi.org/10.1093/genetics/105.2.437

Tsang, L. M., Tsoi, K. H., Chan, S. K. F., Chan, T. K. T., & Chu, K. H. (2016). Strong genetic differentiation among populations of the freshwater shrimp *Caridina cantonensis* in Hong Kong: Implications for conservation of freshwater fauna in urban areas. *Marine and Freshwater Research*, *68*(1), 187–194. https://doi.org/10.1071/MF15377

Visram, S., Yang, M. C., Pillay, R. M., Said, S., Henriksson, O., Grahn, M., & Chen, C. A. (2010). Genetic connectivity and historical demography of the blue barred parrotfish (Scarus ghobban) in the western Indian Ocean. *Marine Biology*, *157*(7), 1475–1487. https://doi.org/10.1007/s00227-010-1422-8

Watterson, G. A. (1975). On the Number of Segregating Sites in Genetical Models without Recombination. *Theoretical Population Biology*, *7*, 256–276.

Weese, D. A., Fujita, Y., Hidaka, M., & Santos, S. R. (2012). The long and short of it: Genetic variation and population structure of the anchialine atyid shrimp *Caridina rubella* on Miyako-Jima, Japan. *Journal of Crustacean Biology*, *32*(1), 109–117. https://doi.org/10.1163/193724011X615389

Wood, L. E., de Grave, S., & Daniels, S. R. (2019). A comparative evolutionary study reveals radically different scales of genetic structuring within two atyid shrimp species (Crustacea: Decapoda: Atyidae). *Zoological Journal of the Linnean Society*, *186*(1), 200–212. https://doi.org/https://doi.org/10.1093/zoolinnean/zly044

Wright, S. (1949). The genetical structure of populations. *Annals of Eugenics*, *15*(1), 323–354. https://doi.org/10.1111/j.1469-1809.1949.tb02451.x

Zhang, W., Jiang, S., Salumy, K. R., Xuan, Z., Xiong, Y., Jin, S., Gong, Y., Wu, Y., Qiao, H., & Fu, H. (2022). Comparison of genetic diversity and population structure of eight *Macrobrachium nipponense* populations in China based on D-loop sequences. *Aquaculture Reports*, *23*, 101086. https://doi.org/10.1016/J.AQREP.2022.101086

Legends to tables and figures:

Table 1. Comparison with other amphidromous species from SWIO with published haplotype networks.

Table 2. Classification of life histories in freshwater organisms.

Figure 1. Concatenated (16S + COI) phylogenetic tree of *Caridina natalensis* obtained by Bayesian Inference. Colours indicate the sampling locality of the specimens. Numbers above branches correspond to Bayesian posterior probabilities. Numbers under branches correspond to Maximum-Likelihood bootstrap numbers. Scale represents genetic p-distance.

Figure 2. Median-joining network obtained from the 16S mitochondrial marker. Circles are color-coded based on sampling locality as figured on the map and are sized according to the relative abundance of each haplotype. Each full line connecting a circle equals one mutation, each bar breaking the line correspond to one additional mutation. Small red circles represent missing or unsampled haplotypes. Photo credit: P. Keith.

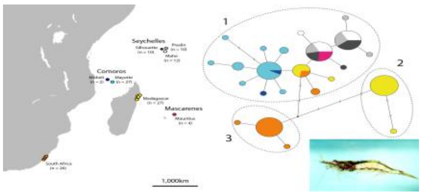
Figure 3. Isolation by distance. Genetic distance $F_{ST}/(1-F_{ST})$ calculated for each population pair from the 16S dataset plotted against geographic distance in kilometres. Regression line is pictured with a solid black line.

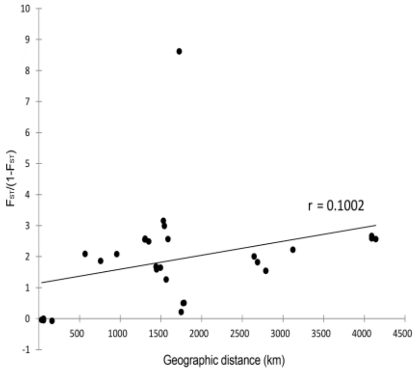
Figure 4. Haplotype networks of the other amphidromous organisms from the South-Western Indian Ocean. A: *Neritina stumpffi* from Abdou et al., 2019; B: *Macrobrachium lar* from Castelin et al., 2013; C: *Septaria borbonica* from Abdou, 2021; D: *Eleotris klunzingerii* from

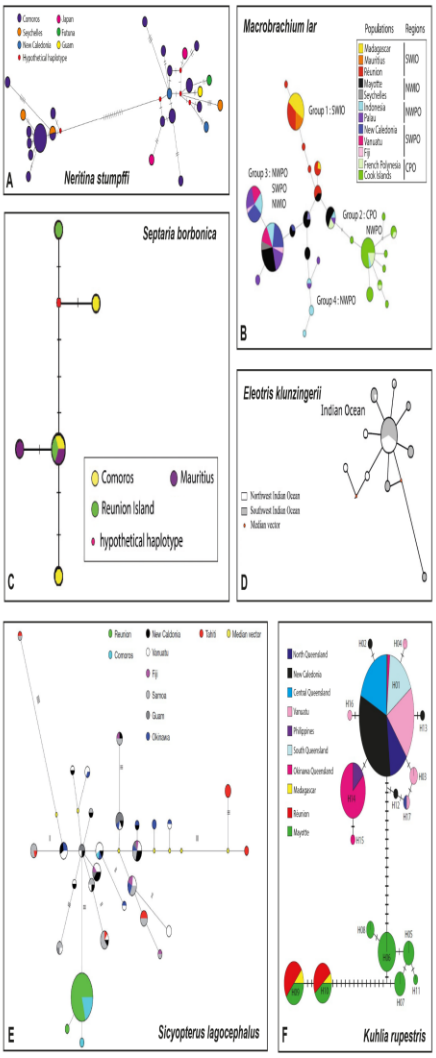
Mennesson et al., 2018; E: *Sicyopterus lagocephalus* from Lord et al., 2012; F: *Kuhlia rupestris* from Feutry et al., 2013.

Figure 5. Oceanic surface circulation in the South-Western Indian Ocean.


MK189893 Caridina typus
NC_024751 Caridina gracilipes
0.89
71
CA1035
CA1037
CA1058
CA1062
CA1063
CA1065
CA1069
CA1077
CA1079
CA1083
CA1089
CA1090
CA1091
CA1092
CA1094
CA1206
CA1207
CA1208
CA1210
0.98
CA1211
62
CA1212
CA1213
CA1214
CA1215
CA1343
CA1344
1
CA1078
100
CA1081
CA1082
CA1093
CA1096
CA1087
CA1088
CA1080
CA1095
CA1209
CA2144
CA2150
1
CA2080
99
CA2082
CA1114
CA1115
CA1116
CA1121
CA1122
CA1186
CA1189
CA1191
0.99
CA1195
CA1200
48
CA2157
CA2158
CA2159
CA2160
CA1120
CA1126
CA1193
CA1196
CA1113
CA1117
CA1118
CA1119
CA1123
CA1124
CA1125
CA1127
CA1128
CA1187
CA1188
CA1190
CA1192
CA1194
CA1197
CA1198
CA1199
CA1112
CA1034
CA1036
CA1057
CA1059
CA1060
CA1061
CA1066
CA1067
CA1070
0.99
CA1071
69
CA1074
CA1075
CA1184
CA1185
CA1226
CA1227
CA1229
CA1230
0.9
CA1068
CA1228
72
CA2079
CA2083
CA2138
CA2139
CA2141
CA2142
CA2143
Madagascar
Mayotte
Mohéli
Comoros Archipelago


Seychelles
Silhouette (n = 10)
Praslin (n = 10)
Mahe (n = 12)
Comoros
Moheli (n = 2)
Mayotte (n = 27)
Madagascar (n = 27)
Mascarenes
Mauritius (n = 4)
South Africa (n = 24)
1,000km
1
2
3


$F_{ST}/(1-F_{ST})$
10
9
8
7
6
5
4
3
2
1
0
-1
r = 0.1002
500
1000
1500
2000
2500
3000
3500
4000
4500
Geographic distance (km)


Comoros
Japan
Seychelles
Futuna
New Caledonia
Guam
Hypothetical haplotype
A
Neritina stumpffi
Macrobrachium lar
Populations
Regions
Madagascar
Mauritius
Réunion
Mayotte
Seychelles
Indonesia
Palau
New Caledonia
Vanuatu
Fiji
French Polynesia
Cook Islands
SWIO
NWIO
NWPO
SWPO
CPO
Group 1 : SWIO
Group 3 : NWPO
SWPO
NWIO
Group 2 : CPO
NWPO
Group 4 : NWPO
B
Septaria borbonica
Comoros
Mauritius
Reunion Island
hypothetical haplotype
C
Eleotris klunzingerii
Indian Ocean
Northwest Indian Ocean
Southwest Indian Ocean
Median vector
D
Reunion
New Caldonia
Tahiti
Median vector
Comoros
Vanuatu
Fiji
Samoa
Guam
Okinawa
E
Sicyopterus lagocephalus
North Queensland
New Caledonia
Central Queensland
Vanuatu
Philippines
South Queensland
Okinawa Queensland
Madagascar
Réunion
Mayotte
H01
H02
H03
H04
H05
H06
H07
H08
H09
H10
H11
H12
H13
H14
H15
H16
H17
F
Kuhlia rupestris

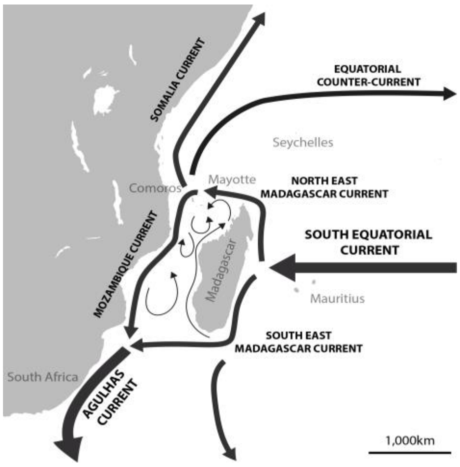


Table 1: Comparison with other amphidromous species from the South Western Indian Ocean (SWIO) with published haplotype networks.

Species	Distribution	Adult habitat	Larval habitat	Pelagic larval duration	Regional genetic structure
DECAPODA					
Caridina natalensis (Atyidae)	Endemic to the SWIO	Freshwater, lower course	Unknown	Unknown	Strong
Macrobrachium lar (Palaemonidae)	Indo-Pacific	Freshwater, ubiquitous	Marine (35.5 g/l) (Atkinson, 1977)	>89 days (Atkinson, 1977)	Strong (Castelin et al., 2013)
GASTROPODA					
Septaria borbonica (Neritidae)	Endemic to the SWIO	Freshwater, lower course	Unknown	Unknown	Weak (Abdou, 2021)
Neritina stumpffi (Neritidae)	Indo-Pacific	Freshwater, lower course	Unknown	Unknown	Weak (Abdo et al., 2019)
TELEOSTEI					
Kuhlia rupestris (Kuhliidae)	Indo-Pacific	Freshwater, ubiquitous	Marine	27–58 days (Feutry et al., 2012)	Weak (Feutr et al., 2013)

Eleotris valadei (Eleotridae)	Endemic to the SWIO	Freshwater, lower course	Marine	38–141 days (Mennesson et al., 2015)	Weak (Mennesson et al., 2018)
Sicyopterus lagocephalus (Gobiidae)	Indo-Pacific	Freshwater, ubiquitous	Marine	130 days (Lord et al, 2010)	Weak (Lord al., 2012)

Table 2: Classification of life histories in freshwater organisms.

Type of life history	**Regional structure**	**Haplotype diversity**	**Planktonic larval phase duration**	**Development conditions**	**Expected distribution**	**Examples (^{F}: fish, ^{G}: gastropod, ^{D}: decapod)**
Land-locked	Highest	Highest	Absent	Freshwater	Small (endemic to one landmass or a few watersheds)	*Caridina africana* (see Wood et al., 2019) ^{D} *Caridina cantonensis* (see Tsang et al., 2016) ^{D} *Caridina zebra* (see Hughes et al., 1996) ^{D} *Macrobrachium australiense* (see Carini & Hughes, 2004) ^{D} *Rhinogobius sp.* (see Maruyama et al., 2001) ^{F}
Reduced amphidromy (may be facultative)	Marked	High	Short to long	Estuarine to marine	Large (across multiple watersheds or short	***Caridina natalensis*** ^{D} *Caridina leucosticta* (see Fujita et al., 2016) ^{D}

					seawater stretches)	*Neritina canalis* (see Abdou et al., 2019) ^{G} *Rhinogobius gigas* (see Liao et al., 2021) ^{F} *Sicyopus zosterophorum* (see Taillebois et al., 2013) ^{F} *Lentipes concolor* (see Heim-Ballew et al., 2020) ^{F} *Eleotris sandwicensis* (see Heim-Ballew et al., 2020) ^{F} *Stenogobius hawaiiensis* (see Heim-Ballew et al., 2020) ^{F}
Common amphidromy	Marked to low	High	Long	Marine	Larger (across big seawater stretches)	***Eleotris klunzingerii*** ^{F} ***Macrobrachium lar*** ^{D} ***Neritina stumpffi*** ^{G} ***Septaria borbonica*** ^{G} *Caridina multidentata* (see Fujita et al., 2016) ^{D} *Caridina typus* (see Bernardes et al., 2017) ^{D} *Cryphiops caementarius* (see Dennenmoser et al., 2010) ^{D} *Macrobrachium digueti* (García-Velazco et al., 2017) ^{D} *Macrobrachium occidentale* (see

						García-Velazco et al., 2014) [D] *Macrobrachium olfersii* (see Rossi & Mantellato, 2013) [D] *Smilosicyopus fehlmanni* (see Taillebois et al., 2013) [F] *Stiphodon percnopterygionus* (see Lord et al., 2015) [F] *Sicyopterus aiensis* (see Lord et al., 2015) [F] *Sicyopterus sarasini* (see Lord et al., 2015) [F]
Super-amphidromy	Lowest	Lowest	Very long	Marine	Widespread (across oceans)	***Sicyopterus lagocephalus*** [F] ***Kuhlia rupestris*** [F] *Atya scabra* (see Oliveira et al., 2019) [D] *Clithon spinosus* (see Myers et al., 2000) [G] *Eleotris fusca* (see Mennesson et al., 2015) [F] *Macrobrachium japonicum* (see Han et al., 2022) [D] *Macrobrachium nipponense* (see Zhang et al., 2022)[D]