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Speciation in the mountains and dispersal by rivers: Molecular phylogeny of *Eulamprus* water skinks and the biogeography of Eastern Australia

Short title: Speciation in mountains, dispersal by rivers

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ABSTRACT

Aim

To develop a robust phylogeny for the iconic Australian water skinks (*Eulamprus*) and to explore the influence of landscape evolution of eastern Australia on phylogeographic patterns.

Location

Eastern and southeastern Australia

Methods

We used Sanger methods to sequence a mitochondrial DNA (mtDNA) locus for 386 individuals across the five *Eulamprus* species to elucidate phylogeographic structure. We also sequenced a second mtDNA locus and four nuclear DNA (nDNA) loci for a subset of individuals to help inform our sampling strategy

for next generation sequencing. Finally, we generated an anchored hybrid enrichment (AHE) approach to sequence 378 loci for 25 individuals representing the major lineages identified in our Sanger dataset. These data were used to resolve the phylogenetic relationships among the species using coalescent-based species tree inference in *BEAST and ASTRAL.

Results

The relationships between *Eulamprus* species were resolved with a high level of confidence using our AHE dataset. In addition, our extensive mtDNA sampling revealed substantial phylogeographic structure in all species, with the exception of the geographically highly restricted *E. leuraensis*. Ratios of patristic distances (mtDNA/nDNA) indicate on average a 30 fold greater distance as estimated using the mtDNA locus ND4.

Main conclusions

The major divergences between lineages strongly support previously identified biogeographic barriers in eastern Australia based on studies of other taxa. These breaks appear to correlate to regions where the Great Escarpment is absent or obscure, suggesting topographic lowlands and the accompanying dry woodlands are a major barrier to dispersal for water skinks. While some river corridors, such as the Hunter Valley, were likely historically dry enough to inhibit the movement of *Eulamprus* populations, our data indicate that others, such as the Murray and Darling Rivers, are able to facilitate extensive gene-flow through the vast arid and semi-arid lowlands of New South Wales and South Australia. Comparing the patristic distances between the mitochondrial and AHE datasets highlights the continued value in analysing both types of data.

Key words: Eastern Australia, Great Dividing Range, gene flow, Murray–Darling Basin, Newer Volcanics Province, Anchored Hybrid Enrichment

INTRODUCTION

Much of the Australian continent is weathered and flat, with little indication of the ancient, eroded landscapes that once existed. In contrast, the margin of eastern Australia is topographically complex, the geological history is younger and relatively well known, and it is from this region that some of the best evidence of Australia's palaeoecology has been derived (Moss & Kershaw, 2000). The Great Divide, and the Great Escarpment to the east (collectively referred to here as the Great Dividing Range) is a

topographic mountain system that extends almost 4000 kilometres down the entire length of the eastern coastline, from the northeastern tip of Queensland (QLD), through New South Wales (NSW) and into Victoria (VIC), before finally fading into the central plain at the Grampians in western VIC. The Great Dividing Range and its biota have been impacted by glacial cycles and the episodic expansion and contraction of forest refuges following the onset of arid conditions in the Miocene. Hypotheses about the location and timing of major vicariance events have been developed based on an understanding of palaeoenvironments and concordant biogeographic patterns of a diverse suite of moisture-dependant taxa (e.g. James & Moritz, 2000; Nicholls & Austin, 2005; Helsewood, Lowe, Crayn, & Rossetto, 2014; Pepper, Barquero, Whiting, & Keogh, 2014; Frankham, Handasyde, & Eldridge, 2015), and more recently, sophisticated spatial analyses have identified evolutionary refugia (Rosauer, Catullo, Van Der Wal, Moussalli, & Moritz, 2015), centres of endemism (Weber, Van Der Wal, Schmidt, McDonald, & Shoo, 2014; Thornhill et al., 2016), and characterised suture zones (Edwards, Crisp, Cook, & Cook, 2017; Peñalba, Mason, Schodde, Moritz, & Joseph, 2017) as well as areas of lineage turnover and lineage range boundaries (Rosauer et al., 2015). In addition, a recently published synthesis provides a detailed review of the geological and landscape evolution of the most common biogeographical barriers in eastern Australia that have long been hypothesised as having been crucial in generating present day diversity of mesic organisms (Bryant & Krosch, 2016). Typically these are dry habitat barriers of open woodland and savannah, developed on lowland regions between mountain ranges.

Water skinks of the genus *Eulamprus* Fitzinger 1843 are among the best-known lizards in Australia. Until recently the generic name was applied to a polyphyletic assemblage (Reeder, 2003; O'Connor & Moritz, 2003), comprising three highly divergent "species groups" (*murrayi*-group, *quoyii*-group and *tenuis*-group) of uncertain relationship. A revision of the Australian *Sphenomorphus*-group skinks confirmed the *E. quoyii* group represented a monophyletic lineage (Skinner, Hutchinson, & Lee, 2013), though their analysis did not include *E. leuraensis*. Subsequently the name *Eulamprus* has been restricted only to species of the *E. quoyii* group as the type species of *Eulamprus* is *Lygosoma quoyii* (Duméril & Bibron, 1839). The *tenuis* group has been assigned to the genus *Concinnia*, while two new genera, *Silvascincus* and *Tumbunascincus*, now accommodate members of the *murrayi* group (Skinner et al., 2013). *Eulamprus sensu stricto* therefore comprises five species, collectively referred to as the "true" water skinks (Greer, 1989) as they typically occur in association with creeks and rivers in dry forests. These are *E. heatwolei* Wells & Wellington, 1984, the yellow-bellied water skink, *E. kosciuskoi* (Kinghorn, 1932), the alpine water skink, *E. leuraensis* Wells and Wellington, 1984, the Blue Mountains water skink, *E. tympanum* (Lönnberg & Andersson, 1913), the highland water skink, and *E. quoyii* (Duméril & Bibron, 1839), the eastern water skink (see Appendix S1 in Supporting Information for details on species' distributions). Their distribution, abundance and ease of maintenance in the lab has led to over a hundred and fifty papers published on various aspects of their biology, including ecology (e.g.

Law & Bradley, 1990; Wilson & Booth, 1998), behaviour (e.g. Stapley & Keogh, 2004; Noble, Wechmann, Keogh, & Whiting, 2013), physiology (e.g. Robert & Thompson, 2000; Murphy, Parker, Murphy, & Thompson, 2011), life history (e.g. Rohr, 1997; Caley & Schwarzkopf, 2004), reproduction (e.g. Schwarzkopf & Shine, 1991; Morrison, Keogh, & Scott, 2002), demography (e.g. Dubey, Sinsch, Dehling, Chevalley, & Shine, 2013), and population genetics and evolution (e.g. Hodges, Rowell, & Keogh, 2007; Dubey & Shine, 2010; O'Connor & Moritz, 2003; Skinner et al., 2013). However, despite this extensive body of work, no phylogenetic hypothesis of relationships among populations from throughout their range has been available for interpreting these data in an evolutionary context.

Here we use detailed new genetic data at three different phylogenetic scales to infer the evolutionary history of *Eulamprus*. We first generated a substantial mtDNA dataset for 386 individuals to provide the dense taxonomic sampling necessary for a comprehensive assessment of phylogeographic structure. To this we added a second mtDNA locus and four nDNA loci for a subset of 84 individuals to help inform our sampling strategy for additional phylogenomic scale assessment. Finally, we used anchored hybrid enrichment (AHE) to sequence 378 nuclear loci for 25 individuals to provide the dense locus sampling required to resolve the deeper relationships and settle long-standing systematic issues within the iconic water skinks.

MATERIALS AND METHODS

Taxonomic sampling

We obtained sequence data from 83 *E. heatwolei,* 61 *E. kosciuskoi,* 5 *E. leuraensis,* 137 *E. quoyii* and 100 *E. tympanum* (Appendix Table S1). We collected specimens from NSW and Victoria (VIC) between 2005 and 2008 (Department of Sustainability and Environment Flora and Fauna Permit No. 10003702 [4.4.2006 – 31.3.2008], NSW National Parks and Wildlife Scientific License No. S12153 [17.12.2007 - 31.12.2008], ANU Animal Ethics Committee: NSW Wildlife Animal Research Authority Proposal F.BTZ.94.06 [21.3.2006 – 31.3.2008] and Australian Museum Animal Ethics Committee: NSW Animal Research Authority 08-08 [21/05/2008 - 31/05/2011]) and the remaining samples were obtained for all unique localities that were available from the collections of Museum Victoria (MV), the South Australian Museum (SAM), and the Australian Museum (AM). Outgroups for analyses include four additional taxa belonging to the *Sphenomorphus* group skink clade; *Eulamprus tenuis*, *Ctenotus leonhardii*, *Hemiergis initialis*, and *Glaphyromorphus darwiniensis*, that represent the four successively nearest lineages to *Eulamprus* recovered by Skinner, Hutchinson and Lee (2013).

Sanger gene sequencing and gene tree estimation

We collected traditional multi-locus data using Sanger sequencing for six loci: two mitochondrial DNA, three single copy protein-encoding nuclear genes and a single nuclear intron. The mtDNA data includes 870 bps of NADH dehydrogenase subunit 4 gene (*ND4*) and the adjacent tRNA genes *Trp* and *Ala*, and 536 bps of the 16S ribosomal RNA gene (*16S*). The nuclear genes consist of 820 bps of recombination activating protein 1 (*RAG1*), 556 bps of prolactin receptor (*PRLR*), 538 bps of intronal ATP Synthetase Subunit B (*ATPSB*) and 725 bps of protein tyrosine phosphatase non-receptor type 12 (*PTPN12*). Details on PCR amplification and cycle sequencing can be found in Appendix S2.

For our large *ND4* dataset, we conducted a maximum likelihood (ML) analysis using RAxML-VI-HPC v7.0.4 (Stamatakis, 2006). We partitioned the dataset by codon position. We then used PartitionFinder2 (Lanfear *et al.* 2016) to establish the best partitioning strategy for the analysis, using unlinked branch lengths, RAxML model of evolution and selected the best model based on the aicc (1 subset partition; [ND4_pos1, ND4_pos2, ND4_pos3]). We implemented the general time-reversible substitution model with gamma-distributed rates among sites (GTR + G), with the best ML tree determined using 20 distinct randomized Maximum Parsimony (MP) starting trees. Bootstrap support was determined using 1000 replicates. Our 6 gene Sanger dataset was used to refine the mitochondrial results so that we could pick samples for our AHE dataset. Initially, we conducted separate ML analyses of individual gene trees in RAxML. We then concatenated the data and partitioned the combined dataset by gene, using the same model conditions described above.

Anchored hybrid enrichment and species tree estimation

The anchored phylogenomics data include 25 *Eulamprus quoyii* group skinks representing all five currently recognised taxa as well as four outgroups (Table 1). Details on extraction, library preparation and assembly can be found in Appendix S3.

To account for differences in phylogenetic estimation as a result of alternate reconstruction methods and data partitions, we used a pluralistic approach to determine relationships among *Eulamprus* skinks. Missing or patchy data may produce undesirable effects (Lemmon, Brown, Stanger-Hall, & Lemmon, 2009), so we initially filtered our set of 378 loci using EAPhy (Blom, 2015), with a focus on retaining only loci for which all samples had data. This resulted in two data sets: a complete set of all 378 loci (all data [AD]; 652,747 bp), and a filtered set of 281 loci with no taxa missing from any alignment (filtered data [FD]; 468,246 bp). For all loci, we generated individual gene trees using RAxML v8.2.21, under GTR + G, with 100 independent tree searches and 100 rapid bootstraps. We then concatenated all loci and

ran a maximum likelihood analysis under the same conditions, with each locus partitioned separately. Because individual loci may differ in their evolutionary history, we implemented a shortcut coalescent (ASTRAL II; Mirarab & Warnow, 2015) and a full Bayesian coalescent method (StarBEAST2; Ogilvie, Bouckaert, & Drummond, 2017). We used RAxML gene trees as our input for ASTRAL analyses, utilizing the 100 bootstraps per locus to undertake multi-locus bootstrapping applied to both the AD and FD datasets. Finally, using StarBEAST2 in BEAST v2.4.7, we ran two independent analyses with chain lengths of 500 million generations for each dataset. We used the results of our ASTRAL analyses to inform the species-level distinctions necessary for StarBEAST analysis. Loci were partitioned individually under the GTR substitution model, with independent trees estimated under a relaxed clock (uncorrelated lognormal) and a birth-death process. After inspecting the runs for stationarity (ESS > 150 for all parameters), we discarded burn-in, and combined the runs, ultimately yielding two species trees. All alignments are available on figshare (DOI 10.6084/m9.figshare.c.4092164).

Comparing nuclear and mitochondrial datasets

In vertebrates, mitochondrial DNA (mtDNA) is known to evolve at a faster rate than nuclear DNA (nDNA) (Allio, Donega, Galtier & Nabholz, 2017). In squamate reptiles, this disparity may result on average in a 25-fold increase in mutation rates, and has considerable impacts on the use of mtDNA and nDNA for phylogenetic reconstruction. To investigate variation among our nuclear and mitochondrial datasets, we plotted the (i) correlation between estimated patristic distances as inferred by our mtDNA (ND4) and nDNA (AHE) maximum-likelihood trees, and (ii) the net phylogenetic informativeness of each locus using PhyDesign (http://phydesign.townsend.yale.edu/). These provide a visual representation of the per-site variation among these data.

RESULTS

Sanger gene sequencing and gene tree estimation

Our dense taxonomic sampling for the mtDNA *ND4* gene provides the basis for clarifying geographic distributions and identifying intraspecific genetic variation (see Appendix Figure S1; for ease of visualisation we constrained the backbone topology to match the AHE tree). *Eulamprus quoyii* has the largest distribution of all the species in the group, ranging from Atherton in far north QLD down the east coast to southern NSW. There are three major lineages within *E. quoyii* (Fig. 2A): a northern lineage from Gladstone to the Atherton Tableland, another lineage around Brisbane, and a third from Kroombit Tops in QLD south to southern NSW and down to SA. *Eulamprus kosciuskoi* comprises seven divergent lineages

restricted to different mountain tops through NSW and eastern VIC (Fig. 2B). *Eulamprus leuraensis* has a very restricted range in the Blue Mountains and Newnes Plateau and is represented in our study by 5 samples from a single locality (Fig 2B). *Eulamprus heatwolei* comprises four major clades (Fig 2C); one (the most divergent) represents populations to the north of the Hunter River, two appear to be very restricted in their geographic distributions in the Blue Mountains NSW and the Cathedral Ranges VIC, while the third is more broadly distributed from Coolah Tops in northern NSW through to VIC with a disjunct population in SA. This third lineage includes other individuals from the Blue Mountains. *Eulamprus tympanum* comprises three major clades (Fig 2D); one broadly distributed through central NSW to the coastal lowlands of southern VIC and into SA, and two genetically divergent, allopatric lineages with much smaller geographic distributions around Dargo in VIC, and the outskirts of the ACT, respectively. Our 6 gene dataset was largely consistent with the *ND4* tree and provided better resolution of the deeper relationships than the mitochondrial data alone. This enabled us to pick appropriate representatives for the AHE dataset.

Anchored hybrid enrichment and species tree estimation
From the original target set of 389 loci, we successfully captured and sequenced 378 across all samples including the outgroups. After filtering with EAPhy to remove loci with missing taxa, the resultant 281 loci had an average contig length of 1568 bp (range: 150–2399 bp). Prior to filtering, gaps and missing data accounted for 2.9% of the total alignment (N = 1,140,683), and filtering resulted in a total alignment including less than 1.1% missing data (N = 285,450).

All phylogenetic methods (maximum likelihood of concatenated data, shortcut coalescent analyses of filtered and unfiltered data, Bayesian coalescent analyses of filtered and unfiltered data) provide a congruent and robust interspecific topology for the genus *Eulamprus*. This consistency among data sets and methods was not previously attainable using a small number of Sanger sequenced loci alone. All individuals sampled remain monophyletic in analyses of phased nuclear data. *Eulamprus tympanum* and *E. heatwolei* are sister taxa, and together are sister to a clade containing *E. leuraensis* and *E. kosciuskoi*. *Eulamprus leuraensis* is nested within *E. kosciuskoi* with robust support, rendering *E. kosciuskoi* paraphyletic. The branch length for the relationship between the *E. tympanum/E. heatwolei* species pair and the *E. kosciuskoi/E. leuraensis* species pair is very short and this is the only relationship for which there is only moderate support. There is strong support for the monophyly of *E. quoyii*, which is inferred as sister to all other *Eulamprus* species in the group. However, there are two deeply divergent lineages within this taxon, the level of divergence commensurate with the differences between other pairs of *Eulamprus* species. With the exception of *E. kosciuskoi*, all species were recovered with 100% bootstrap support as monophyletic.

Comparing nuclear and mitochondrial datasets

Patristic distances for the 22 taxa which overlap between ND4 and AHE datasets confirm a moderate correlation ($r^2 = 0.59$) (Fig.3). Ratios of patristic distances (mtDNA/nDNA) indicate on average a 30 fold greater distance as estimated using ND4. The mitochondrial locus ND4 outperforms all nDNA loci in phylogenetic informativeness, with a peak score nearly an order of magnitude greater than the average AHE locus.

DISCUSSION

A tremendous amount is known about *Eulamprus* ecology and life history however a robust phylogenetic framework has not been available for others to evaluate these data in an evolutionary context. Our comprehensive geographic sampling for all *Eulamprus* species has allowed us to provide the first detailed assessment of the phylogeographic structure of each species in southeastern Australia. In addition, our AHE dataset has allowed us to resolve the species-level relationships among the iconic water skinks.

Phylogeny of Eulamprus *skinks*

Previous hypotheses of relationships within the group based on sections of mtDNA genes and a small number of nuclear loci were unable to provide resolution for interspecific relationships (O'Connor & Moritz, 2003; Skinner et al., 2013). The former study supported the close affinity of *E. kosciuskoi* and *E. leuraensis*, and of *E. quoyii* (inferred to be paraphyletic) to these, however the placement of *E. tympanum* and *E. heatwolei* was ambiguous. Skinner et al. (2013) also recovered *E. quoyii* as the outgroup to the others, but did not recover a sister-group relationship between *E. heatwolei* and *E. tympanum*, instead finding a relationship between *E. heatwolei* and *E. kosciuskoi* (albeit with low support). Using the same data, Pyron et al. (2013) recovered *E. heatwolei* as the sister to *E. tympanum,* with these species sister to *E. kosciuskoi*. *Eulamprus quoyii* was inferred as the outgroup to these, but was placed with *E. leuraensis* (for which they had just a single sequence). Our *BEAST and ASTRAL phylogenies based on the AHE data strongly support the monophyly of *E. quoyii* and place this taxon unambiguously as sister to the remaining species in the group. In line with Pyron et al. (2013) we found strong support for the sister-group relationship between *E. heatwolei* and *E. tympanum.* This accords with the previous taxonomic history of these two species, where they were considered morphological sister-taxa, after being combined together in a single species (see Rawlinson, 1969 & Hutchinson & Rawlinson, 1995). We recovered *E. kosciuskoi* as sister to *E. heatwolei* and *E. tympanum,* though the branch length for this relationship is extremely short and only has moderate support (bootstrap = 82). The close relationship of *E. kosciuskoi*

and *E. leuraensis* also is strongly supported in our AHE phylogeny however *E. leuraensis* is nested within what is currently considered *E. kosciuskoi.*

Despite average mtDNA genetic divergences of up to 8% between species, members of *Eulamprus* are remarkably conservative in many aspects of their biology, including morphology, habitat preference and mating systems (Greer 1989; O'Connor & Moritz 2003). There is extensive overlap in the species ranges (see figure 2), particularly in the Blue Mountains of NSW where the distributions of *E. quoyii*, *E. leuraensis* and *E. heatwolei* overlap, and the Kanangra Plateau, NSW, where the distributions of *E. tympanum* and *E. heatwolei* overlap. At the finer geographic scale, where water skinks co-occur they broadly appear to replace each other at different elevations, with *E. heatwolei* and *E. quoyii* in the foothills, *E. tympanum* in middle elevation areas, and *E. kosciuskoi* on mountain tops (Jenkins & Bartell, 1980). However, there also are areas of habitat sympatry where different species co-occur on the same log (ie. *E. heatwolei* and *E. tympanum*, Hodges et al., 2007).

While differences in habitat preference between the members of the *E. quoyii* group are small, this could have had significant evolutionary consequences given past environmental change (Hodges et al., 2007). The moist forest environments of the east coast have existed in varying patchiness and extent for millions of years, and probably covered much of the continent prior to the onset of arid conditions in the mid-Miocene ~ 15 million years ago (Mya) (Kershaw et al., 1994; Macphail, 2007). This broad climatic shift is thought to have greatly contracted and fragmented formerly extensive and continuous rainforest, profoundly altering the distributions of many species (reviewed in Byrne et al., 2008). O'Connor and Moritz (2003) suggested an ancient divergence between the mainly saxicoline and creek dwelling *E. quoyii* group water skinks and the closely related moist forest genera *Nangura* and *Gnypetoscincus* as well as species in the *Eulamprus tenuis* and *murrayi* complexes (now *Concinnia*, *Silvascincus* and *Tumbunascincus*, see Skinner et al., 2013). More recently a dated phylogeny for the Australian *Sphenomorphus* group estimated this deep divergence to have occurred around 25 (Mya) (Skinner et al., 2013). The crown age of the *E. quoyii* group was inferred to be 12 Mya with subsequent intraspecific divergence around 7 Mya, consistent with mid-late Miocene rainforest fragmentation and the expansion of open forests and woodlands associated with a drier climate (Martin, 2006). This timing is comparable to studies of other taxa from eastern Australia that have dated major interspecific divergences to the late Miocene/Pliocene (Chapple et al. 2011; Moussalli, Hugall, & Moritz, 2005; Byrne et al., 2008; Couper et al., 2008; Dubey & Shine, 2010; Edwards & Melville, 2010). While aridification and rainforest contraction since the mid-Miocene may have driven extinction of rainforest specialists, for species such as *E. quoyii* persisting in now drier forests, these pronounced historical landscape changes may have instead promoted ecological shifts towards more mesic microhabitats (O'Connor & Moritz, 2003; Couper et al., 2008). Considerable differences were found in the genetic signatures of *E. tympanum* and *E.*

heatwolei where they occur in sympatry at Tallaganda State Forest. This has been attributed to palaeoenvironmental changes in the tree line, whereby species with a tolerance for drier conditions associated with higher elevation such as *E. tympanum* are thought to have been less susceptible to extinction during glacial periods compared to *E. heatwolei* that has a preference for more mesic sites associate with lower elevations (Hodges et al., 2007).

Phylogeographic structure

Our detailed mtDNA phylogeny indicates substantial genetic structure within each of the recognised species in the group (see Appendix Figs. S1-S4), with the exception of the geographically limited *E. leuraensis* for which we only had samples from a single locality. Within each species, the majority of intraspecific clades have relatively narrow geographic distributions and are largely allopatric, which may be indicative of broad-scale forest fragmentation in more recent timescales. While the mesic east coast of Australia is generally thought to have been less influenced by the increased dryness, coolness and windiness during glacial cycles, geomorphological and palynological evidence indicates forest refugia during the Last Glacial Maximum (~25 000 yr B.P) were much less extensive than today, and were concentrated in discrete, protected sites along the eastern escarpment (Thom, Hesp & Bryant, 1994). The semi-arid zone is presumed to have extended eastwards into the Eastern Highlands (see Hope, 1989), with widespread semi-arid environments including mobile dune-fields occurring along the eastern margin (Thom et al., 1994). In particular, in some areas (such as the NSW central coast) it is thought that woodland or grass cover was patchy in extent during the LGM exposing bare sand (Thom et al., 1994), whereas for other areas such as the southeast highlands of Victoria there is little evidence for aridity during this period (Kershaw et al., 2007). Unsurprisingly, genetic breaks in *Eulamprus* are largely concordant with lowland dry habitat corridors (see below), suggesting moisture-deficient regions of drier vegetation represent effective barriers to dispersal for the water skinks.

Deep intraspecific divergences that also occur over small geographic distances are seen in *E. kosciuskoi*, a pattern likely related to the distribution of these taxa in geographically subdivided alpine isolates (Gloucester Tops, Barrington Tops, Kosciuszko ranges, and see Appendix Fig. S5). Populations of *E. kosciuskoi* inhabit patchily distributed montane meadows along mountaintops, and these appear to act as "islands" of suitable habitat in a "sea" of lower-altitude woodlands. The "sky island model" predicts that alpine species will have limited gene flow between populations due to their geographic and ecological isolation (Knowles, 2000; McCormack, Huang, & Knowles, 2009). Diversification is expected through the divergent selective pressures generated from altitudinal habitat differences as well as differentiation among different sky islands by genetic drift and/or selection (McCormack et al., 2009). In *E. kosciuskoi*, the northermost samples (New England National Park to Glen Innes, NSW) are very divergent from adjacent, southern New England (Riamukka/Werrikimbe) populations, and similarly in

southern NSW and Victoria there is fragmentation, with distinction of the Brindabellas, Snowy Mountains and Victorian populations, despite their relatively close proximity. A similar phylogeographic pattern was found in the plague skink *Lampropholis delicata*, which has a very similar distribution to *Eulamprus* in mesic forests down eastern Australia (Chapple et al., 2011). This genetically variable species was found to comprise multiple geographically structured non-overlapping clades with a number of divergent populations found on isolated uplands (Kroombit Tops, Blackdown Tableland, Coolah Tops) inland from the main Great Dividing Range (Chapple et al., 2011). Our AHE phylogeny partitions the major clades of *E. kosciuskoi* into a northern and southern group split by the Hunter Valley biogeographic barrier, with *E. leuraensis* affiliated with the southern group. The Hunter Valley barrier comprises extensive lowlands flanking the Hunter River, and is characterised by dry, open woodland and savannah that partitions upland closed forest habitat to the north (e.g. Barrington Tops) and south (e.g. the Blue Mountains) (Bryant & Krosch, 2016). It is also coincident with one of only a handful of regions where the Great Escarpment is absent or obscure (See Fig 5 of Ollier, 1981). Palynological data indicate extensive aeolian instability during the LGM (and presumably at punctuated arid periods of the Neogene) in this region, with evidence of mobile dune-fields found on the terraces of the Hawkesbury River, the lower Hunter River up to Crowdy Head and Point Plomer in the NSW mid north coast (Thom et al., 1994). Disjunctions across the Hunter region have been found in a number of other terrestrial taxa (eg. Chapple et al., 2011; Di Virgilio, Laffan, & Ebach, 2012; Pepper et al., 2014).

Eulamprus quoyii is delineated into a number of clades in our mtDNA phylogeny but partitions into two major lineages in our AHE phylogeny. This deep phylogenetic split is observed around Gladstone on the central QLD coast. This is not one of the commonly identified biogeographic barrriers in the area; the well-known Saint Lawrence Gap (see Bryant & Krosch, 2016) is almost 300 km to the north. However, like the tidal estuaries at Saint Lawrence, Gladstone Harbour also occupies a large estuarine area of Port Curtis. The Boyne and Calliope Rivers which drain the hinterland to the south-west enter the estuary through its south-western coast near Gladstone Harbour (Conaghan, 1966). Much of the landscape to the west of the Port Curtis Estuary consists of a low coastal plain, particularly to the north of the Calliope River. Relief also is subdued along the coast south-east of the Boyne River (Conaghan, 1966). While under present climatic conditions these river systems may be favourable to *Eulamprus*, during arid glacial periods of the Neogene, drier conditions and cooler temperatures are thought to have been particularly pronounced in lowlands (Reeves et al., 2013). Dated alluvial fan sequences from northeast QLD streams show dramatic changes to stream environments during the last glacial cycle (Nott, Thomas, & Price, 2001). The sparser vegetation cover of grasslands and open woodlands on the slopes generated large volumes of sediments into the streams, which are thought to have been much less efficient at transporting sediment as a result of the lower rainfall. This lead to the development of extensive alluvial fan and debris-flow deposits along the base of the escarpment of the east Australian

highlands (Nott, Thomas, & Price, 2001), and may have restricted the movement of *Eulamprus* populations. Another deep, but shallower divergence within *E. quoyii* separates populations either side of the McPherson-Macleay Overlap. This region is biogeographically complex as it is inferred to have driven the disjunction of taxa that inhabit the open and dry forests either side of a chain of upland ranges (Main, McPherson and Border) as well as acting as a dry lowland barrier to dispersal for closed forest species (reviewed in Bryant & Krosch, 2016) as seems the case for *E. quoyii*. It is noteworthy that the deepest divergences within *E. quoyii* all occur in QLD, with much shallower genetic structure along a similar geographic gradient down the NSW coast, as well as through western NSW and into SA (see the Role of Rivers below). The difference in elevation of the Great Escarpment is far greater in the south - up to 1500 m - but less than 50 m in far north Queensland, which suggests this has little to do with high elevation isolates such as seen in *E. kosciuskoi*. These deep splits within *E. quoyii* also directly coincide with a second extensive region where the Great Escarpment is absent or obscure (See Fig 5 of Ollier, 1981). Although there are numerous museum and sight records for *E. quoyii* from this area, which extends more than 350 km from Brisbane to Cania Gorge National Park, it represents a gap in our genetic sampling, other than a divergent lineage represented by a single sample from Fraser Island. Hence, we are unable to resolve the pattern and position of contact and interaction of these two lineages in this region.

The distribution of *E. tympanum* appears to be limited to the north by the Hunter Valley barrier. A broadly distributed clade follows the Great Divide from central NSW to the coastal lowlands of southern VIC and into SA. An additional two divergent lineages with much smaller geographic distributions are found in areas around Dargo in VIC, and the western outskirts of the ACT, respectively. Plateaus such as the Dargo High Plains have been isolated from the main tablelands by erosion (Ollier, 1981), and populations may have been unable to maintain connectivity during inhospitable peri-glacial periods. The population in Namadgi National Park on the western border of the ACT are isolated from those to the east by the low-lying floodplains of the Murrumbidgee River.

The Corangamite water skink *Eulamprus tympanum marnieae* is a recently described subspecies (Hutchinson & Rawlinson, 1995) based on differences in scale counts and distinctive darker colouration that includes bold black longitudinal bars (Wilson & Swan, 2003). Its distribution is restricted to the basalt plains of south-western Victoria between Colac in the southeast and Lake Bolac in the northwest, and is the only water skink occurring within the naturally treeless grasslands of southeastern Australia (Peterson & Robertson, 2011). *Eulamprus tympanum marnieae* also is listed as Endangered on the IUCN red list. We present the first genetic assessment of the subspecies, and our data indicate individuals assigned to the *T. t. marnieae* morphotype are polyphyletic and partition into two geographically adjacent and non-overlapping groups distributed across the basalt plains of Victoria and eastern South Australia (collectively known as the Newer Volcanics Province [NVP]; Boyce, 2013). These groups are nested

within the broadly distributed *E. tympanum* clade mentioned above, which shows little genetic differentiation from central NSW through to eastern SA. The northern group comprises samples from the Hopkins Basin near Lake Bolac, but also includes samples from the Grampians in the west through to Healesville in the east. The southern group comprises individuals directly surrounding Lake Corangamite, and also includes individuals west to Carpenter Rocks in SA. An individual from each group was included in our AHE phylogeny which shows the individual from the "geographic" Lake Corangamite clade (R49610) as sister to the Hopkins Basin individual (D62033), which is nested with the remaining *E. tympanum* samples. Volcanic activity was widespread across eastern Australia throughout the Cenozoic (Wellman & McDougall, 1974), with the most recent phase occurring in the NVP ~ 5000 BP (Boyce, 2013). This volcanism produced extensive lava flows, especially in the Western Plains district of Victoria. The ecological communities associated with this geological substrate are unique (the Southern Volcanic Plain [SVP] is one of Australia's 89 bioregions [http://www.environment.gov.au/land/nrs/science/ibra]), and undoubtedly have been important in shaping the evolution of biota that inhabit the region. Indeed, the grassland legless lizard *Delma impar* appears to have a clade restricted to the volcanics of the NVP (Maldonado, Melville, Peterson, & Sumner, 2012). That the two *E. tympanum* groups share similar dark-coloured morphotypes, but are not each other's closest relatives, suggests potential local adaptation to the peculiar basalt ridges and boulder heaps left by the collapse of lava tunnels (Peterson & Robertson, 2011). Colour variation in reptiles, including melanism, is known to be associated with differences in substrate colour (Norris & Lowe, 1964; Rosenblum, Hoekstra, & Nachman, 2004). Indeed, Hutchinson & Rawlinson (1995) note that melanism is a feature of variation within nominate *E. tympanum*; "A general trend is for rock-dwelling, streamside populations to have a greater development of black dorsal flecking than log-dwelling, forest populations….".

Eulamprus heatwolei has a number of deep lineages. The most divergent clade is distributed along the northern NSW coast, from Newcastle north to New England National Park and appears to be limited to the south by the Hunter Valley barrier. There are two subclades separated by lower elevations that isolate the Barrington massif from the New England Tableland. Two other lineages appear to be very restricted in their geographic distributions in the Blue Mountains NSW and the Cathedral Ranges of central VIC, respectively. Additional sampling of these populations will determine whether these clades reflect true short-range endemism or if they are geographically more widespread. The fourth, more widely distributed clade is distributed from Coolah Tops in northern NSW through to eastern VIC with a disjunct population in SA, with little genetic variation across this wide range (see the Role of Rivers below). We lack sampling between the Cathedral Ranges and eastern VIC to assess the position and nature of any contact between the Cathedral Range and eastern VIC lineages, although there are numerous museum records for the intervening area (Hutchinson & Rawlinson, 1995).

The role of rivers in the distribution of two Eulamprus species

In addition to habitat or landscape barriers that limit gene flow in mesic taxa, riparian corridors also may influence genetic structure by facilitating dispersal and gene flow through otherwise unsuitable habitat (Drake, Blench, Armitage, Bristow, & White, 2011). In inland eastern Australia, the arid and semi-arid regions of western Queensland and New South Wales are traversed by lowland rivers and tributaries of the western flowing Murray-Darling Basin. Our data for *E. quoyii* reveals a clade that extends more than 1200km from coastal northern New South Wales to eastern South Australia through the arid and semi-arid lowlands of the Murray-Darling Basin (see Fig. 2), with very little genetic differentiation across this vast area. These mesic river corridors previously have been inferred to facilitate genetic connectivity between populations of the large terrestrial monitor lizard *Varanus varius* in coastal mesic habitats of eastern Australia with those of South Australia (Smissen et al., 2013), as well as the skink *Lampropholis delicata* which is thought to have reached SA from southern NSW via an inland course rather than from around the southern coast (Chapple et al., 2011). One of our samples (R162652) collected from near Wilcannia in western NSW unambiguously illustrates the Darling River provides the conduit for gene flow across NSW and into SA where a number of other individuals from this clade were sampled. Wilcannia is borderline semi-arid to desert with an average rainfall of just 266mm per year (Australian Government Bureau of Meteorology). That water skinks are maintaining gene flow along this dryland river system, often characterised by waterholes of varying permanence and hydrological connectivity (Bunn, Thoms, Hamilton, & Capon, 2006), is a testament to how remarkably adapted they are to Australian river environments. A second independent example of this long-distance gene flow in *Eulamprus* can be seen in a clade of *E. heatwolei*, where a disjunct population is found on the Fleurieu Peninsula in SA. Interestingly, the distribution of *E. heatwolei* does not extend very far down the present-day Murray River (see Hutchinson & Rawlinson. 1995), or the Murrumbidgee (see Swan, Sadlier, & Shea, 2017), and it does not occur at all on the Lachlan River (see Swan, Sadlier, & Shea, 2017). It is possible that the SA population may have been derived from ephemeral downwash events along Murray and Murrumbidgee Rivers, with any intervening populations now extinct. This is a very different situation to *E. quoyii*, which has a more continuous distribution along the Darling and lower Murray Rivers.

Conclusions

Using both traditional (Sanger) and next-generation sequencing techniques, we have been able to investigate and confidently resolve relationships among *Eulamprus* water skinks at both inter- and intraspecific phylogenetic depths. For the many researchers that use *Eulamprus* as a study system, our phylogeny will allow them to interpret their data in an evolutionary context. In resolving the phylogeny

for *Eulamprus*, we have identified several issues that will need attention. *Eulamprus leuraensis* is nested within what is currently considered *E. kosciuskoi,* rendering the latter taxon paraphyletic. We also found deep genetic structure within *E. quoyii,* and *E. heatwolei* and have found no genetic support for the currently recognised subspecies *E. tympanum marnieae*. This highlights the need for a thorough taxonomic revision of this group and this will be dealt with elsewhere (the taxonomy and nomenclature of *E. quoyii* and *E. heatwolei* are the subject of current morphological studies by G. Shea and H. Pearson). Finally, our results remind us of the duality of mito-nuclear datasets, and the continued value in analysing both. Though rates of molecular evolution and phylogenetic informativeness vary considerably among our sampled mitochondrial and nuclear loci, investigating both provides a means to understand the full evolutionary story.

DATA ARCHIVAL LOCATIONS

Figshare DOI information (10.6084/m9.figshare.c.4092164) is available for:

– Anchored phylogenomic data (nexus format).
– Sanger data alignment, concatenated (nexus format).

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FIGURE CAPTIONS

Figure 1. Phylogenomic analyses provide consistent support of interspecific relationships among *Eulamprus* water skinks, regardless of reconstruction method. Lizard images to the left depict the relative size and appearance of each species. The first and third trees in this figure were constructed using anchored phylogenomics data (nuclear exons—nDNA), resulting in identical topologies between the full (378) and filtered (281) datasets. On these two trees (nDNA starBEAST, nDNA ASTRAL), nodes labelled with a white circle denote fully supported relationships (posterior probability = 1, bootstrap = 100), with all other nodes labelled according to estimated support. Note terminal branch lengths in ASTRAL analysis are fixed, and not to scale. The middle phylogeny has been reconstructed using the mitochondrial locus ND4 (mtDNA), with nodes labelled by a red circle constrained to match the nuclear species tree topology. Intraspecific relationships of the mtDNA tree remained unconstrained, and are used to illustrate the sampling depth, and relative diversity of each species. Labels on the far right of this figure match the nDNA ASTRAL tree, run on phased haplotype data, where each taxon is represented by two terminal tips representing the phased alleles. Sampling data for each individual can be found in Appendix Table S1. Colors designated for each species correspond to sampling maps in Figure 2, and intraspecific mtDNA phylogenies in supplemental materials (Appendix Figs S1–S4).

Fig. 2. Top left is a topographic map of the Australian continent (red = high, green = low, major drainage lines = white). Bottom left map shows finer scale drainage lines (Global Map Australia 1M 2001, Geoscience Australia) overlain on to a digital elevation model image (Shuttle Radar Topography Mission) where light grey equates to areas of high elevation, and dark grey equates to areas of low elevation. Thick dark grey lines indicate biogeographic barriers mentioned in the text. Stippled blue lines indicate the major rivers; Darling River (DR), Lachlan River (LR), Murrumbidgee River (MBR), Murray River (MR). Coloured symbols represent sampling localities for two clades that use rivers to facilitate long distance gene flow. Purple triangles indicate one of the *E. quoyii* clades, while green circles representing our sample localities for one of the *E. heatwolei* clades. State boundaries are shown by thin grey lines. QLD = Queensland, SA = South Australia, NSW = New South Wales, ACT = Australian Capital Territory, VIC = Victoria. Right panel shows the distribution of the five *Eulamprus* species; *E. quoyii* (A, purple), *E. kosciuskoi* and *E. leuraensis* (B, yellow and red, respectively), *E. heatwolei* (C, green) and *E. tympanum* (D, pink). Different coloured shapes on each map refer to major clades within each species, whereas small black + symbols refer to museum locality records. Relevant biogeographic barriers from the left large map have been overlain.

(Photo credit: Stephen Zozaya & Stewart Macdonald).

Fig. 3. The rate of molecular evolution differs considerably among mitochondrial and nuclear loci, providing resolution at differing evolutionary scales. (a) paristic distances as estimated by maximum-likelihood using the mtDNA (*ND4*) and nDNA (AHE) are correlated, providing moderately consistent estimates of molecular distance among taxa (though differing in absolute scale). (b) these distances are, on average, 30-fold greater when estimated using the mitochondrial locus *ND4* alone. (c) at certain evolutionary depths, *ND4* (red line) provides nearly an order of magnitude more phylogenetic information than the average AHE locus (grey lines), however lacks the independent sources of phylogenetic information inherent in next generation multilocus nuclear datasets.
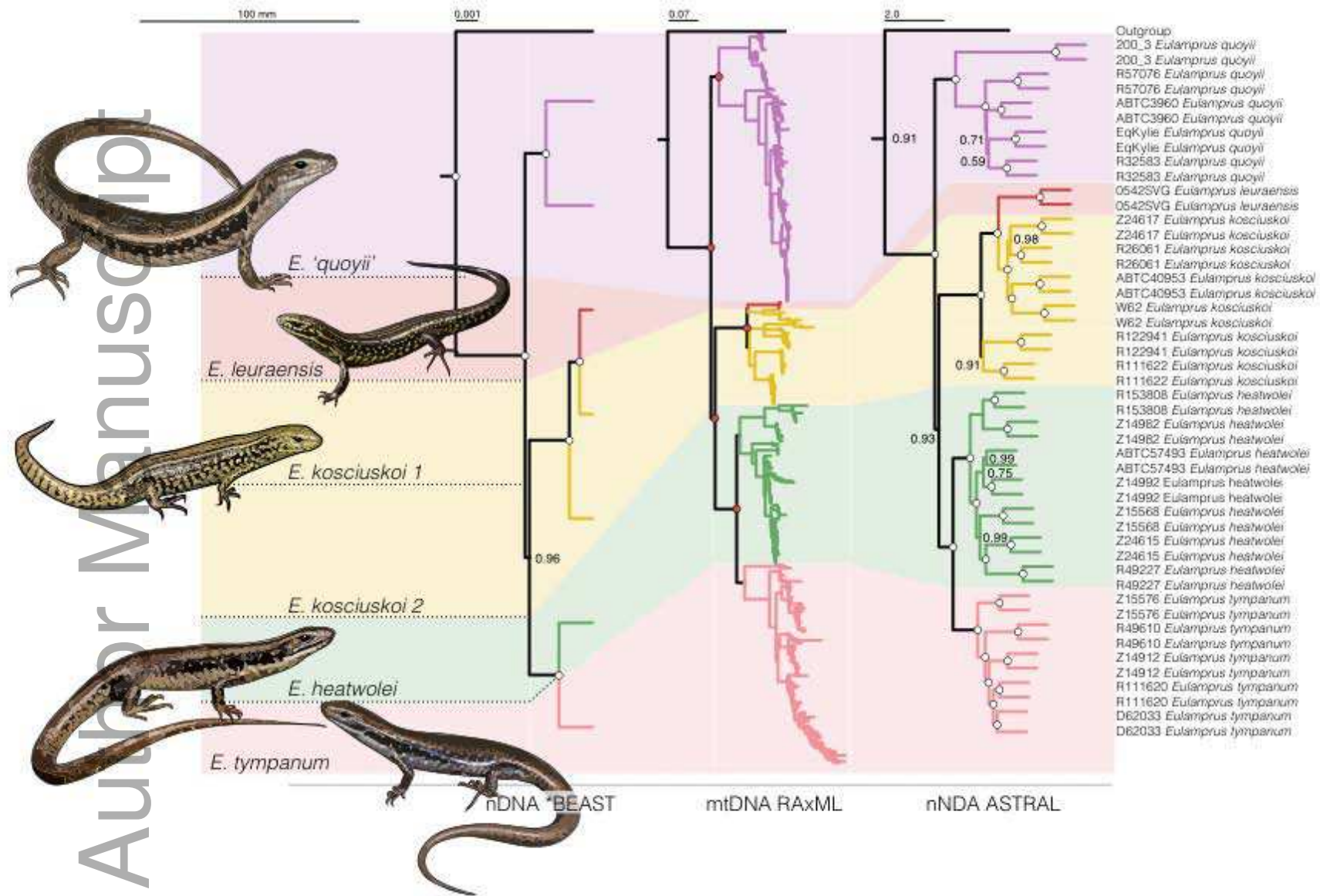
Fig. 2
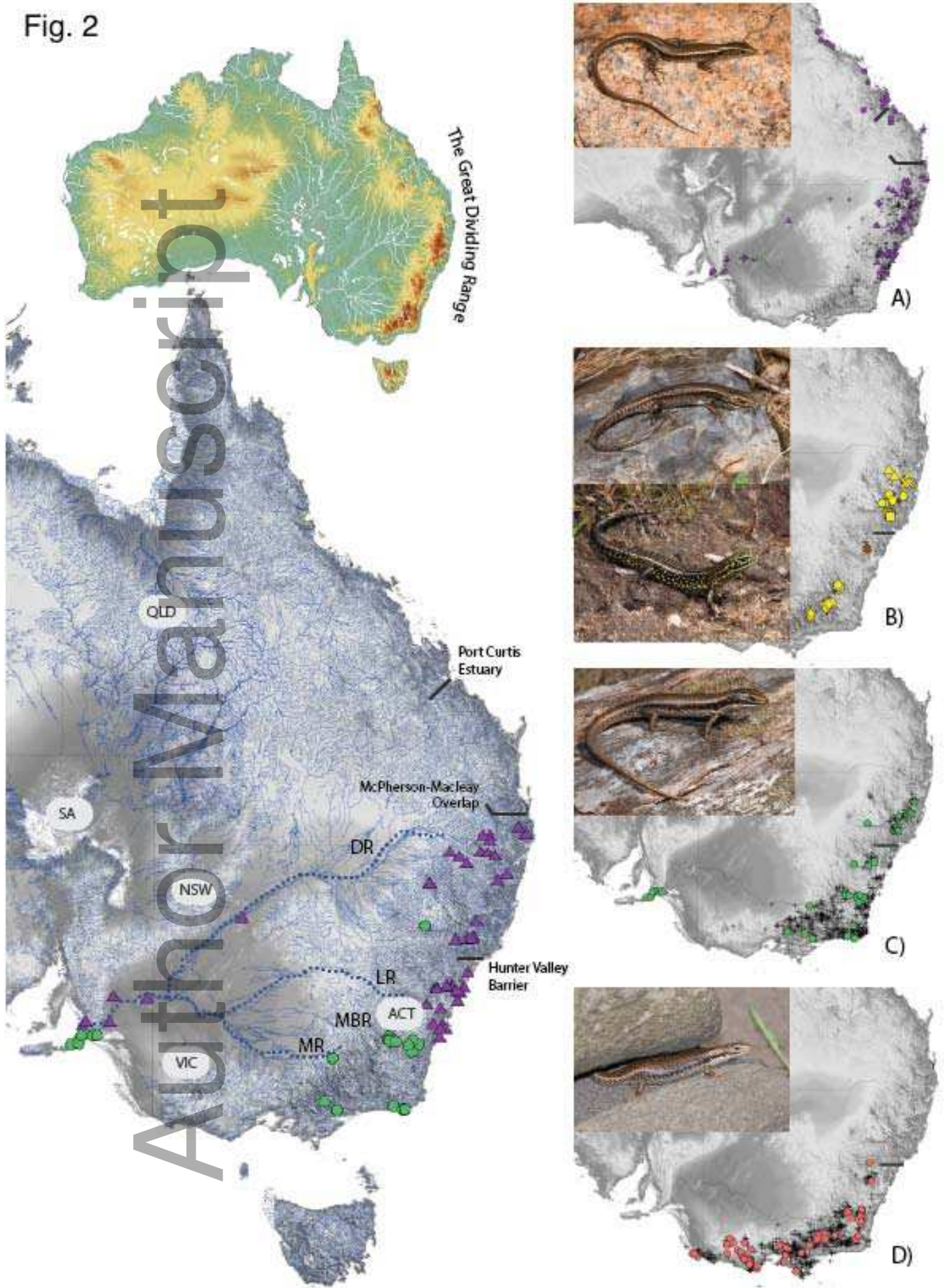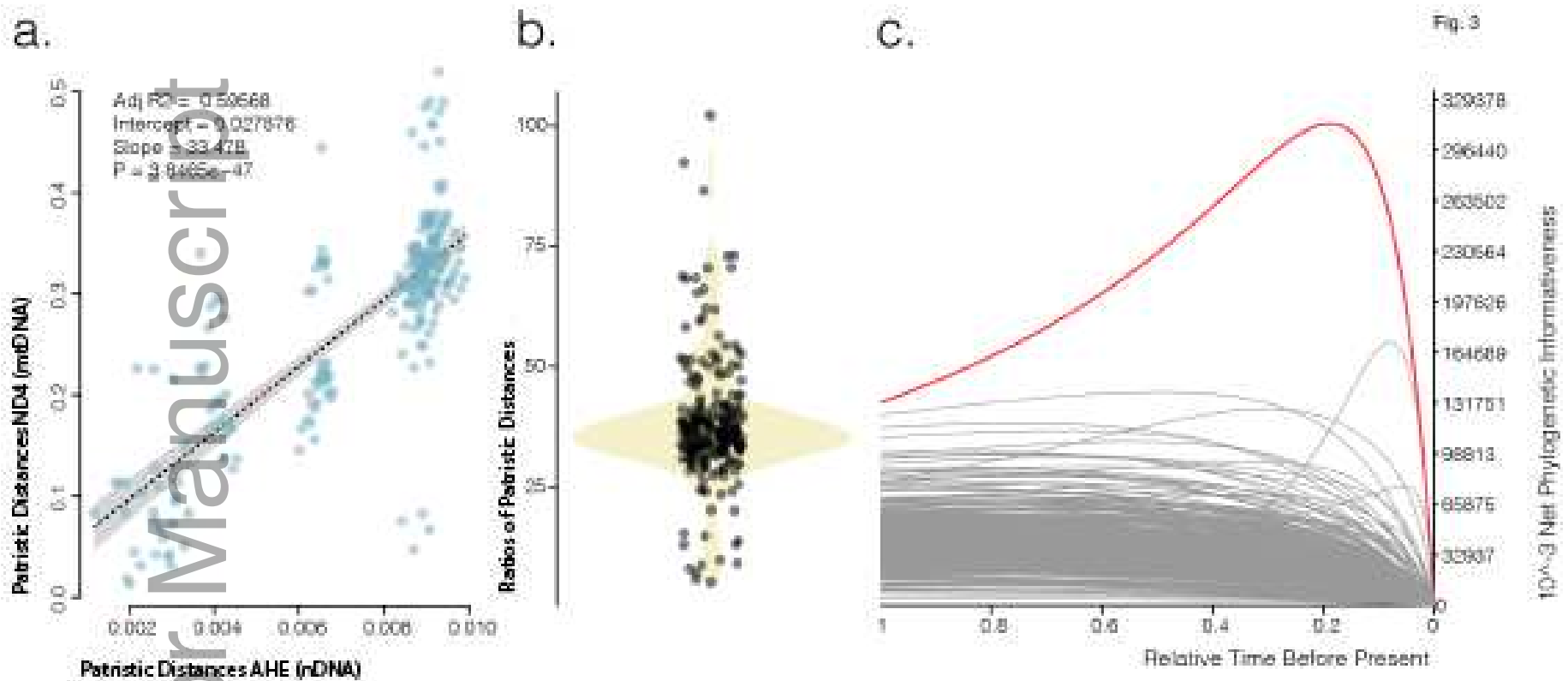
Fig. 3

BIOSKETCH

Mitzy Pepper is a postdoctoral researcher in J. Scott Keogh's lab. Her interests concern the evolution of Australian landscapes, and the associated genetic impacts on the Australian herpetofauna. Joanna Sumner is Manager of Genetic Resources at Museums Victoria. She has research interests in herpetology, conservation genetics, and venom evolution.

Author contributions: JSK, JS and GS conceived the ideas. JS, GS, KH, LS & GP conducted fieldwork and collected samples. JS, KH, IAWS, MP, DR, AL & EL collected molecular data. IGB and MP analysed the data. IGB and MP made the figures. MP lead the writing with assistance from JS, IAWS, JSK and GS.
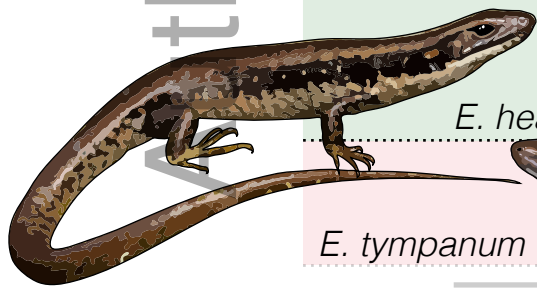
100 mm

0.001

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2.0

Outgroup
200_3 *Eulamprus quoyii*
200_3 *Eulamprus quoyii*
R57076 *Eulamprus quoyii*
R57076 *Eulamprus quoyii*
ABTC3960 *Eulamprus quoyii*
ABTC3960 *Eulamprus quoyii*
EqKylie *Eulamprus quoyii*
EqKylie *Eulamprus quoyii*
R32583 *Eulamprus quoyii*
R32583 *Eulamprus quoyii*
0542SVG *Eulamprus leuraensis*
0542SVG *Eulamprus leuraensis*
Z24617 *Eulamprus kosciuskoi*
Z24617 *Eulamprus kosciuskoi*
R26061 *Eulamprus kosciuskoi*
R26061 *Eulamprus kosciuskoi*
ABTC40953 *Eulamprus kosciuskoi*
ABTC40953 *Eulamprus kosciuskoi*
W62 *Eulamprus kosciuskoi*
W62 *Eulamprus kosciuskoi*
R122941 *Eulamprus kosciuskoi*
R122941 *Eulamprus kosciuskoi*
R111622 *Eulamprus kosciuskoi*
R111622 *Eulamprus kosciuskoi*
R153808 *Eulamprus heatwolei*
R153808 *Eulamprus heatwolei*
Z14982 *Eulamprus heatwolei*
Z14982 *Eulamprus heatwolei*
ABTC57493 *Eulamprus heatwolei*
ABTC57493 *Eulamprus heatwolei*
Z14992 Eulamprus heatwolei
Z14992 Eulamprus heatwolei
Z15568 *Eulamprus heatwolei*
Z15568 *Eulamprus heatwolei*
Z24615 *Eulamprus heatwolei*
Z24615 *Eulamprus heatwolei*
R49227 *Eulamprus heatwolei*
R49227 *Eulamprus heatwolei*
Z15576 *Eulamprus tympanum*
Z15576 *Eulamprus tympanum*
R49610 *Eulamprus tympanum*
R49610 *Eulamprus tympanum*
Z14912 *Eulamprus tympanum*
Z14912 *Eulamprus tympanum*
R111620 *Eulamprus tympanum*
R111620 *Eulamprus tympanum*
D62033 *Eulamprus tympanum*
D62033 *Eulamprus tympanum*

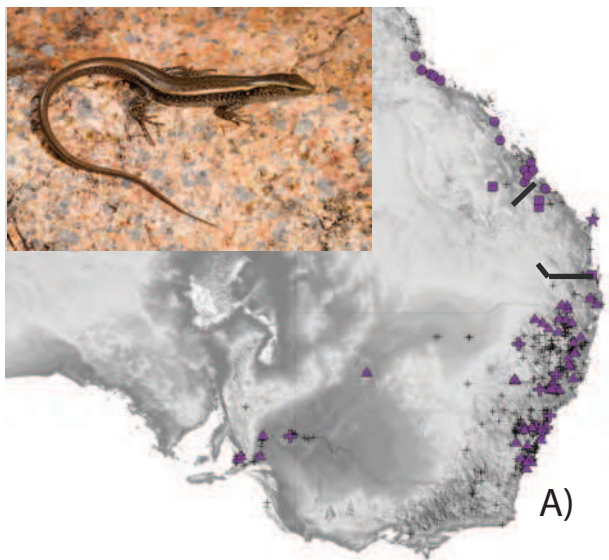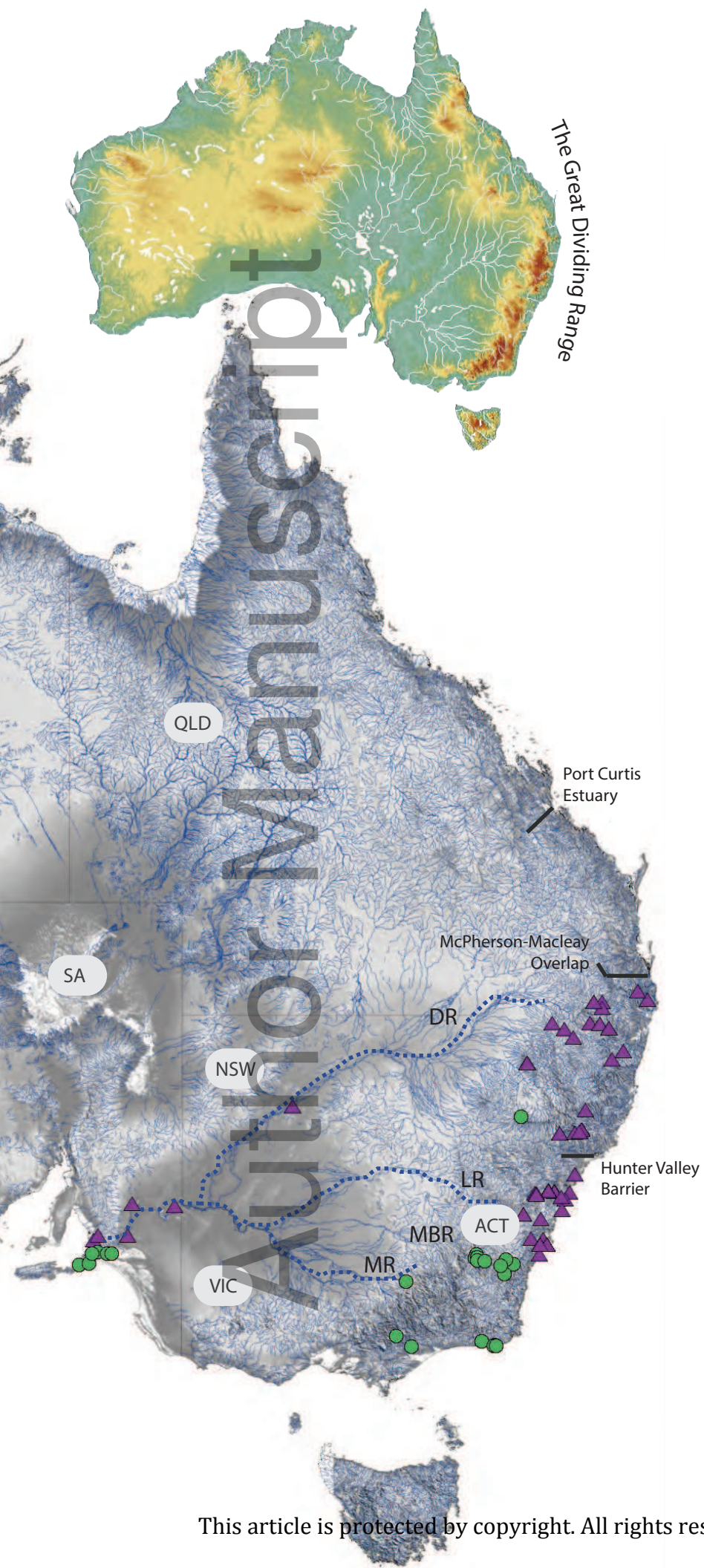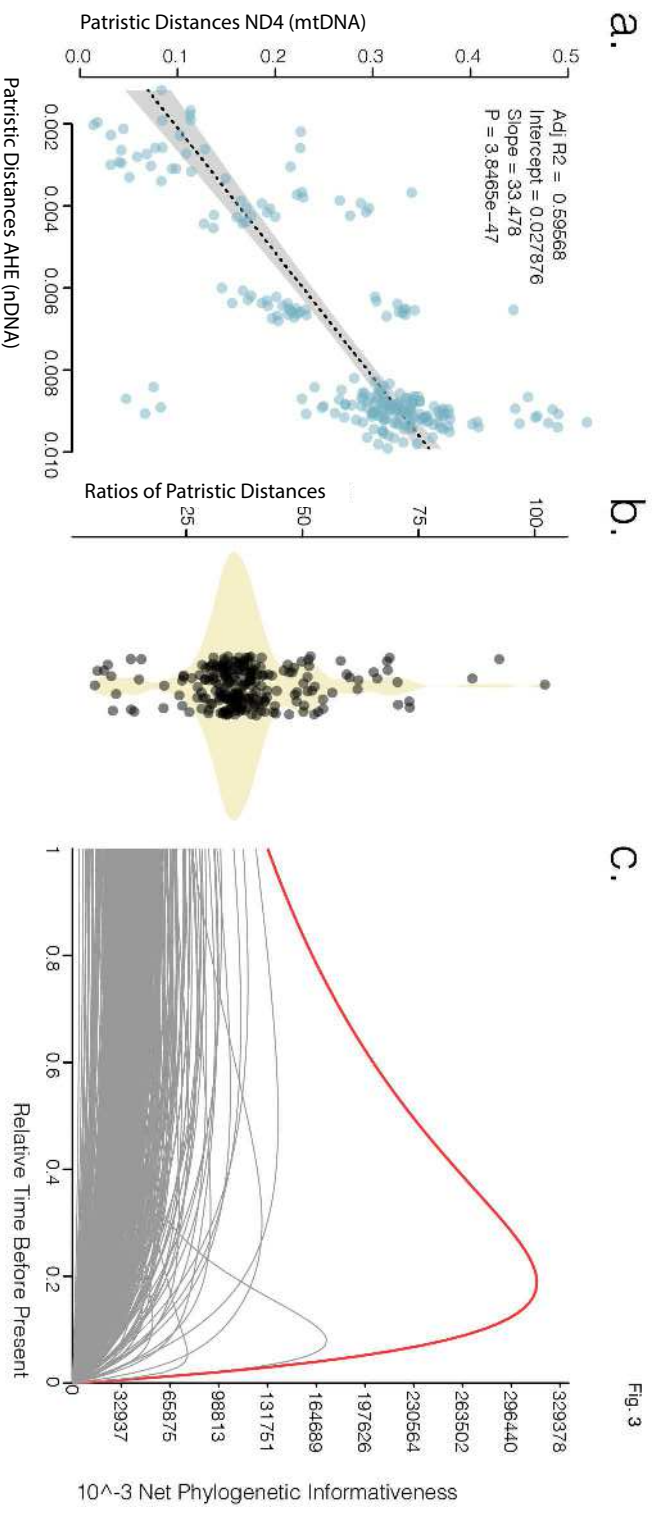E. 'quoyii'

E. leuraensis

E. kosciuskoi 1

0.96

E. kosciuskoi 2

E. heatwolei

E. tympanum

0.91   0.71
        0.59

0.98

0.91

0.93   0.99
        0.75

0.99

nDNA *BEAST   mtDNA RAxML   nNDA ASTRAL

The Great Dividing Range

QLD

Port Curtis
Estuary

McPherson-Macleay
Overlap

SA

DR

NSW

LR

Hunter Valley
Barrier

ACT

MBR

MR

VIC

A)

B)

C)

D)

Author Manuscript

a.

Patristic Distances ND4 (mtDNA)

Adj R2 = 0.59568
Intercept = 0.027876
Slope = 33.478
P = 3.8465e-47

Patristic Distances AHE (nDNA)

b.

Ratios of Patristic Distances

c.

Relative Time Before Present

10^-3 Net Phylogenetic Informativeness

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Fig. 3