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A dated molecular phylogeny of mite harvestmen (Arachnida: Opiliones: Cyphophthalmi) elucidates ancient diversification dynamics in the Australian Wet Tropics

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ABSTRACT

Austropurcellia, a genus of dispersal-limited arachnids endemic to isolated patches of coastal rainforest in Queensland, Australia, has a remarkable biogeographic history. The genus is a member of the family Pettalidae, which has a classical temperate Gondwanan distribution; previous work has suggested that *Austropurcellia* is an ancient lineage, with an origin that predates Gondwanan rifting. Subsequently, this lineage has persisted through major climatic fluctuations, such as major aridification during the Miocene and contraction and fragmentation of forest habitats during the Last Glacial Maximum (LGM). In order to understand *Austropurcellia*'s evolutionary and biogeographic history, we generated DNA sequences from both mitochondrial and nuclear loci and combined this information with previously published datasets for the globally-distributed suborder Cyphophthalmi (i.e., all mite harvestmen). We generated phylogenetic trees using maximum likelihood and Bayesian approaches to date divergences using a relaxed molecular clock. According to our estimates, the family Pettalidae diversified in the late Jurassic, in accordance with Gondwanan vicariance. Within Pettalidae, *Austropurcellia* split from its sister group in the early Cretaceous and began to diversify some 15 Ma later. Therefore, its presence in Australia predates continental rifting—making it one of very few hypothesized examples of Gondwanan vicariance that have withstood rigorous testing. We found a steady rate of diversification within the genus, with no evidence for a shift in rate associated with Miocene aridification. Ages of splits between species predate the Pleistocene, consistent with a "museum" model in which forest refugia acted to preserve existing lineages rather than drive speciation within the group.

1. Introduction

The Australian biota has been shaped by a dramatic geological history, beginning with rifting from the supercontinent Gondwana and continuing through present-day rapid climate change. Australia became isolated from the Gondwanan landmass that would become New Zealand ~60–85 Ma, and rifted from Antarctica ~40–35 Ma (Cook and Crisp, 2005; Hopper, 1996; Rix and Harvey, 2012). Following separation from Gondwana, climate fluctuations have shaped the Australian landscape; although the continent is now dominated by desert habitats, it was once covered by very wet aseasonal forests. Those forest habitats have undergone enormous contraction beginning in the Miocene and continuing through the present. Today, mainland Australia is dominated by arid and semi-arid zones, with forest persisting only in several small and narrow zones close to the southern and eastern coasts (Adam, 1992; BMR Paleogeographic Group, 1990; Cook and Crisp, 2005; Kershaw, 1994; Moritz et al., 2009). Generations of biologists have recognized that vicariance and climatic shifts have both played a role in shaping the Australian biota; however, rigorous tests of biogeographic hypotheses related to these processes have only become possible in the last 20 years with the advent and development of molecular systematics.

Any lineage whose presence in Australia predates the Miocene has endured dramatic shifts in climatic regime, with a variety of

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evolutionary consequences (Byrne et al., 2011). Phylogenies of many Australian vertebrate animal groups such as endemic passerine birds, kangaroos and allies (macropodoids), and bandicoots (peramelids) reveal mesic origins with more recent evolution of lineages specializing in arid habitats, reflecting a history of adaptation to the continent's changing climate (Meredith et al., 2008a,b). On the other hand, many Australian taxa are restricted to forest habitats. Such groups have not adapted to the now-widespread arid habitats of the continent; instead, their history has likely been dominated by extinction and speciation processes as wetter habitats contracted and fragmented. Historically, Pleistocene climatic fluctuations (especially the Last Glacial Maximum [LGM]) have been invoked as a likely driver of diversification in rainforest biota, as in the classic hypothesis of Haffer (1969) in which Amazonian forest contraction and fragmentation were proposed to have led to rapid diversification of fauna. However, dated phylogenies have demonstrated that species-level divergences in many Australian rainforest groups predate the Pleistocene (e.g. Bell et al., 2012; Moreau et al., 2015), and some recent studies have pointed to more ancient Miocene aridification as a probable driver of speciation (e.g. Rix and Harvey, 2012).

Mite harvestmen (Arachnida, Opiliones, Cyphophthalmi) are the subject of this study, and provide outstanding opportunities to test hypotheses related to the history of contraction and fragmentation of Australia's once widespread rainforests, as well as more ancient Gondwanan vicariance. These animals are extremely dispersal-limited, with a typical species range spanning only tens of kilometres in its largest dimension; therefore, their current distribution in space strongly reflects biogeographic history. Currently, two genera of mite harvestmen are recognized in Australia: *Karripurcellia* Giribet 2003 in Western Australia, and *Austropurcellia* Juberthie 1988 in Queensland. Prior to 2007, a handful of Queensland species were described in the New Zealand genera *Neopurcellia* and *Rakaia*; these were subsequently

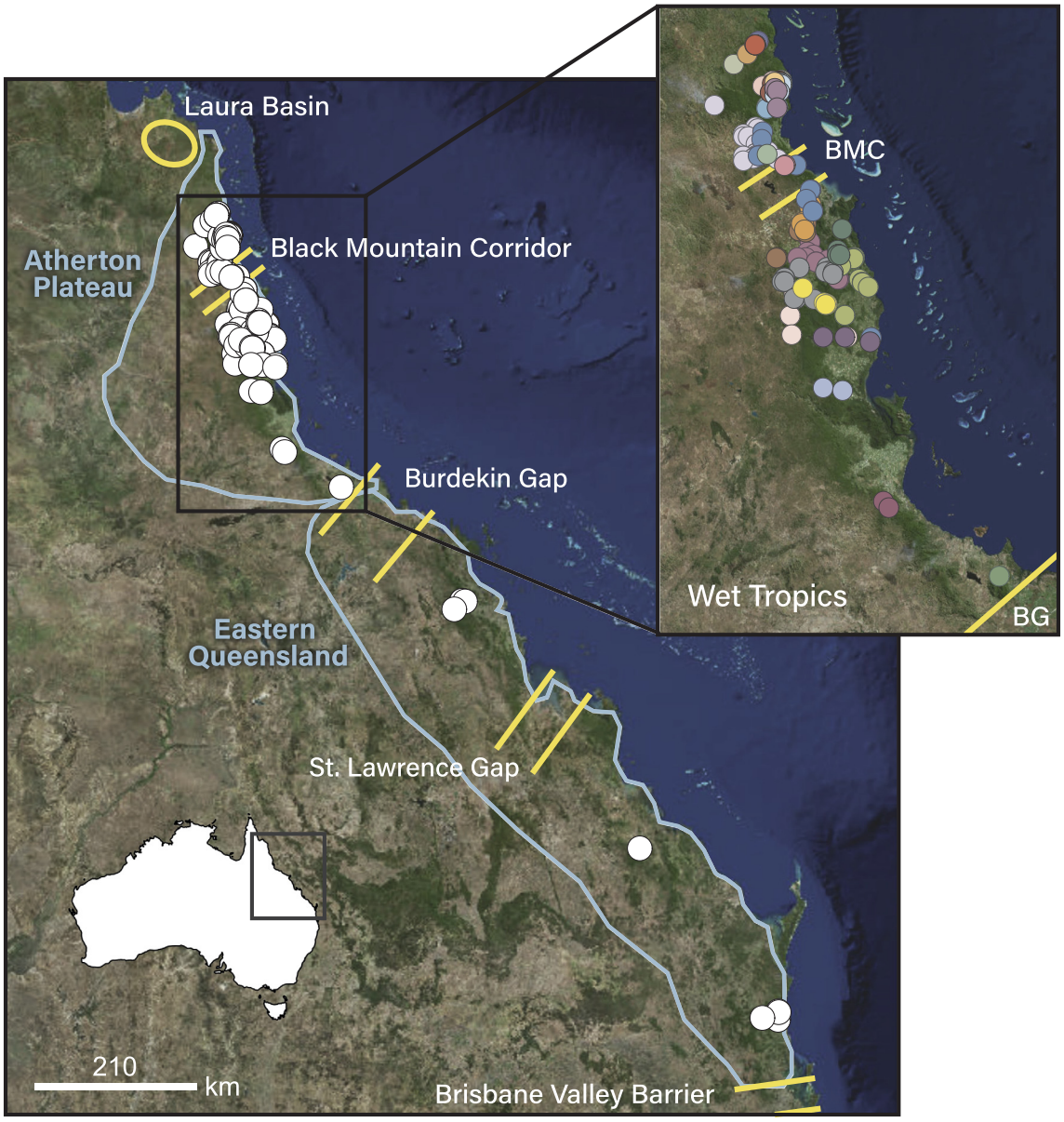

Fig. 1. *Austropurcellia* collection localities, mapped within the context of Queensland's main geographic features. White circles represent all sites from which specimens are known, including those used in this study and museum collections. Yellow lines indicate the approximate location of dry zones, which serve as major biotic transition zones/barriers to dispersal. Coastal areas bounded in blue denote distinct phytogeographical subregions (*sensu* Ebach et al., 2013). Inset map shows sites in the Wet Tropics, which hosts the vast majority of *Austropurcellia*'s diversity; each colour here denotes a different species. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

transferred to *Austropurcellia* by Boyer and Giribet (2007) when phylogenetic analysis of molecular data demonstrated that the Queensland species form a monophyletic group. Currently, there are 25 described species of *Austropurcellia*, 21 of which are endemic to the Wet Tropics (Jay et al., 2016). All but six of these twenty-five species have been described in recent years by our research group (Boyer and Reuter, 2012; Popkin-Hall and Boyer, 2014; Boyer et al., 2015; Jay et al., 2016). These discoveries reflect our own collecting efforts as well as examination of museum material, the bulk of which resulted from decades of Wet Tropics leaf litter arthropod surveys by the Queensland Museum. Given the comprehensive historical nature of collecting campaigns in the Australian Wet Tropics, as well as the restriction of *Austropurcellia* to primary forests, we now feel confident that the majority of the extant diversity of the genus has been described and mapped (Fig. 1).

In the present study, we aim to understand the role that the historical processes of Gondwanan vicariance, Miocene aridification, and Last Glacial Maximum (LGM) climate fluctuations have played in shaping the diversification of *Austropurcellia*. We generate a dated molecular phylogeny for the group in order to answer the following biogeographic questions: 1. Did *Austropurcellia* originate prior to the rifting of Australia from Gondwana? 2. Did diversification rates within *Austropurcellia* accelerate during Miocene aridification, LGM climate fluctuations, or neither?

2. Materials and methods

2.1. Specimen collection and microscopy

Specimens collected by the authors were obtained by leaf-litter sifting and sorting in situ in May-June 2014 and May-June 2015. Once collected, specimens were preserved immediately in 95% ethanol (EtOH). GPS points were taken at each collecting locality. Additional collections were received from collaborators at the Queensland Museum and the Naturkundemuseum – Karlsruhe. Specimen information is summarized in Supplementary Table 1 for new specimens and in the following places for previously published data: Table 1 in Boyer et al., 2015; Table 2 in Giribet et al., 2012; Table 1 in Giribet et al., 2016. The software OpenJUMP v.1.9.1 was used to visualize GPS coordinates.

Initial examination of specimens was performed in an Olympus SZX10 light microscope. For some specimens, scanning electron microscopy was required to identify individuals to species. Species diagnoses were made primarily using the morphology of the anal plate (including the scopula), the shape and degree of division of tarsus IV, and the morphology of the adenostyle as previously detailed by our research group (Boyer et al., 2015; Jay et al., 2016).

2.2. Molecular data

A total of 143 specimens of *Austropurcellia* were used in molecular analyses, including 60 newly sequenced animals. DNA was extracted from the right third leg of each specimen using the Qiagen DNeasy® Tissue Kit, with overnight incubation as described by Boyer et al. (2005). Adult males were preferentially chosen over females and juveniles, as only adult males are identifiable to the species level with morphology alone.

We amplified and sequenced three different loci, including two more conserved nuclear markers (18S and 28S rRNA) and one more variable mitochondrial loci (cytochrome *c* oxidase subunit I [COI]). For each population of each species, three loci were sequenced for one individual specimen (COI, 18S, 28S); we sequenced many additional specimens for the more variable mitochondrial locus (Supplementary Table 1). 18S and 28S rRNAs were amplified using primers detailed by Boyer and Giribet (2007). COI was amplified using primer pair LCO1490-HCOoutout (Folmer et al., 1994; Schwendinger and Giribet, 2005). PCR reactions (25 μL) were carried out with 6 μL of DNA template, 2.5 μL of 2.5 μM primers, 2 μL of dNTPs, and 0.25 μL of PrimeStar Taq Polymerase per reaction. Amplifications were carried out in an ABI 2720 thermal cycler, using conditions summarized in Supplementary Table 2.

Gel electrophoresis (1.5% agarose) was used to visualize amplified DNA fragments. Successful PCR reactions were then cleaned up using Qiagen QIAquick PCR Purification Kit and sequencing was performed by Functional Biosciences, Inc. (Madison, Wisconsin, USA). Forward and reverse chromatograms were assembled and edited in Geneious (v5.5, Biomatters Ltd, Auckland, New Zealand). All new sequences have been deposited in GenBank under accession numbers MH424922-MC425012 (Supplementary Table 1).

2.3. Phylogenetic analysis

Before performing statistical analyses, we fused new data with previously published Cyphophthalmi datasets, including data for the mitochondrial locus 16S and the nuclear protein-coding gene for histone H3 for some taxa (Boyer et al., 2015 [GenBank accession numbers in Table 1]; Giribet et al., 2012 [GenBank accession numbers in Table 2], Giribet et al., 2016 [GenBank accession numbers in Table 1]). Outgroup taxa consisted of two Dyspnoi (*Hesperonemastoma modestum* and *Acropsopilio chilensis*), four Eupnoi (*Caddo agilis, Forsteropsalis turneri*, *Marthana* sp., and *Rhampsinitus* sp.), three Laniatores (*Equitius doriae, Sandokan malayanus*, and *Conomma oedipus*), and the horseshoe crab *Limulus polyphemus* (Giribet et al., 2012; Groh and Giribet, 2015; Sharma and Giribet, 2011). Alignments were inferred *de novo* using MUSCLE v.3.8.81 and trimmed using GBlocks v.0.91b. Tree topology was inferred under a maximum likelihood approach using RAxML v. 8.2.4 using a unique GTR + Γ model for each locus, with 500 independent starts and 1000 rapid bootstrap replicates (Stamatakis, 2006; Stamatakis et al., 2008).

2.4. Estimation of divergence times

Divergence times were inferred using BEAST v.1.8.2 (Drummond and Rambaut, 2007) with a unique GTR + Γ + I model for each partition. COI was partitioned with two separate site models, for first and second positions versus the third codon position. Model selection followed the results of model testing implemented by us in Giribet et al. (2012). Three fossil taxa were used to calibrate the dating: (1) The age of Stylocellidae was constrained using an exponential prior distribution with a mean of 25 Myr and an offset of 105 Myr, reflecting the age of the Cretaceous amber fossil *Palaeosiro burmanicum* (Poinar, 2008). The large mean of this distribution reflects the interpretation of this fossil as a derived member of the family, and specifically as a member of the genus *Meghalaya* (Giribet et al., 2007). (2) The age of Eupnoi was constrained using an exponential prior distribution with a mean of 5 Myr and an offset of 305 Myr, reflecting the Carboniferous age of *Macrogyion cronus*, the oldest known Eupnoi (Garwood et al., 2011). (3) The age of Opiliones was constrained using an exponential prior distribution with a mean of 5 Myr and an offset of 410 Myr, reflecting the age of *Eophalangium sheari*, the oldest known harvestman fossil (Dunlop et al., 2003; Garwood et al., 2014; Sharma and Giribet, 2014).

While we explored adding a fourth calibration for the oldest Dyspnoi (*Ameticos scolos*), this last constraint was omitted, as its inclusion caused the initial likelihood of the starting trees to be too low. This is the result of a commonly encountered error stemming from the incompatibility of ancient calibration points in close temporal vicinity with either of the starting tree defaults in BEAST (random starting trees or UPGMA tree), which manifests as a log likelihood value that is rounded to negative infinity and an ensuing failure of the MCMC chain. Due to the complexity of the dataset analyzed here, providing the maximum likelihood tree topology as a starting tree was trialled, but did not overcome the problem incurred by the *A. scolos* constraint. As

the estimated age of the Dyspnoi outgroups was incidental to this study, *A. scolos* was omitted.

With these calibrations, three different tree priors were used for inference of divergence times: a Yule prior, a birth-death prior, and a birth-death prior with incomplete sampling. As a point of comparison, we conducted a fourth run wherein the age of Cyphophthalmi was constrained using a normal distribution with a mean of 300 Myr and a standard deviation of 7.5 Myr. This constraint reflects the previous interpretation of mite harvestman as a Pangean taxon that began to diversify ca. 300 Ma (ref. Giribet et al., 2012; contra Giribet et al., 2016).

At least two runs were conducted for each of the four parameter sets for 200 M generations; the runs were combined post-burnin. Stationarity was assessed using Tracer v. 1.6 (Rambaut and Drummond, 2009) and 20% of all generations were conservative discarded as burnin.

The speciation-extinction model fitting procedure implemented in the program BAMM v.2.5.0 was used to characterize shifts in net diversification rate over time. To eliminate signs of artificial diversification, we first culled all duplicate species exemplars from our ultrametric phylogeny of Cyphophthalmi, resulting in a tree with 186 taxa spanning the suborder. Analysis, however, focused primarily on the genus *Austropurcellia* in order to determine whether its diversification rates corroborate the patterns of past climatic fluctuation in the Australian Wet Tropics. Markov CMC Monte Carlo simulation was run using four chains for 100 million generations, with priors determined by the R package BAMMtools.

3. Results

3.1. Phylogenetic analysis

We retrieved a monophyletic Cyphophthalmi (100% bootstrap support [BS]), within which a monophyletic Pettalidae (100% BS) is sister group to the remaining families of mite harvestmen (Fig. 3). Within Pettalidae, *Austropurcellia* was retrieved as monophyletic (88% BS). Within *Austropurcellia*, we retrieved several well-supported clades. The basal split within the genus consists of one clade including two Southeast Queensland species + *A. clousei* from the southernmost Wet Tropics (82% BS), and a second clade within which *A. capricornia* from Central Queensland is the sister group to all species from the Wet Tropics north of the Paluma Range (the Wet Tropics Endemic Clade

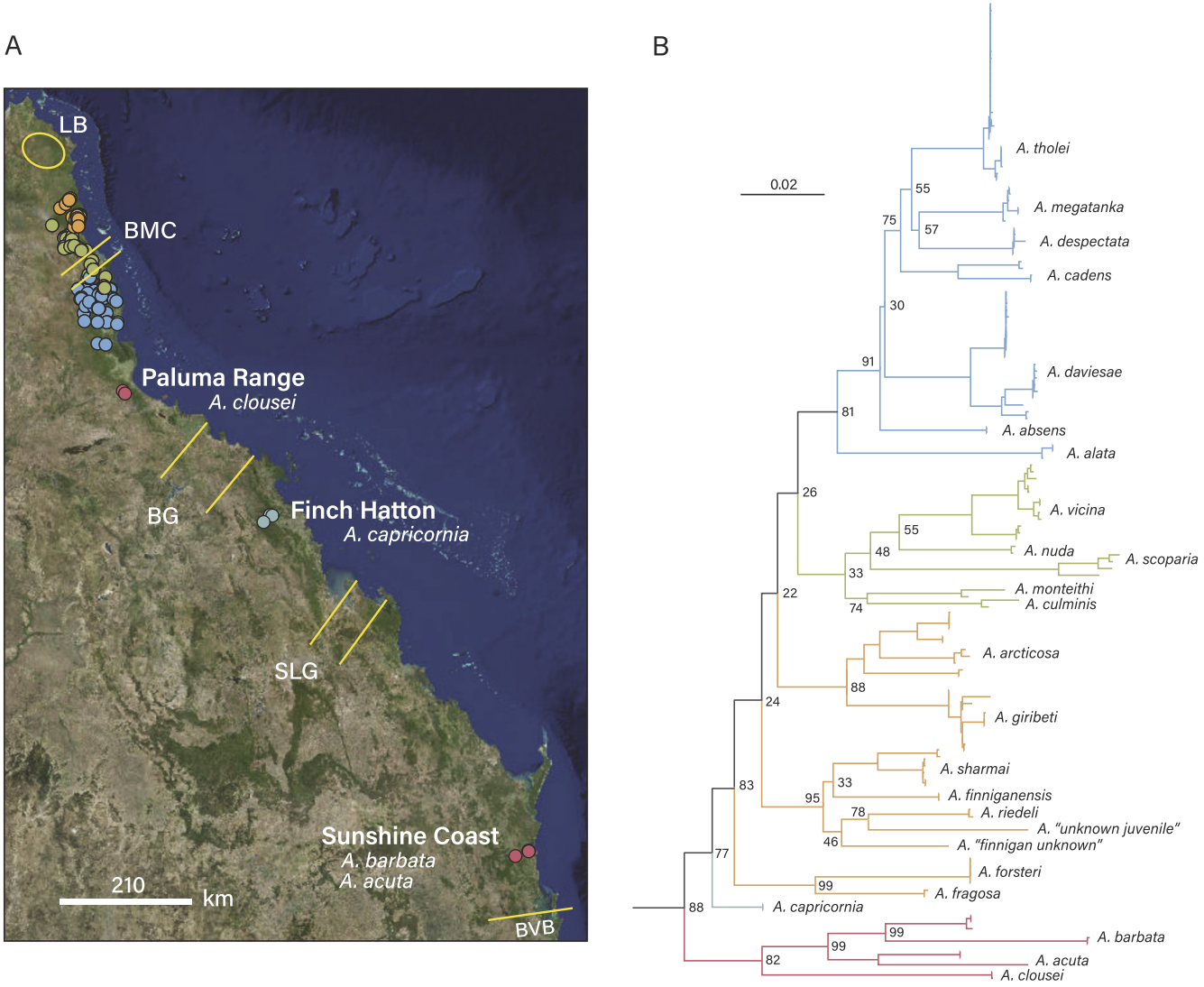

Fig. 2. Geographic distribution and intrageneric relationships of *Austropurcellia* species. (A) Map of Queensland with established biogeographic breaks (full names given in Fig. 1), featuring the locations of specimens included in the phylogenetic analysis at right. (B) Maximum likelihood phylogeny of the genus *Austropurcellia* as inferred by our full RAxML analysis of Cyphophthalmi. Branch colours correspond to (A) and demarcate regional groups and/or monophyletic clades. Node numbers show bootstrap support values.

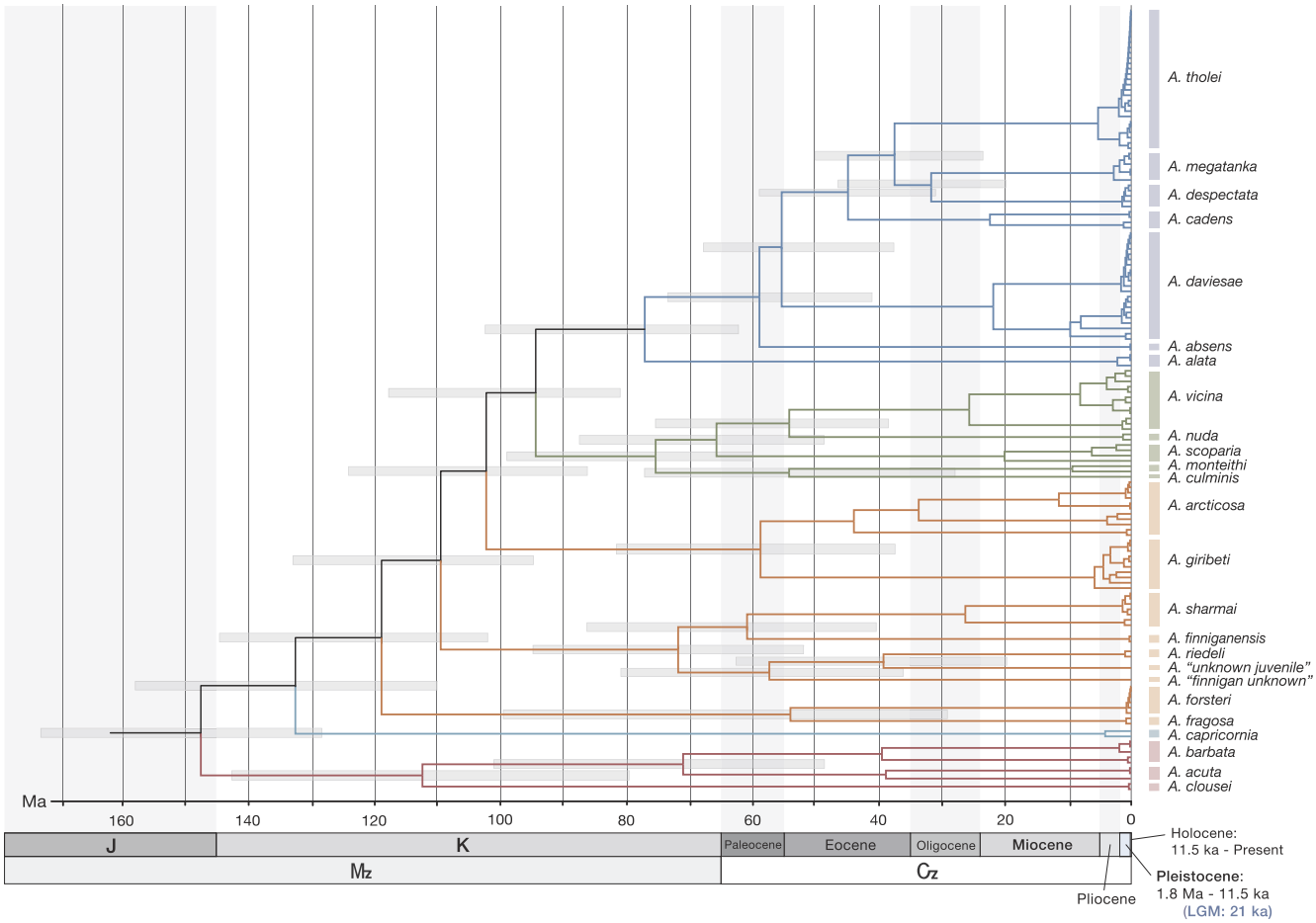

Fig. 3. Estimated divergence dates of *Austropurcellia* species, as recovered by our full BEAST analysis of Cyphophthalmi under the birth-death model with incomplete sampling tree prior (Fig. 5). Branch colours correspond to Fig. 2, representing regional groups and/or monophyletic clades; species are indicated by the coloured bands near their respective terminals. Node bars show 95% confidence intervals for species-level divergences or higher.

sensu Boyer et al., 2015) (77% BS). The Wet Tropics Endemic Clade is supported with 83% BS, and includes several clades that correspond to particular areas within the Wet Tropics. Species from the northernmost Wet Tropics form a monophyletic group (95% BS), and *A. giribeti* and *A. arcticosa*, also from the northern Wet Tropics, are sister taxa (88% BS). We retrieved two other lineages: one poorly-supported group from the north-central Wet Tropics (33% BS), and one well-supported group from the Central Wet Tropics Uplands (81% BS). The lineages from the northern Wet Tropics form a paraphyletic grade with respect to the North-Central and Central Uplands clades; however, these relationships among these major lineages are not supported (22%, 24%, 26% BS) (Fig. 2).

3.2. Diversification times and rates

Estimated branching dates were largely consistent across four sets of tree priors (Fig. 5). Under the birth-death model with incomplete sampling, we inferred the age of the suborder Cyphophthalmi to be 345 Myr (95% HPD [highest posterior density]: 312–373 Mya), which falls solidly within the Carboniferous to end-Devonian under all four tree priors. Diversification times for the families of Cyphophthalmi are as follows: Pettalidae: 210 Mya (95% HPD: 172–253.7 Mya); Stylocellidae: 164 Mya (134–192 Mya); Sironidae: 285 Mya (256–320 Mya); Neogoveidae: 243 Mya (219–265 Mya); and Troglosironidae: 57 Mya (45–70 Mya). Ogoveidae was represented by a single specimen, but diverged from Neogoveidae 266 Ma (245–292 Mya). Divergence dates within the genus *Austropurcellia* were recovered during our full BEAST analysis of Cyphophthalmi as described above in Section 2.4. *Austropurcellia* arose 137 Mya (95% HPD: 120–164 Mya) and diversified 122 Mya (95% HPD: 103–147 Mya; Fig. 3).

By completing a speciation-extinction analysis, we expected to see at least one shift in net diversification rate. If aridification during the Miocene or LGM was acting as a driver of speciation for *Austropurcellia*, we would observe a rate acceleration within the genus. However, BAMM only identified two credible shift sets, and the most probable configuration by far ($P = 0.95$) showed zero shifts. The second best shift configuration ($P = 0.011$) found one rate shift within the European genus *Cyphophthalmus* (Sironidae), which has been previously characterized (Murienne et al., 2009). Even so, under a speciation-extinction model, mite harvestmen exhibit a relatively constant diversification rate over time.

4. Discussion

4.1. Pettalidae diversified in the late Jurassic in accordance with Gondwanan vicariance

The family Pettalidae has a classical Gondwanan distribution (Fig. 4), and has become a textbook example of a taxon whose present-day geography is well-explained by ancient vicariance (Zimmer, 2010). In recent years, some of the most celebrated tales of Gondwanan vicariance have been overturned by molecular clock studies which date lineages endemic to particular continents as originating long after rifting, including the iconic ratite birds (Phillips et al., 2010) and *Nothofagus*, the southern beech (Cook and Crisp, 2005). However, some short-range endemic invertebrates are still upheld as examples of

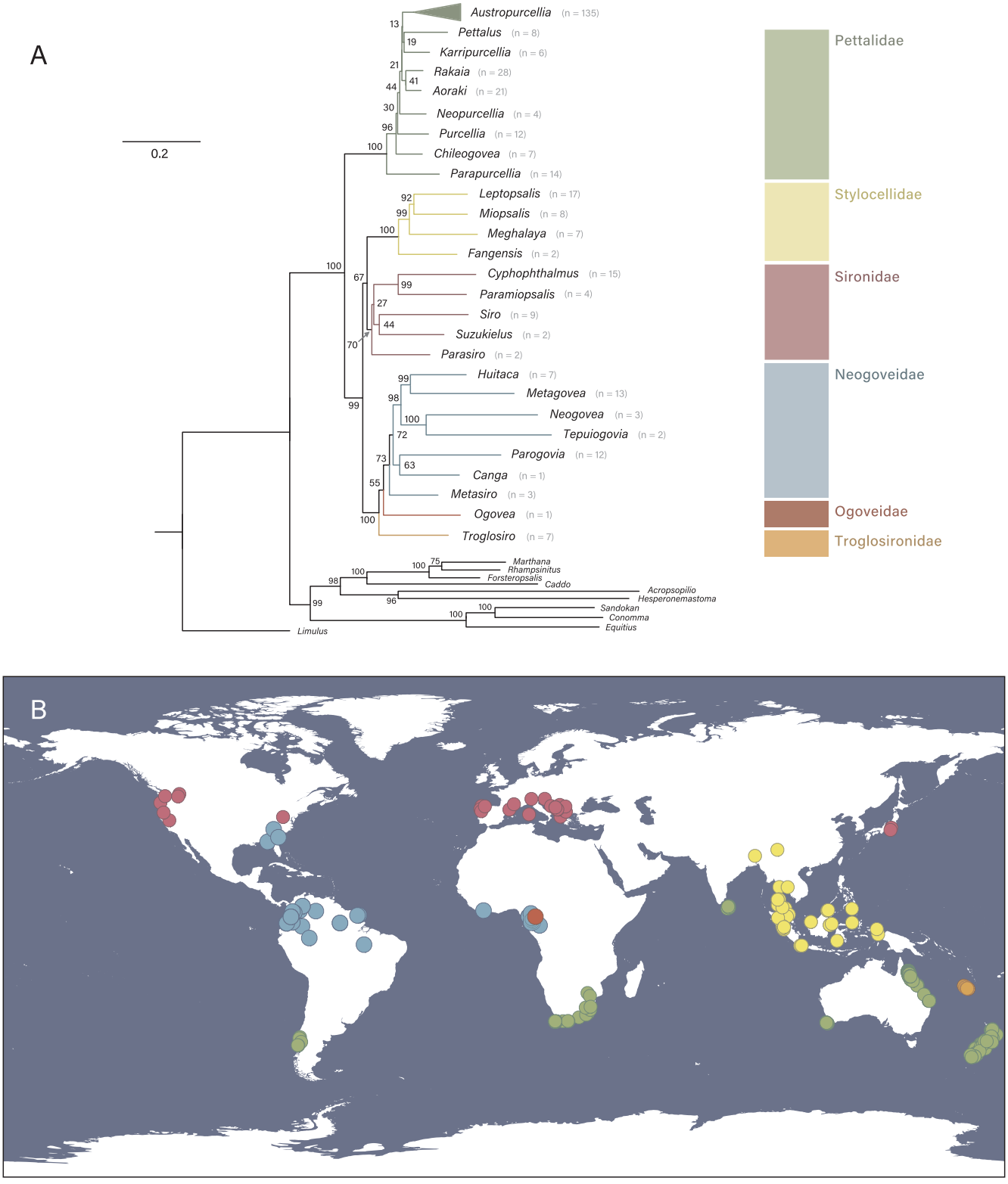

Fig. 4. Phylogeny and sampled distribution of the mite harvestmen. (A) Maximum likelihood phylogeny of the suborder Cyphophthalmi, produced in RAxML. The 331-taxon tree has been condensed to genus level, with the number of respective specimens included in parentheses. All genera were retrieved as monophyletic, with the exception of *Neogovea*. Node numbers show bootstrap support values. Coloured boxes and names at right represent the six families within Cyphophthalmi. (B) Distribution of sampling of the six mite harvestman families. Families are distinguished by colours corresponding to (A).

vicariant taxa, including velvet worms (Allwood et al., 2010; Murienne et al., 2014), certain groups of centipedes (Giribet and Boyer, 2010), and, of course, the pettalids. The first molecular dating of suborder Cyphophthalmi using a relaxed clock approach recovered remarkable correspondence between the divergence times of pettalid lineages and the temporal sequence of landmass fragmentation in both Gondwana and Laurasia (Giribet et al., 2012). Specifically, Cyphophthalmi were inferred to be 332 Myr old in that study; this Permian age is consistent

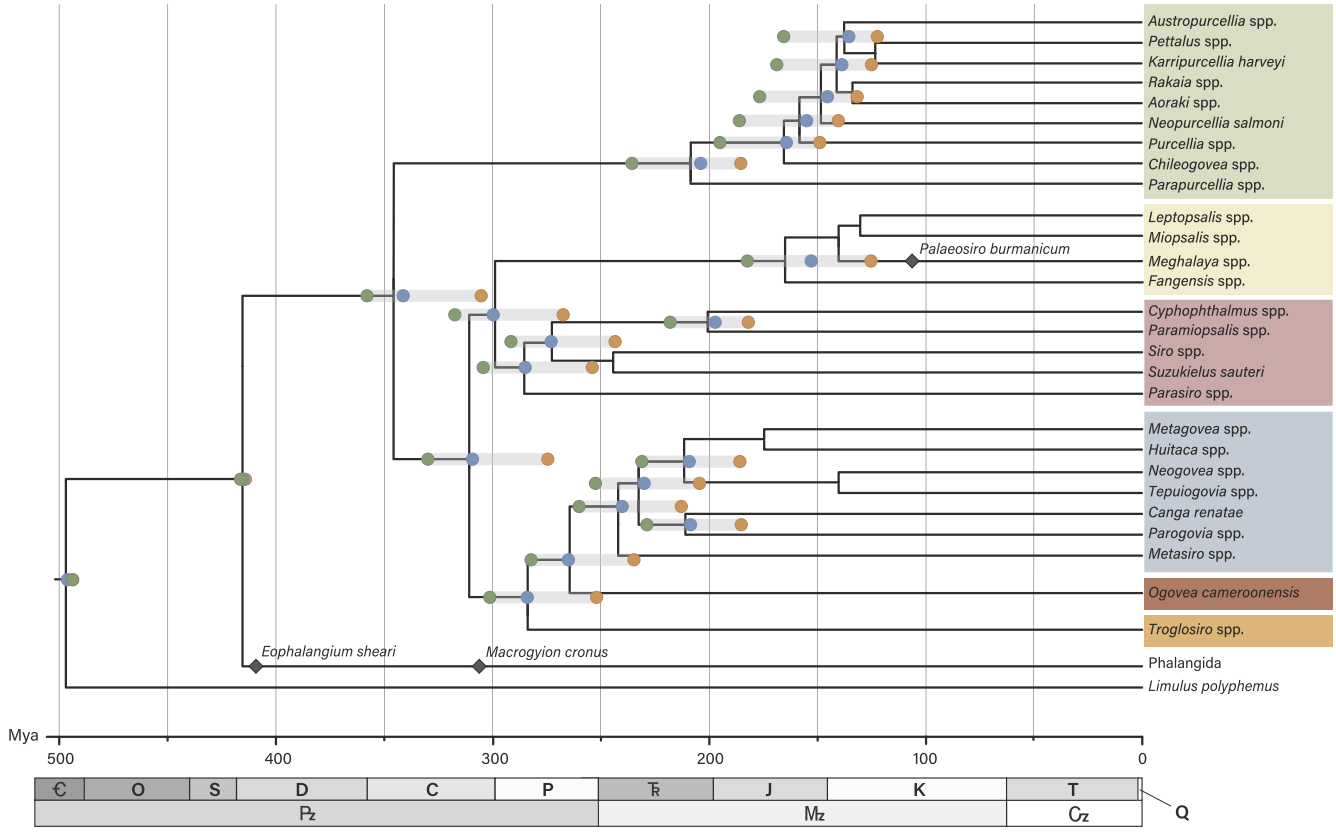

Fig. 5. Divergence dates within the suborder Cyphophthalmi as estimated in BEAST, including a comparison of priors used for the analysis. Time is indicated on the horizontal axis in millions of years (Mya) as well as by geological epoch. Branches were recovered by the analysis using birth-death tree priors with incomplete sampling. Node bars indicate the medians of dates recovered for each set of parameters: green: median node age under Yule tree prior; blue: median node age under birth-death prior with incomplete sampling; orange: median node age under birth-death prior with incomplete sampling and a constraint on the age of Cyphophthalmi. Black diamonds show the three fossil calibration points.

with the characterization of the entire suborder as a Pangean taxon. The 47 sampled Pettalidae in that study were inferred to have diversified in the Late Jurassic (mean age: 183 Mya, 95% HPD: 148–218 Mya), consistent with the age of fragmentation of temperate Gondwana. Age estimates were fairly similar across all four tree priors for the ages of Cyphopthalmi, Pettalidae, and *Austropurcelllia* (Fig. 5).

A more recent assessment of Pettalidae increased the sampling to 117 ingroup terminals, but obtained much older ages for the entire group (Giribet et al., 2016). The diversification of Pettalidae was retrieved as 314 Mya, with the 95% HPD interval spanning the pre-Carboniferous to the early Permian. The split between *Austropurcellia* and its sister taxon was estimated as occurring in the mid-Permian—decidedly pre-rifting—with the earliest divergence within *Austropurcellia* occurring in the mid-Triassic (Giribet et al., 2016). Beyond the dating alone, the ultrametric tree topology recovered by that study was decidedly incongruent with the sequence of Gondwanan breakup (as well as other tree topologies in that study), due to the nested placement of the West Gondwanan genera *Purcellia* and *Chileogovea* within an erstwhile East Gondwanan clade (Ref. Giribet et al., 2012).

A Carboniferous age of Pettalidae is implausible because it implies that the genera of Pettalidae diversified exactly in accordance with the sequence of temperate Gondwanan fragmentation, but over 100 million years before the actual geological fragmentation itself. This scenario of "pre-diversification" is strongly inconsistent with the high fidelity between mite harvestmen distributions and phylogeny (Boyer et al., 2007; Boyer and Giribet, 2007, 2009; Clouse and Giribet, 2010; Clouse et al., 2016), as well as the results of other molecular dating studies (Giribet et al., 2012; Sharma and Giribet, 2014). A more recent dating analysis recovered Cyphophthalmi as end-Jurassic in age, but with a 95% HPD interval that spanned the Late Cretaceous to the Carboniferous (Fernandez et al., 2017); this uninformative estimate likely resulted from the choice of the uncorrelated gamma multipliers clock model, which is prone to high variance and node age underestimates (ref. Lepage et al., 2007; Paradis, 2013; Sharma and Giribet, 2014).

The older ages recovered by Giribet et al. (2016) may be artefactual. As the molecular markers used by most of these studies are identical, we focused on the difference in sampling of outgroup lineages. Giribet et al. (2016) employed 12 outgroup taxa (a horseshoe crab; three Phalangida; and eight non-pettalid Cyphophthalmi); this underrepresentation of basally branching lineages may have incurred errors in estimating substitution rates in basal nodes. The outgroup sampling in our study has been expanded to include: a horseshoe crab; nine Phalangida sampling all basal splits in non-Cyphophthalmi suborders; and 115 non-pettalid Cyphophthalmi.

All of our dating analyses recovered a Jurassic age of Pettalidae and a Carboniferous age of Cyphophthalmi, consistent with earlier estimates (Giribet et al., 2012; Sharma and Giribet, 2014), and wholly congruent with a biogeographic history driven by continental breakup. We found that the inclusion of a constraint enforcing a Permian age of Cyphophthalmi recovered younger ages than under other tree prior models, but the differences were not very large on a geologic scale (i.e., did not render any of the age estimates inconsistent with vicariance scenarios; Fig. 5). Under the birth-death model with incomplete sampling, the split between *Austropurcellia* and its sister group (*Pettalus* + *Karripurcellia*) was estimated as 139 Mya (95% HPD interval: 120–164 Mya), and the diversification of *Austropurcellia* was 123 Mya (95% HPD interval: 103–147 Mya) (Fig. 5).

Our results suggest that the discordant age estimates obtained by Giribet et al. (2016) were likely driven by undersampling of outgroup taxa, and they revalidate the basal diversification of Pettalidae in a

temporal window coincident with the rifting of temperate Gondwana. Therefore, the presence of pettalids on the Australian continent can be attributed to vicariance.

4.2. *Austropurcellia* diversified steadily throughout its history

Our research group has been working toward fully documenting the diversity and distribution of *Austropurcellia* for nearly a decade; prior to 2012, the genus was known only from a handful of localities in the Australian Wet Tropics and one collecting site at Finch Hatton in Central Queensland. We can now state with confidence that the genus is found in humid forested areas throughout the Atherton Plateau and Eastern Queensland zoogeographical subregions (*sensu* Bryant and Krosch, 2016; Ebach et al., 2013), with its northernmost limit occurring at the Laura Basin and its southernmost limit coinciding with the Brisbane Valley, both lowland areas that mark major biogeographic breaks for many forest-restricted taxa (Fig. 1). The Brisbane Valley also represents the northern edge of an area known as the MacPherson-Maclay Overlap (Burbidge, 1960), a north-south transition between the biotas of the Australian temperate and tropical zones. However, while the distribution of *Austropurcellia* is bounded by well-known biogeographic dryland barriers, within the genus we find a spatial distribution of major lineages that does not correspond neatly to geographical regions defined by previous authors (Bryant and Krosch, 2016; Ebach et al., 2013) (Fig. 2).

Within *Austropurcellia*, the basal split occurs between two clades, each of which includes members from both north and south of the Burdekin Gap, a barrier that separates the Atherton Plateau and Eastern Queensland zoogeographical subregions (Fig. 1). One of these lineages includes a species from the Paluma Range (our southernmost sampling site in the Wet Tropics) that is sister group to two species from the far south of Queensland, a result that is counterintuitive from a geographic point of view, but nevertheless corroborates previous results and is not surprising from a morphological perspective (Popkin-Hall and Boyer, 2014; Boyer et al., 2015) (Fig. 2). The other lineage within the genus is comprised of a species from Finch Hatton (just south of the Burdekin Gap in Central Queensland) and its sister group, the speciose Wet Tropics Endemic Clade identified in previous analyses (Boyer et al., 2015; Fig. 2).

In a recent exhaustive review, Bryant and Krosch (2016) summarized molecular clock studies of terrestrial and freshwater organisms whose distributions span major geographic barriers in eastern Australia, including the Black Mountain Corridor, the Burdekin Gap, and the St. Lawrence Gap. They found that divergences across barriers were clustered particularly in the mid-late Miocene (~15 Ma) and the Pliocene-Pleistocene (~6–0.04 Mya). In contrast, within *Austropurcellia*, we find that the basal split occurred at 123 Mya (95% HPD: 104–147 Mya). The two clades each exhibit a geographic divergence between the Wet Tropics and mid-eastern/southeastern Queensland. One such lineage shows tropical *A. clousei* diverging from the southern species *A. acuta* and *A. barbata* 94.7 Mya (95% HPD: 74–114 Mya). In the other, *A. capricornia* in mid-eastern Queensland—between the Burdekin Gap and the St. Lawrence Gap—branches from the remaining *Austropurcellia*, all found in the Wet Tropics, at 109 Mya (95% HPD: 84.5–132 Mya). Each of these early speciation events occurs during the Cretaceous, even at the latest estimated dates within their 95% HPD intervals (Fig. 3), revealing a truly ancient evolutionary history in comparison with other co-distributed groups.

The assassin spiders of Australia (Archaeidae: *Austrarchaea*) provide an excellent point of comparison here: the northeastern and mid-eastern archaeid lineages can be found throughout Queensland's archipelago of coastal forests, thus showing a very similar distribution to *Austropurcellia* (Rix and Harvey, 2012). However, most of the archaeid divergence estimates correspond tightly with geographic barrier formation during the Miocene, and have proven to be much younger than *Austropurcellia*. The exceptions are the northeastern Queensland species, which diverged ca. 51–34 Mya, and the diversification of the mid-eastern *Austrarchaea* ca. 39–26 Mya—but even so, neither of these is old enough to overlap with estimates for *Austropurcellia* (Rix and Harvey, 2012). Even the archaeids of southeastern Australia, which diverged in the Paleocene or early Eocene (95% HPD: 69–46 Mya), diversified largely during the Miocene.

4.3. Ages of speciation events in the Wet Tropics predate Pleistocene glacial cycling

Within the Wet Tropics Endemic Clade, we see a basal paraphyletic grade of species from the far north, in addition to a well-supported clade of species from the central Wet Tropics and an unsupported clade from the north-central Wet Tropics (Fig. 2). These results corroborate findings of previous phylogenetic analyses with less complete taxon sampling (Boyer et al., 2015).

The Australian Wet Tropics of far northern Queensland have become a model system for studying the effect of rainforest contraction and fragmentation on the fauna that is restricted to this habitat (e.g. Schneider and Moritz, 1999; Hoskin et al., 2011; Joseph et al., 1995; Phillips et al., 2004; Bell et al., 2012). Haffer (1969) articulated the classic hypothesis that the high diversity of rainforest animals in the Amazon was driven by allopatric speciation in refugia in the late Quaternary, especially during the LGM; however, when this hypothesis has been tested using molecular clock approaches, researchers have typically found that species' divergence dates predate the Pleistocene significantly. More recently, researchers have turned their attention from the role of LGM refugia as cradles of diversity to their role as museums of diversity, preserving lineages during a period in which widespread extinction occurred outside of refugial areas (e.g. Carnaval et al., 2009). Such differential extinction would have affected dispersal-limited animals more severely than vagile groups, and we have demonstrated previously that the distribution of present-day species richness and phylogenetic diversity of *Austropurcellia* is predicted by LGM climatic suitability, which even out-performs present-day climatic suitability (Boyer et al., 2016).

In order to test further the hypothesis that contraction of rainforest habitat during the LGM resulted in preferential preservation of lineages within refugia, we have dated divergence times among species in the current study. Within *Austropurcellia*, we find that divergences among species do indeed predate Pleistocene glacial cycling (Fig. 3); this is consistent with a model in which the present-day distribution of diversity across the Wet Tropics has been driven by differential extinction, with higher diversity in areas that were more hospitable during the LGM and lower diversity in areas with decreased habitat suitability.

5. Conclusions

Using a variety of dating approaches, we confirm that *Austropurcellia*'s presence in Australia predates Gondwanan rifting, and that diversification within the family Pettalidae is temporally concordant with the breakup of the supercontinent. We do not find any signature of accelerated diversification within the genus coincident with Miocene aridification. Finally, we demonstrate that diversification within the genus long predates the Last Glacial Maximum. Previous researchers have highlighted the potential of the Wet Tropics as a system in which to study not only the evolutionary effects of changing climate during the Quaternary, but also much more ancient diversification processes; for example, Moreau et al. (2015) found evidence for deep divergences among species of *Terrisswalkeris*, a group of endemic earthworms with species ranges comparable in scale to those seen in *Austropurcellia*. Other dispersal-limited animals such as microhylid frogs (Hoskin, 2004), velvet worms (Oliveira and Mayer, 2017), assassin spiders (Rix and Harvey, 2012), and goblin spiders (Edward, 2011) have high diversity within the Wet Tropics. While molecular phylogenetic work has been performed in some of these groups, in no case have

the Wet Tropics lineages been the subject of phylogenetic analyses and molecular dating with dense taxon sampling. A previous generation of evolutionary biologists was surprised to find that LGM refugia have not acted as cradles of rainforest animal diversity; currently, we are discovering that some groups of rainforest animals diversified much earlier than previously suspected. We hope that future work on groups co-distributed with *Austropurcellia* will build our understanding of the evolutionary history of the fauna in this remarkable corner of the world.

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References

Adam, P., 1992. Australian Rainforests. Clarendon Press, Sydney, Australia.
Allwood, J., Gleeson, D., Mayer, G., Daniels, S., Beggs, J.R., Buckley, T.R., 2010. Support for vicariant origins of the New Zealand Onychophora. J. Biogeogr. 37, 669–681.
Bell, R.C., MacKenzie, J.B., Hickerson, M.J., Chavarria, K.L., Cunningham, M., Williams, S., Moritz, C., 2012. Comparative multi-locus phylogeography confirms multiple vicariance events in co-distributed rainforest frogs. Proc. Roy. Soc. B: Biol. Sci. 279, 991–999.
BMR Paleogeographic Group, 1990. Australia: Evolution of a Continent. Bureau of Mineral Resources, Canberra, Australia.
Boyer, S.L., Reuter, C.N., 2012. New species of mite harvestmen from Australia's Wet Tropics, with commentary on historical biogeography of the genus *Austropurcellia* (Opiliones, Cyphophthalmi, Pettalidae). J. Arachnol. 40, 96–112.
Boyer, S.L., Clouse, R.C., Benevides, L., Schwendinger, P., Sharma, P.P., Karunathana, I., Giribet, G., 2007. Biogeography of the world: a case study from cyphophthalmid Opiliones, a globally distributed group of arachnids. J. Biogeogr. 34 (9), 2070–2085.
Boyer, S.L., Giribet, G., 2007. A new model Gondwanan taxon: systematics and biogeography of the harvestman family Pettalidae (Arachnida, Opiliones, Cyphophthalmi), with a taxonomic revision of genera from Australia and New Zealand. Cladistics 23, 337–361.
Boyer, S.L., Giribet, G., 2009. Welcome back, New Zealand: regional biogeography and Gondwanan origin of three endemic genera of mite harvestmen. J. Biogeogr. 36 (6), 1084–1099.
Boyer, S.L., Karaman, I., Giribet, G., 2005. The family Sironidae (Opiliones, Cyphophthalmi) in Europe: a phylogenetic approach to Eastern Mediterranean biogeography. Mol. Phylogenet. Evol. 36 (3), 554–567.
Boyer, S.L., Markle, T.M., Baker, C.M., Luxbacher, A.M., Kozak, K.H., 2016. Historical refugia have shaped biogeographical patterns of species richness and phylogenetic diversity in mite harvestmen (Arachnida, Opiliones, Cyphophthalmi) endemic to the Australian Wet Tropics. J. Biogeogr. 43, 1400–1411.
Boyer, S.L., Baker, C.M., Popkin-Hall, Z.R., Laukó, D.I., Wiesner, H.A., Quay, R.H., 2015. Phylogeny and biogeography of the mite harvestmen (Arachnida: Opiliones: Cyphophthalmi) of Queensland, Australia, with a description of six new species from the rainforests of the Wet Tropics. Inver. Syst. 29, 37–70.
Burbidge, N.T., 1960. The phytogeography of the Australian Region. Aust. J. Bot. 8 (2), 75–211.
Bryant, L.M., Krosch, M.N., 2016. Lines in the land: a review of evidence for eastern Australia's major biogeographical barriers to closed forest taxa. Biol. J. Linn. Soc. 119, 238–264.
Byrne, M., Steane, D.A., Joseph, L., Yeates, D.K., Jordan, G.J., Crayn, D., Aplin, K., Cantrill, D.J., Cook, L.G., Crisp, M.D., Keogh, J.S., Melville, J., Moritz, C., Porch, N., Sniderman, J.M.K., Sunnucks, P., Weston, P.H., 2011. Decline of a biome: evolution, contraction, fragmentation, extinction and invasion of the Australian mesic zone biota. J. Biogeogr. 38, 1635–1656.
Carnaval, A.C., Hickerson, M.J., Haddad, C.F.B., Rodrigues, M.T., Moritz, C., 2009. Stability predicts genetic diversity in the Brazilian Atlantic Forest Hotspot. Science 323 (5915), 785–789.
Clouse, R.M., Giribet, G., 2010. When Thailand was an island – the phylogeny and biogeography of mite harvestmen (Opiliones, Cyphophthalmi, Stylocellidae) in Southeast Asia. J. Biogeogr. 37, 1114–1130.
Clouse, R.M., Sharma, P.P., Stuart, J.C., Davis, L.R., Giribet, G., Boyer, S.L., Wheeler, W.C., 2016. Phylogeography of the harvestman *Metasiro* (Arthropoda, Arachnida, Opiliones) reveals a potential solution to the Pangean paradox. Org. Divers. Evol. 47 (1), 1–20.
Cook, L.G., Crisp, M.D., 2005. Not so ancient: the extant crown group of *Nothofagus* represents a post-Gondwanan radiation. Proc. Roy. Soc. B: Biol. Sci. 272, 2535.
Drummond, A.J., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7, 214. https://doi.org/10.1186/1471-2148-7-214.
Dunlop, J.A., Anderson, L.I., Kerp, H., Hass, H., 2003. Preserved organs of Devonian harvestmen. Nature 425, 916.
Ebach, M.C., Gill, A.C., Kwan, A., Ahyong, S., Murphy, D.J., Cassis, G., 2013. Toward an Australian Bioregionalisation Atlas: a provisional area taxonomy of Australia's biogeographical regions. Zootaxa 3619, 315–342.
Edward, K., 2011. Systematics and biogeography of the goblin spider genus ischnothyreus (Oonopidae) in the Australian Wet Tropics Bioregion. PhD Thesis. The University of Western Australia, Perth, Australia.
Fernández, R., Sharma, P.P., Tourinho, A.L., Giribet, G., 2017. The Opiliones tree of life: shedding light on harvestmen relationships through transcriptomics. Proc. Roy. Soc. B.: Biol. Sci. 284, 20162340.
Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotech. 3, 294–299.
Garwood, R.J., Dunlop, J.A., Giribet, G., Sutton, M.D., 2011. Anatomically modern Carboniferous harvestmen demonstrate early cladogenesis and stasis in Opiliones. Nat. Commun. 2, 444.
Garwood, R.J., Sharma, P.P., Dunlop, J.A., Giribet, G., 2014. A new stem-group Palaeozoic harvestman revealed through integration of phylogenetics and development. Curr. Biol. 24, 1–7.
Giribet, G., Boyer, S.L., 2010. 'Moa's Ark' or 'Goodbye Gondwana': is New Zealand's terrestrial invertebrate fauna ancient, recent, or both? Inver. Syst. 24, 1–8.
Giribet, G., Boyer, S.L., Baker, C.M., Fernandez, R., Sharma, P.P., De Bivort, B.L., Daniels, S.R., Harvey, M.S., Griswold, C.E., 2016. A molecular phylogeny of the temperate Gondwanan family Pettalidae (Arachnida, Opiliones, Cyphophthalmi) and the limits of taxonomic sampling. Zool. J. Linn. Soc. 178, 523–545.
Giribet, G., Sharma, P.P., Bastawade, D.B., 2007. A new genus and species of Cyphophthalmi (Arachnida: Opiliones) from the north-eastern states of India. Zool. J. Linn. Soc. 151, 663–670.
Giribet, G., Sharma, P.P., Benavides, L.R., Boyer, S.L., Clouse, R.M., De Bivort, B.L., Dimitrov, D., Kawauchi, G.Y., Murienne, J., Schwendinger, P.J., 2012. Evolutionary and biogeographical history of an ancient and global group of arachnids (Arachnida: Opiliones: Cyphophthalmi) with a new taxonomic arrangement. Biol. J. Linn. Soc. 105 (1), 92–130.
Groh, S., Giribet, G., 2015. Polyphyly of Caddoidea, reinstatement of the family Acropsopilionidae in Dyspnoi, and a revised classification system of Palpatores (Arachnida, Opiliones). Cladistics 31, 277–290.
Haffer, J., 1969. Speciation in Amazonian forest birds. Science 165, 131–137.
Hopper, S.D., 1996. Gondwanan Heritage: Past, Present, and Future of the Western Australian Biota. Surrey Beatty & Sons, Chipping Norton, NSW, Australia.
Hoskin, C.J., Tonione, M., Higgie, M., MacKenzie, J.B., Williams, S.E., VanDerWal, J., Moritz, C., 2011. Persistence in peripheral refugia promotes phenotypic divergence and speciation in a rainforest frog. Am. Nat. 178 (5), 561–578.
Jay, K.R., Popkin-Hall, Z.R., Coblens, M.J., Oberski, J.T., Sharma, P.P., Boyer, S.L., 2016. New species of Austropurcellia, cryptic short-range endemic mite harvestmen (Arachnida, Opiliones, Cyphophthalmi) from Australia's Wet Tropics biodiversity hotspot. ZooKeys 586, 37–93.
Joseph, L., Moritz, C., Hugall, A., 1995. Molecular support for vicariance as a source of diversity in rain-forest. Proc. Roy. Soc. B: Biol. Sci. 260 (1358), 177–182.
Kershaw, A., 1994. Pleistocene vegetation of the humid tropics of Northeastern Queensland, Australia. Palaeogeogr. Palaeocl. Palaeoecol. 109, 399–412.
Lepage, T., Bryant, D., Philippe, H., Lartillot, N., 2007. A general comparison of relaxed molecular clock models. Mol. Biol. Evol. 24 (12), 2669–2680.
Meredith, R.W., Westerman, M., Springer, M.S., 2008a. Phylogeny and timescale for the living genera of kangaroos and kin (Macropodiformes: Marsupialia) based on nuclear sequences. Aust. J. Zool. 56, 395–410.
Meredith, R.W., Westerman, M., Springer, M.S., 2008b. A timescale and phylogeny for "bandicoots" (Peramelemorphia: Marsupialia) based on sequences for five nuclear genes. Mol. Phylogenet. Evol. 47, 1–20.
Moreau, C.S., Hugall, A.F., McDonald, K.R., Jamieson, B.G.M., Moritz, C., 2015. An ancient divide in a contiguous rainforest: endemic earthworms in the Australian Wet Tropics. PLoS One 10 (9), e0136943.
Moritz, C., Hoskin, C.J., MacKenzie, J.B., Phillips, B.L., Tonione, M., Silva, N., VanDerWal, J., Williams, S.E., Graham, C.H., 2009. Identification and dynamics of a cryptic suture zone in tropical rainforest. Proc. Roy. Soc. B: Biol. Sci. 276, 1235–1244.
Murienne, J., Karaman, I., Giribet, G., 2009. Explosive evolution of an ancient group of Cyphophthalmi (Arachnida: Opiliones) in the Balkan Peninsula. J. Biogeogr. 37 (1), 90–102.
Murienne, J., Daniels, S.R., Buckley, T.R., Mayer, G., Giribet, G., 2014. A living fossil tale of Pangaean biogeography. Proc. Roy. Soc. B: Biol. Sci. 281, 20132648.
Oliveira, I.d.S., Mayer, G., 2017. A new giant egg-laying onychophoran (Peripatopsidae) reveals evolutionary and biogeographical aspects of Australian velvet worms. Org. Divers. Evol. 17, 375–391. https://doi.org/10.1007/s13127-016-0321-3.

Paradis, E., 2013. Molecular dating of phylogenies by likelihood methods: a comparison of models and a new information criterion. Mol. Phylogenet. Evol. 67, 436–444.

Phillips, B.L., Baird, S.J.E., Moritz, C., 2004. When vicars meet: a narrow contact zone between morphologically cryptic phylogeographic lineages of the rainforest skink, Carlia rubrigularis. Evolution 58 (7), 1536–1548.

Phillips, M.J., Gibb, G.C., Crimp, E.A., Penny, D., 2010. Tinamous and moa flock together: mitochondrial genome sequence analysis reveals independent losses of flight among ratites. Syst. Biol. 59, 90–107.

Poinar, G., 2008. Palaeosiro burmanicum n. gen., n. sp., a fossil Cyphophthalmi (Arachnida: Opiliones: Sironidae) in Early Cretaceous Burmese amber. In: Makarov, S.E., Dimitrijevic, R.N. (Eds.), Advances in Arachnology and Developmental Biology. Papers Dedicated to Prof. Dr. Bozidar Curcic. Vienna, Belgrade, Sofia: Faculty of Life Sciences, University of Vienna, and Serbian Academy of Sciences and Arts, pp. 267–274.

Popkin-Hall, Z.R., Boyer, S.L., 2014. New species of mite harvestmen from southeast Queensland, Australia greatly extend the known range of the genus *Austropurcellia* (Arachnida, Opiliones, Cyphophthalmi). Zootaxa 3827 (4), 517–541.

Rambaut, A., Drummond, A.J., 2009. Tracer v. 1.6. Program and documentation available at < http://tree.bio.ed.ac.uk/software/tracer/ > .

Rix, M.G., Harvey, M.S., 2012. Phylogeny and historical biogeography of ancient assassin spiders (Araneae: Archaeidae) in the Australian mesic zone: evidence for Miocene speciation within Tertiary refugia. Mol. Phylogenet. Evol. 62, 375–396.

Schneider, C.J., Moritz, C., 1999. Rainforest refugia and evolution in Australia's Wet Tropics. Proc. Roy. Soc. B: Biol. Sci. 266, 191–196.

Schwendinger, P.J., Giribet, G., 2005. The systematics of the south-east Asian genus Fangensis Rambla (Opiliones: Cyphophthalmi: Stylocellidae). Inver. Syst. 19, 297–323.

Sharma, P.P., Giribet, G., 2011. The evolutionary and biogeographic history of the armoured harvestmen – Laniatores phylogeny based on ten molecular markers, with the description of two new families of Opiliones (Arachnida). Inver. Syst. 25, 106–142.

Sharma, P.P., Giribet, G., 2014. A revised dated phylogeny of the arachnid order Opiliones. Front. Genet. 5, 255.

Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22, 2688–2690. https://doi.org/10.1093/bioinformatics/btl446.

Stamatakis, A., Hoover, P., Rougemont, J., 2008. A rapid bootstrap algorithm for the RAxML web servers. Syst. Biol. 57, 758–771. https://doi.org/10.1080/10635150802429642.

Zimmer, C., 2010. The Tangled Bank: An Introduction to Evolution, first ed. Roberts and Company, Greenwood Village, Colorado.