

Phylogeny and distribution of the mayfly genus *Austrophlebioides*

Campbell & Suter (Ephemeroptera: Leptophlebiidae)

Faye Christidis^{A,C} and John C. Dean^B

^ASchool of Marine and Tropical Biology, James Cook University, Townsville, Queensland 4811, Australia.

^BEnvironment Protection Authority, Freshwater Sciences Unit, Ernest Jones Drive, Macleod, Victoria 3085, Australia.

^CCorresponding author. Email: faye.christidis@jcu.edu.au

Abstract. The mayfly genus *Austrophlebioides* Campbell & Suter, 1988 is endemic to Australia and is widely distributed in eastern Australia and Tasmania. Here, the phylogenetic relationships among species of *Austrophlebioides* are investigated using cladistic analyses based on morphological characters of the nymph and adult, and the first phylogenetic hypothesis for the genus is presented. The results from the phylogenetic analyses support the recognition of three monophyletic species-groups: the ‘*rieki*’, ‘*pusillus*’ and ‘*marchanti*’ clades. The ‘*pusillus*’ clade is the sister-group to the ‘*rieki*’ clade, and the clade comprising these two groups is sister to the ‘*marchanti*’ clade. Minimal overlap was observed in the geographic distribution of the three *Austrophlebioides* clades. The ‘*rieki*’ clade is confined to the Wet Tropics bioregion of north-eastern Queensland. The ‘*pusillus*’ clade is distributed from central-eastern Queensland to Victoria. The ‘*marchanti*’ clade occurs in southern New South Wales, Victoria and Tasmania. Distributional limits of the three clades correspond with the presence of recognised biogeographic barriers (Burdekin Gap, Hunter Valley and Bass Strait) suggesting that vicariance has been important in the differentiation of the group and in determining present-day distributions of species.

Introduction

The mayfly genus *Austrophlebioides* was established by Campbell and Suter (1988) for two Australian species, *A. pusillus* (Harker) and *A. unguicularis* (Ulmer), previously assigned to the genus *Deleatidium* Eaton. Two additional species, *A. marchanti* and *A. booloumbi*, were described by Parnrong and Campbell (1997), and the species *decipiens* Harker was transferred from *Deleatidium* to *Austrophlebioides*. A further three species: *A. rieki*, *A. wooroonooran* and *A. porphyrobranchus*, were described by Christidis and Dean (2005) taking the total number of species formally placed in the genus to eight. Several undescribed species believed to belong to *Austrophlebioides* have also been recognised by Dean (1999), but these are known only from the nymphal stage. Although knowledge of the alpha taxonomy of *Austrophlebioides* has increased in recent years (Parnrong and Campbell 1997; Dean 1999; Christidis and Dean 2005), there have been no phylogenetic studies on the genus and evolutionary relationships among species remain poorly understood.

The genus *Austrophlebioides* is endemic to Australia and is widely distributed in eastern Australia and Tasmania. *Austrophlebioides* is also believed to occur in northern Australia (Dean 1999). The majority of *Austrophlebioides* species appear to have restricted geographic distributions. The nymphs are aquatic and are typically found on stones in cool forest streams. The adults are aerial/terrestrial and live for only a few days. Until recently, the paucity of distributional data for many *Austrophlebioides* species and limited knowledge of the fauna of several regions, including north-eastern Queensland, has hindered the study of biogeographic patterns within the genus.

The main aim of this study was to investigate phylogenetic relationships among species of *Austrophlebioides* using

morphological characters of the nymph and adult, and to propose the first phylogenetic hypothesis for the genus. A secondary aim was to document current knowledge of the geographic distribution of species and investigate biogeographic patterns within the genus in light of the proposed phylogeny.

Materials and methods

Phylogeny

Taxa

Twelve species of the genus *Austrophlebioides* were included in the analysis (Table 1). Excluded from the analysis (owing to insufficient material) were four undescribed species recognised by Dean (1999) (*Austrophlebioides* sp. AV6, *Austrophlebioides* sp. AV9, *Austrophlebioides* sp. AV10, *Austrophlebioides* sp. AV11), an additional undescribed species from Victoria, and *A. unguicularis* and *A. decipiens*. The latter two species are known only from the adult type series lodged at the Stockholm Museum and British Museum respectively. Although the type specimens could not be examined, the figures of the male genitalia in the published descriptions were used to determine the affinities of these two species.

Morphological characters

Characters were obtained from the examination of specimens and from published descriptions (Table 1). For *A. booloumbi* all characters were coded from the species description by Parnrong and Campbell (1997). Nymphal characters were scored from the mouthparts, abdomen and legs. Adult characters were scored from the male genitalia. A total of 21 characters were obtained, 12 of which were binary and 9 were

multistate. Characters 5, 11, 12, and 19 were continuous and for these the presence of clear discontinuities in the variable measured was used to delineate character states. For character 5, most taxa examined fell into two well separated groups: those with a relatively short segment 3 of the labial palp (length/width ratio of 2.2–2.6) and those with a more elongated segment 3 of the labial palp (length/width ratio of 2.9–3.2). In *A. porphyrobranchus* this ratio varied from 2.7 to 2.9, and the proximity of the two states in the final coding of this character (length width ratio of segment 3 of the labial palp: (0) 2.2–2.6; (1) 2.7–3.2) is due to this one species. Characters and character states used in the analyses are listed in Tables 2 and 3, and the data matrices are given in Tables 4 and 5. All characters were equally weighted and multi-state characters were treated as unordered.

Phylogenetic analyses

As not all species of *Austrophlebioides* are presently known from both life history stages, two series of phylogenetic analyses were undertaken. In the first series only those species of *Austrophlebioides* known from both the adult and the nymphal stage were included (Table 4). These were: *A. pusillus*, *A. marchanti*, *Austrophlebioides* sp. AV2; *A. rieki*, *A. wooroonooran*, *A. porphyrobranchus*, and *A. 'armidale'*. Separate analyses were performed on the nymph, adult, and combined (nymph + adult) data. The second series of analyses included all species in the first series plus additional species known from one life history stage (Table 5). Additional taxa were: *A. booloumbi*, described from adult material only; and *Austrophlebioides* sp. AV4, *Austrophlebioides* sp. AV5, *Austrophlebioides* sp. AV7 and *A. 'eungella'*, known only from the nymphal stage.

Parsimony analyses were performed using PAUP* version 4.0b10 (Swofford 2002). The branch-and-bound algorithm, which is guaranteed to find all most parsimonious trees, was used in all analyses with the following options: initial upper bound compute via stepwise; keep minimal trees only; save all optimal trees; collapse zero length branches; addition sequence furthest. The Australian genus *Tillyardophlebia* Dean, 1997 was selected

as the outgroup because of its close affinity to *Austrophlebioides* (Christidis 2005). Both described species of *Tillyardophlebia*, *T. rufosa* Dean, 1997 and *T. alpina* Dean, 1997, were included as outgroups in the analyses. Support for individual nodes was assessed by bootstrap analysis (1000 randomisations, full heuristic search, simple addition, tree bisection reconnection (TBR) branch swapping) (Felsenstein 1985; Hillis and Bull 1993), and by calculating Bremer support values (Bremer 1988) with TreeRot version 3 (Sorenson and Franzosa 2007).

Distribution

Distributional data for species was compiled from localities given in published species descriptions (Harker 1954; Campbell and Suter 1988; Parnrong and Campbell 1997; Christidis and Dean 2005), distributions given in Dean (1999), and from data collected during this study.

Results

Phylogeny

First series of analyses

Combined analysis of adult and nymph. The combined analysis of the adult and nymph data resulted in a single most parsimonious tree (Fig. 1A) with a length of 30 steps, consistency index (CI) 0.866, and retention index (RI) 0.88. Three monophyletic species-groups were identified in the analysis and all three were supported by high bootstrap and Bremer support values. The first comprised *A. marchanti* and *Austrophlebioides* sp. AV2 ('*marchanti*' clade), the second *A. pusillus* and *A. 'armidale'* ('*pusillus*' clade), and the third, *A. rieki*, *A. wooroonooran* and *A. porphyrobranchus* ('*rieiki*' clade). The '*pusillus*' clade was the sister-group to the '*rieiki*' clade, and the clade comprising these two groups was sister to the '*marchanti*' clade.

Adult only analysis. Analysis of adult characters resulted in a single most parsimonious tree (Fig. 1B) with a length of 13 steps, CI, 0.923, RI, 0.933. The tree resulting from the adult data was identical to the combined adult and nymph tree

Table 1. Taxa included in the cladistic analyses and sources from which morphological characters were obtained

Taxon	Specimens examined and literature sourced
Ingroup taxa	
<i>Austrophlebioides booloumbi</i>	Parnrong and Campbell (1997)
<i>Austrophlebioides marchanti</i>	Vic., Badger Ck, 27.xi.1980 (N, M); Vic., Cement Ck, 1.xii.1976 (N); Parnrong and Campbell (1997)
<i>Austrophlebioides porphyrobranchus</i>	Qld, Charmillan Ck, 26.viii.2000 (N, M)
<i>Austrophlebioides pusillus</i>	NSW, Mongarlowe R., 1.xii.1998 (N, M); Vic., Albert R., 3.xi.1977 (M)
<i>Austrophlebioides rieki</i>	Qld, Oliver Ck, 22–23.viii.1999 (N, M)
<i>Austrophlebioides wooroonooran</i>	Qld, Henrietta Ck, 30.v.1999 (N, M)
<i>Austrophlebioides 'armidale'</i>	NSW, Woolomombi R., 25.xi.1998 (N, M); NSW, Dumaresque Ck, 25.xi.1998 (N, M)
<i>Austrophlebioides 'eungella'</i>	Qld, Tributary of Cattle Ck, ii.1998 (N)
<i>Austrophlebioides</i> sp. AV2	Vic., Creek above Pretty Valley Pond, Bogong High Plains, 21.xi.1977 (N), 10.i.1980 (M)
<i>Austrophlebioides</i> sp. AV4	Tas., Meander R., 11.x.1994 (N)
<i>Austrophlebioides</i> sp. AV5	Tas., Douglas Ck, 17.i.1990 (N)
<i>Austrophlebioides</i> sp. AV7	Tas., Douglas Ck, 17.i.1990 (N)
Outgroup taxa	
<i>Tillyardophlebia rufosa</i>	Vic., Badger Ck, 18.i.1980 (N); Dean (1997)
<i>Tillyardophlebia alpina</i>	NSW, Ramshead Ck, 27.i.1984 (N); Dean (1997)

N, nymph; M, male imago; Qld, Queensland; NSW, New South Wales; Vic., Victoria; Tas., Tasmania. AV refers to the Australian Voucher number for undescribed or un-ascribed species.

Table 2. List of morphological characters used in first series of analyses

Nymph	
<i>Labrum</i>	
(1) Subapical setal fringe on dorsal surface of labrum:	(0) arranged in straight row, one to two setae deep and up to three setae deep in middle; (1) arranged in straight row, two to three setae deep and four to five setae deep in middle; (2) merged with apical setal fringe, setae arranged into an elliptical or round setal disc.
(2) Median notch on anterior margin of labrum:	(0) broad U-shaped; (1) V-shaped or narrow U-shaped; (2) greatly reduced; (3) absent.
<i>Mandible</i>	
(3) Serrations on apex of outer incisors of mandible:	(0) absent; (1) present.
(4) Fine setae along apical 1/3 of outer margin of mandible between setal tuft and outer incisor:	(0) absent; (1) present.
<i>Labium</i>	
(5) Length/width ratio of segment 3 of labial palp:	(0) 2.2–2.6; (1) 2.7–3.2.
<i>Legs</i>	
(6) One or two elongated ventral spines in apical third of tarsus:	(0) absent, (1) present on tarsi of all legs; (2) present on tarsi of mid and hind legs only.
(7) Spines along ventral margin of fore tibia:	(0) finely bipectinate; (1) coarsely bipectinate.
(8) Outer margin of hind tibia with:	(0) 25 or fewer spines; (1) 30–40 spines; (2) more than 45 spines.
(9) Doral surface of hind femur:	(0) without area lacking pigmentation near base; (1) with pale round area near base; (2) with pale area in basal half in the form of an elongated streak.
<i>Abdomen</i>	
(10) Lateral margin of abdominal segments:	(0) bare; (1) with sparse to moderately dense fringe of fine setae; (2) with dense fringe of coarser setae.
<i>Adult</i>	
<i>Male genitalia</i>	
(11) Segment 1 of claspers narrowing at:	(0) 0.40–0.50 length of segment; (1) at 0.25–0.30 length of segment.
(12) Penes fused:	(0) at base only or in basal 1/2; (1) in basal 2/3 to 3/4.
(13) Penes, region separating lobes:	(0) V-shaped; (1) broad U-shaped.
(14) Penes in ventral view:	(0) long and narrow; (1) square-shaped, width at apex similar to width at base; (2) triangular-shaped, width at base greater than width at apex.
(15) Dorsal subapical spine on penes:	(0) absent; (1) present, most of spine embedded within lobe, only tip of spine visible on surface of lobe; (2) present, most of spine visible on surface of lobe, only base of spine embedded within lobe.
(16) Pair of large ventral spines near base of penes:	(0) absent; (1) present.
(17) Series of ventral spines on apical 1/2 of penes:	(0) absent; (1) present.
(18) Prominent ventral projection near outer margin of each lobe of penes:	(0) absent; (1) present.
(19) Penes:	(0) long, reaching beyond segment one of claspers; (1) moderately long, reaching beyond 0.6 length of segment one of claspers; (3) short, not reaching 0.5 length of segment one of claspers.

(Fig. 1A), except that relationships among the three species of the ‘rieiki’ clade, *A. rieiki*, *A. wooroonooran* and *A. porphyrobranchus*, were unresolved.

Nymph only analysis. Analysis of nymphal characters resulted in a single most parsimonious trees (Fig. 1C) with a length of 17 steps, *CI*, 0.823, *RI*, 0.842. This tree was identical to the combined adult and nymph tree (Fig. 1A).

Second series of analyses

The second series of analyses, which included additional species known only from one life history stage, resulted in fifteen most parsimonious trees, with a length of 38 steps, *CI*, 0.815, and *RI*, 0.875. One of these trees is shown in Fig. 2A and a strict consensus tree in Fig. 2B. The fifteen trees differed only in the placement of *Austrophlebiooides* sp. AV4 and *A. marchanti* within the ‘*marchanti*’ clade, and the relative positions of species

within the ‘*pusillus*’ clade. The overall topology of all fifteen most parsimonious trees and the strict consensus tree was similar to that obtained in the first series of analyses (Fig. 1A), with the same three major clades identified. *Austrophlebiooides booloumbi* and *A. ‘eungella’* grouped with *A. pusillus* and *A. ‘armidale’* of the ‘*pusillus*’ clade, and the three Tasmanian species, *Austrophlebiooides* sp. AV4, *Austrophlebiooides* sp. AV5, and *Austrophlebiooides* sp. AV7, grouped with *A. marchanti* and *Austrophlebiooides* sp. AV2 of the ‘*marchanti*’ clade. The increased number of most parsimonious trees obtained in the second analysis series was due to the inclusion of taxa for which data was missing for one of the life history stages.

Distribution

The distribution of each species is superimposed on the strict consensus tree in Fig. 2B, and broad areas of distribution of the

Table 3. List of additional morphological characters used in second series of analyses

Nymph	
<i>Labium</i>	
(20) Lateral margins of submentum:	(0) with short spines along basal 2/3 of margins; (1) bare, or with few spines at base.
<i>Legs</i>	
(21) Femur, dark pigmentation near ventral margin:	(0) absent; (1) present.

Table 4. Data matrix used in first series of analyses

Taxa	Characters	
	0000000001	1111111111
	1234567890	123456789
<i>T. rufosa</i>	0101000100	000001000
<i>T. alpina</i>	0101000000	000001000
<i>A. pusillus</i>	0011101111	010220101
<i>A. 'armidale'</i>	0011101111	010210101
<i>A. marchanti</i>	0111011122	001110002
<i>A. sp. AV2</i>	0111011222	001110001
<i>A. wooroonooran</i>	1110120111	110210011
<i>A. rieki</i>	1110120021	110210011
<i>A. porphyrobranchus</i>	1110111111	110210012

three major clades are shown in Fig. 3. The '*marchanti*' clade is present in southern New South Wales and Victoria (South-eastern Forest, *sensu* Cracraft 1991), and is the only clade found in Tasmania (Figs 3A, 2B). *Austrophlebioides* sp. AV4, *Austrophlebioides* sp. AV5 and *Austrophlebioides* sp. AV7 are known only from Tasmania. The mainland species of the '*marchanti*' clade, *A. marchanti* and *Austrophlebioides* sp. AV2, occur in Victoria and southern New South Wales (Figs 3A, 2B). These two species have not been collected from northern New South Wales or further north.

The '*pusillus*' clade is distributed from the Eungella region (Eastern Queensland), just south of the Burdekin Gap, to Victoria (South-eastern Forest) (Fig. 3B). *Austrophlebioides pusillus* is widely distributed and has been recorded from south-eastern Queensland, northern and southern New South Wales, and Victoria (Fig. 2B). Other species of this clade appear to have more restricted distributions. *Austrophlebioides 'eungella'* has only been recorded from the Eungella National Park in central-eastern Queensland, *A. booloumbi* is known only from the Conondale Ranges in south-eastern Queensland, and *A. 'armidale'* has only been collected from northern New South Wales. The distribution of the '*pusillus*' clade partly overlaps with that of the '*marchanti*' clade, with both clades present in southern New South Wales and Victoria (South-eastern Forest).

The 'rieiki' clade is confined to the Wet Tropics bioregion of north-eastern Queensland (Fig. 3C). Within the Wet Tropics, *A. wooroona* and *A. porphyrobranchus* are known only from the Atherton subregion. *Austrophlebia rieiki* was described from material collected from the Daintree subregion, however, nymphs and adults belonging to this or a morphologically similar species have also been collected from the Atherton and Paluma subregions. Based on morphology alone it is difficult to establish whether these specimens represent a single widespread species or several very closely related species.

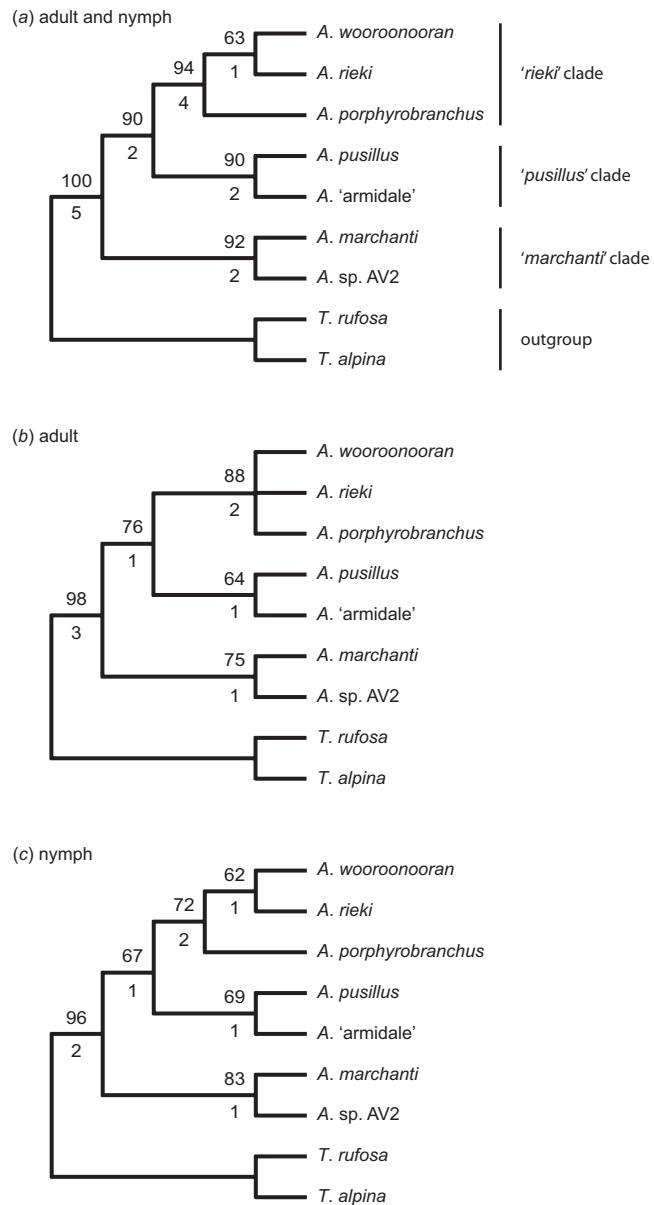


Table 5. Data matrix used in second series of analyses

Taxa	Characters	
	0000000001	11111111122
	1234567890	12345678901
<i>T. rufosa</i>	0101000100	00000100010
<i>T. alpina</i>	0101000000	00000100010
<i>A. pusillus</i>	0011110111	01022010110
<i>A. 'armidale'</i>	0011110111	01021010110
<i>A. marchanti</i>	0111011122	00111000210
<i>A. sp.AV2</i>	0111011222	00111000111
<i>A. wooroonooran</i>	1110120111	11021001110
<i>A. rieki</i>	1110120021	11021001110
<i>A. porphyrobranchus</i>	1110111111	11021001210
<i>A. booloumbi</i>	???????????	010220101??
<i>A. 'eungella'</i>	0011110011	??????????10
<i>A. sp. AV4</i>	0111011122	??????????10
<i>A. sp. AV5</i>	2201011022	??????????01
<i>A. sp. AV7</i>	2301011022	??????????01

Fig. 1. First series of analyses: (a) single most parsimonious tree from analysis of combined nymph and adult data; (b) single most parsimonious tree resulting from analysis of adult data only; (c) single most parsimonious tree from analysis of nymph data only. Bootstrap values are shown above nodes for groups with > 50% support. Bremer support values are shown below nodes.

Discussion

Phylogeny

The cladistic analyses support the recognition of three monophyletic species-groups within the genus *Austrophlebiooides*. These three groups, the ‘*marchanti*’, ‘*pusillus*’ and ‘*rieiki*’ clades, were identified in all analyses. Phylogenetic trees derived from the adult, nymph, and combined data, in the first series of analyses, had very similar topologies and were consistent in the placement of the three clades. The ‘*pusillus*’ clade was the sister-group to the ‘*rieiki*’ clade, and the clade comprising these two groups was sister to the ‘*marchanti*’ clade. Congruence among the phylogenies derived from the different

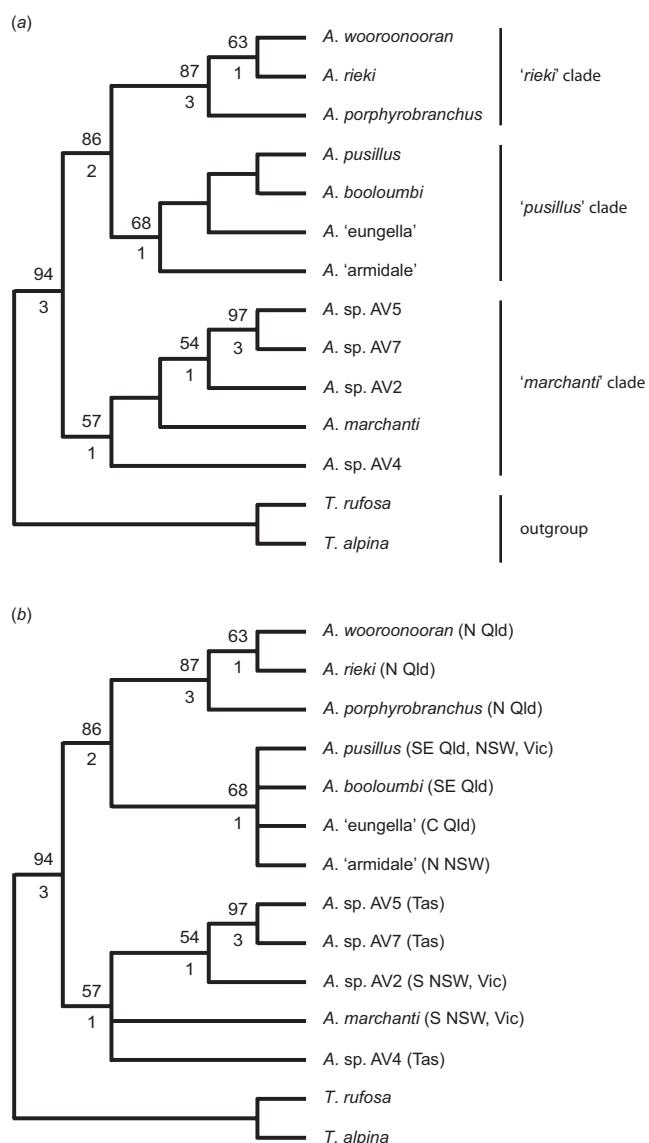


Fig. 2. Second series of analyses: (a) one of fifteen most parsimonious trees resulting from analysis of combined nymph and adult data, with the inclusion of species known from only one life history stage (Table 5); (b) strict consensus tree of fifteen most parsimonious trees. Bootstrap values are shown above nodes for groups with >50% support. Bremer support values are shown below nodes.

life history stages and the combined data, along with high bootstrap support values, suggest that these relationships are robust. Similar patterns were obtained from the second series of analyses, which included additional species known only from one life history stage.

The ‘*marchanti*’ clade comprises *A. marchanti* and *Austrophlebiooides* sp. AV2 and is defined by the following synapomorphic characters of the adult male: penes lobes separated in apical half by a broad U-shaped region (character 13); and penes square-shaped in ventral view, with width at apex similar to the width at base (character 14). The nymphal character that unites species of this clade is the presence of a dense setal fringe on the lateral margins of the abdominal segments (character 10). Several other nymphal characters are shared by species of the ‘*marchanti*’ clade but these are not unique to the clade.

The three Tasmanian species, *Austrophlebiooides* sp. AV4, *Austrophlebiooides* sp. AV5 and *Austrophlebiooides* sp. AV7, based on nymphal characters appear to belong to the ‘*marchanti*’ clade. This placement is supported by the presence of a dense setal fringe on the lateral margins of the abdominal segments (character 10). Other shared derived but not unique characters include: coarsely bipectinate ventral spines on the tibia of the foreleg (character 7), and area lacking pigmentation on the dorsal surface of the hind tibia in the form of an elongated streak (character 9). In addition, *Austrophlebiooides* sp. AV5, *Austrophlebiooides* sp. AV7 and *Austrophlebiooides* sp. AV2 possess the following character state: presence of a dark band of pigmentation near the ventral margin of the femur (character 21). This form of pigmentation is unique to these three species and is absent in *A. marchanti* and *Austrophlebiooides* sp. AV4. Although there is support for placement of the Tasmanian species into the ‘*marchanti*’ clade, information from adult males or from molecular data is required to confirm this placement.

The nymphs of two of the Tasmanian species, *Austrophlebiooides* sp. AV5 and *Austrophlebiooides* sp. AV7, possess several features that are believed to be ancestral within the Gondwanan Leptophlebiidae. In these species, the outer incisor of the right mandible is not serrated as in other *Austrophlebiooides*, and small spines are present along the basal $\frac{2}{3}$ of the lateral margins of the submentum. This may be due to either the retention of primitive character states or possibly to the secondary loss of serration on the outer incisor, and the gain of spines on the lateral margins of the submentum. In contrast, the labrum of both species is highly derived. The setae on the dorsal surface of the labrum are arranged into a broad elliptical disc in *Austrophlebiooides* sp. AV5, and into a round disc in *Austrophlebiooides* sp. AV7. The two species appear to be closely related.

The ‘*pusillus*’ clade is defined by the synapomorphy: presence of a series of fine ventral spines in the apical half of the penes of the adult male (character 17). This character was originally included in the diagnosis of the genus (Campbell and Suter 1988) but was later considered species-specific as it was absent in *A. marchanti* (Parnrong and Campbell 1997). Species comprising the ‘*pusillus*’ clade are: *A. pusillus*, *A. booloumbi*, *A. 'armidale'* and *A. 'eungella'*. The adults of *A. 'eungella'* are unknown at the present time, but the nymph of this species is similar to that of *A. pusillus*. The placement of *A. 'eungella'* within the ‘*pusillus*’ clade was supported by the cladistic analysis. Relationships

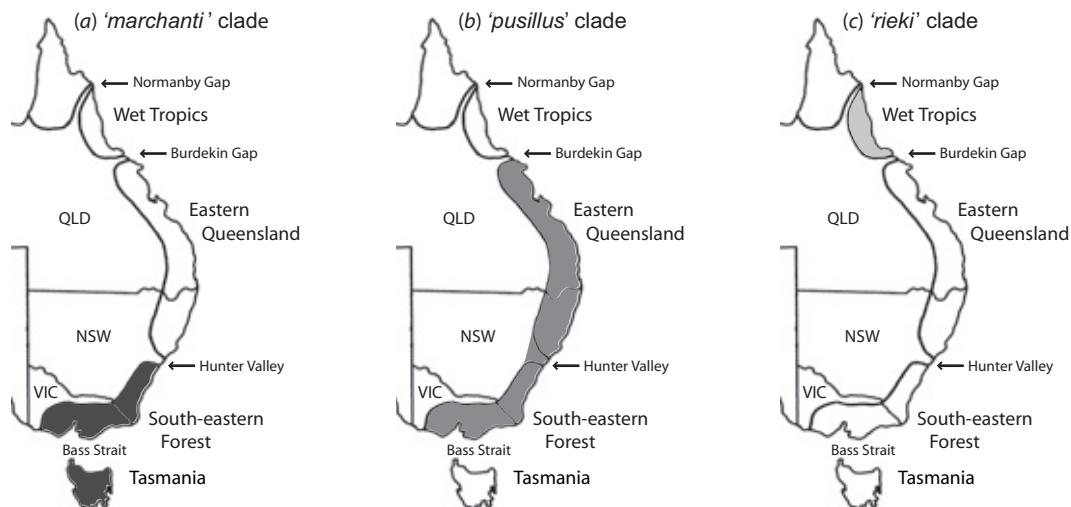


Fig. 3. Broad areas of distribution of the three *Austrophlebioides* clades: (a) ‘*marchanti*’ clade, (b) ‘*pusillus*’ clade, (c) ‘*rieiki*’ clade. Maps also show the major biogeographic regions of eastern Australia and geographic barriers (arrows) (modified from Ford 1987, and Cracraft 1991)

among species within the ‘*pusillus*’ clade were unresolved on the strict consensus tree. This was primarily due to missing data from the adult and nymph stages for several species.

Austrophlebioides unguicularis and *A. decipiens* were not included in the cladistic analyses because material of these species was unavailable. However, illustrations of the male genitalia in the original species description of *A. unguicularis* (Ulmer 1916) and *A. decipiens* (Harker 1954) clearly indicate the presence of fine ventral spines on the penes. On this basis it is proposed that these species also be assigned to the ‘*pusillus*’ group.

The grouping of *A. rieki*, *A. wooroonooran* and *A. porphyrobranchus* from the Wet Tropics bioregion into a monophyletic clade was strongly supported by all analyses. These species share several derived character states. In the adult male, a prominent ventral projection is present on each lobe of the penes (character 18), and segment one of the claspers narrows at ~0.25 to 0.30 of its length (character 11). In the nymph, fine setae are absent from the outer margin of the mandible, between the outer incisor and the median setal tuft (character 4). These fine setae are present in all other *Austrophlebioides* species and have presumably been secondarily lost in the Wet Tropics species. Likewise, the nymphs of *Austrophlebioides* species typically have one to two elongate ventral spines in the apical third of the tarsi. However, in *A. rieki* and *A. wooroonooran* these spines appear to have been reduced or lost from the fore tarsus and are present on the tarsi of the mid and hind legs only. A sister-group relationship between *A. rieki* and *A. wooroonooran* was suggested by the nymph data, with *A. porphyrobranchus* occupying a basal position within the clade. The adults of *A. rieki*, *A. wooroonooran* and *A. porphyrobranchus* are very similar and there was insufficient data in the adult-only phylogeny to resolve relationships among these species.

Distribution

Distributional patterns within the genus *Austrophlebioides* correspond with previously recognised biogeographic regions for

Australia based on areas of endemism of birds (Cracraft 1991) and plants (Crisp *et al.* 1995). There is little overlap in the distribution of the three *Austrophlebioides* clades, with closely related species generally found within the same biogeographic region.

The ‘*rieiki*’ clade is confined to the Wet Tropics bioregion of north-eastern Queensland and is separated from its sister-group the ‘*pusillus*’ clade by the Burdekin Gap, a dry region of open woodland between Townsville and Bowen. The Burdekin Gap has been a major biogeographical barrier for a range of organisms including freshwater and terrestrial invertebrates (Watson and Theischinger 1984; Wang *et al.* 1996; Hugall *et al.* 2003), birds (Ford 1987; Cracraft 1991; Joseph *et al.* 1993), frogs (James and Moritz 2000) and reptiles (Moussalli *et al.* 2005). In one of the few biogeographic studies on Australian freshwater insects, Watson and Theischinger (1984) list several closely related species pairs of Odonata, Plecoptera and Megaloptera whose distributional limits lie on either side of this barrier.

The complete geographic separation of the ‘*rieiki*’ and ‘*pusillus*’ clades suggests that the Burdekin Gap is a significant barrier for these mayflies and has been for a long period of time. Time of divergence of the two clades is difficult to establish, as molecular data are currently unavailable for *Austrophlebioides*. However, genetic studies on vertebrate and invertebrate taxa have dated interspecific divergences across the Burdekin Gap to the mid to late Miocene or Pliocene (Moritz *et al.* 1997; Hugall *et al.* 2003; Moussalli *et al.* 2005).

Species of the ‘*pusillus*’ clade, with the exception of *A. pusillus*, and an undescribed species from Victoria (F. Christidis and J. C. Dean, unpubl. data), are restricted to the Eastern Queensland biogeographic region (*sensu* Cracraft 1991), which includes northern New South Wales. The distribution of these species within Eastern Queensland appears to correspond to recognised subregions (James and Moritz 2000). *Austrophlebioides ‘eungella’* occurs in central-eastern Queensland, *A. booloumbi* in south-eastern Queensland, and *A. ‘armidale’* in

northern New South Wales. Interestingly, *A. 'eungella'*, found just south of the Burdekin Gap, is more closely related to species in south-eastern Queensland than to those in the nearby Wet Tropics bioregion. This suggests a more recent faunal continuity between central-eastern and south-eastern Queensland than between these subregions and the Wet Tropics bioregion, and is consistent with the findings of Joseph *et al.* (1993) for birds, and Hugall *et al.* (2003) for terrestrial snails.

Austrophlebioides pusillus, in contrast to other species within the '*pusillus*' clade, appears to be distributed either side of the Hunter Valley, in Eastern Queensland and the South-eastern Forest. Aspects of the ecology of *A. pusillus*, such as the ability to survive in lowland streams with reduced current and elevated water temperatures, may account for the wider distribution of this species. However, morphological differences among populations have been observed, with adult males collected from northern New South Wales differing in their markings from individuals found south of the Hunter Valley in southern New South Wales and Victoria. Whether *A. pusillus* represents a single widely distributed species or several closely related species with more restricted distributions remains to be established. If the former is true, the presence of *A. pusillus* in both Eastern Queensland and the South-eastern Forest suggests that the Hunter Valley has not been as effective a barrier for this species as for other species of the genus.

The '*marchanti*' clade is distributed in the more southern regions of eastern Australia and in Tasmania. The mainland species of this clade are restricted to southern New South Wales and Victoria (South-eastern Forest), and have not been collected from northern New South Wales, to the north of the Hunter Valley. Similar distributional patterns have been observed for several other mayflies including species of the genus *Coloburiscoides* (Coloburiscidae) (Campbell 1981). The discontinuity between northern New South Wales and southern New South Wales was also noted by Watson and Theischinger (1984) in Odonata, with several species having their distributional limits at or near the Hunter Valley.

The three species of *Austrophlebioides* presently known from Tasmania belong to the '*marchanti*' clade and all are endemic to Tasmania. High levels of endemism in Tasmania have been reported for several other freshwater insects including Plecoptera (Hynes and Hynes 1980), Trichoptera (Neboiss 1977), Psephenidae (Davis 1986) and Blephariceridae (Zwick 1977). The lack of overlap in the distributions of mainland and Tasmania species of *Austrophlebioides* suggest a long period of isolation. Although Tasmania has been connected to the mainland several times since the Miocene and most recently during the Pleistocene, conditions on these land connections are believed to have been unfavourable for the dispersal of several groups of freshwater insects (Tillyard 1936; Hynes and Hynes 1980; Campbell 1981; Davis 1986).

The restricted geographic distribution of the majority of *Austrophlebioides* species, as well as the three clades, is not surprising given the limited dispersal abilities of these mayflies. The nymphs of *Austrophlebioides* are restricted to cool forest streams, and the potentially dispersive adults mostly only live for two to three days. In addition, the adults are relatively soft bodied and prone to desiccation. Distributional patterns within the genus *Austrophlebioides* suggest that vicariance has been

important in the differentiation of the group and in determining present-day distributions of species.

Acknowledgements

We thank Peter Cranston and Richard Pearson for comments on an earlier draft of the manuscript. This research was funded by Land and Water Australia, and James Cook University. Faye Christidis is currently supported by a grant from the Australian Biological Resources Study.

References

- Bremer, K. (1988). The limits of amino-acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* **42**, 795–803. doi:10.2307/2408870
- Campbell, I. C. (1981). Biogeography of some rheophilous aquatic insects in the Australian region. *Aquatic Insects* **3**, 33–43.
- Campbell, I. C., and Suter, P. J. (1988). Three new genera, a new subgenus and a new species of Leptophlebiidae (Ephemeroptera) from Australia. *Journal of the Australian Entomological Society* **27**, 259–273. doi:10.1111/j.1440-6055.1988.tb01172.x
- Christidis, F. (2005). Phylogenetic relationships of the Australian Leptophlebiidae (Ephemeroptera). *Invertebrate Systematics* **19**, 531–539. doi:10.1071/IS05022
- Christidis, F., and Dean, J. C. (2005). Three new species of *Austrophlebioides* Campbell and Suter (Ephemeroptera: Leptophlebiidae: Atalophlebiinae) from the Wet Tropics bioregion of north-eastern Australia. *Australian Journal of Entomology* **44**, 132–143. doi:10.1111/j.1440-6055.2005.00453.x
- Cracraft, J. (1991). Patterns of diversification within continental biotas: hierarchical congruence among the areas of endemism of Australian vertebrates. *Australian Systematic Botany* **4**, 211–227. doi:10.1071/SB9910211
- Crisp, M. D., Linder, H. P., and Weston, P. H. (1995). Cladistic biogeography of plants in Australia and New Guinea: congruent pattern reveals two endemic tropical tracks. *Systematic Biology* **44**, 457–473. doi:10.2307/2413654
- Davis, J. A. (1986). Revision of the Australian Psephenidae (Coleoptera): Systematics, phylogeny and historical biogeography. *Australian Journal of Zoology. Supplementary Series* **119**, 1–97.
- Dean, J. C. (1997). Descriptions of new Leptophlebiidae (Insecta: Ephemeroptera) from Australia. I. *Tillyardophlebia* gen. nov. *Memoirs of Museum Victoria* **56**, 83–89.
- Dean, J. C. (1999). 'Preliminary keys for the identification of Australian mayfly nymphs of the family Leptophlebiidae.' Identification Guide No. 20. (Cooperative Research Centre for Freshwater Ecology: Thurgoona, NSW, Australia.)
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791. doi:10.2307/2408678
- Ford, J. (1987). Hybrid zones in Australian birds. *Emu* **87**, 158–178.
- Harker, J. E. (1954). The Ephemeroptera of eastern Australia. *Transactions of the Royal Entomological Society of London* **105**, 241–268.
- Hillis, D. M., and Bull, J. J. (1993). An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* **42**, 182–192. doi:10.2307/2992540
- Hugall, A., Stanisic, J., and Moritz, C. (2003). Phylogeography of terrestrial gastropods. The case of the *Sphaerospira* lineage and history of Queensland Rainforests. In 'Molecular Systematics and Phylogeography of Mollusks'. (Eds C. Lyndeard and D. R. Lindberg.) pp. 270–301. (Smithsonian Books: Washington, DC, USA.)
- Hynes, H. B. N., and Hynes, M. E. (1980). The endemism of Tasmanian stoneflies (Plecoptera). *Aquatic Insects* **2**, 81–89.
- James, C. H., and Moritz, C. (2000). Intraspecific phylogeography in the sedge frog *Litoria fallax* (Hylidae) indicates pre-Pleistocene vicariance of an open forest species from eastern Australia. *Molecular Ecology* **9**, 349–358. doi:10.1046/j.1365-294x.2000.00885.x

- Joseph, L., Moritz, C., and Hugall, A. (1993). A mitochondrial DNA perspective on the historical biogeography of mideastern Queensland rainforest birds. *Memoirs of the Queensland Museum* **34**, 201–214.
- Moritz, C., Joseph, L., Cunningham, M., and Schneider, C. (1997). Molecular perspective on historical fragmentation of the Australian tropical and subtropical rainforest: implications for conservation. In ‘Tropical Forest Remnants: Ecology, Management and Conservation of Fragmented Communities’. (Eds W. F. Laurance and R. O. Bierregard.) pp. 442–454. (Chicago University Press: Chicago, IL, USA.)
- Moussalli, A., Hugall, A. F., and Moritz, C. (2005). A mitochondrial phylogeny of the rainforest skink genus *Saproscincus*. Wells and Wellington (1984). *Molecular Phylogenetics and Evolution* **34**, 190–202.
- Neboiss, A. (1977). A taxonomic and zoogeographic study of Tasmanian caddis-flies (Insecta: Trichoptera). *Memoirs of the National Museum of Victoria* **38**, 1–208.
- Parnrong, S., and Campbell, I. C. (1997). Two new species of *Austrophlebioides* Campbell and Suter (Ephemeroptera: Leptophlebiidae) from Australia, with notes on the genus. *Australian Journal of Entomology* **36**, 121–127. doi:10.1111/j.1440-6055.1997.tb01444.x
- Sorenson, M. D., and Franzosa, E. A. (2007). TreeRot, version 3. (Boston University: Boston, MA, USA.)
- Swofford, D. L. (2002). ‘PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods) Version 4.0b10.’ (Sinauer Associates: Sunderland, MA, USA.)
- Tillyard, R. J. (1936). The trout-food insects of Tasmania. Part II. A monograph of the mayflies of Tasmania. *Papers and Proceedings of the Royal Society of Tasmania* **1935**, 23–59.
- Ulmer, G. (1916). Results of Dr. E. Mjöberg’s Swedish scientific expedition to Australia. 6. Ephemeroptera. *Arkiv für Zoologi* **10**, 1–18.
- Wang, Q., Thornton, I. W. B., and New, T. R. (1996). Biogeography of the phoracanthine beetles (Coleoptera: Cerambycidae). *Journal of Biogeography* **22**, 31–48.
- Watson, J. A. L., and Theischinger, G. (1984). Regions of taxonomic disjunction in Australian Odonata and other freshwater insects. *Odonatologica* **13**, 147–157.
- Zwick, P. (1977). Australian Blephariceridae (Diptera). *Australian Journal of Zoology. Supplementary Series* **46**, 1–121.

Manuscript received 25 July 2007, accepted 14 November 2007