# Comparative phylogeography of four Indo-Pacific moray eel species (Muraenidae) reveals comparable ocean-wide genetic connectivity despite five-fold differences in available adult habitat

**Joshua S. Reece[1,4,*], Brian W. Bowen[2], David G. Smith[3], Allan Larson[1]**

[1]**Washington University in St. Louis, Department of Biology, Campus Box 1137, One Brookings Drive, St. Louis, Missouri 63130, USA**

[2]**Hawaii Institute of Marine Biology, PO Box 1346, Kaneohe, Hawaii 96744, USA**

[3]**Smithsonian Institution, Museum Support Center MRC 534, 4210 Silver Hill Road, Suitland, Maryland 20746, USA**

[4]*Present address:* **University of California at Santa Cruz, 100 Schaffer Road, Santa Cruz, California 95062, USA**

ABSTRACT: Most coral reef fishes have non-migratory adults and depend on a pelagic larval stage for dispersal. Species with long pelagic larval duration (PLD) can have tremendous dispersal potential and thus display little geographic-genetic differentiation among reef habitats. Restricted adult niche breadth due to habitat specialization can have the opposite effect of fragmenting populations and increasing geographic-genetic differentiation. If long PLD suffices to ensure widespread gene flow among reef populations, we predict similar geographic-genetic homogeneity within species whose adults differ in niche breadth. We tested this hypothesis using a comparative phylogeographic study of 4 sympatric moray eel species that differ in the amount of available habitat within their reported ranges. We generated molecular genetic data for *Echidna nebulosa* (N = 79) and *Gymnomuraena zebra* (N = 67) to measure geographic-genetic structure within these species, whose adult habitat is very restricted for moray eels, and compared these results to identical measurements previously published for habitat generalists *Gymnothorax undulatus* and *Gymnothorax flavimarginatus*. These 4 species share an ocean-wide distribution with adults occupying the same reefs; however, adults of *E. nebulosa* and *G. zebra* are restricted to shallow waters and occupy only 20% of the area occupied by the *Gymnothorax* species. Mitochondrial (632 bp of cytochrome *b* and 596 bp of cytochrome oxidase *I*) genomic sequences revealed high genetic variation ($h$ = 0.995 to 0.998) and low geographic-genetic differentiation (pairwise $\Phi_{ST} < 0.07$ and not significant) for each species across 22000 km of the Indo-Pacific. Nuclear genomic sequences (420 bp of *RAG-1* and 746 bp of *RAG-2*) demonstrated 16 to 25 haplotypes per marker within each species with minimal geographic-genetic differentiation among populations. This suggests that in cosmopolitan and highly dispersive species such as morays, larval life history can ensure widespread gene flow despite a 5-fold difference in the habitat breadth occupied by adult populations.

KEY WORDS: Gene flow · Phylogeography · Connectivity · Reef fish · Leptocephalus · Moray eel

## INTRODUCTION

Many marine organisms disperse exclusively through a pelagic larval stage (Thresher 1984) and the pelagic larval duration (PLD) influences the dispersal ability of these species (Waples 1987, Doherty et al. 1995, Bohonak 1999). Weersing & Toonen (2009) demonstrated that species with very long PLD maintain greater geographic-genetic homogeneity among populations than do species having very short

PLDs. Adult niche breadth can also influence the genetic differentiation among locations within species (Blanchet et al. 2010). It is unclear whether high dispersal ability in reef fishes erases effects of adult habitat fragmentation on geographic-genetic structuring within species. We are unaware of any prior study that directly tests the hypothesis that high pelagic larval dispersal overcomes differences in adult niche breadth to ensure comparable genetic homogeneity among populations in codistributed species of reef fishes.

We used 4 species of moray eel to examine the effect of adult niche breadth on species with high PLD. These species provide a phylogenetically controlled study system of species with similar life histories, a sedentary adult stage, and 2 species each with broad and narrow niche preferences as adults. All 4 species have the thin transparent leptocephalus larvae characteristic of the superorder Elopomorpha, and overlapping geographic ranges that span the entire tropical Indo-Pacific.

Adults of *Gymnothorax undulatus* and *Gymnothorax flavimarginatus* are habitat generalists that occupy reefs and rocky substrates from depths of 1 to 150 m, and feeding generalists that consume fishes, cephalopods and crustaceans (Harmelin-Vivien 1979, Yukihira et al. 1994). *Echidna nebulosa* and *Gymnomuraena zebra* are habitat specialists relative to other morays; they occupy only the shallow portions of the *Gymnothorax* species' range, between 0 and 15 m (but usually <2 m). They are specialist feeders on crustaceans, a small subset of the *Gymnothorax* diet (Hiatt & Strasburg 1960, Yukihira et al. 1994). Detailed habitat data are not available for these or many other coral reef fishes; however, the depth of reef habitat used by a species (Sampayo et al. 2007, Chatfield et al. 2010) and diet (Gaughan & Potter 1997, Scharf et al. 2000, Mehta 2009) are major dimensions by which adult niche breadth varies among coral reef fishes. For both measures, the 2 generalist *Gymnothorax* species have similar niche breadth, and the specialists *E. nebulosa* and *G. zebra* occupy a subset of the *Gymnothorax* niches. Adults of all 4 species are highly philopatric to a small portion of reef habitat; they spawn near that habitat (Moyer & Zaiser 1982, Ferraris 1985) and dispersal occurs entirely through leptocephali. The leptocephali of many species are widely distributed in the ocean and are thought to promote high levels of dispersal through long PLDs and possibly their swimming abilities (Smith 1989, McCleave & Miller 1994, Kuroki et al. 2006, Miller et al. 2006, Miller 2009).

Because *Gymnomuraena zebra* and *Echidna nebulosa* are restricted to the shallower portions of the *Gymnothorax* range, their habitat is more fragmented. Habitat fragmentation can induce genetic differentiation by decreasing gene flow in marine fishes (Deza & Anderson 2010), whereas more continuously distributed habitat promotes geographic and genetic continuity among populations. If high PLD suffices to produce high gene flow among populations, we should observe similar levels of geographic-genetic continuity within species that have similar PLDs but differ dramatically in adult niche breadth and habitat fragmentation.

*Gymnothorax undulatus* and *G. flavimarginatus* both maintain high gene flow throughout their distributions (Reece et al. 2010a). Here, we present comparative phylogeographic analyses using orthologous mitochondrial and nuclear genomic markers for 2 habitat specialists *Echidna nebulosa* and *Gymnomuraena zebra*. We estimate the distributions of each species using geographic information systems and then compare genetic diversity, population coalescence times, and geographic-genetic structure among the 4 species to test the hypothesis that niche breadth/habitat specialization can influence phylogeographic structure despite long PLDs.

## MATERIALS AND METHODS

**Estimating niche breadth using habitat depth.** We used records for museum specimens, field guides, and extensive field sampling to estimate habitat utilization by depth for each species (see Table S1 in the supplement at www.int-res.com/articles/suppl/m437p269_supp.pdf). We examined depth information for 214 individuals of *Gymnothorax flavimarginatus*, 166 individuals of *G. undulatus*, 77 individuals of *Gymnomuraena zebra*, and 163 individuals of *Echidna nebulosa*. The online resource FishBase (www.fishbase.org) and our own collections provided specimen locality and depth data. Museum specimens of moray eels are biased toward small individuals collected in shallow waters by rotenone and dip nets. Maximum depths are thus a much better indication of the depth range of a species. We recorded mean and maximum depths from FishBase and confirmed depths directly from specimen tags in the Smithsonian (National Museum of Natural History, Washington, DC) ichthyology collection. We used the depths occupied by a species to generate areas of suitable habitat by depth as a quantitative measure of niche (habitat) breadth using ArcGIS v.9 and

bathymetric data (database ETOPO1) from the National Oceanic and Atmospheric Association (NOAA) National Geophysical Data Center (www.ngdc.noaa.gov). ETOPO1 data were imported into ArcGIS and rasterized (this translates pixels of an image into a grid of depth information). We binned topographic data into 3 elevation classes: −1 to −15 m, −15 to −150 m, and −1 to −150 m. Range data for each species from FishBase include locality data from multiple collections. Within the polygon encompassing the sympatric ranges of all 4 species, we computed the area in $m^2$ for each depth class. All areas between −1 and −15 m altitude were included for *Echidna nebulosa* and *Gymnomuraena zebra*. All areas between −1 and −150 m were calculated for the 2 *Gymnothorax* species. We express the portion of habitat used by the habitat specialists as a percentage of the total habitat used by the generalist *Gymnothorax* species. Although exact dimensions of adult subpopulations and relative areas occupied undoubtedly changed with global climatic cycles (Reece et al. 2010a), we assume that the generalist *Gymnothorax* species consistently occupied larger portions of the reefs than did the specialists *E. nebulosa* and *G. zebra*.

We assume that PLD does not differ substantially among the 4 species. Morays have among the longest PLDs of coral reef fishes, and reef species with PLDs longer than 45 d almost universally have ocean-wide distributions (Brothers & Thresher 1985, Klanten et al. 2007, Horne et al. 2008, Gaither et al. 2010, Reece et al. 2010a). Our inferences apply only to coral reef fishes that disperse strictly through a pelagic larval phase, and not to species with both long PLDs and migratory adults. PLDs are estimated from otoliths, which contain ringed increments deposited daily in most larval fishes. These rings indicate PLD when counted at time of recruitment to adult habitat, just prior to metamorphosis (see Kuroki et al. 2006). An estimate of PLD for *Echidna nebulosa* of 80 d (Brothers & Thresher 1985) is from a juvenile adult. We examined otoliths from 2 juvenile individuals of each species obtained from the pet trade to make similar estimates. We followed Kuroki et al. (2006) and identified between 60 and 80 distinct rings prior to metamorphosis for both specimens for all 4 species. Prior estimates for morays indicate minimum values of 40 to 80 d (Brothers & Thresher 1985, Smith 1989, Bishop et al. 2000), and even longer PLDs among other anguilliform leptocephali (see review in Miller 2009). Moray larvae occur at high abundance in offshore currents, indicating long PLDs (Leis & Miller 1976, Miller et al. 2006, Miller & McCleave 2007). We

assume that, while difficult to estimate precisely, the PLDs of the 4 species in question are well beyond the 15 to 30 d average for most reef fishes (Lester & Ruttenberg 2005).

**Population genetic analyses.** We obtained tissues through spear fishing, baited lobster traps, museum collections, local fishermen, and the pet trade when reliable records were available for DNA analysis of *Echidna nebulosa* (N = 79) and *Gymnomuraena zebra* (N = 67). Depth data was recorded for all sampled specimens, which were included in the analysis of depth range as described above. Each specimen was identified to a sampling location in one of 11 geographic localities distributed across 3 biogeographic regions: the Indian Ocean, western/central Pacific, and eastern Pacific Ocean. The Sunda Shelf separates the Indian Ocean from the western/central Pacific, and the Eastern Pacific Barrier separates the western/central Pacific from the eastern Pacific (Fig. 1).

In the present study, DNA isolation and sequence generation for *Echidna nebulosa* and *Gymnomuraena zebra* followed previously published methods used for *Gymnothorax undulatus* and *G. flavimarginatus* to provide comparable measurements of population genetic structure (Reece et al. 2010a). We sampled portions of 2 mitochondrial and 2 nuclear (*RAG-1* and *RAG-2*) markers and tested these genetic data for geographic-genetic structure following the methods of Reece et al. (2010a), including reconstruction of haplotype networks in the program TCS v.1.2.1 (Clement et al. 2000), estimates of haplotype and nucleotide diversity in DNAsp v.5.0 (Rozas et al. 2003), pairwise $\Phi_{ST}$ and an Analysis of Molecular Variance (AMOVA) completed in Arlequin v.3.1.1 (Excoffier & Schneider 2005). For AMOVAs, we compared individuals grouped by geographic locality and biogeographic region with all locations combined. Pairwise $\Phi_{ST}$ comparisons included all localities with more than 5 specimens for mtDNA, *RAG-1*, and *RAG-2*, with a B-H (Benjamini & Hochberg 1995) correction for multiple comparisons. We tested for large-scale patterns of isolation by distance using Mantel tests to compare a matrix of pairwise $\Phi_{ST}$ values with a matrix of linear geographic distances between sites in the program Passage v.2.0 (Rosenberg & Anderson 2011).

To ensure comparability of phylogeographic structure among species, we estimated demographic processes and coalescence times for the observed genetic variation. We inferred the history of demographic expansion/contraction using Fu's $F_S$ (Fu 1997) and mismatch distributions of mtDNA. We used 2 metrics to test for the fit of observed data to a

unimodal mismatch distribution that represents a
sudden expansion model (Rogers 1995): (1) Harpend-
ing's Raggedness Index (Harpending 1994), which
examines the maximum number of observed differ-
ences between haplotypes under an expectation of
an expanding population, and (2) the sum of squared
deviations  (Rogers & Harpending 1992), a paramet-
ric bootstrap approach that computes confidence
intervals around an expected unimodal distribution
and tests to see if observed mismatched distributions
fall within or outside those confidence intervals at $\alpha$ =
0.05 (Schneider & Excoffier 1999).

We estimated coalescence times for genetic varia-
tion within *Echidna nebulosa* and *Gymnomuraena
zebra* separately in the program BEAST v.1.5.4
(Drummond & Rambaut 2007). Estimates of coales-
cence times for the 2 *Gymnothorax* species, based
on mutation rates estimated for all 4 markers ana-
lyzed in this study, had been calculated using the
same methods in the previous study (Reece et al.
2010a).

## RESULTS

### Depth range and dispersal capabilities

Depths recorded for species used in this study are
presented in Table S1 in the supplement (www.int-
res.com/articles/suppl/m437p269_supp.pdf). *Gymno-
thorax flavimarginatus* is captured consistently be-
tween 1 and 150 m below sea level (Myers 1991), and
the average depth for specimens examined and cap-
tured for this study was 22 m, with a maximum depth
of 150 m (N = 214). *Gymnothorax undulatus* is also
consistently observed between 1 and 150 m depth
(Mundy 2005, Randall 2007), and we observed mean
and maximum depths of 24 and 150 m depth, respec-
tively (N = 166). *Gymnomuraena zebra* is typically
observed between 0 and 15 m (Sommer et al. 1996,
Mundy 2005), and the average of depth records
examined for this study was 1.9 m, with a maximum
of 15 m (N = 77). The reported depth range for
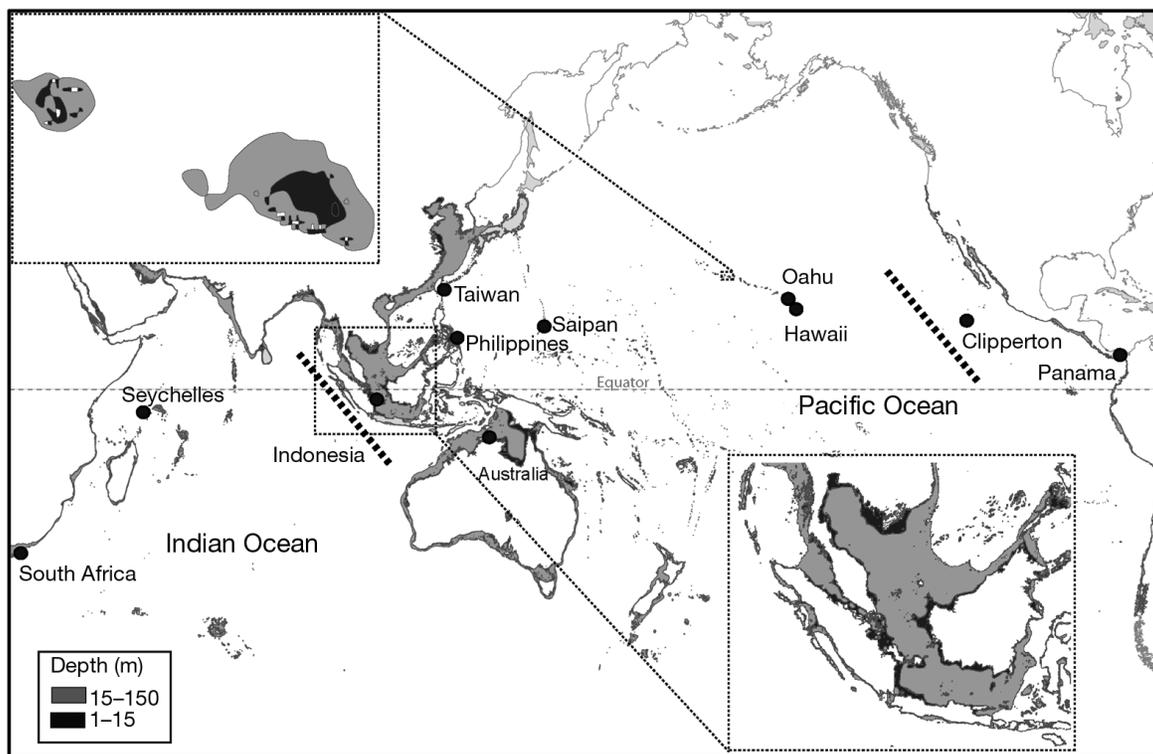*Echidna nebulosa* is also between 1 and 15 m (Kuiter



Fig. 1. Map of sampling localities and potential habitat by depth. Habitat depths are given in grey (15 to 150 m) and black (1 to
15 m) in the geographic range reported for the 4 species (see 'Materials and methods'). The 2 broad-niche species *Gymnotho-
rax undulatus* and *G. flavimarginatus* potentially occupy all areas shaded in grey and black, whereas the 2 narrow-niche spe-
cies *Echidna nebulosa* and *Gymnomuraena zebra* are restricted to black-shaded areas. The top left inset shows the seamount
habitat at Maro reef in the northwestern Hawaiian Islands (sampled in Reece et al. 2010a); the center of the seamount is shallow
habitat in black, surrounded by deeper habitat in grey. The lower right inset shows a continental shelf habitat in a portion of
the Coral Triangle; land is white and bordered by shallow coastal habitat in black, with deeper offshore habitat in grey. Thick
dotted lines represent major biogeographic barriers, the Sunda Shelf to the west and the Eastern Pacific Barrier to the east

Fig. 2. Mitochondrial DNA haplotype networks for *Echidna nebulosa*, *Gymnomuraena zebra*, *Gymnothorax flavimarginatus*, and *Gymnothorax undulatus*. Squares represent observed haplotypes inferred to be ancestral to all other observed haplotypes; circles denote additional observed haplotypes, and small black-filled circles denote unsampled haplotypes inferred to connect observed haplotypes. The size of the circle or square is proportional to the number of individuals sampled for networks within each species; sizes of entire networks between species have been scaled for presentation. Pie-chart shading denotes the 3 oceanic regions delimited by the Sunda Shelf and Eastern Pacific Barrier (Fig. 1): Indian Ocean (black), west/central Pacific (white), and eastern Pacific Ocean (grey)



*Echidna nebulosa*



*Gymnothorax flavimarginatus*



*Gymnomuraena zebra*



*Gymnothorax undulatus*
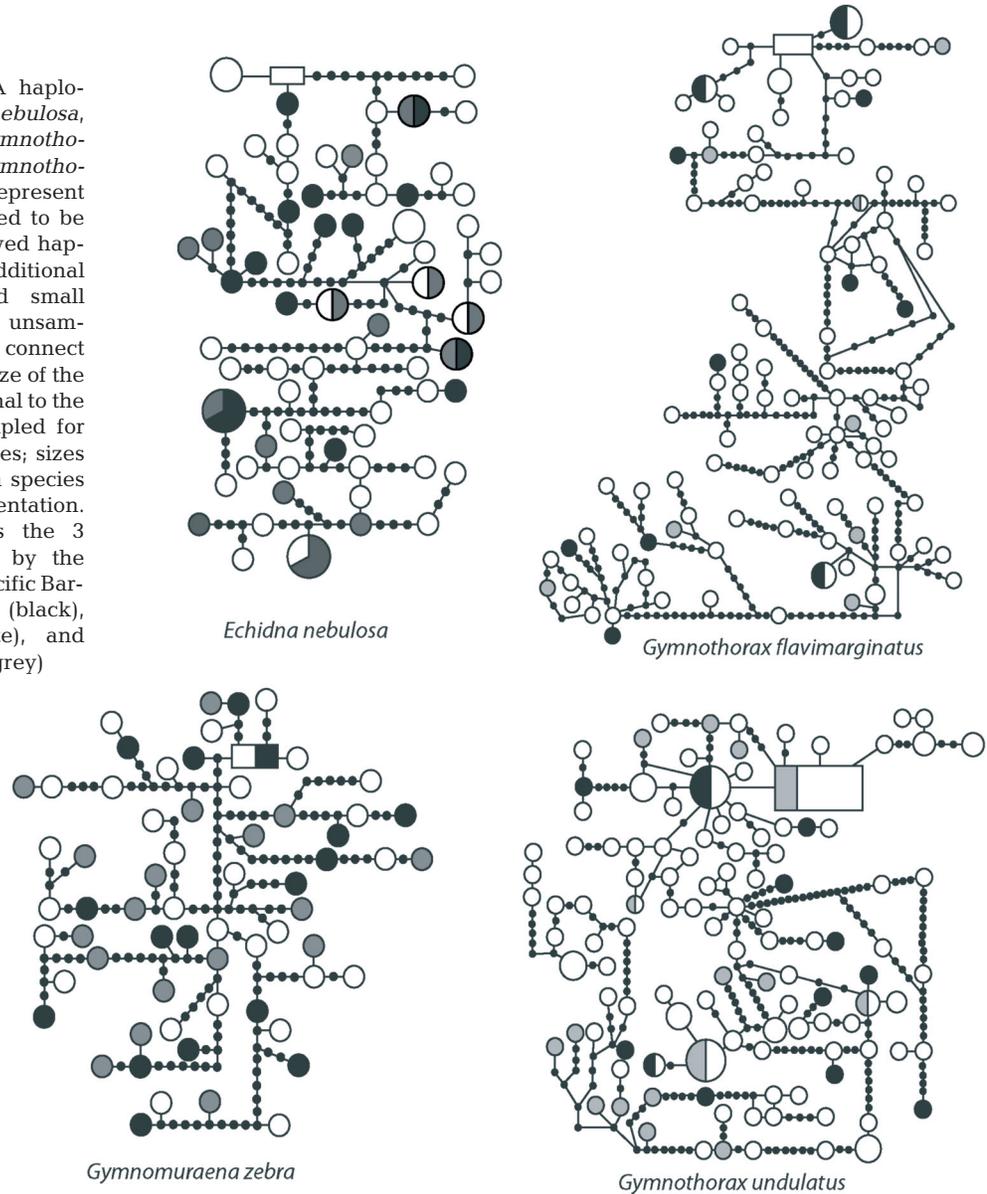
1998, Mundy 2005), and the average depth record was 1.8 m with a maximum of 15 m (N = 163). There is an account of *G. zebra* at 40 m (e.g. Randall 2007); however, this species primarily inhabits shallow waters. We report maximum depth approximations because means reflect collectors' biases more than distributions of individuals.

Within the range of all 4 species, ArcGIS analyses of the bathymetric landscape estimated 650 000 m² of suitable habitat by depth between −1 and −15 m, and $3.25 \times 10^6$ m² of habitat between −1 and −150 m (Fig. 1). Therefore *Echidna nebulosa* and *Gymnomuraena zebra* occupy approximately 20% of the habitat available to the *Gymnothorax* species. Given the tendency of both specialists to inhabit areas of <2 m depth, this is probably an overestimate.

## DNA sequence data

For *Echidna nebulosa* and *Gymnomuraena zebra*, we present new DNA sequences for portions of 2 mitochondrial (632 bp of cytochrome *b* and 596 bp of cytochrome oxidase *I*; treated as a single locus) and 2 nuclear (420 bp of *RAG-1* and 746 bp of *RAG-2*) markers for a total of 2394 bp of DNA. GenBank accession numbers for unique sequences generated in this study are JN008945–JN009084 for *E. nebulosa* and JN020361–JN020488 for *G. zebra*. These data were compared with the previously obtained data for the 2 *Gymnothorax* species (Reece et al. 2010a). Haplotype networks of all 4 species (Figs. 2 & 3) show that genetic relatedness among haplotypes lacks geographic pattern; closely related haplotypes were
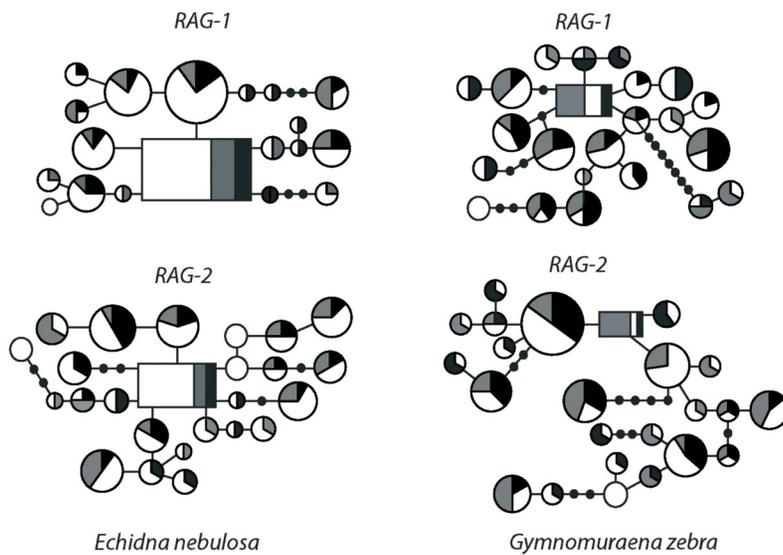
Fig. 3. Nuclear *RAG-1* and *RAG-2* haplotype networks for *Echidna nebulosa* and *Gymnomuraena zebra*. Squares represent inferred ancestral haplotypes; circles are additional haplotypes, and small black-filled circles are missing haplotypes. The size of the circle or square is proportional to the number of individuals sampled for networks within each species. Pie-chart shading denotes the 3 oceanic regions delimited by the Sunda Shelf and Eastern Pacific Barrier (Fig. 1): Indian Ocean (black), west/central Pacific (white), and eastern Pacific Ocean (grey)

observed in disparate geographic regions, indicating low geographic-genetic structure. Table 1 shows the numbers of alleles for each marker and each species, and mtDNA haplotype and nucleotide diversity.

*Echidna nebulosa* and *Gymnomuraena zebra* show evidence of species-wide population growth similar to the 2 *Gymnothorax* species (Table 1), in the form of significantly negative Fu's $F_S$ and mismatched distributions. Estimates of coalescence times for *E. nebulosa* and *G. zebra* from BEAST indicate that geographic patterns of genetic diversity in both species evolved within the last 2.2 to 9.1 million years (Myr) (95% PPD; i.e. the 95% posterior probability distribution, equivalent to a 95% confidence interval surrounding the most probable estimate) and broadly overlap with those reported for the 2 *Gymnothorax* species

(Table 1); this confirms the temporal and demographic comparability of geographic-genetic structure across all 4 species. Pairwise $\Phi_{ST}$ comparisons across locations of mtDNA and both nuclear loci for all 4 species showed no significant differentiation, indicating high gene flow throughout the species' ranges (mtDNA results for *E. nebulosa* and *G. zebra* are presented in Table 2). The AMOVA results demonstrate that >95% of genetic variation in each of the 4 species occurs within populations, indicating little to no geographic population structure. The amount of total genetic variation explained by oceanic regions delimited by the Sunda Shelf and Eastern Pacific Barrier ($\Phi_{CT}$) is 0.3 and 0.1% of the variation for *G. zebra* and *E. nebulosa*, respectively, not significantly different from zero and nearly identical to results from the 2 *Gym-*

Table 1. Results of tests of genetic data from 4 species of moray eel showing the number of individuals sampled, the number of alleles (mtDNA, *RAG-1*, *RAG-2*), mtDNA haplotype (*h*) and nucleotide (π) diversity, coalescence time for standing variation (TMRCA: time to most recent common ancestry) based on all 3 gene regions, portion of genetic variance among biogeographic regions ($\Phi_{CT}$) based on mtDNA, and evidence of demographic expansion in the form of Fu's $F_S$ for mtDNA (significant $\Phi_{CT}$ and $F_S$ values in **bold**). The mismatch distributions' p-values are the result of the sum of squared deviations tests and demonstrate (all p-values > 0.05) that observed mismatched distributions fall within the 95% confidence intervals of data generated under a model of population growth. The values for the Harpending's Raggedness Index (HRI) are given, and are all non-significant at α = 0.05, also indicating that observed data conform to a model of population growth. Mitochondrial DNA was the only marker variable enough to be informative for AMOVA, Fu's $F_S$ and mismatch distributions, but *RAG-1* and *RAG-2* showed no significant structure. A significant negative $F_S$ indicates that the null hypothesis of constant population size is rejected in favor of demographic expansion, while non-significant p-values from mismatch distributions are consistent with a null hypothesis of demographic expansion. Data for *Gymnothorax undulatus* and *G. flavimarginatus* are from Reece et al. (2010a)

| Species | N | No. alleles | *h* | π | TMRCA (Myr) | $\Phi_{CT}$ | $F_S$ | Mismatch distributions p | HRI |
|---|---|---|---|---|---|---|---|---|---|
| *Gymnothorax undulatus* | 165 | 112, 23, 22 | 0.997 | 0.012 | 3.5–8.6 | **0.039** | **−24** | 0.42 | 0.20 |
| *Gymnothorax flavimarginatus* | 124 | 104, 16, 18 | 0.997 | 0.012 | 1.7–3.0 | 0.009 | **−24** | 0.78 | 0.10 |
| *Gymnomuraena zebra* | 67 | 64, 23, 24 | 0.998 | 0.012 | 2.2–5.3 | 0.003 | **−57** | 0.77 | 0.002 |
| *Echidna nebulosa* | 79 | 67, 19, 25 | 0.995 | 0.014 | 4.2–9.1 | 0.001 | **−45** | 0.68 | 0.002 |

Table 2. Pairwise $\Phi_{ST}$ values across geographical localities for *Gymnomuraena zebra* (below diagonal) and *Echidna nebulosa* (above diagonal) for mtDNA. No comparison was significant with or without the Benjamini-Hochberg (B-H) correction for multiple comparisons, indicating no population structure. No pairwise comparison was significant in either of the nuclear markers for either species (data not shown). Negative values have no biological interpretation and are statistically indistinguishable from zero. Blank spaces correspond to localities not shared between the 2 species. The 2 columns on the right represent sample sizes by locality for *E. nebulosa* (Ene, n = 79) and *G. zebra* (Gze, n = 67)

| | Clipperton | Panama | Hawaii | Oahu | Australia | Saipan | Indonesia | Philippines | Taiwan | S. Africa | Ene | Gze |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Clipperton | | −0.39 | −0.50 | −0.41 | −0.43 | −0.58 | −0.49 | −0.31 | −0.38 | −0.45 | 1 | 4 |
| Panama | −0.03 | | −0.03 | −0.02 | 0.00 | −0.05 | −0.05 | −0.01 | −0.04 | −0.02 | 15 | 14 |
| Hawaii | 0.01 | −0.06 | | −0.03 | −0.10 | −0.13 | −0.05 | 0.02 | −0.03 | −0.05 | 9 | 10 |
| Oahu | | | | | −0.03 | −0.06 | −0.06 | −0.06 | −0.07 | −0.04 | 6 | 0 |
| Australia | | | | | | −0.16 | −0.01 | 0.04 | 0.00 | −0.06 | 5 | 0 |
| Saipan | | | | | | | −0.10 | −0.01 | −0.06 | −0.09 | 5 | 0 |
| Indonesia | −0.16 | 0.01 | 0.03 | | | | | −0.02 | −0.08 | −0.05 | 7 | 5 |
| Philippines | −0.07 | −0.01 | 0.00 | | | | −0.04 | | 0.01 | −0.01 | 10 | 13 |
| Taiwan | | | | | | | | −0.08 | | −0.07 | 5 | 2 |
| S. Africa | −0.05 | −0.05 | −0.04 | | | | −0.02 | −0.04 | | | 15 | 14 |
| Seychelles | 0.04 | 0.02 | −0.02 | | | | 0.01 | 0.07 | | 0.03 | 0 | 5 |

*nothorax* species (Table 1). Tests for isolation by distance revealed no significant association between geographic and genetic distances based on a Mantel test (all $R < 0.001$, p-values > 0.3).

Phylogenetic analyses indicate that the 4 species compared in this study have evolved independently for at least 20 Myr (Reece et al. 2010b). Our comparisons of geographic-genetic structuring within these species should be free of any phylogenetic artifacts because the phylogenetic separation among these species greatly exceeds estimates of coalescence times within species.

## DISCUSSION

In *Gymnomuraena zebra* and *Echidna nebulosa*, the distribution of genetic diversity and the relationships among haplotypes reveal almost no geographic structure among ocean basins for either the mitochondrial or nuclear markers (Figs. 2 & 3). Geographic-genetic homogeneity seems even greater for these 2 shallow-water species than for the habitat generalists *Gymnothorax undulatus* and *Gymnothorax flavimarginatus*. As summarized by AMOVA, despite high variation at all loci (Table 1) the total proportion of genetic variation that exists as differences among oceanic regions is <0.4% for *G. zebra* and *E. nebulosa*, compared to 4 and 1% in *G. undulatus* and *G. flavimarginatus*, respectively. Minimum coalescence times estimated for genetic variation within *E. nebulosa* (4.2 Myr) and *G. zebra* (2.2 Myr) span multiple climatic cycles in which the Sunda Shelf would have emerged as a land barrier separat-

ing the Indian Ocean from the Pacific Ocean (Voris 2000), providing ample opportunities for developing geographic structure, yet none was observed. None of the 4 species showed significant associations between genetic and geographic distances (Table 2; see also Table 2 in Reece et al. 2010a) as evaluated by Mantel's tests. *Gymnothorax* species showed similar demographic estimates of population growth to those for *E. nebulosa* and *G. zebra*, nearly identical levels of haplotype and nucleotide diversity, overlapping coalescence times, and only slightly lower levels of connectivity across these barriers despite at least a 5-fold difference in adult habitat (Table 1).

Our new data confirm predictions of our prior study that the leptocephalus larva of moray eels permits ocean-wide exchange of genes among populations, because it is very unlikely that adult moray eels could move long distances through the open ocean between regions. The effectiveness of this mechanism is evident in our findings that the greater habitat fragmentation characteristic of *Echidna nebulosa* and *Gymnomuraena zebra* adults does not diminish the high levels of gene flow throughout the Indo-Pacific, or the maintenance of high levels of genetic variation for both mitochondrial and nuclear genomes. Our estimated coalescence times indicate that current haplotype variation represents at least several million years of accumulation. If the smaller (and shallower) habitat of the *E. nebulosa* and *G. zebra* adult populations had produced greater local extinction and recolonization of populations relative to the *Gymnothorax* species, *E. nebulosa* and *G. zebra* should show lower levels of variation and more recent coalescence times than the *Gymnothorax* spe-

cies. In contrast to this prediction, all 4 moray species show nearly identical levels of nuclear haplotypic variation (Table 1). For all species, the number of mitochondrial haplotypes reported closely tracks sample size (Table 1), with no indication that the species differ greatly in mtDNA variation. The wide dispersal of leptocephalus larvae appears to permit moray species to maintain similarly high levels of genetic variation despite a 5-fold difference in the amount of suitable habitat available to adult individuals. This inference assumes that all 4 species with comparable larval durations disperse similarly, and show low and similar levels of intraspecific variation in PLD and associated dispersal capabilities. Some non-moray anguilliforms show regional variation in PLD (Réveillac et al. 2008), but for the purposes of our interpretations we expect that this variation is minimal relative to the very high PLD of these species.

One potential criticism of our sampling strategy is that we might detect greater phylogeographic structure with more geographic and population sampling. We designed our sampling to investigate large-scale patterns across the Indo-Pacific. If local geographic fragmentation occurred in any of the 4 species, then it should be exaggerated at the ocean-wide scale (22 000 km) of their entire distribution. Transient, local geographic isolation likely does occur in all 4 species, but the range-wide pattern that emerges across reconstructions of each species' history is geographic homogenization of genetic structure. It is likely that geographic structure develops over ecological timescales of thousands to tens of thousands of years, and our results do not suggest that larvae disperse across oceanic basins every generation. However, the effective number of migrants between populations averaged across each species' evolutionary history appears to be sufficient to erase geographic-genetic structure over timescales as short as tens to hundreds of thousands of years (Reece et al. 2010a).

Previous studies have shown that PLD correlates with dispersal only in extreme cases of very long or very short PLDs (e.g. Weersing & Toonen 2009). At intermediate PLDs (15 to 60 d), other aspects of life history, ecology, larval behavior, and niche breadth influence dispersal and population genetic structure (Rocha et al. 2002, Gaither et al. 2010). We show in a comparative framework that in broadly distributed species, high PLDs can indeed supersede differences in habitat and ecological specialization and permit ocean-wide genetic homogeneity. Both specialist and generalist moray eels maintain connectivity across the entire Indo-Pacific, the largest tropical habitat on earth.

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