Highly restricted gene flow between disjunct populations of the skunk clownfish \((Amphiprion akallopisos)\) in the Indian Ocean

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Abstract

The skunk clownfish \((Amphiprion akallopisos)\) has a disjunct distribution, occurring in the Eastern Indian Ocean (EIO) and the Western Indian Ocean (WIO), separated by several thousands of kilometres. Information on connectivity of marine species is very important for the correct spacing of marine protected areas, a powerful instrument for the protection of coral reefs. The population genetic structure of \(A. akallopisos\) was analysed in order to investigate connectivity amongst populations and to explain the disjunct distribution of the species. A fragment of the mitochondrial control region was used to investigate the genetic population structure. Fin clips were collected from 263 individuals at 14 sites in the WIO and three sites in the EIO. The obtained DNA sequences were used to calculate genetic diversity, evaluate demographic history and to construct a haplotype network. An analysis of molecular variance (AMOVA) was conducted to evaluate the significance of the observed genetic population structure. None of the identified 69 haplotypes was shared between the WIO and EIO. Haplotype as well as nucleotide diversity was considerably higher in the EIO than in the WIO. Significant genetic population structure was revealed by an AMOVA with an overall \(\phi_{ST}\)-value of 0.28 \((P < 0.001)\) in the Indian Ocean. The overall AMOVA \((\phi_{ST} = -0.00652)\) was not significant in the EIO, but was significant in the WIO \((\phi_{ST} = 0.016; P < 0.01)\). Demographic analysis indicated population expansion in the EIO and WIO. Population genetic analysis revealed highly restricted gene flow between the EIO and WIO. Genetic diversity was much higher in the EIO than in the WIO, suggesting that the EIO is the geographical origin of the species. Given the large distance between the disjunct populations and the short pelagic larval duration, long-distance dispersal is rather unlikely. A stepping stone model involving islands in the Central Indian Ocean is a more likely scenario for colonization of the WIO.

Introduction

The Indo-Malay Archipelago (IMA), often also referred to as the Indo-Australian Archipelago or Coral Triangle, contains the highest species diversity in the world’s oceans today. The processes that generated this high diversity, however, are not yet fully understood and have been the subject of much discussion, with several hypotheses being offered (Mora et al. 2003; Barber 2009; Carpenter et al. 2011). According to the centre of origin hypothesis, the IMA acts as a centre of species differentiation and exports newly originated species to more peripheral regions of the Indo-Pacific (Briggs 1999, 2000, 2005). An alternative explanation is given by the centre of
overlap hypothesis, according to which many speciation events of coral reef-associated organisms took place during periods of lowered sea levels in the Pleistocene. As a consequence of these low sea levels, the waters of the Indian and Pacific Oceans were no longer directly linked and vicariance could take place. The resulting sister species recolonized the Sunda Shelf when sea levels rose again and currently overlap in the IMA (Woodland 1983; Gaither & Rocha 2013). Yet another hypothesis sees the IMA as a centre of accumulation and explains the high diversity in this region by migration of species from peripheral locations. Speciation takes place when populations become isolated in the periphery of the Indo-Pacific after which the newly formed species migrate back to the centre of accumulation (Jokiel & Martinelli 1992; Bellwood & Meyer 2009). Most probably not just one of these processes took place, but all of them acting together are responsible for the exceptional biodiversity in the IMA (Halas & Winterbottom 2009; Bowen et al. 2013; Cowman & Bellwood 2013).

The spatial distribution of species richness in anemonefishes (Amphiprioninae) follows this overall pattern of coral reef diversity with the highest number of co-occurring species in the IMA, making this group a good model for testing broader hypotheses of processes generating diversity. Within the IMA, this elevated species richness creates a high degree of niche specialization, sometimes only locally (Elliott & Mariscal 2001; Ricciardi et al. 2010). Based on this high species richness and because all basal species in the phylogeny occur there, the IMA was identified as a centre of origin for the Amphiprioninae (Santini & Polacco 2006), providing support for the hypothesis that the IMA acts as a centre of speciation (Briggs 2005). As many as 29 different species of clownfish have been described, all of which occur in the Indo-West Pacific region and live in obligate symbiosis with one or more of 10 species of giant sea anemones (Fautin & Allen 1992). They are the exclusive members of two genera, Premnas and Amphiprion, forming a monophyletic group (Amphiprioninae) within the family Pomacentridae (Cooper et al. 2009). The development of a mutualistic relationship with sea anemones is considered a key innovation that triggered adaptive radiation (Litsios et al. 2012). Anemonefishes are immune against the otherwise lethal venom of the nematocysts and mucus of anemones, which enables them to seek protection from predators in the anemone (Mebs 2009). Both partners benefit from the symbiosis as the fish provide the anemone with nutrients (Holbrook & Schmitt 2005; Roopin & Chadwick 2009; Cleveland et al. 2011) and also protect it from predators (Porat & Chadwick-Furman 2004).

Amphiprion akallopisos has a disjunct distribution (Fautin & Allen 1992). In the Eastern Indian Ocean (EIO), it occurs on the coast of Thailand and Sumatra, as well as in the Java Sea. Here, it is parapatric with its two closest relatives, Amphiprion sandaracinos and Amphiprion perideraion, from which it derived between 1.7 and 1.9 mya, possibly by vicariance during Pleistocene glaciations and sea-level decrease, isolating the Indian Ocean from the Pacific Ocean (Timm et al. 2008). In the Western Indian Ocean (WIO), separated from its EIO populations by more than 4500 km, it is no longer parapatric with its closest relatives but co-occurs with a total of eight other anemonefish species. These form a derived clade within the phylogeny of Amphiprion that was indicated as a recent Indian Ocean adaptive radiation, including many specialized and endemic species (Santini & Polacco 2006). This group is not closely related to A. akallopisos, which forms a more basal clade with its two strictly Pacific sister species (Timm et al. 2008; Litsios et al. 2012). It is currently not clear how this disjunct distribution was formed, whether there is still gene flow between both populations, and which population may have been the source population. Differences in sound production between the EIO and WIO populations were recorded, which may be an indication of isolation (Parmentier et al. 2005). Furthermore, genetic studies have demonstrated the presence of a barrier to dispersal between the EIO and the WIO for a few species (Appleyard et al. 2002; Ridgway & Sampayo 2005; Ragionieri et al. 2009).

For almost every coral reef-associated organism, dispersal is limited to a relatively short juvenile pelagic phase (Hellberg 2007). Because tracking of larvae or eggs in the ocean is extremely difficult, indirect genetic information is mostly used to assess connectivity of these populations (Jones et al. 2009). Originally thought to have open, well-connected populations owing to a pelagic larval duration (PLD) of weeks to months (Roberts 1997), many genetic studies have demonstrated that dispersal of marine species is influenced by a multitude of environmental and behavioural factors (Mora & Sale 2002) and that connectivity in the marine environment is a complex process (Cowen et al. 2000, 2006). Despite these efforts, a general pattern of connectivity amongst populations of coral reef-associated organisms has not yet been identified. Much more empirical data seem to be needed, especially on a more local scale, before levels of connectivity amongst coral reefs can be assessed correctly (McCook et al. 2009). As coral reefs worldwide continue to be under threat from a multitude of environmental and human factors, and correctly spaced marine protected areas (MPAs) seem to provide the best available management tool, information on connectivity is needed (Pandolfi et al. 2003; Mora et al. 2006).
population structure and patterns of gene flow amongst populations of *A. akallopisos* between the WIO and the EIO and amongst populations within both regions and to explain the disjunct distribution of the species.

**Material and Methods**

**Sampling**

A total of 263 *Amphiprion akallopisos* individuals was sampled between 1 and 25 m depth at 14 sites in the WIO and three in the EIO (Table 1 and Fig. 1). Fish were caught in their host anemone while SCUBA-diving with two hand nets. A small piece of the caudal fin was removed and the fish was returned into its host anemone. Samples were preserved in 96% ethanol immediately after the dive.

**DNA extraction, PCR and sequencing**

DNA was extracted with an extraction kit (DNeasy Blood and Tissue kit, QIAGEN, Düsseldorf, Germany) following the manufacturer’s protocol. A fragment of the CR was amplified by PCR using the primers CR-A (5' - TTCCACCTCTAACTCCCAAAGCTAG-3') and CR-E (5' - CCTGAAGTAGAACCAGATG-3') (Lee et al. 1995). The PCR was conducted in an Eppendorf Ep S Mastercy- cler with a volume of 50 μl for each PCR that contained 2 μl DNA template, 10 mM Tris–HCl (pH 9), 50 mM KCl, 4 mM MgCl₂, 0.4 μM of each primer, 0.2 μM deoxy Nucleoside Triphosphates and 1 U Taq polymerase. The following temperature profile was used: 94 °C for 5 min, followed by 35–40 cycles of 1 min at 94 °C, 1.5 min at 45 °C and 1 min at 72 °C. Final extension was conducted at 72 °C for 5 min. Sequencing of both strands was carried out with an ABI 3770XL automated sequencer (Applied Biosystems, Foster City, CA, USA).

**Population genetic analysis**

The sequences were edited using the software CHROMA- SPRO (v. 1.5, Technelysium Ltd, Leicester, UK), i.e. the chromatogram peaks of both strands were compared and the correct representation of nucleotides was checked. A multiple alignment was carried out using Clustal W (Thompson et al. 1994) as implemented in the software BIOEDIT (v. 7.0.0.1; Hall 1999). Haplotype and nucleo- tide diversity, F-statistics, as well as analysis of molecular variance (AMOVA) were calculated with the software ARLEQUIN (v. 3.5; Excoffier & Fischer 2010). Signific- ance of pairwise Fst-values and AMOVA was tested using a Monte-Carlo approach with 1000 and 1023 permutations, respectively, and significance values of pairwise comparisons were Bonferroni corrected. Tests for selective neutrality of the marker, Tajima’s D-test (Tajima 1989), and Fu’s Fst-test (Fu 1997), as well as the sum of squared deviation (SSD) test and Harpending’s raggedness index (HRI) test (Harpending 1994) to test Rogers’ model of sudden population expansion (Rogers 1995) were also conducted with the software ARLEQUIN. A haplotype

**Table 1.** Sample sites, number of samples (n), number of haplotypes (Nh), number of private haplotypes (Ph), haplotype diversity (h) and nucleo- tide diversity (π) for *Amphiprion akallopisos* in the Western Indian Ocean (WIO) and Eastern Indian Ocean (EIO).

<table>
<thead>
<tr>
<th>sample site</th>
<th>code</th>
<th>location</th>
<th>n</th>
<th>Nh</th>
<th>Ph</th>
<th>h</th>
<th>π (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WIO</td>
<td></td>
<td></td>
<td>229</td>
<td>56</td>
<td>56</td>
<td>0.53 ± 0.10</td>
<td>0.58 ± 0.040</td>
</tr>
<tr>
<td>Lamu</td>
<td>La</td>
<td>02°16'S, 40°54'E</td>
<td>8</td>
<td>3</td>
<td>1</td>
<td>0.61 ± 0.164</td>
<td>0.18 ± 0.171</td>
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<tr>
<td>Watamu</td>
<td>Wa</td>
<td>03°22'S, 40°01'E</td>
<td>16</td>
<td>7</td>
<td>5</td>
<td>0.63 ± 0.139</td>
<td>0.29 ± 0.226</td>
</tr>
<tr>
<td>Mombasas</td>
<td>Mo</td>
<td>04°02'S, 39°40'E</td>
<td>18</td>
<td>5</td>
<td>0</td>
<td>0.48 ± 0.138</td>
<td>0.17 ± 0.155</td>
</tr>
<tr>
<td>Diani</td>
<td>Di</td>
<td>04°18'S, 39°35'E</td>
<td>13</td>
<td>4</td>
<td>0</td>
<td>0.42 ± 0.165</td>
<td>0.12 ± 0.120</td>
</tr>
<tr>
<td>Kisite</td>
<td>Ki</td>
<td>04°43'S, 39°22'E</td>
<td>12</td>
<td>7</td>
<td>2</td>
<td>0.83 ± 0.100</td>
<td>0.36 ± 0.267</td>
</tr>
<tr>
<td>Misali</td>
<td>Mi</td>
<td>05°13'S, 39°44'E</td>
<td>15</td>
<td>9</td>
<td>3</td>
<td>0.80 ± 0.108</td>
<td>0.38 ± 0.273</td>
</tr>
<tr>
<td>Stonetown</td>
<td>St</td>
<td>06°09'S, 39°08'E</td>
<td>21</td>
<td>8</td>
<td>3</td>
<td>0.57 ± 0.129</td>
<td>0.32 ± 0.235</td>
</tr>
<tr>
<td>Dongwe</td>
<td>Dw</td>
<td>06°10'S, 39°31'E</td>
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<td>6</td>
<td>0</td>
<td>0.56 ± 0.134</td>
<td>0.17 ± 0.155</td>
</tr>
<tr>
<td>Jambiani</td>
<td>Ja</td>
<td>06°19'S, 39°30'E</td>
<td>11</td>
<td>4</td>
<td>0</td>
<td>0.49 ± 0.175</td>
<td>0.14 ± 0.143</td>
</tr>
<tr>
<td>Dar-es-Salaam</td>
<td>DS</td>
<td>06°40'S, 39°13'E</td>
<td>19</td>
<td>6</td>
<td>1</td>
<td>0.47 ± 0.140</td>
<td>0.27 ± 0.212</td>
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<tr>
<td>Maffia</td>
<td>Mf</td>
<td>07°51'S, 39°47'E</td>
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<td>0.37 ± 0.153</td>
<td>0.14 ± 0.137</td>
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<td>Mt</td>
<td>10°16'S, 40°11'E</td>
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<td>13</td>
<td>5</td>
<td>0.70 ± 0.081</td>
<td>0.26 ± 0.201</td>
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<tr>
<td>Nosy Bé</td>
<td>No</td>
<td>13°20'S, 48°15'E</td>
<td>8</td>
<td>3</td>
<td>2</td>
<td>0.46 ± 0.200</td>
<td>0.13 ± 0.141</td>
</tr>
<tr>
<td>Tulear</td>
<td>Tu</td>
<td>23°21'S, 43°40'E</td>
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<td>7</td>
<td>5</td>
<td>0.57 ± 0.138</td>
<td>0.20 ± 0.174</td>
</tr>
<tr>
<td>EIO</td>
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<td></td>
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<td>13</td>
<td>13</td>
<td>0.98 ± 0.050</td>
<td>3.9 ± 1.94</td>
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<tr>
<td>Padang</td>
<td>Pa</td>
<td>00°57'S, 100°21'E</td>
<td>12</td>
<td>12</td>
<td>8</td>
<td>1.00 ± 0.034</td>
<td>3.3 ± 1.81</td>
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<tr>
<td>Pulau Seribu</td>
<td>PS</td>
<td>05°36'S, 106°33'E</td>
<td>12</td>
<td>11</td>
<td>7</td>
<td>0.98 ± 0.040</td>
<td>2.6 ± 1.46</td>
</tr>
<tr>
<td>Karimunjawa</td>
<td>Ka</td>
<td>05°49'S, 110°27'E</td>
<td>10</td>
<td>8</td>
<td>5</td>
<td>0.96 ± 0.059</td>
<td>2.9 ± 1.64</td>
</tr>
<tr>
<td>total</td>
<td></td>
<td></td>
<td>263</td>
<td>69</td>
<td>49</td>
<td>0.66 ± 0.035</td>
<td>0.77 ± 0.046</td>
</tr>
</tbody>
</table>
Restricted gene flow between fish populations

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network was constructed using the program TCS (v. 1.21; Clement et al. 2000).

Results

Genetic diversity

Based on an alignment of 263 sequences (accession numbers of the European Nucleotide Archive: LN824024–824092) with a length of 337 base pairs, 69 haplotypes were identified. None of the identified haplotypes was shared between the WIO and EIO (Fig. 1). Haplotype diversity was considerably higher in the EIO (h = 0.98) than in the WIO (h = 0.53) (Table 1). In the WIO, one dominant haplotype was present in 68% of the individuals. Nucleotide diversity was also much higher in the EIO (3.9%) than in the WIO (0.58%) (Table 1). Haplotypes in the WIO did not differ by more than five mutational steps from each other and the majority only by one or two mutations. On the contrary, divergence amongst EIO haplotypes was much higher and the majority of them were private haplotypes (Fig. 1). Although none of the haplotypes was shared between the WIO and EIO, the two groups were not reciprocally monophyletic. Two EIO haplotypes only differed by one mutational step from two WIO haplotypes and one WIO haplotype was located between two EIO haplotypes.

Genetic population structure in the Indian Ocean

Significant genetic population structure was revealed by an AMOVA with an overall \( \phi_{ST} \) value of 0.28 (P < 0.001). Genetic differentiation between the EIO and WIO, already demonstrated by the absence of shared haplotypes, was confirmed by the pairwise \( \phi_{ST} \) values (Table 2). The three EIO sample sites (Padang, Karimunjava and Pulau Seribu) displayed high and significant (P < 0.001) pairwise \( \phi_{ST} \) values when compared with the WIO, ranging from 0.27 to 0.57.

Genetic population structure in the Eastern Indian Ocean

In the EIO, an indication of a panmictic population with all sample sites linked by high levels of gene flow is provided by the negative, non-significant value of the overall AMOVA (\( \phi_{ST} = -0.00652 \)). This picture of high gene flow is confirmed by the low pairwise \( \phi_{ST} \) values amongst the subpopulations in the EIO (Table 2). Tajima’s test (\( D = -0.354 \)) was not significant but Fu’s \( F_{S} = -8.65; P < 0.01 \) was. The mismatch distribution of pairwise differences (\( SSD = 0.0049; P = 0.37 \)) and a HRI test (\( HRI = 0.008; P = 0.61 \)) also indicated a recent population expansion in the EIO.

Genetic population structure in the Western Indian Ocean

Within the WIO, the overall AMOVA resulted in a low but significant \( \phi_{ST} \) value of 0.016 (P < 0.01). Pairwise \( \phi_{ST} \) values amongst sample sites (Table 2), however, did not indicate any location or geographical region as being significantly differentiated. Several hierarchical AMOVAs were performed, grouping the WIO populations according to geographical and oceanographical criteria, but these did not reveal any significant \( \phi_{ST} \) value. Fu’s \( F_{S} \)-test and Tajima’s D-test for selective pressure were highly significant for the WIO population (\( D = -2.58, P < 0.0001 \); \( F_{S} = -34.10^{37}, P < 0.0001 \)). In order to discriminate between selective pressure and population expansion, the mismatch distributions of the pairwise differences (sum of squared deviation, \( SSD = 0.000043; P = 1 \)) were calculated and a HRI test (\( HRI = 0.068; P = 0.82 \)) was conducted, both very clearly indicating a recent population expansion in the WIO.

Discussion

Genetic population structure in the Indian Ocean

The absence of shared haplotypes and the results of the AMOVAs and pairwise comparisons indicate highly restricted gene flow between the disjunct populations of *Amphiprion akallopisos* in the EIO and WIO. Although some species are able to disperse across the Indian Ocean (Craig et al. 2007; Horne et al. 2008; Vogler et al. 2012; Muths et al. 2013), the findings of this study provide new evidence for the existence of a barrier to gene flow between the EIO and the WIO (Ridgway & Sampayo 2005). These empirical results also support the predictions from dispersal models stating that larvae generally do not drift over distances of thousands of kilometres.

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**Fig. 1.** Maps of the (A): Indian Ocean and (B): Western Indian Ocean (WIO) showing the sample sites and prevailing currents during the Northeast Monsoon: EACC = East African Coast Current; MC = Mozambique Current; MCE = Mozambique Channel Eddies; NEMC = Northeast Madagascar Current; NMC = North Monsoon Current; SC = Somali Current; SEC = South Equatorial Current; SECC = South Equatorial Counter Current; SEMC = Southeast Madagascar Current (Schott & McCreary 2001; Schouten et al. 2003); see Table 1 for definitions of the sample site codes. Light grey areas along the coast show the continental shelf. (C): Haplotype network: black circles represent haplotypes from in the WIO and white circles haplotypes from the Eastern Indian Ocean; size of the circles corresponds to the number of individuals, with the largest circle representing 156 individuals; each line between small dots or haplotypes represents a mutational step.
and predominantly settle close to their home reef (Cowen et al. 2006).

Genetic population structure in the Eastern Indian Ocean

No genetic differentiation in Amphiprion akallopisos was observed amongst the sample sites in the EIO, which indicates high connectivity, a pattern also found in the anemonefish Amphiprion ocellaris (Nelson et al. 2000; Timm et al. 2008, 2012) and the giant clam Tridacna maxima (Nuryanto & Kochzius 2009) in the same region. The sample sites from the Sunda shelf (Java Sea: Pulau Seribu and Karimunjawa) showed a lower genetic diversity of A. akallopisos than Padang on the Indian Ocean coast of Sumatra, which could be due to a re-colonization event. During the last glacial maximum, when the sea level dropped by about 120 m, the Sunda shelf was exposed and the Java Sea did not exist. At the end of the last glacial the sea level rose again and the Sunda shelf was flooded, re-establishing the Java Sea, enabling re-colonization from populations in the EIO and triggering population growth. This explanation is also supported by Rogers’ model of sudden population expansion (Rogers 1995), which could not be rejected in the present study. The same pattern with respect to genetic diversity and population expansion is also observed in A. ocellaris (Nelson et al. 2000; Timm et al. 2008), A. perideraion (Dohna et al. 2015), T. maxima (Nuryanto & Kochzius 2009) and Tridacna crocea (Kochzius & Nuryanto 2008).

Genetic population structure in the Western Indian Ocean

Significant population structure was detected in the WIO, but no genetic break associated with a particular geographical location or oceanographical feature could be found. Several studies in the WIO have indicated low levels of genetic differentiation and the absence of genetic breaks in invertebrates (Duda & Palumbi 1999; Gopurenko et al. 1999; Fratini et al. 2010; Silva et al. 2010b; He et al. 2011; Vogler et al. 2012) and fish (Dorenbosch et al. 2006; Hoareau et al. 2007; Muths et al. 2013). On the contrary, significant differentiation among populations from the East African coast and from Indian Ocean islands has been discovered, indicating a genetic break (Gopal et al. 2006; Ragionieri et al. 2010; Muths et al. 2012). Another genetic break, possibly influenced by prevailing sea-surface currents, has been indicated amongst populations in the northern (Kenya and Tanzania) and the southern (Mozambique, Madagascar and South Africa) part of the WIO (Silva et al. 2010a; Visram et al. 2010; Muths et al. 2011). The splitting of a branch of the South Equatorial Current (SEC) into the Southeast and Northeast Madagascar Current could cause this. The
latter joins the SEC again, which diverges at the East African coast into the northward East African Coast Current (EACC) and southward Mozambique Current. Additionally, the Mozambique Channel Eddies could be responsible for the separation of populations in the Northern and Southern WIO (Fig. 1). The results of this study do not support these findings, but the sample sites did not fully cover the southern part of the WIO. Another potential oceanographical barrier could be the seasonal convergence of the EACC and Somali Current, which continue into the South Equatorial Counter Current, possibly separating the northernmost sample site (Lamu; Fig. 1). However, also here no genetic break could be detected.

A pattern of population structure without a clear genetic break is known as chaotic genetic patchiness. A number of possible explanations for the phenomenon, such as small-scale pre- or post-settlement selection, sweepstakes reproductive success or variation over time in the larval sources that replenish populations, have been suggested (Selkoe et al. 2006). Testing this hypothesis requires additional research on a small scale involving estimation of self-recruitment by parentage analysis. As the significant population structure in A. akallopisos from the WIO indicates the existence of factors restricting gene flow amongst certain reefs, more research on a large scale with additional sample sites and high-resolution genetic markers will be needed to determine the driving force behind the population structure in the WIO. Potential explanations may be ocean currents creating unidirectional gene flow or a barrier to dispersal too recent to be conclusively detected by the genetic marker used in this study.

**Disjunct distribution of Amphiprion akallopisos**

The genetic diversity of *Amphiprion akallopisos* in the EIO is much higher than in the WIO and the very divergent haplotypes from the EIO are connected by several mutational steps in the network, indicating that the species originated in the EIO, probably in the IMA. This is also supported by several molecular phylogenies using different genetic markers, all showing that *A. akallopisos* is closely related to *A. perideraion* and *A. sandaracinos*, both of which are distributed in the IMA (Santini & Polacco 2006; Timm et al. 2008; Litsios et al. 2012). This clade evolved more than 5 mya in the Miocene (Litsios et al. 2012). It is estimated that *A. akallopisos* diverged from its two sister species between 1.7 and 1.9 mya (Timm et al. 2008). The IMA is a highly dynamic region where sea-level changes due to Pleistocene glacial cycles had a strong impact on marine shallow water habitats (Voris 2000), repeatedly isolating populations. This vicariance-creating mechanism triggered genetic differentiation amongst populations and, leading to speciation in some cases (Kochzius et al. 2003; Kochzius & Nuryanto 2008; Timm & Kochzius 2008; Timm et al. 2008; Nuryanto & Kochzius 2009).

The haplotypes of the WIO show the typical star-like structure of a growing population, with a dominant central haplotype connected by only one mutational step to singletons. Rogers’ model of sudden population expansion (Rogers 1995) could also not be rejected, providing additional support for a founder event by colonization of the WIO from the EIO. As the PLD in anemonefish is only 8–12 days (Fautin & Allen 1992), it is very unlikely that larvae of *A. akallopisos* would have drifted all across the Indian Ocean from the EIO to the WIO, e.g. with the SEC (Fig. 1). Owing to the rather short PLD, dispersal across the Indian Ocean would only be possible in a stepping stone model over several generations. In this scenario, larvae from the EIO would disperse with the seasonally changing North Monsoon Current from Sumatra and the Andaman Sea to the southern tip of the Indian subcontinent. From there, further stepping stones would be the Maldives, Chagos, Saya de Malha submerged banks, Seychelles, Reunion and Mauritius. However, *A. akallopisos* does not occur at any of these potential stepping stones. Therefore, such a stepping stone scenario is only possible if *A. akallopisos* was indeed distributed at these stepping stones in the Central Indian Ocean (CIO), but became extinct in the CIO, with remaining relict populations in the WIO and EIO. Extinction of *A. akallopisos* in the CIO could have been driven by competition with younger species of the genus *Amphiprion* that currently occupy the available *Heteractis magnifica* habitat at these sites, and which belong to an Indian Ocean clade that evolved less than 5 mya in the *Pliocena* (Litsios et al. 2012).

**Acknowledgements**

We would like to thank the institutions and foundations that made this work possible: Fonds Wetenschappelijk Onderzoek Vlaanderen for funding the project ‘Connectivity of Coral Reefs and Mangroves in the Western Indian Ocean’ (grant 1501612N); Vlaamse Interuniversitaire Raad – Universitaire Ontwikkelingssamenwerking for a travel grant to F.H.; the German Federal Ministry of Education and Research (grant no. 03F0390B) for funding this study in the framework of the Science for the Protection of Indonesian Coastal Marine Ecosystems project; the relevant authorities for permits; H.A. Ratsimbazafy for providing samples from Madagascar; R.M. van der Ven (Vrije Universiteit Brussel), M. Sheikh and M.S. Mohammed (State University of Zanzibar) and Janne Timm (University of Bremen), as well as Jelvas M. Mwaura (Kenya Marine and Fisheries Research Institute) for assistance during fieldwork; and K. De Winter, L.
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Otwoma, H.A. Ratsimbazafy, T. Sierens and N. Vanbek-bergen (Vrije Universiteit Brussel) for assistance during laboratory work.

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