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Short Communication

Plio-pleistocene diversification and connectivity between mainland and Tasmanian populations of Australian snakes (*Drysdalia*, Elapidae, Serpentes)

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ABSTRACT

The genus *Drysdalia* contains three recognised species of elapid (front-fanged) snakes, distributed across south-eastern Australia (including Tasmania). Here we aim to clarify the biogeography and phylogeographical relationships of this poorly documented region. We conducted molecular phylogenetic and dating analyses, using mitochondrial genes (*ND4* and *cyt-b*). Our analyses suggest that divergence events among the three extant species, and among major lineages within those species, are congruent with Plio-pleistocene climatic variations. Two highly divergent genetic lineages within *Drysdalia coronoides* occur in Tasmania. Molecular dating suggests that these lineages were isolated from the mainland in the Pleistocene.

1. Introduction

Pliocene and Pleistocene climatic variations had wide-ranging impacts on the genetic structure of modern-day populations of many species within North America and Europe. Studies of the genetic diversity of terrestrial biota throughout the Holartic reveal a general pattern involving southern refugia and northern recolonisation routes (Hewitt, 2004). Populations were isolated in refugia by barriers such as mountains and seas, leading to a diversity of post-glacial recolonisation patterns (e.g. Hewitt, 1999). In contrast to the numerous phylogeographic studies of high-latitude species in the northern hemisphere, the potential impact of Plio-Pleistocene climatic oscillations on species from the southern hemisphere has been examined only rarely except in Patagonia (South America), where the phylogeography of lizards is well documented (genus *Liolaemus*, e.g. Avila et al., 2006; Morando et al., 2007, 2008). Within Australia, for example, the most detailed DNA-based analyses of historical biogeography come from tropical rather than high-latitude areas (e.g. the wet Tropics of northeastern Australia: Hugall et al., 2002; Moritz, 2002; Hewitt, 2004).

In south-eastern Australia, palynological evidence suggests that the Miocene–Pliocene boundary (5 Myr) marked the commencement of a transition in vegetation types: the previously widespread forests were replaced with more open vegetation by the end of the Pliocene (2 Myr; Markgraf et al., 1995; Gallagher et al., 2003; Byrne et al., 2008). During this period, the climate fluctuated between cool–dry and warm–wet, with a general cooling-drying progression during the Pliocene (Gallagher et al. 2003). These climatic oscillations intensified during the Upper Pliocene to Lower Pleistocene (0.7–2 Myr), resulting in rapid fluctuation between cool–dry and warm–wet conditions across south-eastern Australia (Markgraf et al., 1995; Byrne et al., 2008). The amplitude and frequency of these glacial–interglacial cycles increased during the Upper Pleistocene (0.7–0.01 Myr; Markgraf et al., 1995).

These strong climatic variations were accompanied by fluctuations in sea level, leading to periodic flooding of the mainland across southern Australia (Holdgate et al., 2003) and (during times of sea-level minima) to land-bridges between offshore islands and the mainland. For example, during glacial cycles the Bass Strait region of south-eastern Australia emerged as a land bridge connecting mainland Australia to Tasmania, with sea level falling to 150 m below present-day levels (e.g. Schultz et al., 2008). Although we would expect such environmental variation to induce multiple cycles of expansion, contraction and fragmentation of temperate biota in south-eastern Australia (Markgraf et al., 1995), palynological data suggest that the impact of climatic variation on the vegetation was weaker in the southern hemisphere than in the northern hemisphere. That lesser impact may reflect factors such as the lower amplitude of Quaternary change, absence of large ice sheets, and failure of full-glacial environments to persist through interglacial periods (Markgraf et al., 1995).

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Nonetheless, those glacial–interglacial cycles may have had significant impacts on many components of the fauna of south-eastern Australia. Molecular phylogeographic analyses of scincid lizards provide some of the best examples of such effects. For example, Chapple et al. (2005) documented a deep phylogeographic break in skinks of the *Egernia whitii* group between northern and southern Victoria, during the late Miocene–Pliocene. In addition, structuring within both the northern and the southern lineages of these lizards is consistent with the effects of Plio–pleistocene glacial–interglacial cycles. Similar structuring is found in frogs of the *Litoria citropa* species group (Donnellan et al., 1999), *Crinia signifera* (Symula et al., 2008), and *Limnodynastes* (Schaüble and Moritz, 2001). Other studies have documented a genetic break between western and eastern lineages in Victoria, for example in the velvet worms *Planipapillus* (Rockman et al., 2001), and the froglet *C. signifera* (Symula et al., 2008). In contrast, tigersnakes *Notechis scutatus* display a lack of clear structure in south-eastern Australia, coupled with low genetic diversity (Keogh et al., 2005). This latter pattern may reflect a strong reduction of the population during the last glacial maxima, followed by a recent expansion, or continuous gene flow. Clearly, more lineages have to be studied to allow for generalisations about biogeographical patterns linked to Plio-pleistocene climatic variation in south-eastern Australia.

In the present study, we performed molecular phylogenetic and dating analyses within the snake genus *Drysdalia* (Serpentes, Elapidae), using mitochondrial genes. The genus is endemic to south-eastern Australia (including Tasmania), and consequently is an interesting candidate to clarify the impact of Plio-pleistocene climatic variation on fauna in this region. We address two main questions: (i) do patterns of diversification within the genus correlate with major geological and/or climatic changes? and (ii) when were populations of *Drysdalia coronoides* on the island of Tasmania and the mainland last connected?

2. Materials and methods

2.1. Study species

The genus *Drysdalia* comprises three recognised species of small (maximum length <50 cm) proteroglyphous (venomous, front-fanged) snakes. All are viviparous, feed primarily on lizards (Shine, 1981), and are secretive and rarely encountered. Previous phylogenetic studies suggested that these snakes are most closely related to a group of larger species with distributions centred on south-eastern Australia (genera *Austrelaps*, *Hoplocephalus*, *Notechis*, *Tropidechis*: Keogh, 1998; Sanders et al., 2008). The south-western *"D. coronata"* traditionally has been included within *Drysdalia* also (e.g., Shine, 1981), but recent work based on molecular and morphological data resulted in the reallocation of that species to the genus *Elapognathus* (Keogh et al., 2000). The most distinctive species within *Drysdalia* is *D. coronoides*, a relatively heavy-bodied, widely-distributed and variably-colored species, and the only one found in Tasmania (Fig. 1). Dissections of preserved museum specimens suggest life-history divergence between Tasmanian and mainland populations, with less frequent reproduction in the Tasmanian (coldest-climate) populations (Shine, 1981). The other two taxa (*D. mastersii* and *D. rhodogaster*) are more slender-bodied, and are restricted to the south (*mastersii*) and east (*rhodogaster*) of mainland Australia (Fig. 1; Table 1).

2.2. DNA extraction and amplification

Total cellular DNA was isolated from tail clips and livers, and tissues were placed in 200 μL of 5% Chelex containing 0.2 mg/mL of proteinase K, incubated overnight at 56 °C, boiled at 100 °C for 10 min, and centrifuged at 12,000 rpm for 10 min. The supernatant, containing purified DNA, was preleved and stored at −20 °C. Double-stranded DNA amplifications of cytochrome-b (*cyt-b)* were performed with the primer pairs Elapid Cytb Lb/tRNA-ThrA, and those of NADH dehydrogenase 4 (*ND4)* with the primer pairs ND4/LEU (see Sanders et al. 2008). Amplification conditions included a hot start denaturation of 95 °C for 3 min, followed by 35 cycles of 95 °C for 45 s, 50 °C annealing temperature for 45 s, 72 °C for 90 s, and a final extension of 72 °C for 7 min. Amplified products were sequenced with a 3730xl DNA analyzer (Applied Biosystems).

2.3. Phylogenetic analyses

The sequences were aligned by eye. Concordance of the two genes (*cyt-b & ND4)* used to construct the dataset was evaluated with the partition homogeneity test implemented with PAUP* (Swofford, 2001). As the partition homogeneity test revealed no incongruence between the two genes ($P = 0.88$), the analyses were conducted on concatenated sequences (797 bp for *cyt-b* + 703 bp for *ND4*); all codon positions were used. For the combined dataset, trees were rooted using sequences of Old World elapids, i.e. *Naja naja* (*ND4*: EU546997; *cyt-b*: EU547039; from GenBank) and *Bungarus fasciatus* (*ND4*: EU547037; *cyt-b*: EU547086; from GenBank), based on their relationship to Australian elapids (Sanders et al., 2008). Maximum parsimony (MP) analyses on the combined dataset were performed using Paup* 4.0b10 (Swofford, 2001) with 100 random additions of sequences followed by tree bisection and reconnection (TBR) branch swapping, and keeping at most 100 trees at each replicate. Branch support was estimated from 1000 bootstrap re-samples using the same heuristic settings.

For maximum likelihood (ML), models of DNA substitution were selected using the Bayesian Information Criterion (BIC; Schwarz, 1978) implemented in JModeltest 0.1.1 (Guindon and Gascuel, 2003; Posada, 2008). ML heuristic searches and bootstrap analyses (1000 replicates) were performed using PHYML (Guindon and Gascuel, 2003). Bayesian analyses (BA) were performed with the GTR + I + G model, using MrBayes version 3.1.2, which is the closest model to the TrN + I + G (selected by JModeltest 0.1.1) implemented in this software (Huelsenbeck et al., 2001). Two independent runs were performed, each consisting of four parallel MCMC chains of 3 million generations, allowing a good convergence of the independent runs (the average standard deviation of split frequencies being lower than 0.01). Burn-in was assessed by comparing the mean and variance of log likelihoods, both by eye and using the program Tracer v1.4 (http://beast.bio.ed. ac.uk/Tracer). Tree parameters reached stationarity after a burn-in period of 100000 generations. Optimal trees were then sampled every 100 generations to obtain the final consensus BA tree and associated posterior probabilities.

2.4. Dating

The molecular clock hypothesis was tested following Posada and Crandall (1998) by calculating the log likelihood score with molecular clock enforced and comparing it with the log likelihood previously obtained without enforcing the molecular clock, with Paup* 4.0b10 (Swofford, 2001). The molecular clock hypothesis was rejected because the likelihood of the tree without molecular clock was significantly improved (ΔLnL = 65.89, ddl = 38, $P < 0.01$). We used a Bayesian inference implemented in the software BEAST v1.4 (Drummond and Rambaut, 2006) to estimate divergence times between our taxa. Analyses were performed as in the study of Sanders et al. (2008) on the phylogeny of Australasian elapids and sea snakes. A Yule branching process was chosen, this tree prior is being most suitable for trees describing the relationships

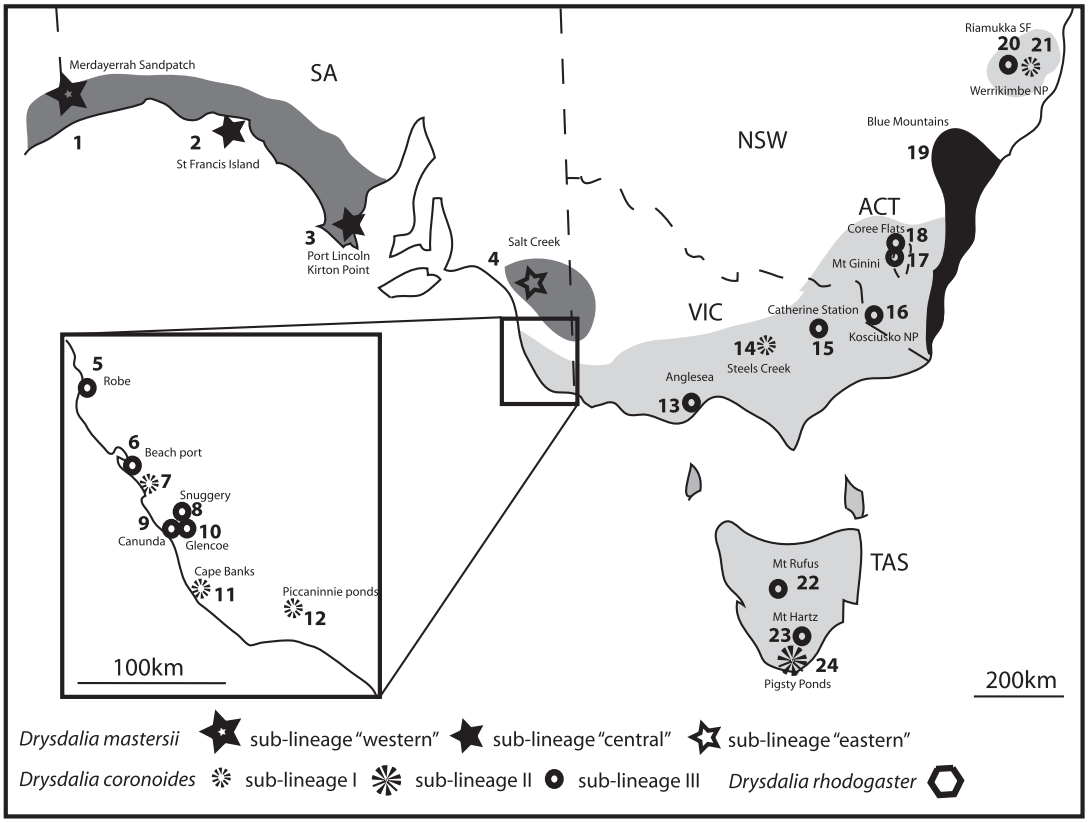

Fig. 1. Geographic ranges of elapid snakes (*Drysdalia*), locations of tissue samples and main mitochondrial lineages (star: *D. mastersii*; circle: *D. coronoides*; hexagone: *D. rhodogaster*), including current distribution of the species (half-tone grey: *D. mastersii*; pale grey: *D. coronoides*; dark grey: *D. rhodogaster*).

between individuals from different species, and a relaxed clock with a uncorrelated and log-normally distributed rate change across branches (Drummond and Rambaut, 2006).

Sanders and Lee (2008) performed molecular dating to estimate the radiation of the Australasian venomous snakes based on two long nuclear genes and several reliable fossils of lizards and snakes allowing a good estimate of the major diversification events (i.e., vipers vs. colubrids + elapids, 34 Myr; Acrochordids vs colubroids, 38 Myr; Scolecophidians vs alethinophidians, 97 Myr; *Shinisaurus* vs *Varanus*, 83 Myr; Scincomorphs vs. lacertoids + toxicoferans, 168 Myr). As no reliable elapid fossils are available as molecular clock calibrations, we performed our analyses as in Sanders et al. (2008). Consequently, two well-supported divergences, with robust molecular dates estimated from Sanders and Lee (2008) were used: the splits between (i) *Laticauda* and all remaining hydrophiines (i.e. the oxyuranines), and (ii) *Micropechis* and other sampled oxyuranines.

As in Sanders et al. (2008), we assigned to the "*Micropechis* – oxyuranines" calibration a lognormal distribution and a hard lower bound of 4.5 Myr corresponding to the lowest value sampled in the posterior distribution of Sanders and Lee (2008); a prior mode of 10 Myr corresponding to the posterior mean; and a 95% confidence interval of 14.2 Myr corresponding to the 95% highest posterior distribution (HPD). Similarly, we assigned to the "*Laticauda* – Oxyuraninae" calibration a lognormal distribution and a hard lower bound of 6.5 Myr, a mode of 13 Myr and a 95% confidence interval of 18 Myr. We also applied a hard upper bound of 30 Myr (following Sanders and Lee, 2008; Sanders et al. 2008) on the divergence between the outgroup (*Naja naja* and *Bungarus fasciatus*) and ingroup to avoid overestimation of divergence dates due to overparameterisation (Sanderson, 2003).

3. Results

3.1. Phylogenetic analyses

The 31 *Drysdalia* samples showed 29 different haplotypes of 1500 bp (combined dataset) and contained 296 variable sites (19.7%), of which 170 were parsimony-informative (11.3%). As the three phylogenetic methods gave identical arrangements of the main branches, the relationship between haplotypes is given only for the ML analysis in Fig. 2 (TreeBASE submission ID number S10371 and Matrix ID 5136). The *Drysdalia* samples formed a monophyletic unit, as did each of the currently-recognised species within the genus (support values for ML, MP, and BA: 100, 100, 1.0).

Our analyses show that *D. rhodogaster* is the sister species to *D. coronoides* and *D. mastersii* (support values for ML, MP, and BA: 100, 100, 1.0; will be in the same order below). Within *D. coronoides*, three divergent sub-lineages are present, one distributed only on the mainland (sub-lineage I; 100, 100, 1.0), one strictly in Tasmania represented by one sample (sub-lineage II), and the remaining lineage both on the mainland and in Tasmania (sub-lineage III; 100, 100, 1.0). Sub-lineage I and II formed a poorly supported (55, 70, 0.82) monophyletic unit. The two mainland sub-lineages (I and III) were sympatric over large areas, with no clear geographic division (see Fig. 1; sample D73646 from Anglesea belonged to sub-lineage III, but was not included in the final analyses due to missing genetic data). Within *D. mastersii*, three sub-lineages ("western", "central", and "eastern") are present and correspond to different geographic distributions (Fig. 1). However, the number of samples for this species is very limited.

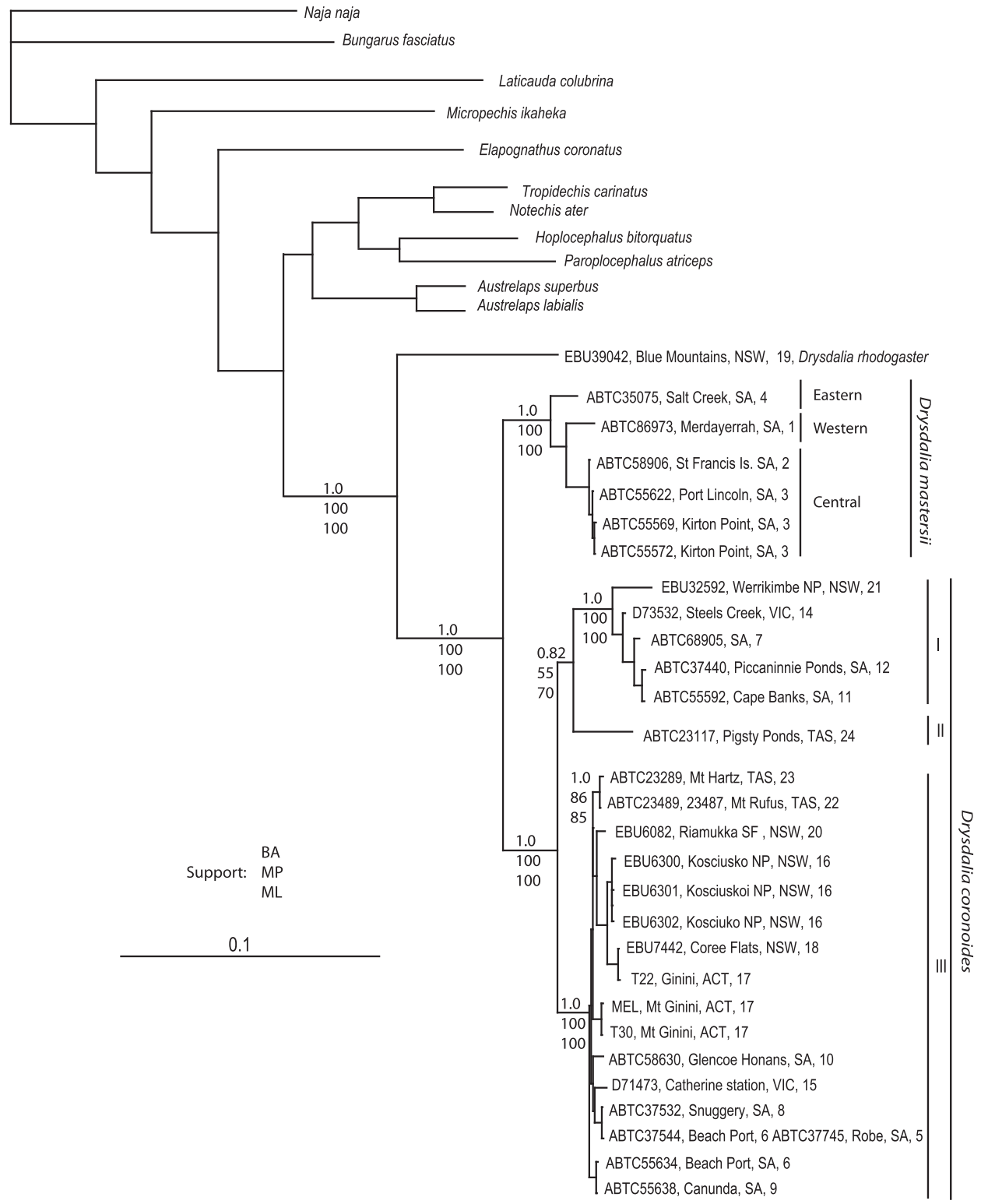

Fig. 2. Phylogeny of the 1501 bp *cyt b* + *ND4* fragment of the elapid snake genus *Drysdalia* in southern Australia analysed using a maximum likelihood (ML) procedure and the TrN + I + G model of substitution. Support values shown for relevant clades only for Bayesian (BA), ML, and maximum parsimony (MP) analyses (percentage of 1000 replications for ML and MP, and posterior probabilities based on 2.9×10^6 generations for BA). Supports for taxa of other genera are not shown, but have been estimated. Codes are as in Table 1.

The Kimura two-parameter genetic distance (K2P) between *D. rhodogaster* and *D. coronoides-mastersii* varied from 12.5% to 13.2%, and the K2P between *D. coronoides* and *D. mastersii* from 6.5% to 7.4%. The distances between the sub-lineages of *D. coronoides* varied from 4.5% to 4.6%.

3.2. Dating

The maximum credibility tree (Supplementary online material 1) was topologically similar to the MRBAYES, MP, and ML trees. Mean posterior estimates for calibration nodes are close to the

Table 1
Species, location (see also Fig. 1), collection code, and Genbank accession numbers of samples used in this study (* sample not used in the phylogenetic and dating analyses due to missing data; see Results section).

Species	Location and coordinates	Coll. Code	GenBank (*cyt-b*, *ND4*)
Drysdalia coronoides	Pigsty Ponds, TAS, 24	ABTC23117	GU062825, GU062855
Drysdalia coronoides	Mt Hartz, TAS, 23	ABTC23289	GU062826, GU062856
Drysdalia coronoides	Mt Rufus, TAS, 22	ABTC23487	GU062828, GU062858
Drysdalia coronoides	Mt Rufus, TAS, 22	ABTC23489	GU062827, GU062857
Drysdalia coronoides	0.6 k SSE Piccaninnie Ponds, SA (380315 S 1405635 E), 12	ABTC37440	GU062829, GU062859
Drysdalia coronoides	16.7 k W Snuggery, SA (373958 S 1401429), 8	ABTC37532	GU062830, GU062860
Drysdalia coronoides	12.5 k ESE Beachport, SA (373026 S 1400857 E), 6	ABTC37544	GU062831, GU062861
Drysdalia coronoides	14.5 k NE Robe, SA (370342S 1395117E), 5	ABTC37745	GU062832, GU062862
Drysdalia coronoides	8.2 k ESE Cape Banks Lighthouse, SA (3756S 14025E), 11	ABTC55592	GU062833, GU062863
Drysdalia coronoides	11.5 k SE Beachport, SA (3730S 14010E), 6	ABTC55634	GU062834, GU062864
Drysdalia coronoides	Canunda CP, SA (37 35 S 14009 E), 9	ABTC55638	GU062835, GU062865
Drysdalia coronoides	5.4 k SSE Glencoe Honans Scrub, SA (374358S 1400857E), 10	ABTC58630	GU062836, GU062866
Drysdalia coronoides	SA (371221S 1395539E), 7	ABTC68905	GU062837, GU062867
Drysdalia coronoides	Catherine Station, VIC (3702S 14650E), 15	D71473	GU062850, GU062880
Drysdalia coronoides	Steels Creek, VIC (3735S 14522E), 14	D73532	GU062843, GU062873
Drysdalia coronoides	Anglesea Heathlands, VIC, 13	D73646	GU066316* (ND4)
Drysdalia coronoides	Riamukka State Forest, NSW, 20	EBU6082	GU062847, GU062877
Drysdalia coronoides	Kosciusko National Park, Spencer Creek crossing on summit rd, 16	EBU6301	GU062845, GU062875
Drysdalia coronoides	Kosciusko National Park, Spencer Creek crossing on summit rd, NSW, 16	EBU6302	GU062848, GU062878
Drysdalia coronoides	Kosciusko National Park, main range walking track from Charlottes Pass, NSW, 16	EBU6300	GU062844, GU062874
Drysdalia coronoides	Coree Flats, NSW, 18	EBU7442	GU062846, GU062876
Drysdalia coronoides	Werrikimbe National Park, NSW, 21	EBU32592	GU062849, GU062879
Drysdalia coronoides	Mt Ginini, ACT, 17	T22	GU062852, GU062882
Drysdalia coronoides	Mt Ginini, ACT, 17	T30	GU062853, GU062883
Drysdalia coronoides	Mt Ginini, ACT, 17	Mel	GU062851, GU062881
Drysdalia mastersii	21 k ENE Salt Creek, SA (360226 S 1395121 E), 4	ABTC35075	GU062838, GU062868
Drysdalia mastersii	Kirton Point Caravan Pk, SA (3443 S 13553 E), 3	ABTC55572	GU062839, GU062869
Drysdalia mastersii	Port Lincoln, SA (3444 S 13552 E), 3	ABTC55622	GU062840, GU062870
Drysdalia mastersii	St Francis Island, W End, SA (323000 S 1331630 E), 2	ABTC58906	GU062841, GU062871
Drysdalia mastersii	Merdayerrah Sandpatch, SA (313944 S 1290513 E), 1	ABTC86984	GU062842, GU062872
Drysdalia mastersii	Kirton Point	Sanders et al. (2008)	EU547076, EU547028
Drysdalia rhodogaster	Blue Mountains, NSW, 19	EBU39042	GU062854, GU062884
Austrelaps labialis		Sanders et al. (2008)	EU547029, EU547077
Austrelaps superbus		Sanders et al. (2008)	EU547030, EU547078
Bungarus fasciatus		Sanders et al. (2008)	EU547086, EU547037
Elapognathus coronatus		Sanders et al. (2008)	EU547069, EU547022
Hoplocephalus bitorquatus		Sanders et al. (2008)	EU547031, EU547079
Laticauda colubrina		Sanders et al. (2008)	EU547040, EU546998
Micropechis ikaheka		Sanders et al. (2008)	EU547042, EU547000
Naja naja		Sanders et al. (2008)	EU547039, EU546997
Notechis ater		Sanders et al. (2008)	EU547034, EU547082
Paroplocephalus atriceps		Sanders et al. (2008)	EU547032, EU547080
Tropidechis carinatus		Sanders et al. (2008)	EU547081, EU547033

priors (see Sanders et al. 2008 for details) and similar to those obtained by Sanders et al. (2008): 13.0 Myr (95% HPD: 14.3–11.0) for *Laticauda* vs. Oxyuraninae, and 9.5 Myr (95% HPD: 11.7–8.6) for *Micropechis* vs. other sampled oxyuranines. Using these rate estimates, the divergence between *D. rhodogaster* and *D. coronoides-mastersii* occurred 4.2 Myr ago (95% HPD: 5.2–2.9), and the divergence between *D. coronoides* and *D. mastersii* 2.3 Myr ago (95% HPD: 2.7–1.4). The splits within *D. coronoides* occurred 1.1 Myr (95% HPD: 1.5–0.7) between sub-lineages I vs. II, and 1.25 Myr (95% HPD: 1.71–0.84) between sub-lineages I + II vs. III. Nevertheless, as sub-lineage I and II formed a poorly supported monophyletic unit (albeit monophyletic in all the analyses; fig. 2 and Supplementary online material 1, the dating of this last split is uncertain (support values for ML, MP, and BA, were, respectively, 55, 70 and 0.82). Within sub-lineage III, the divergence between samples from Tasmania and those from mainland Australia occurred 0.2 Myr ago (95% HPD: 0.1–0.3). Within *D. mastersii*, the split occurred 0.7 Myr (95% HPD: 1.1–0.4) between the "eastern" and "central + western" lineages, and 0.4 Myr (95% HPD: 0.7–0.3) between the "central" and "western" lineages.

4. Discussion

Our study confirms the monophyly of *D. coronoides* and *D. mastersii* (*D. rhodogaster* was represented by one sample), with genetic distances of up to 13.2% between the three species (Fig. 2). Our study also demonstrates significant genetic structure largely corresponding to different geographic areas within *D. mastersii*. However, within *D. coronoides* the distribution of the two sub-lineages present in mainland Australia overlaps substantially, and only the sub-lineages present in Tasmania seem to be distributed across two different areas. However, the number of Tasmanian samples analysed is smaller than for mainland Australia (see Fig. 1).

According to our dating analyses, the first divergence was between *D. rhodogaster* from south-eastern Australia and the two other widely distributed species. This divergence occurred in the Pliocene (5.33–1.81 Myr) at the onset of climatic fluctuations between cool–dry and warm–wet periods (Markgraf et al., 1995). Those fluctuations apparently resulted in the replacement of forest by more open vegetation (e.g. Markgraf et al., 1995; Gallagher et al., 2003). Later, in the Pliocene (5.33–1.81 Myr) and Pleistocene (1.80–0.011 Myr), a succession of breaks occurred, which match well with the divergence of *D. coronoides* and *D. mastersii*, respectively in south-eastern and south-western Australia, and to the different sub-lineages of *D. coronoides*. This period was characterised by intense climatic oscillations, with rapid fluctuations between cool-dry and warm-wet conditions across south-eastern Australia (e.g. Byrne et al., 2008). In addition, these climatic fluctuations affected sea levels, and hence terrestrial connectivity between mainland Australia and Tasmania (Schultz et al., 2008). In keeping

with this scenario, the first differentiation of *Drysdalia* in Tasmania occurred in the Pleistocene (sub-lineage II), as well as the differentiation within sub-lineage III, corresponding with the increased amplitude and frequency of the glacial–interglacial cycles (Markgraf et al., 1995).

The only reptile species endemic to Tasmania are skinks of the genus *Niveoscincus* (Hutchinson et al., 2001). Although these lizards thus have a long insular biogeographical history, all the other Tasmanian species are widespread in mainland Australia, with no insular subspecies described. Consequently, most of these reptile lineages probably dispersed between the mainland and Tasmania or were isolated only recently. The presence of two lineages of *Drysdalia* in Tasmania, one of them apparently endemic and diverging between 1.71–0.84 Myr (95% HPD), is thus of great interest. The apparent restriction of this lineage to a small part of Tasmania raises conservation and management concerns. Nonetheless, more samples are needed to clarify their distributions, as well as to explore the possibility of other lineages in southern Tasmania. The phylogenetic position of sub-lineage II is poorly supported, although its genetic distance to the other sub-lineages is substantial (>4%). Finally, diversification within *D. mastersii* seems to have followed a similar timescale as for *D. coronoides*, with conditions in the Pleistocene coincident with the divergence among populations in eastern, central, and western South Australia.

These analyses suggest that climatic variations during the Plio-pleistocene significantly affected *Drysdalia*, leading to the formation of distinct lineages across the distribution of the genus. In addition, the Pleistocene divergence between the sub-lineages of *D. coronoides* distributed in the mainland (I and III) is not accompanied by any disparity in current distributions, suggesting that a subsequent lack of barriers between these lineages has allowed admixture of the populations (Fig. 1).

Published data on other phylogenetic lineages in this region suggest a complex picture. Some species exhibit a clear genetic structure in southern Australia, with substantial genetic differences across their range: *Drysdalia* (this study), skinks of the *Egernia whitii* group (Chapple et al., 2005), the froglet *C. signifera* (Symula et al., 2008), the open forest frogs *Limnodynastes* (Schaüble and Moritz, 2001), the velvet worms *Planipapillus* (Rockman et al., 2001) in south-eastern Australia, the froglet *C. georgiana* in south-western Australia (Edwards et al., 2007), the short-tailed Grasswren *Amytornis merrotsyi* in South Australia (Christidis et al., 2008) and the skinks *Lerista bougainvillii* in South and south-eastern Australia (Fairbairn et al. 1998). Plausibly, some of these cases reflect the impact of Plio-pleistocene climatic variations, which created unfavorable climatic conditions and hence, created areas of unsuitable habitat that divided formerly continuous distributions and allowed the development of genetically distinctive local populations. In other groups such as *C. signifera*, however, the geographic breaks among lineages are older and consistent with geological events in the Miocene and early Pliocene, such as the secondary uplift of the Great Dividing Range (e.g. Symula et al. 2008). Yet other species lack genetic structure of this type, and instead exhibit low genetic diversity among populations: for example, in the tiger snake *N. scutatus* (Keogh et al., 2005) and in honeyeaters (Joseph and Wilke, 2007). In these latter lineages, climatic variations may have had greater impact on the species involved, drastically reducing population sizes. Hence, the current distributions of these taxa in southern Australia reflect recent post-glacial expansions.

In conclusion, Plio-pleistocene climatic variations appear to have had different effects on different faunal lineages across southern Australia. The geographic ranges of some lineages became fragmented during the Plio-pleistocene, creating genetically distinctive "western" and "eastern" and/or "northern" and "southern" lineages. Other taxa were more vulnerable to climate variations, and experienced drastic reductions in population size followed by recent expansion and hence, now display no clear genetic structure across south-eastern Australia. Therefore, despite an absence of large ice sheets, and the failure of full-glacial environments to persist through interglacial periods in the southern hemisphere (Markgraf et al., 1995), the impact of Plio-pleistocene climatic variations can still be detected among some components of the vertebrate fauna of southern Australia.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2010.04.028.

References

Avila, L.J., Morando, M., Sites, J.W., 2006. Congeneric phylogeography: hypothesizing species limits and evolutionary processes in Patagonian lizards of the *Liolaemus boulengeri* group (Squamata: Liolaemini). Biol. J. Linn. Soc. 89, 241–275.

Byrne, M., Yeates, D.K., Joseph, L., Bowler, J., Cooper, S., Donnellan, S.C., Kearney, M., Keogh, J.S., Leijs, R., Melville, J., Murphy, D., Porch, N., Williams, M.A., Wyrwoll, K.H., 2008. Birth of a biome: synthesizing environmental and molecular studies of the assembly and maintenance of the Australian arid zone biota. Mol. Ecol. 17, 4398–4417.

Chapple, D.G., Keogh, J.S., Hutchinson, M.N., 2005. Substantial genetic substructuring in southeastern and alpine Australia revealed by molecular phylogeography of the *Egernia whitii* (Lacertilia: Scincidae) species group. Mol. Ecol. 14, 1279–1292.

Christidis, L., Horton, P., Norman, J.A., 2008. Subspeciation in the short-tailed Grasswren (*Amytornis merrotsyi*, Maluridae). Emu 108, 275–282.

Donnellan, S.C., McGuigan, K., Knowles, R., Mahony, M., Moritz, C., 1999. Genetic evidence for species boundaries in frogs of the *Litoria citropa* species-group (Anura: Hylidae). Aust. J. Zool. 47, 275–293.

Drummond, A.J., Rambaut, A., 2006. BEAST v1.4. Available at: http://beast.bio.ed.ac.uk/.

Edwards, D.L., Roberts, J.D., Keogh, J.S., 2007. Impact of Plio-Pleistocene arid cycling on the population history of a southwestern Australian frog. Mol. Ecol. 16, 2782–2796.

Fairbairn, J., Shine, R., Moritz, C., Frommer, M., 1998. Phylogenetic relationships between oviparous and viviparous populations of an australian lizard (*Lerista bougainvillii*, Scincidae). Mol. Phylogenet. Evol. 10, 95–103.

Gallagher, S.J., Greenwood, D.R., Taylor, D., Smith, A.J., Wallace, M.W., Holdgate, G.R., 2003. The Pliocene climatic and environmental evolution of southeastern Australia: evidence from the marine and terrestrial realm. Palaeogeogr. Palaeoclim. Palaeoecol. 193, 349–382.

Guindon, S., Gascuel, O., 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52, 692–704.

Hewitt, G.M., 1999. Post-glacial re-colonization of European biota. Biol. J. Linn. Soc. 68, 87–112.

Hewitt, G.M., 2004. Genetic consequences of climatic oscillations in the Quaternary. Phil. Trans. R. Soc. B. 359, 183–195.

Huelsenbeck, J.P., Ronquist, F., Nielsen, R., Bollback, J.P., 2001. Evolution: Bayesian inference of phylogeny and its impact on evolutionary biology. Science 294, 2310–2314.

Hugall, A., Moritz, C., Moussalli, A., Stanisic, J., 2002. Reconciling paleodistribution models and comparative phylogeography in the Wet Tropics rainforest land snail *Gnarosophia bellendenkerensis* (Brazier 1875). Proc. Natl. Acad. Sci. USA 99, 6112–6117.

Hutchinson, M., Swain, R., Driessen, M., 2001. Snakes and Lizards in Tasmania. Fauna of Australia Handbook 9. University of Tasmania, Hobart.

Holdgate, G.R., Wallace, M.W., Gallagher, S.J., Smith, A.J., Keene, J.B., Moore, D., Shafik, S., 2003. Plio-Pleistocene tectonics and eustasy in the Gippsland basin, southeast Australia: evidence from magnetic imagery and marine geological data. Aust. J. Earth Sci. 50, 403–426.

Joseph, L., Wilke, T., 2007. Lack of phylogeographic structure in three widespread Australian birds reinforces emerging challenges in Australian historical biogeography. J. Biogeogr. 34, 612–624.

Keogh, J.S., 1998. Molecular phylogeny of elapid snakes and a consideration of their biogeographic history. Biol. J. Linn. Soc. 63, 177–203.

Keogh, J.S., Scott, I.A.W., Scanlon, J.D., 2000. Molecular phylogeny of viviparous Australian elapid snakes: affinities of *Echiopsis atriceps* (Storr, 1980) and

Drysdalia coronata (Schlegel, 1837), with description of a new genus. J. Zool. 252, 317–326.
Keogh, J.S., Scott, I.A.W., Hayes, C., 2005. Rapid and repeated origin of insular gigantism and dwarfism in Australian tiger snakes. Evolution 59, 226–233.
Markgraf, V., McGlone, M., Hope, G., 1995. Neogene paleoenvironmental and paleoclimatic change in southern temperate ecosystems – a southern perspective. Trends Ecol. Evol. 10, 143–147.
Morando, M., Avila, L.J., Turner, C., Sites, J.W., 2007. Molecular evidence for a species complex in *Liolaemus bibroni* and phylogeography of the closely related *Liolaemus gracilis*. Mol. Phylogenet. Evol. 43, 952–973.
Morando, M., Avila, L.J., Turner, C., Sites, J.W., 2008. Phylogeography between valleys and mountains: the history of populations of *Liolaemus koslowskyi* (Squamata, Liolaemini). Zoologica Scripta 37, 603–618.
Moritz, C., 2002. Strategies to protect biological diversity and the evolutionary processes that sustain It. Syst. Biol. 51, 238–254.
Posada, D., 2008. JModelTest: phylogenetic model averaging. Mol. Biol. Evol. 25, 1253–1256.
Posada, D., Crandall, K.A., 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14, 817–818.
Rockman, M.V., Rowell, D.M., Tait, N.N., 2001. Phylogenetics of Planipapillus, lawn-headed onychophorans of the Australian Alps, based on nuclear and mitochondrial gene sequences. Mol. Phylogenet. Evol. 21, 103–116.
Sanders, K.L., Lee, M.S.Y., Leys, R., Foster, R., Keogh, J.S., 2008. Molecular phylogeny and divergence dates for Australasian elapids and sea snakes (hydrophiinae): evidence from seven genes for rapid evolutionary radiations. J. Evol. Biol. 21, 682–695.
Sanders, K.L., Lee, M.S.Y., 2008. Molecular evidence for a rapid late-Miocene radiation of Australasian venomous snakes. Mol. Phylogenet. Evol. 46, 1165–1173.
Sanderson, M.J., 2003. R8s: inferring absolute rates of molecular evolution and divergence times in the absence of a molecular clock. Bioinform. Appl. Notes 19, 301–302.
Schaüble, C.S., Moritz, C., 2001. Comparative phylogeography of two open forest frogs from eastern Australia. Biol. J. Linn. Soc. 74, 157–170.
Schultz, M.B., Ierodiaconou, D.A., Smith, S.A., Horwitz, P., Richardson, A.M.M., Crandall, A.C.M., 2008. Sea-level changes and palaeo-ranges: reconstruction of ancient shorelines and river drainages and the phylogeography of the Australian land crayfish *Engaeus sericatus* Clark (Decapoda: Parastacidae). Mol. Ecol. 17, 5291–5314.
Shine, R., 1981. Venomous snakes in cold climates: ecology of the Australian genus *Drysdalia* (Serpentes: Elapidae). Copeia 1981, 14–25.
Schwarz, G., 1978. Estimating the dimension of a model. Ann. Statist. 6, 461–464.
Swofford, D.L., 2001. PAUP* Phylogenetic Analysis Using Parsimony (* and other Methods). Version 4.0b1. Sinauer Associates, Sunderland, MA, USA.
Symula, R., Keogh, J.S., Cannatella, D., 2008. Ancient phylogeographic divergence in southeastern Australia among populations of the widespread common froglet, *Crinia signifera*. Mol. Phylogenet. Evol. 47, 569–580.