Molecular Phylogenetics and Evolution 48 (2008) 506–514

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Distribution and phylogenetic relationships of Australian glow-worms *Arachnocampa* (Diptera, Keroplatidae)

Claire H. Baker [a], Glenn C. Graham [a], Kirsten D. Scott [a], Stephen L. Cameron [b], David K. Yeates [b], David J. Merritt [a,*]

[a] School of Integrative Biology, The University of Queensland, Mansfield Place, Brisbane, Qld. 4072, Australia
[b] CSIRO Entomology, PO Box 1700, Canberra ACT 2601, Australia

ARTICLE INFO

Article history:
Received 17 September 2007
Revised 1 April 2008
Accepted 28 April 2008
Available online 3 May 2008

Keywords:
Phylogeny
Conservation
Wet tropics
Bioluminescence
Cave
Troglophile

ABSTRACT

Glow-worms are bioluminescent fly larvae (Order Diptera, genus *Arachnocampa*) found only in Australia and New Zealand. Their core habitat is rainforest gullies and wet caves. Eight species are present in Australia; five of them have been recently described. The geographic distribution of species in Australia encompasses the montane regions of the eastern Australian coastline from the Wet Tropics region of northern Queensland to the cool temperate and montane rainforests of southern Australia and Tasmania. Phylogenetic trees based upon partial sequences of the mitochondrial genes cytochrome oxidase II and 16S mtDNA show that populations tend to be clustered into allopatric geographic groups showing overall concordance with the known species distributions. The deepest division is between the cool-adapted southern subgenus, *Lucifera*, and the more widespread subgenus, *Campara*. *Lucifera* comprises the sister groups, *A. tasmaniensis*, from Tasmania and the newly described species, *A. buffaloensis*, found in a high-altitude cave at Mt Buffalo in the Australian Alps in Victoria. The remaining Australian glow-worms in subgenus *Campara* are distributed in a swathe of geographic clusters that extend from the Wet Tropics in northern Queensland to the temperate forests of southern Victoria. Samples from caves and rainforests within any one geographic location tended to cluster together within a clade. We suggest that the morphological differences between hypogean (cave) and epigean (surface) glow-worm larvae are facultative adaptations to local microclimatic conditions rather than due to the presence of cryptic species in caves.

1. Introduction

In Australia and New Zealand the bioluminescent larvae of flies (Diptera) belonging to the family Keroplatidae, subfamily Arachnocampinae, genus *Arachnocampa* (Matile, 1981) are known as glow-worms. The bioluminescence display produced by high densities of larvae in caves and rainforest settings serves as a major tourist attraction at sites in Australia and New Zealand. Individual larvae construct a snare composed of a horizontal mucous tube hung from the substrate by bracing threads. A series of "fishing lines" made up of silk threads and sticky mucous droplets are hung from the threads. The function of the bioluminescence, produced in terminal cells of the malpighian tubules (Wheeler and Williams, 1915), is to attract flying prey into the fishing lines whereupon they are hauled up by the larva and eaten (Richards, 1960; Broadley and Stringer, 2001).

The genus *Arachnocampa* Edwards comprises nine described species divided into three subgenera (Harrison, 1966; Baker, accepted for publication). A single species, *A. luminosa* Ferguson, is endemic to New Zealand. Eight species are endemic to Australia. Five are newly described (Baker, accepted for publication); their species descriptions are based on morphological criteria. The remaining three Australian species are *A. flava* Harrison, from southeast Queensland (Perkins, 1935; Harrison, 1966); *A. richardsae* Harrison, from central New South Wales (Harrison, 1966); and *A. tasmaniensis* Ferguson from Tasmania (Ferguson, 1925). Six species, all found on mainland Australia, are allocated to subgenus *Campara*; two Australian species, *A. tasmaniensis* and *A. buffaloensis*, are allocated to the newly erected subgenus *Lucifera*. *A. luminosa* from New Zealand is the sole species in the subgenus *Arachnocampa* (Harrison, 1966; Baker, accepted for publication).

The focus of this paper is the distribution and phylogeography of glow-worms. They are of interest in this regard because they are restricted to dark, humid habitats associated with rainforest and caves (Richards, 1960; Stringer, 1967; Baker and Merritt, 2003; Baker, 2004) and are found in a range of climates from cool temperate to tropical. Australia's rainforests are recognised as areas of high endemism because of limited opportunity for gene flow between isolated populations (Harvey, 2002), therefore, glow-worms could be highly structured throughout their distribution. Australian rainforest invertebrates commonly show evidence

* Corresponding author. Fax: +61 7 3365 1655.
E-mail address: d.merritt@uq.edu.au (D.J. Merritt).

1055-7903/$ - see front matter Crown copyright © 2008 Published by Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2008.04.037

of vicariant speciation in rainforest patches (e.g. Bell et al., 2003; Ponniah and Hughes, 2004; Sota et al., 2005) presumably due to a contraction of formerly widespread rainforest into pockets in the eastern montane regions during the aridification through the Miocene and the glacial cycles of the Pleistocene (Morley, 2000; Garrick et al., 2004).

While there is no information available on the dispersal ability of adult glow-worms, it appears to be limited because the adults are very short-lived—2–3 days for females and 4–6 days for males (Baker and Merritt, 2003)—and are sluggish fliers (Richards, 1963). As facultative cave-dwellers, they are classified as troglophiles, compared to the troblobites that are obligate cave-dwellers (Howarth, 1983). Cave glow-worms show markedly less pigmentation, produce longer snares and grow to a larger size than epigean relatives from the same region (Richards, 1960), providing *prima facie* evidence of genetic adaptation to the cave environment. In an allozyme-based study carried out in New Zealand, high levels of polymorphism and heterozygosity between rainforest and cave populations of *A. luminosa* raised the possibility of regular gene flow between glow-worms in each habitat type (Broadley, 1998).

Here we define the distribution of Australian glow-worms, investigate their phylogenetic relationships using 16S and COII gene sequence and assess whether cave populations are genetically distinct from adjacent rainforest populations.

2. Materials and methods

2.1. Sample collection

Larvae of *Arachnocampa* were collected from 35 sites across Australia and three in New Zealand. They included sites of published reports (Ferguson, 1925; Perkins, 1935; McKeown, 1935; Harrison, 1966; Currey, 1966; Goede, 1967; Finlayson, 1982; Department of Conservation, 1994; Eberhard and Spate, 1995) and new sites chosen because of the presence of appropriate habitat. In some cases, locations were identified through information from local residents, National Parks rangers and cavers. In addition to the published records covering 16 sites, many more sites where glow-worms were present have been identified (listed in Baker, 2004) and a subset of these was used for the current molecular analysis (Appendix A, Fig. 1). Larvae were usually collected at night, detected by their bioluminescence, except in caves where they tend to glow continuously. Larvae were collected into absolute ethanol and stored at −20 °C. Larvae of the bioluminescent keroplatid *Orfelia fultoni* were collected from two sites in Alabama, North America (Appendix A). For morphological examination of adults, larvae were chilled and returned alive to the laboratory where they were reared to adulthood and identified to species (Baker and Merritt, 2003; Baker, accepted for publication).

Two outgroups for were chosen for this study (*A. luminosa* and *Orfelia fultoni*) based on their geographic separation from Australian *Arachnocampa* species. Morphologically the New Zealand endemic *A. luminosa* most closely resembles the Australian species, *A. tasmaniensis*. However, they are readily distinguished by the ratio of the length of the basal segment of the fore tarsus to the fore tibia (Harrison, 1966). The American out-group species, *O. fultoni* is placed in the Family Keroplatidae, but within a different genus based on a number of characters (Fulton, 1941). Physiologically, *O. fultoni* differs from *Arachnocampa* spp. in that it glows from different larval tissues and uses different biochemical pathways in light production (Viviani et al., 2002).

2.2. DNA sequencing

Three to ten larvae were collected from each site. DNA was extracted according to Qiagen DNeasy* kit protocols. Three to six individuals from each site were sequenced with forward and reverse primers for both COII and 16S. PCR of the COII DNA fragment was carried out in a 25 μl total reaction volume containing: 20 mM Tris–HCl, 100 mM KCl, 2.8 mM $MgCl_2$, 0.5 mM dNTP's, 0.2 μM each primer MtD16 and MtD20 (Liu and Beckenbach, 1992; Simon et al., 1994) (MtD16 5′attggacatcaatgatattga3′ MtD20 5′gtttaagagac cagtacttg3′), 20 ng DNA, 1 U *Taq* polymerase (Qiagen, Clifton Hill, Victoria, Australia), 16.25 μl ROH_2O. Thermal cycling was performed in PC960 Thermal Cycler (Corbett Research, NSW, Australia) using the following cycling conditions: (94 °C, 2 min; 55 °C, 1 min; 72 °C, 90 s) × 1 cycle, (92 °C, 30 s; 55 °C, 30 s, 72 °C 90 s) × 39 cycles, (72 °C, 5 min; 24 °C, 2 min) × 1 cycle. PCR of the 16S DNA fragment was carried out in a 25 l total reaction volume containing: 20 mM Tris–HCl, 100 mM KCl, 4 mM $MgCl_2$, 0.2 mM dNTP's, primers: 0.2 μM each primer 16SF, 16SR (Lange et al., 2004) (16SF 5′AAGATTTTAATGATCGAACAG, 16SR 5′TGACTGTACAA AGGTAGCATA), 20 ng DNA, 1 U *Taq* polymerase (Qiagen, Clifton Hill), 15.2 μl ROH_2O. Thermal cycling used the following conditions: (94 °C, 2 min; 54 °C, 1 min; 72 °C, 15 min) × 1, (92 °C, 45 s;

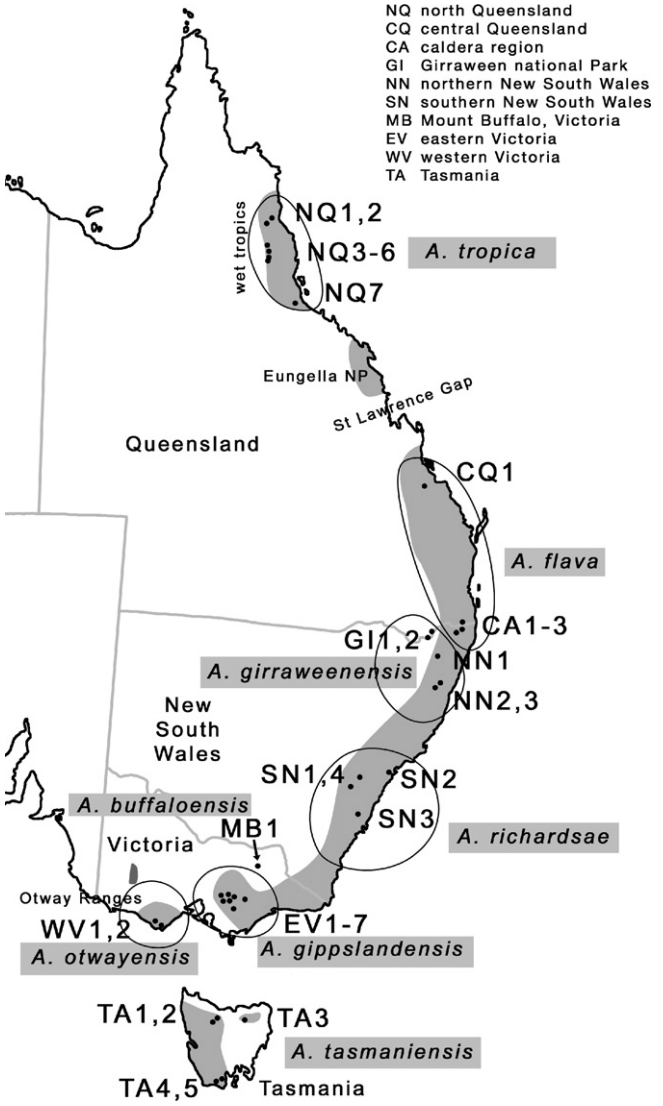

Fig. 1. Map of the eastern coast of Australia showing locations of *Arachnocampa* collections for this study. The lightly shaded regions roughly correspond with the distribution of rainforest. The location codes are spelled out in Appendix A. The same codes are used in Fig. 2. Other geographical features are mentioned in the text. The distributions of described species (in grey boxes) are circled.

54 °C, 1 min; 72 °C, 90 s) × 39, (72 °C, 2 min; 25 °C, 2 min). Amplification for both genes was confirmed by running 5 μl of PCR on a 1.5% TBE agarose gel. Samples were purified in a 96-well plate format using MultiScreen PCR plates (Millipore, NSW, Australia).

Sequencing was performed in the forward and reverse directions in a 12 μl total reaction volume containing 1 μl ABI dye terminator version 3 (Applied Biosystems, Victoria, Australia), 3 μl 5 × dilution buffer, 3.2 pmol of primer and 50 ng PCR product. Cycling conditions for sequencing PCR were (94 °C for 5 min) × 1, (96 °C for 10 min, 50 °C for 5 min, 60 °C for 4 min) × 31, 25 °C for 5 min and hold at 4 °C. Thermal cycling was conducted in a PC960 Thermal Cycler (Corbett Research, NSW, Australia). Sequence clean-up was done using Montage SEQ_{96} sequencing reaction clean-up kits (Millipore, NSW, Australia). Sequences were run on an ABI 3700 DNA sequencer at the Australian Genome Research Facility.

2.3. Phylogenetic analysis

Sequences for both COII and 16S were aligned using Clustal X v1.82 (Thompson et al., 1997) and edited using SeqEd (Myers and Kececioglu, 1992). Aligned sequences were further adjusted in MacClade 4.03 by eye. Phylogenetic relationships of the aligned sequences were analysed in PAUP* (Swofford, 2002). A heuristic search algorithm was used (tree bisection–reconnection branch swapping) for parsimony analysis with the following changes from the default settings: stepwise-addition was increased to 1000 random replicates. A single optimal tree was saved from each replicate. All trees in memory were used (starting tree options) for final heuristic analysis and trees were saved.

Statistical support for the phylogenetic tree internodes was assessed with 1000 bootstrap replicates. Parameters used were the same as the initial parsimony heuristic search with 100 stepwise-addition replicates. Analysis was performed for each gene separately and then for a combined data set of the two genes.

The model of molecular evolution used in the Bayesian analysis was determined using ModelTest v 3.7 (Posada and Crandall, 1998). Models were chosen by AIC and the favoured models were for COII: 6 rate categories plus an invariant and a gamma parameter, for 16S: 6 rate categories plus a gamma parameter. Bayesian analysis was run in MrBayes 3.1 (Huelsenbeck and Ronquist, 2001) for 2 independent runs each with 4 chains for 3 million generations with sampling every 1000 generations. Each of the two data partitions (COII and 16S) was unlinked. At completion, the runs were checked for convergence between each run and the initial burn-in period determined by examining each of the run parameters for convergence. The initial 50,000 generations (50 trees) were discarded as burn-in. The remaining trees were used to calculate the consensus topology and the posterior probabilities for nodal support.

Divergence time estimates were calculated following the method of Brower (1994). Maximum pairwise distances were calculated between each clade from the COII alignments, and divergence times estimated using Brower's (1994) calibration of 2.3% molecular divergence per million years. Similar divergence time estimates (data not shown) were obtained from the 16S and combined data sets however these suggested slightly later divergences due to the generally lower rates of substitution found in 16S versus COII in flies (Cameron et al. 2007).

3. Results

3.1. Distribution and habitat

Colonies of *Arachnocampa* in rainforest were associated with steep embankments and a nearby watercourse, such as a waterfall or small stream. Colonies were present in limestone or granite boulder caves with high humidity (⩾96% RH) and flowing water. Artificial caves such as abandoned mineshafts and railway tunnels where free water is present were recorded as containing glow-worms although it was not practical to systematically search all sites with these characteristics. In these cases the artificial caves were located within or near rainforest.

The ruggedness of suitable terrain and the necessity of surveying at night precluded a comprehensive search of all potential sites. Consequently the determination of distribution limits relied on interviews and anecdotal reports rather than comprehensive searches. The Australian distribution most closely accords with the distribution of rainforest associated with the Great Dividing Range that spans the eastern coast of Australia (Fig. 1). The northern limit appears to be the Wet Tropics region of north-eastern Australia, as surveys of researchers and park rangers who frequent the seasonally wet rainforest north of the Wet Tropics produced no reported sightings. A major distribution disjunction occurs in central Queensland where glow-worms are absent from apparently suitable rainforest at Eungella National Park, north of the St. Lawrence Gap (Fig. 1), based upon both personal surveys and interviews. The known south-western distribution limit is the rainforest of the Otway ranges. They were not found in the Grampian Mountains (personal surveys) and are not present further west, based on interviews. Glow-worms are widely distributed in caves and temperate rainforest of Tasmania (personal surveys and interviews). Wide consultation with park rangers, cavers and field biologists produced no records of glow-worms in the states of Western Australia, the Northern Territory or South Australia.

Natural caves with glow-worms were located at Girraween National Park in southeast Queensland (denoted as GI1, 2 in Figs. 1 and 2 and Appendix A); New South Wales, e.g. Gloucester Cave, Gloucester and Carrai Bat Cave, near Kempsey (not sampled for this study); Mount Buffalo, Victoria (MB1); eastern Victoria (EV3,5,6); and Tasmania (TA1,2,5). The caves at Girraween National Park (GI1, 2) and Mount Buffalo in Victoria (MB1) were the most remote from suitable rainforest habitat.

3.2. Nucleotide sequence

No variability in nucleotide sequence was seen among the 3–6 individuals subject to sequencing from each site, consequently each site was treated as a uniform population with a single haplotype. Distance matrices of each data set showed uncorrected pairwise sequence divergence between sites ranged from 0% to 23% for 16S, 0% to 19% for COII, and 0% to 18% for the combined data. Analysis of 38 haplotypes (1 from each of the sample sites listed in Appendix A) using 16S was performed from an alignment of 414 characters, with 266 constant, 36 variable but parsimony-uninformative, and 112 parsimony-informative. Sequence data for COII were analysed for 39 haplotypes (1 from each of the sample sites listed in Appendix A) from an alignment of 418 characters, with 289 constant, 12 variable but uninformative, and 117 parsimony-informative. The combined analysis of partial COII and partial 16S gene fragments from 37 sites consisted of 833 characters, 529 of which were constant, 50 variable but uninformative, and 254 parsimony-informative. Sequence was not obtained for both gene fragments at every sample site due to contamination with DNA from parasitic wasps and some persistent amplification failures, therefore, 16S, COII and the combined analysis parsimony trees are displayed so all populations could be included (Fig. 2). GenBank accession numbers for all sequences are listed in Appendix A.

All analyses using *O. fultoni* as an outgroup indicated a basal divergence between the New Zealand and the Australian representatives. All analyses placed *A. buffaloensis* and *A. tasmaniensis* as sister species, and separated them from all other Australian mainland

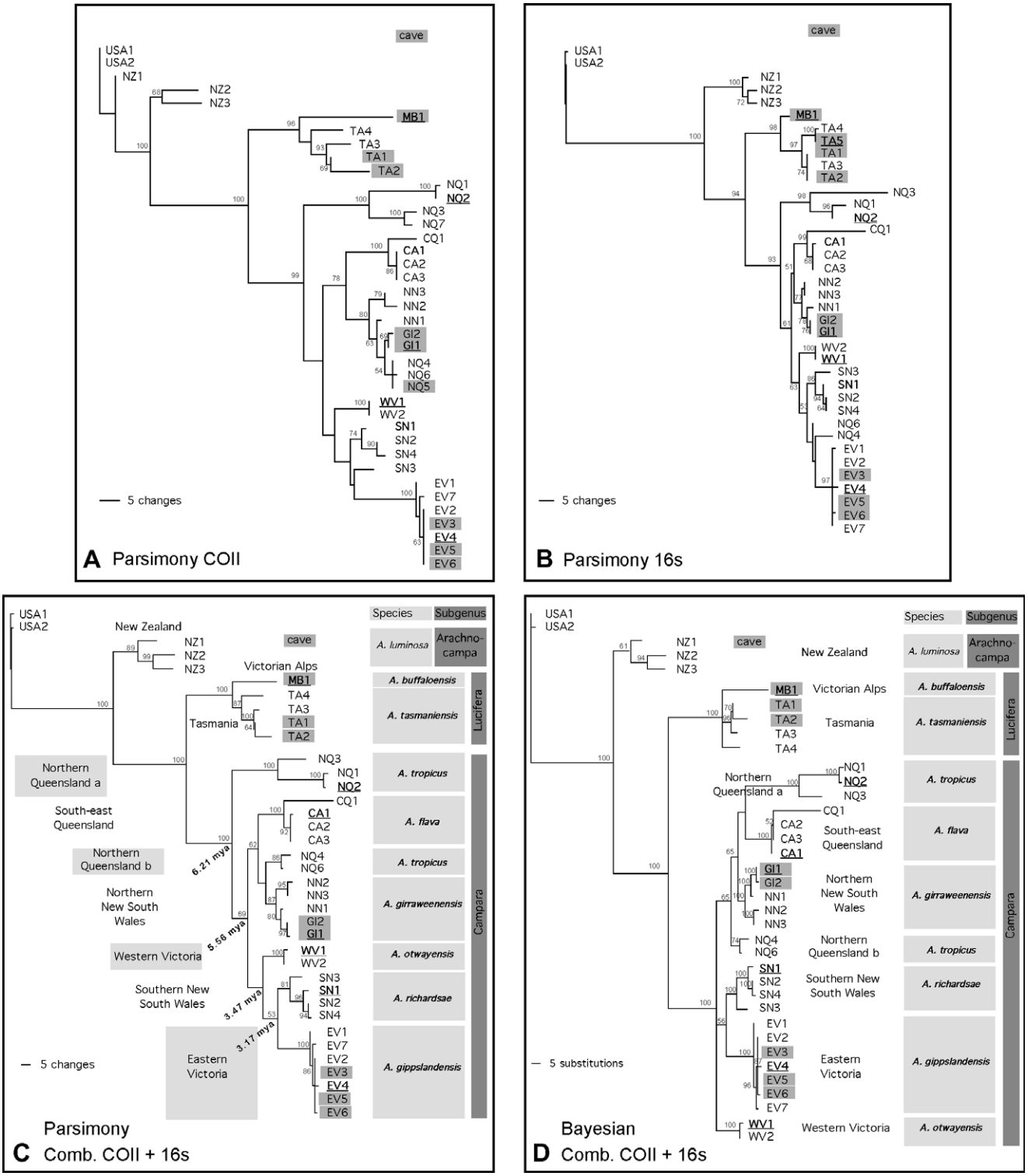

Fig. 2. Parsimony trees generated from partial COII sequence data (A) and partial 16S sequence data (B). (C) Parsimony tree generated from the combined COII and 16S sequence data. (D) Bayesian tree generated from combining the COII and 16S sequence data. In (A–C) bootstrap values from 1000 replicates are shown above each corresponding branch. In (C), estimated divergence times are shown at selected nodes (slanted text; mya = million years ago). In (D), Bayesian posterior probabilities for each node are shown as a percentage. Holotype localities for each species are underlined and in bold. The allocation of glow-worms to subgenera, *Arachnocampa*, *Lucifera* and *Campara*, is shown on the far right adjacent to the species names. Samples taken from natural caves are indicated with a grey background.

populations (Fig. 2). The known distribution of *A. buffaloensis* is restricted to a single cave at an altitude of 1500 m on the edge of a plateau (Baker, accepted for publication). The region experiences regular winter snowfall.

The analyses of the combined data set provided bootstrap support for a number of geographical clusters within subgenus *Campara* that match the known species distributions (Figs. 1 and 2). Parsimony analyses support a "southern" grouping of 3 species located in southern New South Wales (*A. richardsae*) and coastal Victoria (*A. otwayensis* Baker and *A. gippslandensis* Baker). *A. otwayensis* is sister group to *A. gippslandensis* + *A. richardsae*. Coarse estimates of divergence times place the *A. richardsae*/

A. gippslandensis divergence at 3.17 mya and their divergence from *A. otwayensis* at 3.47 mya. Little resolution was evident within the eastern Victorian sites that were sampled (EV1–7). The relationship of the 3 species is less clear using Bayesian analysis due to low support at the nodes and a polytomy at the base of *Campara*.

The remainder of the Australian mainland species is distributed from northern New South Wales to northern Queensland. The species *A. girraweenensis* Baker includes populations found in two granite boulder caves at Girraween National Park (GI1 and 2), on an inland plateau at an altitude of 900 m, and the three northern New South Wales rainforest populations that were analysed. These populations together form a clade in all analyses, supporting their designation as a single species. The one exception is the inclusion of some northern Queensland populations into the *A. girraweenensis* group in the COII parsimony analysis (Fig. 2A, see below).

The species *A. flava* forms a well-supported group encompassing the region from central Queensland (CQ1, Kroombit Tops National Park) to the McPherson Ranges region near the Queensland–New South Wales border (CA1–3). The population at Kroombit Tops in central Queensland (CQ1) forms a clade with the caldera (CA) populations in all analyses, indicating the Kroombit Tops glow-worm is either a member of *A. flava* or is closely related to it. No adult specimens have been obtained from Kroombit Tops for morphological comparison. The coastal component of the McPherson ranges is dominated by an ancient (22 mya) eroded caldera with rainforest clothing the mountains. The type specimen of *A. flava* was taken from a stream-eroded cave on the northern drainage slope of the caldera (CA1).

Glow-worms found in the distinctive area of montane and lowland rainforest known as the Wet Tropics of north Queensland have been designated as a single species, *A. tropica* (Baker, accepted for publication). The larvae are attacked by an undescribed species of parasitoid wasp of the genus *Megastylus* (Hymenoptera: Ichneumonidae, Baker, 2004). Contamination by parasite DNA in two populations (NQ5 and 7) allowed recovery of COII sequence only, consequently NQ5 and NQ7 were not used in the combined analysis. Samples from the Atherton Tableland and Mt Bartle Frere (NQ4, 5 and 6) form a distinct clade (referred to as NQb) that is divergent to samples from further north and south (NQ1, 2, 3 and 7, collectively, NQa). Parsimony analysis placed the more widely distributed clade (NQa) as sister group to all other mainland groups of subgenus *Campara* while Bayesian analysis placed them as sister to the Caldera+Kroombit Tops group. The placement of NQb varied: parsimony analysis of the combined data sets (Fig. 2C) placed NQ4, 5 and 6 as sister to *A. girraweenensis*, whereas Bayesian analysis (Fig. 2D) placed NQ4 and NQ6 as sister to a clade comprising *A. flava*, *A. girraweenensis* and the NQa subset of *A. tropica*.

3.3. Cave vs rainforest habitat

The cave populations sampled for mtDNA analysis are shown highlighted in Fig. 2. Cave populations do not show marked genetic divergence from epigean populations within the same geographic area, for example, the cave populations of *A. girraweenensis* form a clade with northern New South Wales populations, the closest of which was sampled from rainforest 66 km away. In addition, the Eastern Victorian samples from caves and artificial mine adits are very closely related and intermixed in the mtDNA phylogeny.

4. Discussion

4.1. Phylogeography

The geographic focus of Australian glow-worms is the eastern and south-eastern rainforests of the Great Dividing Range/eastern escarpment plus the rainforests of Tasmania. Their absence from the seasonally wet rainforests north of the Wet Tropics and the rainforests of the Northern Territory and Western Australia indicates that these forests may have experienced arid periods sufficient to extinguish any formerly widespread populations or to have acted as barriers to dispersal (see Kikkawa et al., 1981). There is no evidence of sympatry within the genus. Presumably, geographic barriers restrict dispersal and gene flow. The patchiness of suitable rainforest habitat along the eastern coast of Australia could act as such a barrier. Further, the requirement for forested, stream-associated habitats may mean that mountain ranges could restrict gene flow between the major drainage basins. Further fine-scale sampling is required to address these issues.

A coarse dating places the divergences between species within subgenus *Campara* at between 3.17 and 6.21 mya (Fig. 2C), consistent with a diversification during the Pliocene. The chronology appears similar to the radiation of species of eastern Australian rainforest-associated, ground dwelling *Pamborus* beetles (Sota et al., 2005). The most likely explanation for the present-day distribution of subgenus *Campara* is one commonly put forward for Australian rainforests and their fauna: that formerly widespread rainforest was subject to Pliocene/Pleistocene cooling and aridification that resulted in contraction of rainforest and its associated fauna to the coastal and montane regions of the east coast of the Australian mainland (Webb and Tracey, 1981; Kershaw, 1994; Hill, 2004). Inclusion of nuclear gene sequence and longer mtDNA sequence data is needed to provide more accurate estimates of divergence times, especially the deeper splits.

4.1.1. A. richardsae, A. otwayensis and A. gippslandensis

The southern Australian mainland group of three species is distributed in rainforest from mid New South Wales to the Otways region west of Melbourne, Victoria. Each of the three species, which are morphologically distinguishable (Harrison, 1966; Baker, accepted for publication) is allopatrically distributed. The disjunction between eastern and western Victoria is believed to be due to isolation rather than an artefact of sampling point distribution because there are no major tracts of suitable forest between the two localities.

4.1.2. A. tropica

From the current study, mtDNA haplotypes indicate that the species currently designated as *A. tropica* (Baker, accepted for publication) may comprise at least two genetically distinct clades. Their phylogenetic placement in relation to other glow-worms varies according to the data set and analysis used. Bayesian analysis suggests that NQ1–3 is a divergent lineage of *A. flava* (Fig. 2D), and parsimony analyses suggest that it is a separate monophyletic species, sister to all other species in subgenus *Campara* (Fig. 2A–C). Evidently, further genetic and morphological evidence is required to establish how many species are present in the Wet Tropics and to establish their phylogenetic relationship to *A. flava* and other species in subgenus *Campara*.

Arachnocampa tropica occurs in the Queensland Wet Tropics, an area of tropical rainforest composed of mountains, plateaus and lowlands that shows high regional endemism, especially among low-vagility invertebrates (Yeates et al., 2002; Yeates and Monteith, 2008). Pliocene/Pleistocene cycles of rainforest contraction and expansion have restricted gene flow between populations in rainforest refugia, leading to significant population structure in vertebrates (Joseph et al., 1995; Schneider et al., 1998) and speciation in invertebrates (Bouchard et al., 2005). Perhaps the genetic differentiation in glow-worms reflects two cycles of speciation in the area. A parallel occurs in the spiny mountain crayfish (genus *Euastacus*) that inhabits montane mesic forest streams on the mountain-tops of eastern Australia. A molecular phylogeny shows

that within the wet tropics region there is a deep divergence into two clades. The more southern clade shows higher affinity to congeneric species distributed southward into Victoria than to the more northern, but geographically closer, neighbour group (Ponniah and Hughes, 2004; Shull et al., 2005), just as NQb shows higher affinity to more southerly located species than to NQa. A similar result was found in a molecular phylogenetic analysis of flightless, forest beetles of the genus *Pamborus* (Sota et al., 2005). One sister group, present in the wet tropics region, is estimated to have diverged from the remaining lineage in the Oligocene. Other groups were estimated to have diverged in the late Miocene to Pliocene (Sota et al., 2005). It appears that low-vagility montane or rainforest invertebrates of the wet tropics show signs of ancient speciation events that do not have parallels in the sub-tropical to temperate rainforest regions of Australia.

4.1.3. A. flava and A. girraweenensis

The St. Lawrence Gap, a dry corridor that has seen independent evolution of rainforest floristic regions north and south of the gap (Webb and Tracey, 1981), separates *A. tropica* from more southerly species. All analyses place *A. flava* and *A. girraweenensis* as sister species, but with an *A. tropica* clade interspersed in some analyses (see above). They are geographically adjacent: the disjunction between them approximately corresponds to the location of the McPherson ranges near the border between the states of Queensland and New South Wales (Fig. 1), recognised as a biogeographic barrier (Parsons and Bock, 1981; James and Moritz, 2000; Schauble and Moritz, 2001). The ranges are a spur of the Great Dividing Range that heads easterly toward the Pacific coastline. Given the consistent but patchy distribution of rainforest along the Great Dividing and McPherson ranges, the two species must come into close geographic contact, however fine-scale sampling is required to define the demarcation. Populations found in two granite boulder caves at Girraween National Park (GI1 and 2), inland from the caldera on a plateau at an altitude of 900 m, were identified as members of the species *A. girraweenensis* by Baker (accepted for publication). The molecular evidence presented here supports the closer affinity of the Girraween populations with northern New South Wales glow-worms than with *A. flava*.

4.1.4. A. tasmaniensis and A. buffaloensis

The phylogeny supports the current designation (Ferguson, 1925) of the Tasmanian populations as a discrete species (*A. tasmaniensis*) as they are divergent from the mainland Australian populations. Its closest relative is the Mount Buffalo species, *A. buffaloensis*. The sister-group status of the Tasmanian and Mount Buffalo species and their inclusion in a new subgenus, Lucifera (Baker, accepted for publication), is a departure from the pattern of geographically structured species seen in subgenus Campara. Species in Campara are distributed allopatrically in a more or less continuous swathe from the north-east of Australia to southern Victoria. In contrast, *A. tasmaniensis* and *A. buffaloensis* are geographically separated. There are significant present-day geographic barriers between Tasmania and Mount Buffalo. First, Bass Strait is an ocean expanse that has repeatedly receded to form a land-bridge between Tasmania and mainland Australia, most recently during the Pleistocene. Second, mountainous, deeply divided terrain with multiple vegetation types separates Mt Buffalo from the Victorian coast. One possibility is that a cool-adapted ancestor was once more widely distributed in southern Australia and that warming and/or geological events isolated *A. buffaloensis* in the Alps and restricted *A. tasmaniensis* to Tasmania. Alternatively, dispersal across the interconnecting Bassian land-bridge could have occurred during the Pleistocene. The distribution of the eastern Victorian clade of subgenus Campara cuts directly across a track drawn between the Tasmanian and Mt Buffalo distributions. At its closest, the eastern Victorian group is located only 130–170 km from Mt Buffalo.

4.2. Cave vs rainforest populations

In this and other studies it was noted that cave populations show markedly reduced pigmentation, tend to make much longer snares and produce larger mature larvae and adults than rainforest populations (Richards, 1960; Baker, accepted for publication). Given these morphological differences we need to consider whether cave populations belong to the same species as adjacent rainforest populations and whether the morphological differences are polymorphisms or polyphenisms. Polymorphisms are due to genetic differences among individuals, are independent of environment, and are heritable, whereas polyphenisms develop in response to internal or external environmental conditions and individuals are capable of expressing alternative phenotypes (Nijhout, 1994). An example of genetic differentiation in cave populations is the selection for troglomorphic traits that has occurred in the freshwater amphipod *Gammarus minus*. Populations have independently invaded subsurface basins in northern America (Culver and Wilkens, 2000) and evolved the heritable trait of reduced eye size (Fong, 1989). In contrast, the available evidence suggests that epigean/hypogean differences in *Arachnocampa* species are polyphenic traits. First, when *A. flava* was reared through multiple generations in an artificial cave initially seeded with rainforest individuals, the cave individuals achieved a larger body size over 2 years of rearing, too short a time for genetic differentiation to occur (Baker, accepted for publication). Second, some artificial environments such as abandoned mine adits and railway tunnels that have been relatively recently inhabited contain larvae that show typical cave morphology and pigmentation, in marked contrast to nearby epigean larvae (Baker, accepted for publication). In addition, at Mystery Creek cave in southern Tasmania, larvae found directly outside the cave mouth show the characteristic pigmentation of epigean larvae, in marked contrast to the hypogean traits of larvae within the dark zone of the cave (Merritt, personal observations). One definitive test for this hypothesis would be to take eggs from one environment and rear the resulting larvae in the other.

The mitochondrial DNA sequence data presented here can address the question of whether there is a widespread cave species or species group in *Arachnocampa*. If so, the phylogenetic trees would show clustering of cave populations, despite their geographic location. In fact they show the opposite: samples of glow-worms from adjacent geographic locations tend to cluster together, forming clades, no matter what habitat they came from. It must be borne in mind that alternative methods are better suited to addressing the question of localized speciation: microsatellite data or nuclear gene sequence would be more informative. Therefore, it remains possible that species have emerged repeatedly in caves, however the putative speciation events do not produce obvious morphological changes in adults: cave-reared adults show similar morphological traits (e.g. wing venation, tarsus-to-tibia length ratios numbers and distributions of setae) to surface-reared specimens from the same region (Baker, accepted for publication).

It appears most likely that the morphological differences between cave and epigean glow-worm larvae are facultative adaptations to local microclimatic conditions, rather than a sign of deeper genetic differentiation. Repeated gene flow between cave and epigean populations in the history of the genus may explain why troglomorphic traits such as loss of pigmentation and reduction of eyes, commonly seen in cave organisms (Culver and Wilkens, 2000), have not evolved in glow-worms: rather, they are capable of responding morphologically and behaviourally to both habitats. Holsinger (2000) has pointed out that troglophiles should not be

assumed to be intermediate steps in the evolution of troglobites, rather, many troglophiles appear to be well-adapted to cave life without necessarily evolving troglomorphisms. In a study of troglophilic and troglobitic cave arthropods, Caccone (1985) found high levels of gene flow in troglophilic and epigean species whereas, as expected, it was restricted in troglobites. In contrast, mtDNA analysis of Appalachian cave spiders (genus *Nesticus*) showed complete subdivision of populations regardless of whether the species examined is troglobitic, troglophilic or epigean (Hedin, 1997). It is likely that specific ecological, physiological and behavioural characteristics of the species under consideration dictate the level of population structuring that will occur in a cave-restricted population (Caccone, 1985; Holsinger, 2000). Further work is required to determine the level gene flow between hypogean and epigean populations of *Arachnocampa*.

We tentatively conclude that members of the genus *Arachnocampa* have not evolved specific adaptations to caves that would compromise adaptations to epigean ecosystems, and *vice versa*. The single most distinctive trait of glow-worms—the use of bioluminescence to attract prey—appears to be an efficient adaptation allowing them to thrive in both cave and epigean environments.

Acknowledgments

The authors thank Corinna Lange for providing technical assistance in this project. Dr. Stephen Cameron (CSIRO Entomology) assisted with phylogenetic analyses of the mtDNA. The main body of work was funded by the Cooperative Research Centre for Sustainable Tourism and glow-worm tour operators at Natural Bridge, Queensland. Additional support came from Australian Geographic, Xenogen Corporation Pty. Ltd., and Queensland National Parks and Wildlife service. The authors thank the Parks and Wildlife services of the Australian states for providing permission to collect, the Department of Conservation, New Zealand, caving club members and the many individuals who provided information about glow-worm distributions or helped in collecting. They include David Chitty, Arthur Clarke, Neil Collinson, Michael Driessen, Lee Etherington, Bill Goebels, Harry Hines, Deb Hunter, Bevan Jenkins and David Smith.

Appendix A

Regional code, collection site and GenBank accession numbers for animals used in this study. Asterisk indicates sequence available on request. NQ: north Queensland. CQ: central Queensland. GI: Girraween. CA: caldera region. NN: northern New South Wales. SN: southern New South Wales. MB: Mount Buffalo. WV: western Victoria. EV: eastern Victoria. TA: Tasmania. NZ: New Zealand. SP: State Park. NP: National Park. Order corresponds roughly with north to south distribution of *Arachnocampa* spp within Australia, followed by New Zealand. The last two sites are locations of collection of the outgroup species, *Orfelia fultoni*. NA: not sequenced due to parasitism or amplification failures. Species identifications are from Baker (2004, accepted for publication).

Code	Location	Species	Site type	GenBank Accession	
				16S	COII
NQ1	Mossman Gorge	*A. tropica*	Rainforest	*	AY575683
NQ2	Mt Lewis Rd	*A. tropica*	Rainforest	AY576332	AY575684
NQ3	Lamb Range National Park	not identified	Rainforest	AY576333	AY575685
NQ4	Dinner Falls, Mt Hypipamee NP	*A. tropica*	Rainforest	AY576334	AY575686
NQ5	Bartle Frere Cave, Wooroonooran NP	*A. tropica*	Granite boulder cave	NA	AY575687
NQ6	Bartle Frere stream	*A. tropica*	Rainforest	AY576335	AY575688
NQ7	Birthday Creek Falls, Paluma NP	*A. tropica*	Rainforest	NA	AY575689
CQ1	Kroombit Tops	not identified	Rainforest	*	*
CA1	Natural Bridge	*A. flava*	Rainforest	AY576349	AY575703
CA2	Mt Warning NP	*A. flava*	Rainforest	AY576350	AY575704
CA3	Nightcap NP	*A. flava*	Rainforest	AY576351	AY575705
GI1	South Bald Rock, Girraween NP	*A. girraweenensis*	Granite boulder cave	AY576352	AY575706
GI2	Ramsay Creek Cave, Girraween NP	*A. girraweenensis*	Granite boulder cave	AY576353	AY575707
NN1	Washpool NP	*A. girraweenensis*	Rainforest	AY576354	AY575708
NN2	Cleavers Bridge, New England NP	*A. girraweenensis*	Rainforest	AY576355	AY575709
NN3	Crystal Shower Falls, Dorrigo NP	*A. girraweenensis*	Rainforest	*	*
SN1	Newnes Railway tunnel	*A. richardsae*	Railway tunnel	AY576336	AY575690
SN2	Waterfall Springs Cons. Park	*A. richardsae*	Rainforest	AY576338	AY575691
SN3	Fitzroy Falls NP	*A. richardsae*	Rainforest	AY576339	AY575693
SN4	Grand Canyon walk, Blue Mtns NP	*A. richardsae*	Rainforest	AY576337	AY575692
MB1	Underground River Cave, Mt Buffalo NP	*A. buffaloensis*	Granite boulder cave	AY576326	AY575711
WV1	Melba Gully State Park	*A. otwayensis*	Rainforest	AY576348	AY575701
WV2	Grey River picnic area, Angahook-Lorne SP	*A. otwayensis*	Rainforest	AY576347	AY575702
EV1	Upper Yarra Valley mine tunnel	*A. gippslandensis*	Mine adit	AY576340	AY575694
EV2	O'Shannassy Weir	*A. gippslandensis*	Weir tunnel	AY576341	AY575695
EV3	Britannia Creek Cave, State Forest	*A. gippslandensis*	Granite boulder cave	AY576342	AY575696
EV4	Shining Star gold mine, Warburton	*A. gippslandensis*	Mine adit	AY576343	AY575697
EV5	Shiprock Falls, Kilnkurth State Forest	*A. gippslandensis*	Granite boulder cave	AY576344	AY575698
EV6	Labertouche Cave	*A. gippslandensis*	Granite boulder cave	AY576345	AY575699
EV7	Walhalla Mine tunnel	*A. gippslandensis*	Mine adits	AY576346	AY575700
TA1	Marakoopa Cave	*A. tasmaniensis*	Limestone cave	AY576357	AY575712

Appendix A (*continued*)

Code	Location	Species	Site type	GenBank Accession	
				16S	COII
TA2	Sassafras Cave	*A. tasmaniensis*	Limestone cave	AY576358	*
TA3	Derby Mine tunnel	*A. tasmaniensis*	Mine adit	AY576359	*
TA4	Bates Creek Gully, Dover	*A. tasmaniensis*	Rainforest	AY576360	AY575713
TA5	Mystery Creek Cave, Ida Bay	*A. tasmaniensis*	Limestone cave	AY576361	NA
NZ1	Waitomo Cave	*A. luminosa*	Limestone cave	AY576329	*
NZ2	Auckland Waterworks Tramway	*A. luminosa*	Railway tunnel	AY576330	AY575681
NZ3	Te Anau	*A. luminosa*	Limestone cave	AY576331	AY575682
US1	Natural Bridge, Alabama, USA	*Orfelia fultoni*	Deciduous forest	AY576327	AY575679
US2	Dismals Canyon, Alabama, USA	*Orfelia fultoni*	Deciduous forest	AY576328	AY575680

References

Baker, C.H., 2004. Australian Glow-worms (Diptera: Keroplatidae: *Arachnocampa* spp.): distribution, Diversity, Identity and Management. PhD thesis, The University of Queensland.

Baker, C.H., accepted for publication. A new subgenus and five new species of Australian glow-worm (Diptera: Keroplatidae: *Arachnocampa* spp.). Mem. Queensl. Mus.

Baker, C.H., Merritt, D.J., 2003. Life cycle of an Australian glow-worm *Arachnocampa flava* Harrison (Diptera: Keroplatidae: Arachnocampinae). Aust. Entomol. 30, 45–55.

Bell, K.L., Yeates, D.K., Moritz, C., Monteith, G.B., 2003. Molecular phylogeny and biogeography of the dung beetle genus *Temnoplectron* Westwood (Scarabaediae: Scarabaeinae) from Austalia's wet tropics. Mol. Phylogenet. Evol. 41, 741–753.

Bouchard, P., Brooks, D.R., Yeates, D.K., 2005. Mosaic macroevolution in Australian wet tropics arthropods: community assemblage by taxon pulses. In: Bermingham, E., Dick, C.W., Moritz, C. (Eds.), Tropical Rainforests: Past, Present and Future. Chicago University Press, Chicago, pp. 425–469.

Broadley, R.A., 1998. Behavioural ecology and prey attraction of the New Zealand glowworm *Arachnocampa luminosa* (Skuse) (Diptera: Mycetophilidae) in bush and cave habitats. MSc thesis, Massey University.

Broadley, R.A., Stringer, I.A.N., 2001. Prey attraction by larvae of the New Zealand glowworm, *Arachnocampa luminosa* (Diptera: Mycetophilidae). Invertebr. Biol. 120, 170–177.

Brower, A.V.Z., 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. Proc. Natl. Acad. Sci. USA 91, 6491–6495.

Caccone, A., 1985. Gene flow in cave arthropods—a qualitative and quantitative approach. Evolution 39, 1223–1235.

Cameron, S.L., Lambkin, C.L., Barker, S.C., Whiting, M.F., 2007. Utility of mitochondrial genomes as phylogenetic markers for insect intraordinal relationships—a case study from flies (Diptera). Syst. Entomol. 32, 40–59.

Culver, D.C., Wilkens, H., 2000. Critical review of the relevant theories of the evolution of subterranean animals. In: Wilkens, H., Culver, D., Humphreys, W. (Eds.), Ecosystems of the World. Subterranean Ecosystems. Elsevier, Amsterdam, pp. 381–398.

Currey, J.E.B., 1966. Reflections on the Colony of New South Wales. Lansdowne Press, Melbourne.

Department of Conservation and Natural Resources, 1994. Melba Gully State Park Draft Management Plan, National Parks Service—South West Area, Melbourne.

Eberhard, S., Spate, A., 1995. Cave invertebrate survey: towards an atlas of NSW cave fauna. New South Wales Heritage Assistance Program NEP 94 765. National Parks and Wildlife Service, Sydney.

Ferguson, E.W., 1925. Description of a new species of Mycetophilidae (Diptera) with luminous larvae. Proc. Linn. Soc. NSW 50, 487–488.

Finlayson, B., 1982. Granite caves in Girraween National Park, southeast Queensland. Helictite 20, 53–59.

Fong, D.W., 1989. Morphological evolution of the amphipod *Gammarus minus* in caves: quantitative genetic analysis. Am. Midl. Nat. 121, 361–378.

Fulton, B.B., 1941. A luminous fly larva with spider traits. Ann. Ent. Soc. Am. 34, 289–302.

Garrick, R.C., Sands, C.J., Rowell, D.M., Tait, N.N., Greenslade, P., Sunnucks, P., 2004. Phylogeography recapitulates topography: very fine-scale local endemism of a saproxylic 'giant' springtail at Tallaganda in the Great Dividing Range of south-east Australia. Mol. Ecol. 13, 3329–3344.

Goede, J.B., 1967. Tasmanian cave fauna: character and distribution. Helictite 5, 71–86.

Harvey, M.S., 2002. Short-range endemism among the Australian fauna: some examples from non-marine environments. Invertebr. Syst. 16, 555–566.

Harrison, R.A., 1966. Australian glow-worms of the genus *Arachnocampa* Edwards. Pac. Ins. 8, 877–883.

Hedin, M.C., 1997. Molecular phylogenetics at the population/species interface in cave spiders of the southern Appalachians (Araneae: Nesticidae: *Nesticus*). Mol. Biol. Evol. 14, 309–324.

Hill, R.S., 2004. Origins of the southeastern Australian vegetation. Phil. Trans. R. Soc. Lond. B Biol. Sci. 359, 1537–1549.

Holsinger, J.R., 2000. Ecological derivation, colonization, and speciation. In: Wilkens, H., Culver, D., Humphreys, W. (Eds.), Ecosystems of the World. Subterranean Ecosystems. Elsevier, Amsterdam, pp. 399–415.

Howarth, F.G., 1983. Ecology of cave arthropods. Annu. Rev. Ent. 28, 365–389.

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.

James, C.H., Moritz, C., 2000. Intraspecific phylogeography in the sedge frog *Litoria fallax* (Hylidae) indicates pre-Pleistocene vicariance of an open forest species from eastern Australia. Mol. Ecol. 9, 349–358.

Joseph, L., Moritz, C., Hugall, A., 1995. Molecular support for vicariance as a source of diversity in rainforest. Proc. R. Soc. Lond. B Biol. Sci. 260, 177–182.

Kershaw, A.P., 1994. Pleistocene vegetation of the humid tropics of northeastern Queensland, Australia. Palaeogeogr. Palaeoclimatol. Palaeoecol. 109, 399–412.

Kikkawa, J., Monteith, G., Ingram, G., 1981. Cape York Peninsula: major region of faunal interchange. In: Keast, A. (Ed.), Ecological Biogeography of Australia. Dr. W. Junk, The Hague, pp. 1695–1742.

Lange, C.L., Scott, K.D., Graham, G.C., Sallam, M.N., Allsopp, P.G., 2004. Stem borer (Lepidoptera: Noctuidae and Pyralidae) phylogenetics constructed using COII and 16S mitochondrial gene sequence. Bull. Ent. Res. 94, 457–464.

Liu, H., Beckenbach, A.T., 1992. Evolution of the mitochondrial cytochrome oxidase II gene among ten orders of insects. Mol. Phylogenet. Evol. 1, 41–52.

Matile, L., 1981. Description d'un Keroplatidae du Cretace moyen et donnees morphologiques et taxinomiques sur les Mycetophiloidea (Diptera). Ann. Soc. Entomol. Fr. 17, 99–123.

McKeown, K.C., 1935. Insect Wonders of Australia. Angus and Robertson, Sydney.

Morley, R.J., 2000. Origin and Evolution of Tropical Rain Forests. John Wiley and Sons, Chichester.

Myers, E., Kececioglu, J., 1992. SeqEd, version 1.03. Applied Biosystems Inc.

Nijhout, H.F., 1994. Insect Hormones. Princeton University Press, Princeton.

Parsons, P.A., Bock, I.R., 1981. Australian *Drosophila*: diversity, resource utilization and radiations. In: Keast, A. (Ed.), Ecological Biogeography of Australia. Dr. W. Junk, The Hague, pp. 1037–1054.

Perkins, F.A., 1935. "Glow-worms" and other insects collected at Numinbah, Nerang Valley, Easter Camp, Queensland Naturalists' Club, 1935. Qld. Nat. 9, 84–85.

Ponniah, M., Hughes, J.M., 2004. The evolution of Queensland spiny mountain crayfish of the genus Euastacus. I. Testing vicariance and dispersal with interspecific mitochondrial DNA. Evolution 58, 1073–1085.

Posada, D., Crandall, K., 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Richards, A.M., 1960. Observations on the New Zealand glow-worm *Arachnocampa luminosa* (Skuse) 1890. Trans. R. Soc. New Zeal. 88, 559–574.

Richards, A.M., 1963. Glow-worms. Aust. Nat. Hist. 2, 222–224.

Schauble, C.S., Moritz, C., 2001. Comparative phylogeography of two open forest frogs from eastern Australia. Biol. J. Linn. Soc. 74, 157–170.

Schneider, C.J., Cunningham, M., Moritz, C., 1998. Comparative phylogeography and the history of endemic vertebrates in the Wet Tropics rainforests of Australia. Mol. Ecol. 7, 487–498.

Shull, H.C., Perez-Losada, M., Blair, D., Sewell, K., Sinclair, E.A., Lawler, S., Ponniah, M., Crandall, K.A., 2005. Phylogeny and biogeography of the freshwater crayfish *Euastacus* (Decapoda: Parastacidae) based on nuclear and mitochondrial DNA. Mol. Phylogenet. Evol. 37, 249–263.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., Flook, P., 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Ent. Soc. Am. 87, 651–701.

Sota, T., Takami, Y., Monteith, G.B., Moore, B.P., 2005. Phylogeny and character evolution of endemic Australian carabid beetles of the genus *Pamborus* based on mitochondrial and nuclear gene sequences. Mol. Phylogenet. Evol. 36, 391–404.

Swofford, D., 2002. PAUP*: Phylogenetic Analysis Using Parsimony (and other methods) 4.0b10. Sinauer Associates, Sunderland, MA.

Stringer, I.A.N., 1967. The larval behaviour of the New Zealand glow-worm *Arachnocampa luminosa*. Tane 13, 107–117.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The CLUSTALX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25, 4876–4882.

Viviani, V.R., Hastings, J.W., Wilson, T., 2002. Two bioluminescent diptera: the North American *Orfelia fultoni* and the Australian *Arachnocampa flava*. Similar niche, different bioluminescence systems. Photochem. Photobiol. 75, 22–27.

Webb, L.J., Tracey, J.G., 1981. Australian rainforests: patterns and change. In: Keast, A. (Ed.), Ecological Biogeography of Australia. Dr. W. Junk, The Hague, pp. 605–694.

Wheeler, W., Williams, F., 1915. The luminous organ of the New Zealand glow-worm. Psyche 22, 36–43.

Yeates, D.K., Bouchard, P., Monteith, G.B., 2002. Patterns and levels of endemism in the Australian Wet Tropics rainforest: evidence from flightless insects. Invertebr. Syst. 16, 605–619.

Yeates, D.K., Monteith, G.B.M., 2008. The invertebrate fauna of the Wet Tropics: diversity, endemism and relationships. In: Stork, N., Turton, S. (Eds.), Living in a Dynamic Tropical Forest Landscape. Blackwell Publishing, Oxford, pp. 178–191.