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

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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 described 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 supports 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 recover 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 infer the radiation of Macropathinae began during the Lower Jurassic prior to the fragmentation of Gondwana with a potential Pangaean origin for Rhaphidophoridae. Finally, we find 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 (e.g., 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 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, natural caves, and artificial structures such as abandoned mineshafts. Subterranean species are reliant on caves to complete their life cycles forage above-ground on a daily basis (Richards, 1966). In North American cave systems, this delivery of external energy into an otherwise low-energy food web maintains a broader community of egg and guano-feeding invertebrates (Lavoie & Poulson, 2007). Rhaphidophoridae presently contains 10 subfamilies, with the Macropathinae Karny thought to have diversified prior to all others (Allegrucci & Sbordoni, 2019; Dowle et al., 2024; Gorochov, 2001; Hubbell & Norton, 1978). This subfamily comprises the known cave cricket fauna in Australia, New Zealand, South Africa, and South America (Fig. 1). The New Zealand fauna 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., 2019, 2022; Trewick, 2024). In contrast, in Australia almost all species are known from caves, with above-ground aridity dictating their dependence on such habitats (Richards, 1987). 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 has been linked to population declines (Orthopteroid Specialist Group, 1996; Simms et al., 1996; Threatened Species Section, 2022a, 2022c, 2022b, 2022d).

Phylogenetic analyses have shown that the macropathine fauna of neighbouring Australian and New Zealand faunas are not reciprocally monophyletic. Examples include the Tasmanian genus *Parvotettix* Richards being the sister group to all other Macropathinae, including several 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 that predate their island habitats that suggests greater mobility of these flightless, nocturnal insects over water than previously assumed, and potentially further undescribed taxa (Dowle et al., 2024). High rhaphidophorid biodiversity in New Zealand, which is predominantly forested, indicates a capacity for habitat use reduced in arid regions. The macropathine fauna of Australia therefore provides an interesting contrast for which a different pattern of taxonomic diversity might exist in cave and epigean habitats. We expect this diversity might reflect a relatively deep ancestry, echoing past mesic conditions across the continent (Byrne et al., 2011) and now reflected in species utilising cave systems as refugia.

To uncover this diversity, we applied molecular methods to reconstruct a comprehensive phylogeny of the Australian Rhaphidophoridae. The subfamily exhibits a high degree of intraspecific morphological variation (Richards, 1971c), 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 documented genera and species which 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) Examine relationships of the Australian fauna within the subfamily Macropathinae, and finally 4) Enhance understanding of the phylogenetic position of Macropathinae within the broader Rhaphidophoridae.

Materials and methods

Taxon sampling and DNA sequencing

Cave cricket specimens were obtained from museum collections and in the field. Museum specimens were sourced from the South Australian Museum (SAM), 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, 1935), which represents a *nomen dubium* (Beasley-Hall *et al.* submitted), as well as several putatively undescribed species and genera. We also included previously published data for six non-macropathine subfamilies for comparison. Prophalangopsidae Kirby (grigs), Anostostomatidae Saussure (king crickets), and Tettigoniidae Krauss (katydids) were used as outgroups to facilitate fossil calibration of the phylogeny (Supplementary File 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) (Friedrich & Tautz, 1997), and 485 + 689 (*28S* D3-5) (Friedrich & 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 region), 51ºC (*12S* and *16S* rRNAs), or 55ºC (*28S* D3-5 region) 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.

We conducted next-generation sequencing (NGS) on two subsets of our specimens (Supplementary File S1). The first consisted of type specimens housed in ANIC's collection, performed as part of an existing project run by the Commonwealth Scientific and Industrial Research Organisation (CSIRO). These specimens were targeted due to their age, with the aim of obtaining as many of the above Sanger-sequenced genes as possible. 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 and additional nuclear markers to strengthen node support at the backbone of our tree. Library preparation was performed by AGRF using the Illumina DNA Prep (M) workflow. Paired-end sequencing was conducted by AGRF on an Illumina NovaSeq 6000 platform at a depth of 5 Gbp per specimen to generate 150-

base-pair reads. Nuclear *18S* rRNA and histone-3 (*H3*) were also retrieved for specimens sequenced with NGS.

Sanger sequences were processed and aligned using the package *sangeranalyseR* in R v.4.3.0 (Chao et al., 2021; R Core Team, 2024). 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 (https://github.com/FelixKrueger/TrimGalore) for Cutadapt and FastQC (Andrews, 2010; Martin, 2011) 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 GenBank (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 (https://www.geneious.com) using medium sensitivity presets and without trimming before mapping. Sequences were only included in our downstream analysis if less than 50% of their length was represented by missing data.

Phylogenetic analysis

Alignments of protein-coding and rRNA mitochondrial genes, *H3*, *18S*, and two regions of *28S* (aligned separately) were produced separately 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 rRNA (*12S+16S* together), *H3* separated by codon position (1, 2, and 3), *18S* rRNA, and *28S* rRNA (D1+D3-5 regions together), for a total of 17,265 base pairs.

We used maximum likelihood (ML) and Bayesian inference (BI) for phylogenetic inference. 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). Following the analysis, we manually rooted our ML tree at the split between the Prophalangopsidae (included for fossil calibration, see below) and Rhaphidophoridae in FigTree v.1.4.4 (http://tree.bio.ed.ac.uk/software/figtree). BI and divergence dating was performed using BEAST2 v.2.7.5 (Bouckaert et al., 2019) and the package bModelTest for substitution model selection (Bouckaert & Drummond, 2017). We selected 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). Our IQTREE phylogeny was used as a starting tree for BI, scaled using treePL to ensure it was compatible with our fossil calibrations (Sanderson, 2002; Smith & O'Meara, 2012). We ran five separate chains of 100 million generations with a tree logging frequency of 10,000. 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 and resampled to produce 10,000 trees. The maximum clade credibility tree was inferred using TreeAnnotator v.2.7.1

(Bouckaert et al., 2019) with the common ancestors approach to annotate node heights and posterior probability limit of 0.5.

Divergence dating

While several well-dated rhaphidophorid fossils exist—such as †Protroglophilinae Gorochov from the late Eocene—their phylogenetic placement remains unresolved (Allegrucci & Sbordoni, 2019; Gorochov, 2001), meaning there are no appropriate ingroup fossils that can be used to calibrate a phylogeny. As such, we applied two fossil calibrations to our outgroup: 1) the oldest definitive prophalangopsid, †*Aboilus tuzigouensis* Lin & Huang (189.6–196.5 Mya, millions of years ago) to the stem-Prophalangopsidae (Li et al., 2019; Lin & Huang, 2006; Sha et al., 2015), and 2) the earliest known member of the infraorder Tettigoniidea, †*Permohagla tomica* Gorochov (251.3–252.17 Mya) (Gorochov, 2021; Paleobiology Database, 2023), to the root of our phylogeny. We applied calibrations using exponential priors, appropriate for fossil evidence (Ho & Phillips, 2009), with the mean age of each fossil applied as a hard minimum bound. We used the minimum age estimate of the oldest definitive Ensifera (Bethoux et al., 2002; Wolfe et al., 2016), dated to 271.8 Mya, as a soft maximum bound.

Historical biogeography

We used BioGeoBEARS (Matzke, 2013, 2014) in R v.4.4.0 (R Core Team, 2024), a package that uses probabilistic models for ancestral range estimation, to visualise possible historical biogeographic hypotheses for the Macropathinae and relatives. Species distributions were compiled from observations made by Richards (1987) and the Orthoptera Species File as at June

2024 (Song et al., 2015). Species were coded as belonging to Australia, New Zealand (including subantarctic islands), South America, South Africa, East Asia, Southeast Asia, the Mediterranean, and/or North America, though we note that the cryptic nature of the subfamily in forest habitats likely means past sampling has been extremely biassed. Historical biogeographic analyses were run using three models: dispersal-extinction-cladogenesis (DEC) (Ronquist, 1997); a ML implementation of dispersal-vicariance analysis, or DIVA (DIVALIKE) (Ree & Smith, 2008), and Bayesian Analysis of Biogeography (BayArea) (Landis et al., 2013). Each of these models also 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. Models were run with default parameters 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 for a total of 17,265 bp from 116 Australian rhaphidophorids and 189 specimens overall. For samples without mitochondrial genomes, we retrieved up to 4,796 bp. Using ML and BI, we recovered a monophyletic Macropathinae consisting of six major clades (Fig. 2). Sister to all other macropathines in the analysis was *Parvotettix* and an undescribed genus, estimated to have

diverged *ca.* 182.5 Mya (95% highest posterior density [HPD] 106.9–222.4). The ancestor of the South African genus *Spelaeiacris* Péringuey diverged *ca.* 149.2 Mya (95% HPD 85.9–186.5 Mya), and a second Australian lineage emerged *ca.* 127.4 Mya (95% HPD 76.3–154 Mya) containing the southeast Australian *Speleotettix* Chopard and *Cavernotettix* Richards. A diverse group then arose *ca.* 114.2 Mya (95% HPD 69.3–138.5 Mya) containing the New Zealand and South African faunas as well as two unrelated Australian lineages, the Tasmanian *Tasmanoplectron* Richards and Queensland *Australotettix* Richards alongside its undescribed sister genus. The Tasmanian *Micropathus* diverged from the southern Australian *Pallidotettix* Richards and *Novotettix* Richards *ca.* 95.3 Mya (95% HPD 57.47–117.3 Mya). Finally, *Pallidotettix* and *Novotettix* diverged from one another *ca.* 52.3 Mya (95% HPD 32–67.4 Mya). Aemodogryllinae Jacobson and Rhaphidophorinae Walker, 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 Brunner von Wattenwyl, Troglophilinae Karny, Ceuthophilinae Tepper, and Tropidischiinae Scudder.

Several Australian lineages were not recovered as members of described species and instead represent putatively new taxa within *Micropathus* (2 lineages), *Australotettix* (1), *Speleotettix* (4), and *Cavernotettix* (1) (Fig. 2). Three other lineages represent putatively new genera sister to *Micropathus*, *Australotettix*, and *Parvotettix*. A further three lineages were not recovered as nested within described species but also represented by one individual, leaving their species-level identifications uncertain (*Speleotettix* sp. from Abercrombie and *Parvotettix*

spp. from Franklin and Dasher Rivers). These populations may represent new species in their own right but will require further sampling.

Our ML and BI analyses recovered similar relationships among major clades of Macropathinae and with high node support in most cases. However, they differed regarding the placement of a New Zealand clade containing *Talitropsis* Bolívar, *Pharmacus* Pictet & Saussure, *Setascutum* Richards, and *Neonetus* Brunner von Wattenwyl. In our ML analysis, the New Zealand fauna (with the exception of *Macropathus filifer* Walker) was inferred as sister to northeastern Australian species and *Macropathus* was sister to a monophyletic South American fauna (Fig. 2). In contrast, our BI phylogeny recovered a non-monophyletic South American fauna interspersed with New Zealand species (Fig. 3). The placement of Tropidschiinae also differed between our analyses (Figs 2, 3), but relationships at the subfamily level were not the main focus of the present study.

Historical biogeography

The BAYAREALIKE model was the best fit to our dataset based on AIC and AICc scores (AIC = 161.3222; AICc = 161.7222) compared to DEC (AIC = 585.2567; AICc = 585.4534) and DIVALIKE (AIC = 551.7517; AICc = 552.1517) (Fig. S1). 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 was estimated as the ancestral range of the subfamily given the position of *Parvotettix* as sister to all sampled Macropathinae (Fig. S1). At

the root of our tree, Rhaphidophoridae was estimated to have a broad ancestral range spanning Australia, East Asia, the Mediterranean, and North America.

Discussion

Here, we have presented the most comprehensive phylogeny of the Macropathinae to date, representing the majority of known rhaphidophorid biodiversity in the Southern Hemisphere. The Australian and New Zealand Macropathinae had previously been revealed to lack reciprocal monophyly (Allegrucci & Sbordoni, 2019; Beasley-Hall et al., 2018), but through substantially increased sampling our phylogeny indicates the New Zealand, South American, and South African rhaphidophorids all share ancestors with lineages amongst the Australian fauna. We note too that this Australian fauna comprises at least six deep, distinct evolutionary lineages amongst the Macropathinae. The New Zealand macropathines are similarly polyphyletic with at least two lineages; one sharing an ancestor with the subtropical fauna of Australia (Fig. 2). We found that the placement of New Zealand species differed between our ML and BI phylogenetic analyses (Figs 2, 3), a discrepancy also reported by Kim et al. (2024). As relationships in our ML tree more closely mirror those recovered in a recent study focusing on relationships among New Zealand Rhaphidophoridae (Dowle et al., 2024), we focus our discussions on this analysis for the remainder of the study, noting inconsistencies where relevant.

This study has considerably improved our understanding of the relationships between the best-studied macropathine fauna, found in New Zealand, and that of Australia, which was previously poorly sampled in molecular studies. Two other regional groups of Macropathinae

from South America and South Africa were included here, but these are presently only represented by a small number of species with a fairly well-established phylogenetic placement within the subfamily (Allegrucci & Sbordoni, 2019). Previously, New Zealand species (except *Macropathus*) were thought to be sister to *Micropathus* from Tasmania (Allegrucci et al., 2010; Dowle et al., 2024). Inclusion of additional taxa in our molecular sampling instead indicates a sister relationship with *Australotettix* and its undescribed sister genus found in humid (sub)tropical and temperate oceanic climates in northeast Australia. *Tasmanoplectron*, a critically endangered genus restricted to a small, windswept island off the coast of Tasmania, is the sister lineage to this broader clade, which also contains the South American fauna.

We included several other rhaphidophorid subfamilies in our analysis to assess the phylogenetic placement of Macropathinae within the Rhaphidophoridae. Our findings argue against previous morphology-driven hypotheses proposing Macropathinae as the most "primitive" subfamily within the Rhaphidophoridae, being sister to all others (Ander, 1939; Gorochov, 2001; Hubbell & Norton, 1978; Karny, 1930). We note that although this is not the case based on molecular data, the subfamily does appear to have diversified the earliest, at least within the constraints of current sampling (Allegrucci & Sbordoni, 2019; Dowle et al., 2024; Kim et al., 2024). If our topology is correct, the Rhaphidophoridae could be inferred to have had its origins in Pangaea rather than the Southern Hemisphere (Fig. S1), in agreement with the aforementioned molecular studies. However, confidently estimating ancestral ranges of this group is limited by the cryptic nature of the fauna and resulting sampling bias in molecular studies.

Evolutionary history of Macropathinae

Using two fossil calibrations, we inferred a Lower Jurassic origin for Macropathinae *ca.* 182 Mya. Previous age estimates for Rhaphidophoridae have relied on a diverse range of molecular clock calibration methods, but the majority have only included a handful of Northern Hemisphere species and not extensively sampled the family, let alone Macropathinae (e.g., Song et al., 2020; Vandergast et al., 2017). To date, the only such phylogeny is that of Dowle *et al.* (2024), who inferred an origin of *ca*. 97 Mya for the subfamily. This difference may be due to our differing choice of node calibrations: Dowle *et al.* (2024) used a relatively young age of *ca.* 160 Mya to calibrate the stem-Prophalangopsidae and an apparently very narrow distribution on that node. However, †*Aboilus tuzigouensis*, used in the present study, has been dated to at least 189.6 Mya and is considered the earliest definitive prophalangopsid (Lin & Huang, 2006). These choices could lead to an underestimation of the age of major clades. Nonetheless, our median age estimates broadly agree with Dowle et al. (2024) in suggesting the Macropathinae originated prior to the fragmentation of Gondwana *ca*. 175–120 Mya (Fig. S1; McIntyre et al., 2017).

We consider it interesting that Australia has retained so many lineages with deep divergences in our analysis, even when only minimum ages of nodes are considered. This includes those only found on islands on the Bass Strait off the mainland of Tasmania (*C. craggiensis* Richards, *flindersensis* (Chopard), and sp. nov. 1; *P. rangaensis* Richards and *whinrayi* Richards), which we infer diverged millions of years before the isolation of such land

masses over the past 100,000 years due to post-glacial flooding (Lambeck & Chappell, 2001). The 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 (Heads, 2011; McCulloch & Waters, 2019). Dowle *et al.* (2024) suggested the interplay between speciation and extinction processes have resulted in a mismatch between geological history and biology; namely, that a failure to sample non-island sister species has confounded biogeographic signal, either because non-island sister lineages are extinct or unknown to science. At a subfamily-wide level, a combination of both may have led to the patterns we see here; we discuss these influences in an Australia/New Zealand context below.

The spatial range of rhaphidophorid populations is highly constrained by the availability of cool, humid microhabitat, and most species are known from in disjunct pockets of humid subtropical or temperate oceanic climate (Peel et al., 2007). The Australian fauna was therefore likely heavily impacted by the acceleration of the drying of the continent during the late Eocene *ca.* 33 million years ago, which led to a dramatic contraction of appropriate mesic habitat (Byrne et al., 2011). With the exception of species found in ever-wet forests, Richards (1987) considered the fauna to be "struggling for survival". Reduction in diversity following the contraction of suitably humid conditions may explain the relatively high proportion of Australian species now found in caves. An interesting example is the monotypic Australian genus *Pallidotettix,* which occurs across a *ca*. 700 km^2 area of limestone karst in the desert and xeric shrublands of the Nullarbor Plain. This is the only species known outside the continent's

mesic zone and exhibits cave-adapted morphology including pigment loss not found in other Australian taxa (Richards, 1971a). However, we note that all cave-dwelling Australian species should still be considered troglophiles because they forage outside of cave entrances in predictable daily cycles (Richards, 1971b). In contrast, the Rhaphidophoridae of New Zealand and its subantarctic islands are far more speciose and widespread on an archipelago dominated by a temperate oceanic climate (Fitness et al., 2018; Hegg et al., 2022; Trewick, 2024). Consistent with this climate, the majority of New Zealand species are epigean, only a small number are found in cave habitats, and none are exclusive cave dwellers. In addition to extinction processes, we note that the cryptic nature of rhaphidophorids in both forest and subterranean habitats has almost certainly contributed to incomplete sampling of the fauna. This is especially applicable in an Australian context given the fauna's relative abundance in caves, disjunct distributions, and difficulty accessing such locations. Failure to sample for this reason would imply a greater diversity in Australia than currently known and is supported by evidence for several undescribed taxa highlighted here.

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. Food sources range from mosses, liverworts, decaying vegetation, guano, other invertebrates (including conspecifics), mammal carcasses, and small living vertebrates such as chicks (Richards, 1969, 1971a). The abundance of rhaphidophorid biomass also supports predator communities, including harvestmen, cave spiders, and bats (Richards, 1971a). Cave

crickets often span exceptionally small distributions (<10,000 km^2), qualifying them as short-range endemics (SREs) at a higher risk of extinction compared to more widespread species (Harvey, 2002; Richards, 1971c). Several rhaphidophorid species have already been granted conservation listing for this reason. However, only 68 of a described 879 species worldwide have had some form of conservation assessment via 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 widespread taxon is a key step in the management of SREs, without which clear conservation priorities cannot be established. Here, we have used molecular data to determine species boundaries and have highlighted several undescribed Australian taxa.

Our findings have several conservation implications for the Australian Rhaphidophoridae. Five taxa are currently represented in 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, 2022c, 2022b, 2022d). Given all described species in Australia can be typified as SREs, we are of the opinion that many more than this are deserving of conservation listing, particularly in the face of several key threatening processes. These include forestry and land clearing, which risk fragmenting above-ground habitat that would otherwise 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 11 new species. Three additional populations could not be confidently assigned to a new or described species as they were only represented by one individual; these taxa may represent new species in their own right. We also note that the at-risk *Cavernotettix craggiensis*, listed as rare under Tasmanian legislation, requires taxonomic revision given its placement among members of *Speleotettix*. In doing so, we have laid the groundwork for future conservation management of these species; this is particularly important in an Australian context because a species must be given a formal scientific name to be eligible for individual protection under environmental legislation. Relevant descriptions and revisions of these taxa will be the subject of upcoming work by the authors.

Conclusions

Here, we have presented a robust phylogeny of Rhaphidophoridae with a focus on the Southern Hemisphere taxa, almost all of which are within the Macropathinae. Using mitochondrial and nuclear data, we have confidently resolved relationships among eight genera of the Australian fauna and their allies. We find that the Australian fauna is rampantly polyphyletic, with at least three independent colonisations of Tasmania alone (*Micropathus*, *Speleotettix*+*Cavernotettix*, and *Parvotettix*). We have putatively identified at least 11 new species and 3 new genera based on molecular data, which will form the basis of future taxonomic descriptions and revisions by the authors. We confirm the New Zealand, South American, and South African Rhaphidophoridae share ancestors with the Australian fauna, in keeping with the Tasmanian *Parvotettix* being sister to the remainder of the subfamily, and

infer that the polyphyletic New Zealand fauna shares ancestors with crickets found in tropical Australia and South America. Finally, we infer the Macropathinae may have begun to diversify *ca*. 182 Mya following its divergence from an ancestor shared with the Asian Rhaphidophorinae and Aemodogryllinae, though these conclusions are limited by sampling the cryptic nature of the family, the availability of published molecular data, and a lack of ingroup fossils.

Improving understandings of evolutionary relationships and taxonomy within Rhaphidophoridae is necessary for several reasons. First, as discussed above, cave crickets represent an important—yet often cryptic—ecological component of subterranean realms, and identifying species boundaries is necessary for conservation management of short-range endemics, such as those found in Australia. Second, a robust phylogenetic framework can assist in narrowing down characters that are both phylogenetically informative for taxonomists and useful for identification by other 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. 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.

Data availability

The molecular sequences generated in this study will be uploaded to GenBank prior to publication and accession numbers provided in Supplementary File S1.

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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, reproduced under Creative Commons licences.

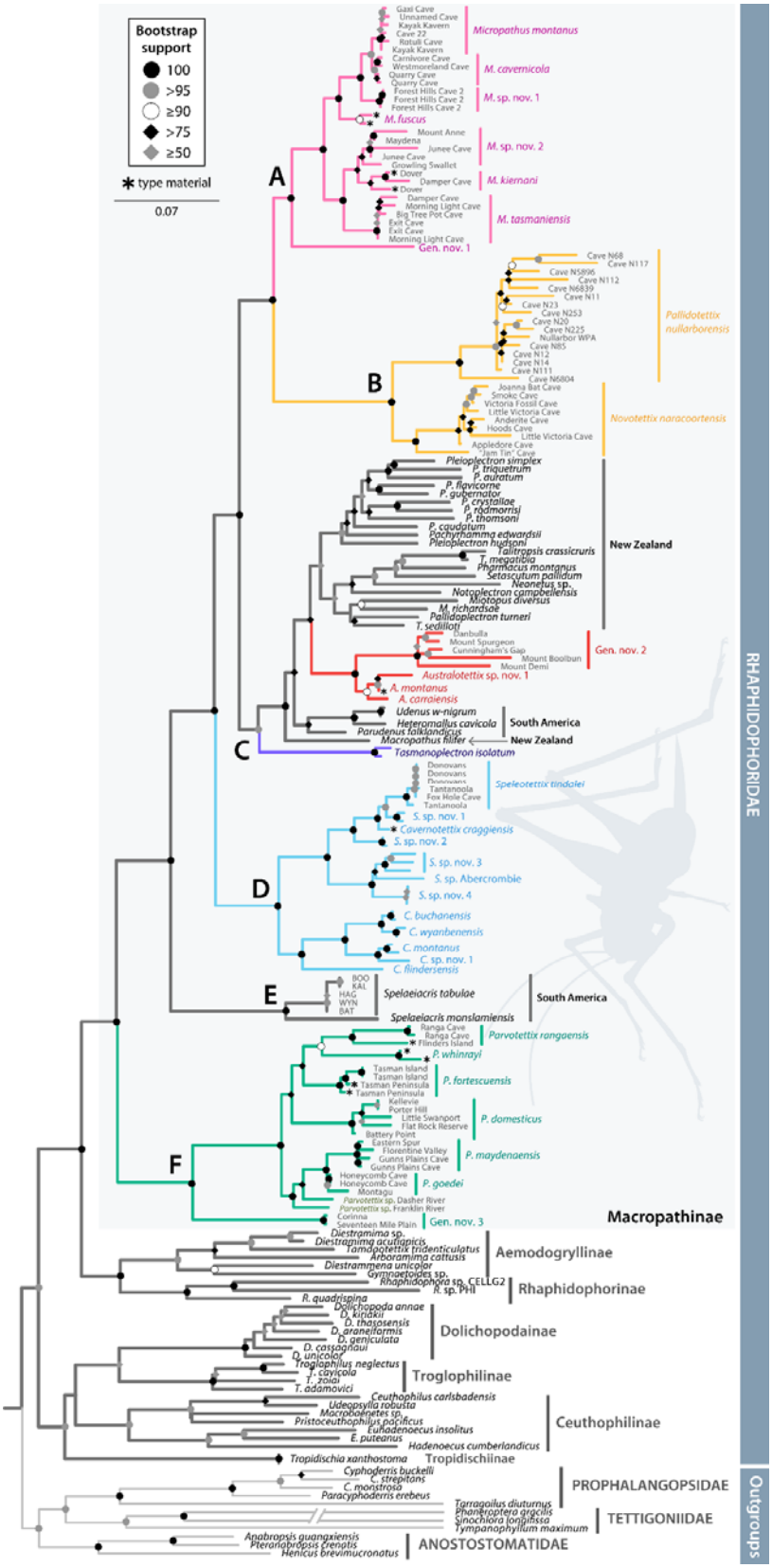

Fig. 2. Maximum likelihood phylogeny of Rhaphidophoridae inferred from mitochondrial (*12S* and *16S* rRNAs, *COI*) and nuclear (*28S* rRNA) data. Taxa for which additional markers were sequenced are listed in Supplementary File S1. Coloured clades indicate Australian taxa. Collection localities/voucher codes are shown at tips for taxa represented by multiple specimens.

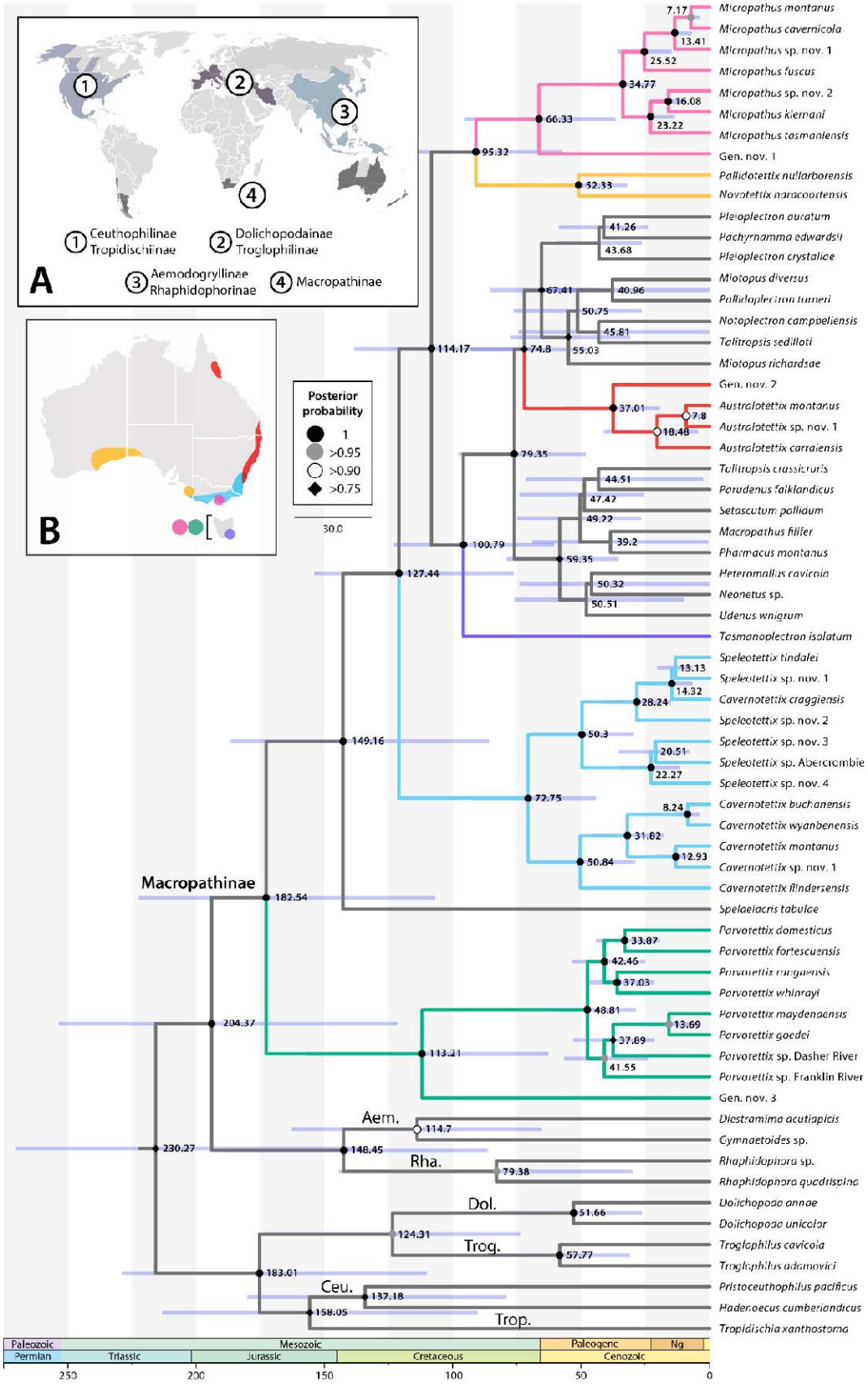

Fig. 3. Time-calibrated Bayesian phylogeny of Rhaphidophoridae constrained by two outgroup fossil calibrations. A) Distribution of sampled subfamilies. B) General distribution of Australian clades of Macropathinae. Median ages are shown at nodes and node bars indicate 95% highest posterior density interval ranges of age estimates.