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Genetic population structure of the lionfish *Pterois miles* (Scorpaenidae, Pteroinae) in the Gulf of Aqaba and northern Red Sea

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Abstract

The aim of this study is to reveal gene flow between populations of the coral reef dwelling lionfish *Pterois miles* in the Gulf of Aqaba and northern Red Sea. Due to the fjord-like hydrography and topology of the Gulf of Aqaba, isolation of populations might be possible. Analysis of 5′ mitochondrial control region sequences from 94 *P. miles* specimens detected 32 polymorphic sites, yielding 38 haplotypes. Sequence divergence among different haplotypes ranged from 0.6% to 9.9% and genetic diversity was high (h=0.85, π=1.9%). AMOVA indicates panmixia between the Gulf of Aqaba and northern Red Sea, but analysis of migration pattern shows an almost unidirectional migration originating from the Red Sea.

Keywords: Gene flow; mtDNA; *Pterois miles*; Coral reef fish; Red Sea

1. Introduction

Like many organisms on coral reefs, fishes have a life history with two totally different phases: adults are relatively strongly site-attached and sedentary (Sale, 1980), whereas larvae of virtually all species are planktonic (Leis, 1991). Individuals of the lionfish *Pterois miles* occur permanently in the same area of the reef and probably never leave their home range (Fishelson, 1975, 1997, personal observation). Therefore, gene flow by adult migration between distant populations is not expected. In contrast, eggs and larval stages of *P. miles* are planktonic (Fishelson, 1975; Thresher, 1984; Leis, 1991) and have the potential to disperse over large areas. *P. miles* produces mucus balls that embody the eggs and float below the surface. In the close relative *Dendrochirus brachypterus*, the mucus balls contain 2000–15,000 eggs and hatching occurs after 36 h under laboratory conditions (Fishelson, 1975). Therefore, it is likely that the pelagic eggs of *P. miles* float as passive drifters with the surface currents before the hatched larvae might start to control their dispersal by active swimming. Studies on late pelagic stages of coral reef fishes have demonstrated that they can swim against strong currents (Leis and Carson-Ewart, 1997).

Genetic homogeneity over large areas is a common feature of tropical marine fishes and can reflect high dispersal capability resulting in high levels of gene flow (Planes et al., 1993; Doherty et al., 1995; Shulman and Birmingham, 1995; Bernardi et al., 2001). However, other studies on tropical marine fishes have shown significant genetic structuring (Planes et al., 1996, 1998).

The fjord-like Gulf of Aqaba is a deep, narrow northern–eastern extension of the Red Sea. It has a length of 180 km and is 6–25 km wide. The depth can reach over 1800 m, but averages 800 m. The Gulf of Aqaba is separated from the Red

Abbreviations: A, adenosine; bp, base pair(s); BSA, bovine serum albumin; C, cytidine; dNTP, deoxyribonucleoside triphosphate; G, guanosine; h, haplotype diversity; M, population parameter M; mtDNA, mitochondrial DNA; ML, maximum likelihood; MP, maximum parsimony; NJ, neighbour joining; T, thymidine; π, nucleotide diversity; γ, number of effective immigrants; Θ, population parameter Θ.

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Sea by a shallow sill of 242–270 m depth at the Straits of Tiran. Deserts and mountains, with a hot and dry climate, flank the semi-enclosed basin. A high evaporation rate results in a high salinity of 41‰ and a thermohaline circulation that drives water exchange with the Red Sea proper (Reiss and Hottinger, 1984). Calculations of the residence time of the upper 300 m vary from 4 months to 1 or 2 years. The inflow of Red Sea water reaching the northern tip of the gulf is estimated to 1% of that at the Straits of Tiran (Wolf-Vecht et al., 1992 and references therein). Simulation of wind-driven circulation by Berman et al. (2000) suggests a series of gyres distributed along the Gulf of Aqaba. Due to the curved shoreline at the northern tip of the gulf, more gyres develop in this part than in the south.

Gyres can cause retention of larvae of tropical shore fishes at their natal reef (Johannes, 1978; Swearer et al., 1999) and therefore restriction in gene flow between populations in the Gulf of Aqaba and the Red Sea proper might be possible. Restriction of faunal exchange between the Gulf of Aqaba and northern Red Sea is indicated by analysis of species composition of shore fish communities from several sites in the Red Sea (Khalaf and Kochzius, 2002), and Klausewitz (1989) suggested isolated development of the deep-sea fishes in the gulf.

Due to the special, fjord-like hydrographic and topographic situation in the Gulf of Aqaba, isolation of populations in the gulf might be possible. Investigations on the genetic population structure with molecular markers can give indications on connectivity of populations. The molecular marker applied in this study is a partial sequence of the 5′ mitochondrial control region. The mutation rate of the control region is much higher than the mutation rate of all mitochondrial genes in fishes (Lee et al., 1995) and therefore a suitable marker for investigations on the genetic structure of populations (Avise et al., 1987; Parker et al., 1998).

This study aims to investigate the genetic population structure of *P. miles* to reveal gene flow between populations in the Gulf of Aqaba and northern Red Sea.

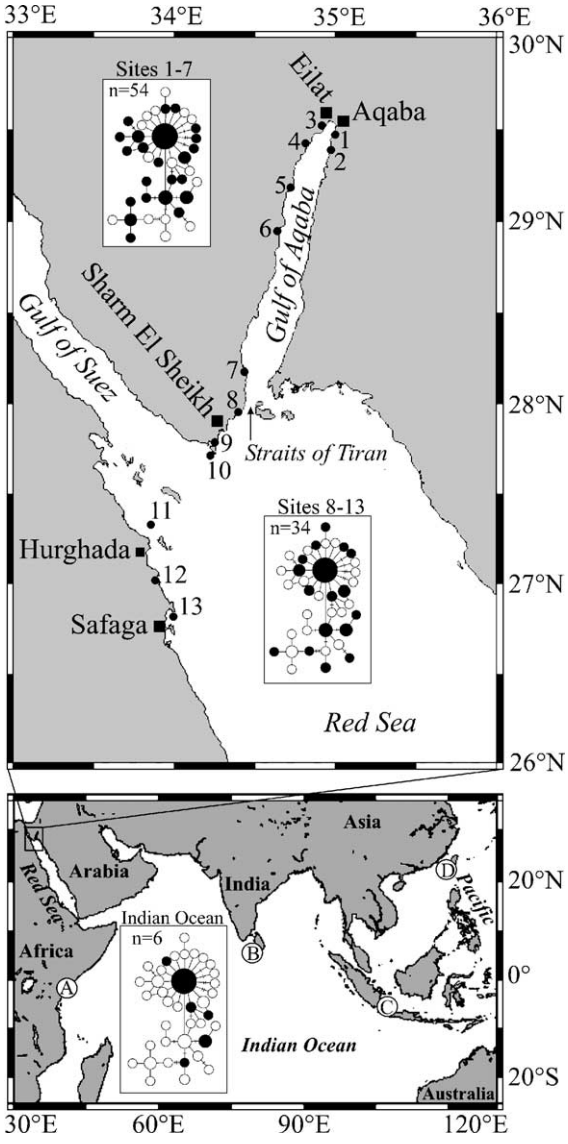

Fig. 1. Maps of the Indian Ocean, Red Sea, and Gulf of Aqaba showing the sample sites (A: Kenya; B: Sri Lanka; C; Indonesia; Northern Red Sea: ● 1–13; see also Table 1) for the lionfishes *Pterois miles* and *P. volitans* (D: Taiwan). Solid circles in the minimum spanning network indicate the presence of a given haplotype in a putative population.

2. Materials and methods

2.1. Sampling and DNA extraction

Fin clips of *P. miles* were collected in the field from August to October 1998 at 13 sites in the Gulf of Aqaba and northern Red Sea (Fig. 1, Table 1). *P. miles* is encountered frequently on coral reefs and in seagrass-dominated habitats (Khalaf and Kochzius, 2002). Due to its venomous spines, *P. miles* has virtually no predators and shows no escape behaviour. While diving, clips of 1–2 cm length from the feathery pectoral fin, depending on size of the fish, were cut off with a pair of scissors. In most cases this was possible without catching the fish. If necessary, lionfishes were caught with aquarium hand nets and released after fin clipping.

P. miles specimens from Kenya, Sri Lanka, and Indonesia, and the sibling species *Pterois volitans* (Kochzius et al., 2003) from Taiwan were obtained from colleagues or purchased from an aquarium shop (Fig. 1, Table 1). Total DNA was extracted from 100–300 mg of tissue according to the Chelex® method as described in Söller et al. (2000).

2.2. Primers, polymerase chain reaction (PCR), and sequencing

Amplification of a 222 bp 5′ mitochondrial control region fragment was conducted with the primers L-CR-01 (5′-TGTTTTATCACCATATCTAGGGTT-3′) and H-CR-02 (5′-GAAATGGACTTGTTGGTCGG-3′). The primers have

Table 1
Sample sites of *Pterois miles* and *Pterois volitans*

Number in map	Sampling site	Putative population	Sequenced samples
P. miles			
1	Marine Science Station (MSS), Aqaba (Jordan)	Gulf of Aqaba	12
2	Saudi Arabian Border (Jordan)	Gulf of Aqaba	6
3	Interuniversity Institute (IUI), Eilat (Israel)	Gulf of Aqaba	8
4	Fjord, Taba (Egypt)	Gulf of Aqaba	9
5	Ras Burka (Egypt)	Gulf of Aqaba	10
6	Nuweiba (Egypt)	Gulf of Aqaba	5
7	Nahalet El Tel, Nabq (Egypt)	Gulf of Aqaba	4
8	Ras Nasrani, Sharm El Sheikh (Egypt)	Northern Red Sea	2
9	Marsa Bareika, Ras Mohammad (Egypt)	Northern Red Sea	4
10	Ras Mohammad (Egypt)	Northern Red Sea	8
11	Umm Gamar and Fanadir, Hurghada (Egypt)	Northern Red Sea	6
12	Makadi Bay (Egypt)	Northern Red Sea	6
13	Tobya Arba, Safaga (Egypt)	Northern Red Sea	8
A–C	Kenya, Sri Lanka, Indonesia	Indian Ocean	6 ∑94
P. volitans			
D	Taiwan	Western Pacific	5

been designed on the basis of eight *Sebastes* spp. (Scorpaenidae) sequences (Rocha-Olivares et al., 1999). Fifty-microliter PCRs contained 10 mM Tris–HCl (pH 9.0), 50 mM KCl, 6 mM $MgCl_2$, 0.2 mM each dNTP (PeqLab, Erlangen), 0.2 μM each primer, 1 U Taq polymerase (Biomaster, Köln), 4 μl BSA (20 mg/ml; MBI Fermentas, St. Leon-Rot), and 4 μl supernatant of the DNA extraction. Thermal cycling profile began at 95 °C for 5 min and was held at 85 °C until Taq polymerase was added (hot start), subsequently followed by 40 cycles of 94 °C (50 s), 50 °C (60 s), 72 °C (90 s), and a final step of 5 min at 72 °C for termination of the PCR.

PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, Hilden). Both strands were sequenced using the DyeDeoxy Terminator chemistry (PE Biosystems, Foster City) and an ABI Prism 310 automated sequencer (Applied Biosystems, Weiterstadt) according to the manufacturer's recommendations.

2.3. Genetic diversity and phylogenetic analysis

Both strands of a sequence were aligned with the programme Sequence Navigator (version 1.0.1; Applied Biosystems) and confirmed by eye. Multiple alignment of sequences was done using Clustal V implemented by Sequence Navigator (version 1.0.1; Applied Biosystems).

The hypothesis of neutral evolution was tested by Tajima's *D* test (Tajima, 1989) and Fu's Fs test (Fu, 1997) with 10,000 permutations as implemented in the programme ARLEQUIN (version 2.000; Schneider et al., 2000). Analysis of Rogers' (1995) model of sudden population expansion was also conducted with ARLEQUIN. Estimates of genetic variation were obtained in the form of haplotype diversity *h* (Nei, 1987), nucleotide diversity π (Nei and Jin, 1989), and mean number of nucleotide differences among all haplotypes in a putative population with the software ARLEQUIN (version 2.000; Schneider et al., 2000).

Phylogenetic analysis was performed with the software PAUP* (version 4.0b10; Swofford, 1998), using neighbour-joining (NJ), maximum likelihood (ML), and maximum parsimony (MP). The computer programme ModelTest (version 3.06; Posada and Crandall, 1998) was used to determine the best-fit model of DNA evolution, which was used for the NJ and ML analysis. ML and MP analysis was done using heuristic searches, and analysis was restricted to 10,000 trees in MP. Evaluation of statistical confidence in nodes was based on 1000 non-parametric bootstrap replicates in NJ and MP, and 100 non-parametric bootstrap replicates in ML analysis (Felsenstein, 1985). *P. volitans* was used as outgroup for all trees because it is very closely related to *P. miles* (Kochzius et al., 2003). The relationship of haplotypes was also inferred by a minimum spanning network with the programme ARLEQUIN (version 2.000; Schneider et al., 2000).

2.4. Analysis of population structure

Significance of population structure was tested by the analysis of molecular variance (AMOVA; Excoffier et al., 1992) as implemented in ARELQUIN, which takes into account distances between haplotypes, gamma shape parameter, and geographic distribution. Φ statistics of AMOVA quantify population structure at each level in a given hierarchy. For this hierarchical analysis, the samples were grouped into three putative populations: Gulf of Aqaba (sites 1–7) northern Red Sea (sites 8–13), and Indian Ocean (sites A–C) (Fig. 1). The significance of Φ statistics was tested by comparisons to null distributions constructed from 10,000 random permutations of the original data matrix.

Migration between the three putative populations was analysed with the software MIGRATE 1.7.3 (Beerli, 2003), which is a maximum likelihood estimator based on the coalescent theory. It uses a Markov chain Monte Carlo approach to investigate possible genealogies with migration events. We used the following search strategy, considering the recommendation of Beerli and Felsenstein (2001): 10 short chains of 10,000 steps each were run, followed by three long chains of 20,000 steps each, sampling every 20th step. To increase the probability of

Table 2
Measures (±S.D.) of genetic diversity in putative populations of *Pterois miles*

	n	Number of haplotypes	Haplotype diversity *h*	Nucleotide diversity π (%)	Mean pairwise difference (%)	Number of sites with substitutions *S*
Gulf of Aqaba	54	23	0.841±0.047	1.97±1.14	3.27±1.71	28
Northern Red Sea	34	17	0.870±0.052	1.72±1.03	2.86±1.54	20
Indian Ocean	6	6	1.000±0.096	1.85±1.29	3.07±1.85	8

receiving reliable results from the searches of the maximum likelihood surface, we used 10 "heated" chains. In addition, we incorporated a t_i/t_v ratio of 5.56 and base frequencies (A=0.41, C=0.19, G=0.14, T=0.26) obtained with the software ModelTest in the analysis. Furthermore, a stepping stone migration model with exchange between the Gulf of Aqaba and the northern Red Sea, as well as migrations in both directions between the northern Red Sea and Indian Ocean was applied. The number of effective immigrant (γ) was calculated with the following formula: $\gamma=\Theta M$ (Beerli and Felsenstein, 2001). The population parameters Θ and M were computed with MIGRATE 1.7.3 (Beerli, 2003).

3. Results

3.1. Polymorphic sites

The PCR products had a length of 222 bp and after removing of areas of ambiguity as well as missing data, mtDNA control region sequences of 166 bp were obtained from 94 *P. miles* specimens (54 from the Gulf of Aqaba, 34 from the northern Red Sea, and 6 from the Indian Ocean) and 5 *P. volitans* specimens from Taiwan (Table 1). Among the 94 *P. miles* specimens, 32 polymorphic sites were detected, yielding 38 haplotypes. These polymorphisms included 27 transitions and 8 transversions. The haplotype

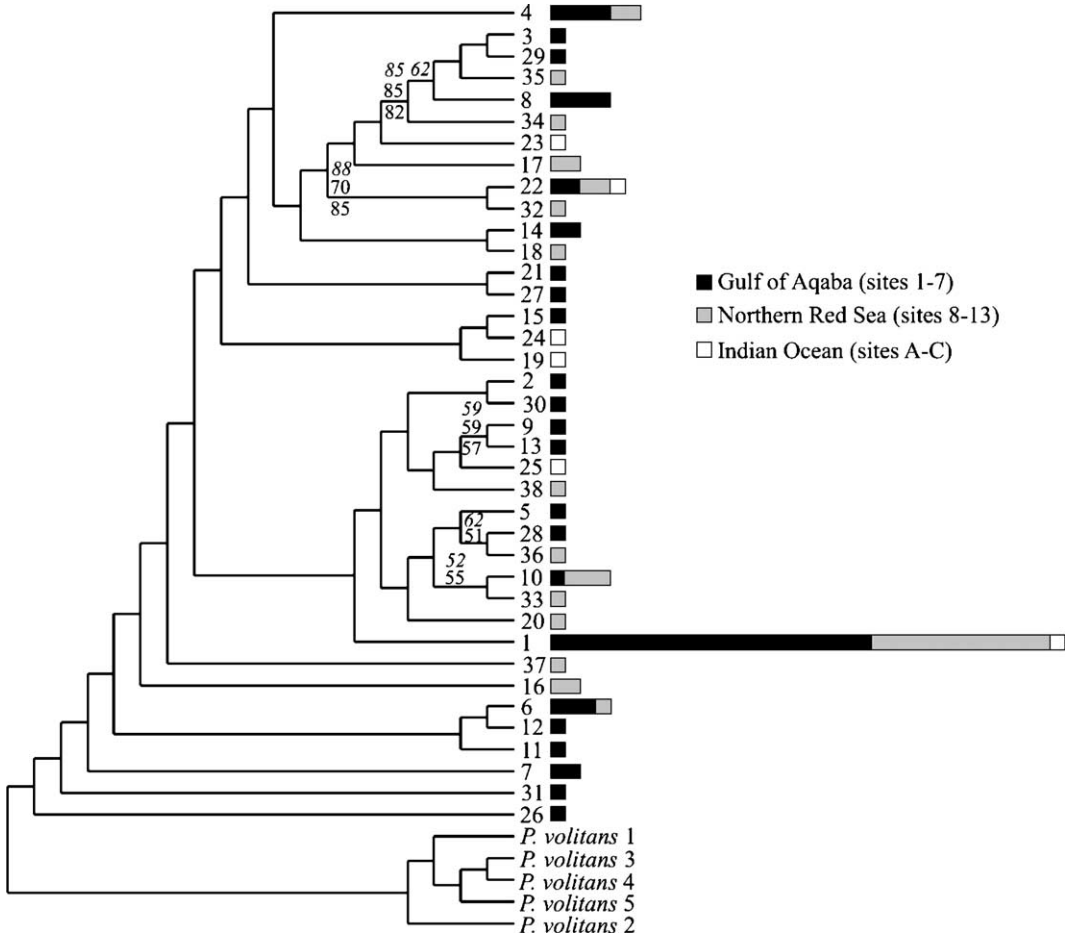

Fig. 2. Neighbour-joining tree of mtDNA control region haplotypes in the lionfish *Pterois miles*. Numbers at the tip of branches correspond to one haplotype. Bootstrap values for NJ (italics) and ML are shown above branches, for MP below branches. Bars represent the frequency and geographical distribution of each haplotype (one square: n=1).

Table 3
Hierarchical analysis of molecular variance (AMOVA) of mtDNA control region haplotypes of *Pterois miles*

Source of variation	*df*	Sum of squares	Variance	% Variation	Φ Statistic	*p*
Among putative populations	2	2.512	−0.03094	−2.01	Φ_{ct}=−0.02010	0.76
Among sample sites within putative populations	11	22.300	0.08123	5.28	Φ_{sc}=0.05174	0.09
Within sample sites	80	119.114	1.48892	96.73	Φ_{st}=0.03267	0.07

sequences are available from EMBL database (accession nos. AJ628896–AJ628938). The alignment is available from the EMBL server at ftp://ftp.ebi.ac.uk/pub/databases/embl/align/ALIGN_000670.dat.

3.2. Genetic diversity

Mean pairwise nucleotide difference of mtDNA control region haplotypes of *P. miles* was 3.10±1.62%. All putative regional populations showed high levels of haplotype diversity (h) ranging from 0.84 to 1.00, and nucleotide diversities (π) ranging from 1.72% to 1.97%. Mean pairwise nucleotide difference between haplotypes within putative populations ranged from 2.86% to 3.27% (Table 2).

The null hypothesis of neutrality of the mtDNA control region mutations was rejected for Tajima's D test (D=−1.58; p=0.03) and Fu's Fs test (Fs=−26.27; p<0.0001). These results indicate rather population growth than selection because Rogers' (1995) model of sudden population expansion could not be rejected (p=0.69).

3.3. Phylogeographic patterns and genetic population structure

The best-fit ML model obtained from ModelTest (version 3.06; Posada and Crandall, 1998) analysis was the HKY85 model (Hasegawa et al., 1985) with a t_i/t_v ratio of 5.33, a gamma distribution shape parameter of 0.28, and the following base frequencies: A=0.40, C=0.20, G=0.14, T=0.26. Parameters obtained from this analysis were used for the construction of the NJ and ML phylogeny.

The evolutionary relationships among the 38 *P. miles* haplotypes are shown in a NJ tree rooted with five *P. volitans* haplotypes (Fig. 2). ML and MP trees showed several polytomies (trees not shown) and bootstrap analysis only supported branches also confirmed in NJ analysis (Fig. 2). Only a few minor branches were supported by the bootstrap analysis and no geographic pattern could be observed.

The minimum spanning network of 38 haplotypes revealed a partly star-like structure, with many haplotypes originating from the most abundant haplotype (Fig. 1). The dominant haplotype 1 accounted for 36% of all *P. miles* specimens and appears in all putative populations (Fig. 2).

Several groupings of sample sites into putative populations have been investigated, but none of these has shown substantial differences in the analysis of gene flow with the programmes AMOVA and MIGRATE. Therefore, results based on the following three putative populations are presented: Gulf of Aqaba (sites 1–7), northern Red Sea (sites 8–13), and Indian Ocean (sites A–C) (Table 1).

Only 8.8% of the pairwise Φ_{ST} comparisons between all sample sites show significant differences in the AMOVA analysis, mainly between sites in the Gulf of Aqaba. All molecular variance determined from the AMOVA was attributed to variance among sites within putative populations or within sites, and no significant population structure was detected (Table 3).

The mean values for Θ and numbers of effective immigrants per generation of six different runs with the software MIGRATE are summarised in Table 4. The numbers of effective immigrants per generation show values that indicate panmixia, but also suggest unequal migration between the putative populations. The migration pattern is characterised by a higher number of migrants entering (1) the Red Sea from the Indian Ocean, and (2) the Gulf of Aqaba from the Red Sea. However, values for the exchange between the Red Sea and Indian Ocean have to be taken with caution because the number of individuals analysed from the Indian Ocean is rather low.

4. Discussion

None of the analytical tools revealed a separation of the putative *P. miles* populations in the Gulf of Aqaba, northern Red Sea, and Indian Ocean. Haplotype 1 was dominant (Figs. 1 and 2) and neither NJ, ML, and MP trees (Fig. 2) nor the minimum spanning network revealed clades with a

Table 4
Estimated population parameters and migration rate (±S.D.) between putative populations of the lionfish *Pterois miles*

Migration from	*n*	Θ	Effective migrants/generation		
			Gulf of Aqaba	Northern Red Sea	Indian Ocean
Gulf of Aqaba	54	0.004±0.002	–	0.9±0.9	–
Northern Red Sea	34	0.021±0.008	197.0±75.1	–	4.8±3.9
Indian Ocean	6	0.015±0.003	–	15.3±5.1	–

geographic pattern (Fig. 1). In addition, the number of effective migrants per generation was high, indicating panmixia (Table 4). However, an unequal migration pattern was observed, with higher migration from the Indian Ocean into the Red Sea and from the Red Sea into the Gulf of Aqaba. This migration pattern can be caused by (1) different population sizes, leading to a higher gene flow from the larger populations into the smaller ones, (2) oceanographic conditions, (3) or a combination of both effects.

Analysis of genetic population structure in invertebrates revealed a different picture of gene flow between the Red Sea and Indian Ocean. Studies on the crown-of-thorns starfish *Acanthaster planci* (Benzie et al., 2000) and mud crab *Scylla serrata* (Fratini and Vannini, 2002) have shown a genetic isolation of Red Sea populations from the Indian Ocean. However, there are currently no studies on gene flow between population of fishes in the Red Sea and Indian Ocean for comparison available. As mentioned before, the exchange between populations of *P. miles* in the Red Sea and Indian Ocean has to be taken with caution, due to the low sample size and wide geographic distribution of collection sites in the Indian Ocean that have been pooled. For a study of this relationship in *P. miles*, more samples from the Indian Ocean are required.

The revealed population structure in the northern Red Sea and Gulf of Aqaba indicates a high level of gene flow and panmixia. However, analysis of migration between the Gulf of Aqaba and northern Red Sea shows almost unidirectional gene flow from the Red Sea into the Gulf of Aqaba. This might be due to the oceanographic conditions in regard to water exchange between the Gulf of Aqaba and Red Sea proper. The thermohaline circulation in the Gulf of Aqaba consists of inflow from the Red Sea proper in the upper layers through the Straits of Tiran, and outflow of water with higher salinity in the lower layer (Berman et al., 2000). The eggs of *P. miles* are embedded in mucus balls that drift passively under the surface (Fishelson, 1975). Therefore it is likely that they enter the Gulf of Aqaba from the Red Sea with the inflow of water in the upper layer, but cannot drift out of the gulf with the lower layer. In addition, oceanographic simulations indicate that the circulation in the Gulf of Aqaba is made up of a series of gyres along its main axis (Berman et al., 2000) which can also cause retention of larvae.

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