GENETIC POPULATION STRUCTURE, GENE FLOW, AND EVOLUTIONARY HISTORY OF SELECTED ORNAMENTAL FISH IN THE RED SEA

Tawfiq Froukh

Dissertation submitted as a partial fulfillment of the requirements for the degree
Doctoral of Natural Sciences (Dr. rer. nat.)

Faculty of Biology and Chemistry
University of Bremen

Bremen 2007

Table of Contents

Abstract

The ornamental fishery is expanding rapidly in the Red Sea, and concerns about the possibility of overexploitation were raised. Marine protected areas (MPAs) were addressed as a potential solution to prevent overexploitation. However, the sources of stock recruitment are not well understood. This thesis aims to reveal the genetic population structure and the demographic connectivity in the endemic fish species of the Red Sea *Larabicus quadrilineatus*, and in the two common fish species *Chromis viridis* and *Pseudanthias squamipinnis*.

The fish samples were obtained from five locations in the Red Sea. For comparison, additional samples of the two common species were obtained from two locations in the Indo-Malay Archipelago. Partial sequence of the mitochondrial control region was used as a molecular marker in the three studied species.

The studied species exhibited high genetic diversity as inferred from the haplotype and nucleotide indices. Analysis of molecular variance (AMOVA) detected significant genetic variation between northern and central/southern populations of *L. quadrilineatus* ($\Phi_{CT} = 0.01$; $P < 0.01$), and between the populations in the Gulf of Aqaba and the Red Sea proper of *P. squamipinnis* ($\Phi_{ST} = 0.02$; $P < 0.01$). In addition, AMOVA detected significant genetic variation between the Red Sea and the Indo-Malay Archipelago for both *C. viridis* ($\Phi_{CT} = 0.462$; $P < 0.001$) and *P. squamipinnis* ($\Phi_{ST} = 0.78$; $P < 0.001$). Migration analysis in the Red Sea revealed (1) higher migration into the Gulf of Aqaba for all species; (2) higher northward migration for *C. viridis* and *L. quadrilineatus*; and higher southward migration in the Red Sea proper for *P. squamipinnis*. A significant relationship between the genetic versus the geographic distances was shown only for *L. quadrilineatus*, and as a consequence the mean larval dispersal distance based on the isolation-by-distance model was estimated to be between 0.44 and 5 km. Estimates of the effective population size were the highest (1) in Hodeidah (southern Red Sea) for both *L. quadrilineatus* and *C. viridis*; and (2) in Tor (northern Red Sea) for *P. squamipinnis*.

The results in this thesis were discussed in relation to the oceanographic factors and the biological features of the studied fishes. The historical event Last Glacial Maximum proved its influence on the population demographic history and the currents effective population size of the studied species.

In order to enable a sustainable ornamental fishery on the studied species in the Red Sea, the

results of this thesis suggest that (1) populations in the Red Sea should be managed as one stock for *C. viridis*; (2) populations of *L. quadrilineatus* northern and southern Red Sea should be managed as two stocks; and (3) populations in the Gulf of Aqaba and in the Red Sea proper should be managed separately for *P. squamipinnis*. The rather low larval dispersal distance of about 5 km needs to be considered in the design of MPAs to enable connectivity and self-seeding in *L. quadrilineatus*.

Chapter 1

Review of the thesis

1. Introduction

Ornamental marine fishes are traded globally with an annual estimate of 20-24 million individuals belonging to 1,471 species (Wabnitz *et al.* 2003). There is a growing interest in this kind of trade especially in the Caribbean Sea, the Philippines and Hawaii. In the Red Sea the relative size of ornamental fishery was classified as a small industry (up to 50,000 fish annually; Wood 2001). The aquarium trade in the Red Sea has started 1984 in Egypt and expanded rapidly to Saudi Arabia, Yemen, Eritrea, and Djibouti. This rapid expansion was due to: (1) undisturbed coral reefs, which support a large number of highly marketable aquarium fishes; and (2) the availability of direct flights to Europe and to the United States of America (PERSGA/GEF 2003).

Overexploitation can be the main negative impact of aquarium trade. Many of the targeted reef fishes play a major role in the equilibrium of ecological processes occurring in the coral reef environment. For example, surgeon fish play an essential role in controlling the growth of marine algae. This control is important because an increased algal growth reduces the settlement efficiency of coral larvae (Vine 1974). Another example are trigger fish which play a vital role in controlling the numbers of certain invertebrates like sea urchin and starfish. This control is important because very high numbers (outbreaks) of sea urchins and starfishes destroy corals (Sweatman 1995).

The Regional Organization for the Conservation of the Environment of the Red Sea and Gulf of Aden (PERSGA) addressed marine protected areas (MPAs) as a potential solution to protect these resources against overexploitation (Gladstone *et al.* 2003). However, the sources of stock recruitment are not well understood, including the larval transport in and out the MPAs, the question whether MPAs will be self-seeding or whether they accumulate recruits from surrounding areas, and whether MPAs can exchange recruits.

This thesis aims to provide data on the genetic population structure and gene flow of three ornamental fishes in the Red Sea using molecular tools. Such data on the connectivity among populations are necessary to identify populations that export larvae to areas of exploitation. The selected ornamental fishes are the blue green chromis *Chromis viridis*, fourline wrasse *Larabicus quadrilineatus*, and sea goldie *Pseudanthias squamipinnis*.

1.1 Population genetics and species conservation

Populations and/or stocks are the natural focal units for conservation and management. Identifying population boundaries can have far-reaching management implications (Waples

and Gaggiotto 2006). In marine species it is difficult to identify populations and migration among them directly by mark recapture methods using tags, due to the high mortality in their early life stages and the large areas of dispersal (Thorrold *et al.* 2002). Indirect methods using the techniques of molecular genetics are applied to discriminate among marine species and populations; analyse migration pattern among populations; and estimate their effective population size (Burton 1996; Neigel 1997). Molecular techniques use the variation of distinct alleles at a defined locus, known as molecular markers, to understand the genetic structure of populations. Many types of molecular markers are used for this purpose (Parker *et al.* 1998).

The mitochondrial DNA (mtDNA) of vertebrates is a common molecular marker, used in phylogenetics, population genetics, and species identification because of four peculiar properties: (1) it has rapid rate of evolution estimated as 5 to 10 times higher compared with the nuclear genomes; (2) it is maternally inherited, reflecting the female-specific part in the evolution and history of a certain taxon; (3) it lacks homologous recombination system, avoiding the effect of intermolecular recombination on the rate of mtDNA mutations and thus has a significant impact on the interpretation of mtDNA diversity; and (4) it has high copy number in a cell estimated as more than 1000 copies, playing a key role in all fundamental questions of mitochondrial genetics e.g. the recombination and segregation of mtDNA sequences (Zischler 1999).

MtDNA includes a small non-coding region known as “control region”, which serves as the origin of replication in the mtDNA. D-loop is much more variable than the rest of the mtDNA and is therefore very useful molecular marker for the study of very recently divergent populations (Parker *et al.* 1998).

1.2 Physical and biological features of the Red Sea

The Red Sea is a portion of the Syrian-African rift system, which extends from Mozambique to Turkey. The Red Sea is connected to the Mediterranean through the Suez Canal, and to the Indian Ocean through the Strait of Bab-el-Mandab.

According to Morcos (1970), the Red Sea has a total length of 1932 km, an average width of 280 km, and an average depth of 491 m. The range of total surface area of the Red Sea is 438,000–450,000 km^2 and the volume is 215,000–251,000 km^3. The Red Sea has unique features that make it vulnerable to the impact of human activities. These features include: (1) warm and most saline water of the world’s oceans; (2) no permanent inflow of rivers or streams; (3) prevailing north-westerly winds; (4) partially isolated from the Indian Ocean; (5) located in an arid tropical zone; and (6) sparse, widely variable rainfall (Edwards 1987).

The Red Sea is diverse with the following habitats: sandy shores, rocky shores, mangroves, coral reefs, lagoon and seagrasses, pelagic zones, deep benthic habitat, and deep axial trough. The Red Sea fauna and flora include: seaweeds, phytoplankton, zooplankton, fishes, turtles, dugongs, whales and dolphins (Head 1987). The high level of the Red Sea fauna and flora endemism is estimated as 70% for crinoids, 13.7% for fishes, 9% for algae, and 8.5% for corals (Head 1987; Goren 1993).

Fishes constitute the most diverse group of the Red Sea fauna with about 1,284 fish species compared to more than 4,000 fish species in the Indo-Malay Archipelago, an evolutionary centre of origin for dominant species (Goren 1993; Briggs 2005). The majority of the Red Sea fishes inhabit coral reefs. Little of information are available about the fish communities of the entire Red Sea. However, differences in fish richness, assemblages and abundances among different regions of the Red Sea and Gulf of Aqaba are recorded (Sheppard *et al*. 1992; Khalaf and Kochzius 2002 a&b; PERSGA/GEF 2003).

1.3 Genetic studies in the Red Sea

Ridgway and Sampayo (2005) listed 31 genetic studies conducted in the Western Indian Ocean, three of them include comparisons to the Red Sea. However, nine references have been found which use genetic tools to infer connectivity among marine populations between the Red Sea and other waters. Six of them are comparisons of Lessepsian fishes between the northern Red Sea as well as Gulf of Aqaba and the Mediterranean. The genetic differentiations in populations of the Lessepsian rabbitfishes *Siganus rivulatus* and *Siganus luridus* were tested using mitochondrial and nuclear markers (Bonhomme *et al.* 2003; Hassan *et al.* 2003; Azzurro *et al.* 2006). In these three studies, no genetic differentiation between the Mediterranean and the northern Red Sea was revealed because of the high number of migrants that colonised the Mediterranean. The absence of genetic structure was recorded as well in the Lessepsian fishes *Atherinomorus lacunosus* and *Upeneus moluccensis* (Bucciarelli *et al.* 2002; Hassan and Bonhomme 2005).

The hypothesis “Red Sea to the Mediterranean invasion” was tested on the mussel *Brachidontes pharaonis* using the molecular marker cytochrome oxidase I (Shefer *et al.* 2004). The study showed panmixing among the Mediterranean, Gulf of Suez and the northern Red Sea, due to the drift of the larvae from northern Red Sea to the Mediterranean. Three other studies revealed homogenisation of the mudcrab *Scylla serrata* between one location in the southern Red Sea and the Indo-West Pacific (Gopurenko *et al.* 1999; Fratini & Vannini 2002; Gopurenko 2001).

Two studies compared populations of marine organisms in the northern Red Sea and Gulf of Aqaba. Kochzius & Blohm (2005) investigated the genetic population structure of the lion fish *Pterois miles* in the Gulf of Aqaba and northern Red Sea. They have performed an analysis on 166 bp of the mitochondrial DNA control region, and concluded panmixia between the Gulf of Aqaba and northern Red Sea and unidirectional migration from the Red Sea to the Gulf of Aqaba. Maier *et al.* (2005) investigated the genetic structure of the scleractinian coral *Seriatopora hystrix* from northern Red Sea and Gulf of Aqaba using microsatellite markers. They detected moderate genetic differentiation accompanied with isolation by distance.

2. Objectives and outline of the thesis

In this thesis, I want to test hypotheses concerning population connectivity and evolutionary history of three ornamental fishes in Red Sea using molecular genetic methods. The chosen fishes *C. viridis*, *L. quadrilineatus*, and *P. squamipinnis* are among the most traded ornamental fishes in the Red Sea. In addition, they are widely distributed in the Red Sea compared to other ornamental species (PERSGA/GEF 2003). Genetic investigations of marine populations are virtually the only tool for larval tracking because water current patterns were proved as misleading predictors for larval dispersal (Benzie 1999). The investigations in this thesis will provide estimates of demographic connectivity and thus levels of dispersal capabilities. Such information will be essential for the establishment of marine protected areas. Establishing marine protected areas depending only on life history traits (biological factors) of the protected species might be misleading. This is because connectivity among populations might be reflected from the historical and oceanographic factors beside the biological factors. Therefore, comparative genetic population structure will provide a test for the effect of these factors on population connectivity.

Genetic population structure (Chapters 2 and 3)

Chapter 2 presents the connectivity pattern among populations of the ecologically important endemic fourline wrasse *Larabicus quadrilineatus* along the entire Red Sea coastline of the Arabian Peninsula by determining the levels of genetic differentiation and estimating the amount of gene flow among its populations. In chapter 3, I displayed the biological, historical and oceanographic factors that affect the genetic structure of *C. viridis* and *P. squamipinnis* at

two geographic scales: (1) within the Red Sea; and (2) between the Red Sea and the Indo-Malay Archipelago.

Molecular phylogeny (Chapter 4)

I exposed in this chapter the phylogenetic relationship between the two closely related fish species *C. viridis* and *C. atripectoralis*. In addition, the phylogeography of *C. viridis* in the Indo-Malay Archipelago is discussed.

3. Synoptic discussion

Implications for genetic population structure

The main aim of the thesis was to investigate the genetic population structure of three ornamental fishes in the Red Sea: blue green chromis *C. viridis*, sea goldie *P. squamipinnis*, and cleaner wrasse *L. quadrilineatus*.

The study shows dissimilar genetic structure in these fishes throughout the same ocean basin. While panmixing was revealed for *C. viridis*, a significant genetic structure was disclosed for *P. squamipinnis* (between the Red Sea proper and the Gulf of Aqaba) and *L. quadrilineatus* (between northern and central/southern Red Sea).

These results led to the question: What factors could be responsible for the genetic structure between populations in one fish species and no genetic structure in others in a certain geographic region?

The number of distinct populations in marine species might be determined by the interaction between the physical factors of the habitat, and the ecology as well as biology of larvae. The physical factors include oceanographic factors such as oceanic gyres and currents, and geographic distances. These factors structure the populations of marine organisms by producing geographical clustering and regional divergence (Planes 2002).

Although the studied species live in the same habitat, they are likely none equivalently affected by the physical factors in the Red Sea. Three examples are shown in the thesis: (1) the ecological differences between northern and southern Red Sea, such as increase of turbidity and decrease of coral variety and reef development in the South (Sheppard *et al.* 1992; Roberts *et al*. 1992), which led to differences in fish communities at about 20 N° was only congruent to the genetic differentiation between northern and southern populations of *L. quadrilineatus*; (2) the circulation pattern and the water exchange between the Gulf of Aqaba

and northern Red Sea drive only the genetic structure for *P. squamipinnis*; and (3) the geographic distance was only a factor for the genetic structure in *L. quadrilineatus*.

The biological and ecological features in the early life stages of the studied species must be largely responsible for their different population structures. The features like PLDs, swimming speeds, and the fecundity rates are pointed as possible factors influencing the sensitivity of species to the physical barriers in the marine realm (Zardoya *et al*. 2004). Therefore, knowledge on these features is needed for the full interpretation of the genetic data.

Implications for demographic history and evolution

Historical events are important factors leading to the present genetic structure and speciation in marine organisms. Isolation between marine populations, and hence speciation, is accompanied by extreme changes in the ecological conditions such as decrease in temperature and increase in salinity (Goren 1986). The harsh conditions (low temperature and high salinity) in the Red Sea during the Pleistocene (Siddal 2003), are suggested in this thesis as a historical factors influencing the genetic structure of the studied fishes.

The diversity indices, neutrality tests, and the mismatch distributions of the studied fishes showed that their population size was reduced, most probably as a consequence of the worse ecological conditions in the Red Sea during sea-level low stands. A sudden population expansion has occurred after the ecological changes took place (increase in temperature and decrease in salinity). The Range of expansion, estimated using MIGRATE, was higher from the region of largest effective population size, which is Hodeidah for *C. viridis* and *L. quadrilineatus* and Tor for *P. squamipinnis*. The data on *P. squamipinnis* give signatures supporting the hypothesis that part of the Red Sea fauna survived during the Pleistocene. Unfortunately, the hypothesis of land bridge that might have emerged during the Pleistocene separating the Red Sea completely from the Indian Ocean could not be discussed because it was not possible to obtain samples from Gulf of Aden (Wildman *et al.* 2004; Winney *et al.* 2004; Fernandes *et al.*2006). Further analyses on other marine species in the Red Sea with additional collection sites from the Gulf of Aden would be necessary for more conclusions on the status of the Red Sea and its fauna during the Pleistocene.

Additionally, signatures of the historical processes were shown in: (1) the isolation between the Red Sea and the Indo-Malay Archipelago for populations of *C. viridis* and *P. squamipinnis*; (2) the isolation between the Indonesian and the Philippine populations of *C. viridis*; and (3) the indication of at least one cryptic or incipient species in the blue green

damselfishes, as inferred from the phylogenetic analysis of the sibling species *C. viridis* and *C. atripectoralis*.

Implications for management and conservation

The effective management of marine ecosystems must match the population/stock structure and the dispersal capability of the target species (Palumbi 2004). The derived information on stock structure based on morphological, geographical, and/or behavioral data rather than genetic data are misleading (Avise *et al.* 1987).

The studied species are among the most traded ornamental fishes in the Red Sea. The lack of basic knowledge on the studied species might infer that the stocks have no boundaries. Therefore, population decline is not noticed and hence, stocks are not regulated in response to over fishing.

For *C. viridis*, the observed genetic homogeneity was interpreted as support for the view that this species should be managed in the Red Sea as one stock. For *L. quadrilineatus*, the proofed separation between the populations northern and southern Red Sea should be taken into consideration by managing these populations as two different stocks. The rather low larval dispersal distance of about 5 km needs to be considered in the design of MPAs to enable connectivity and self seeding. Finally, *P. squamipinnis* should be managed as two stocks according to the separation between the Red Sea proper and the Gulf of Aqaba.

References

Avise JC, Arnold J, Ball RM, Bermingham E, Lamb T, Neigel JE, Reeb CA, Saunders NC (1987) Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics*, **18**, 489–522.

Azzurro E, Golani D, Bucciarelli G, Bernardi G (2006) Genetics of the early stages of invasion of the Lessepsian rabbitfish *Siganus luridus*. *Journal of Experimental Marine Biology and Ecology*, **333**, 190-201.

Benzie JAH (1999) Genetic structure of coral reef organisms: ghosts of dispersal past. *American Zoology*, **39**, 131-145.

Bonhomme F, Baranes A, Golani D, Harmelin-Vivien M (2003) Lack of mitochondrial differentiation between Red Sea and Mediterranean populations of the Lessepsian rabbitfish, *Siganus rivulatus* (Perciforms: Siganidae). *Scientia Marina*, **67**, 215-217.

Briggs JC (2005. The marine East Indies: diversity and speciation. *Journal of Biogeography*, **32**, 1517-1522.

Bucciarelli G, Golani D, Bernardi G (2002) Genetic cryptic species as biological invaders: the case of a Lessepsian fish migrant, the hardyhead silverside *Atherinomorus lacunosus*. *Journal of Experimental Marine Biology and Ecology*, **273**, 143–149.

Burton RS (1996) Molecular tools in marine ecology. *Journal of Experimental Marine Biology and Ecology*, **200**, 85-101.

Edwards FJ (1987) Climate and Oceanography. In: *Key Environments. Red Sea* (eds Edwards AJ, Mead SM), pp. 45-69. Pergamon Press, Oxford, UK.

Fernandes C, Rohling E, Siddall M (2006) Absence of post-Miocene Red Sea land bridges: biogeographic implications. *Journal of Biogeography*, **33**, 961-966.

Fratini S, Vannini M (2002) Genetic differentiation in the mud crab *Scylla serrata* (Decapoda: Portunidae) within the Indian Ocean. *Journal of Experimental Marine Biology and Ecology*, **272**, 103-116.

Gladstone W, Krupp F, Younis M (2003) Development of a network of marine protected areas in the Red Sea and Gulf of Aden region. *Ocean and Coastal Management*, **46**, 741-761.

Gopurenko D (2001) Genetic structure within the distruíbution of the Indo-West Pacific mud crab *Scylla serrata*. PhD thesis, Griffith University, Queensland, Australia.

Gopurenko D, Hughes JM, Keenan CP (1999) Mitochondrial DNA evidence for rapid colonisation of the Indo-West Pacific by the mudcrab *Scylla serrata*, *Marine Biology*, **134**, 227-233.

Goren M (1986) A suggested model for the recolonisation process of the Red Sea at the post glacial period. In: Indo-Pacific fish biology: proceedings of the second international conference on Indo-Pacific fishes (eds Uyeno T, Arai R, Taniuchi T, Matsuura K), pp 648-654. Ichthyological Society of Japan, Tokyo.

Goren M (1993) Statistical aspects of the Red Sea Ichthyofauna. *Israel Journal of Zoology*, **39**, 293-298.

Hassan M, Bonhomme F (2005) No reduction in neutral variability of mitochondrial and nuclear genes for a Lessepsian migrant, Upeneus moluccensis. *Journal of Fish Biology*. **66**, 3-865.

Hassan M, Harmelin-Vivien M, Bonhomme F (2003) Lessepsian invasion without bottleneck: examples of two rabbitfish species (*Siganus rivulatus* and *Siganus luridus*). *Journal of Experimental Marine Biology and Ecology*, **291**, 219-232.

Head S (1987) Key Environment Red Sea (eds Edwards A, Head S), pp 1-21. Pergamon Press, Oxford, UK.

Khalaf MA, Kochzius M (2002a) Changes in trophic community structure of shore fishes at an industrial site in the gulf of Aqaba, Red Sea. *Marine Ecology Progress Series*, **239**, 287-299.

Khalaf MA, Kochzius M (2002b) Community structure and biogeography of shore fishes in the Gulf of Aqaba, Red Sea. *Helgoland Marine Research*, **55**, 252-284.

Kochzius M, Blohm D (2005) Genetic population structure of the lionfish *Pterois miles* (Scorpaenidae, Pteroinae) in the Gulf of Aqaba and northern Red Sea. *Gene*, **347**, 295-301.

Maier E, Tollrian R, Rinkevich B, Nürnberger B (2005) Isolation by distance in the scleractinian coral *Seriatopora hystrix* from the Red Sea. *Marine Biology*, **147**, 1109-1120.

Morcos SA (1970) Physical and chemical oceanography of the Red Sea. *Oceanography Marine Biology Annual Review*, **8**, 73-202.

Neigel JE (1997) Population genetics and demography of marine species. In: Marine Biodiversity (eds Ormond RFG, Gage JD, Angel MV), pp.274-292, Cambridge University press.

Palumbi SR (2004) Marine reserves and ocean neighborhoods: the spatial scale of marine populations and their management. Annual Review of Environment and Resources, **29**, 31-68.

Parker PG, Snow AA, Schug MD, Booton GC, Fuerst PA (1998) What molecules can tell us about populations: Choosing and using a molecular marker. *Ecology*, **79**, 361-382.

PERSGA/GEF (2003) Current status of the ornamental fish trade: their assemblages, collections, practices, export, conservation issues and management in The Red Sea and Gulf of Aden. PERSGA Technical Series Nr. 9. PERSGA, Jeddah.

Planes S (2002) Biogeography and larval dispersal inferred from population genetic analysis. In: Coral reef fishes (eds Sale PF), pp. 201-220. Academic Press, New York.

Ridgway T, Sampayo EM (2005) Population genetic status of the western Indian Ocean: what do we know? *Western Indian Ocean Journal of Marine Sciences*, **4**, 1-9.

Roberts CM, McClean CJ, Veron JEN, Hawkins JP, Allen GR, McAllister DE, Mittermeier CG, Schueler FW, Spalding M, Wells F, Vynne C, Werner TB (2002) Marine biodiversity hotspots and conservation priorities for tropical reefs. *Science*, **295**, 1280-1284.

Shefer S, Abelson A, Mokady O, Geffen E (2004) Red to Mediterranean Sea bioinvasion: natural drift through the Suez Canal, or anthropogenic transport? *Molecular Ecology*, **13**, 2333-2343.

Sheppard C, Price A, Roberts C (1992) *Marine ecology of the Arabian region*. Academic Press, London, UK.

Siddall M, Rohling E, Almogi-Labin A, Hemleben C, Melschner D, Schmelzer I, Smeed D (2003) Sea-level fluctuations during the last glacial cycle. *Nature*, **423**, 853-858.

Sweatman HPA (1995) A field study of fish predation on juvenile crown-of-thorns starfish. *Coral Reefs*, **14**, 47-53.

Thorrold SR, Jones GP, Hellberg ME, Burton RS, Swearer SE, Neigel JE, Morgan SG, Warner RR (2002) Quantifying larval retention and connectivity in marine populations with artificial and natural markers. *Bulletin of Marine Science*, **70**, 291-308.ö

Vine PJ (1974) Effects of algal grazing and aggressive behaviour of the fishes *Pomacentrus lividus* and *Acanthurus shoal* on coral reef ecology. *Marine Biology*, **24**, 131-136.

Wabnitz C, Taylor M, Green E, Razak T (2003) From Ocean to Aquarium. UNEP-WCMC, Cambridge, UK.

Waples RS, Gaggiotti O (2006) Waht is a population? An empirical evaluation of some genetic methods identifying the number of gene pools and their degree of connectivity. *Molecular Ecology*, **15**, 1419-1439.

Wildman D, Bergman T, Al-Aghbari A, Sterner K, Newman T, Phillips-Conroy J, Jolly C, Disotell T (2004) Mitochondrial evidence for the origin of hamadryas baboons. *Molecular Phylogenetics and Evolution*, **32**: 287-296.

Winney B, Hammond R, Macasero W, Flores B, Boug A, Biquand V, Biquand S, Bruford W (2004) Crossing the Red Sea: phylogeography of the hamadryas baboon, *Papio hamadryas hamadryas*. *Molecular Ecology*, **13**, 2819-2827.

Wood E (2001) Collection of coral reef fish for aquaria: global trade, conservation issues and management strategies. Marine Conservation Society, Ross-on-Wye, UK.

Zardoya R, Castilho R, Grande C, Favre-Krey L, Caetano S, Marcato S, Krey G, Patarnello T (2004) Differential population structuring of two closely related fish species, the mackerel (*Scomber scombrus*) and the chub mackerel (*Scomber japonicus*), in the Mediterranean Sea. *Molecular Ecology*, **13**:1785-1798.

Zischler H (1999) Mitochondrial DNA: diversity analysis and pitfalls. In: Methods and tools in Biosciences and Medicine DNA profiling and DNA fingerprinting (eds Epplen JT, Lubjuhn T), pp.117-131. Birkhäuser Verlag Basel, Switzerland.

Chapter 2

Genetic population structure of the endemic fourline wrasse (*Larabicus quadrilineatus*) suggests limited larval dispersal distances in the Red Sea

Tawfiq Froukh, Marc Kochzius

Published in *Molecular Ecology* (doi: 10.1111/j.1365-294X.2007.03236.x)

Abstract

The connectivity among marine populations is determined by the dispersal capabilities of adults as well as their eggs and larvae. Dispersal distances and directions have a profound effect on gene flow and genetic differentiation within species. Genetic homogeneity over large areas is a common feature of coral reef fishes and can reflect high dispersal capability resulting in high levels of gene flow. If fish larvae return to their parental reef, gene flow would be restricted and genetic differentiation could occur. *Larabicus quadrilineatus* (Labridae) is considered as an endemic fish species of the Red Sea and Gulf of Aden. The juveniles of this species are cleaner fish that feed on ectoparasites of other fishes. Here, we investigated the genetic population structure and gene flow in *L. quadrilineatus* among five locations in the Red Sea to infer connectivity among them. To estimate genetic diversity, we analysed 369 bp of 237 mitochondrial DNA control region sequences. Haplotype and nucleotide diversities were higher in the southern than in the northern Red Sea. Analysis of molecular variance (AMOVA) detected the highest significant genetic variation between northern and central/southern populations ($\Phi_{ct} = 0.01$; $p < 0.001$). Migration analysis revealed a several fold higher northward than southward migration, which could be explained by oceanographic conditions and spawning season. Even though the Φ_{st} value of 0.01 is rather low and implies a long larval dispersal distance, estimates based on the isolation by distance model show a very low mean larval dispersal distance (0.44 to 5.1 km) compared to other studies. In order to enable a sustainable ornamental fishery on the fourline wrasse, the results of this study suggest that populations in the northern and southern Red Sea should be managed separately as two different stocks. The rather low larval dispersal distance of about 5 km needs to be considered in the design of MPAs to enable connectivity and self seeding.

Keywords: aquarium trade, Arabian Peninsula, conservation, effective migrants, isolation by distance, neutrality test, population expansion

Introduction

The connectivity among marine populations is determined by the dispersal capabilities of adults as well as their eggs and larvae. Fishes on coral reefs have a life history with two totally different phases: larvae of virtually all species are planktonic (Leis 1991), whereas adults are relatively strongly site-attached and sedentary (Sale 1980). Dispersal distances and directions have a profound effect on gene flow and genetic differentiation within species. Genetic homogeneity over large areas is a common feature of coral reef fishes and can reflect high dispersal capabilities resulting in high levels of gene flow (Planes *et al.* 1993, Doherty *et al.* 1995, Shulman and Bermingham 1995, Bernardi *et al.* 2001). If fish larvae return to their parental reef, gene flow would be restricted and genetic differentiation could occur (Planes *et al.* 1996, Planes *et al.* 1998). However, the scale of dispersal might be limited by fish behaviour or oceanographic factors even in fishes that have long pelagic larval durations (Sponaugle *et al.* 2002). On the one hand, studies on late pelagic stages of coral reef fishes have demonstrated that they can swim against strong currents (Leis and Carson-Ewart 1997). On the other hand, gyres can cause retention of larvae at their parental reef (Johannes 1978, Swearer *et al.* 1999).

Connectivity of populations can be inferred by investigating the genetic population structure with molecular markers, such as the mitochondrial control region. The control region is a suitable marker for such studies (Avise *et al.* 1987, Parker *et al.* 1998,), because it has a much higher mutation rate than any of the mitochondrial genes (Lee *et al*. 1995).

However, only a few studies used genetic tools to infer gene flow in the Red Sea and therefore connectivity among populations in this region is virtually unknown. Most of these studies are restricted to the northern Red Sea and Gulf of Aqaba, including fishes (Hassan *et al*. 2003, Kochzius & Blohm 2005) and a stony coral (Maier *et al*. 2005). Only one study compares northern and southern Red Sea populations of a eulittoral bivalve (Shefer *et al.* 2004) at the African coastline.

The fourline wrasse *Larabicus quadrilineatus* (Labridae) occurs on coral reefs and is considered as an endemic fish species of the Red Sea and Gulf of Aden. The juveniles of this species are cleaner fish that feed on ectoparasites and mucus of other fishes, whereas adults appear to feed mainly on coral polyps (Randall 1983). Several studies supported the view that the interaction between cleaner fish and its client is a mutual relationship and plays a very important ecological role. Studies by Grutter (1999) and Bshary (2003) found a significant reduction of client fish after the experimental removal of cleaner fish. In particular, Bshary

(2003) conducted a field experiment on the impact of the cleaner wrasse *Labroides dimidiatus* on reef fish distribution in the Red Sea at Ras Mohammed National Park, Egypt. He showed significant decline in fish diversity after 4-20 months of cleaner fish removal, and inferred that cleaner fish are a key organism for local reef fish diversity.

This study aims to investigate connectivity among populations of the ecologically important endemic fourline wrasse *L. quadrilineatus* along the entire Red Sea coastline of the Arabian Peninsula by determining the levels of genetic differentiation and estimating the amount of gene flow among its populations. This covers almost the whole species range. Such data on migration rates will uncover the degree of connectivity among populations in the Red Sea and will give insights in dispersal and evolutionary processes in the Red Sea.

Materials and Methods

Tissues and fin clips of 237 individuals from five locations in the Red Sea (Figure 1) were collected in November and December 2003 either by SCUBA diving or by obtaining them from local ornamental fishermen. Samples were placed immediately in absolute ethanol and were stored in the laboratory at –75 °C.

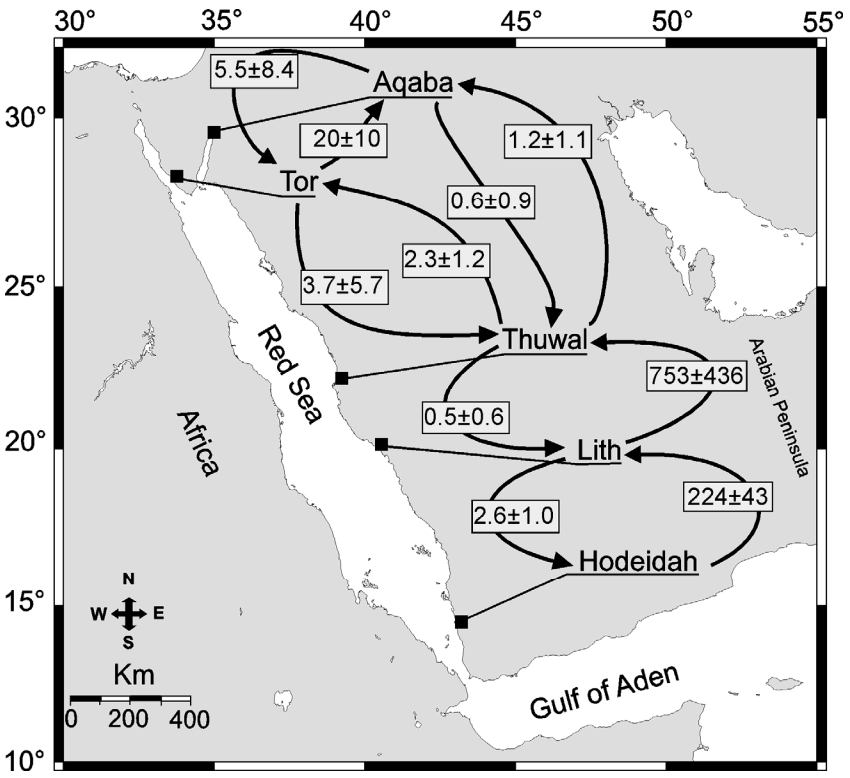

Figure 1 Map of the Red Sea showing collections sites and the mean number of effective migrants ± standard deviation of *Larabicus quadrilineatus* among these sites. The results are mean values of six runs with the programme MIGRATE

DNA was extracted with Chelex® as described in Söller *et al*. (2000). A fragment of 369 bp from the 5´ end of the mitochondrial control region was amplified with the universal primer CR-A and the specific primer XAN-DL-R (Fauvelot *et al*. 2003). Each PCR reaction with a volume of 50 µl contained 10 mM Tris-HCl (pH 9), 50 mM KCl, 2 mM $MgCl_2$, 0.2 mM each dNTP, 0.2 µM each primer, 2 U Taq polymerase and 2 µl of the DNA template. The following temperature profile was used for the polymerase chain reaction: hot start at 95 °C for 5 min followed by 35 cycles of 94 °C (45 s), 50 °C (45 s), 72 °C (60 s), and a final extension at 72 °C for 5 min.

PCR products were purified with the QIAquick PCR purification kit (Qiagen, Hilden). Sequencing of both strands was done with the same primers used for PCR and an ABI Prism 310 automated sequencer following the manufacturer’s protocol (Applied Biosystems, Foster City, CA). The two sequenced strands were aligned with the programme SEQUENCE NAVIGATOR (version 1.0.1; Applied Biosystem) and confirmed by eye. A multiple alignment of all sequences was done with the programme CLUSTAL W (Thompson *et al.* 1994) as implemented in BioEdit (version 7.0.4.1; Hall 1999).

The number of haplotypes and polymorphic sites was derived using the programme COLLAPSE (version 1.2; available at http://darwin.uvigo.es) and verified by DNASP (version 4.10.3; Rozas *et al.* 2003). Mean nucleotide und haplotype diversities were calculated using ARLEQUIN (version 3.0.1; Schneider *et al*., 2000).

A haplotype network was constructed using the median-joining network method as implemented in NETWORK (version 4.1.1.1 available at www.fluxus-engineering.com; Bandelt *et al*. 1999). This method begins by combining the minimum spanning trees (MSTs) within a single network (minimum spanning network, MSN) using an analogous algorithm to that proposed by Excoffier and Smouse (1994). Then, median vectors (which represent missing intermediates haplotypes) are added to the network using the parsimony criterion.

Tajima’s *D* test (Tajima 1989) and Fu’s *Fs* test (Fu 1997) with 10,000 permutations as implemented in ARLEQUIN (version 3.01; Schneider *et al.*, 2000) were used to test the hypothesis of neutrality of the marker. Negative Tajima’s *D*-values can indicate selection, but also population bottlenecks or population expansions (Tajima 1989). We used mismatch distributions to evaluate the hypothesis of recent population growth with 99,999 permutations as implemented in ARLEQUIN 3.0.1 (Rogers and Harpending 1992). This distribution is commonly unimodal in populations that have passed through a recent demographic expansion, and is multimodal in stable populations.

Collection sites were regarded as populations and the hypothesis of panmixing was tested using AMOVA (Excoffier *et al.* 1992), as well as the exact test of population differentiation (10,000 permutations; Raymond & Rousset 1995). Alternative groupings between populations were tested using AMOVA to find the highest significant grouping. AMOVA is similar to other approaches based on the analysis of variance of gene frequencies, but it takes into account the number of mutations between molecular haplotypes. The significance of Φ-values was tested using a non-parametric permutation procedure with 10,000 permutations. The exact test of population differentiation evaluates the hypothesis of a random distribution of different haplotypes among pairs of populations.

The amount of connectivity among the collection sites was measured by estimating: (1) the number of effective migrants among populations using the programme MIGRATE, and (2) the mean larval dispersal distance based on the slope of the relationship of genetic and geographic distances. Based on the linear distribution of collection sites along the Red Sea coast of the Arabian Peninsula we hypothesised a stepping stone model for estimating migration rates only between adjacent collection sites. Due to the detected isolation by distance in *L. quadrilineatu*s, the slope of the regression of $F_{ST}/(1-F_{ST})$ to geographical distance was considered appropriate to estimate the mean larval dispersal distance (Rousset 1997).

Migration among collection sites was evaluated using the programme MIGRATE (version 2.0.3; Beerli & Felsenstein 1999, 2001; Beerli 2005). MIGRATE estimates migration rates M, and effective population sizes Θ. The effective number of migrants among collection sites (γ) was calculated as follows: $\gamma=\Theta M$. The programme MIGRATE uses a maximum-likelihood framework based on the coalescence theory and investigates possible genealogies with migration events using a Markov chain Monte Carlo approach (Beerli & Felsenstein 2001). A transition/transversion ratio of 3.93 and the following base frequencies were incorporated in the analysis: A = 0.32, C = 0.21, G = 0.16, T = 0.31. These parameters were obtained with the programme MODELTEST (version 3.7; Posada & Crandall 1998). We performed six independent runs, each run with 10 short chains of 5000 steps and 3 long chains of 50,000 steps with a sampling increment of 100. In addition to that, heating was used for 4 chains.

The relationship between genetic and geographic distances among populations was examined using Reduced Major Axis (RMA) regression. Correlation of pairwise genetic distances ($F_{ST}/(1-F_{ST})$; Rousset 1997) and geographic distances among populations was assessed using the Mantel test (Mantel 1967) as implemented in the isolation by distance web service IBDWS (version 2.6; Bohonak 2002; http://phage.sdsu.edu/%7Ejensen/) with 30,000

permutation to test significance. Mean larval dispersal distance was estimated from the slope of the relationship of genetic and geographic distances. The slope of the regression of $F_{ST}/(1-F_{ST})$ to geographical distance is related to the inverse of $4D\sigma^2$, where D refers to the density of individuals and σ^2 refers to the variance of parental position relative to offspring position (Rousset 1997). By substituting values of slope and appropriate adult densities (D), estimates of σ^2 are obtained. Adult densities were taken from Khalaf & Kochzius (2002). Assuming that dispersal follows a symmetrical two sided exponential distribution centred at zero, estimate of σ^2 may then be related to average deviation from the mean (standard deviation or mean dispersal distance) as $\sigma^2=2d^2$, where d refers to the mean dispersal distance.

Results

Sequences of a 369 bp fragment of the mtDNA control region were obtained for 237 individuals sampled from 5 locations. Fifty seven polymorphic sites revealed 91 haplotypes. Three haplotypes were shared among all populations, eight were shared by two or three populations, and all others were either unique to Aqaba (10), Tor (18), Thuwal (11), Lith (19), or Hodeidah (22). Haplotype diversity ranged from 0.80 to 0.95, nucleotide diversity from 0.38% to 0.82%, and the mean number of nucleotide differences ranged from 1.4 to 3.0 (Table 1).

Table 1 Mitochondrial DNA control region sequences variation of Larabicus quadrilineatus populations in the Red Sea and their genetic diversity indices.

Sample site	n	No. of haplotypes	No. of variable sites	Haplotype diversity h ± SD	Nucleotide diversity π% ± SD	Mean number of pairwise nucleotide differences ± SD
Aqaba	52	16	16	0.80 ± 0.05	0.38 ± 0.27	1.4 ± 0.87
Tor	48	26	21	0.92 ± 0.03	0.64 ± 0.40	2.3 ± 1.30
Thuwal	46	18	18	0.86 ± 0.03	0.54 ± 0.35	1.9 ± 1.10
Lith	39	25	25	0.95 ± 0.02	0.82 ± 0.48	3.0 ± 1.60
Hodeidah	52	32	30	0.95 ± 0.02	0.79 ± 0.47	2.8 ± 1.50

AMOVA analysis rejected the null hypothesis that the studied populations are homogeneous in the Red Sea (Φ_{st} = 0.01; p = 0.01; Table 2). The great majority of variation was within (98.99 %) rather than among populations (1.01%). Alternative groupings between populations with AMOVA showed the highest significant differentiation when we arranged populations in two groups: northern (Aqaba and Tor) and central/southern (Thuwal, Lith and Hodeidah; Table 2) Red Sea. The exact test of population differentiation was significant ($p < 0.05$) between the northern and central/southern Red Sea, but not within these groups ($p > 0.05$).

Table 2 Hierarchical analysis of molecular variance (AMOVA) of mtDNA control region sequences in *Larabicus quadrilineatus* from the Red Sea. *0.05≥*p*≥0.01, ** 0.01>*p*≥0.001, ***$p<0.001$

Region groupings	Φ_{ST}	Φ_{CT}	% variance among groups
No grouping	0.010**	—	1.01
(Aqaba) (Tor, Thuwal, Lith, Hodeidah)	0.008	0.003	-0.34
(Aqaba, Tor) (Thuwal, Lith, Hodeidah)	**0.015**	**0.012*** **	**1.18**
(Aqaba, Tor, Thuwal) (Lith, Hodeidah)	0.014	0.011**	1.11
(Aqaba, Tor, Thuwal, Lith) (Hodeidah)	0.007	0.005	-0.50
(Aqaba, Tor) (Thuwal) (Lith, Hodeidah)	0.012	0.011**	1.07
(Aqaba, Tor) (Thuwal, Lith) (Hodeidah)	0.011	0.005	0.47

The median-joining network (not shown) of the 91 haplotypes contained many loops and was therefore not very informative. Three of the haplotypes were shared among all populations and represented 43 % of all haplotypes. The proportion of haplotype 1 decreases from North to South, whereas the proportion of haplotype 3 increases.

The negative and significant values of Tajima's *D* and Fu's *Fs* tests rejected the selective neutrality hypothesis and implied selection, population bottleneck or population expansion. Mismatch distribution was unimodal which is consistent with the model of sudden population expansion from a small number of fish (Figure 2). Both sum of square deviations (SSD) and raggedness index (r) tests suggested that the observed distribution curves do not significantly differ from the simulated distribution curves under a model of demographic expansion (Table 3).

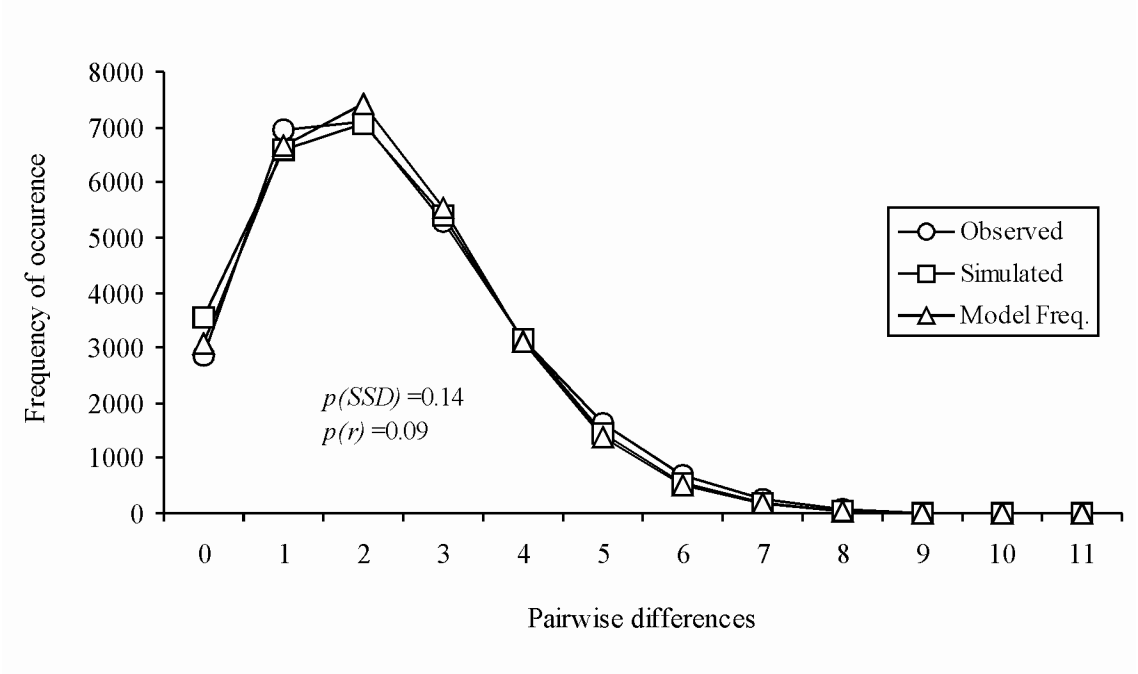

Figure 2 Mismatch distribution of mtDNA sequences of *Larabicus quadrilineatus*. The curves represent the frequency distribution of pairwise differences.

Table 3 Neutrality tests and the estimated parameters of mismatch distribution for populations of *Larabicus quadrilineatus* from the Red Sea. *D* = Tajima's D; *p(D) = p* value for *D*; *F* = Fu's *Fs*; *p(F) = p* value for *Fs*; Mismatch = mean and standard deviation of mismatch distribution; *p(SSD) = p* value of sum of square deviations (*SSD*) between the observed and the expected mismatch; *p(r) = p* value of the raggedness index of the observed distribution. Some values are missing because the least squares procedures to fit the model mismatch distribution and the observed distribution did not converge after 1,800 steps. Significant *p* values are marked with *

Sampling site	*D*	*p(D)*	*F*	*p(F)*	Mismatch	*p(SSD)*	*p(r)*
Aqaba	-1.870	0.011*	-11.883	<0.001*	1.39 ± 1.55	—	—
Tor	-1.505	0.045*	-24.953	<0.001*	2.34 ± 1.90	—	—
Thuwal	-1.521	0.043*	-12.294	<0.001*	1.96 ± 2.09	0.351	0.024*
Lith	-1.568	0.038*	-22.191	<0.001*	2.98 ± 2.94	0.685	0.333
Hodeidah	-1.889	0.010*	-26.481	<0.001*	2.85 ± 2.82	—	—
Aqaba&Tor	-1.911	0.006*	-27.610	<0.001*	1.86 ± 1.88	—	—
Thuwal, Lith & Hodeidah	-1.986	0.003*	-26.746	<0.001*	2.59 ± 2.74	0.363	0.265
All	-2.142	0.001*	-26.808	<0.001*	2.29 ± 2.45	0.137	0.085

Results of MIGRATE analysis indicated a several fold higher northward than southward migration (Figure 1). The effective population sizes are higher in the southern Red Sea than in the northern Red Sea (Table 4).

Table 4 Proportions of theta values (effective population sizes) of *Larabicus quadrilineatus* from five populations in the Red Sea. Theta values (Θ) were estimated using the programme MIGRATE.

	Θ	Proportion (%)
Aqaba	0.000413	4.5
Tor	0.000436	4.7
Thuwal	0.000082	1.0
Lith	0.001937	21.0
Hodeidah	0.006348	68.8

According to the Mantel test (r = 0.66; *p* = 0.017), the relationship between genetic and geographical distances was significantly correlated (Figure 3). The mean larval dispersal distance was estimated from the slope of the Reduced Major Axis (RMA) regression of genetic and geographic distances. Dispersal distances based on a slope of 0.0488 per 1000 km and on adult densities of 100, 120, 500 and 13320 adults km^{-2} are 5.1 km, 4.6 km, 2.3 km, and 0.44 km per generation, respectively.

Discussion

Genetic population structure

This study on *Larabicus quadrilineatus* revealed significant levels of genetic differentiation among populations. The used marker, a fragment of the mitochondrial control region, obtained 91 haplotypes from 237 individuals. All collection sites, including Aqaba and Hodeidah (1700 km distance), were dominated by three ancestral haplotypes.

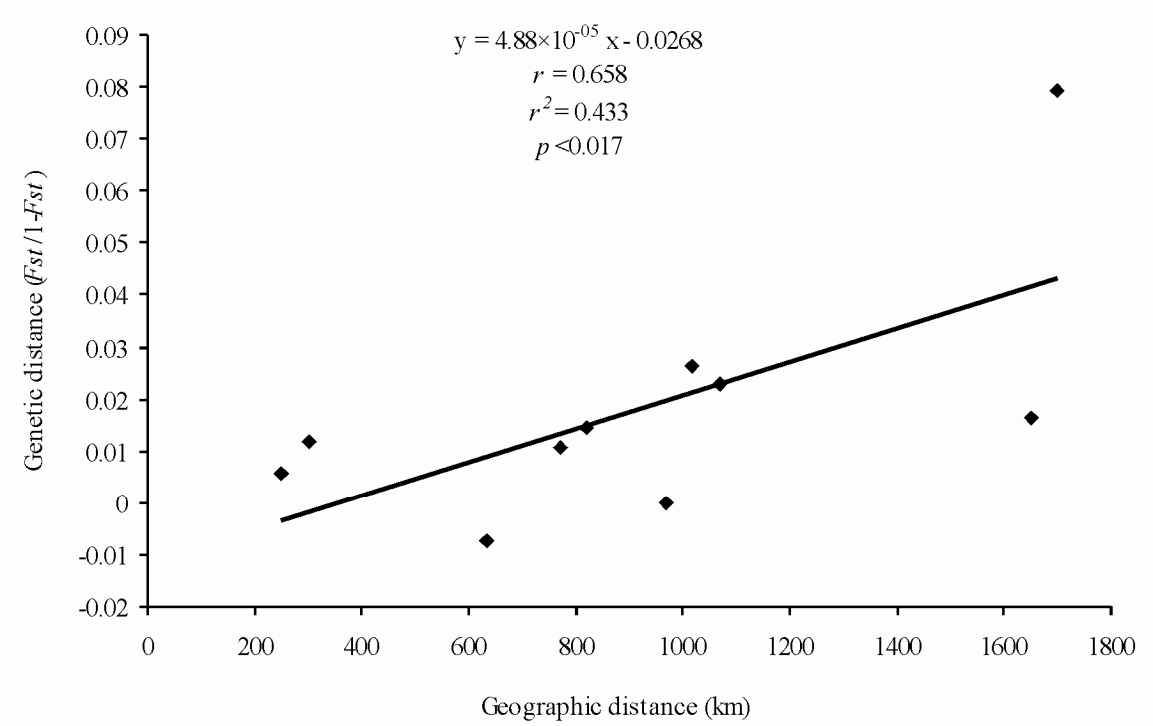

Figure 3 Relationship between genetic versus geographic distances in *Larabicus quadrilineatus* using Reduced Major Axis (RMA) regression.

The detected genetic differentiation between the northern (Aqaba and Tor) and central/southern (Thuwal, Lith and Hodeiadah) Red Sea is inferred from the significant estimate of Φ-values and the significant pairwise variances. This genetic structure is supported by the MIGRATE analysis, which revealed a higher number of migrants within the northern (Aqaba and Tor) and within the central/southern (Thuwal and Lith; Lith and Hodeidah) Red Sea, compared to the number of migrants between North and South. The genetic differentiation observed between the northern and central/southern regions is congruent to findings in differences of fish communities in the Red Sea at about 20 N°. This is due to ecological differences between the northern and southern Red Sea, such as an increase of turbidity and decrease of coral variety and reef development in the South (Sheppard *et al.* 1992; Roberts *et al*. 1992).

The lack of population structure within northern region of the Red Sea coincides with studies on fishes by Kochzius and Blohm (2005) and Hassan *et al.* (2003), which suggest high gene flow and panmixing in the Gulf of Aqaba and northern Red Sea.

Historical demography

The median joining network did not reveal any geographic cluster. All haplotypes of all populations were scattered throughout the network. This lack of structure is likely due to

incomplete separation of haplotypes between the northern and central/southern Red Sea caused by migration among these populations or a population expansion. Tajima’s *D* and Fu’s *Fs* tests rejected the neutrality hypothesis for all collection sites. These tests cannot distinguish between selection and changes in population size. We performed Roger’s test to detect departure from the null hypothesis of sudden demographic expansion (Rogers & Harpending 1992). Our data showed a unimodal distribution of pairwise differences which indicates a recent population expansion. Therefore, a sudden demographic expansion rather than selection seems to be the reason for the rejection of the neutrality tests.

This pattern of a shallow population structure and indication of a sudden population expansion could be explained by the demographic history of *L. quadrilineatus*. Paleoceanographic studies revealed that during the Pleistocene water exchange between the Red Sea and Indian Ocean at the shallow sill of Bab-el-Mandab was restricted (Siddall *et al*. 2003) or even interrupted (Braithwaite 1987). However, the extent of environmental changes in the Red Sea is still controversial. Some authors assume hypersalinity that killed most marine life (Sheppard *et al*. 1992) or even conditions comparable with the present day Dead Sea (Braithwaite 1987), while others suggest survival of the fauna (Goren 1986, Klausewitz 1989, Rohling *et al*. 1998). However, in any case the population size of organisms in the Red Sea was reduced and a population expansion either took place from a reduced surviving population in the Red Sea or by re-colonisation from the Gulf of Aden, which is proposed as a refuge of the Red Sea fauna during the glacials (Klausewitz 1989).

The estimated effective population sizes were higher in the southern Red Sea (Lith and Hodeidah) than northern Red Sea (Aqaba and Tor) (Table 4). This difference might be caused by the northward directed re-colonisation from the Gulf of Aden after the last sea-level low stand (Klausewitz 1989).

Direction of gene flow and mean larval dispersal distance

The estimated effective number of migrants among the collection sites using the programme MIGRATE was several fold higher to the North than to the South. This pattern of migration in the Red Sea seems to be influenced by a combination of two factors: currents and spawning season. In summer (April to October) the currents are directed to the North, with a deflection to the Arabian Peninsula. In contrast, during winter (November to March) currents are southward directed and show a deflection to the African coast (Morcos, 1970). There are no published studies about the spawning season of *L. quadrilineatu*s, but several studies indicate that the spawning season of fishes in the Red Sea is during the summer months (Wahbeh &

Ajiad 1985, Froukh 2001, Zekeria 2003). This finding shows, that oceanographic conditions and timing of spawning are important factors for determining the direction of dispersal in a marine organism.

Even though the Φ_{st} value of 0.01 is rather low and implies a long larval dispersal distance, estimates based on the isolation by distance model show a very low mean larval dispersal distance (0.44 to 5.1 km) compared to other studies. Buonaccorsi *et al.* (2004) estimated 10 km as a larval dispersal distance per generation of the grass rockfish (*Sebastes rastrelliger*) from the Californian coastline based on 100 individuals km^{-2}. Based on a two dimensional stepping stone model and assuming a population size of 500 individuals km^{-2}, Kinlan & Gaines (2003) estimated mean dispersal distances from genetic isolation by distance slopes. Their estimates of dispersal for 28 fish ranged from few kilometres (2.3 km for *Axoclinus nigricaudus*; Tripterygiidae) to several hundred kilometres (527 km for *Sciaenops ocellatus*; Sciaenidae). The majority of fish species have a mean dispersal distance of more than 20 km.

The discrepancy between low Φ_{st} value and low mean larval dispersal distance in *L. quadrilineatus* could be explained by the historical demography of the species, causing a shallow genetic structure due to population expansion. This shows that larval dispersal distances on ecological time scales can be much smaller than on evolutionary time scales (Palumbi 2003).

Implications for conservation

Cleaner fishes are very popular and are therefore collected for marine ornamental trade in high numbers. *L. quadrilineatus* is exported by several companies in Egypt, Saudi Arabia, Yemen and Djibouti to Europe and North America. It is one of the top ten fishes exported from Saudi Arabia (Khalaf & Ali 2005, Wood 2001). Due to the role of cleaner fishes in maintaining the health and diversity of their clients, concerns have been raised about the impact of removing large quantities of cleaner fishes on the populations of their clients and reef health in general (Wood 2001). Khalaf & Ali (2005) recommended a regular monitoring of *L. quadrilineatus* to prevent overexploitation.

Marine protected areas (MPAs) are considered to be an appropriate tool to prevent overexploitation and to ensure the sustainable use of living marine resources. It is proposed that MPAs should to be arranged in a network and that the spatial distribution should match the dispersal capabilities of the species to be protected (Palumbi 2003).

The estimated larval dispersal distance for *L. quadrilineatus* suggests a space of less than 4.6 km among marine protected areas to inter-connect them by larval dispersal and to enable

larvae to settle in protected areas. Gladstone *et al.* (2003) proposed a regional network of 12 MPAs, eight in the Red Sea and four in the Gulf of Aden. The size of two MPAs in the Red Sea was undefined, one in Jordan has a length of 7 km, one in Sudan has an area of 12 km^2 and the remaining MPAs range in their sizes between 300 km^2 and 3310 km^2. The network of MPAs proposed by Gladstone *et al.* (2003) can not connect populations of *L. quadrilineatus* on an ecological time scale in the Red Sea and Gulf of Aden, because a maximum larval dispersal distance of about 5 km can not facilitate exchange between these distant locations. However, seven of the established or proposed MPAs are large enough to enable self seeding in *L. quadrilineatus*.

In order to enable a sustainable ornamental fishery on the fourline wrasse, the results of this study suggest that populations in the northern and southern Red Sea should be managed separately as two different stocks. The rather low larval dispersal distance of about 5 km needs to be considered in the design of MPAs to enable connectivity and self seeding. On the one hand, a network of small MPAs could be arranged along the coastline with a maximum distance of about 5 km to enable connectivity among them. Currently, only the network of MPAs along the coasts of Egypt, Israel, and Jordan in the Gulf of Aqaba (Kochzius 2002), and to some extent along the Egyptian coast of the northern Red Sea proper fulfil this requirement. On the other hand, a number of large MPAs with a minimum size of 5 km in diameter are self seeding and can serve as a source of recruits for areas where ornamental fishery takes place. However, only seven declared MPAs in the Red Sea and Gulf of Aden reported by Gladstone *et al.* (2003) are large enough to enable self seeding. Further studies on other fishes and invertebrates from more locations are needed to draw a generalised picture of connectivity among populations in the Red Sea in order to design a functioning network of MPAs.

References

Avise JC, Arnold J, Ball RM Jr, *et al*. (1987) Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics*. **18**, 489–522.

Bandelt HJ, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, **16,** 37-48.

Beerli P, Felsenstein J (1999) Maximum likelihood estimation of a migration rates and population numbers of two populations using a coalescent approach. *Genetics*, **152**, 763-773.

Beerli P, Felsenstein J (2001) Maximum likelihood estimation of a migration matrix and effective population size in *n* subpopulations by using a coalescent approach. *Proceedings of the National Academy of Sciences of the USA*, **98**, 4563-4568.

Beerli, P. (2005) Comparison of Bayesian and maximum likelihood inference of population genetic parameters. *Bioinformatics*, **22**, 341-345.

Bernardi G, Holbrook SJ, Schmitt RJ (2001) Gene flow at three spatial scales in a coral reef fish, the three-spot dascyllus, *Dascyllus trimaculatus*. *Marine Biology* **138**, 457-465.

Bohonak AJ (2002) IBD (Isolation By Distance): a program for analyses of isolation by distance. *Journal of Heredity*, 93, 153-154.

Buonaccorsi VP, Westerman, Stannard J *et al.* (2004) Molecular genetic structure suggests limited larval dispersal in grass rockfish, *Sebastes rastrelliger*. *Marine Biology*, **145**, 779-788.

Braithwaite CJR (1987) Geology and paleogeography of the Red Sea region. In: *Key environments. Red Sea* (eds. Edwards AJ, Head SM), pp. 22-44. Pergamon Press, Oxford.

Bshary R (2003) The cleaner wrasse, *Labroides dimidiatus*, is a key organism for reef fish diversity at Ras Mohammed National Park, Egypt. *Journal of Animal Ecology*, **72**, 169-176.

Doherty PJ, Planes S, Mather P (1995) Gene flow and larval duration in seven species of fish from the Great Barrier Reef. *Ecology* **76**, 2373-2391.

Excoffier L, Smouse PE (1994) Using allele frequencies and geographic subdivision to reconstruct gene trees within a species: molecular variance parsimony. *Genetics*, **136**, 343-359.

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131**, 479-491.

Fauvelot C, Bernardi G, Planes S (2003) Reduction in the mitochondrial DNA diversity of coral reef fish provides evidence of population bottlenecks resulting from holocene sea-level change. *Evolution*, **57**, 1571-1583.

Froukh T (2001) Taxonomical and ecological studies on fish larvae from the Gulf of Aqaba. M.Sc. thesis, University of Jordan.

Fu Y. X. (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, **147**, 915-925.

Gladstone W, Krupp, F, Younis M (2003) Development of a network of marine protected areas in the Red Sea and Gulf of Aden region. *Ocean & Coastal Management*, **46**, 741-761.

Goren M (1986) A suggested model for the recolonisation process of the Red Sea at the post glacial period. *In:* T. Uyeno, R. Arai, T. Taniuchi, K. Matsuura ed. *Indo-Pacific fish biology: proceedings of the second international conference on Indo-Pacific fishes*. Tokyo: Ichthyological Society of Japan, 648-654.

Grutter AS (1999) Cleaner fish really do clean. *Nature*, **398**, 672–673.

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, **41**, 95-98.

Hassan M, Harmelin-Vivien M, Bonhomme F (2003) Lessepsian invasion without bottleneck: examples of two rabbitfish species (*Siganus rivulatus* and *Siganus luridus*). *Journal of Experimental Marine Biology and Ecology*, **291**, 219-232.

Johannes RE (1978) Reproductive strategies of coastal marine fishes in the tropics. *Environmental Biology of Fishes* **3**, 65-84

Khalaf MA, Ali MA (2005) Status of the ornamental fish trade in the Red Sea and Gulf of Aden. *In: Sea to Sea Second Regional Forum*, *13-16 February 2005 Cairo*, 139-153.

Khalaf MA, Kochzius M (2002) Community structure and biogeography of shore fishes in the Gulf of Aqaba, Red Sea. *Helgoland Marine Research*, **55**, 252-284.

Kinlan BP, Gaines SD (2003) Propagule dispersal in marine and terrestrial environments: a community perspective. *Ecology*, **84**, 2007-2020.

Klausewitz W (1989) Evolutionary history and zoogeography of the Red Sea ichthyofauna. *Fauna of Saudi Arabia* **10**, 310-337.

Kochzius M (2002) Coral reefs in the Gulf of Aqaba. In: Status of Coral Reefs of the World: 2002 (ed. Wilkinson C), p. 52. Australian Institute of Marine Science.

Kochzius M, Blohm D (2005) Genetic population structure of the lionfish *Pterois miles* (Scorpaenidae, Pteroinae) in the Gulf of Aqaba and northern Red Sea. *Gene*, **347**, 295-301.

Lee W-J, Conroy JC, Howell WH, Kocher TD (1995) Structure and evolution of Teleost mitochondrial control regions. *Journal of Molecular Evolution*, **41**, 54-66.

Leis JM (1991) The pelagic stage of reef fishes: the larval biology of coral reef fishes. In: The ecology of fishes on coral reefs (ed Sale PF), pp. 183-230. Academic Press, San Diego.

Leis JM, Carson-Ewart BM (1997) *In situ* swimming speeds of the late pelagic larvae of some Indo-Pacific coral-reef fishes. *Marine Ecology Progress Series* 159: 165-174.

Maier E, Tollrian R, Rinkevich B, Nürnberger B (2005) Isolation by distance in the scleractinian coral *Seriatopora hystrix* from the Red Sea. *Marine Biology*, **147**, 1109-1120.

Mantel N (1967) The detection of disease clustering and generalized regression approach. *Cancer Research*, **27**, 209–220.

Morcos SA (1970) Physical and chemical oceanography of the Red Sea. *Oceanography Marine Biology Annual Review*, **8**, 73-202.

Palumbi SR (2003) Population genetics, demographic connectivity, and the design of marine reserves. *Ecological Applications*, **13**, S146-S158.

Parker PG, Snow AA, Schug MD, Booton GC, Fuerst PA (1998) What molecules can tell us about populations: choosing and using a molecular marker. *Ecology*, **79**, 361-382.

Planes S, Bonhomme F, Galzin R (1993) Genetic structure of *Dacyllus aruanus* populations in French Polynesia. *Marine Biology* **117**, 665-674.

Planes S, Galzin R, Bonhomme F (1996) A genetic metapopulation model for reef fishes in oceanic islands: the case of the surgeonfish, *Acanthurus triostegus*. *Journal of Evolutionary Biology* **9**, 103-117.

Planes S, Parroni M, Chauvet C (1998) Evidence of limited gene flow in three species of coral reef fishes in the lagoon of New Caledonia. *Marine Biology* **130**, 361-368.

Posada D, Crandall K (1998) *Modeltest*: testing the model of DNA substitution. *Bioinformatics* **14**, 817-818.

Randall JE (1983) Red Sea reef fishes. Immel, London.

Raymond M, Rousset F (1995) An exact test for population differentiation. *Evolution*, **49**, 1280-1283.

Roberts CM, Shepherd ARD, Ormond RFG (1992) Large-scale variation in assemblage structure of Red Sea butterflyfishes and angelfishes. *Journal of Biogeography*,**19,** 239–250.

Rogers AL, Harpending H (1992) Population growth makes waves in the distribution of pairwise genetic differences. *Molecular. Biology and Evolution*. **9**, 552-569.

Rohling EJ, Fenton M, Jorissen FJ, Bertrand P, Gnassen G, Caulet JP (1998) Magnitudes of sea-level lowstands of the past 500,000 years. *Nature* **394**, 162-165.

Rousset F (1997) Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. *Genetics*, **145,** 1219-1228.

Rozas, J., Sánchez-DelBarrio, J. C., Messegyer, X. and Rozas, R. (2003) DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics*, **19,** 2496-2497.

Sale PF (1980) The ecology of fishes on coral reefs. *Oceanography and Marine Biology: An Annual Review* 18, 367-421.

Schneider S, Roessli D, Excoffier L (2000) *Arlequin, version 2.000: a software for population genetics data analysis*. Genetic and Biometry Laboratory, Department of Anthropology, University of Geneva, Switzerland.

Shefer S, Abelson A, Mokady O, Geffen E (2004) Red to Mediterranean Sea bioinvasion: natural drift through the Suez Canal, or anthropogenic transport? *Molecular Ecology*, **13**, 2333-2343.

Sheppard C, Price A, Roberts C (1992) *Marine ecology of the Arabian region*. Academic Press, London.

Shulman MJ, Birmingham E (1995) Early life histories, ocean currents, and the population genetics of Caribbean reef fishes. *Evolution* **49**, 897-910.

Siddall M, Rohling EJ, Almogi-Labin A, Hemleben Ch, Melschner D, Schmelzer I, Smeed DA (2003) Sea-level fluctuations during the last glacial cycle. *Nature* **423**, 853-858.

Söller R, Warnke K, Saint-Paul U, Blohm D (2000) Sequence divergence of mitochondrial DNA indicates cryptic biodiversity in *Octopus vulgaris* and supports the taxonomic distinctiveness of *Octopus mimus* (Cephalopoda: Octopodidae). *Marine Biology*, **136**, 29–35.

Sponaugle S, Cowen RK, Shanks A, Morgan SG, JM. Leis, J. Pineda, G.W. Boehlert, M.J. Kingsford, K.C. Lindeman, C. Grimes, and J.L. Munro. 2002. Predicting self-recruitment in marine populations: biophysical correlates and mechanisms. *Bulletin of Marine Science* **70**, Suppl.:341-375.

Swearer SE, Caselle JE, Lea DW, Warner RR (1999) Larval retention and recruitment in an island population of a coral-reef fish. *Nature* **402**, 799-802.

Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, **125**, 585-593.

Thompson JG, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignments through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Research*, **22**, 4673–4680.

Wahbeh MI, and Ajiad A (1985) Reproductive biology and growth of the goatfish, *Parupeneus barberinus* (Lacepede), in Aqaba, Jordan. *Journal of Fish Biology*, **26**, 583-590.

Wood E (2001) Collection of coral reef fish for aquaria: global trade, conservation issues and management strategies. Marine Conservation Society, Ross-on-Wye, UK.

Zekeria ZA (2003) Butterflyfishes of the southern Red Sea. PhD thesis, University of Groningen.

Chapter 3

Comparative genetic population structure of two reef fishes at different geographical scales in the Red Sea and Indo-Malay Archipelago: biological, physical and Historical factors

Tawfiq Froukh, Marc Kochzius

Molecular Ecology (submitted)

Abstract

We compared in this study the genetic population structure for two ornamental fishes (*Chromis viridis* and *Pseudanthias squamipinnis*) within and between the Red Sea and the Indo-Malay Archipelago. Fragments of 459 bp (from 266 individuals of *C. viridis*) and 411 bp (from 200 individuals of *P. squamipinnis*) of the mtDNA control region were analysed. Both species exhibited significant genetic structure between the Red Sea and the Indo-Malay Archipelago, and panmixing within the Red Sea proper. However, only *P. squamipinnis* exhibited significant genetic structure between the Gulf of Aqaba and the Red Sea proper. The MIGRATE analysis revealed: (1) higher migration from the Red Sea proper to the Gulf of Aqaba in the two species; and (2) higher southward migration for *P. squamipinnis* but higher northward migration for *C. viridis* in the Red Sea proper. The biological, physical, and historical factors were used to explain the obtained results.

Keywords: Pomacentridae, Serranidae, PLD, Strait of Bab-el-Mandab, Strait of Tiran; re-colonisation.

Introduction

The genetic connectivity among populations of reef fishes is determined by functional, historical, and physical factors (Planes, 2002). The functional factors are related to the ecology and the biology of fishes, including reproduction, behaviour, and pelagic larval duration (PLD). Most reef fishes are either pelagic or demersal spawners. They vary in their dispersal capabilities due to the following differences: (1) demersal spawners guard their eggs in nests with parental care until hatching, while pelagic spawners release their eggs into the water column without parental care; (2) eggs and larvae of pelagic spawners are smaller; (3) larvae of demersal spawners have a combination of better swimming abilities and well developed functional fins, eyes, guts, and sensory systems; (4) larvae of demersal spawners are predominantly inshore distributed, while larvae of pelagic spawners are predominantly offshore distributed; (5) PLD is typically less than 25 days for demersal spawners and around 20 to 50 days for pelagic spawners (Thresher, 1984; Thresher, 1991; Shulman, 1998). The historical factors include colonisation (founder effect), re-colonisation, and extinction as a result of sea level fluctuations. The physical factors include oceanographic features such as eddies and gyres. These three factors act on the genetic structure none equivalently, historical factors are certainly predominant over other ones (Planes, 2002). For example, Shulman and Bermingham (1995) investigated the genetic spatial structure in eight Caribbean reef fishes differing in length of larval life. The results suggest that genetic differentiation in Caribbean reef fishes is not a consequence of PLD, but rather induced by oceanic currents as well as historical events. However, Doherty et al. (1995) compared seven species of coral reef fishes from the Great Barrier Reef; one without and the others with pelagic larval stage. Five species showed significant regional differentiation and two species with the longest PLDs did not. They demonstrated a significant correlation between the logarithm of genetic variation and the PLD.

The Indo-Pacific region, which stretches from the Red Sea eastward across the Indo-Malay Archipelago and the Polynesian Islands to the western coast of America, contains the world's largest fish fauna, estimated at over 4000 species (Planes, 2002). The Indo-Malay Archipelago is the hotspot and the centre of marine biodiversity, and the Red Sea is considered an important secondary centre of evolution (Klausewitz, 1989; Roberts et al., 2002). The Red Sea is a long, narrow body of water separating north-east Africa from the Arabian Peninsula. It is connected to the Indian Ocean through the Strait of Bab-el-Mandab, and to the Mediterranean through the Suez Canal (Figure 1). Although the Red Sea is usually

considered as an integral part of the Western Indian Ocean Province of the Indo-West Pacific zoogeographic region, it was suggested to be a zoogeographic province on its own due to the high level of endemism (Briggs 1995, Head, 1987). Only a few studies were performed on the genetic population structure of reef fishes in the Red Sea, including the lionfish *Pterios miles* (Kochzius and Blohm, 2005), the rabbitfish *Siganus rivulatus* and *Siganus luridus* (Hassan et al., 2003), and the fourline wrasse *Larabicus quadrilineatus* (Froukh and Kochzius, 2007). These studies have shown no genetic differentiation on a spatial scale of less than 400 km.

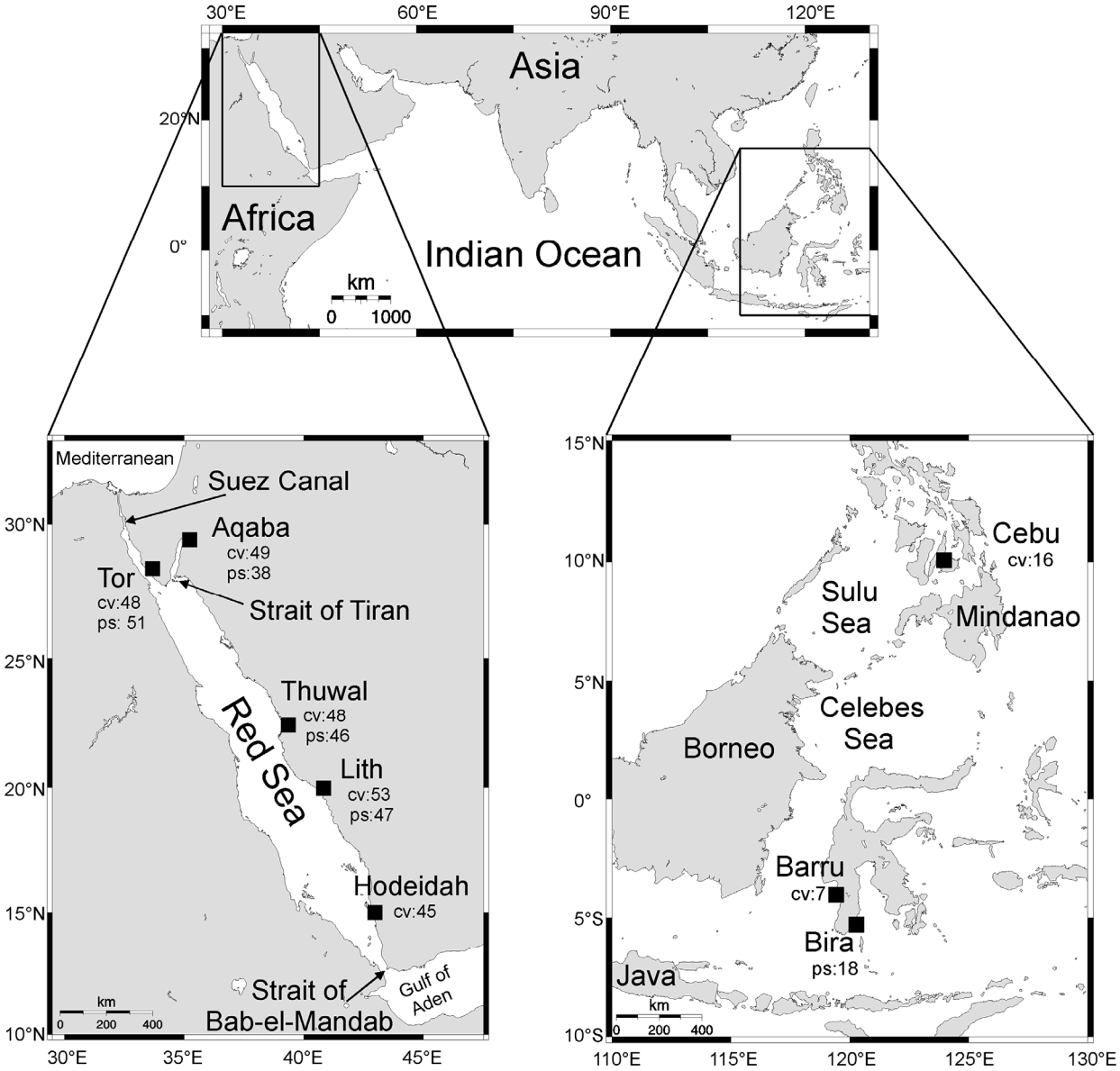

Figure 1 Map of the Indo-West Pacific showing the collections sites in the Red Sea and Indo-Malay Archipelago. The number of collected samples are indicated below the name of the collection site; cv= *Chromis viridis*, ps= *Pseudanthias squamipinnis*.

In this study, we investigate gene flow and genetic population structure in two reef fishes, blue green chromis *Chromis viridis* (Cuvier, 1830) and sea goldie *Pseudanthias squamipinnis* (Peters, 1855). These two fishes vary in their life history traits: the blue green chromis is a demersal spawner, whereas the sea goldie is a pelagic spawner (Thresher, 1984). They are not important as food fish, but are exported as ornamentals in the aquarium trade (Wood, 2001). *P. squamipinnis* dominates fish assemblage on reefs in the northern Red Sea, while *C. viridis* is more abundant in the central Red Sea at latitudes between 20°N and 21°N. No individuals of *P. squamipinnis* were sighted in the southern Red Sea along the coastline of Yemen, and in the Gulf of Aden along the coastline of Djibouti (Khalaf and Kochzius, 2002; PERSGA/GEF, 2003).

The purpose of this study is to investigate the genetic population structure of these two fishes at two geographic scales: (1) within the Red Sea; and (2) between the Red Sea and the Indo-Malay Archipelago. In addition, we want to test: (1) the role of historic and oceanographic factors on structuring the populations of these two fishes; and (2) whether the differences in the life history traits between these two species affect their genetic structures.

Materials and Methods

Adults of *C. viridis* (243 specimens) and *P. squamipinnis* (182 specimens) were collected in December 2003, January 2004 and February 2005 at five collection sites in the Red Sea: Aqaba, Tor, Thuwal, Lith, and Hodeidah (Figure 1). Populations of *C. viridis* (7 specimens) and *P. squamipinnis* (18 specimens) were collected from Barru and Bira (Indonesia) respectively, and an additional population of *C. viridis* (16 specimens) was collected from Cebu (Philippines; Figure 1). Tissue samples (fin clips and muscles) were placed immediately in absolute ethanol and were stored in the laboratory at -75°C.

Total DNA was extracted from each tissue sample using Chelex® as described by Söller et al., (2000). A fragment of the mitochondrial control region was amplified by PCR, using the universal primer CR-A and the specific primer XAN-DL-R (Fauvelot et al., 2003). PCR conditions were as follows: 95°C for 5 minutes, followed by 35 cycles of 94°C for 45 seconds, 50°C for 45 seconds, and 72°C for 60 seconds, followed by a final extension at 72°C for 5 minutes. The reaction mix of 50 µl contained 10 mM Tris-HCl (pH 9), 50 mM KCl, 5.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.2 µM each primer, 2 U Taq polymerase, and 2 µl of the DNA template. Amplified PCR products were purified with the QIAquick PCR purification kit (Qiagen, Hilden). Both strands were sequenced with the same primers used for PCR, the

DyeDeoxy Terminator chemistry (PE Biosystems, Foster City), and an ABI automated sequencer. Sequences were edited with the programme Sequence Navigator (version 1.0.1; Applied Biosystem), while a multiple alignment of all sequences was done with Clustal W (Thompson et al., 1994) as implemented in BioEdit (version 7.0.4.1; Hall, 1999).

The programmes ARLEQUIN (version 3.1; Excoffier et al., 2005) and DnaSP (version 4.10.3; Rozas et al., 2003) were used to obtain the following data: (1) genetic variation as nucleotide diversity (the probability that two randomly chosen homologous nucleotides are different) and haplotype diversity (the probability that two randomly chosen haplotypes are different). The haplotype and nucleotide diversities were calculated as mean values with standard deviations. We wanted to test the hypothesis that haplotype and nucleotide diversities in a certain population are the same for the two species. Therefore, standard deviations (SD) and sample sizes (n) were used to estimate the confidence intervals at a significance level alpha=0.05 as $\pm 1.96 \times SD/\sqrt{n}$. The hypothesis was accepted if confidence intervals overlap; (2) population differentiation based on pairwise F_{ST} estimates; (3) genetic variance among collection sites was computed with AMOVA (Excoffier et al., 1992), the significance of Φ_{STs} was tested using a non-parametric permutation procedure with 10,000 permutations; (4) Tajima's *D* test (Tajima, 1989) and Fu's *Fs* test (Fu, 1997) were used to examine neutrality of the marker with 10,000 permutations; and (5) the mismatch distribution (Harpending, 1994) was calculated with 10,000 permutations to test the sudden population expansion under Rogers' model (Rogers, 1995). Mismatch distribution is unimodal in populations that have passed through a recent demographic expansion, and is multimodal in stable populations.

The phylogenetic analysis was carried out using the programme PAUP* (version 4.0b10; Swofford, 1998). The programme MODELTEST (version 3.7; Posada and Crandall, 1998) was used to determine the best-fit model of DNA evolution. Gaps in the sequences were deleted for the neighbour-joining (NJ) and the maximum likelihood (ML) methods, while for the maximum parsimony (MP) method gaps were treated as a fifth base. The haplotypes from the Indo-Malay Archipelago and representative haplotypes from the Red Sea were used in constructing the phylogenetic trees. Statistical confidence in nodes was evaluated based on 10,000, 1000, and 100 non-parametric bootstrap replicates in NJ, MP, and ML analyses, respectively (Felsenstein, 1985). We used *Chromis atripectoralis* and *Plectropomus maculatus* as outgroups for *C. viridis* and *P. squamipinnis*, respectively. The sequences of the mtDNA control region of *C. atripectoralis* (DQ212281.1, DQ212246.1, DQ212256.1,

DQ212260.1, and DQ212275.1) and *Plectropomus maculatus* (NC008449 and DQ101270) were obtained from GenBank.

The Haplotype networks were constructed using the median-joining network method as implemented in Network (version 4.1.1.1; www.fluxus-engineering.com; Bandelt et al., 1999). This method begins by combining the minimum spanning trees (MSTs) within a single network (minimum spanning network, MSN) using an analogous algorithm to that proposed by Excoffier and Smouse (1994). Then, median vectors (which represent missing intermediates haplotypes) are added to the network using the parsimony criterion.

The relationship between genetic and geographic distances among populations was examined using the Reduced Major Axis (RMA) regression. Correlation of pairwise genetic distances (Φ_{ST}) and geographic distances among populations was assessed using the Mantel test (Mantel, 1967) as implemented in the Isolation By Distance Web Service IBDWS (version 3.02; Jensen et al., 2005; http://ibdws.sdsu.edu/) with 30,000 permutations to test significance.

We used the likelihood approach in the programme MIGRATE (version 2.0.3; Beerli and Felsenstein, 1999, 2001; Beerli, 2005) to estimate migration rates (M) and effective population sizes (Θ) in each collection site in the Red Sea. The effective number of migrants among collection sites (γ) was calculated as follows: $\gamma=\Theta M$. We performed 6 independent runs, each with 10 short chains of 5000 steps and 3 long chains of 50,000 steps with a sampling increment of 500, and an adaptive heating scheme of 10 chains.

Results

We analysed 266 specimens of *C. viridis* from seven collection sites. A fragment of 459 bp of the mtDNA control region revealed 112 polymorphic sites and 149 haplotypes. No haplotypes were shared between the Red Sea, Indonesia, and the Philippines. All of the specimens from Indonesia (7) and the Philippines (16) had unique haplotypes. The Red Sea exhibited a total of 126 haplotypes, four haplotypes were shared among all collection sites, 22 haplotypes were shared between two, three or four collection sites, and the remaining haplotypes were unique to Aqaba (25), Tor (17), Thuwal (18), Lith (18), and Hodeidah (22). No exclusive shared haplotypes were observed in the northern (Aqaba and Tor) or southern collection sites (Thuwal, Lith, and Hodeidah).

The analysis of 200 specimens of *P. squamipinnis* from five collection sites using 411 bp of the mtDNA control region revealed 97 polymorphic sites and 109 haplotypes. No haplotypes

were shared between the Red Sea and Indonesia. All of the specimens from Indonesia (18) were unique haplotypes. The Red Sea showed 91 haplotypes, three haplotypes were shared among all collection sites, six haplotypes were shared between two or three collection sites, and all other haplotypes were unique to Aqaba (16), Tor (27), Thuwal (23), and Lith (16). Two haplotypes were exclusively shared between the northern collection sites (Aqaba and Tor), but no exclusively shared haplotypes could be observed in the South (Thuwal and Lith).

The total haplotype and nucleotide diversities in the Red Sea were 0.98 and 0.032 for *C. viridis* and 0.94 and 0.008 for *P. squamipinnis*, respectively. In Indonesia and the Philippines, haplotype diversities were all 1.00 for the two species. Nucleotide diversity for *C. viridis* was 0.034 in Indonesia and 0.039 in the Philippines. *P. squamipinnis* showed a nucleotide diversity of 0.019 in Indonesia. The hypothesis that haplotype and nucleotide diversities for the two species are similar was rejected to all populations except Indonesia (Figure 2).

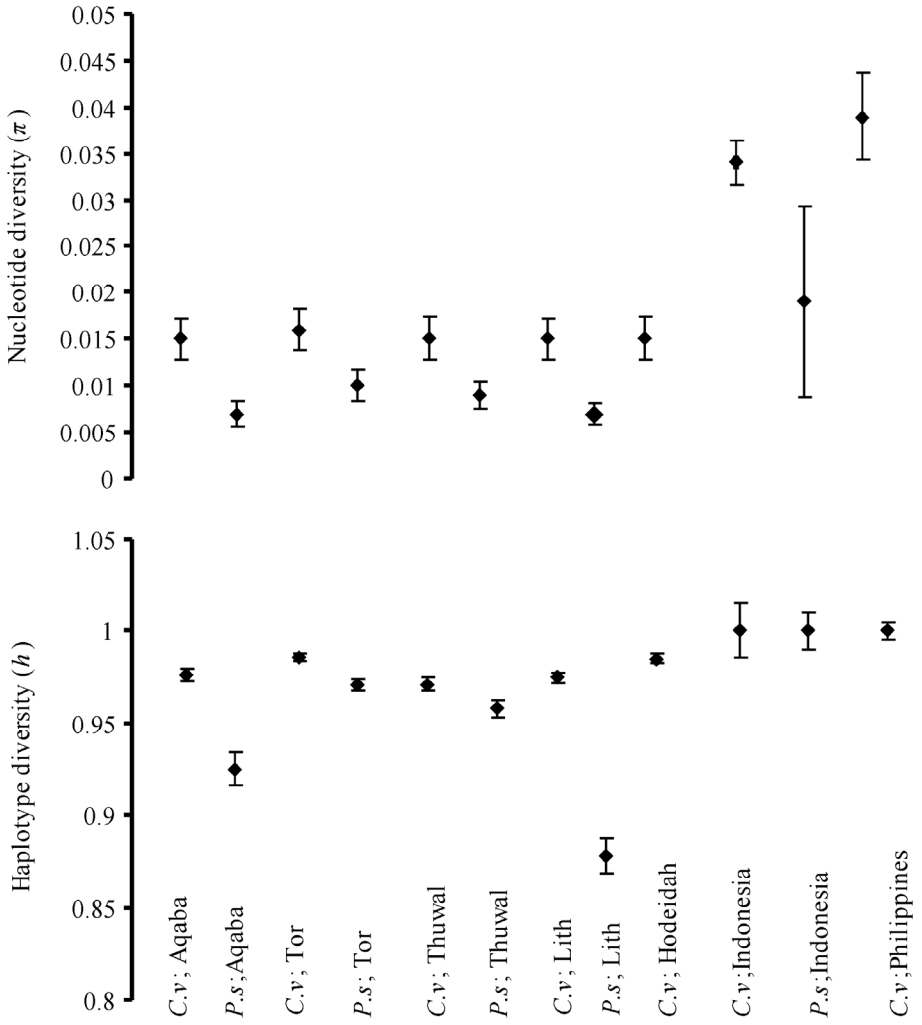

Figure 2 Comparison between nucleotide and haplotype diversities of *Chromis viridis* (*C.v*) and *Pseudanthias squamipinnis* (*P.s*) from the collection sites in the Red Sea and the Indo-Malay Archipelago.

The best-fit evolutionary model for *C. viridis* was the Jukes-Cantor-model with an equal rate of substitutions and an equal base frequencies, while for *P. squamipinnis* it was the Hasegawa-Kishino-Yano-model with a transition/transversion ratio of 8.5, gamma distribution shape parameter of 0.13, and the following base frequencies: A=0.37; C=0.21; G=0.15; and T=0.27. These parameters were used for the NJ and ML methods. The three methods (NJ, MP, and ML) show similar well-supported trees for the two species. Two main clades without phylogeographic structure within each clade were revealed. The first clade represents the haplotypes of the Red Sea and the second clade represents the haplotypes of the Indo-Malay Archipelago. Figure 3 shows the ML trees for the two species with the bootstrap values of the three methods.

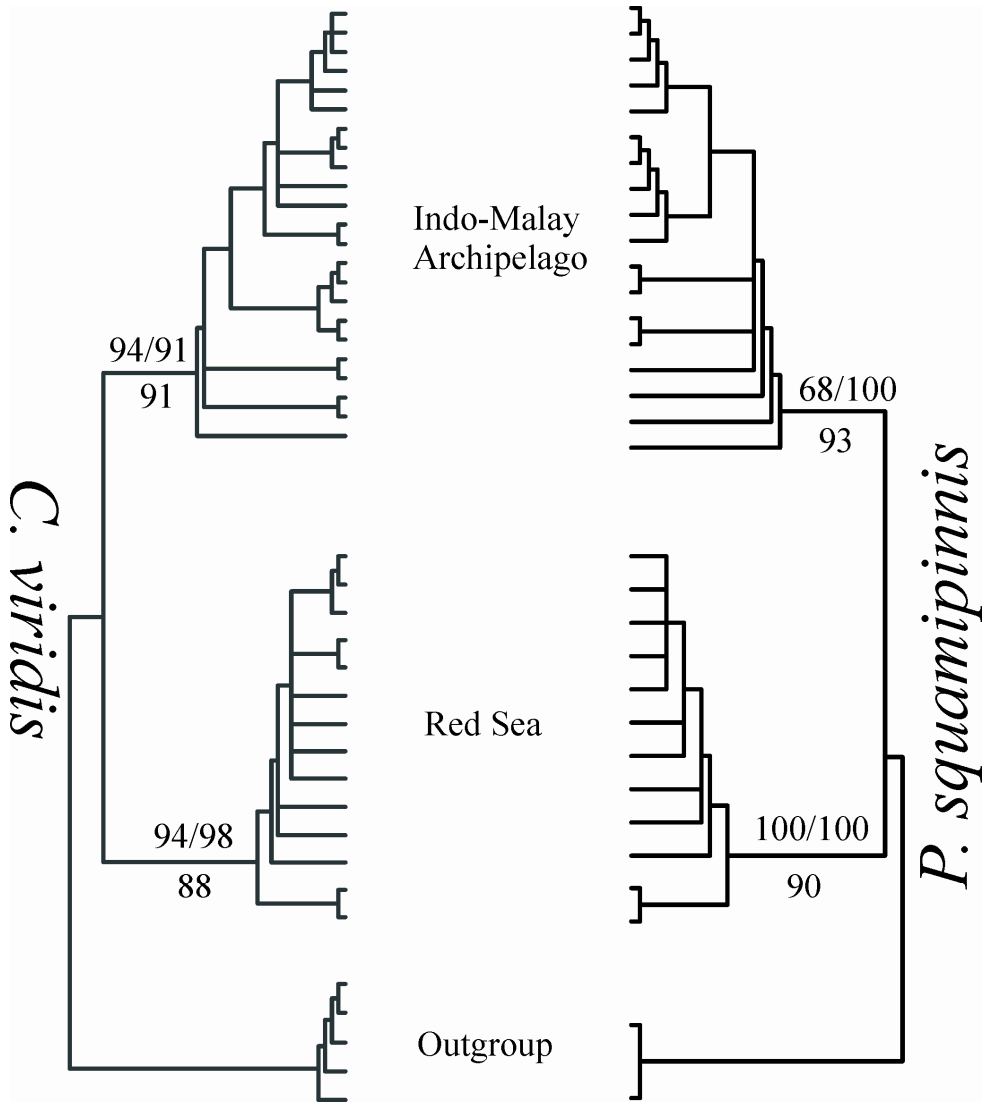

Figure 3 Phylogenetic trees for *Chromis viridis* and *Pseudanthias squamipinnis* from the Red Sea and the Indo-Malay Archipelago based on a fragment of the mitochondrial control region using the ML method. Bootstrap values for NJ (10,000 replicates)/MP(1,000 replicates) are indicated above branches and ML(100 replicates) are indicated below branches.

The median joining networks (not shown) for the two species contained many loops and were therefore not very informative. However, a network for the two populations of *C. viridis* from Indonesia and the Philippines revealed their separation into 2 clades (Figure 4).

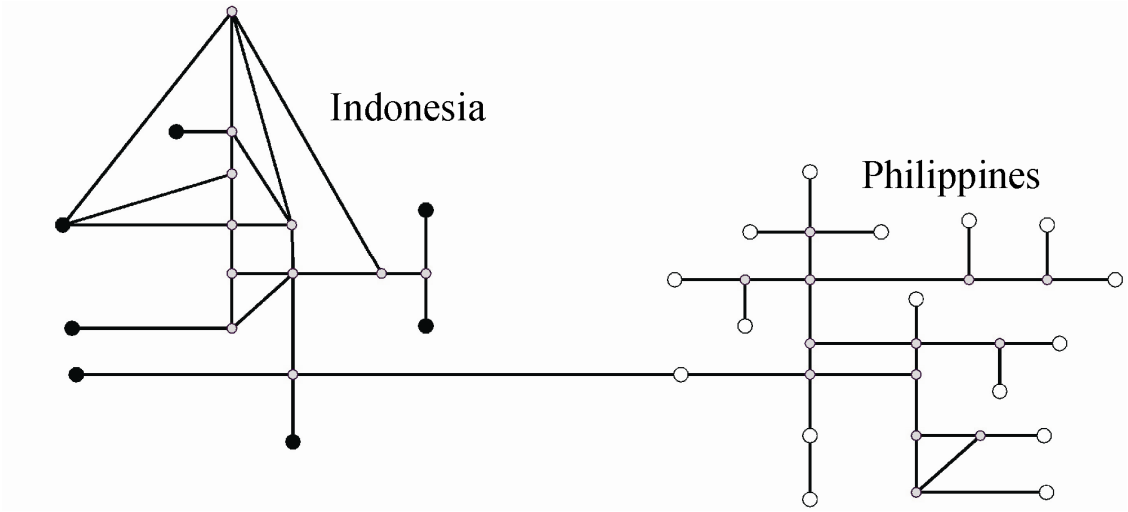

Figure 4 Median joining network describing the genetic relationship among 25 specimens of *Chromis viridis* in Indonesia and the Philippines. White circles: Indonesia, black circles: the Philippines, grey circles: median vectors.

AMOVA was run twice for each species, the first run included all populations (Red Sea, Indonesia, and the Philippines) and the second run included the Red Sea populations only. In the first run, significant Φ_{ST} values were obtained for the two species, while in the second run only *P. squamipinnis* exhibited significant Φ_{ST} value. The result of AMOVA for *C. viridis* indicated a molecular variance of 46% due to differences among all populations (Red Sea, Indonesia and the Philippines), but no molecular variance among Red Sea populations. Results of the AMOVA for *P. squamipinnis* showed a molecular variance of 77.78% among populations in the Red Sea and Indonesia, but a very low but still significant variance of 1.86% due to differences among Red Sea populations (Table 1).

Table 1 Analysis of genetic population structures of *Chromis viridis* and *Pseudanthias squamipinnis* in the Red Sea, Indonesia, and the Philippines based on AMOVA.

Species	Population groupings	% of variation	Φ_{ST} values	*p*-value
Chromis viridis (Red Sea, Indonesia and Philippines)	Among populations	45.97	0.460	<0.001
	Within populations	54.03		
Chromis viridis (Red Sea)	Among populations	-0.15	-0.001	0.578
	Within populations	100.15		
Pseudanthias squamipinnis (Red Sea and Indonesia)	Among populations	77.78	0.778	<0.001
	Within populations	22.22		
Pseudanthias squamipinnis (Red Sea)	Among populations	1.86	0.019	0.006
	Within populations	98.14		

Within the Red Sea, pairwise Φ_{ST}-values for *C. viridis* were not significant, while all three pairwise Φ_{ST}-values involving Aqaba were significant in *P. squamipinnis.* Pairwise Φ_{ST}-values between the Indo Malay Archipelago and the populations in the Red Sea were significant in the two species. In *C. viridis*, Φ_{ST}-values were also significant between Indonesia and the Philippines (Table 2).

Table 2 Pairwise population differentiation (Φ_{ST} values) of *Chromis viridis* (above diagonal) and *Pseudanthias squamipinnis* (below diagonal) among collection sites in the Red Sea, Indonesia, and the Philippines. *0.05≥*p*≥0.01, **0.01>*p*≥0.001, ***p<0.001

	Aqaba	Tor	Thuwal	Lith	Hodeidah	Indonesia	Philippines
Aqaba		0.002	0.003	0.008	-0.006	0.799***	0.787***
Tor	0.033**		-0.011	-0.004	-0.001	0.793***	0.783***
Thuwal	0.061***	0.000		-0.006	-0.0004	0.805***	0.791***
Lith	0.018*	0.004	0.011		0.000	0.804***	0.793***
Hodeidah	—	—	—	—		0.800***	0.785***
Indonesia	0.935***	0.924***	0.930***	0.939***			0.406***

For *C. viridis*, Tajima's *D* and Fu *Fs* values were significant for the Red Sea and not significant for Indonesia and the Philippines. For *P. squamipinnis*, Tajima's *D* and Fu *Fs* values were significant for the Red Sea, and only Fu *Fs* value was significant for Indonesia. Mismatch distributions for the two species were consistent with Rogers' model of sudden population expansion because the sum of square deviation values and the raggedness indices were all not significant (Table 3).

Table 3 Neutrality tests and the estimated parameters of mismatch distribution for populations of *Chromis viridis* and *Pseudanthias squamipinnis* in the Red Sea, Indonesia, and the Philippines. *D* = Tajima's D; *p(D)* = *p* value for *D*; *F* = Fu's *Fs*; *p(F)* = *p* value for *Fs*; Mismatch = mean ± variance of mismatch distribution; *p(SSD)* = *p* value of sum of square deviations (*SSD*) between the observed and the expected mismatch; *p(r)* = *p* value of the raggedness index of the observed distribution. Significant *p* values are marked with *.

	D	*p(D)*	*F*	*p(F)*	Mismatch	*p(SSD)*	*p(r)*
Chromis viridis							
Red Sea	-1.455	0.038*	-24.711	<0.000*	6.32 ± 14.02	0.160	0.472
Indonesia	-0.528	0.335	-0.964	0.176	14.29 ± 32.01	0.373	0.220
Philippines	-0.460	0.352	-5.645	0.098	16.65 ± 43.04	0.800	0.876
Pseudanthias squamipinnis							
Red Sea	-1.832	0.006*	-26.021	<0.000*	3.37 ± 6.10	0.286	0.334
Indonesia	-1.083	0.131	-12.394	<0.000*	7.67 ± 9.16	0.063	0.997

The total migration rate in *P. squamipinnis* is four times higher than in *C. viridis* within the Red Sea, and two times higher within the Red Sea proper. The migration rate of *P. squamipinnis* is higher southward than northward, while it is higher northward than southward for *C. viridis*. The number of migrants per generation into the Gulf of Aqaba is

1061 for *P. squamipinnis* and 89 for *C. viridis*, while only 1.3 individuals of *P. squamipinnis* and 30 individuals of *C. viridis* are migrating in the opposite direction (Figure 5).

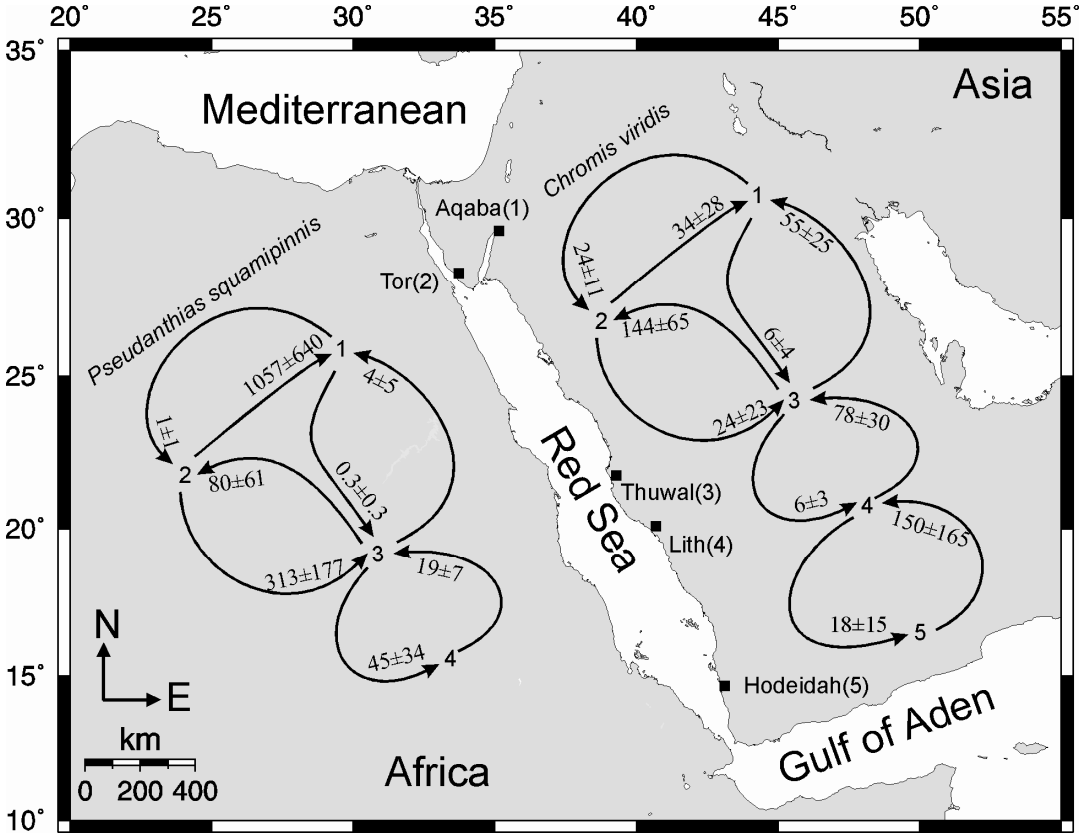

Figure 5 Map of the Red Sea showing numbers of effective migrants/generation ± standard deviation of *Chromis viridis* and *Pseudanthias squamipinnis* among the collection sites in the Red Sea. The results are the mean values of six runs using the programme MIGRATE.

Hodeidah showed the largest effective population size for *C. viridis*, whereas Tor showed the largest effective population size for *P. squamipinnis* (as inferred from theta values, Table 4).

Table 4 Proportions of theta values (Θ = effective population sizes) of *Chromis viridis* and *Pseudanthias squamipinnis* from collection sites in the Red Sea. Theta values (Θ) were estimated using the programme MIGRATE and are represented as the mean values of six independent runs ± SD.

	Θ	Proportion %
Chromis viridis		
Aqaba	0.014±0.007	12.2
Tor	0.017±0.011	14.8
Thuwal	0.013±0.006	11.3
Lith	0.020±0.005	17.4
Hodeidah	0.051±0.039	44.3
Pseudanthias squamipinnis		
Aqaba	0.011±0.006	2.5
Tor	0.369±0.235	84.0
Thuwal	0.049±0.034	11.2
Lith	0.010±0.003	2.3

According to the Mantel test, genetic and geographic distances were not significantly correlated for the two species among the six populations in the Red Sea and Indonesia or the Philippines. A significant correlation between genetic and geographic distances (r=0.976; $p<0.05$) was obtained for *C. viridis* only when all populations (Red Sea, Indonesia and the Philippines) are included in the analysis (Table 5).

Table 5 Mantel tests for *Chromis viridis* and *Pseudanthias squamipinnis* in the Red Sea, Indonesia, and the Philippines using the software Isolation By Distance Web Service (IBDWS, version 3.02). r = correlation factor; * = significant *p*-value.

Species	Population	r	*p*-value
Chromis viridis	Red Sea	-0.0234	0.4460
	Red Sea and Indonesia	0.9927	0.1210
	Red Sea and Philippines	0.9936	0.1010
	Red Sea, Indonesia and Philippines	0.9760*	0.0450
Pseudanthias squamipinnis	Red Sea	-0.0916	0.5390
	Red Sea and Indonesia	0.9964	0.1290

Discussion

Genetic diversity, neutrality, and demographic expansion

The results of the two studied fishes revealed shallow mtDNA genealogies which are characterised by: (1) three to four predominant haplotypes and many unique haplotypes with one or two mutations difference, and (2) high haplotype diversity *h* and low nucleotide diversity π (Figure 2). This pattern could be due to the high variance in reproductive success for relatively few females which leads to the survival of only few haplotypes (Shields and Gust 1995). Alternatively, populations with high *h* and low π are attributed to expansion after a period of low effective population size (Grant and Bowen, 1998). Our results support this scenario because Rogers' model of sudden population expansion was accepted. Because the confidence intervals of the haplotype and nucleotide diversities do not overlap, *C. viridis* is significantly much more genetically diverse than *P. squamipinnis* (Figure 2). Compared to other coral reef fishes in the Red Sea (*Pterois miles* and *Larabicus quadrilineatus*), our results were the highest for haplotype diversity and the lowest for nucleotide diversity (Kochzius and Blohm, 2005; Froukh and Kochzius, 2007). The DNA polymorphism of the used molecular marker can be explained by the neutral mutation hypothesis for *C. viridis* in the Indo-Malay Archipelago. The rejection of the neutral mutation hypothesis for *C. viridis* in the Red Sea and for *P. squamipinnis* in the Red Sea and in Indonesia is likely due to a sudden population expansion rather than selection, because the data could not reject Rogers' model of sudden population expansion.

Genetic structure within the Indo-Malay Archipelago

In the Indo-Malay Archipelago, a phylogeographic structure between the *C. viridis* populations in Indonesia and the Philippines was observed. This structure is likely a signature of the isolation of marine basins caused sea-level low stands during the glacial periods (Voris, 2000). The present-day islands between the Sulu and the Celebes Seas were connected by land bridges which would have acted as barriers for marine organisms, possibly assisting allopatric diversification or even speciation (McManus, 1985). Such a process of speciation in the Indo-Malay Archipelago was e.g. shown for the sibling lionfish species *Pterois miles* and *P. volitans* (Kochzius et al. 2003).

Genetic structure between the Red Sea and the Indo-Malay Archipelago

Concordant results for the two studied species are shown for the phylogenetic separation between the Red Sea and the Indo-Malay Archipelago. In a study by Kochzius and Blohm (2005) on the lionfish *P. miles*, no separation was revealed between the Red Sea and the Indian Ocean (pooled sequences from Indonesia, Kenya and Sri Lanka). Unfortunately, no other previous studies compared fish populations between the Red Sea and the Indo-Malay Archipelago that we can use for comparison. However, other studies on invertebrates revealed a separation between populations in the Red Sea and populations in the Indo-West Pacific costal waters (Gopurenko et al., 1999); coasts of east Africa (Fratini and Vannini, 2002); and Sri Lanka (Shefer et al., 2004). This separation could also be attributed to sea-level low stand during the glacial periods, when the water exchanges between the Red Sea and Indo-West Pacific at the shallow sill at the Bab-el-Mandab was restricted (Siddall et al. 2003) or even interrupted (Braithwaite 1987).

Genetic structure and gene flow within the Red Sea

Within the Red Sea proper, no genetic differentiation was observed for the two studied species. However, the higher southward than northward migration in *P. squamipinnis*, and the higher northward than southward migration in *C. viridis* could be explained by their relative abundance and effective population size in the North and South, respectively. The relative abundance of *P. squamipinnis* in Tor (northern) is 84.6% compared to 10.4% of *C. viridis*, while of *C. viridis* in Thuwal and Lith (southern) is 81.2% compared to 3% of *P. squamipinnis* (PERSGA/GEF, 2003). A similar pattern can be observed in the effective population size, with higher values for Tor in *P. squamipinnis* and for Thuwal and Lith in *C.*

viridis (Table 4). It is most likely that the two species exhibit a higher migration from the region of higher abundance and effective population size.

Contradictory results for the two studied species are revealed about the hypothesis of disconnecting populations across the Strait of Tiran. For *P. squamipinnis*, genetic differentiation is observed between the Gulf of Aqaba and the Red Sea proper, whereas for *C. viridis* homogenisation is observed. Such incongruent results may be important to reveal: (1) the historical differences in gene flow and/or effective population size among species; and (2) the sensitivity to barriers and oceanographic factors. Several hypotheses might explain the observed pattern.

A. Biological factors (life history traits)

Genetic differentiation or homogenisation could be created from the differences in dispersal capabilities. Species exhibiting homogenisation might have higher dispersal capability. Larvae of the two studied species spend their pelagic duration in the offshore waters and therefore, dispersal is likely to occur during this stage. The dispersal capabilities of larvae might be related to the PLDs and their swimming abilities. Unfortunately, no data are available on PLDs of the two studied fishes from the Red Sea. Even though some studies provide estimates of PLDs to *C. viridis* from Papua New Guinea, Marshall Islands, Palau and Lizard Island (Thresher et al., 1989; Wellington and Victor, 1989; Wilson and McComrick, 1999), we could not rely on these estimates because of the significant intraspecific variation in PLDs of *C. viridis* both spatially and temporally (Bay et al., 2006). Therefore, we can not explain the observed pattern based on this hypothesis. The swimming abilities of larvae might be an alternative explanation, in which faster swimmers may show a genetic homogenisation (Stobutzki, 1998). The swimming speed of reef fish larvae at the end of their pelagic stages are strongly correlated with their body size (Bellwood and Fisher, 2001). Data on the swimming speed of larvae are available for *C. viridis* only (Leis and Carson-Ewart, 2000; Leis and Fisher, 2006). However, body sizes of *C. viridis* and *P. squamipinnis* at the settlement stage are 6.6-8.0 and 11-15 millimetres (mm), respectively (Avise and Shapiro, 1986; Wellington and Victor; 1989) and therefore, late-stage larvae of *C. viridis* seem to be faster than *P. squamipinnis*, which might explain the homogenisation in *C. viridis*.

B. Oceanographic factors

The obtained genetic structure of *P. squamipinnis* might be linked to the circulation patterns and the water exchanges between the Gulf of Aqaba and northern Red Sea. The Gulf of

Aqaba is separated from the Red Sea by a 242-270 m deep of the Strait of Tiran (Reiss and Hottinger, 1984). Water exchanges between the Gulf of Aqaba and northern Red Sea are characterised by an inflow into the Gulf in the upper 70 m depth and an outflow from the Gulf below 70 m depth (Manasrah et al., 2004). This circulation pattern explains the higher migration into the Gulf of Aqaba than into the northern Red Sea. Three studies have compared the genetic structure of fishes between Gulf of Aqaba and the Red Sea across the Strait of Tiran that can be used for comparisons. A study by Hassan et al., (2003) on the rabbitfish *Siganus rivulatus* and *Siganus luridus* did not reveal genetic structure between the Gulf of Aqaba and northern Red Sea. Studies on *Pterois miles* and *Larabicus quadrilineatus* found no genetic differences between the Gulf of Aqaba and northern Red Sea either, but revealed higher migration from the Red Sea into the Gulf of Aqaba (Kochzius and Blohm, 2005; Froukh and Kochzius, 2007). The migration pattern of *Pterois miles* and *Larabicus quadrilineatus* in these two studies are concordant to our results. The chain of cyclonic and anti-cyclonic eddy pairs along the main axis of the Gulf of Aqaba can cause larval retention to the Gulf of Aqaba (Manasrah et al., 2004). This can explain the genetic separation of the Gulf from northern Red Sea for *P. squamipinnis*.

This raises the question why the circulation patterns and the water exchanges between the Gulf of Aqaba and northern Red Sea lead to a significant genetic structure in *P. squamipinnis* and not in *C. viridis*? The existences of marine barriers (currents, gyres, etc.) can cause genetic differentiation in different species. For example, the ecological differences between the northern and southern Red Sea lead to differences in fish communities in the Red Sea at about 20N°, which was congruent to the genetic differentiation between northern and southern populations of *Larabicus quadrilineatus* (Froukh and Kochzius, 2007) but not in the fishes of the current study. Another example is the genetic differentiation for three sparid fishes and the genetic homogenisation for two other sparid species across the Strait of Gibraltar between the Mediterranean Sea and the Northeast Atlantic Ocean (Bargelloni et al., 2003). It seems that differences in larval behaviour and swimming speed of *P. squamipinnis* and *C. viridis* are responsible for the different genetic structures.

C. Historical factors

Another hypothesis to explain the genetic differentiation in *P. squamipinnis* and the genetic homogenisation in *C. viridis* within the Red Sea is the consequences of historical events. The limited water exchanges between the Red Sea and the Indian Ocean at the shallow Strait of Bab-el-Mandab during the Pleistocene has worsened the ecological conditions (high salinity)

in the Red Sea (Siddall et al., 2003). During these harsh conditions, the Red Sea organisms were either capable to survive in certain restricted areas within the Red Sea (Goren, 1986, Klausewitz, 1989, Rohling et al., 1998) or migrated to the Gulf of Aden which served as a refuge (Klausewitz, 1989). In both cases reduction in population size followed by expansion has occurred. Under this scenario, the genetic homogenisation of *C. viridis* is the result of recent re-colonisation from the Gulf of Aden without the possibility of accumulating detectable genetic differences in the Red Sea. Whereas the genetic differentiation of *P. squamipinnis* is the result of population expansion from the survived individuals in the Red Sea with a sufficient time to build genetic differences between Gulf of Aqaba and the Red Sea proper. The proposed hypothesis that *C. viridis* found a refuge to the Gulf of Aden but *P. squamipinnis* stayed in the Red Sea could be supported by the following arguments. First, the available data show that *P. squamipinnis* is the most abundant fish in Aqaba and Tor (northern Red Sea). In addition, *P. squamipinnis* does not occur in Yemen, the most southern part of the Red Sea, and Djibouti (Gulf of Aden). *C. viridis* is the most abundant fish in Djibouti and in Yemen (Khalaf and Kochzius, 2002; PERSGA/ALECSO, 2003; PERSGA/GEF, 2003; Maha Ebeid, personal communication). We have to emphasis here that the absence of *P. squamipinnis* in Yemen and Djibouti is the most possible reason behind the high Φ_{st}-values between the Red Sea and the Indonesian population (based on the independence of Φ_{st}-values on the geographical distances). Similar high Φ_{st}-values (more than 0.9) were observed from mtDNA data between populations of sparid fishes across the Strait of Gibraltar (Bargelloni et al., 2003). Therefore, our high Φ_{st}-values for *P. squamipinnis* are not odd. The second reason is the estimated effective population size using the programme MIGRATE which was higher for *C. viridis* in southern Red Sea (Hodeidah in Yemen) and higher for *P. squamipinnis* in northern Red Sea (Tor).

Conclusion and consequences for conservation

While these results underline the importance of comparing genetic population structure of various species, it could not be firmly settled whether the observed pattern is caused by the current ecological differences between species or by the consequences of historical events. For a better understanding to the observed difference further studies on the biology (PLDs, and the swimming speeds) of demersal and pelagic fishes across transition zones (Strait of Tiran or Strait of Bab-el-Mandab) are required. In addition to that, fast evolving nuclear markers are needed to get better estimates of gene flow across short geographical distances.

However, the obtained results are helpful for the implementation of conservation strategies. On the one hand, the presence of structure for the *P. squamipinnis* between the Red Sea proper and the Gulf of Aqaba suggests a management strategy considering two stocks. On the other hand, *C. viridis* might be managed as one stock due to the absence of structure in the Red Sea and Gulf of Aqaba. However, since the lack of genetic structure could be due to a recent re-colonisation from the Gulf of Aden without the possibility of accumulating detectable genetic differences in the Red Sea, this has to be taken with caution. For a final assessment of the genetic population structure in *C. viridis*, genetic markers with a higher resolution, such as microsatellites should be applied.

References

Avise JC, Shapiro DY (1986) Evaluating kinship of newly settled juveniles within social groups of the coral reef fish *Anthias squamipinnis*. Evolution 40: 1051-1059.

Bandelt HJ, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. Mol. Biol. Evol. 16: 37-48.

Bargelloni L, Alarcon JA, Alvarez MC, Penzo E, Magoulas A, Reis C, Patarnello T (2003) Discord in the family Sparidae (Teleostei): divergent phylogeographical patterns across the Atlantic-Mediterranean divide. J. Evol. Biol. 16: 1149-1158.

Bay LK, Buechler K, Gagliano M, Caley MJ (2006) Intraspecific variation in the pelagic larval duration of tropical reef fishes. J. Fish Biol. 68: 1206-1214.

Beerli P (2005) Comparison of Bayesian and maximum likelihood inference of population genetic parameters. Bioinformatics 22: 341-345.

Beerli P, Felsenstein J (1999) Maximum likelihood estimation of a migration rates and population numbers of two populations using a coalescent approach. Genetics 152: 763-773.

Beerli P, Felsenstein J (2001) Maximum likelihood estimation of a migration matrix and effective population size in n subpopulations by using a coalescent approach. P. Natl. Acad. Sci. USA 98: 4563-4568.

Bellwood DR, Fisher R (2001) Relative swimming speeds in reef fish larvae. Mar. Ecol. Prog. Ser. 211:299-303.

Braithwaite CJR (1987) Geology and paleogeography of the Red Sea region. In: Key environments. Red Sea (eds. Edwards AJ, Head SM), pp. 22-44. Pergamon Press, Oxford.

Briggs JC (1995) Global biogeography. Elsevier, Amsterdam, London, New York, Tokyo.

Doherty PJ, Planes S, Mather P (1995) Gene flow and larval duration in seven species of fish from the great barrier reef. Ecology 76: 2373-2391.

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1:47-50.

Excoffier L, Smouse PE (1994) Using allele frequencies and geographic subdivision to reconstruct gene trees within a species: molecular variance parsimony. Genetics 136: 343-359.

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131: 479-491.

Fauvelot C, Bernardi G, Planes S (2003) Reduction in the mitochondrial DNA diversity of coral reef fish provides evidence of population bottlenecks resulting from holocene sea-level change. Evolution 57: 1571-1583.

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783-791.

Fratini S, Vannini M (2002) Genetic differentiation in the mud crab Scylla serrata (Decapoda: Portunidae) within the Indian Ocean. J. Exp. Mar. Biol. Ecol. 272: 103-116.

Froukh T, Kochzius M (2007) Genetic population structure of the endemic fourline wrasse (*Larabicus quadrilineatus*) suggests limited larval dispersal distances in the Red Sea. Mol. Ecol. (doi: 10.1111/j.1365-294X.2007.03236.x).

Fu YX (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147: 915-925.

Gopurenko D, Hughes JM, Keenan CP (1999) Mitochondrial DNA evidence for rapid colonisation of the Indo-West Pacific by the mudcrab *Scylla serrata*. Mar. Biol. 134: 227-233.

Goren M (1986) A suggested model for the recolonisation process of the Red Sea at the post glacial period. In: Indo-Pacific fish biology: proceedings of the second international conference on Indo-Pacific fishes (Uyeno T, Arai R, Taniuchi T, Matsuura K. eds.), pp 648-654. Ichthyological Society of Japan, Tokyo.

Grant WS, Bowen BW (1998) Shallow population histories in deep evolutionary lineages of marine fishes: insights from Sardines and Anchovies and lessons for conservation. J. Hered. 89:415-426.

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41: 95-98.

Harpending HC (1994) Signature of Ancient population growth in a low resolution mitochondrial DNA mismatch distribution. Hum. Biol. 66: 591-600.

Hassan M, Harmelin-Vivien M, Bonhomme F (2003) Lessepsian invasion without bottleneck: examples of two rabbitfish species (*Siganus rivulatus* and *Siganus luridus*). J. Exp. Mar. Biol. Ecol. 291: 219-232.

Head S (1987) Key Environment Red Sea. 1st edn. Pergamon Press, Oxford.

Jensen JL, Bohonak AJ, Kelley ST (2005) Isolation by distance, web service, BMC. Genetics 6: 13. v. 3.0.1 http://ibdws.sdsu.edu/

Khalaf MA, Kochzius M (2002) Community structure and biogeography of shore fishes in the Gulf of Aqaba, Red Sea. Helgoland Mar. Res. 55: 252-284.

Klausewitz W (1989) Evolutionary history and zoogeography of the Red Sea ichthyofauna. Fauna of Saudi Arabia 10: 310-337.

Kochzius M, Blohm D (2005) Genetic population structure of the lionfish *Pterois miles* (Scorpaenidae, Pteroinae) in the Gulf of Aqaba and northern Red Sea. Gene 347: 295-301.

Kochzius M, Söller R, Khalaf MA, Blohm D (2003) Molecular phylogeny of the lionfish genera *Dendrochirus* and *Pterois* (Scorpaenidae, Pteroinae) based on mitochondrial DNA sequences. Mol. Phylogenet. Evol. 28: 396-403.

Leis JM, Carson-Ewart BM (2000) Behaviour of pelagic larvae of four coral-reef fish species in the ocean and atoll lagoon. Coral Reefs 19: 247-257.

Leis JM, Fisher R (2006) Swimming speed of settlement-stage reef-fish larvae measured in the laboratory and in the field: a comparison of critical speed and *in situ* speed. In: Proceedings of the 10th International Coral Reef Symposium, Okinawa (Suzuki, Y., Nakamori, T., Hidaka, M., Kayanne, H., Casareto, B. E., Nadaoka, H., Yamano, H., & Tsuchiya, M. eds.) pp: 438-445.

Manasrah R, Badran M, Lass HU, Fennel W (2004) Circulation and winter deep-water formation in the northern Red Sea. Oceanologia 46: 5-23.

Mantel N (1967) The detection of disease clustering and generalized regression approach. Cancer Res. 27: 209–220.

McManus JW (1985) Marine speciation, tectonics and sea level changes in Southeast Asia. In: Proceedings of the Fifth International Coral Reef Congress, Tahiti (Gabrie, C., & Salvat, B. eds.) pp: 133-138.

PERSGA/ALECSO (2003) Survey of habitats in Djibouti and plans for their protection. PERSGA Technical Series No. 5. PERSGA, Jeddah.

PERSGA/GEF (2003) Current status of the ornamental fish trade: their assemblages, collections, practices, export, conservation issues and management in the Red Sea and Gulf of Aden. PERSGA Technical Series Nr. 9. PERSGA, Jeddah.

Planes S (2002) Biogeography and larval dispersal inferred from population genetic analysis. In: Coral reef fishes (Sale, P.F. eds.), pp. 201-220. Academic Press, New York.

Posada D, Crandall K (1998) Modeltest: testing the model of DNA substitution. Bioinformatics 14: 817-818.

Reiss Z, Hottinger L (1984) The Gulf of Aqaba. Ecological Micropaleontology. Springer, Berlin.

Roberts CM, McClean CJ, Veron JEN, et al (2002) Marine biodiversity hotspots and conservation priorities for tropical reefs. Science 295: 1280-1284.

Rogers AL (1995) Genetic evidence for a Pleistocene population explosion. Evolution 49: 608-615.

Rohling E, Fenton M, Jorissen F, Bertrand P, Gnassen G, Caulet J (1998) Magnitudes of sea-level lowstands of the past 500,000 years. Nature 394: 162-165.

Rozas J, Sánchez-DelBarrio, Messegyer JC, Rozas R (2003) DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19: 2496-2497.

Siddall, M., Rohling, E., Almogi-Labin, et al (2003) Sea-level fluctuations during the last glacial cycle. Nature 423: 853-858.

Shefer S, Abelson A, Mokady O, Geffen E (2004) Red to Mediterranean Sea bioinvasion: natural drift through the Suez Canal, or anthropogenic transport? Mol. Ecol. 13: 2333-2343.

Shields GF, & Gust JR (1995) Lack of geographic structure in mitochondrial DNA sequences of Bering Sea walleye Pollock, *Theragra chalcogramma*. Mol. Mar. Biotechnol. 4: 69-82.

Shulman MJ (1998) What can population genetics tell us about dispersal and biogeographic history of coral-reef fishes. Aust. J. Ecol. 23: 216-225.

Shulman MJ, Bermingham E (1995) Early life histories, ocean currents, and the population genetics of Caribbean reef fishes. Evolution 49: 897-910.

Söller R, Warnke K, Saint-Paul U, Blohm D (2000) Sequence divergence of mitochondrial DNA indicates cryptic biodiversity in *Octopus vulgaris* and supports the taxonomic distinctiveness of *Octopus mimus* (Cephalopoda: Octopodidae). Mar. Biol. 136: 29–35.

Stobutzki IC (1998) Interspecific variation in sustained swimming ability of late pelagic stage reef fish from two families (Pomacentridae and Chaetodontidae). Coral Reefs 17: 111-119.

Swofford DL (1998) PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4 Sinauer, Sunderland.

Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 125: 585-593.

Thompson JG, Higgins DG, Gibson TJ (19949 CLUSTAL W: improving the sensitivity of progressive multiple sequence alignments through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Res. 22: 4673–4680.

Thresher RE (1984) Reproduction in reef fishes. TFH Publications, New Jersey.

Thresher RE (1991) Geographic variability in the ecology of coral reef fishes: evidence, evolution, and possible implications. In: The ecology of fishes on coral reefs (Sale, P.F. eds.), pp. 401-436. Academic Press, San Diego, California.

Thresher RE, Colin PL, Bell LJ (1989) Planktonic duration, distribution and population structure of western and central Pacific damselfishes (Pomacentridae). Copeia 420-434.

Voris HK (2000) Maps of Pleistocene sea leveles in Southeast Asia: shorelines, river systems, time durations. J. Biogeog. 27: 1153-1167.

Wellington GM, Victor BC (1989) Planktonic larval duration of one hundred species of Pacific and Atlantic damselfishes (Pomacentridae). Mar. Biol. 101: 557-567.

Wilson DT, McCormick MI (1999) Microstructure of settlement-marks in the otoliths of tropical reef fishes. Mar. Biol. 134: 29-41.

Wood E (2001) Collection of coral reef fish for aquaria: global trade, conservation issues and management strategies. Marine Conservation Society, Ross-on-Wye, UK.

Chapter 4

Deep evolutionary lineages in the blue green damselfish indicate cryptic or incipient species

Tawfiq Froukh, Marc Kochzius

Proceedings of the Royal Society B: Biological Sciences (submitted)

Abstract

The blue green damselfishes were described as complex of two species (*Chromis viridis* and *C. atripectoralis*) mainly based on the colouration of pectoral fin base. In this study, we analysed the mitochondrial control region of 88 blue green damselfishes from Indonesia, the Philippines, Red Sea, and the Great Barrier Reef. The phylogenetic analysis revealed four major monophyletic clades. Two clades grouped exclusively: (1) the *C. atripectoralis* from the Great Barrier Reef; and (2) the *C. viridis* from the Red Sea. The remaining individuals of *C. viridis* from Indonesia and the Philippines were grouped into two clades without phylogeographic structure. The obtained results suggest that the blue green damselfishes are a complex of three species, including a cryptic or incipient one.

Keywords: sequence divergence, evolutionary origin, Pomacentridae, evolutionary lineages, molecular phylogeny, coral re

Introduction

Many reef fishes consist of complexes of co-occurring species. Understanding the processes of speciation in reef fishes remains a challenging topic in evolutionary biology (Choat 2006). Colour patterns of reef fishes affect mainly their mate selection, and thus play a role on their speciation processes, for example in butterflyfishes (McMillan *et al.* 1999) and the three-spot damselfish (*Dascyllus trimaculatus*) species complex (Bernardi *et al.* 2002, 2003). Allopatric speciation in fish was recorded within separated ocean basins due to the fluctuations of the sea level during glacials (McManus 1985; McMillan and Palumbi 1995; Kochzius *et al.* 2003). Speciation is complicated by the presence of cryptic species which do not differ in the colour patterns but differ genetically (Knowlton 2000).

Here, we study the speciation in the damselfishes (Pomacentridae) blue green chromis *Chromis virids* (Cuvier 1830) and the black-axil chromis *C. atripectoralis* (Welander and Schultz 1951). *C. virids* usually occurs in schools which may include more than 100 individuals without being mixed with other colonial species. These schools are associated with large heads of *Acropora* corals that provide shelters from predators and nocturnal retreat (Fishelson *et al.* 1974; Allen and Randall 1980). Throughout the Indo-Pacific region, *C. viridis* occurs abundantly from the Red Sea and the east African coasts to French Polynesia and New Caledonia in the south Pacific, and Line Islands to Ryukyu in the north Pacific (Lieske and Myers 1994). While it was thought that the blue green damselfishes in these regions are one species, studies of Welander and Schultz (1951) have revealed that they are a complex of two species. *C. atripectoralis* was described as a sister species of *C. viridis*, which occurs in the same regions as *C. viridis* but not in the Red Sea (Lieske and Myers 1994). The black area at the pectoral fin base in *C. atripectoralis* distinguishes it from *C. viridis* which is pigmented with black dots forming a dusky area only at the upper pectoral fin base (Welander and Schultz 1951). However, similarities in their overall morphology make it very difficult to distinguish them. Genetic studies for these two species are scarce. Jang-Liaw *et al.* (2002) presented a hypothesis of relationship for 48 pomacentrid species including only *C. viridis*, while Quenouille *et al.* (2004) presented the same hypothesis for 103 pomacentrid species including both *C. viridis* (collected from Japan) and *C. atripectoralis* (collected from Australia). Molecular tools have proven useful to reveal marine cryptic species where traditional techniques can not detect them (Knowlton 2000). We used the mitochondrial control region as a molecular marker to evaluate the phylogenetic relationship of the damselfishes *C. viridis* and *C. atripectoralis*.

Materials and Methods

Fin clips of *C. viridis* were collected at the Red Sea in December 2003, Indonesia (Barru) in May 2004, and the Philippines (Cebu) in February 2006. Additional sequences of *C. atripectoralis* were obtained from the GenBank DQ212240-DQ212281 (Figure 1).

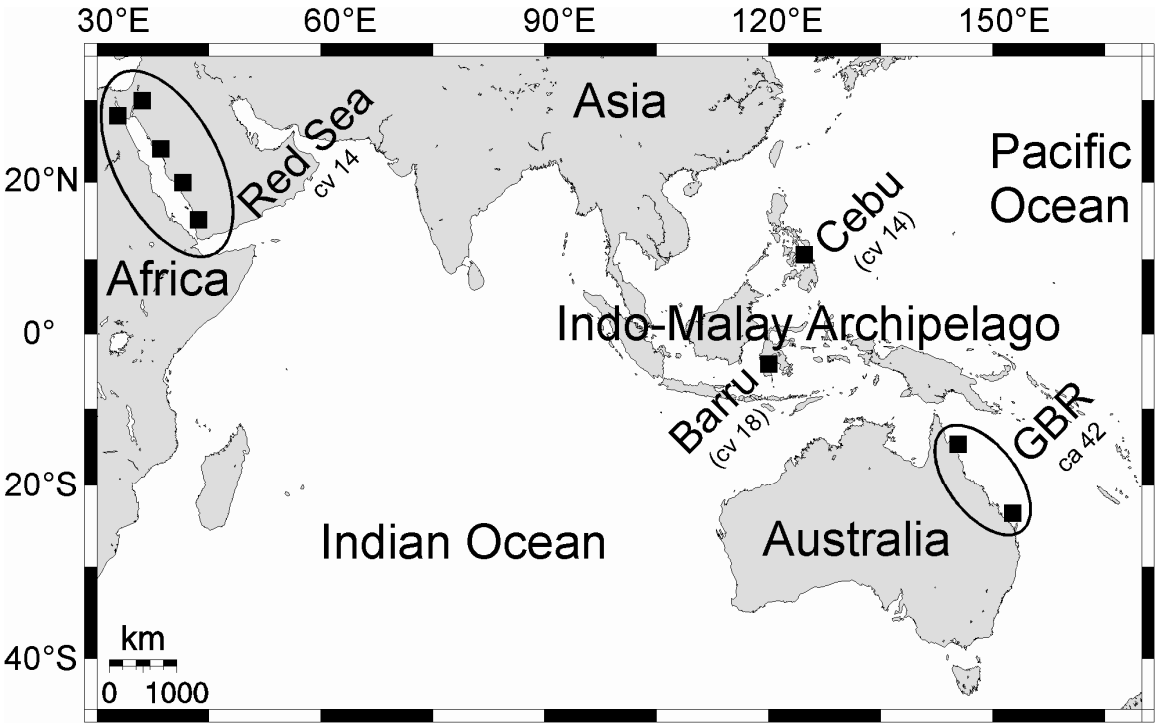

Figure 1 Map of the Indo-West Pacific showing the collection sites of *Chromis viridis* (cv) in the Red Sea and Indo-Malay Archipelago, and of *Chromis atripectoralis* (ca) in the Great Barrier Reef (GBR) (Bay *et al.* 2006). The number of the obtained sequences is indicated below the name of the region.

The total DNA was extracted from fin clips using Chelex® as described by Söller *et al.* (2000). A fragment of the mitochondrial control region was amplified by PCR, using the universal primer CR-A and the specific primer XAN-DL-R (Fauvelot *et al.* 2003). PCR conditions were as follows: 95°C for 5 minutes, followed by 35 cycles of 94°C for 45 seconds, 50°C for 45 seconds, and 72°C for 60 seconds, finished by a final extension at 72°C for 5 minutes. The reaction mix contained 10 mM Tris-HCl (pH 9), 50 mM KCl, 5.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.2 µM each primer, 2 U Taq polymerase, 2 µl of the DNA template, and a double distilled water to 50 µl total reaction volume. Amplified PCR products were purified with the QIAquick PCR purification kit (Qiagen, Hilden). Both strands were sequenced with the same primers used for PCR under the DyeDeoxy Terminator chemistry (PE Biosystems, Foster City) and an ABI automated sequencer. Sequences were edited with the programme SEQUENCE NAVIGATOR (version 1.0.1; Applied Biosystem), while a

multiple alignment of all sequences was done with the programme CLUSTALW (Thompson *et al.* 1994) as implemented in BIOEDIT (version 7.0.4.1; Hall 1999).

The phylogenetic analysis was carried out using the programme PAUP* (version 4.0b10; Swofford 1998). The programme MODELTEST (version 3.7; Posada and Crandall 1998) was used to determine the best-fit model of DNA evolution. Gaps in the sequences were deleted for the neighbour-joining (NJ) and the maximum likelihood (ML) methods, while for the maximum parsimony (MP) method gaps were treated as a fifth base. Statistical confidence in nodes was evaluated based on 10,000, 1000, and 100 non-parametric bootstrap replicates in NJ, MP (10,000 trees were kept at each replicate), and ML analyses, respectively (Felsenstein 1985). Only the haplotypes of *C. atripectoralis* sequences (33 sequences out of 42 sequences) were used in the analyses. Five sequences of *C. nitada*, obtained from the GenBank (DQ250522-DQ250526) were used as outgroup. Alternative ML topologies with and without enforcing a molecular clock were compared using a Shimodaira-Hasegawa test (Shimodaira and Hasegawa 1999).

A haplotype network was constructed using the median-joining network method as implemented in NETWORK (version 4.2.0.1; www.fluxus-engineering.com; Bandelt *et al.* 1999). This method begins by combining the minimum spanning trees (MSTs) within a single network (minimum spanning network, MSN) using an analogous algorithm to that proposed by Excoffier and Smouse (1994). Then, median vectors (which represent missing intermediates haplotypes) are added to the network using the parsimony criterion.

Results

The best-fit evolutionary model was the Hasegawa-Kishino-Yano with a transition/transversion ratio of 3.55, gamma distribution shape parameter of 0.58, proportion of invariable sites of 0.35, and the following base frequencies: A=0.36; C=0.23; G=0.15; and T=0.26. These parameters were used for the NJ and ML methods. The three methods (NJ, MP, and ML) showed similar trees. Figure 1 shows a ML tree with the bootstrap values of the three methods. The phylogenetic analyses revealed four well-supported clades, and no subclades were observed within any of them. Clade 1 contains most of the individuals from the Philippines and seven individuals from Indonesia, whereas all of *C. viridis* individuals from the Red Sea clustered in clade 2. Clade 3 contains most of the Indonesian individuals and three individuals from the Philippines. All of the *C. atripectoralis* individuals from the Great Barrier Reef (GBR) clustered in clade 4 (Figure 1A). DNA sequence divergences

between and within clades were calculated based on the maximum likelihood evolutionary model (Table 1). Clade 3 has the smallest sequence divergence within haplotypes and the largest sequence divergence to all other clades. ML topologies with and without enforcing a molecular clock were significantly different based on Shimodaira-Hasegawa test (-lnL= 2323.57; p<0.0001; Shimodaira and Hasegawa 1999).

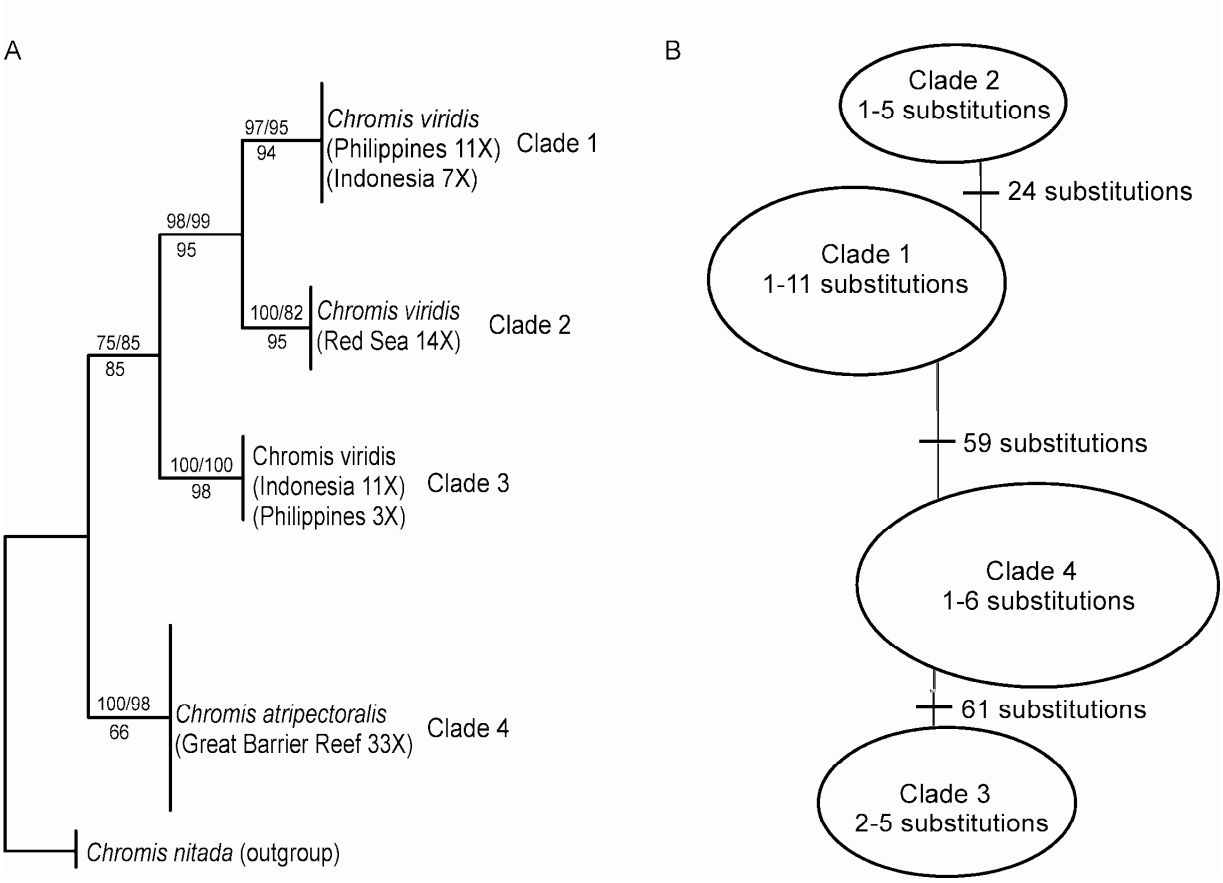

Figure 2 A. Molecular phylogenetic tree of the blue green damselfishes (*Chromis viridis* and *C. atripectoralis*) from the Indo-Malay Archipelago and the Red Sea based on the mitochondrial control region using the maximum likelihood. Bootstrap values are: NJ (10,000 replicates)/MP(1000 replicates) indicated above branches and ML(100 replicates) indicated below branches. X refers to the number of individuals per corresponding location. B. Median joining network for all sequences based on the mitochondrial control region using the programme NETWORK. Branch lengths are independent on the number of nucleotide substitutions. Numbers of substitutions and the range of substitutions between and within clades are indicated.

The haplotype network reinforced the phylogentic tree by clustering the same haplotypes into four distinct clades (Figure 1B). The clades 3 and 4 are separated by the highest number of nucleotide substitutions (61) and the caldes 1 and 2 are separated by the lowest number of nucleotide substitutions. Nucleotide substitutions between haplotypes within each clade are relatively low, not exceeding 11 nucleotide substitutions.

Table 1 Average DNA sequence divergence (± standard deviation) between and within clades of the bluegreen damselfishes (Pomacentridae) *Chromis viridis* and *C. atripectoralis*.

Taxa	Sequence divergences (%) ± SD
Clade 1-clade 2	12.8 ± 1.4
Clade 1-clade 3	45.4 ± 2.2
Clade 1-clade 4	30.4 ± 2.5
Clade 2-clade 3	30.4 ± 1.3
Clade 2-clade 4	28.8 ± 1.9
Clade 3-clade 4	36.7 ± 1.8
Clade 1	4.3 ± 1.8
Clade 2	1.5 ± 0.8
Clade 3	1.1 ± 0.5
Clade 4	2.6 ± 0.9

Discussion

Clades 1, 2, and 3 could be considered as three deep evolutionary lineages of *C. viridis*. The presence of Indonesian and Philippine individuals in two separated clades (1 and 3) without phylogeographic structure might be due to a diversification of *C. viridis* outside the Indo-Malay Archipelago and subsequent mixing in this region. These evolutionary lineages may be explained by the limited exchange among the isolated ocean basins (Red Sea, Indian Ocean, and Pacific Ocean) during glacials, when the sea level dropped up to 120 m (McManus 1985; Voris 2000). The clades in the phylogenetic tree could be assigned to these ocean basins: clade 1 to the Indian Ocean; clade 2 to the Red Sea, and calde 3 to the Pacific Ocean. Because samples from the Indian Ocean are missing, it is difficult to assign clade 1 and 3 to a certain ocean basin. However, clade 2 shows a clear origin in the Red Sea and is closely related to clade 1 than clade 3. Therefore, we can assume origination of clade 1 in the Indian Ocean and of clade 3 in the Pacific Ocean. Samples from the Indian Ocean are needed to verify this scenario.

Additionally, clade 3 seems to represent cryptic or incipient species while clades 1 and 2 are two evolutionary lineages of *C. viridis*. We could not calculate the time of divergence between clades because the molecular clock was rejected, indicating variation in the substitution rates among lineages for the used molecular marker (Huelsenbeck and Rannala 1997).

Welander and Schultz (1951) discriminated *C. atripectoralis* from *C. viridis* on the basis of two characters: (1) the black axil of the pectoral fin; and (2) more branched pectoral rays usually 17 or 18 in *C. atripectoralis* and 15 or 16 in *C. viridis*. However, the number of pectoral rays in the two species is overlapping as follows: 18-20 in *C. atripectoralis* and 17-19 in *C. viridis* (Randall *et al.* 1997; Allen and Randall 1980). Therefore, *C. viridis* is mainly discriminated by the black dots forming a dusky area only at the upper pectoral-fin base. These elusive dots should be carefully examined on a larger number of individuals to test if the different evolutionary lineages correspond to this minor differences in morphology and coloration. Previous studies have shown that colourations play an important role in the speciation of coral reef fishes (Messmer *et al.* 2005 a and b; McMillan *et al.* 1999; Bernardi *et al.* 2002; Rocha 2004).

A larger sample size and an additional collection sites in Indonesia, the Philippines and in the Indian Ocean are needed to describe the evolutionary processes of blue green damselfishes in more details.

References

Allen, G. R. & Randall J. E. 1980 A review of the damselfishes (Teleostei: Pomacentridae) of the Red Sea. *Israel J. Zool.* **29**, 1-98.

Bandelt, H. J., Forster, P., & Röhl, A. 1999 Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol*. **16**, 37-48.

Bay, L. K., Crozier, R. H., & Caley, M. J. 2006 The relationship between population genetic structure and pelagic larval duration in coral reef fishes on the Great Barrier Reef. *Mar. Biol*. **149**, 1247-1256.

Bernardi, G., Holbrook, S. J., Schmitt, R. J., Crane, N. L. & DeMartini, E. 2002 Species boundaries, populations and colour morphs in the coral reef three-spot damselfish (*Dascyllus trimaculatus*) species complex. *Proc Roy Soc Lond B* **269**, 599–605

Bernardi, G., Holbrook, S. J., Schmitt, R. J. & Crane, N. L. 2003 Genetic evidence for two distinct clades in a French Polynesian population of the coral reef three-spot damselfish *Dascyllus trimaculatus*. *Mar. Biol*. **143**, 485-490.

Choat, J. H. 2006 Phylogeography and reef fishes: bringing ecology back into the argument. *J. Biogeogr*. **33**, 967-968.

Cuvier, G. L. 1830 *Historie Naturelle des Poissons*. Vol. 5, 420 and 497.

Excoffier, L., & Smouse, P. E. 1994 Using allele frequencies and geographic subdivision to reconstruct gene trees within a species: molecular variance parsimony. *Genetics* **136**, 343-359.

Fauvelot, C., Bernardi, G. & Planes, S. 2003 Reduction in the mitochondrial DNA diversity of coral reef fish provides evidence of population bottlenecks resulting from holocene sea-level change. *Evolution* **57**, 1571-1583.

Felsenstein, J. 1985 Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783-791.

Fishelson, L., Popper, D. & Avidor, A. 1974 Biosociology and ecology of pomacentrid fishes around the Sinai Peninsula (northern Red Sea). *J Fish Biol*. **6**, 119–133.

Hall, T. A. 1999 BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* **41**, 95-98.

Huelsenbeck, J. P., & Rannala, B. 1997 Phylogenetic methods come of age: testing hypotheses in an evolutionary context. *Science* **276**, 227-232.

Jang-Liaw, N. H., Tang, K. L., Hui, C. F. & Shao, K. T. 2002 Molecular phylogeny of 48 species of damselfishes (Perciformes: Pomacentridae) using 12S mtDNA sequences. *Mol. Phylogenet. Evol.* **25**, 445-454.

Knowlton, N. 2000 Molecular genetic analyses of species boundaries in the sea. *Hydrobiologia* **420**, 73-90.

Kochzius, M., Söller, R., Khalaf, M. A. & Blohm, D. 2003 Molecular phylogeny of the lionfish genera *Dendrochirus* and *Pterois* (Scorpaenidae, Pteroinae) based on mitochondrial DNA sequences. *Mol. Phylogenet. Evol.* **28**, 396-403.

Lieske, E. & Myers, R. 1994 *Coral reef fishes*. HarperCollins Publishers, London.

McManus, J. W. 1985 Marine speciation, tectonics and sea-level changes in Southeast Asia. *Proceedings of the Fifth International Coral Reef Congress*, Tahiti **4**, 133-138.

McMillan, W. O., & Palumbi, S. R. 1995 Concordant evolutionary patterns among Indo-West Pacific butterflyfishes. *Proc Roy Soc Lond B* **260**, 229-236.

McMillan, W. O., Weigt, L. A. & Palumbi, S. R. 1999 Color pattern evolution, assortative mating, and genetic differentiation in brightly colored butterflyfishes (Chaetodontidae). *Evolution* **53**,247–260.

Messmer, V., Herwerden, L., Munday, P. L. & Jones, P. 2005a Phylogeography of colour polymorphism in the coral reef fish *Pseudochromis fuscus*, from Papua New Guinea and the Great Barrier Reef. *Coral Reefs* **24**, 392-402.

Messmer, V., Jones, P., Herwerden, L. & Munday, P. L. 2005b Genetic and ecological characterisations of colour dimorphism in a coral reef fish. *Environ. Biol. Fish.* **74**, 175-183.

Posada, D. & Crandall, K. 1998 *Modeltest*: testing the model of DNA substitution. *Bioinformatics* **14**, 817-818.

Quenouille, B., Bermingham, E. & Planes, S. 2004 Molecular systematics of the damselfishes (Teleostei: Pomacentridae): Bayesian phylogenetic analyses of mitochondrial and nuclear DNA sequences. *Mol. Phylogenet. Evol.* **31**, 66-88.

Randall, J. E., Allen, G. R., & Steene, R. C. 1997 *Fishes of the Great Barrier Reef and Coral Sea*. Crawford House Publishing, Bathurst.

Rocha, L. A. 2004 Mitochondrial DNA and colour pattern variation in three western Atlantic Halichoeres (Labridae), with the revalidation of two species. *Copeia* **4**,770–782.

Söller, R., Warnke, K., Saint-Paul, U. & Blohm, D. 2000 Sequence divergence of mitochondrial DNA indicates cryptic biodiversity in *Octopus vulgaris* and supports the taxonomic distinctiveness of *Octopus mimus* (Cephalopoda: Octopodidae). *Mar. Biol.* **136**, 29–35.

Swofford, D. L. 1998 *PAUP*. Phylogenetic analysis using parsimony (*and other methods).* Version 4 Sinauer, Sunderland.

Thompson, J. G., Higgins, D. G. & Gibson, T. J. 1994 CLUSTAL W: improving the sensitivity of progressive multiple sequence alignments through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Res*. **22**, 4673–4680.

Voris, H. K. 2000 Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time duration. .*J Biogeogr*, **27**, 1153-1167.

Welander, A. D. & Schultz, P. S. 1951 *Chromis atripectoralis*, a new damselfish from the tropical Pacific, closely related to *C. caeruleus*, family Pomacentridae. *Journal of the Washington Academy of Sciences*, **41**, 107-110.

Acknowledgment

I want to thank my promoters and supervisors, Prof. Dietmar Blohm and Dr. Marc Kochzius, who have been supportive throughout the study. They were always on my side whenever I needed help. Their critical thinking and wide experience have helped me in looking at my results from different angles. I am greatly indebted to them for the assistance in all logistic, administrative, and scientific issues.

I want to thank the German academic exchange service (DAAD) for the financial support of my PhD research.

I would like to thank all people who helped me in obtaining the fish materials, especially Dr. Maroof Khalaf from the Marine Science Station, Aqaba, Jordan, and Kamal Al-Dahodie from Jeddah, Saudi Arabia. I would like also to thank the following people for their various help in the lab work, statistical analyses, and their comments: Frank Meyerjürgens, Dr. Sacha Todt, Janne Timm, Marco Ewald, Rijuta Kotenkar, Selma Kurt, and Nidal Odat.

Many thanks to my family: my parents, my nine brothers and three sisters with their spouses and their thirty three kids for encouragement and the unlimited love.

Last but certainly not least, I want to thank my wife Nour Abu Dabasi, who took the full responsibilities of our newly established life and to our son Tarik while I was in the critical moments of my study. Thank you very much for your support and your love!

Declaration

I verify that the work presented in this thesis is authentically and essentially my own and without any other help. I did not use any other sources and auxiliary equipments than the mentioned.

Bremen, 16.03.2007

_____

Tawfiq Froukh