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

A R T I C L E   I N F O

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

A B S T R A C T

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. buffalensis, 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. buffalensis, 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...
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 troglobites 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 in 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 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 MgCl₂, 0.5 mM dNTP’s, 0.2 μM each primer MtD16 and MtD20 (Liu and Beckenbach, 1992; Simon et al., 1994) (MtD16 5’atcagactaataagatggagtaa3’, MtD20 5’tgtaagacgcatctgtg’), 20 ng DNA, 1 U Taq polymerase (Qiagen, Clifton Hill, Victoria, Australia), 16.25 μl ROH2O. 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, 3 min) × 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 MgCl₂, 0.2 mM dNTP’s, primers: 0.2 μM each primer 16SF, 16SR (Lange et al., 2004) (16SF 5’AGATTTTAAATATGCAAAAG, 16SR 5’TGACTGTACAA AGGTACATA), 20 ng DNA, 1 U Taq polymerase (Qiagen, Clifton Hill), 15.2 μl ROH2O. Thermal cycling used the following conditions: (94°C, 2 min; 54°C, 1 min; 72°C, 15 min) × 1, (92°C, 45 s;
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 SEQ96 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 Kecicioglu, 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 mineshfts 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, caves 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. buffalensis and A. tasmaniensis as sister species, and separated them from all other Australian mainland
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 *Camara* 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. gippสlansdensis 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 (G1 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 COI 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 sympathy 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 finescale 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 Pamborini (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. richardsiae, A. otwayensis and A. gippสlansdensis

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 con-
generic species distributed southward into Victoria than to the
more northern, but geographically closer, neighbour group (Ponn-
iah and Hughes, 2004; Shull et al., 2005), just as NQb shows higher
affinity to more southerly located species than to NQA. A similar re-
sult 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 di-
verted 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 rainfor-
est invertebrates of the wet tropics show signs of ancient specia-

dation 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 be-
tween them approximately corresponds to the location of the
McPherson ranges near the border between the states of Queens-
land and New South Wales (Fig. 1), recognised as a biogeographic
barrier (Parsons and Bock, 1981; James and Moritz, 2000; Schaub
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 boul-
der caves at Girraween National Park (GII 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. tas-
maniensis) as they are divergent from the mainland Australian pop-
ulations. Its closest relative is the Mount Buffalo species, A. buffa-
loensis. The sister-group status of the Tasmanian and Mount
Buffalo species and their inclusion in a new subgenus, Luciferia (Ba-
er, accepted for publication), is a departure from the pattern of
geographically structured species seen in subgenus Campara. Spe-
cies in Campara are distributed allopatrically in a more or less con-

tinuous swathe from the north-east of Australia to southern
Victoria. In contrast, A. tasmaniensis and A. buffaloesis are geo-
graphically 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 ter-
rain 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. buffaloesis in the
Alps and restricted A. tasmaniensis to Tasmania. Alternatively, dis-
peral 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
snakes and produce larger mature larvae and adults than rainforest
populations (Richards, 1960; Baker, accepted for publication). Gi-
ven 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 poly-
morphisms 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
capability of expressing alternative phenotypes (Nijhout, 1994).
An example of genetic differentiation in cave populations is the selec-
tion for troglomorphic traits that has occurred in the freshwater
amphipod Gammarus minus. Populations have independently in-
vaded 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/
hygopian 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, ac-
cepted for publication). Second, some artificial environments such
as abandoned mine adits and railway tunnels that have been rela-
tively recently inhabited contain larvae that show typical cave
morphology and pigmentation, in marked contrast to nearby epi-
gean larvae (Baker, accepted for publication). In addition, at Mys-
tery 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 ad-
dress 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 geo-

draphic 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 sui-
ted to addressing the question of localized speciation: microsatel-
lite data or nuclear gene sequence would be more informative.
Therefore, it remains possible that species have emerged repeat-
edly in caves, however the putative speciation events do not pro-
duce obvious morphological changes in adults: cave-reared adults
show similar morphological traits (e.g. wing venation, tarsus-to-ti-
bia 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 be-
tween cave and epigean glow-worm larvae are facultative adapta-
tions to local microclimatic conditions, rather than a sign of deeper
genetic differentiation. Repeated gene flow between cave and epi-
gean populations in the history of the genus may explain why trog-
lomorphic 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 troglobites appear to be well-adapted to cave life without necessarily evolving troglomorphisms. In a study of troglobiphilic and troglobitic cave arthropods, Caccone (1985) found high levels of gene flow in troglobiphilic 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 troglobiotic, troglobiphilic 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 Gobels, Harry Hines, Deb Hunter, Bevan Jenkins and David Smith.

**Appendix A**


<table>
<thead>
<tr>
<th>Code</th>
<th>Location</th>
<th>Species</th>
<th>Site type</th>
<th>GenBank Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>NQ1</td>
<td>Mossman Gorge</td>
<td>A. tropica</td>
<td>Rainforest</td>
<td>*</td>
</tr>
<tr>
<td>NQ2</td>
<td>Mt Lewis Rd</td>
<td>A. tropica</td>
<td>Rainforest</td>
<td>AY576332</td>
</tr>
<tr>
<td>NQ3</td>
<td>Lamb Range National Park</td>
<td>not identified</td>
<td>Rainforest</td>
<td>AY576333</td>
</tr>
<tr>
<td>NQ4</td>
<td>Dinner Falls, Mt Hypipamee NP</td>
<td>A. tropica</td>
<td>Rainforest</td>
<td>AY576334</td>
</tr>
<tr>
<td>NQ5</td>
<td>Bartle Frere Cave, Woorooolooran NP</td>
<td>A. tropica</td>
<td>Granite boulder cave</td>
<td>NA</td>
</tr>
<tr>
<td>NQ6</td>
<td>Bartle Frere stream</td>
<td>A. tropica</td>
<td>Rainforest</td>
<td>AY576335</td>
</tr>
<tr>
<td>NQ7</td>
<td>Birthday Creek Falls, Paluma NP</td>
<td>A. tropica</td>
<td>Rainforest</td>
<td>NA</td>
</tr>
<tr>
<td>CQ1</td>
<td>Krombit Tops</td>
<td>not identified</td>
<td>Rainforest</td>
<td></td>
</tr>
<tr>
<td>CA1</td>
<td>Natural Bridge</td>
<td>A. flavo</td>
<td>Rainforest</td>
<td>AY576349</td>
</tr>
<tr>
<td>CA2</td>
<td>Mt Warning NP</td>
<td>A. flavo</td>
<td>Rainforest</td>
<td>AY576350</td>
</tr>
<tr>
<td>CA3</td>
<td>Nightcap NP</td>
<td>A. flavo</td>
<td>Rainforest</td>
<td>AY576351</td>
</tr>
<tr>
<td>GI1</td>
<td>South Bald Rock, Girraween NP</td>
<td>A. grraweenensis</td>
<td>Granite boulder cave</td>
<td>AY576352</td>
</tr>
<tr>
<td>GI2</td>
<td>Ramsay Creek Cave, Girraween NP</td>
<td>A. grraweenensis</td>
<td>Granite boulder cave</td>
<td>AY576353</td>
</tr>
<tr>
<td>NN1</td>
<td>Washpool NP</td>
<td>A. grraweenensis</td>
<td>Rainforest</td>
<td>AY576354</td>
</tr>
<tr>
<td>NN2</td>
<td>Cleavers Bridge, New England NP</td>
<td>A. grraweenensis</td>
<td>Rainforest</td>
<td>AY576355</td>
</tr>
<tr>
<td>NN3</td>
<td>Crystal Shower Falls, Dorrigo NP</td>
<td>A. grraweenensis</td>
<td>Rainforest</td>
<td></td>
</tr>
<tr>
<td>SN1</td>
<td>Newnes Railway tunnel</td>
<td>A. richardsae</td>
<td>Railway tunnel</td>
<td>AY576336</td>
</tr>
<tr>
<td>SN2</td>
<td>Waterfall Springs Cons. Park</td>
<td>A. richardsae</td>
<td>Rainforest</td>
<td>AY576338</td>
</tr>
<tr>
<td>SN3</td>
<td>Fitzroy Falls NP</td>
<td>A. richardsae</td>
<td>Rainforest</td>
<td>AY576339</td>
</tr>
<tr>
<td>SN4</td>
<td>Grand Canyon walk, Blue Mtns NP</td>
<td>A. richardsae</td>
<td>Rainforest</td>
<td>AY576337</td>
</tr>
<tr>
<td>MB1</td>
<td>Underground River Cave, Mt Buffalo NP</td>
<td>A. buffalonensis</td>
<td>Granite boulder cave</td>
<td>AY576326</td>
</tr>
<tr>
<td>WV1</td>
<td>Melba Gully State Park</td>
<td>A. otwayensis</td>
<td>Rainforest</td>
<td>AY576348</td>
</tr>
<tr>
<td>WV2</td>
<td>Grey River picnic area, Angahook-Lorne SP</td>
<td>A. otwayensis</td>
<td>Rainforest</td>
<td>AY576347</td>
</tr>
<tr>
<td>EV1</td>
<td>Upper Yarra Valley mine tunnel</td>
<td>A. gippslandensis</td>
<td>Mine adit</td>
<td>AY576340</td>
</tr>
<tr>
<td>EV2</td>
<td>O'Shanassy Weir</td>
<td>A. gippslandensis</td>
<td>Weir tunnel</td>
<td>AY576341</td>
</tr>
<tr>
<td>EV3</td>
<td>Britannia Creek Cave, State Forest</td>
<td>A. gippslandensis</td>
<td>Granite boulder cave</td>
<td>AY576342</td>
</tr>
<tr>
<td>EV4</td>
<td>Shining Star gold mine, Warburton</td>
<td>A. gippslandensis</td>
<td>Mine adit</td>
<td>AY576343</td>
</tr>
<tr>
<td>EV5</td>
<td>Shiprock Falls, Klinkurst State Forest</td>
<td>A. gippslandensis</td>
<td>Granite boulder cave</td>
<td>AY576344</td>
</tr>
<tr>
<td>EV6</td>
<td>Labertouche Cave</td>
<td>A. gippslandensis</td>
<td>Granite boulder cave</td>
<td>AY576345</td>
</tr>
<tr>
<td>EV7</td>
<td>Walhalla Mine tunnel</td>
<td>A. gippslandensis</td>
<td>Mine adits</td>
<td>AY576346</td>
</tr>
<tr>
<td>TA1</td>
<td>Marakoopa Cave</td>
<td>A. tasmaniensis</td>
<td>Limestone cave</td>
<td>AY576357</td>
</tr>
</tbody>
</table>
Appendix A (continued)

<table>
<thead>
<tr>
<th>Code</th>
<th>Location</th>
<th>Species</th>
<th>Site type</th>
<th>GenBank Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>TA2</td>
<td>Sassafras Cave</td>
<td>A. tasmaniensis</td>
<td>Limestone cave</td>
<td>AY576358</td>
</tr>
<tr>
<td>TA3</td>
<td>Derby Mine tunnel</td>
<td>A. tasmaniensis</td>
<td>Mine adit</td>
<td>AY576359</td>
</tr>
<tr>
<td>TA4</td>
<td>Bates Creek Gully, Dover</td>
<td>A. tasmaniensis</td>
<td>Rainforest</td>
<td>AY576360</td>
</tr>
<tr>
<td>TA5</td>
<td>Mystery Creek Cave, Ida Bay</td>
<td>A. tasmaniensis</td>
<td>Limestone cave</td>
<td>AY576361</td>
</tr>
<tr>
<td>NZ1</td>
<td>Waitomo Cave</td>
<td>A. luminosa</td>
<td>Limestone cave</td>
<td>AY576329</td>
</tr>
<tr>
<td>NZ2</td>
<td>Auckland Waterworks Tramway</td>
<td>A. luminosa</td>
<td>Railway tunnel</td>
<td>AY576330</td>
</tr>
<tr>
<td>NZ3</td>
<td>Te Anau</td>
<td>A. luminosa</td>
<td>Limestone cave</td>
<td>AY576331</td>
</tr>
<tr>
<td>US1</td>
<td>Natural Bridge, Alabama, USA</td>
<td>Orfila fultoni</td>
<td>Deciduous forest</td>
<td>AY576327</td>
</tr>
<tr>
<td>US2</td>
<td>Dismals Canyon, Alabama, USA</td>
<td>Orfila fultoni</td>
<td>Deciduous forest</td>
<td>AY576328</td>
</tr>
</tbody>
</table>


