# Molecular phylogenetics illuminates the evolutionary history and hidden diversity of Australian cave crickets (Orthoptera: Rhaphidophoridae)

Beasley-Hall, P. G.[1]*, Trewick, S. A.[2], Eberhard, S. M.[3], Zwick, A.[4], Reed, E. H.[1], Cooper, S. J. B.[1,5], Austin, A. D.[1]

[1] School of Biological Sciences, The University of Adelaide, Adelaide, SA, Australia

[2] Wildlife & Ecology, Massey University Manawatū, Palmerston North, New Zealand

[3] Subterranean Ecology Pty Ltd., Coningham, TAS, Australia

[4] Australian National Insect Collection, CSIRO National Research Collections Australia, Canberra, ACT, Australia

[5] Evolutionary Biology Unit, South Australian Museum, Adelaide, SA, Australia

*** Corresponding author**

Perry Beasley-Hall, School of Biological Sciences, Darling Building, The University of Adelaide, Adelaide, South Australia 5000, Australia

Email: perry.beasley-hall@adelaide.edu.au

**Abstract**

Cave crickets (Orthoptera: Rhaphidophoridae) are a globally distributed group of insects found in dark, humid microhabitats including natural caves, alpine scree, and forest litter. Ten extant subfamilies are currently recognised and Macropathinae, which comprises the entirety of the fauna in South America, South Africa, Australia, and New Zealand, is thought to be the most ancient of these. The New Zealand rhaphidophorid fauna comprises high phylogenetic diversity throughout its mesic zone, with most species occurring above-ground. In contrast, the Australian fauna is poorly known, with an apparently greater relative proportion of species utilising caves as refugia. A robust phylogenetic framework is needed to underpin future taxonomic work on the group and potentially contrasting patterns of taxonomic diversity within Macropathinae. Here, we performed fossil-calibrated phylogenetic analysis using whole mitochondrial genomes and nuclear markers to reconstruct the evolutionary history of Macropathinae, with a focus on the Australian fauna. By dramatically increasing taxon sampling relative to past studies, we recovered the Australian fauna as rampantly polyphyletic, with the remaining Macropathinae nested amongst six distinct Australian lineages. Deep divergences between major clades imply additional Australian lineages remain undetected, either due to extinction or sampling bias, and have likely confounded past biogeographic signal. We inferred the radiation of Macropathinae began during the Lower Cretaceous prior to the fragmentation of Gondwana with a potential Pangaean origin for Rhaphidophoridae. Finally, we found evidence for several undescribed taxa of Australian Rhaphidophoridae, all of which qualify as short-range endemics, and discuss the conservation implications of these restricted distributions.

**Introduction**

Insects are the most biodiverse group of animals on Earth and are represented by over one million described species (Stork, 2018), yet their true diversity is poorly known. Enumerating this extraordinary diversity is necessary for conservation, but this endeavour is complicated by the fact that certain species are less likely to be discovered than others. Large-bodied,

geographically widespread species are more likely to be given formal scientific names, and therefore conservation attention, particularly those with visual appeal (Barua et al., 2012; Stork et al., 2008). In contrast, subterranean insects typically possess "unaesthetic" troglomorphic traits that include the elongation of sensory appendages, eye loss, and pigment reduction. These features can result in morphologically cryptic species which may only be detected through molecular phylogenetic analysis, further exacerbating a lack of taxonomic knowledge (King et al., 2022). The resulting lack of taxonomic knowledge may result in respective habitats receiving inadequate conservation effort relative to actual need. Indeed, subterranean environments—which include terrestrial and groundwater ecosystems in karstic caves, lava tubes, calcretes, and interstitial habitats—are often overlooked in conservation management efforts, despite being the largest non-marine ecosystem in the world (Mammola et al., 2022; Vaccarelli et al., 2023). Increasing taxonomic knowledge has been identified as an urgent first step towards conserving subterranean ecosystems worldwide (Wynne et al., 2021). To this end, here we use molecular phylogenetics and historical biogeography to explore the biodiversity and evolutionary history of an understudied group of Australian cave insects.

Rhaphidophoridae Walker, commonly referred to as camel or cave crickets, is a globally distributed, largely nocturnal family of Orthoptera found on every continent except Antarctica (Cigliano et al., 2022). Rhaphidophorids are wingless and may be found in animal burrows, alpine scree, forest litter, caves, and artificial structures such as abandoned mines. Subterranean species are reliant on caves to complete their life cycles but typically forage above-ground on a nightly basis (Richards, 1966). Rhaphidophoridae presently contains 10 subfamilies, with Macropathinae Karny thought to have diversified prior to all others (Allegrucci and Sbordoni, 2019a; Dowle et al., 2024; Gorochov, 2001; Hubbell and Norton, 1978). This Gondwanan subfamily comprises the known cave cricket fauna in Australia, New Zealand, South Africa, and South America (Fig. 1). Within Macropathinae, New Zealand fauna (also called cave wētā or tokoriro) is the best studied and the most biodiverse, with most species known from forests and the alpine zone and only a handful found in caves (Fitness et al., 2018; Hegg et al., 2022, 2019; Richards, 1972; Trewick, 2024). In contrast, in Australia almost all species are

predominantly recorded from caves, with above-ground aridity—and consequently less expansive wet forest habitats, especially on the mainland—implicated in determining their dependence on subterranean habitats (Richards, 1987). In the cooler, wetter island state of Tasmania, which supports extensive tracts of wet forests, aridity may be less of a factor versus the distribution of cave bearing rock types. No taxonomic research has been conducted on the Australian fauna for almost fifty years; the resulting knowledge gap is concerning because five Australian species are formally listed as threatened and human disturbance, including clearing of native forest, has been linked to population declines (Orthopteroid Specialist Group, 1996; Simms et al., 1996; Threatened Species Section, 2022a, 2022b, 2022c, 2022d).

Phylogenetic analyses have shown that the neighbouring Australian and New Zealand macropathine faunas are not reciprocally monophyletic. Examples include the Tasmanian genus *Parvotettix* Richards being the sister group to the remainder of the subfamily, including all other Australian genera (Beasley-Hall et al., 2018) and the New Zealand *Macropathus* Walker being sister to representatives from South America (Allegrucci et al., 2010). Further, recent time-calibrated phylogenetic work has highlighted several New Zealand lineages with divergence times pre-dating their island habitats, suggesting not only that a large proportion of the fauna remains to be discovered, but also that these flightless, nocturnal insects may possess a greater propensity for over-water dispersal than previously assumed (Dowle et al., 2024). Like Tasmania, high rhaphidophorid diversity in New Zealand—which has a cool, wet climate that supports wet forests—indicates that aridity, or native forest logging and clearing, reduces available habitat for these insects. The macropathine fauna of Australia therefore provides an interesting contrast in which a different pattern of taxonomic diversity might exist in cave and epigean habitats. We expect this diversity could reflect a relatively deep ancestry, echoing past mesic conditions across the continent (Byrne et al., 2011) and the utilisation of cave systems as refugia in the present day, especially in the warmer and drier regions of mainland Australia.

To paint a clearer picture of rhaphidophorid diversity in Australia, we applied molecular methods to reconstruct a comprehensive phylogeny for the group. The subfamily

Macropathinae exhibits a high degree of intraspecific morphological variation (Richards, 1971a), necessitating the use of molecular data to strengthen future taxonomic inference. A robust phylogenetic framework for Australian macropathines will not only enable the discovery and description of species previously unknown to science but also assist with documentation of genera and species that have awaited formal scientific names for decades (Richards, 1987). Here, we specifically sought to: 1) resolve relationships among the eight described Australian genera; 2) identify Australian lineages representing undescribed taxa for future taxonomic study; 3) determine relationships of the Australian fauna within the broader subfamily Macropathinae; and finally 4) enhance understanding of the phylogenetic position and biogeography of Macropathinae at the familial level.

## Materials and methods

### *Taxon sampling and DNA sequencing*

Australian cave cricket specimens were obtained from museum collections and in the field (see Fig. S1 for sampling map). Museum specimens were sourced from the South Australian Museum (SAMA), the Tasmanian Museum and Art Gallery (TMAG), the Queensland Museum (QM), the Australian Museum (AM), the Melbourne Museum (MM), and the Australian National Insect Collection (ANIC). We sampled all 22 described species of Australian Rhaphidophoridae with the exception of *Speleotettix chopardi* (Karny), which represents a *nomen dubium* (Beasley-Hall *et al.* submitted), as well as several putatively undescribed species and genera. We also integrated published GenBank data from six other rhaphidophorid subfamilies to infer the placement of Macropathinae at the family level, namely Aemodogryllinae Jacobson and Rhaphidophorinae Walker (East and Southeast Asia), Dolichopodainae Brunner von Wattenwyl and Troglophilinae Karny (Mediterranean), and Ceuthophilinae Tepper and Tropidischiinae Scudder (North America). Additional GenBank data for Prophalangopsidae Kirby (grigs), Anostostomatidae Saussure (king crickets), Tettigoniidae Krauss (katydids), and Caelifera Ander (grasshoppers and allies) were used as outgroups to facilitate fossil calibration and root the

tree. A complete list of taxa included in the present study, as well as relevant citations and accession numbers, is available in Supplementary Table S1.

For all specimens except those housed at ANIC (see below), DNA was extracted from mesothoracic leg tissue using a Gentra Puregene Tissue Kit (QIAGEN). The mitochondrial genes *12S* rRNA, *16S* rRNA, and cytochrome oxidase I (*COI*) and nuclear *28S* rRNA (D1 and D3-5 regions) were amplified using the following primers: 12Sai + 12Sbi (*12S*) (Simon et al., 1994), 16sar + 16sbr (*16S*) (Cunningham et al., 1992), LCO1490 + HCO2198 (*COI*) (Folmer et al., 1994), 686 + 427 (*28S* D1 region) (Friedrich and Tautz, 1997), and 485 + 689 (*28S* D3-5 regions) (Friedrich and Tautz, 1997). PCRs were performed using a standard hot-start protocol consisting of a preheat step at 95ºC for 10 minutes, denaturation at 95ºC for 45 seconds, annealing at 48ºC (*COI*, *28S* D1), 51ºC (*12S* and *16S* rRNAs), or 55ºC (*28S* D3-5) for 45 sec, extension at 72ºC for 1 min, 72ºC for 10 min, and 25ºC for 2 min 20 sec. Denaturation, annealing, and extension steps were repeated for 39 cycles. Bi-directional Sanger sequencing was outsourced to the Australian Genome Research Facility (AGRF, Melbourne, Australia) under standard PCR cycling conditions.

Sanger sequences were processed and aligned using the package *sangeranalyseR* in R v.4.3.0 {}. Sequences were trimmed by quality scores using a Phred score cutoff of 0.001 (Q30) and modified Mott trimming. A signal ratio cutoff of 0.33 and minimum fraction call of 0.75 were selected for base calling. Next-generation sequencing data were processed using the Trim Galore wrapper (github.com/FelixKrueger/TrimGalore) for Cutadapt and FastQC {} for quality trimming, filtering, and adapter removal. Quality-controlled reads were then *de novo* assembled using SPAdes with k-mer lengths of 33, 55, 77, 91, and 121, and the identity of resulting contigs was verified using a MegaBLAST search (Camacho et al., 2009) against the nucleotide collection of the GenBank repository (Sayers et al., 2023). For specimens with smaller contigs requiring scaffolding against a reference, reads were mapped against the closest available relative in GenBank with the *Map to Reference* tool in Geneious Prime v.2023.1.2 (geneious.com) using medium sensitivity presets and without trimming before mapping.

We conducted next-generation sequencing (NGS) on two subsets of our specimens (Table S1). The first consisted of type specimens housed at ANIC, sequenced as part of an existing project run by the Commonwealth Scientific and Industrial Research Organisation (CSIRO). ANIC specimens were extracted in-house using a modified Qiagen DNeasy Blood and Tissue kit protocol in 384-well format and sequenced using a miniaturised genome skimming approach centred around the Qiagen QiaSeq UltraLow Input library kit. Pools of 384 libraries were sequenced on an Illumina NovaSeq S1 flow cell. Our second subset was selected after initial analysis to provide whole mitogenome data for at least one specimen per major clade, as well as additional nuclear markers (*18S* rRNA and *histone-3* [H3]) to strengthen node support at the backbone of our tree. This subset also included museum specimens that did not successfully PCR amplify for Sanger sequencing. Library preparation was performed by AGRF using the Illumina DNA Prep (M) (fresh specimens) or IDT xGen Library Prep workflow (museum specimens). Paired-end sequencing was conducted by AGRF on an Illumina NovaSeq 6000 or NovaSeq X Plus platform, respectively, to generate 150-base-pair reads. Coding regions, tRNAs, and rRNAs in resulting mitogenome sequences were annotated using Prokka v.1.14.6 (Seemann, 2014). Annotated mitogenomes were processed for GenBank submission with GB2sequin (Lehwark and Greiner, 2019).

***Phylogenetic analysis***

Alignments of mitochondrial protein-coding genes, mitochondrial rRNAs, *H3*, *18S*, and two regions of *28S* (aligned separately) were produced using MUSCLE v.3.8.425 (Edgar, 2004) with default settings. Alignments were then concatenated and divided into nine partitions: mitochondrial protein-coding genes separated by codon position (1, 2, and 3), mitochondrial rRNAs (*12S*+*16S* together), *H3* separated by codon position (1, 2, and 3), *18S* rRNA, and finally *28S* rRNA (D1+D3-5 regions together) for a total of 18,146 base pairs.

We used maximum likelihood (ML) and Bayesian inference (BI) for phylogenetic analysis and divergence dating. ML analysis was performed in IQ-TREE v.1.6.12 (Nguyen et al., 2015)

using ModelFinder for substitution model selection (*-m MFP+MERGE*) and 1000 ultrafast bootstrap replicates (Hoang et al., 2018; Kalyaanamoorthy et al., 2017). We performed Bayesian inference using BEAST2 v.2.6.4 (Bouckaert et al., 2019). We selected the package bModelTest for substitution model selection (Bouckaert and Drummond, 2017), the birth-death tree prior—suitable for datasets containing inter- and intraspecific data (Ritchie et al., 2017)—and the optimised relaxed clock (Douglas et al., 2021). To assist the analysis through the burn-in stage, we set starting clock rates of 1.0e-6 and 1.0e-9 for mitochondrial and nuclear genes, respectively (Bouckaert, 2015). We ran three separate chains of 100 million generations with a tree logging frequency of every 10,000 generations. Convergence of the stationary distribution and effective sample size values (>200) of parameters were assessed using Tracer v.1.7.2 (Rambaut et al., 2018). Log files and trees were combined in LogCombiner v.2.7.5 with a 10% burn-in. A maximum clade credibility tree was inferred using TreeAnnotator v.2.7.1 (Bouckaert et al., 2019) with the common ancestors approach to node height annotation and a posterior probability limit of 0.5.

***Divergence dating***

To date the timings of cladogenesis within Rhaphidophoridae, we constrained our BI analysis with our ML topology as a starting tree such that only branch lengths were inferred throughout the run. We chose to do this because our initial BI analysis (described above) recovered the South American fauna as polyphyletic with poor node support, whereas it was inferred as a well-supported monophyletic group in our ML analysis in agreement with several previous studies (Allegrucci and Sbordoni, 2019b; Dowle et al., 2024; Kim et al., 2024). As such, we had reason to believe our ML tree reflected a more plausible set of relationships within Rhaphidophoridae. To constrain our BI analysis, we first scaled the ML starting tree using treePL to ensure compatibility with our chosen fossil calibration (Smith and O'Meara, 2012). Our dated Bayesian analysis was run in BEAST2 as for the uncalibrated analysis but only required three chains to reach convergence.

While several well-dated rhaphidophorid fossils exist, namely †Protroglophilinae Gorochov from the late Eocene, their phylogenetic placement remains unresolved (Allegrucci and Sbordoni, 2019a; Gorochov, 2001) and there are therefore no appropriate ingroup fossils to calibrate the molecular clock for the family. As such, we calibrated the node leading to Prophalangopsidae, Tettigoniidae, and Anostostomatidae using †*Pseudaboilus wealdensis* Gorochov, Jarzembowski & Coram, which has been rigorously assessed for its appropriateness in calibrating the orthopteran molecular clock and used in a recent phylogenomic analysis of the order (Song et al., 2020). This fossil has been dated to a minimum age of 127.5 Mya (Barremian, Lower Cretaceous) and is conservatively considered stem-(Tettigonioidea Krauss+Hagloidea Handlirsch) (Gorochov et al., 2006; Song et al., 2020). We applied the calibration using an exponential distribution with 127.5 Mya as a hard minimum bound and 271.8 Mya, the minimum age estimate of the oldest definitive Ensifera Chopard (Bethoux et al., 2002; Wolfe et al., 2016), as a soft maximum.

***Historical biogeography***

We used BioGeoBEARS (Matzke, 2014, 2013) in R v.4.4.0 (R Core Team, 2024), a package using probabilistic models for ancestral range estimation, to visualise historical biogeographic hypotheses for Macropathinae and relatives. Species distributions were compiled from observations made by Richards (Richards, 1987) and the Orthoptera Species File as at June 2024 (Cigliano et al., 2022). Species were coded as belonging to Australia (including Tasmania), New Zealand (including subantarctic islands), South America, South Africa, Southeast Asia, the Mediterranean, and/or North America. Historical biogeographic analyses were run using three models: dispersal-extinction-cladogenesis (DEC) (Ronquist, 1997), a ML implementation of dispersal-vicariance analysis (DIVALIKE) (Ree and Smith, 2008), and Bayesian Analysis of Biogeography (BAYAREALIKE) (Landis et al., 2013). Each of these models assumes a different set of possible sympatric and/or vicariant processes associated with cladogenetic events (Matzke, 2013). Founder-event speciation can also be incorporated into BioGeoBEARS models, but we chose not to use this parameter (*j*) because our primary goal was to estimate ancestral ranges rather than the mode of speciation or diversification. We ran these models with default

parameters using our time-calibrated phylogeny and their relative probabilities were assessed using the Akaike (AIC) and corrected Aikaike (AICc) information criteria.

## Results

### *Phylogenetic analysis*

We recovered mitochondrial (*12S* rRNA, *16S* rRNA, and *COI*; whole mitogenomes where possible) and nuclear (*18S* rRNA, two subunits of *28S* rRNA, and *H3*) molecular markers from 131 representatives of Australian Rhaphidophoridae. For samples for which we recovered whole mitogenomes, up to 18,094 bp were sequenced; for those represented by multi-locus Sanger data only, we recovered up to 5,966 bp. Using ML and BI, we recovered a monophyletic Macropathinae consisting of six major clades (Figs. 2, 3). Sister to all other macropathines in the analysis was *Parvotettix* and an undescribed genus, the most recent common ancestor (MRCA) of which diverged from the rest of the subfamily *ca.* 119.1 Mya (95% highest posterior density [HPD] 88.91–159.87 Mya). The MRCA of the South African genus *Spelaeiacris Péringuey* diverged *ca*. 99.78 Mya (95% HPD 71.78–136.75 Mya), and a second Australian lineage emerged *ca.* 85.16 Mya (95% HPD 62.53–115.59 Mya) containing the southeast Australian *Speleotettix* Chopard and *Cavernotettix* Richards. A widespread group then arose *ca*. 67.97 Mya (95% HPD 56.9–105.02 Mya) containing the New Zealand and South African faunas as well as two unrelated Australian lineages, the Tasmanian *Tasmanoplectron* Richards and Queensland *Australotettix* and relatives. The Tasmanian *Micropathus* Richards diverged from the MRCA of the southern Australian *Pallidotettix* Richards and *Novotettix* Richards *ca.* 63.74 Mya (95% HPD 46–87.98 Mya), which themselves diverged from one another *ca*. 31.55 Mya (95% HPD 21.39–44.14 Mya). Aemodogryllinae and Rhaphidophorinae, representing the Indomalayan realm in our analysis, were recovered as the sister clade to Macropathinae. Sister to this broader group was a Laurasian clade containing Dolichopodainae, Troglophilinae, Ceuthophilinae, and Tropidischiinae.

Several Australian lineages were not recovered as members of described species and/or genera and represent putatively new taxa (Figs. 2, 3). These include two species of *Micropathus* (clade Australia A); a new genus sister to *Micropathus* (Australia A); three lineages representing new *Australotettix* Richards, if not new genera in their own right (Australia C); a new genus related to *Australotettix* (Australia C); four species of *Speleotettix* (Australia E); two species of *Cavernotettix* (Australia E); and finally a new genus sister to *Parvotettix* (Australia F). Additional lineages were suggestive of new species within existing genera but were only represented by one sample, leaving their identifications less certain (e.g., *Parvotettix* spp. from Franklin and Dasher Rivers, Fig. 3).

Our ML and BI analyses recovered similar relationships among major clades of Macropathinae and with high node support in most cases. However, they differed with respect to two clades (Figs. 2, 3; see white node symbols). In our ML analysis, the New Zealand fauna (except for *Macropathus filifer* Walker) was inferred as sister to northeastern Australian species (Australia C) and *Macropathus* was sister to a monophyletic South American clade (New Zealand C, Fig. 2). In contrast, an initial BI phylogeny recovered a non-monophyletic South American fauna interspersed by New Zealand species but with low node support, prompting us to constrain the topology of our downstream time-calibrated BI tree. Interestingly, the same ML/BI conflict was reported by (Kim et al., 2024) for the New Zealand and South American Rhaphidophoridae. The position of Tropidschiinae also differed between our ML and BI analyses (Fig. 3), but the Laurasian taxa were nonetheless recovered as monophyletic and relationships among non-macropathine subfamilies were not the main focus of the present study.

***Historical biogeography***

The DEC model was the best fit to our dataset based on AIC and AICc scores (AIC = 78.82414; AICc = 79.02086) compared to DIVALIKE (86.22179; 86.41851) and BAYAREALIKE (435.22473; 435.42146) (Fig. S2). In historical biogeographic analysis, a *range* refers to the geographic range of a taxon and can consist of one or more *areas*. BioGeoBEARS infers the relative probability of different ancestral ranges; here and below we refer to most-probable ancestral ranges. In our

analysis, Australia, New Zealand, South America, and South Africa (i.e., Gondwana) was estimated as the ancestral range of the subfamily (Fig. S2). At the root of our tree, Rhaphidophoridae was estimated to have a broad ancestral range spanning all areas except the Mediterranean.

**Discussion**

Here we present a comprehensive molecular phylogenetic analysis of Rhaphidophoridae with a focus on Macropathinae, a subfamily representing the majority of rhaphidophorid biodiversity in the Southern Hemisphere. Our results expand on previous findings demonstrating the Australian and New Zealand faunas are both non-monophyletic, with the former thought to consist of at least two distantly related clades based on limited sampling (Allegrucci and Sbordoni, 2019a; Beasley-Hall et al., 2018). In the current study, we recovered Macropathinae as a monophyletic group containing a diverse, polyphyletic Australian fauna consisting of at least six major lineages, with the remainder of the Southern Hemisphere fauna nested between or within those groups. We recovered the New Zealand fauna as similarly polyphyletic and sharing a most recent common ancestor with South American species (Fig. 2), in agreement with a recent phylogenetic study focusing on the Rhaphidophoridae of New Zealand (Dowle et al., 2024).

Here, we were primarily interested in examining the relationships and biogeography of Macropathinae, but we also included additional representatives of the family to assess the placement of Macropathinae within the broader Rhaphidophoridae. Our findings are inconsistent with morphology-driven hypotheses proposing Macropathinae as the most "primitive" subfamily within Rhaphidophoridae and being sister to all others (Ander, 1939; Gorochov, 2001; Hubbell and Norton, 1978; Karny, 1930). However, our results agree with other recent phylogenetic studies in that the subfamily appears to have diversified prior to other major groups of Rhaphidophoridae, supporting its "ancient" status (Allegrucci and Sbordoni, 2019a; Dowle et al., 2024; Kim et al., 2024). We recovered similar relationships among Northern Hemisphere subfamilies as per a recent global phylogeny for the family, with

the Southeast and Eastern Asian subfamilies Aemodogryllinae and Rhaphidophorinae being inferred as the closest relatives of Macropathinae (Kim et al., 2024).

***Biogeography of Macropathinae***

Previous age estimates for Rhaphidophoridae have largely been derived from order-level studies that have not extensively sampled the group, with a tendency to focus on a small number of Northern Hemisphere taxa (Song et al., 2020; Vandergast et al., 2017). Two recent studies have expanded sampling of Rhaphidophoridae to more closely examine its age and diversification. Kim *et al.* (2024) focused largely on the Southeast Asian and Mediterranean Rhaphidophoridae and constrained their tree using secondary age estimates sourced from Song *et al.* (2020). Dowle et al. (2024) focused on the New Zealand Macropathinae and used both a fossil and geological calibration. While the age estimates of Dowle *et al.* (2024) were slightly older than Kim *et al*. (2024) and the present study, all estimated an age of around 140 Mya for the most recent common ancestor of Macropathinae.

Our results support the hypothesis that the macropathine fauna radiated prior to the fragmentation of Gondwana, with the earliest representatives appearing in Australia. Firstly, the South African, South American, and New Zealand faunas are all nested between, or within, Australian clades in the tree (Figs. 2, 3). Secondly, we infer Macropathinae diverged from the remainder of Rhaphidophoridae in the Lower Cretaceous 134.56 Mya (95% HPD 103.11–182 Mya) prior to the divergence of the MRCA of the Tasmanian *Parvotettix* and an undescribed sister genus *ca.* 119.1 Mya (95% HPD 88.91–159.87 Mya), well before the separation of Australia from Antarctica and New Zealand *ca*. 75 Mya (McIntyre et al., 2017). Not all cladogenetic events in our tree reflect the breakup of Gondwana: for example, we estimated the South African *Spelaeiacris* diverged from the Australian (excluding clade F), New Zealand, and South American Rhaphidophoridae *ca*. 99.78 Mya, placing it after the separation of Africa from West Gondwana *ca*. 110 Mya (McIntyre et al., 2017). Long distance dispersal via rafting has been proposed to resolve this apparent contradiction between inferred divergence times and geological events, e.g., in the case of New Zealand wētā (Dowle et al., 2024). At the family

level, we estimated a late Jurassic age of *ca.* 149.66 Mya (95% HPD 115.68–199.63 Mya) for Rhaphidophoridae. As for previous studies, this order and timing of diversification is consistent with the family originating prior to the separation of Pangaea, with major cladogenetic events reflecting the separation of the supercontinent into Laurasia and Gondwana (Allegrucci and Sbordoni, 2019a; Kim et al., 2024).

Our estimates of lineage age do not necessarily justify the interpretation of vicariance mediated by continental drift as the single, or even primary, driver of diversification of Macropathinae. It is clear that dispersal, extinction, and sampling have influenced biogeographic signal for Rhaphidophoridae (Dowle et al., 2024), and many other taxa. The diverse Australian rhaphidophorid fauna is separated by deep divergences in our analysis, even when only minimum ages of nodes are considered, suggesting that more closely related taxa have not been sampled. For example, we inferred several island species (*Cavernotettix craggiensis* Richards, *flindersensis* (Chopard), and sp. nov. 2; *Parvotettix rangaensis* Richards and *whinrayi* Richards) diverged from their closest relatives millions of years before the isolation of such land masses over the past ~14,000 years due to post-glacial flooding (Lambeck and Chappell, 2001). This observation of apparently "old" taxa on "young" islands has also been documented in the New Zealand Rhaphidophoridae (Dowle et al., 2024) and discussed throughout the broader phylogenetic literature (McCulloch and Waters, 2019). Dowle *et al.* (2024) suggested the interplay between speciation and extinction processes have resulted in a mismatch between geological history and biology. In other words, a failure to sample non-island sister species has likely confounded biogeographic signal, either because those lineages are extinct or as-yet unknown to science. A combination of both may have led to the patterns we see in our tree. In addition to extinction processes resulting from extensive aridification and Pleistocene climatic changes to the Australian continent (Byrne et al., 2008; Weij et al., 2024), the cryptic nature of rhaphidophorids in both forest and subterranean habitats has almost certainly contributed to incomplete sampling of the fauna worldwide. This is especially applicable in an Australian context given the apparent high abundance of the group in caves, the disjunct distributions of species in relictual forest, and the inaccessibility of such locations

for sampling. Failure to sample for this reason would imply a greater diversity in Australia than currently known and is supported by the discovery of several undescribed taxa in our phylogeny.

***Conservation and taxonomic implications***

Rhaphidophorids are an important faunal component of caves in the Australian mesic zone where they play a prominent ecological role as predators, omnivorous scavengers, and detritivores. The abundance of rhaphidophorid biomass also supports predator communities (Richards, 1971b) and they are the key food source sustaining populations of the iconic Tasmanian cave spider *Hickmania troglodytes* (Higgins & Petterd) (Driessen, 2009). Further, all described Australian cave cricket species are known from exceptionally limited distributions (<10,000 $km^2$), qualifying them as short-range endemics (SREs) at a higher risk of extinction compared to more cosmopolitan taxa (Harvey, 2002; Richards, 1971a). However, only 68 of a described 879 species globally have had some form of conservation assessment as per the IUCN Red List, and over half of those 68 are considered at risk of extinction (Cigliano et al., 2022; IUCN, 2023). As such, establishing which species are undescribed *vs*. a disjunct population of a more widespread taxon is a key step in the management of these SREs, without which clear conservation priorities cannot be established.

Our findings have several conservation implications for the Australian Rhaphidophoridae. Five species are currently represented in federal or state threatened species lists: *Cavernotettix craggiensis*, *Micropathus kiernani* Richards, *Parvotettix rangaensis*, *P. whinrayi*, and *Tasmanoplectron isolatum* Richards, all found in Tasmania (Orthopteroid Specialist Group, 1996; Threatened Species Section, 2022a, 2022b, 2022c, 2022d). Given all described Australian species can be typified as SREs many more are likely in need of conservation listing, particularly in the face of several key threatening processes. These include native forest logging and land clearing, which risk fragmenting wet forest habitat that provides essential food sources above-ground, and also act as corridors to connect cave populations; predation from rats; and human disturbance via recreational and tourist activities in caves

(IUCN, 2023; Richards, 1987; Simms et al., 1996). Here, we have revealed a far higher diversity in Australia than the eight genera and 22 species currently known, highlighting at least three new genera and 15 new species (Figs. 2, 3). "Gen. nov. 1" in Fig. 2 was described as the monotypic genus *Eburnocauda* Beasley-Hall & Iannello while the present study was in review, bringing the total number of rhaphidophorid genera in Australia to nine (Iannello and Beasley-Hall, 2024). Finally, the phylogeny presented here has highlighted the need to redescribe *Micropathus tasmaniensis* Richards, the southern portion of which was recovered as a new species (*Micropathus* sp. nov. 2). Our findings have increased the known biodiversity of Rhaphidophoridae in Australia by at least 50%, but a far greater number of taxa almost certainly remain undiscovered.

**Conclusions**

We have presented a robust phylogeny of the Southern Hemisphere cave cricket subfamily Macropathinae. By substantially expanding upon past sampling of the Australian fauna, we have confidently resolved relationships among all described genera. We have also identified at least 15 new species and three new genera based on molecular data, which will form the basis of future taxonomic work. The Australian fauna is rampantly polyphyletic and consists of five major unrelated lineages, three of which have independent evolutionary origins in Tasmania. We have demonstrated that the New Zealand and South African Rhaphidophoridae share ancestors with the Australian fauna, and that South American biota is in turn nested within a polyphyletic New Zealand fauna. We estimate Macropathinae began to diversify across Gondwana in the Lower Cretaceous *ca*. 119 Mya—following the divergence from a common ancestor shared with the Asian Rhaphidophorinae and Aemodogryllinae *ca*. 134 Mya—and the Rhaphidophoridae itself originated *ca*. 150 Mya across Pangaea.

Improving understandings of evolutionary relationships and taxonomy within Rhaphidophoridae is important for several reasons. First, cave crickets represent a valuable, yet often cryptic, ecological component of subterranean realms. Identifying species boundaries is necessary for conservation management of these short-range endemics such as the

understudied Australian biota. Second, robust phylogenetic frameworks may assist in identifying characters that are informative for future taxonomy and identification by non-scientist stakeholders (e.g., park managers, naturalists, and speleologists). Finally, groups such as the Rhaphidophoridae can shed further light on the link between changing climatic conditions, species distributions, and lineage diversity. For example, by comparing the Australian and New Zealand fauna in the present study, we have highlighted that cave crickets are not simply a "relictual" fauna only found underground—as is often the narrative associated with Northern Hemisphere species—but can be highly diverse in cool, wet forests, with many lineages still awaiting taxonomic description even in the relatively restricted mesic zone of Australia.

## References


Allegrucci, G., Ketmaier, V., Di Russo, C., Rampini, M., Sbordoni, V., Cobolli, M., 2017. Molecular phylogeography of Troglophilus cave crickets (Orthoptera, Rhaphidophoridae): A combination of vicariance and dispersal drove diversification in the East Mediterranean region. J. Zool. Syst. Evol. Res. 55, 310–325. https://doi.org/10.1111/jzs.12172

Allegrucci, G., Rampini, M., Gratton, P., Todisco, V., Sbordoni, V., 2009. Testing phylogenetic hypotheses for reconstructing the evolutionary history of Dolichopoda cave crickets in the eastern Mediterranean. J. Biogeogr. 36, 1785–1797. https://doi.org/10.1111/j.1365-2699.2009.02130.x

Allegrucci, G., Sbordoni, V., 2019a. Insights into the molecular phylogeny of Rhaphidophoridae, an ancient, worldwide lineage of Orthoptera. Mol. Phylogenet. Evol. 138, 126–138. https://doi.org/10.1016/j.ympev.2019.05.032

Allegrucci, G., Todisco, V., Sbordoni, V., 2005. Molecular phylogeography of Dolichopoda cave crickets (Orthoptera, Rhaphidophoridae): a scenario suggested by mitochondrial DNA. Mol. Phylogenet. Evol. 37, 153–164. https://doi.org/10.1016/j.ympev.2005.04.022

Allegrucci, G., Trewick, S.A., Fortunato, A., Carchini, G., Sbordoni, V., 2010. Cave crickets and cave weta (Orthoptera, Rhaphidophoridae) from the southern end of the World: a molecular phylogeny test of biogeographical hypotheses. J. Orthoptera Res. 19, 121–130.

Ander, K., 1939. Vergleichend-anatomische und phylogenetische Studien über die Ensifera (Saltatoria). Opusc. Entomol. 2.

Barua, M., Gurdak, D.J., Ahmed, R.A., Tamuly, J., 2012. Selecting flagships for invertebrate conservation. Biodivers. Conserv. 21, 1457–1476. https://doi.org/10.1007/s10531-012-0257-7

Beasley-Hall, P.G., Tierney, S.M., Weinstein, P., Austin, A.D., 2018. A revised phylogeny of macropathine cave crickets (Orthoptera: Rhaphidophoridae) uncovers a paraphyletic Australian fauna. Mol. Phylogenet. Evol. 126, 153–161. https://doi.org/10.1016/j.ympev.2018.04.024

Bethoux, O., Nel, A., Lapeyrie, J., Gand, G., Galtier, J., 2002. Raphogla rubra gen. n., sp. n., the oldest representative of the clade of modern Ensifera (Orthoptera: Tettigoniidea, Gryllidea). Eur. J. Entomol. 99, 111–116. https://doi.org/10.14411/eje.2002.019

Bouckaert, R.R., 2015. Help, BEAST acts weird! (Or how to set up rates) [WWW Document]. BEAST2. URL https://www.beast2.org/2015/06/23/help-beast-acts-weird-or-how-to-set-up-rates.html (accessed 12.10.24).

Bouckaert, R.R., Drummond, A.J., 2017. bModelTest: Bayesian phylogenetic site model averaging and model comparison. BMC Evol. Biol. 17, 42. https://doi.org/10.1186/s12862-017-0890-6

Bouckaert, R.R., Vaughan, T.G., Barido-Sottani, J., Duchêne, S., Fourment, M., Gavryushkina, A., Heled, J., Jones, G., Kühnert, D., Maio, N.D., Matschiner, M., Mendes, F.K., Müller, N.F., Ogilvie, H.A., Plessis, L. du, Popinga, A., Rambaut, A., Rasmussen, D., Siveroni, I., Suchard, M.A., Wu, C.-H., Xie, D., Zhang, C., Stadler, T., Drummond, A.J., 2019. BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. PLOS Comput. Biol. 15, e1006650. https://doi.org/10.1371/journal.pcbi.1006650

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. https://doi.org/10.1111/j.1365-2699.2011.02535.x

Byrne, M., Yeates, D.K., Joseph, L., Kearney, M., Bowler, J., Williams, M. a. J., Cooper, S., Donnellan, S.C., Keogh, J.S., Leys, R., Melville, J., Murphy, D.J., Porch, N., Wyrwoll, K.-H., 2008. Birth of a biome: insights into the assembly and maintenance of the Australian arid zone biota. Mol. Ecol. 17, 4398–4417. https://doi.org/10.1111/j.1365-294X.2008.03899.x

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., Madden, T.L., 2009. BLAST+: architecture and applications. BMC Bioinformatics 10, 421. https://doi.org/10.1186/1471-2105-10-421

Cigliano, M.M., Braun, H., Eades, D.C., Otte, D., 2022. Orthoptera Species File version 5.0/5.0 [WWW Document]. URL http://orthoptera.speciesFile.org (accessed 10.8.22).

Cunningham, C.W., Blackstone, N.W., Buss, L.W., 1992. Evolution of king crabs from hermit crab ancestors. Nature 355, 539–542. https://doi.org/10.1038/355539a0

deWaard, J.R., Ratnasingham, S., Zakharov, E.V., Borisenko, A.V., Steinke, D., Telfer, A.C., Perez, K.H.J., Sones, J.E., Young, M.R., Levesque-Beaudin, V., Sobel, C.N., Abrahamyan, A., Bessonov, K., Blagoev, G., deWaard, S.L., Ho, C., Ivanova, N.V., Layton, K.K.S., Lu, L., Manjunath, R., McKeown, J.T.A., Milton, M.A., Miskie, R., Monkhouse, N., Naik, S., Nikolova, N., Pentinsaari, M., Prosser, S.W.J., Radulovici, A.E., Steinke, C., Warne, C.P., Hebert, P.D.N., 2019. A reference library for Canadian invertebrates with 1.5 million barcodes, voucher specimens, and DNA samples. Sci. Data 6, 308. https://doi.org/10.1038/s41597-019-0320-2

Douglas, J., Zhang, R., Bouckaert, R., 2021. Adaptive dating and fast proposals: Revisiting the phylogenetic relaxed clock model. PLOS Comput. Biol. 17, e1008322. https://doi.org/10.1371/journal.pcbi.1008322

Dowle, E.J., Trewick, S.A., Morgan-Richards, M., 2024. Fossil-calibrated phylogenies of Southern cave wētā show dispersal and extinction confound biogeographic signal. R. Soc. Open Sci. 11, 231118. https://doi.org/10.1098/rsos.231118

Driessen, M.M., 2009. Baseline monitoring of the Tasmanian Glow-worm and other cave fauna: Exit Cave and Mystery Creek Cave – Tasmania (No. Nature Conservation Report 09/02). Parks and Wildlife Service, Tasmania.

Edgar, R.C., 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics 5, 113. https://doi.org/10.1186/1471-2105-5-113

Fenn, J.D., Song, H., Cameron, S.L., Whiting, M.F., 2008. A preliminary mitochondrial genome phylogeny of Orthoptera (Insecta) and approaches to maximizing phylogenetic signal found within mitochondrial genome data. Mol. Phylogenet. Evol. 49, 59–68. https://doi.org/10.1016/j.ympev.2008.07.004

Fitness, J., Morgan-Richards, M., Hegg, D., Trewick, S.A., 2018. Reinstatement of the New Zealand cave wētā genus Miotopus Hutton (Orthoptera: Rhaphidophoridae) and description of a new species. Eur. J. Taxon. https://doi.org/10.5852/ejt.2018.468

Flook, P.K., Klee, S., Rowell, C.H., 1999. Combined molecular phylogenetic analysis of the Orthoptera (Arthropoda, Insecta) and implications for their higher systematics. Syst. Biol. 48, 233–253. https://doi.org/10.1080/106351599260274

Flook, P.K., Rowell, C.H., Gellissen, G., 1995. The sequence, organization, and evolution of the Locusta migratoria mitochondrial genome. J. Mol. Evol. 41, 928–941. https://doi.org/10.1007/BF00173173

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. Biotechnol. 3, 294–299.

Friedrich, M., Tautz, D., 1997. An episodic change of rDNA nucleotide substitution rate has occurred during the emergence of the insect order Diptera. Mol. Biol. Evol. 14, 644–653. https://doi.org/10.1093/oxfordjournals.molbev.a025804

Goldberg, J., Trewick, S.A., 2011. Exploring Phylogeographic Congruence in a Continental Island System. Insects 2, 369–399. https://doi.org/10.3390/insects2030369

Gorochov, A.V., 2001. The higher classification, phylogeny and evolution of the superfamily Stenopelmatoidea. Biol. Wetas King Crickets Their Allies, CABI Books 3–33. https://doi.org/10.1079/9780851994086.0003

Gorochov, A.V., Jarzembowski, E.A., Coram, R.A., 2006. Grasshoppers and crickets (Insecta: Orthoptera) from the Lower Cretaceous of southern England. Cretac. Res. 27, 641–662. https://doi.org/10.1016/j.cretres.2006.03.007

Harvey, M.S., 2002. Short-range endemism amongst the Australian fauna: some examples from non-marine environments. Invertebr. Syst. 16, 555–570. https://doi.org/10.1071/is02009

Hegg, D., Morgan-Richards, M., Trewick, S.A., 2022. High alpine sorcerers: revision of the cave wētā genus Pharmacus Pictet & de Saussure (Orthoptera: Rhaphidophoridae: Macropathinae), with the description of six new species and three new subspecies. Eur. J. Taxon. 808, 1–58. https://doi.org/10.5852/ejt.2022.808.1721

Hegg, D., Morgan-Richards, M., Trewick, S.A., 2019. Diversity and distribution of Pleioplectron Hutton cave wētā (Orthoptera: Rhaphidophoridae: Macropathinae), with the synonymy of Weta Chopard and the description of seven new species. Eur. J. Taxon. https://doi.org/10.5852/ejt.2019.577

Hoang, D.T., Chernomor, O., von Haeseler, A., Minh, B.Q., Vinh, L.S., 2018. UFBoot2: Improving the Ultrafast Bootstrap Approximation. Mol. Biol. Evol. 35, 518–522. https://doi.org/10.1093/molbev/msx281

Hubbell, T.H., Norton, R.M., 1978. The systematics and biology of the cave-crickets of the North American tribe Hadenoecinni (Orthoptera Saltatoria: Rhaphidophoridae: Dolichopodinae). Misc. Publ. Mus. Zool. Univ. Mich. 156, 1–125.

Iannello, S., Beasley-Hall, P.G., 2024. Systematics and biology of Eburnocauda gen. nov., a cave cricket from Australian granite pseudokarst (Orthoptera: Rhaphidophoridae). Aust. J. Taxon. 73, 1–9.

IUCN, 2023. Rhaphidophoridae. The IUCN Red List of Threatened Species. Version 2023-1 [WWW Document]. IUCN Red List Threat. Species. URL https://www.iucnredlist.org/search/stats?taxonomies=101861&searchType=species (accessed 6.19.24).

Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., von Haeseler, A., Jermiin, L.S., 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat. Methods 14, 587–589. https://doi.org/10.1038/nmeth.4285

Karny, H.H., 1930. Phylogenetische und tiergeographische Erwägungen zur Systematik der Rhaphidophorinen. (Orth. Fam. Gryllacridae). Arch. Für Klassif. Phylogenetische Entomol. 1, 57–76.

Kim, D.-Y., Kim, S., Song, H., Shin, S., 2024. Phylogeny and biogeography of the wingless orthopteran family Rhaphidophoridae. Commun. Biol. 7, 1–11. https://doi.org/10.1038/s42003-024-06068-x

King, R.A., Fagan-Jeffries, E.P., Bradford, T.M., Stringer, D.N., Finston, T.L., Halse, S.A., Eberhard, S.M., Humphreys, G., Humphreys, B.F., Austin, A.D., Cooper, S.J.B., 2022. Cryptic diversity down under: defining species in the subterranean amphipod genus Nedsia Barnard & Williams, 1995 (Hadzioidea: Eriopisidae) from the Pilbara, Western Australia. Invertebr. Syst. 36, 113–159. https://doi.org/10.1071/IS21041

Lambeck, K., Chappell, J., 2001. Sea Level Change Through the Last Glacial Cycle. Science 292, 679–686. https://doi.org/10.1126/science.1059549

Landis, M.J., Matzke, N.J., Moore, B.R., Huelsenbeck, J.P., 2013. Bayesian Analysis of Biogeography when the Number of Areas is Large. Syst. Biol. 62, 789–804. https://doi.org/10.1093/sysbio/syt040

Lehwark, P., Greiner, S., 2019. GB2sequin - A file converter preparing custom GenBank files for database submission. Genomics 111, 759–761. https://doi.org/10.1016/j.ygeno.2018.05.003

Liu, C., Chang, J., Ma, C., Li, L., Zhou, S., 2013. Mitochondrial genomes of two Sinochlora species (Orthoptera): novel genome rearrangements and recognition sequence of replication origin. BMC Genomics 14, 114. https://doi.org/10.1186/1471-2164-14-114

Lu, H.-J., Huang, Y., 2012. Phylogenetic relationship among some groups of orthopteran based on complete sequences of the mitochondrial COI gene. Dong Wu Xue Yan Jiu Zool. Res. 33, 319–328. https://doi.org/10.3724/SP.J.1141.2012.03319

Lu, X., Lin, L.I., Liu, J., Liang, L.L., Bian, X., 2022a. Study of the Subfamily Anabropsinae (Orthoptera: Anostostomatidae) in China V: Two new species of Anabropsis (Apteranabropsis) from Guangxi and phylogenetic analysis of the genus Anabropsis. Zootaxa 5141, 227–248. https://doi.org/10.11646/zootaxa.5141.3.2

Lu, X., Liu, J., Huang, X., Bian, X., 2022b. Contribution to the Chinese subfamily Rhaphidophorinae Walker, 1869 (Orthoptera: Rhaphidophoridae: Rhaphidophorinae) IV: Seven new species of Rhaphidophora and one new mitogenome. Zootaxa 5087, 129–153. https://doi.org/10.11646/zootaxa.5087.1.6

Luong, L.T., Chapman, E.G., Harwood, J.D., Hudson, P.J., 2013. Linking predator-prey interactions with exposure to a trophically transmitted parasite using PCR-based analyses. Mol. Ecol. 22, 239–248. https://doi.org/10.1111/mec.12095

Mammola, S., Meierhofer, M.B., Borges, P.A.V., Colado, R., Culver, D.C., Deharveng, L., Delić, T., Di Lorenzo, T., Dražina, T., Ferreira, R.L., Fiasca, B., Fišer, C., Galassi, D.M.P., Garzoli, L., Gerovasileiou, V., Griebler, C., Halse, S., Howarth, F.G., Isaia, M., Johnson, J.S., Komerički, A., Martínez, A., Milano, F., Moldovan, O.T., Nanni, V., Nicolosi, G., Niemiller, M.L., Pallarés, S., Pavlek, M., Piano, E., Pipan, T., Sanchez-Fernandez, D., Santangeli, A., Schmidt, S.I., Wynne, J.J., Zagmajster, M., Zakšek, V., Cardoso, P., 2022. Towards evidence-based conservation of subterranean ecosystems. Biol. Rev. 97, 1476–1510. https://doi.org/10.1111/brv.12851

Matzke, N.J., 2014. Model Selection in Historical Biogeography Reveals that Founder-Event Speciation Is a Crucial Process in Island Clades. Syst. Biol. 63, 951–970. https://doi.org/10.1093/sysbio/syu056

Matzke, N.J., 2013. Probabilistic historical biogeography: new models for founder-event speciation, imperfect detection, and fossils allow improved accuracy and model-testing. Front. Biogeogr. 5. https://doi.org/10.21425/F5FBG19694

McCulloch, G.A., Waters, J.M., 2019. Phylogenetic divergence of island biotas: Molecular dates, extinction, and "relict" lineages. Mol. Ecol. 28, 4354–4362. https://doi.org/10.1111/mec.15229

McIntyre, S.R.N., Lineweaver, C.H., Groves, C.P., Chopra, A., 2017. Global biogeography since Pangaea. Proc. R. Soc. B Biol. Sci. 284, 20170716. https://doi.org/10.1098/rspb.2017.0716

Mugleston, J.D., Song, H., Whiting, M.F., 2013. A century of paraphyly: A molecular phylogeny of katydids (Orthoptera: Tettigoniidae) supports multiple origins of leaf-like wings. Mol. Phylogenet. Evol. 69, 1120–1134. https://doi.org/10.1016/j.ympev.2013.07.014

Nguyen, L.-T., Schmidt, H.A., von Haeseler, A., Minh, B.Q., 2015. IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. Mol. Biol. Evol. 32, 268–274. https://doi.org/10.1093/molbev/msu300

Orthopteroid Specialist Group, 1996. Tasmanoplectron isolatum. IUCN Red List Threat. Species 1996 e.T21504A9292775. https://doi.org/10.2305/IUCN.UK.1996.RLTS.T21504A9292775.en

R Core Team, 2024. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rambaut, A., Drummond, A.J., Xie, D., Baele, G., Suchard, M.A., 2018. Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. Syst. Biol. 67, 901–904. https://doi.org/10.1093/sysbio/syy032

Ree, R.H., Smith, S.A., 2008. Maximum Likelihood Inference of Geographic Range Evolution by Dispersal, Local Extinction, and Cladogenesis. Syst. Biol. 57, 4–14. https://doi.org/10.1080/10635150701883881

Richards, A.M., 1987. Distribution and relationships of the Australian Rhaphidophoridae (Orthoptera): pointers to past climatic changes, in: Evolutionary Biology of Orthopteroid Insects. Elsevier, pp. 438–449.

Richards, A.M., 1972. Revision of the Rhaphidophoridae (Orthoptera) of New Zealand. Part XIV. Three alpine genera from the South Island. J. R. Soc. N. Z. 2, 151–174.

Richards, A.M., 1971a. The Rhaphidophoridae (Orthoptera) of Australia. Part 10. A new genus from southeastern Tasmania with New Zealand affinities. Pac. Insects 13, 589–595.

Richards, A.M., 1971b. An ecological study of the cavernicolous fauna of the Nullarbor Plain Southern Australia. J. Zool. Proc. Zool. Soc. Lond. 164, 1–60.

Richards, A.M., 1966. Activity rhythms in Rhaphidophoridae (Orthoptera) from Australia and New Zealand. Helictite 4, 64–66.

Ritchie, A.M., Lo, N., Ho, S.Y.W., 2017. The Impact of the Tree Prior on Molecular Dating of Data Sets Containing a Mixture of Inter- and Intraspecies Sampling. Syst. Biol. 66, 413–425. https://doi.org/10.1093/sysbio/syw095

Ronquist, F., 1997. Dispersal-Vicariance Analysis: A New Approach to the Quantification of Historical Biogeography. Syst. Biol. 46, 195–203. https://doi.org/10.1093/sysbio/46.1.195

Sayers, E.W., Cavanaugh, M., Clark, K., Pruitt, K.D., Sherry, S.T., Yankie, L., Karsch-Mizrachi, I., 2023. GenBank 2023 update. Nucleic Acids Res. 51, D141–D144. https://doi.org/10.1093/nar/gkac1012

Seemann, T., 2014. Prokka: rapid prokaryotic genome annotation. Bioinforma. Oxf. Engl. 30, 2068–2069. https://doi.org/10.1093/bioinformatics/btu153

Simms, R., Lawrence, R., Buswell, C., Jevons, A., Kraehenbuehl, P., Maurer, H., Laidlaw, J., 1996. Cave crickets at Naracoorte: a report on the cave (camel) cricket Novotettix naracoortensis (Richards) (Orthoptera: Rhaphidophoridae) in the Naracoorte karst area. South Australian Speleological Council.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., Flook, P., 1994. Evolution, Weighting, and Phylogenetic Utility of Mitochondrial Gene Sequences and a Compilation of Conserved Polymerase Chain Reaction Primers. Ann. Entomol. Soc. Am. 87, 651–701. https://doi.org/10.1093/aesa/87.6.651

Smith, S.A., O'Meara, B.C., 2012. treePL: divergence time estimation using penalized likelihood for large phylogenies. Bioinformatics 28, 2689–2690. https://doi.org/10.1093/bioinformatics/bts492

Song, H., Amédégnato, C., Cigliano, M.M., Desutter-Grandcolas, L., Heads, S.W., Huang, Y., Otte, D., Whiting, M.F., 2015. 300 million years of diversification: elucidating the patterns of orthopteran evolution based on comprehensive taxon and gene sampling. Cladistics 31, 621–651. https://doi.org/10.1111/cla.12116

Song, H., Béthoux, O., Shin, S., Donath, A., Letsch, H., Liu, S., McKenna, D.D., Meng, G., Misof, B., Podsiadlowski, L., Zhou, X., Wipfler, B., Simon, S., 2020. Phylogenomic analysis sheds light on the evolutionary pathways towards acoustic communication in Orthoptera. Nat. Commun. 11, 4939. https://doi.org/10.1038/s41467-020-18739-4

Stork, N.E., 2018. How Many Species of Insects and Other Terrestrial Arthropods Are There on Earth? Annu. Rev. Entomol. 63, 31–45. https://doi.org/10.1146/annurev-ento-020117-043348

Stork, N.E., Grimbacher, P.S., Storey, R.I., Oberprieler, R.G., Reid, C. a. M., Slipinski, S.A., 2008. What determines whether a species of insect is described? Evidence from a study of tropical forest beetles. Insect Conserv. Divers. 1.

Terry, M.D., Whiting, M.F., 2005. Mantophasmatodea and phylogeny of the lower neopterous insects. Cladistics 21, 240–257. https://doi.org/10.1111/j.1096-0031.2005.00062.x

Threatened Species Section, 2022a. Craggy Island cave cricket (Cavernotettix craggiensis): Species management profile for Tasmania's Threatened Species Link.

Threatened Species Section, 2022b. Southern sandstone cave cricket (Micropathus kiernani): Species management profile for Tasmania's Threatened Species Link.

Threatened Species Section, 2022c. Ranga cave cricket (Parvotettix rangaensis): Species management profile for Tasmania's Threatened Species Link.

Threatened Species Section, 2022d. Whinray's cave cricket (Parvotettix whinrayi): Species management profile for Tasmania's Threatened Species Link.

Trewick, S.A., 2024. Two new genera of tokoriro (Orthoptera: Rhaphidophoridae: Macropathinae) from Aotearoa New Zealand. Zootaxa 5481, 531–546. https://doi.org/10.11646/zootaxa.5481.5.3

Vaccarelli, I., Colado, R., Pallarés, S., Galassi, D.M.P., Sánchez-Fernández, D., Di Cicco, M., Meierhofer, M.B., Piano, E., Di Lorenzo, T., Mammola, S., 2023. A global meta-analysis reveals multilevel and context-dependent effects of climate change on subterranean ecosystems. One Earth 6, 1510–1522. https://doi.org/10.1016/j.oneear.2023.09.001

Van Dam, M.H., Matzke, N.J., 2016. Evaluating the influence of connectivity and distance on biogeographical patterns in the south-western deserts of North America. J. Biogeogr. 43, 1514–1532. https://doi.org/10.1111/jbi.12727

Vandergast, A.G., Weissman, D.B., Wood, D.A., Rentz, D.C.F., Bazelet, C.S., Ueshima, N., 2017. Tackling an intractable problem: Can greater taxon sampling help resolve relationships within the Stenopelmatoidea (Orthoptera: Ensifera)? Zootaxa 4291, 1–33. https://doi.org/10.11646/zootaxa.4291.1.1

Weckstein, J.D., Johnson, K.P., Murdoch, J.D., Krejca, J.K., Takiya, D.M., Veni, G., Reddell, J.R., Taylor, S.J., 2016. Comparative phylogeography of two codistributed subgenera of cave crickets (Orthoptera: Rhaphidophoridae: Ceuthophilus spp.). J. Biogeogr. 43, 1450–1463. https://doi.org/10.1111/jbi.12734

Weij, R., Sniderman, J.M.K., Woodhead, J.D., Hellstrom, J.C., Brown, J.R., Drysdale, R.N., Reed, E., Bourne, S., Gordon, J., 2024. Elevated Southern Hemisphere moisture availability during glacial periods. Nature 626, 319–326. https://doi.org/10.1038/s41586-023-06989-3

Wolfe, J.M., Daley, A.C., Legg, D.A., Edgecombe, G.D., 2016. Fossil calibrations for the arthropod Tree of Life. Earth-Sci. Rev. 160, 43–110. https://doi.org/10.1016/j.earscirev.2016.06.008

Wynne, J.J., Howarth, F.G., Mammola, S., Ferreira, R.L., Cardoso, P., Lorenzo, T.D., Galassi, D.M.P., Medellin, R.A., Miller, B.W., Sánchez-Fernández, D., Bichuette, M.E., Biswas, J., BlackEagle, C.W., Boonyanusith, C., Amorim, I.R., Borges, P.A.V., Boston, P.J., Cal, R.N., Cheeptham, N., Deharveng, L., Eme, D., Faille, A., Fenolio, D., Fišer, C., Fišer, Ž., ?Ohukani?ōhi?a Gon, S.M., Goudarzi, F., Griebler, C., Halse, S., Hoch, H., Kale, E., Katz, A.D., Kováč, Ľ., Lilley, T.M., Manchi, S., Manenti, R., Martínez, A., Meierhofer, M.B., Miller, A.Z., Moldovan, O.T., Niemiller, M.L., Peck, S.B., Pellegrini, T.G., Pipan, T., Phillips-Lander, C.M., Poot, C., Racey, P.A., Sendra, A., Shear, W.A., Silva, M.S., Taiti, S., Tian, M., Venarsky, M.P., Pakarati, S.Y., Zagmajster, M., Zhao, Y., 2021. A conservation roadmap for the subterranean biome. Conserv. Lett. 14, e12834. https://doi.org/10.1111/conl.12834

Xiao, B., Feng, X., Miao, W.-J., Jiang, G.-F., 2012. The complete mitochondrial genome of grouse locust Tetrix japonica (Insecta: Orthoptera: Tetrigoidea). Mitochondrial DNA 23, 288–289. https://doi.org/10.3109/19401736.2012.674123

Zhou, Z., Shi, F., Zhao, L., 2014. The First Mitochondrial Genome for the Superfamily Hagloidea and Implications for Its Systematic Status in Ensifera. PLOS ONE 9, e86027. https://doi.org/10.1371/journal.pone.0086027

Zhou, Z., Zhao, L., Liu, N., Guo, H., Guan, B., Di, J., Shi, F., 2017. Towards a higher-level Ensifera phylogeny inferred from mitogenome sequences. Mol. Phylogenet. Evol. 108, 22–33. https://doi.org/10.1016/j.ympev.2017.01.014

Zhu, Q., Zhou, Z., Zheng, X., Wang, T., Ma, L., Shi, F., 2022. Phylogeny and phylogeography of Diestramima cave crickets (Orthoptera: Rhaphidophoridae): speciation driven by multiple dispersal and vicariance events. Syst. Entomol. 47, 179–201. https://doi.org/10.1111/syen.12524

Zong, J.-S., Qin, Y.-Y., Li, K., He, Z.-Q., 2021. Report of a new genus Arboramima belonging to tribe Diestramimini, with a new species A. cattusis from Guangxi, China (Orthoptera: Rhaphidophoridae: Aemodogryllinae). Zootaxa 4981, 593600. https://doi.org/10.11646/zootaxa.4981.3.9

## Acknowledgements

The authors have no conflict of interest to disclose. This work was funded by a National Taxonomy Research Grant Program fellowship awarded to PGBH by the Australian Government Department of Climate Change, Energy, the Environment and Water (no. 4-H3JJWE), with co-funding from the Australian Speleological Federation, South Australian Museum, Environment Institute, and The University of Adelaide. We would like to heartily thank the staff, curators, and collection managers of institutions around Australia for sending us material: Simon Grove and Kirrily More (Tasmanian Museum and Art Gallery); You Ning Su and Federica Turco (Australian National Insect Collection); Matthew Shaw and Ben Parslow (South Australian Museum); and Karin Koch (Queensland Museum). Finally, we acknowledge The University of

Adelaide's Phoenix High Performance Computing service for contributing resources for the phylogenetic analyses presented here.

Specimens were collected under the following permits held by PGBH unless otherwise stated: AA-0001076 via Parks Victoria and FS/14-3694/1_2024 via the Victorian Department of Energy, Environment and Climate Action (Victoria); U26509-1 and Y27154-2 via the South Australian Department for Environment and Water, the latter held by ER (South Australia); SL102683 via the New South Wales Department of Planning and Environment, held by the Australian Museum (New South Wales); and TFA 22452 via the Tasmanian Department of Natural Resources and Environment, held by SME (Tasmania).

**Data availability**

The molecular sequences generated in this study have been uploaded to the GenBank repository. Accession numbers and associated specimen metadata are available in Supplementary Table S1.

## Figures

**Fig. 1.** Morphological diversity of Macropathinae from throughout the Southern Hemisphere. TAS = Tasmania; QLD = Queensland. Images © Jessa Thurman, Keith Martin-Smith, Greg Tasney, commoncopper via iNaturalist, Peter Swart, and Benjamin Silva Ahumada. Photographs reproduced under Creative Commons licences.

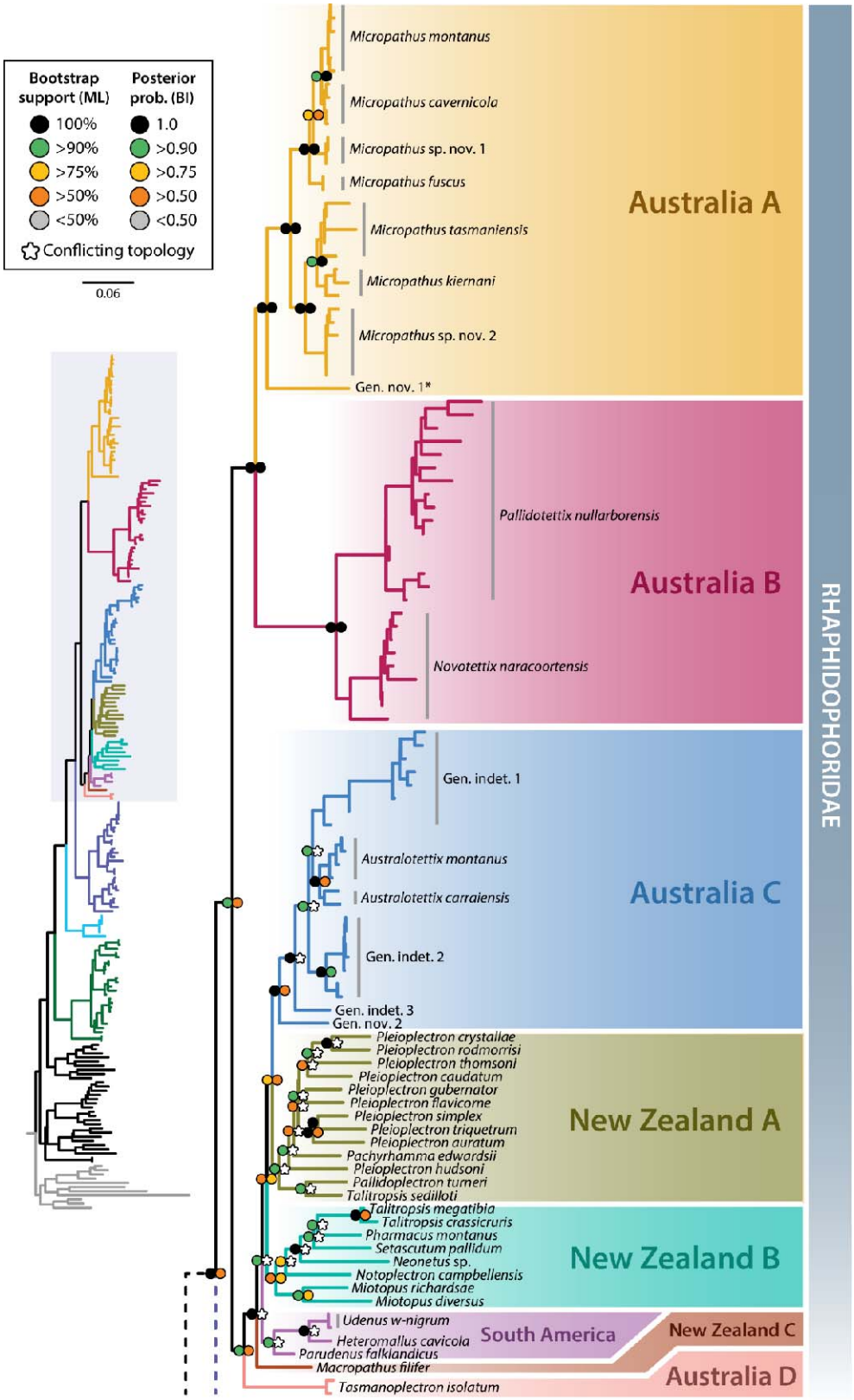


**Fig. 2.** Maximum likelihood phylogeny of Macropathinae (part) inferred from mitochondrial and nuclear data. *Described as *Eburnocauda* while the present study was in preparation (Iannello and Beasley-Hall, 2024). Conflicting topologies refer to disagreement between maximum likelihood and Bayesian analyses. Phylogeny continued in Fig. 3. A map showing sampling locations is available in Fig. S1.

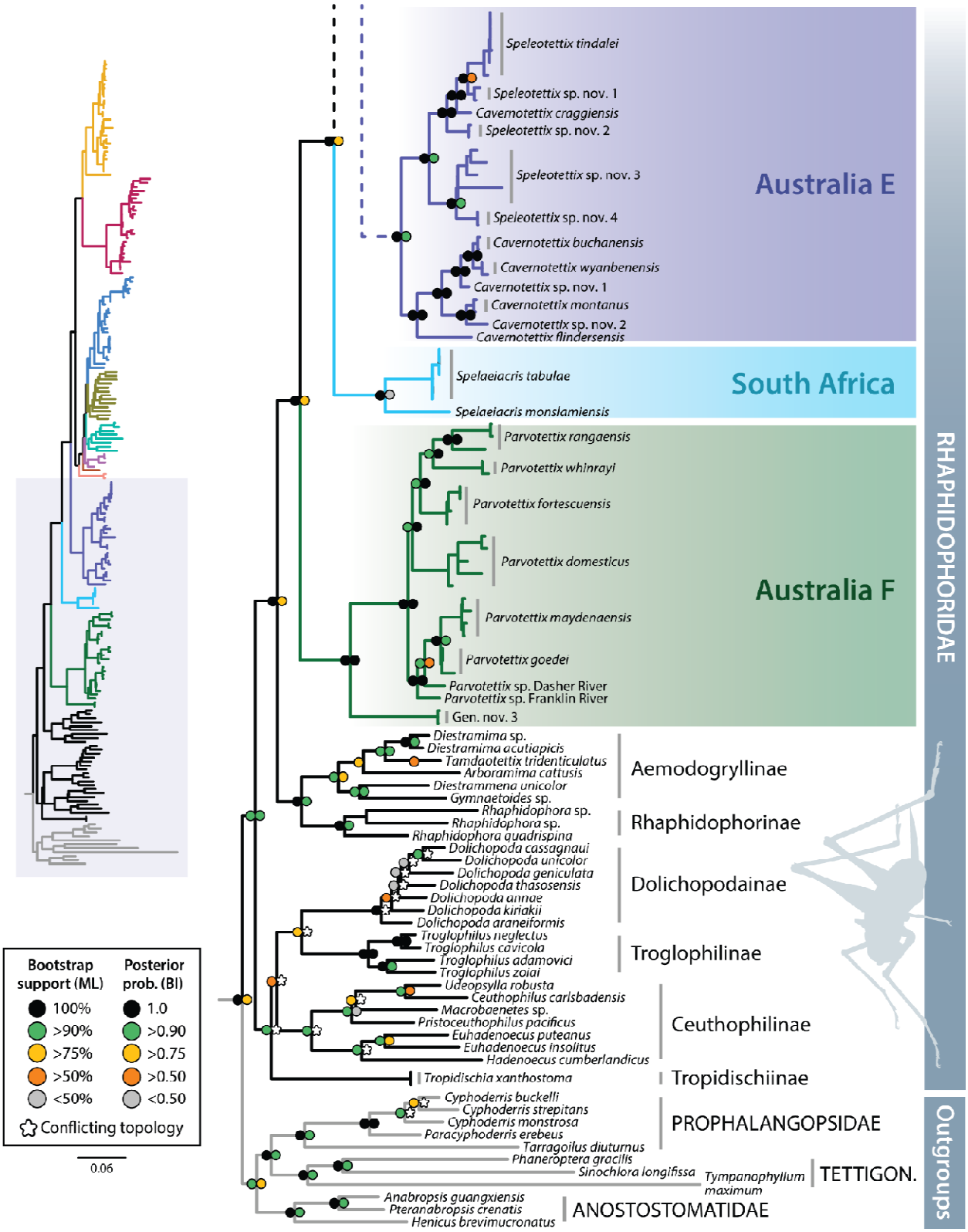


**Fig. 3.** Phylogeny of Macropathinae (coloured branches) and related subfamilies of Rhaphidophoridae (black branches) inferred from mitochondrial and nuclear data, continued from Fig. 2. Conflicting topologies refer to disagreement between maximum likelihood and Bayesian analyses. TETTIGON. = Tettigoniidae. A map showing sampling locations is available in Fig. S1. The caeliferan outgroup used in the analysis is not shown for clarity.

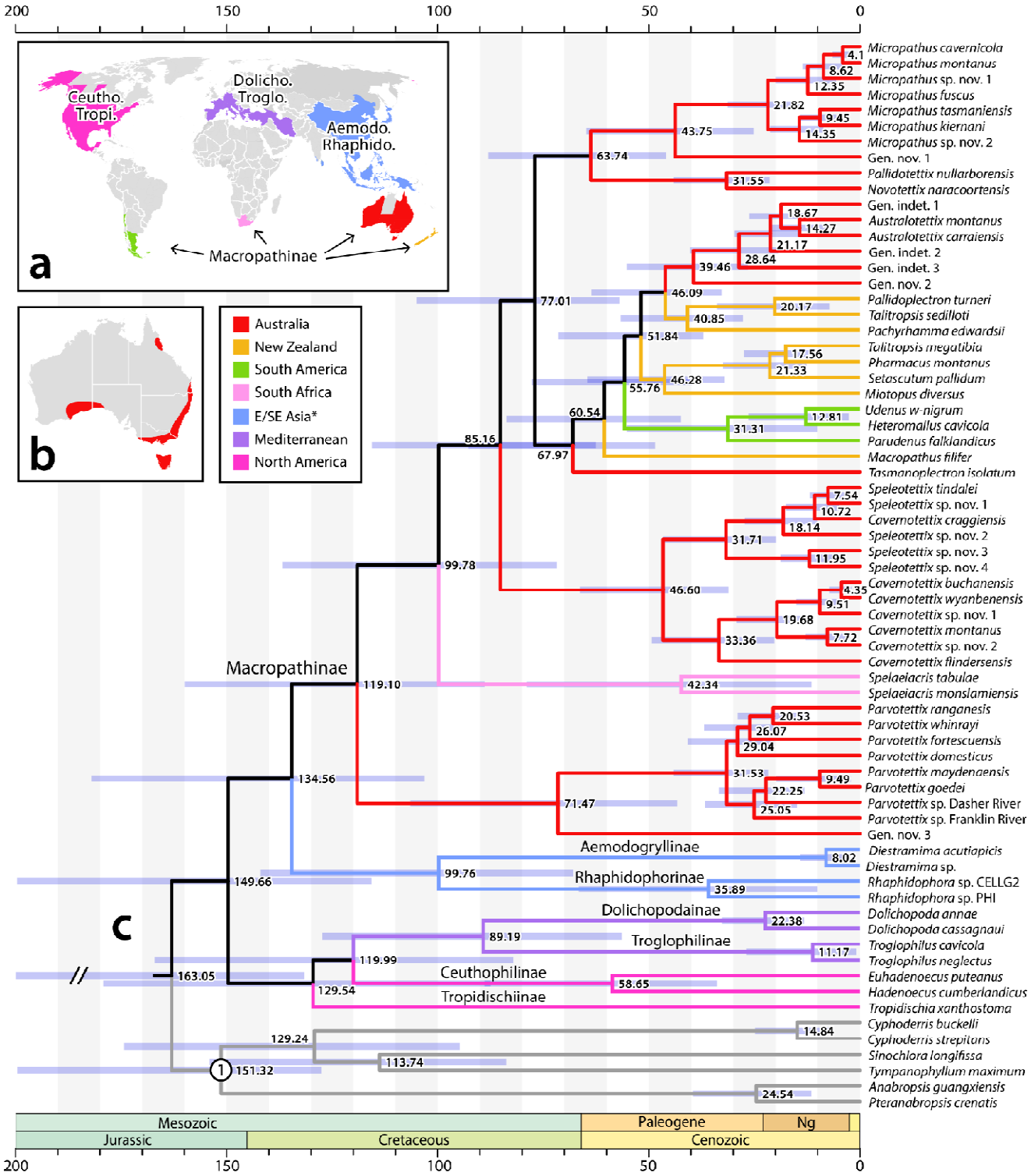


**Fig. 4.** a) Distribution of rhaphidophorid subfamilies examined here. b) Distribution of Macropathinae in Australia. c) Time-calibrated Bayesian phylogeny of Rhaphidophoridae using a single fossil calibration (node 1). Estimated timings of cladogenesis are shown at nodes; node bars indicate 95% HPD interval ranges. *Aemodogryllinae is also found in East Asia, but only Southeast Asian species were included in our analysis.