Digging deep: a revised phylogeny of Australian burrowing cockroaches (Blaberidae: Panesthiinae, Geoscapheinae) confirms extensive non-monophyly and provides insights into biogeography and evolution of burrowing

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ABSTRACT

Soil-burrowing cockroaches (Blaberidae: Geoscapheinae) are large insects endemic to Australia. Originally thought to represent a monophyletic group, these enigmatic species have in fact evolved burrowing behaviour, associated morphological modifications, and dietary transitions to dry leaf-litter feeding multiple times from the wood-feeding Panesthiinae in a striking example of parallel evolution. However, various relationships within these two subfamilies remain unresolved or poorly understood, notably the apparent paraphyly of Panesthiinae with respect to Geoscapheinae, the position and diversification of certain species within major clades, and several aspects of the overall group's biogeography and morphological evolution. Here, we investigate the phylogeny of Australian members of these two subfamilies using whole mitochondrial genomes paired with nuclear ribosomal markers and highly conserved genes from the bacterial endosymbiont *Blattabacterium*. Using the resulting robust, fossil-calibrated phylogeny from these three sources we confirm the non-monophyly of both subfamilies and recover Geoscapheinae as polyphyletic within a paraphyletic Panesthiinae. The non-monophyly of natural groups, at all levels from subfamily to species, has been driven by repeated, independent acquisitions of burrowing forms in Geoscapheinae from panesthiine ancestors that colonised the continent on two separate occasions during the Miocene. We additionally find morphological variation within Geoscapheinae itself is correlated with species distributions: older soil-burrowing clades living in comparatively arid environments have additional morphological reductions beyond obvious fossorial adaptations compared to those in comparatively temperate, younger burrowing clades. Ultimately, the results presented here demonstrate connections among phylogeny, biogeography, and morphology throughout Australian representatives of these two subfamilies, factors that could not be previously consolidated using existing phylogenetic frameworks. Given the discordance between molecular data implemented here and the existing taxonomic classification, we find no support for retaining Geoscapheinae as a discrete taxonomic grouping. Finally, we discuss the taxonomic implications of these results and present a roadmap for future research on Geoscapheinae and their panesthiine relatives.

INTRODUCTION

Soil-burrowing cockroaches (Blattodea: Blaberidae: Geoscapheinae) are a subfamily of large insects found exclusively in Australia. Geoscapheines subsist on fallen leaf litter, twigs and seed capsules and construct winding, permanent burrows in sandy soil; such structures are not only utilised as permanent homes for these long-lived animals, but also as food stores and nurseries for their young (Rugg & Rose, 1984a). As for many burrowing animals, members of the subfamily exhibit subterranean-adapted characteristics such as aptery, reduced compound eyes and ocellar spots, and fossorial legs with shovel-like tibiotarsi for digging (Fig. 1; Roth, 1977). The bulk of the described species of Geoscapheinae are distributed in the eastern states of Australia, with two species found in South Australia and one of these, *Geoscapheus robustus* Tepper, also occurring in Western Australia (Roth 1977). An undescribed soil-burrowing species also occurs in the Northern Territory (HAR, JAW, pers. obs.). While their unique behaviour and taxonomy have been relatively well documented over the decades, evolutionary relationships among geoscapheines remain poorly understood.

Geoscapheines have long been phylogenetically allied with Panesthiinae, a subfamily comprising Australian and Asian wood-feeding cockroaches (Maekawa *et al.,* 2003; Pellens *et al.,* 2007; Legendre *et al.,* 2014, 2015, 2017; Wang *et al.,* 2017). Panesthiines feed on rotting wood that they also use as a burrowing substrate and range from gregarious to subsocial, although their associations tend to be in groups as opposed to distinct bi- or uniparental familial units typical of geoscapheines (Rugg & Rose, 1984b; O'Neill *et al.,* 1987; Matsumoto, 1992). In Australia 11 panesthiine species are known, most of which belong to the genus *Panesthia*; all are endemic with the exception of *Ancaudellia marshallae* Roth that is also found in Papua New Guinea (Roth, 1977). *Salganea* Stål was also recorded in Australia by Roth (1977), but this was likely in error (HAR, pers. obs.). Though Panesthiinae and Geoscapheinae can be separated by morphological, behavioural, and reproductive differences, a recent molecular study has shown that geoscapheines are in fact derived panesthiines and that burrowing behaviour in this subfamily evolved multiple times in parallel from wood-feeding ancestors, rendering both groups non-monophyletic (Lo *et al.,* 2016). Continental aridification of Australia was likely a major influence that drove some lineages of ancestral panesthiines to evolve soil-burrowing, as the shrinking availability of wet, cool environments to

which they were pre-adapted led to strong selective pressures to maintain similarly humid and cool conditions underground (Maekawa *et al.,* 2003; Lo *et al.,* 2016; Beasley-Hall *et al.,* 2018).

Despite increased understanding of the phylogenetic placement of geoscapheines, uncertainty remains concerning the relationships among some lineages of Geoscapheinae and Australian Panesthiinae, as well as the evolution of particular morphological characters presumably associated with their diversification. Certain key nodes in the phylogeny of Lo *et al.* (2016) were not well supported, and relationships therein were inferred using only ~2100 base pairs of four molecular markers (*18S*, *ITS1*, *COII* and *12S*). One example is the relationship of the wood feeder *Panesthia sloanei* Shaw to a clade of soil burrowers including the largest geoscapheine cockroach, *Macropanesthia rhinoceros* Saussure. *Panesthia sloanei* is found on rainforest mountaintops across northern Queensland and, while individuals of those populations are morphologically very similar to one another, they represent at paraphyletic series of at least two separate lineages (Lo *et al.,* 2016). A similar situation exists concerning *Panesthia tryoni* in relatively high-altitude rainforest areas of more southern areas of Queensland and northern New South Wales. Uncertainty also remains regarding the phylogenetic position of wood-feeding species more generally, such as *Panesthia australis* Brunner von Wattenwyl and *Panesthia obtusa* Shaw. These are the only members of the Australian Panesthiinae that exhibit putatively plesiomorphic morphological characteristics (i.e., possessed by the ancestral taxon that originally colonised Australia, such as the retention of all male genital phallomeres), but are presently allied with Geoscapheinae based on molecular evidence. In the phylogeny of Lo *et al.* (2016) these two taxa formed a sister group with members of the burrowing genus *Geoscapheus* Tepper, although support for this grouping was weak. Finally, an understudied species*, Panesthia parva* Shaw, has not been previously included in phylogenetic studies. *Panesthia parva* can be found in dead standing trees in savannah woodlands typified by high temperatures and long periods without rain, contrasting starkly with other panesthiines that rely on rotting wood in more mesic biomes (Roth, 1977; JAW, pers. obs.). Whether this species might represent a key transitional form between wood-feeding *Panesthia* and soil-burrowing species or an alternate arid-adapted life history strategy remains to be determined. Crucially, in the absence of reliable and robust tree topologies, our ability to estimate the timing and frequency of shifts from

wood-feeding to soil-burrowing is weakened. Such data are also necessary for biogeographic analyses that rely on a phylogenetic framework, particularly relevant for a group potentially very sensitive to climatic fluctuations. A need therefore remains for an updated phylogeny of these two subfamilies to clarify their systematics and evolutionary history.

Here, we present a revised phylogeny of Geoscapheinae and the Australian lineages of Panesthiinae using mitochondrial genomes (hereafter mitogenomes), ribosomal nuclear data and 31 protein-coding genes from the endosymbiont *Blattabacterium cuenoti* (Mercier) to clarify evolutionary relationships within these two subfamilies. *Blattabacterium* Hollande & Favre is a strictly vertically transmitted, intracellular mutualist present in the fat body of almost all cockroaches, and this relationship is thought to have existed for over 150 million years (Lo *et al.,* 2003; Evangelista *et al.,* 2019). The phylogeny of this bacterium is known to be highly concerted with that of its hosts, and provides an ideal additional locus with which to infer host evolutionary relationships (Lo *et al.,* 2003; Maekawa *et al.,* 2005; Kinjo *et al.,* 2018; Arab *et al.,* 2020). In this study we specifically aim to investigate: 1) how many times soil-burrowing behaviour and associated fossorial morphology has evolved in parallel within Geoscapheinae and Panesthiinae, including clarifying the phylogenetic position of *P. sloanei, P. australis, P. obtusa, P. tryoni* ssp. and *P. parva*; 2) the timing of diversification events using up-to-date fossil calibration data; 3) the relationship between morphology and biogeography using our revised phylogenetic framework; and lastly 4) provide a template to inform and facilitate future taxonomic work on the group in a phylogenetic context based on natural groupings..

METHODS

Taxon sampling

Taxa were selected with a focus on Australian Geoscapheinae and Panesthiinae (32 species, 4 subspecies), of which we recovered sequence data for 29 taxa for the two subfamilies combined (Table 1). Outgroups were obtained from GenBank representing Ectobiidae and multiple subfamilies within Blaberidae; mitochondrial and nuclear data from additional members of Geoscapheinae were

recovered from Lo *et al.* (2016) to ensure taxon sampling was as comprehensive as possible (Table 1).

DNA sequencing and mitogenome assembly

DNA sequencing was outsourced to Macrogen (Seoul, South Korea) or performed in-house at the Okinawa Institute of Science & Technology Graduate University (hereafter OIST) using Illumina technology, following the methods outlined in Bourguignon *et al.* (2018). To exclude bacterial sequences in our mitochondrial assemblies (e.g. belonging to *Blattabacterium*), sequencing results were first filtered with mitochondrial reference sequences from Bourguignon *et al.* (2018) using SAMtools and BWA (Li *et al.,* 2009; Li & Durbin, 2009). Filtered reads were then assembled *de novo* using Velvet (v.1.2.10, Zerbino & Birney, 2008) with default settings, and the *k* value was manually optimised for each assembly. Contigs produced in Velvet were imported into Geneious (v.10.1.3, http://geneious.com) for a second round of *de novo* assembly using default settings and a 95% similarity cut-off between reads. As some of our sequencing runs were pooled, a custom BLAST database was constructed to isolate our resulting contigs to Panesthiinae or Geoscapheinae. Read Mapper in Geneious was used to assemble contigs according to a reference sequence belonging to the closest possible sister taxon with at least 90% similarity; this was aided using mitogenomes from Bourguignon *et al.* (2018) and, as additional assemblies were completed, our own mitogenomes were used as reference sequences. The resulting consensus sequences were annotated using the MITOS web server with default settings (Bernt *et al.,* 2013) and duplicated or split genes were corrected by hand. The same process was applied to our nuclear data (*18S + ITS1*) following a similar methodology.

Blattabacterium markers were obtained by first assembling draft bacterial genomes using the TCSF-IMRA method designed for the genus by Kinjo *et al.* (2015) (whole genomes presented in Beasley-Hall *et al.,* unpublished data). Bacterial annotation was performed using Prokka with default settings (Seemann, 2014). A set of 31 highly conserved housekeeping genes per Wu & Eisen (2008) were then extracted using these annotations or, if the gene was not recovered, by mapping the gene

of interest from *Blattabacterium cuenoti* str. BPAY (Kinjo *et al.,* 2015) to our data with Geneious'
Read Mapper using default settings.

Phylogenetic analyses

We combined mitochondrial *12S + COII* and nuclear *18S + ITS1* data from Lo *et al.* (2016) with data
generated in this study to create per-gene alignments in the MUSCLE add-on implemented in
Geneious with default settings and excluded sections of overlapping genes. Mitochondrial, nuclear,
and *Blattabacterium* datasets consisted of whole genomes (14,890bp), nuclear rRNA data (919bp),
and 31 highly conserved genes (23,110bp), respectively. Protein-encoding alignments were checked
by eye to ensure they conformed to the appropriate reading frame using invertebrate mitochondrial,
standard, and bacterial genetic codes for each dataset. We tested for substitution saturation using
DAMBE6 (Xia 2017) and in its absence retained third codon positions in the alignments. Maximum
likelihood (ML) and Bayesian methods were employed for phylogenetic inference of the
concatenated mitochondrial and nuclear dataset. For ML analyses we opted for a partitioning scheme
consisting of 1st and 2nd codon positions, 3rd codon positions, tRNAS+rRNAs, and nuclear rRNAs
based on previous phylogenetic studies of termites and cockroaches (Bourguignon *et al.,* 2014,
2018). RAxML (Stamatakis, 2014) was used running 1000 bootstrap replicates and default settings
with the partitions described above. We performed the same methods as above for our
Blattabacterium dataset but partitioned our data by 1st, 2nd, and 3rd codon positions.

Bayesian phylogenetic analyses on both our mitochondrial+nuclear and *Blattabacterium*
datasets were performed in BEAST2 (v. 2.4.5, Bouckaert *et al.,* 2014), with the bModelTest package
(Bouckaert and Drummond, 2017) employed for substitution model selection, the inference of
invariant sites, and gamma rate heterogeneity. We chose a relaxed lognormal molecular clock and
the birth-death tree prior to account for intraspecific sampling in the dataset (Ritchie *et al.,* 2017).
Our analyses were performed over 100 million generations and convergence of the stationary
distribution was checked using ESS values of >200 in TRACER (v. 1.7.1, Rambaut *et al.,* 2018). The
maximum clade credibility tree from the combined runs was produced using TreeAnnotator using a
10% burn-in (Bouckaert *et al.,* 2014).

One fossil calibration, '*Gyna*' *obesa* Piton, was applied to the host and endosymbiont phylogenies in BEAST2 to infer divergence times using an exponential distribution and soft maximum bounds to reflect uncertainty in the calibration (Ho & Phillips, 2009). This fossil cockroach was also used by Bourguignon *et al.* (2018) to calibrate the Gyninae+Panchlorinae+Blaberinae with an age range of 56–145 Mya in their order-level phylogeny, but it has since undergone a redescription by Evangelista *et al.* (2017) based on morphological evidence. Following a reassessment of the holotype, the subfamily assignment was questioned and is now considered *incertae sedis* within Blaberidae. As a result, following Evangelista *et al.* (2019) we included '*Gyna*' *obesa* to represent the stem-Blaberidae with a minimum age of 57.7 Mya. We selected 145 Mya as a soft maximum bound to represent the first modern cockroach (Lin 1980). Fossils used by Lo *et al.* (2016), i.e., *Epilampra* sp., *Zetobora brunneri* Scudder, and *Pycnoscelus gardneri* Cockerell, were unfortunately unsuitable for this study due to differences in their outgroup sampling compared to the present study. Importantly, these three fossils were also considered unreliable for use as molecular clock calibrations by Evangelista *et al.* (2017), owing to vagueness in their respective taxonomic placements.

Historical biogeography analyses

We used BioGeoBEARS (v. 0.2.1, Matzke, 2013) to estimate ancestral ranges of the Geoscapheinae and Australian Panesthiinae. Our input tree was constructed by subsampling the fossil-calibrated phylogeny presented here so that every described species in it corresponded to a single tip. Species distributions were compiled from Roth (1977, 1979) and validated against the Cockroach Species File Online (Beccaloni, 2014). Species were coded as belonging to the Palaearctic, Indo-Malayan, and/or Australasian biogeographic realms, with species on the Australian mainland (including Lord Howe Island) coded specifically to assess colonisations of the continent. We ran BioGeoBEARS using three different models with default parameters to take into account how biogeography could have evolved on the phylogeny: Dispersal-Extinction-Cladogenesis (DEC) (Ree *et al.,* 2008) and maximum likelihood implementations of Dispersal-Vicariance Analysis (DIVALIKE) (Ronquist, 1997) and BayArea, also called Bayesian Analysis of Biogeography (BAYAREALIKE) (Matzke,

2014). Models were executed with default settings and their relative probabilities were assessed using the Akaike information criterion (AIC) and corrected Akaike (AICc). We did not include models containing a jump dispersal parameter (+J) due to an ongoing debate surrounding its use (Ree & Sanmartín, 2018).

Ancestral character state reconstructions

Seven morphological characters were mapped onto our phylogeny using ancestral state reconstructions (ASRs) to test the hypothesis that morphological variation within Geoscapheinae can be correlated with factors such as habitat preference or divergence time. We considered the following characters: oothecal membrane (coded as present/absent), wing and tegmina form (fully developed/reduced/apterous), male L2d, L1, and R2 phallomeres (fully developed/reduced/absent), ocellar spots (fully developed/reduced/absent), and holes in the anterolateral corners of the abdominal tergites (present/absent). We also considered characters such as male pronotal morphology (e.g., the presence of marginal and/or postmarginal tubercles, and texture of the pronotal disc floor), cerci morphology (shape and the presence of setae), the projection angle of the supra-anal plate, tergite morphology, and colour of the pronotum and thoracic nota. These characters proved uninformative in our phylogenetic framework; we did not assess leg morphology as their adaptive significance has been examined previously by Beasley-Hall *et al.* (2018). ASRs were performed in a maximum likelihood framework with the *rayDISC* command implemented in *corHMM* in R (Beaulieu *et al.,* 2013; R Core Team, 2020). Each character was analysed using an asymmetric (ARD) model of evolution and marginal reconstruction of ancestral states. In all cases, probabilities at the root were fixed corresponding to the presumed plesiomorphic state of a given character, e.g. the retention of wings or the oothecal membrane. We performed two ASRs for each morphological character: one permitting reversions with default settings and the parameters detailed above, and another with reversions not permitted. To do this we made use of a unidirectional rate matrix from Sauquet *et al.* (2015) that prohibits derived characters reverting back to the ancestral state in two-state characters (i.e. rates of 1 to 0 set to zero, where 0, absence of a character, is the ancestral state). For three-state characters, we extended this rate matrix to only allow losses and not gains (rates of 0 to 1 and 1 to 2 set to zero, where 2, fully developed, is the ancestral state).

RESULTS

Phylogenetic relationships and historical biogeography

Our results strongly supported the non-monophyly of both Geoscapheinae and Panesthiinae. The major Australian groups were separated into two lineages that diverged ~29 Mya: lineage 1, containing Geoscapheinae and a number of Australian Panesthiinae, and lineage 2, exclusively containing Australian panesthiines (Fig. 2). Lineage 2 also included non-soil-burrowing species of panesthiines (*P. ancaudellioides, P. cribrata, P. lata, P. matthewsi*, and *P. parva*; Figs. 2, S1) as a monophyletic group sister to a clade comprising species of *Panesthia* and *Ancaudellia* found throughout South East Asia and greater Australasia (Lo *et al.,* 2016; Figs. 1, 2). *Ancaudellia marshallae* Roth 1977 was recovered in the sister clade to lineage 2 and is found in both Papua New Guinea (PNG) and Australia, suggesting another possible connection among Australian and SE Asian Panesthiinae, though in the present study we only considered a specimen sampled from PNG.

The earliest cladogenesis event within lineage 1 was the split into two main clades around 23 Mya, with one clade containing a paraphyletic *P. sloanei*+the soil-burrowing clade including eight species of *Macropanesthia* rendered paraphyletic by *Geoscapheus woodwardi* and *Neogeoscapheus hanni*. This clade (G) was sister to the rest of Geoscapheinae (clades A-F), itself also rendered paraphyletic by several species of Panesthiinae (i.e., *P. tryoni* ssp., *P. australis*, and *P. obtusa*). We recovered two major lineages of *P. sloanei* within clade G separated by ~19 Mya (95% HPD 14.56–25.65 Mya). Clade F (*G. dilatatus*+*G. robustus*) was the second geoscapheine grouping to diverge in our phylogeny at ~21.24 My (95% HPD 15.93–28.88 Mya) and was not found to form a sister group with any wood-feeding lineage. The next divergence was that of clade E, which exclusively contained *P. australis* and *P. obtusa* with no soil-burrowing relatives and split from its sister clade (itself comprising clades A to D) ~15.57 My (95% HPD 11.99–21.07 Mya). The remaining clades in our phylogenies consisted of soil-burrowing species with close phylogenetic affinities to members of the wood-feeding taxa *P. tryoni tryoni* and *P. tryoni tegminifera*. *P. tryoni tryoni* was divided into three distinct lineages which we define here as the northern, southern, and Kroombit Tops lineages. These clades (A, B, C, and D) began to diversify between approximately 7 and 11 My. This study

represents the first phylogenetic assessment of *M. monteithi* and *P. parva,* which are placed in lineage 1's clade A and lineage 2, respectively.

The DEC model was the best fit to our data of the ancestral range estimation analyses conducted here (AICC = 73.47, AICc = 73.72) compared to DIVALIKE (AICC = 75.41, AICc = 75.66) and BAYAREALIKE (AICC = 108.17, AICc = 108.42). The scenario inferred by this model chiefly supported two independent colonisations of the Australian mainland (Fig. 2). The most recent common ancestor (MRCA) of lineage 1 and 2 was inferred as an equivocal state between the Indo-Malayan realm, Australasian realm, and the Australian mainland, with states on branches leading to lineage 1 and 2 immediately after cladogenesis (at "corners" of splits) corresponding to the Australian mainland and a combination of the Indo-Malayan and Australasian realms, respectively. The MRCA of at the root of our tree was inferred to be an Indo-Malayan taxon, though this state represented less than 35% of the relative probability of all areas or area combinations at this node.

Co-evolution of cockroach hosts and their endosymbionts

We were able to retrieve *Blattabacterium* genomic data for a reduced subset of the taxa represented in our broader mitogenome and nuclear dataset presented in Fig. 2. The phylogeny of *Blattabacterium* strains was entirely congruent with our reduced taxon tree in Figure 3 but provided different node ages with narrower 95% HPD intervals than those inferred from host data and markedly better node support than whole mitochondrial genomes and nuclear markers. Under this alternate reduced-taxon framework, using *Blattabacterium* data the age of lineage 1 was inferred as ~13.14 Mya (95% HPD 10.51–18.05 Mya) and the split from panesthiine ancestors continued in this lineage until ~4 Mya (95% HPD 2.76–5.80 Mya).

Morphological evolution

If no reversions have occurred from the ancestral state, soil-burrowing behaviour was inferred to have evolved at least seven times in our main analysis (Fig. 2, see Figs. S2 to S9 for additional ancestral state reconstructions). In the case that reversions are permitted, soil-burrowing was inferred to have evolved just once in the MRCA of lineage 1, with a regain of the ancestral wood-feeding

habit having occurred in *P. australis* and *obtusa* in clade E (Fig. S2). Lo *et al.* (2016) previously argued that reversion from the soil-burrowing state to wood-feeding was improbable given the specialised nature of soil-burrowing behaviour (i.e. a convergence on fossorial forelegs paired with a rounded body form and the loss of wings), but we are unable to rule out such reversions in the present study.

Based on the data at hand, the oothecal membrane has been lost on at least two occasions in Geoscapheinae (Figs. 4, S3): in the ancestor of the clade comprising *M. kraussiana*, *M. heppleorum*, *M. rhinoceros*, *M. mutica*, *G. woodwardi* and *N. hanni* (in clade G) and in the ancestor of *G. dilatatus* and *G. robustus* (clade F). This reconstruction was identical under both scenarios of permission of reversals. The differentiation between wing and tegmina forms is also present in the two subfamilies, although not to such a clear-cut degree as oothecal membrane loss. Wings may be fully macropterous and functional, or with increasing brachyptery to full aptery (no evidence of wings being present in adults). We do not consider reversions to the ancestral state likely for wings, given the loss and complete re-evolution of the trait is very unlikely (Trueman *et al.,* 2004); under this assumption the reduction or loss of wings has occurred on at least three occasions in our dataset (Fig. S4). Additionally, wing loss is known to be common in Blaberidae (Djernæs *et al.,* 2020).

All geoscapheines, as well as *P. sloanei, P. tryoni tryoni*, and *P. tryoni tegminifera*, have lost their L2d male genital phallomeres, whereas they are present to some degree in all members of clade E (*P. australis, P. obtusa*) and lineage 2 (*P. ancaudellioides, P. parva, P. matthewsi, P. cribrata, P. lata* in Fig. S3). The loss of the L2d phallomere has occurred at least three times, in the most recent common ancestor of clades A+B+C+D, F, and G (Fig. S5), with a secondary gain of a fully developed L2d from an ancestrally reduced L2d in clade E, if reversions are permitted. Broad patterns also emerge regarding the L1 and R2 phallomeres (Figs. S6, S7). These structures are fully developed in clades A+B+C+D whereas they are reduced in most members of clade E and lost or reduced to some degree in all members of F and G, as well in species of *Salganea*, and have been lost up to four (L1) or three (R2) separate occasions depending on whether reversions are permitted or not. Reduction or absence of the ocelli has occurred in *P. sloanei, P. tryoni tryoni*, and *P. tryoni*

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tegminifera, whereas they are fully developed in species without burrowing relatives, the exception being *P. matthewsi* (Roth) (Fig. S8); this loss could have happened once in the MRCA of lineage 1, with a subsequent regain in *P. australis* and *obtusa*, or three times independently in clades A+B+C+D, F, and G. We note, however, that presence or absence of ocelli is generally presented in relation to type material (JAW, pers. obs.). It is therefore possible that such observations are not consistent across populations (Roth 1977; Rose *et al.,* 2014). More detailed observations of compound eye morphology within Geoscapheinae is warranted to test the hypothesis that eyes and ocelli are universally reduced or lost across soil-burrowing species. Finally, the presence of holes on the anterolateral corners of the fifth to seventh tergite is an apparently ancestral trait in *Panesthia* that has been lost in all members of lineage 1 aside from clade E and *M. mackerrasae*, *M. kinkuna*, and *N. dahmsi,* and *P. sloanei* (Fig. S9). This trait has been lost twice in lineage 2 and relatives, and up to five times in lineage 1 if reversions are not permitted to the ancestral state.

DISCUSSION

Phylogenetic relationships

The phylogenies presented here are consistent with previously estimated relationships among members of Geoscapheinae and Panesthiinae (Maekawa *et al.,* 2003; Lo *et al.,* 2016; Legendre *et al.,* 2014, 2015, 2017; Djernæs *et al.,* 2020), albeit with some minor differences. We recovered eight major Australian clades compared to Lo *et al.*'s (2016) seven; the incongruence being the position of two clades containing the wood feeder *P. australis* and the soil burrower *G. dilatatus* (clades E and F, Fig. 2). The topology recovered in our *Blattabacterium* phylogeny in Figure 3 is also congruent with our reduced taxon dataset derived from host mitochondrial genome and nuclear rRNA data.

An early divergence event within lineage 1 is that of clade G, which includes multiple lineages of *P. sloanei* in serial paraphyly. Each of these is found on rainforest mountaintops in northern Queensland (with the exception of Mossman Gorge). These results are similar to those of Lo *et al.* (2016), who inferred four different *P. sloanei* lineages. A difference between these two analyses is our inclusion of a sample from Windsor Tableland, which does not appear closely related to any other *P. sloanei* lineage. We acknowledge that the suite of *P. sloanei* localities included here,

and in Lo *et al.* (2016), is not representative of all known populations. Indeed, these lineages might represent distinct species themselves. Further details on the biogeography of this clade are provided below.

Clade F (i.e., *G. dilatatus+G. robustus*) was not recovered as sister to any wood-feeding lineage. This contrasts with the weak support found by Lo *et al.* (2016) for an affinity of these species with *P. australis+P. obtusa* (herein clade E). Clade E is strongly supported as the sister to the larger clade comprising clades A, B, C, and D, though we note that there remains weak support for the placement of clade F as sister to this larger grouping. *Panesthia tryoni tryoni* is notably polyphyletic throughout Geoscapheinae, with populations from Queensland being split into three separate groups from Kroombit Tops National Park (clade A), Lamington National Park and Mary Cairncross Scenic Reserve ('South' lineages in clade B), and the Dalrymple Heights region ('North') (clade D). Representatives of this third clade were found to be sister to *G. rugulosus* in Lo *et al.* (2016), but we recovered it as a monophyletic group in our analysis with high node support (Fig. 2). Similar to the case for clade G, the soil-burrowing species *Parapanesthia gigantea* and *M. saxicola* were recovered nested among wood-feeding species, in this case *P. t. tryoni* (South) and *P. t. tegminifera* from northern New South Wales and southern Queensland (Figs. 2, S1). This polyphyly of *P. tryoni tryoni* was also documented by Lo *et al.* (2016).

The highly concerted phylogenetic congruence between most cockroach hosts and their *Blattabacterium* endosymbionts, as presented here, has been documented previously (Clark *et al.,* 2001; Lo *et al.,* 2003; Garrick *et al.,* 2017; Arab *et al.,* 2020). However, such congruence has never been robustly demonstrated within Geoscapheinae or Australian Panesthiinae. The results presented here provide strong support for the phylosymbiotic nature of *Blattabacterium* for these two subfamilies for the first time. The dates inferred from the *Blattabacterium* phylogeny (Fig. 3) differ considerably from the host-derived datasets presented here (Figs. 2, 3). While these age estimates do not rule out that divergence times of ancestral Panesthiinae likely overlapped with aridification events of the Australian continent, the timescales of evolution inferred from host mitogenome + nuclear and *Blattabacterium* datasets (Fig. 3) are nonetheless markedly different, with comparatively

younger ages derived from the bacterial data. Previous work has shown that phylogenies inferred from cockroach mitochondrial and *Blattabacterium* genes produce differing branch lengths (Arab *et al.,* 2020), but the exact mechanisms underlying these differences remain unclear.

Biogeographic history of Geoscapheinae

Based on previous detailed analyses of Australian and Asian Geoscapheinae and Panesthiinae (Maekawa *et al.,* 2003; Lo *et al.,* 2016), the two major lineages recovered in our major phylogeny (Fig. 2) likely represent independent invasions from Asia into Australia as they are nested within a number of other Asian lineages. This scenario was also supported by our ancestral range estimation analysis. An alternative scenario, in which only one invasion of Australia occurred, appears less likely, since it would necessitate the re-colonisation of many locations in Asia by members of the genera *Panesthia* and *Ancaudellia*.

The evolutionary history of Geoscapheinae presented in Figure 2 is congruent with previous hypotheses that the drying of Australia exerted a strong selection pressure on wood-feeding lineages to transition to subterranean habitats (Maekawa *et al.,* 2003; Lo *et al.,* 2016; Beasley-Hall *et al.,* 2018). Aridification of the Australian continent began ~35 Mya once the landmass separated entirely from Antarctica as it moved north, making conditions progressively drier (McLoughlin 2001). Australian mesic biomes, once dominant across the continent, began to decline and were succeeded by more xeric environments beginning ~25 Mya (Byrne *et al.,* 2011). Drier habitats, such as sclerophyllous woodlands, are thought to have undergone two major periods of expansion ~15 Mya and ~7 Mya with severe periods of aridity subsequently occurring during the Pliocene ~3 Mya, and the evolutionary timescale shown in Figure 2 indicates that ancestral geoscapheines would have experienced both of these 'bursts' given the age of the subfamily.

The divergence of the clade containing Geoscapheinae and Australian Panesthiinae from Asian ancestors occurred ~ 29 Mya (95% HPD 22.31–38.81 Mya) in our main dataset (Fig. 2), in agreement with the age previously suggested by Lo *et al.* (2016) of ~26 Mya. The age range of this

divergence event is consistent with Australian geoscapheines being descendant of an Asian lineage of wood-dwelling Panesthiinae that dispersed to the continent following the collision of the Sahul and Sunda shelves ~25 Mya prior to the diversification of burrowing forms spurred by bursts of continental aridification (Maekawa *et al.,* 2003). This hypothesis is in conflict with alternate scenarios of Geoscapheinae evolving in Australia and colonising Asia secondarily or via Gondwanan vicariance (Maekawa *et al.,* 2003).

Inferring the environment inhabited by ancestral panesthiines is fraught with unknowns. There is limited knowledge concerning Asian members of Panesthiinae, comprising primarily *Panesthia*, a genus containing 56 described species and 10 subspecies (Wang *et al.,* 2014). The majority of attention on Asian *Panesthia* has focussed on the subspecies of *P. angustipennis* (Maekawa *et al.,* 1999; Maekawa & Matsumoto 2003). However, a possible scenario based on present habitats in Asia (Indo-Malayan realm in Fig. 2) is that the ancestors of lineage 1 initially occupied relictual mesic, wet forest environments when they first arrived in Australia from ~29 Mya onwards, the age of the split from lineage 2 and its sister clade. Paleobotanic evidence supports extensive wet forests being present in eastern Australia during this time (White 1986). Following the onset of increasing aridification ~25 Mya, wood-feeding taxa present in relatively low elevation areas would presumably have been under strong selection to evolve the soil-burrowing habitat to maintain the required humid environments underground as wet rotting wood availability decreased. Legs preadapted for burrowing into wood would presumably assist burrowing into soil, eventually becoming the exaggerated, shovel-like tibiotarsi modern geoscapheines possess, and xeric leaf litter would have supplanted rotting logs as a readily available food source (exceptions to this include *P. lata* and *P. parva*). Our recent biogeographic analyses also support this conclusion; soil-burrowing behaviour and forms consistently correlate with drier climates, indicating such environments might have played a role in the evolution of these traits (Beasley-Hall *et al.,* 2018).

At higher elevations, rainforested areas have persisted as refugia for species such as *P. sloanei* and *P. tryoni*. These two species have noteworthy biogeographic patterns, being distributed primarily on mountaintops on Australia's northeast coast in Queensland around the Wet Tropics

region and across the Great Dividing Range, respectively. *P. sloanei* represents at least four distinct lineages: those present near Paluma (elevation ~900m), Mossman Gorge/Mt. Lewis (~900m), Windsor Tableland (~1200m), and Ravenshoe/Dinden National Park (~1200m). A number of these *P. sloanei* lineages separated ~19 Mya, suggesting a scenario of vicariance with lineages becoming isolated on mountaintops as opposed to subsequent long-range dispersal events. Geographically distinct lineages of *P. tryoni* diverged from one another ~13 Mya, presumably via similar processes. Large apterous or tegminiferous *Panesthia* similar to *P. sloanei* and *P. tryoni* spp. also occur in Papua New Guinea (*P. heurni*) and Vietnam (*P. triangulifera*) in high altitude, mesic mountain environments (Roth 1979). Deep-time rainforest contractions in the Australian Wet Tropics are known to have influenced current patterns of diversity in other insects (Bell *et al.,* 2004; Moreau *et al.,* 2015) and Southern Hemisphere invertebrates with limited capacity for dispersal (either through wing loss or strict habitat fidelity) becoming isolated on rainforested mountaintops through vicariance processes are relatively well-documented. These include dung beetles, which are notably thought to have experienced elevation-related wing loss (Bell *et al.,* 2004; Endo *et al.,* 2014), alpine grasshoppers (Slatyer *et al.,* 2014), springtails (Greenslade & Slatyer, 2017), millipedes (Decker 2016a, 2016b), and freshwater crustaceans (Hatley & Murphy, 2016).

The ancestor of lineage 2 is thought to have diversified at a later point compared to that of lineage 1, ~12 Mya in the dataset of Lo *et al.* (2016) and ~14 Mya in the present study after diverging from its sister clade ~25 Mya. The ancestral habitat of this lineage is unclear but might have been wet sclerophyll forest given the timing of this divergence, which is now the habitat of its most widespread species *P. cribrata.* Under this scenario, *P. ancaudellioides* would have colonised the rainforests of northern Queensland (also occupied by *P. sloanei*) when it diverged ~10 Mya. Alternatively, the ancestral habitat of lineage 1 might have been rainforest, implying that the ancestors of taxa such as *P. cribrata* shifted from this habitat to wet sclerophyll.

Further adaptation presumably occurred in lineage 2 taxa such as *P. lata* and *P. parva. Panesthia lata* is found in poorly forested environments on the Lord Howe archipelago and is notable in this lineage as it is essentially apterous, possessing very small tegmina, and feeds on dead

leaves of plants such as banyan, *Cyperus*, and sallywood (HAR, pers. obs.). This species is assumed to have evolved from wood-feeding ancestors following its arrival in the Lord Howe archipelago. In contrast to other panesthiines, *P. lata* does not make use of rotting wood as a nesting substrate, instead is found under rocks and around the bases of trees, though it can consume it in controlled conditions (HAR, pers. obs.). Similarly, *P. parva* does not require moist rotting logs and can be found within dry dead standing trees. *Panesthia parva* is found in areas that experience very hot temperature and long periods of dry weather during the southern Queensland winter or monsoon dry season further north. Colonies of *P. parva* have been maintained for up to a year surviving on dry pieces of wood (*Callitris* sp.) with only occasional water supplied by hand (JAW, pers. obs.). Such a niche is distinctly different to those preferred by other members of Panesthiinae and it is possible that *P. parva* is capable of metabolic water production or restricting water loss. *Panesthia parva* therefore appears to have adapted to drier conditions via a different mechanism to ancestral geoscapheines.

The dates derived from our *Blattabacterium* analysis (Fig. 3) are considerably younger than those in our mitochondrial and nuclear tree but provide a similar narrative concerning the evolution of Geoscapheinae. This would require a markedly later date of colonisation of Australia by a wood-feeding ancestor, albeit during a time period in which the aridification of the continent was still ongoing. As this analysis only considered taxa for which we were able to obtain high-quality *Blattabacterium* data, we were unable to test any hypotheses concerning the diversification of lineage 2. Nonetheless, these endosymbiont-derived timings of cladogenesis in lineage 1 cannot be ruled out in the present study.

Morphological evolution in Geoscapheinae and Australian Panesthiinae

Morphological characters of Geoscapheinae and Australian Panesthiinae have not been previously assessed in a phylogenetic context, especially considering that generic assignments poorly reflect the evolutionary relationships of these insects (Humphrey *et al.,* 1998; Lo *et al.,* 2016; Djernæs *et al.,* 2020). We examined seven characters considered informative following their consistent and extensive documentation in the literature, coded as present/fully developed, reduced, or absent against our phylogenetic framework: the oothecal membrane, wings and tegmina, three male genital

phallomeres (L1, first left phallomere; L2d dorsal second left phallomere; R2, second right phallomere) following McKittrick (1964), ocellar spots, and holes in the anterolateral corners of the abdominal tergites. The characters we assessed were mapped onto the main topology (Fig. 4), which includes every described species of the Australian Panesthiinae and Geoscapheinae (except *P. tepperi* Kirby, which was not examined by Roth (1977) and may not represent a valid Australian species (JAW, pers. obs.)), as well as Asian panesthiines assessed in Lo *et al.* (2016) to indicate the presumed ancestral states of the two subfamilies. Ancestral state reconstructions were performed for each of these characters, with reversions either permitted or not permitted (Figs. S2–9).

The first major trend that emerges from such comparisons is that phylogenetic relationships recovered for the Australian Panesthiinae and Geoscapheinae are generally reflective of both their morphology and the geographic regions they occupy, with some notable exceptions. Clades with the most extensive character reductions (clades F and G) have diverged earlier from their panesthiine ancestors than their more derived relatives (~19 Mya and older), which is also reflected in the ages of taxa with more moderate reductions (clade E, ~16 Mya). Notably, species with more marked character reductions (e.g. clades F and G in Fig. 2) tend to inhabit drier environments across wide geographic distributions, an extreme example being *G. robustus*, which is present as a presumably relictual population in Western Australia (Fig. 4). Such patterns of reduction and loss could be a product of these burrowers occupying drier habitats for a longer period of time than their relatives. *Macropanesthia mutica* and *N. hanni*, which are the earliest diverging geoscapheines in clade G, retain their oothecal membranes and have present (or reduced forms) of two of the three male genital phallomeres (as opposed to complete loss). These species might represent a transitional state between the morphology of *P. sloanei* and greater arid-adapted burrowers with more extensive character reduction in this clade. In contrast, species that are found largely in subtropical or temperate environments (clades A+B+C+D+E in Fig. 4) have smaller geographic distributions, more recent divergence times, and have not experienced such dramatic morphological reductions.

Abiotic variables being consistently associated with the presence of certain traits imply that these factors have played a role in the evolution of such characters. Although there are exceptions to

this rule in cockroaches (e.g., Lee *et al.,* 2017; Beasley-Hall *et al.,* 2019), burrowing behaviour in Geoscapheinae has been correlated with a consistent bias towards hotter, drier environmental tolerances in these species compared to their panesthiine relatives, suggesting arid environments have exerted selective pressures necessary to drive ancestral Geoscapheinae underground (Beasley-Hall *et al.,* 2018). The only existing study on the biogeography of this system focussed on the acquisition of burrowing behaviour (which was considered a single character) and its association with modelled fundamental niches, not additional patterns of loss within burrowing species themselves. In contrast, the findings presented here suggest that the occupation of more arid biomes, and/or the divergence time from panesthiine ancestors in geoscapheine species, are responsible for additional character losses within the subfamily after burrowing behaviour was already established.

Why have these traits been lost when species encounter drier environments? The loss or reduction of wings and ocellar spots in subterranean species are well documented (reviewed by Culver & Pipan 2014), but the variation in reproductive characters within Geoscapheinae is less well understood. Oothecal membrane loss in Geoscapheinae is thought to be related to the microhabitat of cockroach burrows; the relative humidity in these environments is assumed to be very high and stable, negating the need for an ootheca (Walker and Rose 1998). Why the loss of this trait appears to be consistently linked to losses or reductions of male genital morphology is unknown.

The evolutionary processes responsible for the loss or reduction of the aforementioned morphological traits could be neutral or adaptive, in that relaxation of selection on their maintenance has occurred or species are able to reduce their energy investment in the maintenance of certain structures, respectively (*e.g.* Tierney *et al.,* 2015). In contrast to burrowing species in clades F and G which have lost this suite of characters, species that have presumably not inhabited burrows for as long a time period (clades A+B+C+E) might have diverged too recently from their panesthiine ancestors for the loss of these traits to occur, whether via neutral or adaptive processes. Comparative scenarios involving apparently insufficient divergence times can be found in still-functioning vision systems of subterranean animals expected to be blind (Friedrich *et al.,* 2011; Niemiller *et al.,* 2012).

Alternatively, these species might simply not be subjected to as strong selective pressures to lose these structures due to their preference for comparatively temperate biomes.

A second major pattern that emerges from our morphological comparisons involves similarities between clade D and lineage 2, which have generally retained the complete suite of presumably ancestral morphological characters examined here, whereas panesthiine taxa belonging to clades also containing geoscapheines (*P. sloanei*, *P. tryoni tryoni*, and *P. tryoni tegminifera*) can be considered 'geoscapheine'-like in that a number of characters related to vision, reproduction, and flight are reduced in these species (Fig. 4). These latter taxa are apterous or near apterous, have reduced or absent L2d phallomeres, and have lost their ocellar spots. No such morphological combination exists in either *P. australis*+*P. obtusa* in clade D or lineage 2, though such characters are seen in isolation in some species such as *P. matthewsi* and *P. parva*; *P. lata* is another exception, having lost its wings and modified its diet. The similar morphologies and geographic distributions of these 'geoscapheine'-like panesthiines imply these species have experienced analogous selective pressures, and indeed, many of these lineages are restricted to mountainous rainforest as stated earlier.

Taxonomy of Geoscapheinae and future directions

Geoscapheinae have had a tumultuous taxonomic history. Panesthiinae was formally erected by Kirby (1904), and Geoscapheusidae was then synonymised with the subfamily by Princis (1965). Subsequently, Roth (1982) performed a comprehensive revision of Panesthiinae in which he proposed Geoscapheini as a distinct tribe with Panesthiini, Ancaudelliini, Salagneini, and Caepariini erected for the remaining panesthiines. Four genera (*Macropanesthia*, *Geoscapheus*, *Neogeoscapheus*, and *Parapanesthia*) were placed in Geoscapheini on the basis of the laterocaudal angle of the seventh tergite being directed dorsally. Rugg & Rose (1984c) later elevated the subfamily Geoscapheinae based on differences in characters between two species and those of Panesthiinae, namely the aforementioned tergite morphology and the presence of 'true' ovoviviparity, i.e. the lack of a membrane protecting the internally incubated ootheca (compared with the 'false' ovoviviparity of Panesthiinae). The four geoscapheine genera were further defined by

the morphology of the margins of the sixth tergite: specifically, a lack of spines at the posterior margin but not the laterocaudal angle (*Geoscapheus*), a lack of spines and tubercles in both of these locations (*Macropanesthia*), a thickened posterior margin with a fringe of tubercles (*Parapanesthia*), or at least one spine on the posterior margin facing sublaterally (*Neogeoscapheus*) (Rose *et al.,* 2014).

The subfamilial and generic assignments of Roth (1977) and Rugg & Rose (1984c) conflict with protein electrophoretic and allozyme frequency data (Humphrey *et al.,* 1998) and a then-preliminary molecular phylogeny (Maekawa *et al.,* 2003). Humphrey *et al.* (1998) noted that, while their dataset conflicted with tergite-based generic assignments, biogeography was nonetheless reflected in their trees. These topologies are also strikingly similar to the phylogeny reconstructed here using molecular data: this includes the placement of *M. monteithi* with *M. mackerrasae*, and the recovery of *M. heppleorum+M.rhinoceros+G. woodwardi*, *Parapanesthia pearoni+N. dahmsi, N. hirsutus+M. lithgowae+N. barbarae,* and *Parapanesthia gigantea+M. saxicola* as close relatives. Maekawa *et al.* (2003) inferred different topologies to both this study and that of Humphrey *et al.* (1998) likely due to limited taxon sampling, with Geoscapheinae similarly rendering Panesthiinae paraphyletic but with only a single evolution of burrowing forms. Overall, none of the morphological characters mentioned here are synapomorphies defining both Geoscapheinae and Australian members of Panesthiinae in either our phylogenies or that of Humphrey *et al.* (1998), Maekawa *et al.* (2003), or Lo *et al.* (2016). It appears to be the case that many morphological traits cannot be easily used to define the Geoscapheinae because of convergent morphology occurring in Australian panesthiines as well, excluding *P. australis* and *P. obtusa*.

The findings presented confirm the non-monophyly of almost every taxonomic group within Geoscapheinae. Geoscapheinae are polyphyletic within a paraphyletic Panesthiinae; this is due to rampant morphological convergence that has previously undermined taxonomic assignments. Within Geoscapheinae the genera *Geoscapheus*, *Macropanesthia*, *Neogeoscapheus* and *Parapanesthia* are all recovered as polyphyletic and all but *Geoscapheus* have at least one non-monophyletic species therein. The panesthiine genera *Ancaudellia, Caeparia*, and *Panesthia* are similarly not reciprocally

monophyletic (Fig. 2). Given this striking incongruence with current taxonomic assignments it is clear a revision of both of these subfamilies is appropriate. Regarding the classification of the group, the least impactful taxonomic change would involve subsuming all members of Geoscapheinae into Panesthiinae, a conclusion also reached by Djernæs *et al.* (2020). This proposal is similar to that of Roth (1977), albeit without tribal status for the former Geoscapheinae given the polyphyly of the group. Within the wider Panesthiinae, *Ancaudellia* and *Caeparia* should likely be subsumed within *Panesthia* as they are both nested amongst its members in our phylogeny (Fig. 2). At the species level, *P. angustipennis* and *P. cribrata* are each likely to represent species complexes.

CONCLUSIONS

The results of this study are consistent with ancestral Asian wood-feeding lineages of Panesthiinae dispersing to Australia at least twice, with one of those colonisation events giving rise to soil-burrowing cockroaches presently classified as Geoscapheinae. We find that soil burrowing has evolved at least seven times from this ancestral state and that the diversification of burrowing forms was ongoing from approximately 17 to 3 Mya. These transitions were likely driven by bursts of aridification the Australian continent experienced beginning ~25 Mya, and this parallel evolution of burrowing traits in geoscapheines has rendered soil-burrowing associated morphology alone phylogenetically misleading. Within Geoscapheinae, we demonstrate additional character reductions have occurred related to reproductive and tergite morphology in clades that diverged from their panesthiine ancestors earlier and inhabit more arid biomes than younger burrowing clades found in more temperate environments. We also identify a distinction between panesthiines with putatively plesiomorphic traits that have close soil-burrowing relatives compared to those with more 'geoscapheinae'-like morphology that are nested within, or gave rise to, geoscapheine clades.

Ultimately, our revised phylogenetic framework is reflective of both biogeography and morphology of these species, and this is the first time such factors have been unified in the context of the evolutionary history of these insects. Given this new phylogenetic evidence, we propose the morphology and habitat preferences of Geoscapheinae no longer warrant subfamilial status for the group.

DATA AVAILABILITY STATEMENT

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The molecular data that support the findings of this study are openly available via the GenBank repository. Relevant accession numbers can be found in Table 1 of this manuscript.

AUTHOR CONTRIBUTIONS

PGBH and NL conceived and designed the study. HAR and JAW performed taxon sampling. YK and TB assisted with the sequencing and assembly of *Blattabacterium* molecular data. PGBH analysed the molecular data, performed the relevant analyses, and drafted the manuscript. All authors finalised the manuscript prior to submission.

CONFLICTS OF INTEREST STATEMENT

The authors have no conflict of interest to disclose.

SHORT SUPPORTING MATERIAL LEGENDS

Table S1. Site model selection results from bModelTest implemented in BEAST2 for the main mitogenome and nuclear marker phylogeny.

Table S2. Site model selection results from bModelTest implemented in BEAST2 for the reduced taxon mitogenome phylogeny and *Blattabacterium* phylogeny.

Fig. S1. Maximum-likelihood phylogeny inferred from whole mitogenomes and nuclear markers of the Australian Panesthiinae and Geoscapheinae.

Fig. S2. Ancestral state reconstruction of wood-feeding behaviour.

Fig. S3 Ancestral state reconstruction of the presence or absence of the oothecal membrane.

Fig. S4 Ancestral state reconstruction of the wings being absent, reduced/tegminiferous, or fully developed.

Fig. S5 Ancestral state reconstruction of the male L2d genital phallomere being absent, reduced, or fully developed.

Fig. S6. Ancestral state reconstruction of the male L1 genital phallomere being absent, reduced, or fully developed.

Fig. S7. Ancestral state reconstruction of the male R2 genital phallomere being absent, reduced, or fully developed.

Fig. S8. Ancestral state reconstruction of the ocellar spots being absent, reduced, or fully developed.

Fig. S9. Ancestral state reconstruction of holes in the anterolateral corners of the abdominal tergites being present or absent.

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Figure 1: *Macropanesthia mutica* Rose, Walker & Woodward, a geoscapheine, surrounded by leaf litter in captivity. *Macropanesthia* displays morphology common to all geoscapheines, including the loss of wings and ocellar spots, reduced compound eyes, "shovel-like" protibiotarsi for digging and a generally ovoid body shape. Photograph by Braxton Jones.
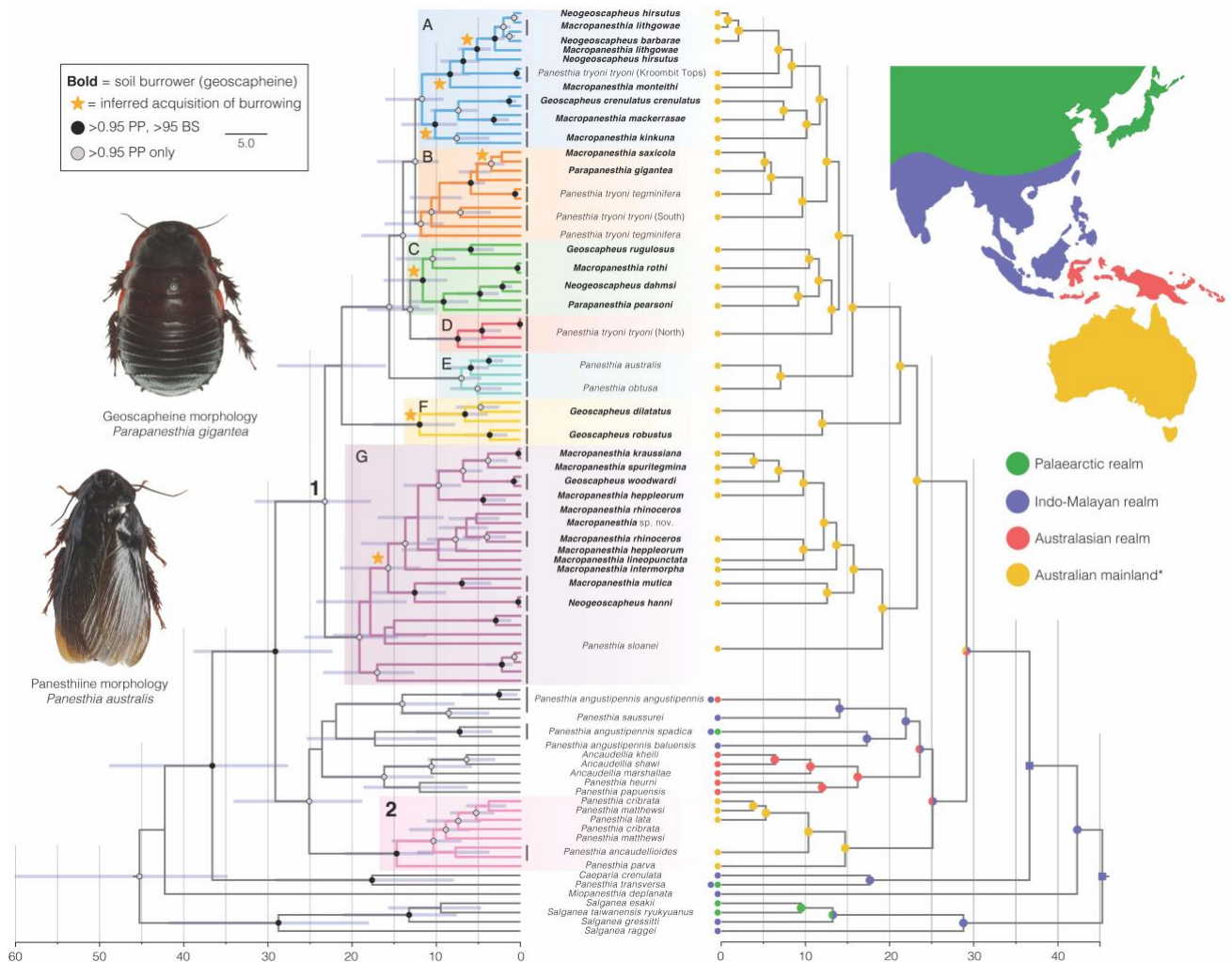
Figure 2: Left, phylogeny of Geoscapheinae and Panesthiinae produced in BEAST2 and RAxML using *"Gyna" obesa* Piton (1940) as a fossil calibration with mitogenome and nuclear molecular markers. Stars represent the inferred earliest possible acquisition of burrowing. PP = posterior probability, BS = bootstrap support. Scale bar is in substitutions/site/million years and node bars represent the 95% HPD of node ages. Scale axis is in millions of years.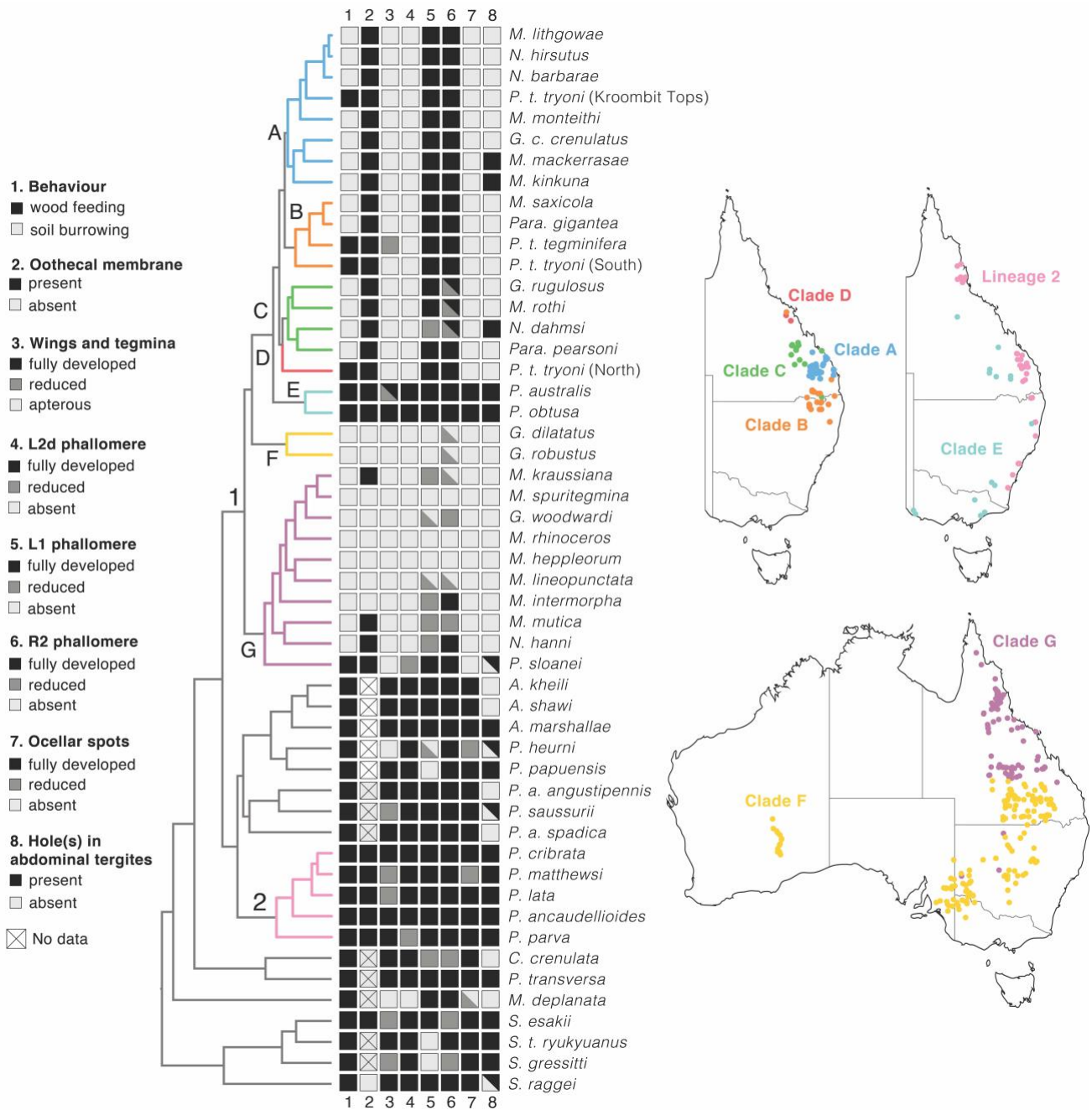 Right, most probable ancestral ranges for Geoscapheinae and Panesthiinae inferred using a DEC model in BioGeoBEARS, with the phylogeny pruned to retain one representative per taxon in the tree. Circles at nodes represent the most probable ancestral areas or combinations thereof and, unless specified by squares, the relative probability of area(s) at each node is >33%. Circles at tips represent present-day distributions. * Includes Lord Howe Island to account for the distribution of *Panesthia lata*. Photographs by DigiVol (https://volunteer.ala.org.au) and CBG Photography Group, Centre for Biodiversity Genomics (https://biodiversitygenomics.net/).
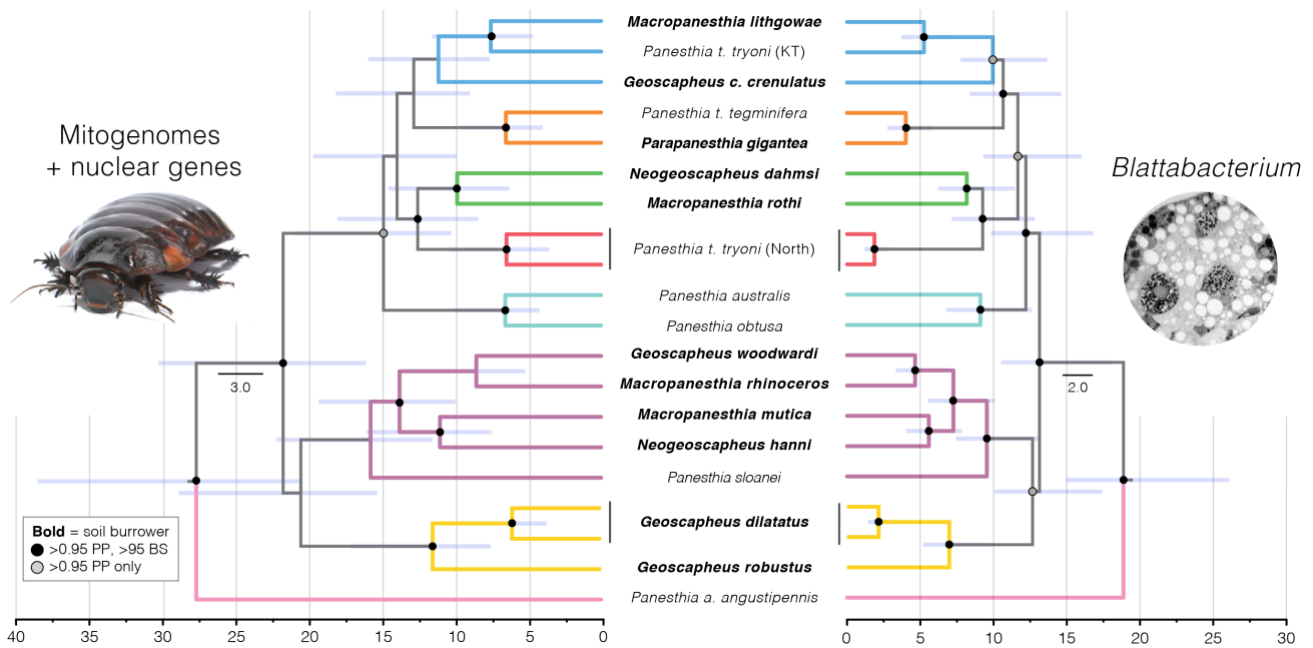
Figure 3: Time-calibrated whole mitogenome + nuclear marker and 31-gene *Blattabacterium* phylogenies of Geoscapheinae and Australian Panesthiinae inferred using BEAST2 and RAxML. Congruent relationships among Geoscapheinae are reflected in both our mitogenome and Blattabacterium dataset when taxon sampling is equalised. KT = Kroombit Tops population. *Macropanesthia rhinoceros* photograph by Yi-Kai Tea, *Blattabacterium* image adapted from Lo et al. (2007).

Figure 4: Morphological characters of the Geoscapheinae and Australian Panesthiinae are largely reflective of their phylogenetic relationships and geographic distributions. Combinations of characters are synapomorphies for certain groups, but no set of characters are a synapomorphy for all taxa shown here. Phylogeny sourced from Fig. 2. Geographic distribution data were sourced from HAR's personal records and morphological characters are per Roth (1977, 1979a, 1979b, 1982).

Table 1. Geoscapheine and panesthiine taxa sampled in this study. Taxa in black were sequenced as part of this study and where possible were paired with single-gene data from Lo *et al.* (2016); those in grey were not sequenced in the present study and retrieved from GenBank. Australian regional abbreviations: Queensland (QLD), West Australia (WA), South Australia, (SA), Victoria (VIC). All species aside from those in *Panesthia* in the ingroup belong to the Geoscapheinae. Identifications of specimens to species level were performed by HAR and JAW.

Genus	Species	Collection locality or reference	GenBank accession no.		
			Mitogenome (TBA)	*Blattabacterium* markers	Nuclear rRNA
Geoscapheus	*crenulatus crenulatus*	Rainbow Beach, QLD	MW996579		*18S:* MW365869*; ITS1:* MW365805
	dilatatus (non-tuberculate form)	Mitchell, QLD	MW354074		*18S:* MW365870; *ITS1:* MW365806
	dilatatus (tuberculate form)	Patchewollock, VIC	MW354075	MW938770 - MW939358	*18S:* MW365871; *ITS1:* MW365807
	robustus	Laverton, WA	MW996606		*18S:* MW365872; *ITS1:* MW365808
	rugulosus	Blackdown National Park, QLD	MW996580		*18S:* MW365873; *ITS1:* MW365809
	woodwardi	"Mt. Cornish" Station, Muttaburra, QLD	MW996581		*18S:* MW365874; *ITS1:* MW365810
Macropanesthia	*heppleorum*	Yuleba, QLD	MW996582		*18S:* MW365875; *ITS1:* MW365811
	intermorpha	Lo *et al.* (2016)	KU577617–KU577895 (*12S, COII*)		KU577617–KU577895 (*ITS1, 18S*)
	kinkuna	Coonarr, QLD	MW996583		*18S:* MW365876; *ITS1:* MW365812
	kraussiana	Isisford, QLD	MW996584		*18S:* MW365877; *ITS1:* MW365813
	lineopunctata	Lo *et al.* (2016)	KU577617–KU577895 (*12S, COII*)	MW938770 - MW939358	KU577617–KU577895 (*ITS1, 18S*)
	lithgowae	Nudley State Forest, QLD	MW354066		*18S:* MW365878; *ITS1:* MW365814
	mackerrasae	Base of Mt. Woowoonga, QLD	MW996585		*18S:* MW365879; *ITS1:* MW365815
	monteithi	Archookoora, QLD	MW996586		*18S:* MW365880; *ITS1:* MW365816
	mutica	Dinden State Forest, QLD	MW354067		*18S:* MW365881; *ITS1:* MW365817
	rhinoceros	Bourguignon *et al.* (2018, 2020), Lo *et al.* (2016)	MG882202, KU577617–KU577895 (*12S, COII*)	CP059200.1	KU577617–KU577895 (ITS1, 18S)
	rothi	Agnes Water, QLD	MW354068	MW938770 - MW939358	*18S:* MW365882;

Genus	Species	Locality/Source	12S, COII	Mitogenome	18S / ITS1
					ITS1: MW365818
	sp. nov. "Kirrama" *saxicola* *spuritegmina*	Lo *et al.* (2016)	KU577617–KU577895 (*12S, COII*)		KU577617–KU577895 (*ITS1, 18S*)
Neogeoscapheus	*barbarae*	Lo *et al.* (2016)	KU577617–KU577895 (*12S, COII*)		KU577617–KU577895 (*ITS1, 18S*)
	dahmsi	Taroom, QLD	MW996587	MW938770 - MW939358	*18S*: MW365883; *ITS1*: MW365819
	hanni	Mt. Molloy, QLD	MW996588		*18S*: MW365884; *ITS1*: MW365820
	hirsutus	Bundaberg, QLD	MW996589		*18S*: MW365885; *ITS1*: MW365821
Panesthia	*ancaudellioides*	Dawson Gully, QLD	MW354069		*18S*: MW365886; *ITS1*: MW365822
	australis	Kalangadoo, SA (b2,34)	MW996591		*18S*: MW365888; *ITS1*: MW365824
		Wiseleigh, VIC	MW354070		*18S*: MW365887; *ITS1*: MW365823
	matthewsi	Mt. Walsh, QLD	MW354071		*18S*: MW365889; *ITS1*: MW365825
	obtusa	Injune, QLD	MW996592		*18S*: MW365890; *ITS1*: MW365826
	parva	Walkamin, QLD	MW996593		*18S*: MW365891; *ITS1*: MW365827
	sloanei	Mt. Lewis, QLD	MW996596	MW938770 - MW939358	*18S*: MW365894; *ITS1*: MW365829
		Paluma Range, QLD	MW996597		*18S*: MW365895; *ITS1* not recovered
		Windsor Tableland, QLD	MW996594		*18S*: MW365892; *ITS1* not recovered
		Mossman Gorge, QLD	MW996595		*18S*: MW365893; *ITS1*: MW365828
	tryoni tegminifera	Killarney, QLD	MW996598		*18S*: MW365896; *ITS1*: MW365830
		Dorrigo National Park, QLD	MW996599		*18S*: MW365897; *ITS1*: MW365831
		Kroombit Tops National Park, QLD	MW996600		*18S*: MW365898; *ITS1*: MW365832
	tryoni tryoni	Cathu, QLD (b2_15)	MW996602		*18S*: MW365900; *ITS1*: MW365834
		Eungella, QLD (b2_14)	MW996601		*18S*: MW365899; *ITS1*: MW365833

		Sarina, QLD (r4bv_20)	MW354072		*18S*: MW365901; *ITS1*: MW365835
		Lamington National Park, QLD (b2_11)	MW996603		*18S*: MW365902; *ITS1*: MW365836
		Mary Cairncross Scenic Reserve, QLD (b2_12)	MW996604		*18S*: MW365903; *ITS1*: MW365837
Parapanesthia	*gigantea*	Warwick, QLD	MW354073	MW938770 - MW939358	*18S*: MW365904; *ITS1*: MW365838
	pearsoni	Blackdown National Park, QLD	MW996605		*18S*: MW365905; *ITS1*: MW365839

Outgroup (non-Australian taxa)

Paneshia	*angustipennis angustipennis*	Le Niol, Mahe Island, Seychelles	MW996590	-	Not recovered
Ancaudellia	*kheili*			-	
	marshallae			-	
	shawi			-	
Caeparia	*crenulata*			-	
Miopanestha	*deplanata*			-	
	ancaudellioides			-	
	angustipennis angustipennis			-	
	angustipennis baluensis			-	
Paneshia	*angustipennis spadica*	Lo *et al.* (2016); Tokuda *et al.* (2013)	KU577617–KU577895 (*12S*, *COII*)	NC_020510.1	KU577617–KU577895 (*ITS1*, *18S*)
	cribrata			-	
	heurni			-	
	lata			-	
	papuensis			-	
	saussurii			-	
	transversa			-	
	essakii			-	
Salganea	*gressiti*			-	
	raggei			-	
	taiwanensis ryukyuanus			-	
Diploptera	*punctata* (Diplopterinae)	Bourguignon *et al.* (2018)	MG882143	-	-
Blattella	*germanica* (Ectobiidae)	Xiao *et al.* (2012); Lopez-Sanchez *et al.* (2009)	NC_012901.1	NC_013454.1 – NC015679.1	-
Epilampra	*maya* (Epilamprinae)		MG882194	see Bourguignon *et al.* (2020)	-
Galiblatta	*cribosa* (Epilamprinae)	Bourguignon *et al.* (2018, 2020)	MG882232	-	-
Gyna	*capucina* (Gyninae)		MG882152	see Bourguignon *et al.* (2020)	-
Nauphoeta	*cinerea* (Oxyhaloinae)	Dumans *et al.* (2017); Kambhampati *et al.* (2013)	KY212743	NC_022550.1 – NC_022551.1	-
Neolaxta	*mackerrasae* (Perisphaerinae)	Bourguignon *et al.* (2018, 2020)	MG882201	see Bourguignon et al. (2020)	-
Paranauphoeta	*circumdata* (Paraneuphoetinae)		MG882225		-

Pycnoscelus	sp. (Pycnoscelinae)	MG882200	-	-
Rhabdoblatta	sp. (Epilamprinae)	MG882228		-
Schultesia	*lampyridiformis* (Zetoborinae)	MG882163	see Bourguignon et al. (2020)	-