Phylogeography of the olive sea snake, *Aipysurus laevis* (Hydrophiinae) indicates Pleistocene range expansion around northern Australia but low contemporary gene flow

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Abstract

Pleistocene sea-level fluctuations profoundly changed landmass configurations around northern Australia. The cyclic emergence of the Torres Strait land bridge and concomitant shifts in the distribution of shallow-water marine habitats repeatedly sundered east and west coast populations. These biogeographical perturbations invoke three possible scenarios regarding the directions of interglacial range expansion: west to east, east to west, or bidirectional. We evaluated these scenarios for the olive sea snake, *Aipysurus laevis*, by exploring its genetic structure around northern Australia based on 354 individuals from 14 locations in three regions (Western Australia, WA; Gulf of Carpentaria, GoC; Great Barrier Reef, GBR). A 726-bp fragment of the mitochondrial DNA ND4 region revealed 41 variable sites and 38 haplotypes, with no shared haplotypes among the three regions. Population genetic structure was strong overall, $\Phi_{ST} = 0.78$, $P < 0.001$, and coalescent analyses revealed no migration between regions. Genetic diversity was low in the GBR and GoC and the genetic signatures of these regions indicated range or population expansions consistent with their recent marine transgressions around 7000 years ago. By contrast, genetic diversity on most WA reefs was higher and there were no signals of recent expansion events on these reefs. Phylogenetic analyses indicated that GBR and GoC haplotypes were derived from WA haplotypes; however, statistical parsimony suggested that recent range expansion in the GBR-GoC probably occurred from east coast populations, possibly in the Coral Sea. Levels of contemporary female-mediated gene flow varied within regions and reflected potential connectivity among populations afforded by the different regional habitat types.

Keywords: *Aipysurus*, mitochondrial DNA, phylogeography, Pleistocene, population genetics, sea snake

Received 19 January 2007; revision accepted 13 April 2007

Introduction

Palaeoclimatic events during the Pleistocene epoch have profoundly influenced species’ distributions (Webb & Bartlein 1992) and have had genetic consequences for species and populations (Hewitt 2000). Over the past two million years, at least 10 major glaciation cycles have given rise to prolonged periods of global cooling, interrupted by warmer conditions similar to present-day climates (Hays et al. 1976). In the marine realm, Quaternary sea-level fluctuations and the associated cyclic emergence and subsidence of land bridges, repeatedly sundered and rejoined populations resulting in cyclic range contractions and expansions, local extinctions of species and populations, and influenced population genetic patterns and processes (Hewitt 2000).

Northern Australia is surrounded by extensive shallow-water marine habitats that stretch over its vast continental shelf. Consequently, Pleistocene sea-level fluctuations resulted in extreme shifts in the locations of coastlines and changed levels of connectivity among populations of shallow-water marine species. During glacial maxima, when sea levels were more than 100 m below present...
levels (BPL), Australia’s continental shelf was exposed (Fig. 1, Inset A). Northern Australia and New Guinea were connected by the massive Torres Strait land bridge (Voris 2000); the Great Barrier Reef (GBR) did not exist (Davies 1994); and the region that is now the Gulf of Carpentaria (GoC) was dry until a fresh water or brackish lake, Lake Carpentaria, formed at sea levels around 75 m BPL (Torgersen et al. 1985). As sea levels rose, the Torres Strait land bridge gradually narrowed; however, it still connected Australia and New Guinea at sea levels just 10 m BPL (Voris 2000) and separated east and west coast marine habitats until around 7000 years ago when sea levels reached present levels. At this time, the east coast continental shelf was also transgressed and the current manifestation of the GBR formed (Davies 1994). Ashmore, Scott and other reefs that lie at the edge of the continental shelf in the Timor Sea, and reefs on the Marion and Queensland plateaus in the Coral Sea (Fig. 1), persisted during glacial maxima then became increasingly isolated as sea levels rose.

The cyclic emergence and marine transgression of Australia’s continental shelf is predicted to have left strong imprints in the genetic signatures of tropical marine species around northern Australia, particularly those reliant on coastal or shallow-water marine habitats (Chenoweth et al. 1998b). Vicariant events and population bottlenecks during lowered sea stands, followed by range expansions and demographic growth, are expected to have left congruent genetic signatures among co-occurring marine species (Avise et al. 1987; Avise 2000). Documented patterns of population genetic structure around northern Australia have revealed varying degrees of genetic divergence congruent with vicariance between eastern and western Australian marine populations for invertebrate (Benzie et al. 1992, 2002; Benzie & Stoddart 1992; Williams & Benzie 1997, 1998; Benzie 1999; Gopurenko & Hughes 2002) and vertebrate species (Keenan 1994; Norman et al. 1994; Chenoweth et al. 1998a,b).

Despite this broadly congruent pattern of east–west divergence, these studies also revealed divergent patterns...
of genetic diversity and population structure. For example, decreasing genetic diversity from east to west around northern Australia for the crown-of-thorns starfish (*Acanthaster planci*, Benzie 1999) and the tiger prawn (*Penaeus monodon*, Benzie et al. 1992, 2002), and strong affinities between west coast and Pacific Ocean populations (compared with Indian Ocean populations) for the parrotfish (*Chlorurus sordidus*, Bay et al. 2004) and the holothurian (*Holothuria nobilis*, Uthicke & Benzie 2003) suggest that populations in the west were established by dispersal from the east. In contrast, strong genetic affinities between green turtle, *Chelonia mydas*, rookeries in the GoC and the Indian Ocean, and large differences between GoC and east coast rookeries, suggest that GoC rookeries were established from Indian Ocean populations (Norman et al. 1994). Alternatively, the intraspecific phylogeny and pattern of genetic diversity (highest in the GoC) of the estuarine barramundi, *Lates calcarifer*, suggested vicariance and subsequent second intergradation between east and west coast populations (Chenoweth et al. 1998a,b). The reasons for these diverse responses of species to a shared biogeographical history are unclear but may be related to taxon-specific dispersal potentials or life-history traits (e.g. Doherty et al. 1995; Ayre & Hughes 2000) and/or divergent responses to changing water temperatures or current flows associated with eustasy (Reid et al. 2006). However, they may also reflect the effects of stochastic forces on marine populations or species (Palumbi 1996).

Irrespective of their underlying causes, the documented genetic signatures can be grouped into three broad alternate biogeographical hypotheses: east to west dispersal, west to east dispersal, and bidirectional dispersal from east and west coasts. These competing hypotheses generate predictions regarding the genetic signatures of populations based on their relative ages and recent demographic histories. For example, genetic diversities are predicted to be higher in older populations, in which more time has elapsed for mutations to accumulate, or in admixtures of previously isolated populations, although high diversity may also be attributable to larger effective population sizes (Palumbi 1997). In contrast, populations that have recently undergone range or demographic expansions tend to have low nucleotide diversities and low to intermediate haplotype diversities (Grant & Bowen 1998). Moreover, phylogeographical analyses of the relationships amongst haplotypes are predicted to reveal the geographical locations of basal (old) and derived (young) populations, the effects of vicariant events (Avise et al. 1987; Avise 2000), and signals of recent population expansion, such as poorly resolved phylogenies.

Specific predictions associated with west to east dispersal therefore include Western Australian (WA) populations having basal haplotypes (possibly in several structured clades) and higher genetic diversity, whereas populations in the GoC and GBR would be characterized by low genetic diversities, derived haplotypes with poorly resolved relationships, and an excess of rare haplotypes. The reverse pattern would be expected for east to west dispersal. Patterns for bidirectional dispersal are more complex to predict: nonetheless, it is expected that the GoC would share haplotypes with (or derived from) both GBR and WA populations, and have higher genetic diversity due to admixture. The strength of this pattern will depend on the time since east and west coast lineages diverged, the subsequent degree and frequency of mixing between them, and the life-history traits of the taxa studied.

The olive sea snake, *Aipysurus laevis* (Lacepede 1804), has many characteristics that make it ideal for exploring these alternate biogeographical hypotheses. The genus *Aipysurus* comprises seven species that all occur in Australian waters. Six aipysurids are endemic to Australasian waters (including New Caledonia and New Guinea) and four are endemic to WA: only *Aipysurus eydouxii* has a range that extends into Southeast Asia (Heatwole & Cogger 1994; Heatwole 1999). *Aipysurus laevis* is the most abundant, widespread sea snake species on Australian coral reefs and also occurs over soft sediment habitats where it is sometimes captured in trawls. Its Australian range extends from Shark Bay, WA around northern Australia to the southern GBR, and includes some Timor and Coral Sea reefs (Fig. 1, Inset A). Beyond Australia, its range extends to New Caledonia, the Loyalty and Chesterfield Islands (Ineich & Laboute 2002) and the south coast of New Guinea (O’Shea 1996).

All hydrophiine sea snakes are viviparous and give birth at sea. Viviparity therefore distinguishes *A. laevis* from marine species with a dispersive larval phase (the focus of previous studies, such as those outlined above) and from marine reptiles that lay eggs on land (e.g. sea turtles), potentially allowing insights into the effects of divergent life-history strategies on population genetic structure. Hydrophiine sea snakes tend to occur in dense aggregations throughout their ranges with large discontinuities, and potentially low dispersal, among populations. Adult *A. laevis* have small home ranges, with some evidence of site fidelity (Burns & Heatwole 1998); however, movement patterns have not been well documented. Juvenile *A. laevis* are secretive during their first year of life (Zimmerman & Shohet 1994) but it is unclear whether they disperse during this period. *Aipysurus laevis* exhibits the aggregated distribution typical of hydrophiines at several spatial scales. For example, in the Swains region (Fig. 1) patchiness occurs at the level of individual reefs: *A. laevis* is abundant on some reefs, yet absent on adjacent reefs, despite reefs being in very close proximity (Lukoschek et al. 2007). At a larger scale, *A. laevis* aggregations occur at many reefs in the southern GBR, yet similar aggregations are not found on reefs in the northern or central regions (Dunson 1975). In
WA, *A. laevis* occurs at some offshore reefs in the Timor Sea and some coastal locations; however, it is notably absent at others, such as the Rowley Shoals (Fig. 1). Trawling by-catch studies also indicate a patchy distribution in the GoC (Stobutzki et al. 2000).

Pleistocene shifts in the shallow-water habitats preferred by *A. laevis*, combined with its extant patchy distribution, provide an interesting system for evaluating the relative contributions of historical Pleistocene forces and contemporary patterns of gene flow around northern Australia. A hierarchical sampling design and mitochondrial DNA sequencing were used to: (i) investigate the intraspecific phylogeny and population genetic structure of *A. laevis* throughout its range and test the three competing biogeographical hypotheses regarding the direction(s) of range expansion events as sea levels rose; (ii) evaluate the relative contributions of historical and contemporary female-mediated gene flow, over small, medium and large spatial scales, using both equilibrium and coalescent models; and (iii) test whether isolation by distance or discrete barriers to gene flow are influencing contemporary population genetic structure.

**Materials and methods**

**Sampling design and collection of tissue samples**

Samples were collected from 354 olive sea snakes, comprising 148 females, 199 males and 7 samples for which sex was not determined. The hierarchical sampling design used allowed population genetic inferences at large, medium and small spatial scales throughout most of this species’ range. Samples were obtained from a total of 14 locations in three geographical regions (Fig. 1). Sample sizes for each location ranged from 8 to 54 individuals (Table 1), with the exception of Broome (WA) where only one sample was obtained, and was therefore excluded from some analyses. One sample was also obtained from the Torres Strait (Fig. 1) but it had the most common haplotype found in the GoC and was grouped with Mornington Island (GoC) for analyses.

Tissue samples from the GBR and WA were collected from live snakes caught in fish catch bags on SCUBA or snorkel. Small tissue samples (about the size of a scale) were obtained from the flattened ventral surface of the paddle tail using a surgical biopsy punch sterilized in 100% ethanol or a sterile scalpel blade. Snakes were sexed by examining the paddle tail for presence of hemipenes and scale tubercles (males), or absence of these features (females), and subsequently released. Juveniles are rarely seen but easily distinguished from adults by their size and a strong banding pattern that has completely disappeared in adult snakes: juveniles were not sampled in this study. Previously sampled snakes were recognizable by the small notches visible on the ventral surface of the tail: these marks ensured that individuals were not resampled. Observations of sampled snakes confirmed that snakes did not suffer obvious adverse effects from this capture and biopsy sampling procedure. Tissue samples from the GoC were obtained from trawl fishery by-catch studies, conducted in 1996, 1997 and 2001 by Australia’s Commonwealth Scientific and Industrial Research Organization (CSIRO) (Stobutzki et al. 2000). Sea snakes were frozen immediately after capture and returned to CSIRO where they were identified and muscle tissue obtained for molecular analyses. Tissue samples from two individuals of each of three congeneric species also were collected as follows: *Aipysurus fuscus* from live snakes on WA reefs, and *Aipysurus daboisi* and *Aipysurus eydouxii* from the CSIRO. All tissue samples were stored at room temperature in 70% ethanol.

**DNA extraction, mitochondrial DNA amplification and sequencing**

Total genomic DNA was extracted from muscle tissue using a modified organic protocol (Lukoschek & Keogh 2006). A fragment of the mitochondrial genome (850 bp) that targeted the 3' half of the ND4 gene and most of the adjacent tRNA cluster comprising the histidine and serine tRNA genes was amplified in 40 µL polymerase chain reactions (PCR) containing 10 ng template DNA, 2 U *Taq*-polymerase (*QIAGEN*), 4 µL 10× *QIAGEN* buffer, 100 mM MgCl$_2$, 10 mM dNTPs, and 2 pmol of each primer: ND4 (L) 5′-TGACTACCAAAAGCTCATGTAGAAGC-3′ and LEU (H) 5′-TACTTTTACTGATTGACCCA-3′ (Forstner et al. 1995). PCR amplifications of double-stranded products, and gel purifications and cycle sequencing of amplicons, were performed following the protocols described in Lukoschek & Keogh (2006).

**Data analysis**

**Phylogenetic analyses.** Sequence data were edited and aligned by eye using *sequencher* 4.2.2 (Gene Codes Corporation). Three single indels occurred in the histidine and serine tRNA genes that did not complicate alignment. Following alignment, the ND4 region was translated into amino acid sequence using the vertebrate mitochondrial genetic code. No premature stop codons were observed, indicating that the sequences obtained were mitochondrial in origin and not nuclear copies. Phylogenetic analyses were conducted using parsimony, maximum-likelihood (ML) and Bayesian approaches. The data set comprised one sequence representing each unique haplotype, and phylogenies were rooted using sequences from the three congeners. The computer program *modeltest* 3.06 (Posada & Crandall 1998) selected the Tamura–Nei model (Tamura & Nei 1993) based on Akaike Information...
Table 1  Population summary statistics for Aipysurus laevis: latitude and longitude of sampling locations (Lat. – Long), sample size (n), number of haplotypes (N), haplotypic (h ± SE) and nucleotide (π ± SE (%)) diversities. Results of tests of population expansion: Fu’s FS (F̄S) and Tajima’s D (D̄) and corresponding significance levels (P̄FS and P̄D)

<table>
<thead>
<tr>
<th>Region</th>
<th>Location</th>
<th>Lat – Long</th>
<th>n</th>
<th>N</th>
<th>h ± SE</th>
<th>π ± SE (%)</th>
<th>F̄S</th>
<th>P̄FS</th>
<th>D̄</th>
<th>P̄D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Great Barrier Reef</td>
<td>Keppel Island</td>
<td>23°11'S, 150°58'E</td>
<td>48</td>
<td>5</td>
<td>0.23 ± 0.08</td>
<td>0.04 ± 0.04</td>
<td>-1.64</td>
<td>0.001</td>
<td>-1.66</td>
<td>0.012</td>
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<tr>
<td></td>
<td>21104 Turtlehead Reef</td>
<td>21°06'S, 151°15'E</td>
<td>36</td>
<td>5</td>
<td>0.30 ± 0.10</td>
<td>0.06 ± 0.06</td>
<td>-3.137</td>
<td>0.004</td>
<td>-1.49</td>
<td>0.045</td>
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<td></td>
<td>21109 Whitemtit Reef</td>
<td>21°07'S, 151°05'E</td>
<td>38</td>
<td>4</td>
<td>0.15 ± 0.07</td>
<td>0.03 ± 0.03</td>
<td>-3.791</td>
<td>&lt; 0.001</td>
<td>-1.720</td>
<td>0.011</td>
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<tr>
<td></td>
<td>21441 D-J Reef</td>
<td>21°33'S, 151°46'E</td>
<td>34</td>
<td>7</td>
<td>0.46 ± 0.10</td>
<td>0.14 ± 0.10</td>
<td>-3.673</td>
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<td>-1.630</td>
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<td>21258 Mystery Cay</td>
<td>21°23'S, 152°01'E</td>
<td>37</td>
<td>6</td>
<td>0.26 ± 0.09</td>
<td>0.04 ± 0.05</td>
<td>-5.311</td>
<td>&lt; 0.001</td>
<td>-1.75</td>
<td>0.008</td>
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<td></td>
<td>Central GBR</td>
<td>18°–20°S, 146°–148°E</td>
<td>8</td>
<td>3</td>
<td>0.71 ± 0.12</td>
<td>0.16 ± 0.13</td>
<td>0.204</td>
<td>0.467</td>
<td>1.104</td>
<td>0.846</td>
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<tr>
<td></td>
<td>Region Total</td>
<td></td>
<td>201</td>
<td>16</td>
<td>0.55 ± 0.03</td>
<td>0.12 ± 0.10</td>
<td>-10.16</td>
<td>&lt; 0.001</td>
<td>-1.85</td>
<td>0.007</td>
</tr>
<tr>
<td>Gulf of Carpentaria</td>
<td>Mornington Island</td>
<td>15–17°S, 138–140°E</td>
<td>8</td>
<td>3</td>
<td>0.61 ± 0.16</td>
<td>0.05 ± 0.05</td>
<td>-2.185</td>
<td>&lt; 0.001</td>
<td>-1.06</td>
<td>0.039</td>
</tr>
<tr>
<td></td>
<td>Vanderlin Island</td>
<td>15–16°S, 137–138°E</td>
<td>34</td>
<td>6</td>
<td>0.52 ± 9.21</td>
<td>0.06 ± 0.06</td>
<td>-5.839</td>
<td>&lt; 0.001</td>
<td>-1.75</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>Groote Eylandt</td>
<td>13–15°S, 136–137°E</td>
<td>15</td>
<td>8</td>
<td>0.73 ± 0.12</td>
<td>0.55 ± 0.33</td>
<td>-3.379</td>
<td>0.009</td>
<td>-2.12</td>
<td>0.005</td>
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<td></td>
<td>Groote Eylandt (excluding ALH17)*</td>
<td></td>
<td>14</td>
<td>7</td>
<td>0.69 ± 0.14</td>
<td>0.14 ± 0.11</td>
<td>-5.925</td>
<td>&lt; 0.001</td>
<td>-1.89</td>
<td>0.012</td>
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<tr>
<td></td>
<td>Region Total</td>
<td></td>
<td>57</td>
<td>12</td>
<td>0.58 ± 0.07</td>
<td>0.18 ± 0.13</td>
<td>-11.050</td>
<td>&lt; 0.001</td>
<td>-2.36</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Region Total (excluding ALH17)*</td>
<td></td>
<td>56</td>
<td>11</td>
<td>0.57 ± 0.07</td>
<td>0.07 ± 0.07</td>
<td>-14.010</td>
<td>&lt; 0.001</td>
<td>-2.08</td>
<td>&lt; 0.002</td>
</tr>
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<td>Western Australia</td>
<td>Ashmore Reef</td>
<td>12°15'S, 123°05'E</td>
<td>54</td>
<td>4</td>
<td>0.46 ± 0.06</td>
<td>0.44 ± 0.26</td>
<td>1.814</td>
<td>0.833</td>
<td>0.512</td>
<td>0.726</td>
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<td></td>
<td>Hibernia Reef</td>
<td>12°01'S, 123°20'E</td>
<td>9</td>
<td>4</td>
<td>0.58 ± 0.18</td>
<td>0.61 ± 0.37</td>
<td>-0.061</td>
<td>0.457</td>
<td>-0.53</td>
<td>0.347</td>
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<tr>
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<td>Cartier Islet</td>
<td>12°33'S, 123°34'E</td>
<td>9</td>
<td>3</td>
<td>0.67 ± 0.10</td>
<td>0.14 ± 0.12</td>
<td>-0.108</td>
<td>0.382</td>
<td>0.196</td>
<td>0.696</td>
</tr>
<tr>
<td></td>
<td>Scott Reef</td>
<td>14°01'S, 121°40'E</td>
<td>23</td>
<td>4</td>
<td>0.32 ± 0.12</td>
<td>0.10 ± 0.09</td>
<td>-2.269</td>
<td>0.006</td>
<td>-1.88</td>
<td>0.011</td>
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<tr>
<td></td>
<td>Broome</td>
<td>17°55'S, 122°05'E</td>
<td>1</td>
<td>1</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
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<tr>
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<td>Region Total</td>
<td></td>
<td>96</td>
<td>10</td>
<td>0.63 ± 0.03</td>
<td>0.52 ± 0.30</td>
<td>-1.117</td>
<td>0.351</td>
<td>-1.12</td>
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<tr>
<td></td>
<td>Region Total (excluding ALH38)†</td>
<td></td>
<td>95</td>
<td>9</td>
<td>0.62 ± 0.03</td>
<td>0.46 ± 0.26</td>
<td>-0.805</td>
<td>0.401</td>
<td>-0.06</td>
<td>0.537</td>
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<tr>
<td>All</td>
<td>Total</td>
<td></td>
<td>354</td>
<td>38</td>
<td>0.81 ± 0.02</td>
<td>0.59 ± 0.32</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Total (excluding ALH17 &amp; ALH38)‡</td>
<td></td>
<td>352</td>
<td>36</td>
<td>0.82 ± 0.02</td>
<td>0.49 ± 0.27</td>
<td></td>
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</tr>
</tbody>
</table>

*Values excluding Groote Eylandt individual with unusual haplotype, ALH17; †values excluding Broome individual with unusual haplotype, ALH38; ‡values excluding both unusual haplotypes, ALH17 and ALH38.

Criterion as the most appropriate model of molecular evolution for the data set. The Tamura–Nei model with the rate matrix (1.0, 19.9, 1.0, 1.0, 36.3) and proportion of invariant sites (I = 0.6933) was used in the ML analyses implemented in PAUP* (Swofford 2000). An unweighted parsimony analysis was also conducted using PAUP*.

Bayesian analyses were implemented in the computer program MrBayes version 3.0 (Huelsenbeck & Ronquist 2001) using the default value of four Markov chains per run and the same character state frequencies, proportion of invariant sites and substitution rates used for ML analyses. Five replicate analyses (each 1 000 000 generations, sampled every 100 generations, that recovered 10 000 sampled trees) were run to ensure that overall tree space was well sampled. The Markov chain reached stationarity after approximately 100 000 generations (1000 sampled trees), so the first 2000 trees were discarded as the burn-in phase and the remaining 8000 trees were used to construct a 50% majority rule consensus tree and estimate Bayesian posterior probabilities. Branch supports were evaluated using unweighted parsimony bootstrap (10 000 replicates) and ML bootstrap (500 nonparametric ML bootstrap replicates) tests, and Bayesian posterior probabilities. All analyses recovered trees with very similar topologies; thus, the ML best tree is presented with all three measures of branch support shown.

Sequence divergence (p) values were estimated among Aipysurus laevis regional phylogroups and corrected for within phylogroup diversity (following Avise & Walker 1998). Dates of divergences among regions were calculated using a conventional mitochondrial DNA (mtDNA) clock of 2% sequence divergence between a pair of lineages per million years (Brown et al. 1979). As reptilian lineages have been shown to have lower mtDNA rates of evolution, divergence times were also estimated using a rate of 0.5% sequence divergence between a pair of lineages per million years.
years, which is the lower end of the range of reptilian mtDNA rates (0.47–1.32%) estimated by Zamudio & Greene (1997).

Gene genealogies and population genetic analyses
Gene genealogies were estimated with statistical parsimony criteria in tcs 1.13 (Clement et al. 2000) and the geographical origins of sampled haplotypes were mapped onto the resulting parsimony network. Population genetic analyses were conducted using the computer program arlequin 3.01 (Excoffier et al. 2005). Levels of DNA polymorphism within locations and regions were summarized with haplotype and nucleotide diversity statistics (Nei 1987).

A hierarchical analysis of molecular variance (AMOVA) framework (Excoffier et al. 1992) was used to estimate three molecular variance components (between regions, between locations within regions, and within locations) and investigate population subdivision. AMOVA based on raw haplotype frequencies yields a statistic $F_{ST}$ analogous to Weir & Cockerham’s (1994) unbiased estimator $\theta$, which is not affected by sample size. AMOVA analyses were also conducted taking percent sequence divergence (based on the Tamura–Nei model) into account ($\phi_{ST}$). Pairwise $F_{ST}$ and $\phi_{ST}$ values were calculated for the 78 possible comparisons among the 13 locations (Broome, WA individual excluded) and the three comparisons between geographical regions. $P$ values were adjusted with sequential Bonferroni corrections for multiple comparisons (Rice 1989). Tajima’s $D$ tests (Tajima 1989a) were used to assess evidence of population expansion. Significantly, negative Tajima’s $D$ values indicate excesses of low-frequency haplotypes, generally ascribed to population expansion following a severe reduction in population size (bottleneck), whereas positive values indicate secondary contact among previously differentiated lineages (Tajima 1989b). Fu’s $F$ values were also calculated as an alternative test for population expansion (Fu 1996, 1997).

Coalescent estimation of migration and population isolation.
Migration and population divergence between adjacent populations were analysed using MDIV, a Bayesian Markov chain Monte Carlo method that jointly estimates migration rates and isolation times between pairs of populations (Nielsen & Wakeley 2001). Comparisons were conducted on two spatial scales, between regions (two comparisons) and between locations within regions (WA, six comparisons; GoC, three comparisons; GBR, three comparisons, using the four Swain reef samples pooled as one location). All data sets were analysed using the finite-sites model and analyses for each of the 14 comparisons were conducted three times to ensure that parameter space was well sampled and to avoid being trapped in local optima. The first analysis (of each set of three) comprised 2 000 000 replicates with the first 200 000 replicates discarded as burn-in, while the second and third comprised 5 000 000 replicates and the first 300 000 were discarded as burn-in. The likelihood-of-divergence times was estimated for values from 0.02 to 5 in increments of 0.02, while migration likelihood was estimated for values from 0.02 to 10 using the same increments. Estimated migration rates within the GoC appeared to be much larger than 10 from the first run; thus, the range of possible values was adjusted to 0.02–20 for the second and third runs. The parameter with the highest probability (maximum likelihood) was taken as the best estimate and the 90% credibility limits were the upper and lower values that encompassed 90% of the area under the curve of the integrated likelihood function around the maximum-likelihood estimate. Divergence times were evaluated as significantly different from zero when $\pm 2 \log L$ (where $L$ is the likelihood function) of the best estimate, did not include zero (Nielsen & Wakeley 2001). Replicate parameter estimates and credibility limits for each comparison were highly congruent and were averaged over runs.

Isolation by distance. Mantel tests of correlations between genetic and geographical distance matrices, implemented in the computer program ISOLATION BY DISTANCE WEB SERVICE (IBDWS) (Jensen et al. 2005), were used to test for significant relationships between genetic and geographical distance matrices. Reduced Major Axis (RMA) regressions (implemented in IBDWS) were used to evaluate isolation by distance (IBD) and estimate the slopes and intercepts of relationships. RMA regression has been shown to better estimate slopes in IBD analyses than ordinary least squares regression (Hellberg 1994) and is more appropriate when both the dependent and independent variables are measured with error (Sokal & Rohlf 1995). Genetic distances were defined as $\phi_{ST}/1 – \phi_{ST}$ (Rousset 1997). Geographical distances were measured as the shortest over-water distances between pairs of locations in the Universal Transverse Mercator (UTM) projected co-ordinate system, and were $\log_{10}$ transformed prior to analyses (Slatkin 1993). A global IBD analysis was conducted across all 13 locations (Broome individual excluded) and separate analyses were conducted within each geographical region. The shortest geographical distances between pairs of locations within regions generally traversed suitable marine habitats for A. laevis (water depths less than 50 m); however, in the GBR, the Swain reefs and Keppel Islands are separated by a deep-water channel (> 100 m) approximately 150 km wide (Fig. 1). To test whether distances through suitable habitats correlated better with gene flow than the shortest distances for the GBR, a partial Mantel test was conducted that used geographical distances across shallow water habitats to the north as an indicator matrix (shown on Fig. 1) (Bohonak 2002).
Results

Genetic diversity

Sequence analysis of 726 bases of the ND4-tRNA fragment revealed 41 variable sites that defined 38 putative haplotypes (GenBank Accession nos EF506638–75) among 354 individual olive sea snakes (Table 1). Thirty-three variable sites were transitions, five were transversions and three were indels. The minimum number of base pair differences between any two haplotypes ranged from one to three, with the exception of two divergent haplotypes, each sampled from just one individual: ALH17 (Groote Eylandt, GoC) and ALH38 (Broome, WA) that differed by a minimum of 8 bp to all other sampled haplotypes. As genetic diversity measures were artificially inflated by the inclusion of these divergent haplotypes, two sets of genetic diversity measures (including and excluding the divergent haplotypes) were calculated for WA, Groote Eylandt and the GoC (Table 1), and values excluding these unusual haplotypes are reported below.

Overall nucleotide diversity was low (% $\pi = 0.49 \pm 0.2$ SE), with the lowest within-region nucleotide diversities occurring in the GBR (% $\pi = 0.12 \pm 0.10$ SE) and GoC (% $\pi = 0.07 \pm 0.07$ SE) and the highest occurring in WA (% $\pi = 0.46 \pm 0.30$ SE) (Table 1). Within-location (reef) nucleotide diversities were also lowest in the GBR (range 0.03–0.16) and GoC (range 0.05–0.14); by comparison, nucleotide diversities in WA reefs were almost an order of magnitude higher (range 0.10–0.61) (Table 1). Overall haplotype diversity ($h = 0.81$) was high (Table 1); however, within-region haplotype diversities were lower and followed a similar pattern to nucleotide diversities, with lowest haplotype diversities occurring in the GBR ($h = 0.55 \pm 0.03$ SE) and GoC ($h = 0.57 \pm 0.07$ SE) compared with WA ($h = 0.62 \pm 0.03$ SE). Within-location haplotype diversities ranged from 0.15 to 0.71, with the lowest haplotype diversities occurring in the Swain reefs and Keppel Island, southern GBR (range 0.15–0.46) (Table 1), however, the central GBR had the highest haplotype diversity (0.71).

Phylogeny and tests of biogeographical hypotheses

Monophyly of Aipysurus laevis was strongly supported by parsimony and likelihood bootstrap values and Bayesian posterior probabilities; however, intraspecific clades were poorly resolved (Fig. 2). ML bootstrap values were low (51–63%) and parsimony bootstrap values over 50% supported just one clade (comprising four WA haplotypes): only Bayesian posterior probabilities provided strong support (> 80%) for most intraspecific clades. Nonetheless, the three inference methods recovered very similar best trees with haplotypes from WA forming two clades basal to a derived monophyletic clade comprising all GBR and GoC haplotypes except haplotype ALH17. The two divergent haplotypes, ALH17 and ALH38, were consistently basal to all other haplotypes: however, this may reflect the divergence of these haplotypes rather than their true phylogenetic position (Felsenstein 1978). Analyses were conducted with these two haplotypes excluded (tree not shown) and recovered identical relationships among all other haplotypes as the best tree (Fig. 2).

Sequence divergence

Sequence divergences among the 38 haplotypes ranged from 0.14% to 1.89% (mean = 0.64%) and from 0.14% to 1.11% (mean = 0.54%) when divergent haplotypes were excluded. Within-region sequence divergences (divergent haplotypes excluded) were lower in the GBR (0.31%) and GoC (0.22%) than in WA (0.41%). The uncorrected between-region divergence for the GBR vs. GoC (0.58%) was lower than the between-region divergence for WA vs. GoC (0.66%); however, after correction for within-region variation, between-region divergences were almost identical (GBR vs. GoC, 0.32%; GoC vs. WA, 0.34%). Based on corrected sequence divergences and a divergence rate of 2% per million years, estimated divergence times were 160,000 years between the GBR and GoC and 170,000 years between the GoC and WA: the slower rate of 0.5% per million years recovered estimated between-region divergences of 640,000 and 680,000 years, respectively.

Gene genealogies and population expansion

Statistical parsimony revealed that each geographical region was characterized by a unique suite of haplotypes. Within each region, there were a few common haplotypes and numerous rare haplotypes, which differed by just 1 or 2 bp from the common haplotypes and formed the star-shaped patterns typically associated with recent population expansion (Fig. 3). Again, the WA haplotypes comprised two clades but, unlike the phylogenetic tree, one clade was linked to the GoC haplotypes while the other clade was linked to the GBR haplotypes. However, a minimum-spanning network (not shown) recovered an alternative link between the two WA clades (shown in Fig. 3) to the link between the WA and GBR clades. There was further population subdivision within the GBR, with few shared haplotypes between the Swain reefs and Keppel Islands (Figs 3 and 4). These two locations were characterized by one common haplotype (87.5% of Keppel Island individuals shared ALH1 and 84% of Swain reef individuals shared ALH3), and numerous rare haplotypes (Figs 3 and 4). By contrast, the central GBR had no unique or rare haplotypes (Fig. 4). Both common haplotypes found in the Swains and Keppels occurred in the central GBR.
and the most common central GBR haplotype (ALH5) also occurred at some Swain reefs, though not the Keppels (Figs 3 and 4). Fu’s F and Tajima’s D tests showed congruent signals of population expansion for all locations in the GoC (including and excluding ALH17) and all GBR locations, except the central GBR (Table 1). By contrast, Scott Reef was the only location in WA with an excess of low frequency haplotypes (Fig. 4) and where population expansion was indicated from Fu’s F and Tajima’s D tests (Table 1).

Population genetic structure, migration and isolation: estimates from equilibrium and coalescent models

The genetic structure in the haplotype tree was confirmed by hierarchical AMOVA, which revealed strong (and statistically highly significant) population subdivision at all levels (among regions, among locations within regions, and within locations) based on both raw haplotypes frequencies and taking percent sequence divergences into account (Table 2). While genetic variation was similarly structured in both sets of analyses, some differences are worth noting: the analysis treating haplotypes as equidistant partitioned less variation among regions (33.9%) than when sequence divergence among haplotypes was included (63.9%), confirming that molecular distances are larger for pairs of haplotypes drawn from different regions than from the same region (Excoffier et al. 1992) and suggesting some underlying separation of evolutionary lineages. Conversely, molecular variation based on haplotype frequencies alone was larger among locations within regions (27.3% cf. 16.1%) and among individuals within locations (38.8% cf. 20.0%) (Table 2) than those based on sequence divergence.

Global $F_{ST}$ values were very high for raw haplotype frequencies ($F_{ST} = 0.61$, $P < 0.001$) and when accounting for evolutionary distances amongst haplotypes ($\theta_{ST} = 0.78$, $P < 0.001$), as were comparisons among pairs of regions
Estimates of migration rate and divergence time between adjacent regions revealed that migration rates were very low (GBR vs. GoC, $M = 0.03$; GoC vs. WA, $M = 0.02$) and that divergence times were significantly different from zero (Table 3). $F_{ST}$ and $\phi_{ST}$ values between locations encompassed a wide range ($F_{ST}$: $-0.052$–$0.794$; $\phi_{ST}$: $-0.061$–$0.957$) and, while all comparisons between regions and pairs of locations from different regions were highly significant ($P > 0.001$), there were striking differences in patterns of divergence between locations within regions (ESM1). In the GoC, $F_{ST}$ and $\phi_{ST}$ values for all comparisons between the three locations were extremely low ($F_{ST}$: $-0.052$–$0.001$; $\phi_{ST}$: $-0.061$–$0.020$), divergence times were not significantly different from zero, and migration rates were high (8.76–12.19), with upper credibility limits approaching 20 (Table 3). The four Swain reefs were also not significantly differentiated from each other ($F_{ST}$: $-0.008$–$0.052$; $\phi_{ST}$: $-0.003$–$0.034$) and were therefore combined into one population for $\operatorname{mdiv}$ estimates of within-GBR divergence times and migration rates. $F_{ST}$ and $\phi_{ST}$ values among the Swains, Keppels and central GBR were much higher ($F_{ST}$: $0.289$–$0.794$; $\phi_{ST}$: $0.401$–$0.799$), migration rates were low, and divergence times were significantly different from zero (Table 3). Comparisons between pairs of locations in WA revealed a more complex picture: a number of comparisons were not significant, even though (some) corresponding $F_{ST}$ and $\phi_{ST}$ values were not small ($F_{ST}$ = $0.005$–$0.307$; $\phi_{ST}$: $0.122$–$0.675$; ESM1). Moreover, estimated divergence times and migration rates did not directly reflect the $F$-statistics or distances between reefs. In particular, Scott Reef, which lies isolated from the Ashmore/Cartier/Hibernia cluster (Fig. 1), was differentiated from Ashmore and Cartier reefs, based on $F$-statistics and divergence times, and migration rates were low (Table 3, ESM1); yet, migration rates between Scott and Hibernia reefs were highest in this region and corresponding $F$-statistics were low (Table 3).
Isolation by distance

The global Mantel test revealed a significant correlation between the genetic and geographical distance matrices ($r = 0.44$, $P < 0.0001$) and RMA regression recovered a positive relationship between genetic and geographical distances across all 13 locations (Fig. 5). However, the relationship ($r^2$) only explained 19.2% of the variance and the pattern of regression of genetic vs. geographical distance across the entire region sampled showed greatly increased variance in genetic distances at large geographical distances (Case IV from Hutchison &

Table 2 Hierarchical analysis of molecular variance (AMOVA) of 13 locations of *Aipysurus laevis* in three regions (Great Barrier Reef, Gulf of Carpentaria and Western Australia) based on raw haplotype frequencies ($F_{ST}$) and percent sequence divergence ($\phi_{ST}$). $P$ values based on 10 000 Markov chain permutations

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>SS</th>
<th>Variance component</th>
<th>Percentage Variation</th>
<th>$F_{ST}$</th>
<th>$P$</th>
<th>Variance component</th>
<th>Percentage Variation</th>
<th>$\phi_{ST}$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among regions $F_{CT}$</td>
<td>2</td>
<td>43.14</td>
<td>0.167</td>
<td>33.9</td>
<td>0.338</td>
<td>&lt; 0.0001</td>
<td>403.90</td>
<td>1.83</td>
<td>63.9</td>
<td>0.639</td>
</tr>
<tr>
<td>Among locations within regions $F_{SC}$</td>
<td>10</td>
<td>35.72</td>
<td>0.134</td>
<td>27.3</td>
<td>0.413</td>
<td>&lt; 0.0001</td>
<td>122.15</td>
<td>0.46</td>
<td>16.1</td>
<td>0.447</td>
</tr>
<tr>
<td>Within locations $F_{ST}$</td>
<td>340</td>
<td>64.97</td>
<td>0.191</td>
<td>38.8</td>
<td>0.612</td>
<td>&lt; 0.0001</td>
<td>194.31</td>
<td>0.57</td>
<td>20.0</td>
<td>0.800</td>
</tr>
<tr>
<td>Total</td>
<td>352</td>
<td>143.83</td>
<td>0.492</td>
<td></td>
<td></td>
<td></td>
<td>720.36</td>
<td>2.86</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NB: Individual from Broome excluded for AMOVA’s.

Fig. 4 Proportional abundances of *Aipysurus laevis* mtDNA haplotypes for each of 13 sampled locations in three regions. The haplotypes unique to each region, Western Australia, Gulf of Carpentaria, Great Barrier Reef, are shown in the same order along the X-axis for each location within the same region. Graphs are aligned to allow immediate among-location comparisons of haplotype frequencies (as proportions). Numbers in brackets following location names correspond to numbers marking locations on the map. Sample sizes ($n$) for each location are given below the location name.
Table 3 Coalescent model based estimates of migration and time of divergence between pairs of populations as indicated by mDiv analyses. $M$ is the estimated number of migrants per generation scaled by effective population size [$M = (\text{migration rate})*2\times N_e$], Nielsen & Wakeley (2001) and $T$ is the estimated time of divergence between two populations. $M$ and $T$ are presented with upper and lower 90% credibility limits. $T = 0$ indicates that ±log(L) of the best estimate includes zero. $T \neq 0$ indicates that ±2log(L) of the best estimate did not encompass zero; thus, divergence time is significantly different from zero (Nielsen & Wakeley 2001).

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Time</th>
<th>Credibility limits</th>
<th>$T$</th>
<th>$M$</th>
<th>Credibility limits</th>
<th>$\Phi_{ST}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Between regions</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GBR — Gulf of Carpentaria</td>
<td>0.82</td>
<td>0.36—4.01</td>
<td>$T \neq 0$</td>
<td>0.03</td>
<td>0.00—0.29</td>
<td>0.78*</td>
</tr>
<tr>
<td>Gulf of Carpentaria — WA</td>
<td>0.76</td>
<td>0.32—3.89</td>
<td>$T \neq 0$</td>
<td>0.02</td>
<td>0.00—0.33</td>
<td>0.510*</td>
</tr>
<tr>
<td><strong>Within Regions</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within Great Barrier Reef</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Keppel Is — Swain Reefs</td>
<td>1.03</td>
<td>0.26—4.47</td>
<td>$T \neq 0$</td>
<td>0.35</td>
<td>0.07—0.77</td>
<td>0.683*</td>
</tr>
<tr>
<td>Central GBR — Swain Reefs</td>
<td>0.93</td>
<td>0.08—4.49</td>
<td>$T \neq 0$</td>
<td>0.65</td>
<td>0.08—2.88</td>
<td>0.614*</td>
</tr>
<tr>
<td>Central GBR — Keppel Is.</td>
<td>0.61</td>
<td>0.04—4.49</td>
<td>$T \neq 0$</td>
<td>0.70</td>
<td>0.03—5.67</td>
<td>0.438*</td>
</tr>
<tr>
<td>Within Gulf of Carpentaria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mornington Is — Groote Eylandt</td>
<td>0.23</td>
<td>0—4.45</td>
<td>$T = 0$</td>
<td>12.49</td>
<td>2.91—19.80</td>
<td>-0.061</td>
</tr>
<tr>
<td>Mornington Is — Vanderlin Is.</td>
<td>0.18</td>
<td>0—4.49</td>
<td>$T = 0$</td>
<td>8.76</td>
<td>1.54—19.43</td>
<td>0.020</td>
</tr>
<tr>
<td>Vanderlin Is — Groote Eylandt</td>
<td>0.02</td>
<td>0—4.51</td>
<td>$T = 0$</td>
<td>11.57</td>
<td>3.42—19.72</td>
<td>-0.031</td>
</tr>
<tr>
<td>Within Western Australia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hibernia Reef — Scott Reef</td>
<td>0.11</td>
<td>0.01—4.67</td>
<td>$T = 0$</td>
<td>2.63</td>
<td>0.79—9.41</td>
<td>0.122</td>
</tr>
<tr>
<td>Hibernia Reef — Ashmore Reef</td>
<td>0.33</td>
<td>0.03—4.49</td>
<td>$T = 0$</td>
<td>1.32</td>
<td>0.25—7.54</td>
<td>0.276</td>
</tr>
<tr>
<td>Hibernia Reef — Cartier Islet</td>
<td>2.73</td>
<td>0.18—4.61</td>
<td>$T = 0$</td>
<td>0.38</td>
<td>0.00—6.37</td>
<td>0.676</td>
</tr>
<tr>
<td>Ashmore Reef — Cartier Islet</td>
<td>0.74</td>
<td>0.07—4.48</td>
<td>$T \neq 0$</td>
<td>0.18</td>
<td>0.00—3.95</td>
<td>0.322</td>
</tr>
<tr>
<td>Scott Reef — Ashmore Reef</td>
<td>2.65</td>
<td>0.38—4.67</td>
<td>$T \neq 0$</td>
<td>0.56</td>
<td>0.07—2.11</td>
<td>0.561*</td>
</tr>
<tr>
<td>Scott Reef — Cartier Islet</td>
<td>3.70</td>
<td>1.28—5.00</td>
<td>$T \neq 0$</td>
<td>0.10</td>
<td>0.00—0.62</td>
<td>0.918*</td>
</tr>
</tbody>
</table>

Reduced Major Axis regressions showing relationships between Aipysurus laevis genetic and geographical distances for all 13 locations combined (Broome not included) and separately for each region. Comparisons between locations within and between regions are indicated by different symbols shown in the key. Mantel tests indicated that there was a significant global effect of isolation by distance (IBD) but no effect of IBD within each region.

Fig. 5 Reduced Major Axis regressions showing relationships between Aipysurus laevis genetic and geographical distances for all 13 locations combined (Broome not included) and separately for each region. Comparisons between locations within and between regions are indicated by different symbols shown in the key. Mantel tests indicated that there was a significant global effect of isolation by distance (IBD) but no effect of IBD within each region.

GoC ($r = -0.45, P < 0.506$) because of pannixia, or WA ($r = 0.39, P < 0.375$) because of the anomalies between $\Phi_{ST}$ values and geographical distances outlined above. Partial Mantel tests for the GBR indicated that genetic distances were significantly correlated with geographical distances that traversed suitable sea snake habitats ($r = 0.773, P < 0.017$) but not with the shorter over-water distances between locations ($r = -0.47, P < 0.928$; Figs 1 and 5).

Discussion

This study revealed strong population subdivision for the olive sea snake, Aipysurus laevis, at several spatial scales across the three regions that comprise most of its Australian range. Low nucleotide and low to moderate haplotype diversities across much of this species’ range strongly supported recent population expansions consistent with regional Pleistocene histories. Low haplotype and nucleotide diversities are uncommon in marine organisms but have been documented for sharks (Duncan et al. 2006; Stow et al. 2006) and some teleost fish species (Grant & Bowen 1998). The genetic patterns revealed in the intraspecific phylogenies, geographical distribution of genetic diversity and structure, and signals of range expansion, are explored in relation to the patterns predicted by the competing hypotheses. Considered separately, each line of evidence did not unequivocally support one biogeographical hypothesis.
over both others; however, when taken in combination, the genetic patterns revealed in this study most strongly supported dispersal from east and west coasts, albeit at different temporal scales. Interestingly, while signals of recent expansion events corresponded with Pleistocene habitat shifts around northern Australia, genetic structure also reflected regional habitat types. These results imply that unsuitable habitats present strong barriers to contemporary female-mediated gene flow for *A. laevis*, with implications for the conservation of this potentially low dispersal marine species.

**Evaluation of biogeographical hypotheses**

**Tree and network genealogies.** Phylogenetic reconstruction indicated that haplotypes in the GoC and GBR were derived with respect to WA haplotypes, suggesting interglacial colonization of the GBR-GoC from WA and supporting west to east dispersal. Tree-based phylogenies are, however, not necessarily as suitable as network methods for evaluating relationships within poorly resolved intraspecific genealogies (Posada & Crandall 2001). Population genetic theory predicts that older haplotypes will have more descendant haplotypes associated with them (Posada & Crandall 2001) and consequently occupy interior positions within a network (Crandall et al. 1994). The most common haplotype sampled in the central GBR (ALH5) held an interior position in the statistical parsimony network (Fig. 3) and was basal to all other GBR-GoC haplotypes in the phylogenetic tree (Fig. 2); thus, both analyses support its ancestral position in the GBR-GoC clade. However, in contrast to the phylogenetic tree, the network placed WA haplotypes in exterior (tip) positions (Fig. 3) suggesting that they were younger and derived from the GBR-GoC, thus supporting east to west dispersal.

**Genetic diversity and population expansion.** The high genetic diversities on most WA reefs are inconsistent with recent colonization from east coast populations (Table 1) and, although high diversities may be attributable to larger effective population sizes (Palumbi 1997), there is no evidence that WA reefs support larger *A. laevis* populations than other locations sampled. Nonsignificant Tajima's *D* and Fu's *F* tests for Ashmore, Cartier and Hibernia reefs indicated that, rather than having been recently colonized, populations persisted on these reefs during the Pleistocene. Patterns of endemism in the genus *Aipysurus* also support the persistence of *Aipysurus* species on reefs in this region. *Aipysurus apraefrontalis* (WA endemic) is the sister species to the widespread *A. duboisi*, and *Aipysurus fuscus* (WA endemic) is the sister species to *A. laevis* (Lukoschek & Keogh 2006). These sister-species pairs are reciprocally monophyletic and within-clade divergences (between each widespread and restricted species pair) are considerably shallower (younger) than between-clade divergence (Lukoschek & Keogh 2006) suggesting that neo-endemics were derived *in situ* in WA waters from widespread species.

The GoC and southern GBR locations had low genetic diversities and highly significant Tajima’s *D* and Fu’s *F* tests (Table 1), supporting recent expansion events in these regions, and the two star phylogenies that characterized the Kepps and Swain reefs, with almost no shared haplotypes (Figs 3 and 4), suggest that inshore and offshore southern GBR locales were colonized by different maternal lineages, probably following the most recent marine transgression 10 000 years ago. Early studies (with limited sampling) documented the Swains as genetically distinct from the rest of the GBR for various taxa (reviewed by Benzie 1994); however, recent studies, with inshore and offshore sampling, did not document a major inshore-offshore genetic divide in the southern GBR (Doherty et al. 1994; Worheide et al. 2002). Rather, a genetic divide (when it existed) separated the southern GBR from northern and central regions (Doherty et al. 1994; FitzSimmons et al. 1997b; Worheide et al. 2002; Dethmers et al. 2006; Smith-Keune & van Oppen 2006) attributable to cyclic the retraction and expansion of northern and southern GBR populations to the Queensland (northern) and Marion (southern) plateaus, respectively (Fig. 1). It is likely that *A. laevis* retracted to the Marion plateau during Pleistocene perturbations, as it currently occurs at several Marion plateau reefs (Heatwole 1975); however, its present and historical occurrence on Queensland plateau reefs is unclear. In contrast to the southern GBR and GoC, the central GBR had high genetic diversity and no signals of recent population expansion (Table 1). Higher haplotype diversity may be a sampling artefact: the eight snakes from the central GBR were sampled from a broad geographical range (18° S to 20° S) rather than discrete reefs (because of the absence of large *A. laevis* aggregations on reefs in the central or northern GBR). High genetic diversity may, however, also indicate that the central GBR population is older or larger than southern populations (Palumbi 1997) or represents an admixture of divergent lineages (Chenoweth et al. 1998b). Based on our data, it is not possible to determine whether the central GBR is part of a larger population; however, the ancestral position of the most common central GBR haplotype (ALH5) (Figs 2 and 3), suggests that, rather than being a region of secondary contact, the central GBR was colonized from populations in the Coral Sea, with subsequent range expansion into the southern GBR and the GoC.

**Timing of divergences.** Corrected sequence divergences and rates of mtDNA evolution of 2% and 0.5% per million years, suggest that *A. laevis* regional lineages diverged between 160 000 and 650 000 years ago. These dates place
lineage divergences well within the Pleistocene and divergence times based on the 2% rate of mtDNA evolution correspond with the penultimate glacial maximum, which occurred approximately 150 000 years ago (Williams et al. 1998). Both estimates place divergences before the most recent glaciation and marine transgression cycle of Australia’s continental shelf (10 000–17 000 years ago): divergence rates of 20% per million years would be needed for lineages to have diverged this recently, which seems unlikely given the slower rates of mtDNA evolution documented for reptilian lineages (Zamudio & Greene 1997).

These dates not only provide a first approximation to the timing of lineage divergences: perhaps more importantly, they also indicate that regional lineages have been isolated for similar periods of time. This result is potentially reconcilable with both the east to west and west to east dispersal hypotheses: three isolated lineages may have persisted in either eastern or western Australian waters and independently colonized the GBR, GoC and WA. However, given the other lines of evidence, the similar between-region divergences most strongly supports dispersal from both east and west coasts: the GoC and GBR having been colonized by two east coast matrilines that were isolated around the same time that east and west coast lineages were isolated from each other.

Population subdivision reflects historical processes and contemporary gene flow

The fixed differences among regional haplotypes indicate that, since being colonized, between-region gene flow has been insufficient to overcome the effects of local genetic drift, thus allowing the development of regional lineages through mutation and lineage assortment (Gopurenko et al. 1999). Equilibrium and coalescent models confirmed strong population subdivision and extremely limited contemporary among-region female-mediated gene flow. Global $F_{ST}$ and $\phi_{ST}$ values were high, as were values between locations pairs from different regions (Table 3, ESM1), and migration between regions was effectively zero (Table 3). Within regions, however, marked differences in the extent of population subdivision and divergence times reflected differences in regional habitat types.

All sea snake species (with the notable exception of the pelagic Pelemodon melanopleurus) are associated with benthic habitats and all need to surface to breath; sea snakes are therefore essentially confined to shallow water habitats. Large deep-water expanses are therefore predicted to present barriers to dispersal, and the genetic data of this study confirmed this prediction. Regional differences in gene flow reflected the potential connectivity afforded sea snakes by regionally different habitat types. The GoC is a large shallow water lagoon (water depths mostly < 20 m and rarely > 70 m) and all lines of evidence indicated extremely high gene flow (panmixia) for A. laevis in this region. By contrast, the Timor Sea oceanic shoals bioregion (Fig. 1) comprises isolated reefs separated by deep water. Water depths around Ashmore, Hibernia and Cartier reefs are generally less than 200 m but reach 2000 m around Scott Reef. The overall genetic picture in this region is one of low gene flow among populations that have been diverging for some time (Table 3). Only Scott Reef had a genetic signature consistent with recent population expansion, possibly following colonization from the Ashmore/Cartier/Hibernia cluster facilitated by the southwest-flowing Leeuwin Current (Simpson 1991).

Strong population subdivision between the central GBR, Keppels and Swain reefs is partially attributable to historical processes (described above) but also to low contemporary gene flow among these locations (Table 3). The Swains region is geographically unusual within the GBR, being comparatively isolated from inshore reefs by a deep-water channel which lacks reefs (Fig. 1), is intruded by colder Tasman Sea waters in winter (Burrage et al. 1996) and appears to act as barrier to female-mediated gene flow for A. laevis. Restricted gene flow across this channel has also been reported for the live-bearing damselfish, Acanthochromis polyacanthus (Doherty et al. 1994); thus, the Swain reefs may be genetically isolated for taxa that give birth to live young. By contrast, high levels of gene flow have been reported throughout the GBR for many organisms with dispersive larvae including reef fish (Dudgeon et al. 2000; van Herwerden et al. 2003; Bay et al. 2004), echinoderms (Benzie & Stoddart 1992) and molluscs (Benzie & Williams 1992). Genetic and geographical distances were better correlated over suitable habitats in the GBR than for over-water distances, suggesting a gradient of more recent gene flow along the mosaic of reefs that form the GBR. There was no effect of isolation by distance in the panmictic GoC or among populations on isolated WA reefs.

Caveats

The above discussion comes with several caveats that will be addressed in turn. (i) Limited sampling at the extremes of A. laevis’ current range. Aipysurus laevis has not been reported as occurring on the north coast of New Guinea (O’Shea 1996) and is unlikely to have occurred there during lowered sea levels as much New Guinea’s north coast shallow water habitat was lost (the north coast mostly comprises a narrow continental shelf that descends steeply into deep water). If A. laevis persisted in waters to the west and east of Australia during glaciation cycles (as suggested by our data), its distribution would have repeatedly been sundered by the emergence of the Torres Strait land-bridge with potentially greater divergences between east and west coast lineages than recovered in this study. The two divergent haplotypes (AL17 and AL38)
suggest the existence of un-sampled lineages: further sampling of *A. laevis* in coastal WA, southern New Guinea, New Caledonia, and on Coral Sea reefs may recover these and other divergent lineages and potentially an unequivocal genetic signal regarding this species’ biogeographical history. (ii) Small sample sizes. Four locations had small sample sizes and haplotypes frequencies may not have been representative for those locations; however, despite some minor inconsistencies in patterns of gene flow among WA reefs, these small sample sizes are unlikely to have influenced the overall interpretations for WA, the GoC, or the three regions combined. As outlined above, the small sample size from a large area in the central GBR makes interpretation of the high genetic diversity in this location equivocal: further sampling throughout the northern and central GBR is needed to resolve patterns of genetic diversity and gene flow in this region. (iii) Mitochondrial DNA coding region. The ND4 gene is potentially under selection and may have undergone one or more selective sweeps in the past that resulted in the observed low genetic variation. The noncoding mitochondrial control region is often used in intraspecific phylogeography studies, as it tends to be highly variable and is regarded as not being under selection. However, the control region has undergone a gene duplication event within the mitochondrion in all advanced snake lineages (Douglas et al. 2006) making amplification of homologous fragments more difficult. Moreover, Ashton & de Queiroz (2001) compared the utility of the control region and the ND2 gene for resolving intra- and interspecific phylogenetic relationships among vipersnakes and found that the control region had lower levels of intraspecific variation than ND2 third codon bases, suggesting either a lower mutation rate or selective constraints. Mitochondrial DNA is also maternally inherited and does not reflect male-mediated gene flow; thus, further research incorporating nuclear markers is needed to verify the patterns of genetic variation and relationships among regional lineages, and the strong geographical structuring recovered in this study.

**Further sampling at the extreme of this species’ range is needed to confirm and determine the specific details of this scenario. Despite the ability of *A. laevis* to undergo range expansion into new marine habitats, strong population subdivision exists among regions around northern Australia. Contemporary female-mediated gene flow subsequent to colonization has therefore been extremely restricted across this large spatial scale. Similar large-scale genetic subdivision across deep ocean basins has been documented for viviparous hammerhead sharks (Duncan et al. 2006) and among rookeries for highly philopatric marine turtles (FitzSimmons et al. 1997a) and suggests that the three regions sampled (GBR, GoC, WA) comprise distinct management units (MU) that warrant separate conservation strategies (Moritz 1994).**

**Acknowledgements**

This study was funded by the Reef CRC as part of its Marine Wildlife Program — Assessing Threats to Vertebrate Biodiversity. V.L. was Task Leader and was supported by an APA and a CRC Postgraduate Scholarship. We are grateful to P. Doherty and the Australian Institute of Marine Sciences for in-kind ship time to collect samples in the Swain Reefs on R.V. Lady Basten, and the Australian Customs Service and DEH for getting V.L. to Timor Sea reefs on A.C.V. Roebuck Bay, and to their crews for maximum assistance in the field. D. Milton, G. Fry and others at CSIRO Marine Research provided the GoC tissue samples and Chelonia Wildlife Rescue provided the only Broome sample. The Keppel Island Dive Shop and Digital Dimensions shared many useful tips on catching and sexing sea snakes. Many thanks to C. Dudgeon, E. Hutchinson, S. Lowe, and other volunteers for assistance catching and sampling sea snakes. We thank B. Evans, J.S. Keogh, C. Riginos and two anonymous reviewers for helpful comments on earlier drafts of the manuscript. This research was conducted under JCU Animal Ethics Permit A653–01, GBRMPA Permit G01/224 and EA Permit E2002/0031.

**References**


Supplementary material

The following supplementary material is available for this article:

**ESM 1** Pairwise FST (above diagonal) and $\phi_{ST}$ (below diagonal) values for 78 pairwise comparisons between 13 populations. $\phi_{ST}$ values were calculated using the Tamura–Nei model of substitution with alpha-shape gamma correction (0.015). Significance was tested after 10,000 permutations. Cells shaded light grey indicate nonsignificant comparisons after $P$ values were adjusted using sequential Bonferroni corrections. All other comparisons were highly significant ($P < 0.001$).

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