Molecular Phylogenetics and Evolution 49 (2008) 629–638

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

High population connectivity across the Indo-Pacific: Congruent lack of phylogeographic structure in three reef fish congeners

John B. Horne [a,*], Lynne van Herwerden [a], J. Howard Choat [a], D.R. Robertson [b,1]

[a] School of Tropical and Marine Biology, Molecular Ecology and Evolution Laboratory, James Cook University, Townsville, Qld 4811, Australia
[b] Smithsonian Tropical Research Institute, Balboa, Republic of Panama

ARTICLE INFO

Article history:
Received 26 June 2008
Revised 20 August 2008
Accepted 24 August 2008
Available online 6 September 2008

Keywords:
Coral reef fish
Acanthuridae
Naso brevirostris
Naso unicornis
Mitochondrial control region
Stochastic variation
Comparative phylogeography

ABSTRACT

We used the mitochondrial control region and a comparative approach to study the genetic population structure of two surgeonfishes, *Naso brevirostris* and *Naso unicornis*, across their Indo-central Pacific ranges. Our purpose was to compare our results with those of a previous study of *Naso vlamingii* [Klanten, S.O., van Herwerden, L., Choat J.H., 2007. Extreme genetic diversity and temporal rather than spatial partitioning in a widely distributed coral reef fish. Mar. Biol. 150, 659–670] another widely distributed Indo-central Pacific *Naso* species. We found no evidence of a barrier to gene flow between the Indian and Pacific Oceans for either species, consistent with what was shown for *N. vlamingii*. Overall, both target species lacked spatial population partitions and probably have complex patterns of gene flow on several spatial scales. Despite the lack of geographic population structure distinct clades were observed in *N. brevirostris*, similar to those found in *N. vlamingii*. Coalescence times for intraspecific clades of *N. brevirostris* and *N. vlamingii* approximate each other, suggesting parallel evolutionary histories. A bimodal mismatch distribution in *N. brevirostris* indicates that a biogeographic barrier separated *N. brevirostris* populations sometime during its species history. *Naso unicornis*, in contrast, lacked genetic structure of any kind, although it has what could represent a single surviving clade. Congruent lack of spatial population structure among all three species suggest that such patterns are not due to stochastic processes of DNA mutation and are most likely driven by ecological and environmental factors.

1. Introduction

Reef fish species tend to have broad geographic distributions comprising patchily distributed adult populations connected by pelagic larvae (Sale, 1991). Adult distributions reflect the spatial patterning of suitable reef habitats, which are often separated by hundreds or even thousands of kilometers of deep open Ocean. The application of molecular markers has provided the means to assess the extent to which widespread reef fish species may be partitioned into distinct populations, as well as the spatial scale of partitioning and the temporal pattern of population connectivity between isolated adult populations. An important finding of reef fish phylogeographic studies has been the identification of a degree of spatial structuring among populations greater than might be expected for species with large species ranges and high larval dispersal potential (Swearer et al. 2002; Planes and Fauvelot, 2002; Bernardi et al. 2003; Taylor and Hellberg 2003; Thacker et al 2007). This degree of spatial structuring has promoted interest in the nature, types, abundance and effectiveness of barriers that prevent or limit larval dispersal (Rocha et al., 2007; Hellberg 2007; Floeter et al. 2008) and the behavior of dispersing larvae (Leis 2007; Leis et al. 2007). In contrast, however, an increasing number of phylogeographic studies are also providing evidence of widespread reef fishes in which geographic population structure is minimal or altogether lacking. Some examples of reef fishes with little genetic population structure include holocentrids (Craig et al. 2007), scarine labrids (Bay et al. 2004), aulostomids (Bowen et al. 2001) and acanthurids (Klanten et al., 2007). Such studies suggest that the larvae of some reef fishes readily cross very large open ocean barriers that impede dispersal in other species (Lessios and Robertson 2006) and that larval movement across oceanic barriers may be highly variable among different reef fish taxa.

The molecular approach is an effective method for studying connectivity between discontinuous populations provided that the genetic diversity of the populations is effectively represented in the sample. However, it can be difficult to capture the molecular signal of populations and assess their connectivity, particularly when the effective population size is large and genetic diversity is high, as is the case with many benthic marine organisms (Palumbi 2003; Hellberg, 2007; Hedgecock et al. 2007). When much of a population's diversity remains unsampled, the molecular method is less effective. Thus, species with strong geographic structural

* Corresponding author. Fax: 61 07 4724 1770.
E-mail address: john.horne@jcu.edu.au (J.B. Horne).
[1] Mailing address: STRI, Unit 0948, APO AA 34002, USA.

1055-7903/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2008.08.023

differences between populations and low genetic diversity are ideal for this type of research, while those that lack structure and have high genetic diversity yield results that are more difficult to interpret. Moreover, in the absence of strong population structure stochasticity, such as homoplasy, may confound true population patterns. Therefore, weak genetic population structure must be interpreted cautiously, particularly in studies where only one molecular marker is used.

Because weak population structure obtained from a single molecular marker may be confounded by stochastic variation, validation of population patterns through an assessment of their congruence is often called for. In most cases the extent of congruence can be assessed using independent data sets, such as additional unlinked molecular markers within a single species or through comparative phylogeography. The second approach traces independent species histories through a single molecular marker. Comparative phylogeography has a number of applications (Bermingham and Moritz, 1998) and can help determine the extent to which homoplasy and other stochastic signals are confounding genetic population structure in certain reef fish species.

One of the most extreme examples of a reef fish with minimal population structure is *Naso vlamingii*. The population genetics of this species, inferred from the mitochondrial control region, is characterized by: (i) an extremely high nucleotide and haplotype diversity, (ii) a lack of geographic population structure resembling panmixia on a very large spatial scale and (iii) the presence of a temporal genetic structure presumed to have arisen from episodic isolation during a long species history (Klanten et al., 2007). Notwithstanding the biological interest of the *N. vlamingii* findings, because this study was based on a single marker there is the possibility that stochastic variation rather than a biologically relevant process is causing the observed genetic patterns. The purpose of the current study was to evaluate the biological relevance of these unusual genetic patterns by studying the same mitochondrial marker in two other equally widespread members of the genus *Naso*.

The first species chosen for this study was *Naso brevirostris*, which is sister to *N. vlamingii* (Klanten et al., 2004) and similar in terms of its geographic distribution and foraging ecology (Randall, 2001; Choat et al. 2002). The second species was *Naso unicornis*, which is also widespread but belongs to a separate clade from *N. vlamingii* (Klanten et al., 2004). *Naso unicornis* also differs from *N. brevirostris* and *N. vlamingii*, in being a benthic rather than a pelagic forager (Randall, 2001; Choat et al. 2002). All three species have similar life histories and have large pelagic larvae that persist in the pelagic environment for approximately 90 days (B. Victor, personal communication), giving all three species a high larval dispersal potential. In addition, the phylogenetic lineages of our two target species are comparable in age to that of *N. vlamingii* (Klanten et al., 2004). Ergo, these two species were chosen because they and *N. vlamingii* potentially share parallel evolutionary histories and might therefore have congruent population structures.

In light of the possibility that these three species may have similar population structures, the primary questions posed in this study were: (i) Do other *Naso* species exhibit elevated levels of genetic diversity as observed in *N. vlamingii?* (ii) Are other widely distributed *Naso* species characterized by a lack of geographical population partitioning across their equally wide species ranges? (iii) Do either of the target species in this study have a temporal rather than spatial population structure suggesting historical isolation across an ancient biogeographic barrier? Using a comparative approach, this study addresses the uncertainty surrounding the intriguing genetic partitioning observed in *N. vlamingii*, highlights the usefulness of comparative phylogeography as a tool for studying reef fish populations and provides insight into the population connectivity of these widespread reef fishes.

2. Methods

2.1. Sample collection and storage

Naso brevirostris and *N. unicornis* samples were collected at various locations in the tropical Indo-Pacific across their species distributions between March 2000 and February 2004 (Fig. 1). The majority of our samples originated at sites from the Seychelles Islands, Cocos Keeling, Christmas Island, Western Australia, the Great Barrier Reef, Solomon Islands and the Society Islands of French Polynesia. However, the *N. unicornis* data set was supplemented by a small number of samples from: Oman, Rodriguez Island, Reunion Island, Taiwan and Kimbe bay in Papua New Guinea. These supplementary samples were included only in the phylogenetic analysis and median joining haplotype network but due to the small sample size, these samples were excluded from population level analyses. Samples were either captured in the field by spearing or purchased at local fish markets. Fin clippings were stored in 80% ethanol or in salt-saturated dimethyl sulfoxide. One hundred and two *N. brevirostris* and 107 *N. unicornis* individuals were included in the final analysis.

2.2. Laboratory procedures

Total genomic DNA was extracted from fin clippings via proteinase K digestion followed by a standard salt-chloroform, DNA precipitation (Sambrook et al. 1989). The non-coding mitochondrial control region was PCR amplified using *Naso* specific, NA1 primers: **NA1-F** 5′-AGC ATT CTG AAC TAA ACT AC-3′ and **NA1-R** 5′-TGT CCC TTG ACT CTC AAT A-3′ (Klanten et al., 2007). DNA was amplified in 20 μL PCR reactions containing 2.5 mM Tris–Cl (pH 8.7), 5 mM KCL, 5 mM $(NH_4)_2SO_4$, 200 μM each dNTP, 3.5 mM $MgCl_2$, 10 μM each primer, 1 U of Taq Polymerase (Qiagen Ltd.) and 10 ng of template DNA. Thermocycling was carried out with an initial denaturation of 94 °C for 2 min, 35 cycles of denaturation, annealing and extension (94 °C for 30 s, 50 °C for 30 s, 72 °C for 90 s) and a final extension of 72°C for 10 min. PCR products were confirmed by gel electrophoresis on 1.5% agarose gels and purified by standard isopropanol purification. PCR products were sequenced with the NA1 forward primer using ABI (Applied Biosystems Incorporated) technologies at Macrogen sequencing service Seoul, South Korea. Sequences from this study can be obtained from GenBank Accession Nos. FJ216727-FJ216935.

2.3. Phylogenetic analysis

Sequences were first aligned using a Clustal W alignment (Higgins et al., 1994) implemented in BioEdit version 7.0.9.0 (Hall, 1999). Sequences were then further aligned and edited visually in BioEdit. Appropriate substitution models, chosen according to log likelihood score, were selected for both species using mrModeltest, version 2.2 (Nylander, 2004) in PAUP* (Swofford, 1999). PAUP* was also used for maximum likelihood analysis. Further, phylogenetic analysis was carried out in MrBayes, version 3.1 (Huelsenbeck and Ronquist, 2001). A Markov chain Monte Carlo (MCMC) with four chains for one million generations was performed, recording trees once every 100 generations. The first 26,000 and 170,000 trees were discarded as post-burn-ins for *N. brevirostris* and *N. unicornis*, respectively, when stationary was reached. *Naso unicornis* trees were rooted with *N. brevirostris* as out group and *N. brevirostris* trees were rooted with *N. vlamingii* as out group. Trees were sorted according to likelihood scores and majority rule consensus trees, for each species, were generated from the best 500 trees.