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Authors

Edwards, Danielle L
Melville, Jane

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ORIGINAL ARTICLE

Phylogeographic analysis detects congruent biogeographic patterns between a woodland agamid and Australian wet tropics taxa despite disparate evolutionary trajectories

Danielle L. Edwards* and Jane Melville

Department of Sciences, GPO Box 666, Museum Victoria, Melbourne, Vic. 3001, Australia

ABSTRACT

Aim To test the congruence of phylogeographic patterns and processes between a woodland agamid lizard (*Diporiphora australis*) and well-studied Australian wet tropics fauna. Specifically, to determine whether the biogeographic history of *D. australis* is more consistent with a history of vicariance, which is common in wet tropics fauna, or with a history of dispersal with expansion, which would be expected for species occupying woodland habitats that expanded with the increasingly drier conditions in eastern Australia during the Miocene–Pleistocene.

Location North-eastern Australia.

Methods Field-collected and museum tissue samples from across the entire distribution of *D. australis* were used to compile a comprehensive phylogeographic dataset based on *c.* 1400 bp of mitochondrial DNA (mtDNA), incorporating the *ND2* protein-coding gene. We used phylogenetic methods to assess biogeographic patterns within *D. australis* and relaxed molecular clock analyses were conducted to estimate divergence times. Hierarchical Shimodaira–Hasegawa tests were used to test alternative topologies representing vicariant, dispersal and mixed dispersal/vicariant biogeographic hypotheses. Phylogenetic analyses were combined with phylogeographic analyses to gain an insight into the evolutionary processes operating within *D. australis*.

Results Phylogenetic analyses identified six major mtDNA clades within *D. australis*, with phylogeographic patterns closely matching those seen in many wet tropics taxa. Congruent phylogeographic breaks were observed across the Black Mountain Corridor, Burdekin and St Lawrence Gaps. Divergence amongst clades was found to decrease in a north–south direction, with a trend of increasing population expansion in the south.

Main conclusions While phylogeographic patterns in *D australis* reflect those seen in many rain forest fauna of the wet tropics, the evolutionary processes underlying these patterns appear to be very different. Our results support a history of sequential colonization of *D. australis* from north to south across major biogeographic barriers from the late Miocene–Pleistocene. These patterns are most likely in response to expanding woodland habitats. Our results strengthen the data available for this iconic region in Australia by exploring the understudied woodland habitats. In addition, our study shows the importance of thorough investigations of not only the biogeographic patterns displayed by species but also the evolutionary processes underlying such patterns.

Keywords

Agamidae, Australian wet tropics, biogeography, *Diporiphora australis*, eastern Australia, lizards, phylogeography.

*Correspondence: Danielle L. Edwards, Museum of Zoology, The University of Michigan, Ann Arbor, MI 48109-1079, USA.
E-mail: daned@umich.edu

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INTRODUCTION

The eastern seaboard is not only one of the most biologically diverse regions of Australia (Blakers *et al.*, 1984; Strahan, 1995; Cogger, 2000; Slatyer *et al.*, 2007) but also one of the most studied biogeographic systems on the continent (McGuigan *et al.*, 1998; Schneider *et al.*, 1998; James & Moritz, 2000; Hurwood & Hughes, 2001; Schäuble & Moritz, 2001; Hugall *et al.*, 2002; Moritz, 2002; Ponniah & Hughes, 2004; Wong *et al.*, 2004; Hoskin *et al.*, 2005; Moussalli *et al.*, 2005; Nicholls & Austin, 2005; Graham *et al.*, 2006; Bell *et al.*, 2007). Habitats and rainfall gradients vary dramatically along the eastern margin of Australia, from wet rain forest fragments to open woodlands, with a large proportion of Australia's vertebrate diversity occurring in this region (Mackey *et al.*, 2008). Dramatic shifts in the distributions of forest types from the mid-Miocene to the present have been driven by climatic changes, with a general reduction in the extent of rain forest and an expansion of open forests and woodlands associated with a drier climate (Martin, 2006). These changes in rain forest distributions have established north-eastern Australia, in particular, as a model system for the study of speciation processes. It is thought that the intraspecific divergences observed in many taxa have resulted from the historical isolation of rain forest fragments (McGuigan *et al.*, 1998; Schneider *et al.*, 1998; James & Moritz, 2000; Hurwood & Hughes, 2001; Schäuble & Moritz, 2001; Hugall *et al.*, 2002; Moritz, 2002; Ponniah & Hughes, 2004; Wong *et al.*, 2004; Hoskin *et al.*, 2005; Moussalli *et al.*, 2005; Nicholls & Austin, 2005; Graham *et al.*, 2006; Bell *et al.*, 2007).

Numerous phylogeographic studies of north-eastern Australia have shown that the same biogeographic barriers occur across many rain forest taxa. These barriers partition the north-eastern seaboard into discrete biogeographic regions (see Fig. 1). The Black Mountain Corridor (BMC) is one of the most studied barriers, with divergences across this corridor observed in most rain forest vertebrate and invertebrate species studied. The BMC is thought to be a barrier that is specific to rain forest taxa, with the majority of divergences observed across the BMC believed to be ancient (i.e. mid–late Miocene–Pliocene) (Joseph *et al.*, 1995; Schneider *et al.*, 1998, 1999; Hugall *et al.*, 2002; Bell *et al.*, 2007). The Burdekin Gap is another well-studied barrier that consists of a belt of dry savanna vegetation which connects the semi-arid interior of the continent with the coastal woodland and splits the rain forest to the north and south (Joseph & Moritz, 1994). Divergences associated with the Burdekin Gap have generally been considered younger than those for the BMC (late Miocene–Pleistocene). However, there are similar degrees of variation in admixture across both these barriers, providing mixed evidence regarding the importance of the Burdekin Gap in shaping diversity patterns in the region (James & Moritz, 2000; Pope *et al.*, 2000; Schäuble & Moritz, 2001; Wong *et al.*, 2004; Brown *et al.*, 2006; Schiffer *et al.*, 2007; Toon *et al.*, 2007). The biogeographic barriers occurring further south, such as the St Lawrence Gap and the McPherson Range, are less well studied. The St Lawrence Gap may not be as influential in generating divergences as the BMC or Burdekin Gap (Joseph & Moritz, 1993; Hugall *et al.*, 2002); however, Quaternary and older splits within taxa have been observed (McGuigan *et al.*, 1998; Stuart-Fox *et al.*, 2001; Moussalli *et al.*, 2005). The McPherson Range, on the other hand, is generally considered to have affected only wet-restricted species (McGuigan *et al.*, 1998; Donnellan *et al.*, 1999; James & Moritz, 2000). Further north, the Carpentarian Barrier, extending south of the Gulf of Carpentaria, and the Torresian Barrier, delimiting the northern edge of the wet tropics rain forest, have been associated with late Pleistocene divergences in several bird species groups (Jennings & Edwards, 2005; Lee & Edwards, 2008). However, the importance of these barriers across woodland taxa is unclear.

Rain forest habitat has been of particular conservation concern, both from habitat destruction and a climate change perspective, resulting in many phylogeographic studies focusing on rain forest species (Moritz, 1994, 2002; Moritz & Faith, 1998; Moritz *et al.*, 2001; Graham *et al.*, 2006). Conversely, woodland habitats of north-eastern Australia have received more limited conservation attention, with studies focused on fire management rather than extensive conservation genetic studies as conducted in the rain forest habitats in the region, despite significant reductions and fragmentation as a result of human activity (Glanzig, 1995; Cogger *et al.*, 2003). Thus far, there have been only a few studies investigating genetic patterns in the woodlands and open forests of north-eastern Australia. Most of these studies have been conducted on moisture-dependent frog species (James & Moritz, 2000; Schäuble & Moritz, 2001) where genetic patterns are more complex and harder to predict than those displayed by rain forest restricted taxa (James & Moritz, 2000). A handful of studies on broadly distributed northern Australian bird species, incorporating woodland habitats, have identified phylogeographic structure associated with the Carpentarian and Torresian barriers (Jennings & Edwards, 2005; Lee & Edwards, 2008). However, there have been virtually no phylogeographic studies of vertebrates occurring across the woodlands of the north-eastern seaboard. Thus, more phylogeographic studies of woodland species are needed to untangle the complex biogeographic history of the north-eastern forests and provide a much needed genetic framework for conservation management in these disappearing habitats.

Australian agamid lizards, which form the subfamily Amphibolurinae, are a large adaptive radiation covering most habitats across continental Australia, from sand deserts to cool temperate forest. A few ancestral species, such as species in the *Hypsilurus* and *Physignathus* genera, show distributions restricted to wetter forest habitats (Schulte *et al.*, 2003; Hugall *et al.*, 2008). However, the greatest diversity of Amphibolurinae species occurs in arid and semi-arid Australia, providing an ideal model system to study evolution and diversification throughout Australia's drier environments (Schulte *et al.*, 2003; Hugall *et al.*, 2008). *Diporiphora australis* (Steindachner, 1867) is a common species occurring along the north-eastern

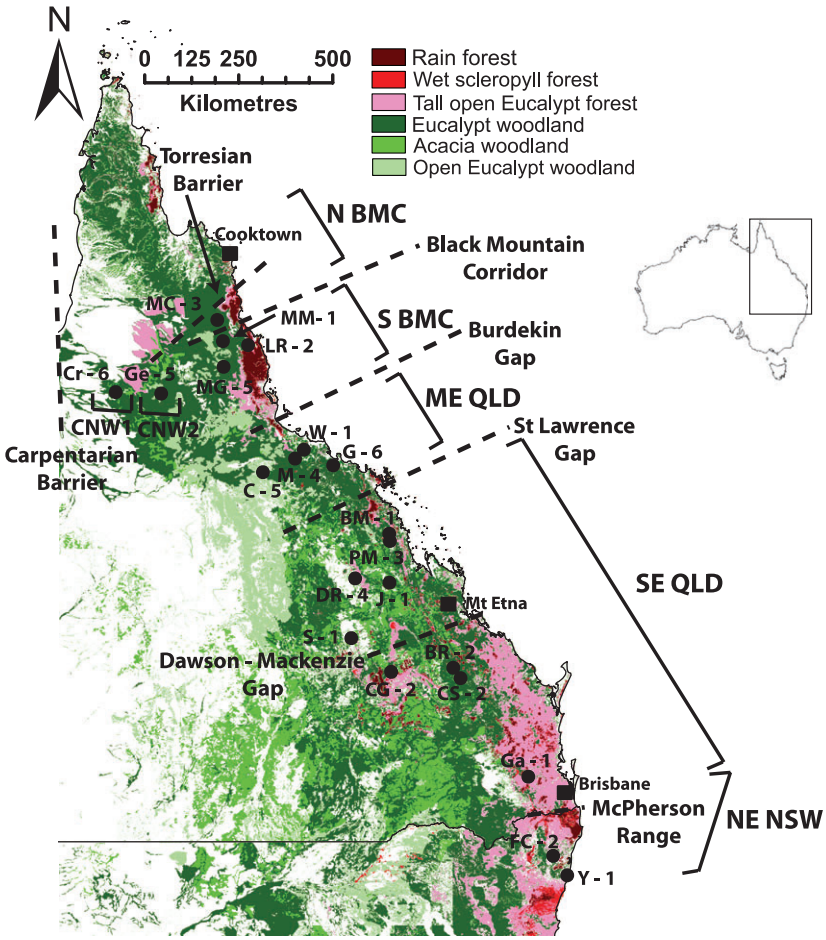

Figure 1 Map of the north-eastern seaboard of Australia showing the sample locations of *Diporiphora australis* across the entire distribution of the species. A map of continental Australia is inset. Sample locations (closed circles) are shown with an abbreviated site name (see Appendix S1 for expanded names) and sample sizes. These sites overlay the major vegetation types for the area obtained from http://www.environment.gov.au/erin/nvis/mvg/. Forested habitats supporting wetter vegetation types are represented in pink/red tones and dry open woodland habitats are shown in green tones. Major biogeographic barriers observed in taxa across the region are also shown, namely the Carpentarian Barrier, Torresian Barrier, Black Mountain Corridor, Burdekin Gap, St Lawrence Gap, Dawson–Mackenzie Gap and the McPherson Range. Major cities are shown as points of reference (closed squares), as is the site of collection of *D. australis* fossil material – Mount Etna. Biogeographic regions across north-eastern Australia are also identified: North Black Mountain Corridor (N BMC), South Black Mountain Corridor (S BMC), Central North-west Regions (C NW1 and C NW2), Mid-east Queensland (ME QLD), South-east QLD (SE QLD) and North-east New South Wales (NE NSW).

margin of Australia (Fig. 1), with broad habitat preferences across its distribution but generally preferring woodland habitats.

While relatively understudied, *D. australis* shows a high degree of morphological variability across its distribution, particularly in body patterning, size and secondary sexual coloration in males (Cogger, 2000). Based on this variability, Cogger (2000) suggested that *D. australis* may form a complex of up to five species. However, details of the distributions of such species are not available, making predictions regarding the species biogeography difficult to formulate. If patterns within *D. australis* follow those found in moisture-restricted woodland frog species (James & Moritz, 2000; Schäuble & Moritz, 2001) and the 'species' is indeed a complex, phylogeographic breaks may correspond to those seen in rain forest taxa. Counter to this argument is evidence from a middle Pleistocene fossil (205–170 ka) record at Mount Etna (see Fig. 1) in central Queensland suggesting that the '*Diporiphora australis–Diporiphora bilineata*' lineage moved into areas previously occupied by rain forest specialists in response to expanding woodland communities (Hocknull *et al.*, 2007). This fossil evidence also provides support for the notion that species occupying recently derived environments, such as woodlands in north-eastern Australia, will tend to have recent biogeographic histories (Moritz *et al.*, 2005). These two scenarios would lead to different phylogeographic structuring in *D. australis*. Under a simultaneous vicariance scenario, lineages within *D. australis* would be expected to be equally divergent, with little resolution of relationships between lineages. Alternatively, under a hypothesis of sequential

colonization north to south, one might expect sequentially younger southern lineages branching off from older northern lineages accompanied by signatures of expansion within younger lineages.

Given the general trend of increasing expansion of woodland communities from north to south along the eastern seaboard throughout the late Miocene–Pleistocene (Martin, 2006), it is likely that *D. australis* has expanded in a north–south direction in response to these changes in habitat. In order to test this hypothesis and the hypothesis that woodland species do not share a vicariance-based biogeographic history with rain forest species, we undertook a detailed phylogeographic study across the distribution of *D. australis* using the *c.* 1400 bp of mitochondrial DNA (mtDNA), incorporating the entire protein-coding gene *ND2* (NADH dehydrogenase subunit two) and the genes encoding $tRNA^{Trp}$, $tRNA^{Ala}$, $tRNA^{Asn}$, $tRNA^{Cys}$ and $tRNA^{Tyr}$, to the beginning of the protein-coding gene *COI* (subunit I of cytochrome *c* oxidase). We used phylogenetic analyses and relaxed molecular clock dating to decipher the divergence history of the species, and used hierarchical Shimodaira–Hasegawa tests to compare alternative biogeographic scenarios. We also used a variety of phylogeographic techniques to infer the underlying evolutionary processes shaping the phylogeographic history of *D. australis.*

MATERIALS AND METHODS

Tissue samples

A total of 58 individuals were sampled from 22 sites across the entire range of *D. australis*, incorporating one to five animals per site (Fig. 1). For field-caught samples (16 sites), two voucher specimens, from which liver tissue was extracted, were collected per site and deposited at Museum Victoria (NMV). The remaining one to three genetic samples from each site were non-lethally collected (tail-tips). Samples from the Australian Museum (AM) and the Queensland Museum (QM) were also included in the study. Further details of voucher specimens and tissue samples are available in Appendix S1 in Supporting Information.

Molecular genetic methods

Genomic DNA was extracted from the tail and liver samples using a modified chloroform method, suspended in TE buffer and stored at −20 °C (see Shoo *et al.*, 2008, for details). Targeted DNA was amplified using a touch-down polymerase chain reaction (PCR) profile [94 °C for 5 min – 1×; 94 °C for 30 s, 70–45 °C (decreasing in 5 °C increments) for 20 s, 72 °C for 90 s – 2×; 94 °C for 30 s, 40 °C for 30 s, 72 °C for 45 s – 40×; 72 °C for 4 min – 1×; 4 °C held]. Primers used to amplify *ND2* and the genes encoding $tRNA^{Trp}$, $tRNA^{Ala}$, $tRNA^{Asn}$, $tRNA^{Cys}$ and $tRNA^{Tyr}$ were Metf-1 (5′-AAGCAGTTGGG CCCATRCC-3′ – complement of H4419b) (Macey *et al.*, 2000) and H5934 (5′-AGRGTGCCAATGTCTTTGTGRTT-3′) (Macey *et al.*, 1997) or CO1r.aga (5′-ACRGTTCCRATRT-CTTTRTGRTT-3′) (Macey *et al.*, 2000). Targeted fragments were amplified in 40-μl reactions comprising *c.* 100 ng template DNA, 4 μL of 10× reaction buffer, 3 mM $MgCl_2$, 0.5 mM dNTPs, 10 pmol of each primer and 2 units of Hot Start *Taq* polymerase (Fermentas International Inc., Burlington, ON, Canada). The PCR products were purified using a Sureclean PCR cleanup kit (Bioline, London, UK) or gel purified using GFX columns (GE Healthcare, London, UK) and then sent to Macrogen Inc. (Seoul, Korea) for sequencing. Internal primers, *ND2*f.17 (5′-TGACAAAAAATTGCNCC-3′) (Macey *et al.*, 2000) and *ND2*f.dip (5′-AAATRATAGCCTACTCAT-3′) (Shoo *et al.*, 2008), were used in addition to PCR primers to obtain reliable sequence across the entire gene. DNA sequence data were then edited using SEQUENCHER 4.1.4 (Gene Codes Corporation, Ann Arbor, MI, USA). Sequences were aligned individually using CLUSTALX (Thompson *et al.*, 1997). Alignments were then checked by eye. Protein-coding regions were translated using the mammalian genetic code option in SEQUENCHER 4.1.4 and a clear reading frame was observed in all sequences. Thus, sequences were assumed to be genuine mitochondrial copies and not nuclear paralogues. Haplotype sequences have been lodged in GenBank (GU555966–GU556023: see Appendix S1 for individual numbers).

Phylogenetic analysis

Maximum parsimony (MP) and Bayesian analyses of haplotype sequences were used to assess overall phylogenetic structure and support for major clades in PAUP* 4.0b10 (Swofford, 2002) and MRBAYES v.3.1.2 (Ronquist & Huelsenbeck, 2003), respectively. A heuristic search was conducted for MP analyses, using a tree bisection–reconnection (TBR) branch-swapping method and stepwise addition. Bootstrap values for the MP trees were calculated from 1000 bootstrap replicates. Bayesian analyses were conducted using the GTR+I+Γ model using default priors for Markov chain Monte Carlo (MCMC) analyses in MRBAYES v.3.1.2 (Ronquist & Huelsenbeck, 2003). Four independent runs of four chains each were run for 4×10^6 generations sampling every 100 generations, with burn-in set at 400,000 generations. Convergence of posterior probabilities and stationarity of likelihood scores between the runs were assessed in TRACER v.1.3 (Rambaut & Drummond, 2005). Pairwise genetic distances were calculated for each major clade identified in phylogenetics analyses using both Jukes–Cantor (MEGA v.4.0.2; Tamura *et al.*, 2007) and D_{XY} (DNASP v.4.50.3; Rozas *et al.*, 2003) methods. Outgroup samples were selected from across the *Diporiphora* genus and associated genera, with *Amphibolurus muricatus* selected as the basal outgroup for the analyses. All outgroup sequences have previously been published (*Diporiphora bilineata*, AF128473; *Amphibolurus nobbi*, AY132999; *Diporiphora albilabris*, AY133003; *Diporiphora arnhemenica*, AY133004; *Caimanops amphiboluroides*, AF128472; *Diporiphora magna*, AY133009; *Diporiphora winneckii*, AY133012; *Amphibolurus muricatus*, AF128468). For specific information on each of

these sequences refer to Melville *et al.* (2001) and Schulte *et al.* (2003).

Divergence analyses

We estimated divergence times for *D. australis* clades using the relaxed molecular clock method in the program BEAST v.1.4.5 (Drummond & Rambaut, 2007). Five calibration points were used, including five fossil points and one biogeographic calibration point (see Shoo *et al.*, 2008; Melville *et al.*, 2009). As fossils are a minimum estimate of age, we used lognormal distribution for calibrations for fossils, and a normal distribution for the biogeographic calibration. Previously published sequences were included in our analyses to allow the calibration of our dataset (GenBank accession numbers for each of these sequences accompany the BEAST output tree in Appendix S2). Our analyses employed a GTR+I model of evolution, using an uncorrelated lognormal relaxed molecular clock without a lognormal distribution on the substitution rate across the tree. A Yule speciation process tree prior was employed and the analysis was run for 10 million generations. The output was viewed in TRACER v.1.3 (Rambaut & Drummond, 2005) to check that stationarity had been reached, to examine the coefficient of variation to determine the appropriateness of a lognormal clock and to assess the autocorrelation of rates from ancestral to descendant lineages (Drummond *et al.*, 2006).

Testing biogeographic hypotheses

We sought to test the biogeographic history of *D. australis* by determining whether the phylogenetic structure fitted a model of vicariance better than a model of sequential colonization from north to south (Fig. 2). If a sequential colonization history could be inferred, we also sought to determine whether species expansion was seeded from inland or coastal populations in its northern distribution (Fig. 2b). MODELTEST 3.7 (Posada & Crandall, 1998) and the Akaike information criterion were used to select the model of nucleotide substitution and parameters for all likelihood analyses. The observed tree was obtained using a heuristic search, TBR branch-swapping methods and stepwise addition of sequences

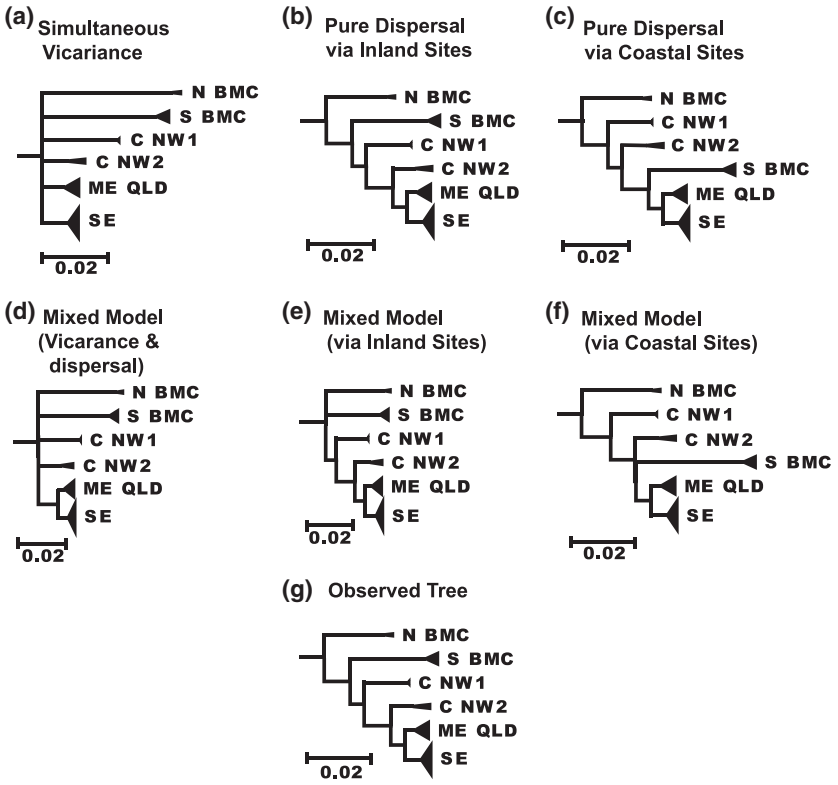

Figure 2 Trees constructed to represent alternative biogeographic hypotheses for *Diporiphora australis*. We sought to test the following hypotheses: (a) a simultaneous vicariance; (b) sequential colonization of southern populations via northern inland sites around the Burdekin Gap; (c) sequential colonization of southern populations via northern coastal sites across the Burdekin Gap; (d) vicariance of most populations with dispersal across the St Lawrence Gap; (e) vicariance across the Black Mountain Corridor and sequential dispersal around the Burdekin Gap via inland sites; and (f) vicariance across the Black Mountain Corridor and sequential dispersal across the Burdekin Gap via coastal sites. A maximum likelihood phylogram (g) was constructed from the observed data to test against alternative hypotheses; this was not found to differ from the topology of the MP/Bayesian phylogenies represented in Fig. 3. Names of clades for the most part reflect the biogeographic regions these clades are found in, as defined in Fig. 1. Exceptions are SE, which also includes haplotypes from both the SE QLD and NE NSW bioregions.

using PAUP* 4.0b10 (Swofford, 2002). To simplify analyses, only the previously published '*D. bilineata*' (AF128473) sequence was used as an outgroup (Melville *et al.*, 2001), as phylogenetic analyses indicated this to be the sister lineage of *D. australis*.

Trees representing alternative colonization scenarios for *D. australis* were constructed using MACCLADE v.4.0.8 (Maddison & Maddison, 2000). These tests were set up to initially test between hypotheses of simultaneous vicariance (Fig. 2a) and pure dispersal. Due to some uncertainty in the tree topology and bootstrap support for basal branches we wanted to test competing pure dispersal hypotheses, with dispersal into areas south of the Burdekin Gap via either inland (CNW2) or coastal (S BMC) populations (Fig. 2b and c, respectively). This then compares scenarios where dispersal occurs inland around the BMC and then across the Burdekin Gap at the coast (Fig. 2c), or where dispersal occurs across the BMC on the coast and then inland around the Burdekin Gap (Fig. 2b). Additionally, due to the same uncertainty of bootstrap support for basal branches in the *D. australis ND2* phylogeny, we also wanted to test if a mixed dispersal/vicariance model was a more appropriate fit for the data. Therefore, we tested three mixed vicariance + dispersal models.

1. Where northern populations [Northern Black Mountain Corridor (N BMC), Southern Black Mountain Corridor (S BMC), Central North-west 1 (C NW1) and Central North-west 2 (C NW2)] are allopatric, with dispersal from Mid-east Queensland (ME QLD) to the South-east (SE) portions of the species distribution (Fig. 2d).

2. Where S BMC and N BMC populations are allopatric and southern portions of the species distribution are colonized via dispersal from C NW → ME QLD → SE (Fig. 2e).

3. Where N BMC and C NW populations are allopatric and southern portions of the species distribution are colonized via dispersal from S BMC → ME QLD → SE (Fig. 2f).

The topologies created by these alternative hypotheses were then compared with the unconstrained/observed tree (Fig. 2g) using Shimodaira–Hasegawa (SH) tests (Shimodaira & Hasegawa, 1999) in PAUP* 4.0b10 (Swofford, 2002), with full optimization and 1000 bootstrap replicates. Alternative hypotheses were rejected if they were found to be significantly different from the observed data at the $\alpha = 0.05$ significance level.

Phylogeographic analyses

A number of summary statistics and statistical phylogeographic analyses were used to reconstruct the population history of *D. australis*. Hudson's 'nearest neighbour' statistic (*Snn*) was calculated to provide information on population differentiation within the S BMC, ME QLD and SE (including SE a and b) clades (Hudson, 2000) using DNASP v.4.50.3 (Rozas *et al.*, 2003). Other clades could not be analysed due to insufficient population diversity. The statistical significance of *Snn* was assessed by permuting values 10,000 times employing the coalescent simulation options in DNASP v.4.50.3 (Rozas *et al.*, 2003). Hudson's *Snn* is specifically designed for haplotypic data, where values close to 1 suggest little or no gene flow and values close to 0.5 suggest panmictic populations (Hudson, 2000).

Summary statistics were employed to detect violations of neutrality/demographic expansion in all the major clades identified within the *D. australis* mtDNA data. Tajima's *D* (D_T) (Tajima, 1989), Fu's F_S (Fu, 1997) and R_2 (Ramos-Onsins & Rozas, 2002) were all calculated using DNASP v.4.50.3 (Rozas *et al.*, 2003). To test the significance of these statistics, random samples were generated under the hypotheses of both selective neutrality and demographic equilibria using coalescent simulations and permuted 10,000 times (Rozas *et al.*, 2003). Mismatch distributions were computed by comparing empirical distributions with those based on both a demographic expansion model (DEM) and a spatial expansion model (SEM) by the generalized least squares method in ARLEQUIN v.3.11 (Excoffier *et al.*, 2005). The statistical significance of τ (DEM) and τ (SEM) was calculated by a bootstrapping procedure using 1000 replicates. Clades with inadequate sample sizes were excluded from mismatch analyses (N BMC, C NW1 and C NW2).

Coalescent-based estimates of theta (θ) were obtained using a maximum likelihood (ML) approach ($\theta_{ML} - \theta = 2N_f\mu$ where N_f is the effective female population size for mitochondrial data and μ is the mutation rate) and an exponential population growth parameter (g) in LAMARC v.2.1.2b (Kuhner, 2006; Kuhner & Smith, 2007). The θ parameter was estimated initially with g held at zero and with initial starting θ obtained using Watterson's (1975) estimate (termed θ_{NG}). The θ parameter was then estimated jointly with g (initial value of $g = 0.1$ – termed θ_G). The search strategy employed for all runs consisted of 15 short chains (10,000 steps) and five long chains (200,000 steps) each sampling every 20th step, with a burn-in of 1000 genealogies, random starting trees, transition/transversion ratio = 2, and empirical base frequencies with each estimate run five times for replication. Significant growth/decline was assumed if $3 \times SD \pm$ mean estimate of g did not overlap, with mean and SDs of all parameters calculates from the five replicated runs (Lessa *et al.*, 2003). These estimates were not calculated for N BMC, C NW1 or C NW2 clades due to insufficient haplotypic diversity in these clades.

RESULTS

Phylogenetic analyses

A 1379-bp fragment of the mtDNA gene *ND2* from a total of 58 individuals of *D. australis* yielded 35 haplotypes with 161 variable sites, 132 of which were parsimony informative. Total haplotype diversity (HD) was 0.978 ± 0.007 and nucleotide diversity (π) was 0.0259 ± 0.000005. Phylogenetic analyses reveal six major mtDNA clades within *D. australis*, which correspond to the major biogeographic regions within north-eastern Australia (Figs 1 & 3). The topologies of trees obtained using MP and Bayesian methods are congruent. Posterior probability/bootstrap support for the phylogenetic

Table 1 Table of pairwise distances between *Diporiphora australis* clades identified in Fig. 3 calculated using a Jukes–Cantor model (below the diagonal) and D_{XY} (above the diagonal).

Clades	N BMC	S BMC	C NW1	C NW2	ME QLD	SE	SE a	SE b
N BMC	–	0.0476	0.0423	0.0458	0.0481	0.0450	0.0452	0.0447
S BMC	0.0492	–	0.0427	0.0449	0.0439	0.0439	0.0438	0.0440
C NW1	0.0435	0.0439	–	0.0319	0.0343	0.0339	0.0342	0.0340
C NW2	0.0473	0.0463	0.0326	–	0.0221	0.0234	0.0228	0.0239
ME QLD	0.0497	0.0452	0.0351	0.0225	–	0.0153	0.0139	0.0167
SE	0.0464	0.0452	0.0347	0.0237	0.0154	–	–	–
SE a	0.0466	0.0452	0.0350	0.0232	0.0140	–	–	0.0068
SE b	0.0461	0.0453	0.0345	0.0243	0.0168	–	0.0068	–

N BMC, Northern Black Mountain Corridor; S BMC, Southern Black Mountain Corridor; C NW, Central North-west; ME QLD, Mid-east Queensland; SE, South-eastern populations including SE QLD and NE NSW bioregions (see Fig. 1 for geographic distributions).

relationships between the N BMC, S BMC and C NW1 clades is low, but each clade is strongly supported as monophyletic (Figs 1 & 3). The C NW2 clade is supported as basal to both the ME QLD and SE clades, which are together resolved as sister lineages.

Table 1 summarizes the pairwise distances between the six clades defined in the phylogenetic analyses. Pairwise differences between clades increase with geographic distance. The deepest pairwise distances occur between geographically proximal clades north and south of the BMC (Jukes–Cantor = 4.9%; D_{XY} = 4.8%). There is a trend for pairwise distances between geographically proximal clades to decline with increasing latitude (i.e. low divergences in more southerly populations), with as little as 1.5% Jukes–Cantor distance and 1.5% D_{XY} between the ME QLD and SE clades. Pairwise distances between C NW2 and C QLD are shallower (Jukes–Cantor = 2.3%; D_{XY} = 2.2%) than those between C NW1 and ME QLD (Jukes–Cantor = 3.5%; D_{XY} = 3.4%). The shallowest distances occur between two lineages within the SE clade: SE a (BM, PM, DR, J and S; see Fig. 1) and SE b (BR, CG, CS, FC, Ga and Y; see Fig. 1), with 0.7% Jukes–Cantor and D_{XY} between them.

Divergence analyses

The relaxed lognormal clock analysis of the mtDNA dataset produced the same in-group topology as the phylogenetic analyses (Fig. 3). There was a slight tendency towards a positive correlation in the rate of parent to child branches, with a covariance of 0.145 but zero was included in the 95% highest posterior density (HPD) (−0.39 to 0.25); thus, this autocorrelation was not considered significant (Drummond *et al.*, 2006). The coefficient of rate variation was estimated to be 0.46 (95% HPD: 0.40–0.52), indicating that the dataset is not strictly clock-like and that a lognormal relaxed clock is appropriate. The complete BEAST output tree is supplied in Appendix S2.

Estimated divergences suggest the age of the *D. australis* lineage is in the vicinity of 8.9–16 million years ago (Ma). Divergence estimates show sequentially younger divergences in a north–south direction across the species distribution between clades in adjacent biogeographic regions. Initial divergences associated with the BMC are estimated at 4.7–9.2 Ma, with estimated divergences between coastal northern clades (S BMC) and northern inland clades (C NW1 and 2) ranging from 2.6 to 6 Ma. Divergences associated with the Burdekin Gap are estimated at between 1.3 and 3.2 Ma, with younger divergences associated with the St Lawrence Gap (0.3–1.3 Ma).

Testing alternative biogeographic hypotheses

MODELTEST 3.7 (Posada & Crandall, 1998) and the Akaike information criterion were used to select the GTR+I+Γ model of evolution with the following parameters: nucleotide frequencies A = 0.364, C = 0.316, G = 0.098, T = 0.222; N_{ST} = 6; substitution rates A → C = 1.207, A → G = 15.573, A → T = 1.355, C → G = 0.240, C → T = 15.573, G → T = 1.000; gamma distribution shape parameter = 0.917, proportion of invariant sites = 0.425. A single likelihood tree with a likelihood score of −ln *L* 3466.752 was obtained using these parameters in PAUP* v.4.0b10 (Swofford, 2002), and used to represent the observed data in hypothesis testing (Fig. 2c). The topology of the observed tree was no different from the phylogenetic trees found using Bayesian and MP methods (Fig. 3). SH test results reject both the mixed and simultaneous vicariance hypotheses in favour of a dispersal topology (Table 2). Topologies representing a mixed dispersal/vicariance model, with dispersal via coastal populations, are also rejected. SH tests could not distinguish between topologies representing a pure dispersal model or a mixed dispersal/vicariance model with dispersal via inland populations. A pure dispersal model with dispersal via coastal populations could also not be rejected.

Phylogeographic analyses

A summary of the phylogeographic analyses carried out on the *D. australis* mtDNA clades is outlined in Table 3. Hudson's 'nearest neighbour' statistic, which was used to calculate population differentiation, suggests that populations within the S BMC and ME QLD clades and between the SE a and SE b

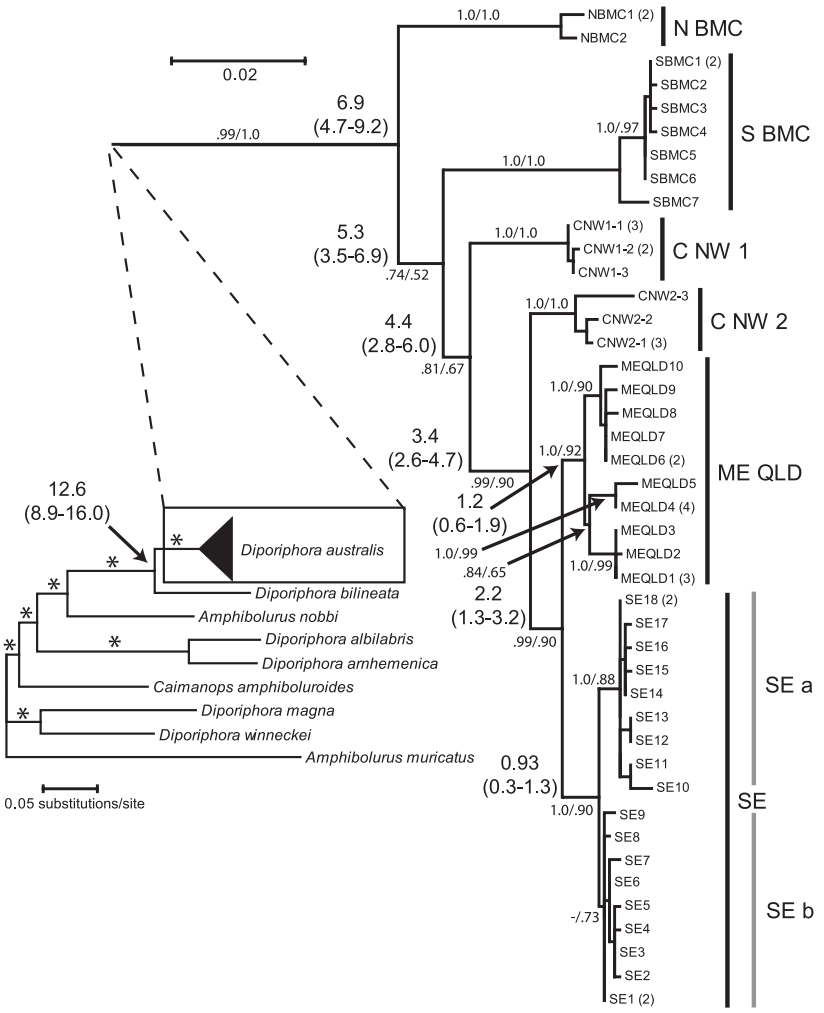

Figure 3 Phylogram of *Diporiphora australis ND2* haplotypes obtained from Bayesian analysis. Inset is a detailed diagram of the outgroups used and their relationship to *D. australis*. Branch support from Bayesian posterior probabilities and maximum parsimony bootstrap values (MP) are shown on clade nodes, respectively (where * indicates high posterior probabilities/bootstrap values for outgroup taxa). Estimates of divergence amongst clades and their associated errors are shown in brackets on nodes of interest. Six major clades are shown, each with an abbreviated name symbolizing the biogeographic region that they represent (refer to Fig. 1 for site and biogeographic barrier information), with the number of individuals per haplotype shown in brackets where this is more than one. N BMC, north of the Black Mountain Corridor; S BMC, south of the Black Mountain Corridor; C NW1, Cr site; C NW2, Ge site; ME QLD, sites between the Burdekin and St Lawrence Gaps; SE, sites south of the St Lawrence Gap, also encompasses the NE NSW bioregion outlined in Fig. 1; SE a is BM, PM, DR J and S sites; SE b is CG, BR, CS, Ga, FC and Y sites. The scale bar on trees represents substitutions/site.

Table 2 Summary of Shimodaira–Hasegawa tests comparing alternative biogeographic hypotheses. Models tested comprise scenarios where simultaneous vicariance, pure dispersal and various mixed models of dispersal and vicariance explain the history of *Diporiphora australis* in north-eastern Australia. Refer to Fig. 2 for explicit information on the hypotheses tested.

Hypothesis	−ln *L*	Diff −ln *L*	*P*-value
Simultaneous vicariance	3512.5953	45.8431	0.004
Dispersal via inland sites	3466.7521	Best	–
Dispersal via coastal sites	3482.8386	16.0865	0.105
Mixed model (MM)	3494.2788	27.5267	0.017
MM via inland sites	3469.2907	2.5386	0.653
MM via coastal sites	3494.1553	27.4031	0.02

clades are highly differentiated (*Snn* = 1.000; $P = 0.017$, $P \geq 0.001$ and $P \geq 0.001$, respectively). When the SE clade was considered as a whole, estimates suggested that populations are moderately differentiated (*Snn* = 0.650; $P \geq 0.001$). Demographic/neutrality summary statistics only detect statistically significant population expansion in the SE clade, and this is only detected using Fu's F_S-test of demographic expansion (−8.41; $P \geq 0.01$). Alternatively, statistically significant demographic (DEM = 11.88; $P \geq 0.001$) and spatial (SEM = 11.49; $P \geq 0.05$) expansion was only detected in the ME QLD clade using mismatch analyses. Coalescent estimates of the *g* parameter show statistically significant population growth in the S BMC, ME QLD and SE clades (*g* = 71.61, 172.18, 1620.51, respectively). There is a trend for increasingly large *g* estimates in clades moving from north to south.

DISCUSSION

Our results show that phylogeographic structure between six mtDNA clades of *D. australis* corresponds to several major biogeographic barriers in north-eastern Australia (Figs 1 & 3), with sequentially younger divergences moving from north to south. Phylogenetic analyses and hypothesis testing support the hypothesis that the biogeographic history of *D. australis* has followed a scenario of sequential colonization from north to south across known biogeographic barriers in north-eastern Australia, seeded from inland populations in the C NW region.

Table 3 Results of tests of neutrality/demography (Tajima's D_T, Fu's F_S and R_2), mismatch analyses based on both models of demographic expansion (DEM) and spatial expansion (SEM), and coalescent analyses for the whole *Diporiphora australis ND2* dataset and the major clades within the species.

			Neutrality/demographic tests			Mismatch distributions				Coalescent simulations		
Group	N	h	D_T	Fu's F_S	R_2	DEM	τ	SEM	τ	θ_{NG}	θ_G	g
Whole species	58	35	0.0355 n.s.	−1.0136 n.s.	0.1095 n.s.	–	–	–	–	–	–	–
Clades												
N BMC	5	2	−0.8165 n.s.	0.0902 n.s.	0.4 n.s.	–	–	–	–	–	–	–
S BMC	8	6	−1.5218 n.s.	−1.0797 n.s.	0.2293 n.s.	n.s.	1.63672 (0–3.3)	n.s.	1.63583 (0.3–3)	0.003622 (0.000024)	0.0039456 (0.000038)	**71.509 (57–86)**
C NW1	6	3	1.3926 n.s.	0.0203 n.s.	0.2833 n.s.	–	–	–	–	–	–	–
C NW2	5	3	−1.031 n.s.	2.8785 n.s.	0.3168 n.s.	–	–	–	–	–	–	–
ME QLD	16	8	0.4112 n.s.	1.6004 n.s.	0.1463 n.s.	$P < 0.001$	**11.875 (2.6–15.5)**	$P < 0.05$	**11.49458 (7–15.3)**	0.0067058 (0.000054)	0.0082808 (0.000145)	**172.177 (166–178)**
SE	19	14	−0.2711 n.s.	**−8.40975** $P < 0.01$	0.1171 n.s.	n.s.	8.55469 (2.3–13.7)	n.s.	1.63817 (0.47–12)	0.008761 (0.000171)	0.0334124 (0.00228)	**1620.505 (1411–1830)**

The significance of neutrality/demographic and mismatch analyses were conducted using 10,000 permutations and 10,000 bootstrap replicates, respectively. The significance of the g parameter (an exponential population growth parameter) was assessed by repeating parameter estimates five times, with growth only inferred as significant where $g \pm 3 \times SD(g) > 0$ (Lessa *et al.*, 2003). Values in bold indicate significant results, while 'n.s.' indicates values that were not statistically significant at $\alpha = P < 0.05$. Clades are defined in Table 1.

N, number of individuals; h, number of haplotypes; θ_{NG}, theta estimated assuming no population growth; θ_G, theta estimated assuming population growth has occurred.

Diporiphora australis probably expanded into the southern parts of its distribution throughout the Plio-Pleistocene, most likely in response to expanding woodland habitats. Our results support evidence from a Quaternary fossil record from Mount Etna suggesting that the presence of taxa preferring drier habitats in this region is a relatively recent occurrence.

Patterns of divergence and the biogeography of north-eastern woodlands

Detailed palaeoclimatic records for north-eastern Australia (Macphail, 1997; Martin, 2006) have allowed comprehensive research on the evolution of refugial rain forest isolates throughout the Neogene–Quaternary (Moritz *et al.*, 2005). As a result, there have been many molecular studies of rain forest taxa seeking to understand spatial and temporal divergence patterns across such refugia (Moritz *et al.*, 2005). However, until now there have been virtually no studies of the phylogeographic structure of vertebrates occurring in the open forest and woodland habitats of north-eastern Australia, despite the ongoing human-induced fragmentation and reductions of these habitats (Glanzig, 1995; Melzer *et al.*, 2000; Cogger *et al.*, 2003). The few phylogeographic studies in these drier habitats have found that the history of these species may be more complicated and difficult to predict than that of rain forest restricted species (James & Moritz, 2000).

The reconstruction of the biogeographic history of the north-eastern Australian woodlands is still in its infancy; however, there is striking evidence that phylogeographic breaks in the region are not restricted to rain forest elements, but have broader applicability. For example, three frog species display biogeographic histories with varying degrees of congruence with rain forest taxa (James & Moritz, 2000; Schäuble & Moritz, 2001). Our results provide the first evidence that the BMC, St Lawrence Gap and the Dawson–Mackenzie barriers may have a role to play in delimiting phylogeographic structure in woodland fauna, despite very different evolutionary trajectories between rain forest and woodland fauna. For rain forest species, it has always been assumed that dry woodland and savanna expansion and contraction of rain forest across these breaks has caused the congruent divergences of rain forest taxa (Moritz *et al.*, 2005; Graham *et al.*, 2006). These conditions would be expected to provide little resistance to gene flow in woodland species, yet this is not the case.

Our results demonstrate, for the first time, that biogeographic barriers present in rain forest taxa also occur in broadly distributed dry forest species. Phylogenetic structure in *D. australis* is largely predicted by pre-defined biogeographic regions in north-eastern Australia. Divergence across the BMC in *D. australis* is estimated to have occurred during the late Miocene–early Pliocene period (4.7–9.2 Ma). The estimated divergence for *D. australis* across the BMC shows strong temporal congruence with divergences observed across this barrier within rain forest taxa using multiple genetic markers (Dolman & Moritz, 2006) and single loci (Schneider *et al.*,

1998, 1999; Moritz *et al.*, 2000, 2005). Our divergence estimates of the late to early Pliocene for *D. australis* across the Burdekin Gap also correspond to those of some rain forest taxa (Schneider *et al.*, 1999; Hugall *et al.*, 2002; Wong *et al.*, 2004; Dolman & Moritz, 2006) and three woodland frog species studied (James & Moritz, 2000; Schäuble & Moritz, 2001). Additionally, our divergence estimates for *D. australis* fall within the age limits of the '*D. bilineata–D. australis*' lineage within the Agamidae using multi-locus data (Hugall *et al.*, 2008). Although these independent studies support our age estimates and spatial arrangement of phylogeographic structure our results therefore need to be treated with caution (Edwards & Beerli, 2000; Liu *et al.*, 2008), as we used a single genetic marker in the current study. Further research using more markers would be required to confirm our divergence estimates and biogeographic patterns.

Deep phylogeographic breaks (2.8–6.9 Ma, late Miocene to mid-Pliocene) are also observed within *D. australis* from the northern QLD coastal regions (N BMC and S BMC) and directly inland from the C NW region (Fig. 1). Common phylogeographic breaks in many taxa have been associated with the Carpentarian Barrier and Torresian Barrier (Jennings & Edwards, 2005; Lee & Edwards, 2008), and BMC (Schneider *et al.*, 1998, 1999; Moritz *et al.*, 2000, 2005; Dolman & Moritz, 2006). However, the disjunction between coastal and inland *D. australis* mtDNA clades does not correspond to the traditional positions of these barriers. It is possible that the patterns identified in our study suggest complex convergence between these barriers in the C NW region. Finer-scale sampling in this area in the future could resolve the interactions of the Carpentarian Barrier and Torresian Barrier in woodland species.

Divergence estimates across the St Lawrence Gap between the *D. australis* ME QLD and SE lineages in the late Pliocene–early Pleistocene (1.3–3.2 Ma) provide the first evidence of this barrier in woodland species, as none of the previous phylogeographic studies of woodland taxa have found evidence of this barrier (James & Moritz, 2000; Schäuble & Moritz, 2001). There is also discrepancy in the importance of the St Lawrence Gap in rain forest species, with divergences ranging from the late Miocene into the Pleistocene (Stuart-Fox *et al.*, 2001; Moussalli *et al.*, 2005). The McPherson Gap, on the other hand, did not show phyogeographic structure in *D. australis*, which is in accordance with previous studies that have only found divergences in wet-restricted species (McGuigan *et al.*, 1998). Yet we did find evidence of a mid- to late Pleistocene divergence across the little known Dawson–Mackenzie phylogeographic break, which is regarded as important in the speciation of birds in north-eastern Australia (Joseph & Omland, 2009) but has received no support thus far in molecular studies. Given the low level of congruence with other taxa and our use of a single marker, one might consider the potential for error in our estimates of the age of these southern clades. However, independent fossil evidence suggests that '*D. australis–D. bilineata*' type animals did not appear in the SE QLD region until the mid-Pleistocene (Hocknull *et al.*, 2007), which can be directly correlated with our dating estimates for these clades.

Our results show that there is no mtDNA gene flow across most of these well-known biogeographic barriers in north-eastern Australia in *D. australis*. This raises the following questions. Can these barriers continue to be considered rain forest specific? If not, why do these barriers also cause disruptions to gene flow in woodland elements? Do species currently occupying woodland environments represent radiations in response to expanding habitat, recent habitat shifts or a combination of both? It is possible that shifts of climate and vegetation are much more complex than simply the expansion and contraction of woodlands and rain forest in opposition to each other. There is evidence from Mount Etna that arid-zone species have been found in this area in the past; for example, *Tympanocryptis* species now found in more semi-arid and arid non-forest habitats were observed in this area during glacial–arid phases (Hocknull *et al.*, 2007). The Burdekin Gap has also been described as a connection between the arid interior and the coast (Joseph & Moritz, 1994). It is possible that there have been periods of extreme aridity during the Pleistocene when all forests/woodlands contracted and were intersected at the points of these biogeographic barriers by sparser arid-like vegetation. The incursion of arid-like vegetation could provide a mechanism for genetic divergence in woodland species across these barriers. In order to adequately answer these questions more woodland species need to be considered for molecular analysis, and historical woodland habitat and species distributions need to be modelled under climatic extremes. Such research would also allow conservation agencies to address the applicability of conservation priorities based on rain forest biogeography to woodland systems.

Dispersal versus vicariance: support for alternative biogeographic hypotheses

While understanding the spatial and temporal patterns of divergence in a region can provide information on sustained restrictions to gene flow, likely refugia and some information on evolutionary processes, it does not allow a complete understanding of the underlying evolutionary history of a region. Fortunately, a large amount of molecular information has been synthesized for rain forest species in north-eastern Australia, which has shown that the history of rain forests and their associated faunas has been shaped by vicariant contraction followed by expansion of rain forest isolates (Moritz *et al.*, 2005). In addition, the specific locations of rain forest refuges during glacial maxima have been identified along the eastern seaboard of Australia (Hugall *et al.*, 2002; Graham *et al.*, 2006). Using evidence from phylogenetic hypothesis testing and phylogeographic techniques, we provide the first such information for a woodland species in the north-eastern Australian region.

Hypotheses tests, using alternative mtDNA tree topologies, showed a history of sequential dispersal into southern areas across known biogeographic barriers in *D. australis*, rather

than a history of pure vicariant speciation as seen in rain forest fauna. Sequential southern dispersal is further supported by our evidence of increasingly large demographic expansion in southern populations. The mid-Pleistocene fossil of '*D. australis–D. bilineata*' identified by Hocknull *et al.* (2007) also lends support to a southern expansion in *D. australis*, probably in response to the sweeping southerly expansion of woodland habitats. Hocknull *et al.* (2007) identified that a major floral and faunal turnover had occurred between rain forest and woodland elements during this period in conjunction with increasingly drier climatic conditions in SE QLD. Palaeoclimatic and palynological studies also suggest that woodland environments spread from north to south along the north-eastern margin of Australia primarily during the Pleistocene, due to the establishment of cyclical aridity in Australia (Macphail, 1997; Martin, 2006; Hocknull *et al.*, 2007).

Subsequent hierarchical tests were unable to distinguish between a mixed model of allopatric divergence in northern populations with sequential dispersal to the south and a pure dispersal model with southern dispersal via the C NW region. However, we were also unable to reject a mixed model of vicariant northern divergence with southern dispersal via the S BMC region. Colonization of southern areas via the S BMC region may be supported by the fact that demographic expansion could be detected in the S BMC clade, a pattern that has been noted in many other rain forest taxa (Joseph & Moritz, 1994; Schneider *et al.*, 1998; Pope *et al.*, 2000). However, it is unlikely that the recent expansion within the S BMC clade would have resulted in the colonization of southern clades given the large divergences between clades in the northern portion of *D. australis*'s range. Further, the high nodal support for the C NW2 clade as basal to the ME QLD and SE clades would also contradict this hypothesis. Therefore, we conclude that a history of either allopatric vicariance in the north followed by sequential colonization of southern regions, or a model of purely sequential colonization via the C NW region is the most likely historical scenario explaining the biogeographic history of *D. australis*.

CONCLUSIONS

Diporiphora australis presents an important test case for investigations of phylogeographic structure in woodland species in comparison to rain forest species in one of the most studied biogeographic systems in the world, the north-eastern Australian coast. We were able to show that *D. australis* shows similar patterns of divergence, not only in a spatial context but also temporally. Despite these congruent patterns, we were able to determine that *D. australis* had undergone an expansion from north to south, resulting in the sequential colonization of newly formed woodland environments across known biogeographic barriers in north-eastern Australia. Our results further highlight the need for broader considerations of the habitat diversity and the importance of detailing evolutionary processes underlying biogeographic pattern in phylogeographic studies. We were able to show that despite the presumption that species living in recently derived environments will have a 'young' history when compared with rain forest systems (Moritz *et al.*, 2005), woodland species can in fact have deep spatial and temporal divergence patterns similar to those seen in wet-restricted species. This study also suggests that even with congruent divergence patterns, species history may have disjunct evolutionary trajectories. As such, our results are the first step in the formation of a genetic framework for the conservation of woodland habitats in north-eastern Australia.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Haplotype, specimen and locality data for *Diporiphora australis*.

Appendix S2 Complete BEAST output tree for *Diporiphora australis*.

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BIOSKETCHES

Danielle Edwards studies the evolution, systematics and conservation genetics of Australian herpetofauna. She is particularly interested in comparative phylogeography, ecological diversification and responses to historical climates. She is currently a post-doctoral fellow in Lacey Knowles's lab at the University of Michigan.

Jane Melville studies the evolution, systematics and ecomorphology of Australian agamids. Her research focuses on understanding adaptive responses of agamids to rapid environmental change and the evolutionary and developmental cues underlying these changes.

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