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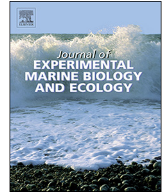

Genetic population structure of the mangrove snails *Littoraria subvittata* and *L. pallescens* in the Western Indian Ocean

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A B S T R A C T

Littoraria snails are an important component of the food chain in the mangrove ecosystem. This study intends to examine the influence of the Western Indian Ocean currents and isolation-by-distance (IBD) on the genetic diversity and structure of *Littoraria subvittata* and *Littoraria pallescens*, which are the most dominant species of *Littoraria* along the East African coast. A fragment of the mitochondrial COI gene from 334 individuals of *L. subvittata* and 134 of *L. pallescens* collected from mangroves sites in Kenya, Tanzania, Mozambique and Madagascar, was used in the analysis. Low values of nucleotide diversity (*L. subvittata*: 0.13 ± 0.10%, *L. pallescens*: 0.12 ± 0.00%) and high to moderate haplotype diversity (*L. subvittata*: 0.57 ± 0.03, *L. pallescens:* 0.55 ± 0.05) were recorded for both species. An analysis of molecular variances (AMOVA) detected a significant genetic difference among populations of *L. subvittata* (Φ_{st}: 0.093, $P < .001$) and was supported by significant IBD, while *L. pallescens* showed panmixia (Φ_{st}: 0.004, $P > .05$). The spatial analysis of molecular variance (SAMOVA) did not detect population clusters in *L. subvittata*. In contrast, SAMOVA revealed slight but significant genetic structuring between two groups of populations in *L. pallescens*. These results may indicate that *L. subvittata* is sensitive to impacts of population geographic IBD compared to *L. pallescens*. The differences in genetic structure of populations between the two species may be linked to their larval potential differences in crossing the oceanic barriers such as currents and eddies during dispersal.

1. Introduction

The snails *L. subvittata* and *L. pallescens* dominate the East African coast, with *L. subvittata* being limited to this region of the Western Indian Ocean (WIO), while *L. pallescens* is distributed throughout the tropical Indo-West Pacific (Torres et al., 2008). *Littoraria subvittata* is monomorphic with diagonal dark stripes while *L. pallescens* is a polymorphic species, varying from dark brownish, yellow to reddish in colour (Cook, 1990; Reid, 1986). The polymorphism in *L. pallescens* is thought to be controlled genetically or by predators selection (Cook, 1992).

Both species are often found on leaves or barks of mangrove trees. Like other littorinids (Christensen et al., 2001) these species probably obtain their food from leaves, roots, and trunks of mangrove trees where they feed on microepiphytes algae, epidermal plant cells and fungi. Both species belong to the subgenus *Littorinopsis*, which is ovoviviparous, brood the eggs in their mantle cavity for few days and undergo planktonic development (Reid et al., 2010). *Littoraria pallescens* has a clear growth pattern with a one-year life cycle (Sanpanich et al., 2008). There is limited information on the life cycle of *L. subvittata.*

Some studies have indicated that species with a large effective population size and planktonic development, such as *L. subvittata* and *L. pallescens*, are expected to have limited genetic population structuring (Burton, 2009; Collin, 2001; Kyle and Boulding, 2000). However, despite large effective population sizes and planktonic development in some species, sharp genetic breaks have been observed in populations separated by small geographic distances (Palumbi, 2003; Sá-Pinto et al., 2012). In general, genetic population structure is known to be governed by many factors, such as larval behaviour, effective population size, environmental factors, recruitment success of larvae, selection coefficient, mutation rate, migration rate, pattern of ocean currents, and isolation-by-distance (IBD) (Chiu et al., 2013; Hedgecock, 1986; Johnson and Black, 1998; Miller et al., 2013; Palumbi, 1994). Some of the above mentioned factors could lead to deviation in the expected pattern of genetic population structuring in the case of a large population size and planktonic larval development. Although it may be difficult to be certain which factor exactly is influencing genetic population structure of a particular species, molecular genetic methods can help marine ecologists to exclude some of these factors and suggest the potential factors that are likely to cause the observed pattern of genetic diversity and structuring (Haig, 1998).

Molecular information, such as genetic diversity and genetic population structure, is important for conservation purposes in an area like the WIO, where environmental degradation is high and marine resources are threatened by human activities (Nehemia et al., 2019; Ridgway and Sampayo, 2005). The majority of studies focused on

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genetic population structure of marine invertebrates along the WIO have reported extensive gene flow in various species (Fratini and Vannini, 2002; Silva et al., 2010b, 2013; Fratini and Vannini, 2002; Fratini et al., 2016). Nevertheless, some studies have indicated the existence of a genetic break due to oceanic eddies and currents (eg. Ragionieri et al., 2010; Silva et al., 2010b) and IBD (Madeira et al., 2012). The genetic structuring of various marine species in the WIO is influenced by oceanographic features that include ocean currents, counter-currents and eddies (Madeira et al., 2012; Otwoma and Kochzius, 2016). Apart from oceanographic features, genetic structuring and gene flow in marine species can be influenced by suitable habitats and life-history traits (Palumbi, 1994; Wang et al., 2016).

This study intends to determine the influence of oceanographic features and IBD on gene flow of *L. subvittata* and *L. pallescens,* using mitochondrial cytochrome oxidase I (COI) sequences. The sequences that assess IBD in which close populations are more similar than distant ones, can help to increase the confidence in the significance of slight genetic differentiation (Palumbi, 2003). Mitochondrial cytochrome oxidase I (COI) sequences are widely applied to detect genetic structure in marine molluscs and many studies conducted in the Indo-West Pacific have utilised this marker (Crandall et al., 2008; Kochzius and Nuryanto, 2008; Nuryanto and Kochzius, 2009; Nehemia et al., 2017; Nehemia and Kochzius, 2017). Unlike other markers used in population genetic studies, mtDNA COI data obtained from different sites and regions can easily be combined (Keyse and Crandall, 2014). The COI sequences of *L. subvittata* from previous study conducted along the Tanzanian coast (Nehemia et al., 2017) are included in this study. The results of this study will, therefore, increase the availability of population genetic data for future research in the WIO.

2. Material and methods

2.1. Sampling of populations

Samples of *L. subvittata* and *L. pallescens* were collected during low tide in natural mangroves dominated by *Avicennia marina*, *Sonneratia alba,* and *Rhizophora mucronata* along the coast of the WIO between 2014 and 2016 (Fig. 1). Samples of *L. subvittata* were collected in Kenya, Tanzania, Madagascar and Mozambique, while *L. pallescens* were collected in Tanzania and Madagascar (Table 1).

2.2. DNA extraction and amplification

About 30 mg of tissue was taken from the foot of *L. subvittata and L. pallescens* and DNA was extracted following the protocol of the E.Z.N.A.® Tissue DNA Kit (Omega Bio-Tek, California, USA). DNA was stored in the freezer for further analysis. The primers LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACT TCAGGGTGACCAAAAAATCA-3′) were used to amplify a fragment of the mitochondrial cytochrome oxidase I (COI) gene (Folmer et al., 1994). The PCR was prepared in a final volume of 50 µl, containing 2 µl of DNA extract, 1 mM PCR reaction buffer, 0.2 µM of each primer, 3.5 µl of 10 mg/ml BSA, 1 U Taq polymerase, 3 mM $MgCl_2$ and 0.2 mM dNTPs. The PCR temperature profile was as follows: initial denaturation of five minutes at 94 °C, 35 cycles at 94 °C for one minute, 41 °C for 1.5 minutes and 72 °C for one minute, and a final extension of five minutes at 72 °C. The PCR was performed in a BIO-RAD T100TM thermocycler (USA) and both strands of the PCR product were sequenced with an ABI 3770XL automated sequencer (Applied Biosystems, Foster City, USA).

2.3. Genetic and statistical analysis

Sequences were first confirmed as mtDNA COI of *L. subvittata* and *L. pallescens* by using online local alignment search tool (BLAST) at GenBank. Sequences of *L. subvittata* for site N-2, N-3, N-5, N-7, N-11, N-12 and N-13 (Fig. 1) were obtained from a previous study (Nehemia et al., 2017).The software Chromaspro (v. 1.5; Technelysium) was then used for editing and assembling the sequences of the two strands. The sequence alignment was performed by using CLUSTAL W (Thompson et al., 1994) as implemented in the software MEGA 6 (Tamura et al., 2013). The online program FaBox 1.41 was used for collapsing the sequences into haplotypes. The software Arlequin (v. 3.5.2.2; Excoffier and Lischer, 2010) was used to characterise the genetic variability of the COI sequences by calculating haplotype and nucleotide diversity (Nei and Jin, 1989) for each sample site. The program Squint Alignment Editor (v. 1.02) (Goode and Rodrigo, 2007) was used to translate nucleotides into an amino acid sequence in order to check for the presence of pseudogenes. Analysis of molecular variance (AMOVA) was performed using Arlequin to assess the genetic population structure by calculating the overall fixation index Φ_{st} among all sample sites. The same analysis was used to calculate pairwise Φ_{st}-values among sample sites and significance values were sequential Bonferroni corrected (Rice, 1989). SAMOVA 2.0 was used for spatial analysis of molecular variance which is used to define the groups of populations that are geographically homogeneous and maximally differentiated from each other (Dupanloup et al., 2002). The values for K ranged from 2 to 15 for *L. subvittata* and 2–7 for *L. pallescens* and the initial condition was set to 400. The configuration with the highest variance among clusters (F_{ct}) was retained as the best grouping of populations.

Analysis of neutrality of the marker and mismatch distribution was performed by calculating Tajima's *D* (Tajima, 1989), Fu's *Fs* (Fu, 1997), the sum of square deviations (SSD) (Rogers and Harpending, 1992) and raggedness index (Rogers, 1995) using Arlequin. Tajima's *D* test is powerful in detecting selective sweeps and Fu's Fs is sensitive in detecting demographic expansion and genetic hitchhiking (Fu, 1997). A non-significant SSD and raggedness index supports the hypothesis of population expansion. The software PopArt (Leigh and Bryant, 2015) was used to assess the genealogical relationship at the intraspecific level and to make an inference about biogeography by constructing a haplotype network. We evaluated the hypothesis of isolation by distance among the populations by plotting the geographical distances against pairwise Φ_{st} values. The geographical distances (log) were measured as the shortest path by sea between two sample sites. Mantel test was used to determine the significant correlation between the increase in genetic and geographic distance (Mantel, 1967).

3. Results

3.1. Haplotypes and nucleotide diversities

An alignment of 334 sequences with a length of 680 bp was obtained for *L. subvittata,* while the alignment for *L. pallescens* had a length of 604 bp with 134 sequences (GenBank accession nos. MG826398 - MG826531 and MG826532 - MG826717). The number of haplotypes obtained from COI sequences of *L. subvittata* was 45, and for *L. pallescens* 18. Low nucleotide diversity and moderate to high haplotype diversity were recorded for both species (Tables 2 and 3). The mean nucleotide and haplotype diversity for *L. subvittata* was 0.13 ± 0.10% and 0.57 ± 0.03, respectively, and for *L. pallescens* 0.12 ± 0.00% and 0.55 ± 0.045, respectively. The highest nucleotide and haplotype diversity for *L. subvittata* was recorded at Inhambane. Higher nucleotide diversity was also observed in populations of Vilankulo and Ile St. Marie (Table 2). For *L. pallescens*, the highest nucleotide diversity was observed at Mtwara followed by Toliara (Table 3). The highest value of haplotype diversity for *L. subvittata* was recorded at Inhambane, followed by Unguja - Ukuu and Ile St. Marie (Table 2). For *L. pallescens* the highest haplotype diversities were observed at Toliara and Mtwara (Table 3).

The number of haplotypes at each site ranged from three to seven for *L. subvittata* and two to eight for *L. pallescens*. Among 45 haplotypes detected for *L. subvittata*, 68.9% were singletons and the most dominant haplotype (h1) represents 65% of the total sequences and was present at

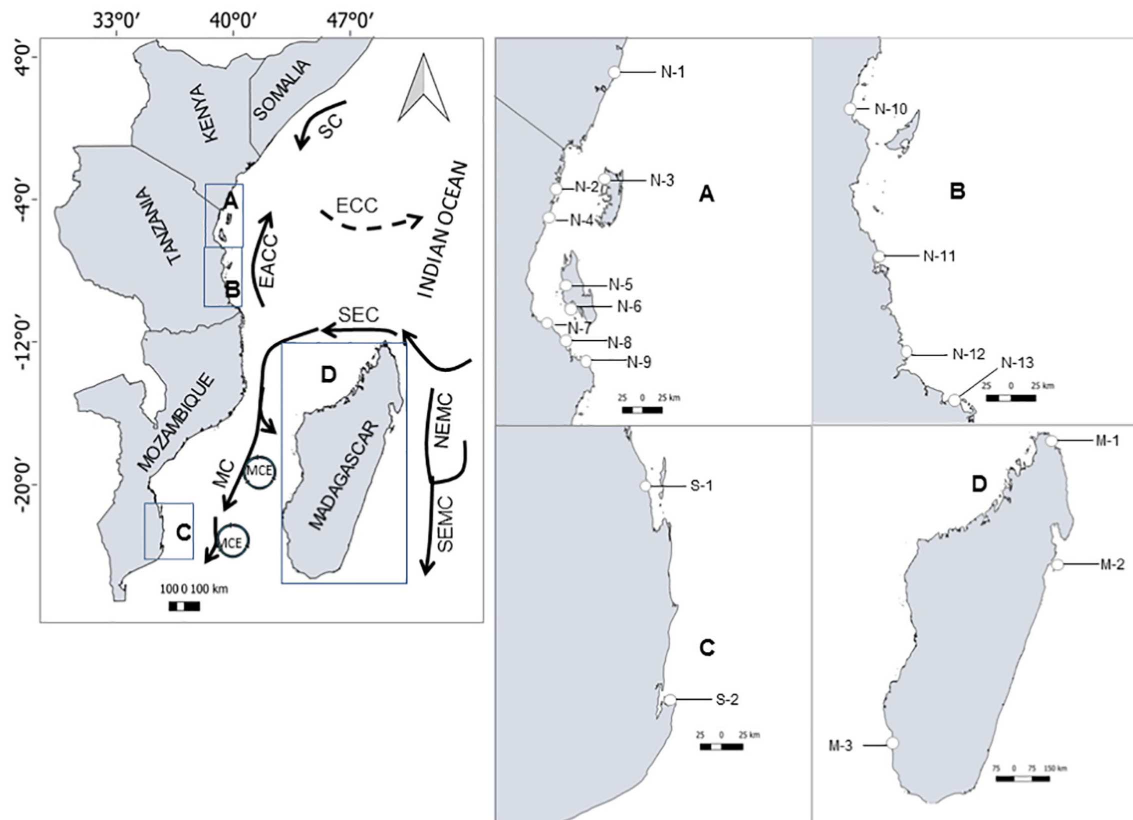

Fig. 1. A map indicating dominant ocean currents in the Western Indian Ocean with A, B, C, and D representing the regions with samples sites. Solid lines represent permanent Ocean currents; East African Coast current (EACC), South Equatorial Current (SEC), Mozambique Current (MC), Northeast Madagascar Current (NEMC) and Southeast Madagascar Current (SEMC). The dotted lines indicate seasonal ocean currents; Northern Equatorial Counter Current (NECC) and MCE is the Mozambique Channel Eddies. The numbers represent the samples sites of which details are given in Table 1.

Table 1
Sample sites and samples collected in the Western Indian Ocean.

Sites	Codes	*Littoraria subvittata*	*Littoraria pallescens*
Mtwapa	N-1	19	–
Tanga - Lumbachia	N-2	21	17
Pemba - Chakechake	N-3	21	–
Pangani	N-4	16	–
Unguja - Fujoni	N-5	23	–
Unguja - Ukuu	N-6	21	–
Bagamoyo - Kaole	N-7	21	28
Dar Es Salaam - Kunduchi	N-8	29	–
Dar Es Salaam - Kigamboni	N-9	20	20
Rufiji	N-10	21	–
Kilwa	N-11	20	18
Lindi-Mbanula	N-12	21	9
Mtwara - Ng'wale	N-13	21	9
Inhambane	S-1	17	–
Vilankulo	S-2	20	–
Nosy Be	M-1	–	17
Ile St. Marie	M-2	23	–
Toliara	M-3	–	16

all sample sites (S 1 Table). Among the 18 haplotypes identified for *L. pallescens*, 72.2% were singletons and the dominant haplotype represents 64.2% of the total sequences (S 2 Table).

3.2. Genetic population structure

Low but significant genetic differentiation among all populations was observed in *L. subvitata* (Φ_{st}: 0.093, $P < .001$), while the genetic differentiation among all populations of *L. pallescens* was much lower and non-significant (Φ_{st}: 0.004, $P > .05$). Pairwise AMOVA followed by sequential Bonferroni correction revealed genetic differentiation of populations of *L. subvitata* collected at Ile St. Marie, Inhambane, and Vilankulo from most of the other populations studied (Table 4). However, no genetic differentiation was detected in *L. pallescens* through pairwise AMOVA followed by sequential Bonferroni correction (Table 5). Two-group structure were generated by SAMOVA in *L. pallescens* (Madagascar (Toliara) versus Madagascar (Nosy Be)-Tanzania: K = 2, F_{ct} = 0.07, $P < .001$). For *L. subvittata* no genetic population clusters detected (Madagascar versus Mozambique-Tanzania-Kenya: F_{ct}: 0.17, $P = .06$). Analysis of Isolation by distance (IBD) among populations through a Mantel test detected significant correlation between the increase in genetic (pairwise Φ_{st} values) and geographic distance (log) only in *L. subvittata* ($r = 0.56$, $P < .01$) (Fig. 2 a) and not for *L. pallescens* ($r = 0.17$, $P > .05$) (Fig. 2 b).The haplotype network analysis for *L. subvittata* (Fig. 3) and *L. pallescens* (Fig. 4) resulted in a 'star-like' genealogy with rare haplotypes connected by a few mutational steps to the central haplotype.

3.3. Mismatch distribution and demographic history

The snail *L. subvittata* showed negative Fu's *Fs*-values, with all samples from Tanzania being significant from zero, except for Kilwa and Mtwara. In contrast, all populations of *L. subvittata* from Madagascar and Kenya had *Fs*-values not significant from zero (Table 2). All populations of *L. subvitata* showed non-significant negative Tajima's *D*-values, with the exception of samples from Tanga, Unguja, Bagamoyo, Kigamboni and Mtwara, which had significant negative *D*-values. Populations of *L. pallescens* exhibited non-significant values of Fu's *Fs*, except for Bagamoyo and Toliara. All populations of *L. pallescens* showed negative Tajima's *D*-values, with the exception of

Table 2
Nucleotide (π) and haplotype (*h*) diversity as well as parameters of neutrality tests and mismatch distribution for cytochrome oxidase subunit I sequences in *Littoraria subvittata* from the Western Indian Ocean; for site codes see Table 1.

Codes	π (%)	*h*	Fu's *Fs*	*P*	Tajima's *D*	*P*	SSD	*P*	Raggednes index	*P*
N-1	0.14 ± 0.00	0.64 ± 0.10	−1.290	0.11	−1.025	0.18	0.0004	0.99	0.0597	0.81
N-2	0.14 ± 0.1	0.55 ± 0.12	−2.238	0.03	−1.608	0.04	0.0006	0.94	0.0538	0.90
N-3	0.14 ± 0.11	0.56 ± 0.12	−2.361	0.02	−1.365	0.07	0.0206	0.40	0.1157	0.66
N-4	0.04 ± 0.05	0.24 ± 0.14	−1.615	0.01	−1.498	0.05	0.0037	0.49	0.3263	0.56
N-5	0.08 ± 0.07	0.46 ± 0.13	−4.262	0.00	−1.821	0.01	0.0072	0.36	0.1432	0.49
N-6	0.15 ± 0.12	0.75 ± 0.08	−3.366	0.00	−0.804	0.22	0.0256	0.18	0.1782	0.10
N-7	0.04 ± 0.05	0.27 ± 0.12	−2.820	0.00	−1.727	0.02	0.0049	0.51	0.2814	0.53
N-8	0.08 ± 0.08	0.32 ± 0.11	−3.633	0.00	−2.233	0.00	0.0038	0.43	0.1871	0.31
N-9	0.06 ± 0.06	0.36 ± 0.13	−2.135	0.01	−1.441	0.07	0.0035	0.57	0.2683	0.53
N-10	0.15 ± 0.12	0.67 ± 0.11	−3.366	0.01	−1.222	0.09	0.0018	0.86	0.0598	0.72
N-11	0.15 ± 0.12	0.62 ± 0.11	−1.088	0.16	−0.866	0.22	0.0088	0.63	0.0615	0.83
N-12	0.15 ± 0.11	0.64 ± 0.10	−2.209	0.03	−1.276	0.10	0.0005	0.91	0.0522	0.83
N-13	0.09 ± 0.09	0.35 ± 0.13	−0.973	0.12	−1.630	0.04	0.0084	0.51	0.2539	0.57
S-1	0.22 ± 0.16	0.79 ± 0.08	−1.600	0.12	−0.072	0.49	0.0058	0.69	0.0666	0.65
S-2	0.18 ± 0.13	0.67 ± 0.10	−1.626	0.10	−0.856	0.21	0.0152	0.46	0.1012	0.60
M-2	0.19 ± 0.14	0.71 ± 0.07	−1.335	0.17	−0.679	0.28	0.0152	0.24	0.1165	0.24

Kilwa. The values of Tajima's *D* were not significant from zero for all populations of *L. pallescens*. The populations of *L. pallescens and L. subvittata* have non-significant values for the SSD and raggedness index analysis. Mismatch distribution analysis showed an unimodal distribution for both species.

4. Discussion

4.1. Haplotypes and genetic diversities

The analysis of COI sequences in the present study indicated that *L. subvittata* and *L. pallescens* in the WIO have a low nucleotide and moderate to high haplotype diversity, with a star-like haplotype network. This could indicate a rapid population growth since Pleistocene (Grant and Bowen, 1998). The hypothesis of rapid population growth is supported by the existence of an excess of rare haplotypes in both species. Otherwise, these rare mutations would already have been eliminated by genetic drift (Zane et al., 2006). Low genetic diversity of other marine invertebrates in the WIO has been suggested to be caused by recently expanded population size following a period of lower effective population size due to bottlenecks or founder events (Fratini et al., 2016; Silva et al., 2010a). The pattern of low nucleotide diversity obtained in the present study can be explained by the same factors.

4.2. Population genetic structure and demographic history

The minimum spanning network did not show clades of haplotype being restricted to certain geographic regions in the species distribution, suggesting a weak or no phylogeographic structure. The AMOVA and pairwise differentiation support the scenario of lacking phylogeographic structure, especially in *L. pallescens*. Nevertheless, pairwise comparisons for samples of *L. subvittata* collected from Vilalankulo and Inhambane in Mozambique and Ile St. Marie in Madagascar showed evidence of genetic differentiation from other populations collected at other sites (Table 4). This pattern of genetic structure observed is supported by significant IBD results, suggesting that *L. subvittata* is more sensitive to the influence of IBD as compared to *L. pallescens*. The previous study carried on the Tanzania coastline (Nehemia et al., 2017) indicated absence of genetic structuring which may be indicating higher gene flow for *L. subvittata* over short geographical distance. A significant IBD has been associated with a pattern of genetic differentiation observed in the study (Villamor et al., 2014) and it increases the confidence in the significance of slight genetic differentiation obtained (Palumbi, 2003). The lack of a positive correlation in *L. pallescens* between genetic and geographic distance may be explained by contemporary high mixing of larvae associated with high levels of ongoing gene flow between geographically separated populations during the planktonic stage (Silva et al., 2013). The constraints of mitochondrial DNA when testing for IBD have recently reported including a significant IBD resulted from lumping distinct evolutionary clusters (Teske et al., 2018). However, we did not find such constraints to influence the IBD results obtained in this study.

In addition, the differences in population genetic differentiation observed between the two species may be associated with the differences in their larval dispersal potentials, evolutionary historic events and habitat preferences. *Littoraria subvittata* are more abundant in the middle and landside in mangroves while *L. pallescens* are more abundant at sea side and middle in mangroves (Torres et al., 2008, Field observations). Frequent availability of tidal waves at the sea side may be facilitating larval dispersal over long geographical distance in *L. pallescens* compared to *L. subvittata*. Differences in habitats and environmental differences among localities can also lead to genetic divergence (Palumbi, 1994). Passive movement of pelagic larvae by oceanic currents could result in large dispersal distances and a

Table 3
Nucleotide (π) and haplotype (*h*) diversity, as well as parameters of neutrality tests and mismatch distribution for cytochrome oxidase subunit I sequence in *Littoraria pallescens* from the Western Indian Ocean; for the site codes see Table 1.

Codes	π (%)	*h*	Fu's *Fs*	*P*	Tajima's *D*	P	SSD	*P*	Raggedness index	*P*
N-2	0.08 ± 0.08	0.49 ± 0.08	1.233	0.64	1.238	0.92	0.0189	0.18	0.2364	0.18
N-7	0.12 ± 0.10	0.53 ± 0.11	−2.719	0.01	−1.194	0.11	0.0002	1.00	0.0726	0.72
N-9	0.15 ± 0.12	0.60 ± 0.10	−1.401	0.13	−1.501	0.06	0.0174	0.17	0.1515	0.25
N-11	0.06 ± 0.07	0.37 ± 0.11	0.796	0.48	0.796	0.77	0.0047	0.34	0.2058	0.43
N-12	0.04 ± 0.05	0.22 ± 0.17	−0.264	0.16	−1.088	0.18	0.3067	0.10	0.3580	0.31
N-13	0.23 ± 0.18	0.81 ± 0.12	−1.686	0.05	−1.038	0.21	0.0051	0.77	0.0857	0.64
M-1	0.11 ± 0.10	0.55 ± 0.12	−1.055	0.087	−0.673	0.26	0.0076	0.45	0.1155	0.55
M-3	0.22 ± 0.16	0.81 ± 0.09	−4.530	0.001	−0.414	0.37	0.0130	0.37	0.1294	0.22

Table 4
Pairwise Φ_{st}-values of *Littoraria subvitatta* populations from the Western Indian Ocean based on cytochrome oxidase subunit I sequences; for site codes see Table 1.

Codes	N-1	N-2	N-3	N-4	N-5	N-6	N-7	N-8	N-9	N-10	N-11	N-12	N-13	S-1	S-2	M-2
N-1	0															
N-2	0.040	0														
N-3	0.094*	0.082*	0													
N-4	0.026	0.032	0.068	0												
N-5	0.069	0.007	0.074	0.003	0											
N-6	−0.012	0.026	0.104*	0.037	0.063*	0										
N-7	0.074*	0.05	0.079	−0.001	0.008	0.087*	0									
N-8	0.048	0.013	0.072	−0.010	−0.004	0.057*	−0.004	0								
N-9	0.073	−0.000	0.078	0.013	−0.019	0.044	0.016	−0.013	0							
N-10	0.082*	0.079*	0.104*	0.075	0.081*	0.052	0.087	0.053*	0.038	0						
N-11	0.015	0.041	0.131*	0.072	0.090	−0.015	0.124	0.107*	0.091	0.134*	0					
N-12	0.078*	−0.009	0.117*	0.080	0.040	0.037	0.107	0.074*	0.033	0.121*	0.027	0				
N-13	0.050	−0.014	0.078	0.011	−0.011	0.031	0.03	0.008	−0.018	0.084*	0.044	−0.000	0			
S-1	0.037	0.105	0.206*	0.183*	0.203*	0.192	0.248*	0.210*	0.205	0.023*	0.005	0.094	0.145*	0		
S-2	0.208*	0.081*	0.253*	0.262*	0.205*	0.243*	0.287*	0.238*	0.199	0.161*	0.146*	0.050	0.148*	0.192	0	
M-2	0.072	0.050	0.110*	0.086	0.085*	−0.006	0.099*	0.050	0.057	0.053	0.125*	0.104*	0.081*	0.160*	0.140*	0

Statistical significant differences of adjusted *P*-values after sequential Bonferroni ($p < .001$) are indicated by *.

Table 5
Pairwise Φ_{st}-values of *Littoraria pallescens* populations from the Western Indian Ocean based on cytochrome oxidase I sequences; for site codes see Table 1.

Codes	N-2	N-7	N-9	N-11	N-12	N-13	M-1	M-3
N-2	0							
N-7	0.049	0						
N-9	−0.022	−0.002	0					
N-11	−0.017	−0.003	−0.033	0				
N-12	0.0585	−0.042	−0.036	−0.047	0			
N-13	−0.022	0.043	−0.015	0.006	0.011	0		
M-1	−0.043	0.004	−0.036	−0.035	−0.008	−0.022	0	
M-3	0.0296	0.055	0.020	0.028	0.009	0.008	0.019	0

homogeneous population (Bohonak, 1999).

The slight but significant restricted gene flow observed through SAMOVA in *L. pallescens* between Madagascar (Toliara) and the rest populations may be associated with the oceanic eddies during dispersal. A unique patterns of currents and eddies in the Mozambique Channel (Fig. 1) can cause the isolation of local marine invertebrate populations (Madeira et al., 2012) and play a major role in shaping genetic structuring and larval dispersal in various fauna (Tsang et al., 2012). Toliara is located in the southern west of Madagascar and is under the influence of the Mozambique Channel eddies that produces a series of anticyclonic mesoscale eddies interacted by smaller cyclonic eddies (Hancke et al., 2014). Mozambique Channel eddies has been suggested to cause an oceanographic barrier for dispersal between the northern and southern East African coast (Otwoma and Kochzius, 2016, and may be responsible for the slight restricted gene flow observed in *L. pallescens*. It would be interesting for further study to include samples of *L. pallescens* from Mozambique and Kenya to confirm the gene flow observed for this species.

Most of the populations of both species have non-significant but negative Tajima's *D*-values, probably supporting the null hypothesis of neutrality of the COI marker. However, most populations of *L. subvittata* sampled from Tanzania have significant negative Fu's *Fs*-values, suggesting rapid population expansion. Fu's *Fs* has been regarded as more powerful than Tajima's *D* in detecting traces of signatures of past population expansion (Fu, 1997). Although some populations have non-significant Tajima's *D* and Fu's *Fs*-values, particularly for *L. pallescens*, analysis of mismatch distribution and Rogers' test (Rogers and Harpending, 1992; Rogers, 1995) supported the hypothesis of sudden population expansion for all populations of both species. The mismatch analysis exhibited a unimodal distribution for both species. This hypothesis is further supported by the presence of a star-like topology in the haplotype network. Signs of population expansion have been reported in many marine invertebrates in the Western Indian Ocean (e.g. Madeira et al., 2012; Silva et al., 2010a, 2013; Otwoma and Kochzius, 2016).

The reasons for demographic changes have been suggested to be a reduction of habitats during low sea-level stands that resulted in population bottlenecks, followed by recolonisation of new habitats after rising of the sea level (Kochzius and Nuryanto, 2008; Silva et al., 2010a). During the Tertiary period (65 million years ago) the earth's climate became cooler, with frequent oscillations that increased in amplitude and consequently led to the series of major ice ages of the Quaternary (2.4 Myr ago until present) (Hewitt, 2000). Mangrove ecosystems, which are the habitat for both species studied have been reported to have shrunk on a global scale during the period of low sea-

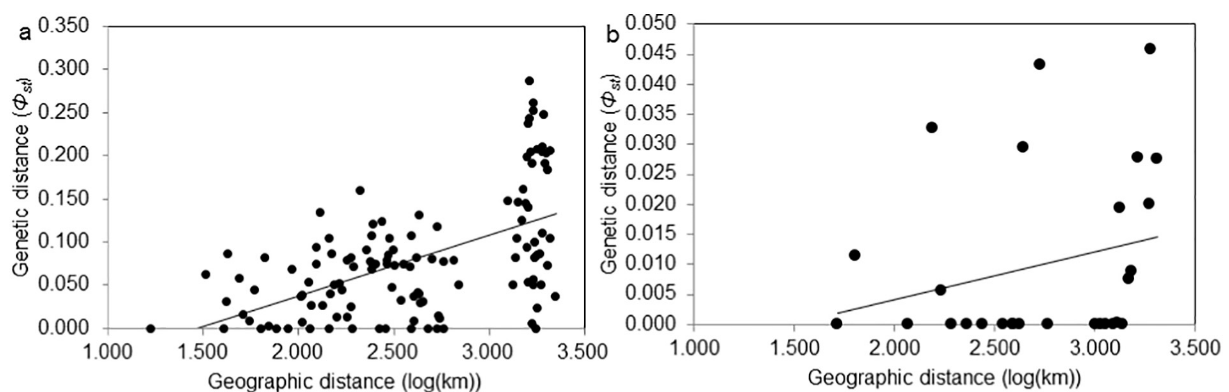

Fig. 2. Isolation by distance in a) *Litoraria subvittata* and b) *L. pallescens*. The pairwise Φ_{st} values are plotted against geographic distance.

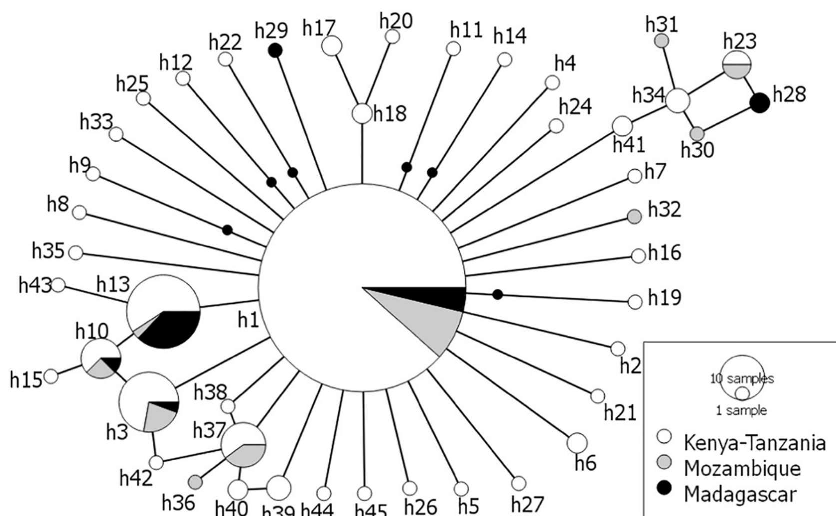

Fig. 3. Minimum spining network indicating haplotype distribution of *Littoraria subvittata* based on cytochrome oxidase subunit I sequences. The haplotype network indicates percentage of haplotypes from Kenya (blue), Tanzania (grey), Mozambique (pale) and yellow (Madagascar). The central haplotype is representing 216 individuals. The size of the other circles corresponds to the number of individuals as indicated at the bottom right side of the haplotype network. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

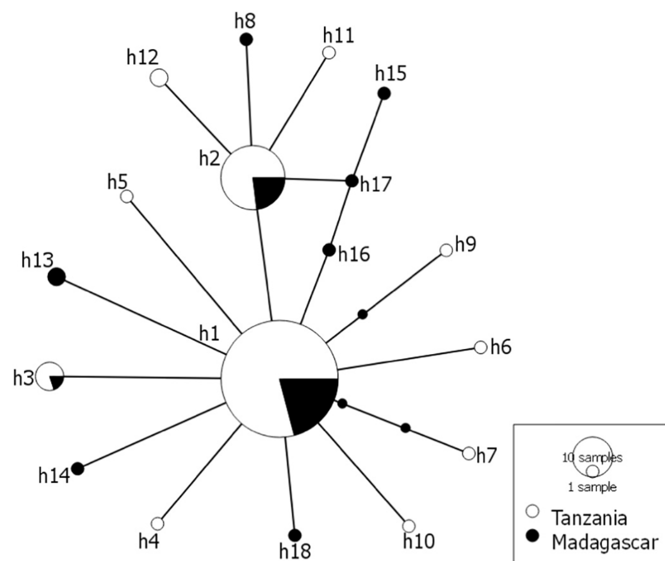

Fig. 4. Minimum spanning network indicating haplotype distribution of *Littoraria pallescens* based on cytochrome oxidase subunit I sequences for populations from Tanzania and Madagascar, Western Indian Ocean. The central haplotype is representing 104 individuals. The size of the other circles corresponds to the number of individuals as indicated at the bottom right side of the haplotype network.

level stands (Woodroffe and Grindrod, 1991).

5. Conclusion

This study has revealed that *L. subvittata* and *L. pallescens* have a low to moderate haplotype diversity and low nucleotide diversity, showing a similar genetic diversity as other invertebrate species in the WIO (Silva et al., 2010b; Fratini et al., 2016). However, the population genetic differentiation of these two species is different, with *L. subvittata* displaying higher genetic differentiation than *L. pallescens*. This might indicate that *L. subvittata* and *L. pallescens* are influenced differently by oceanographic features such as ocean currents, eddies and IBD.

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Appendix A. Supplementary data

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