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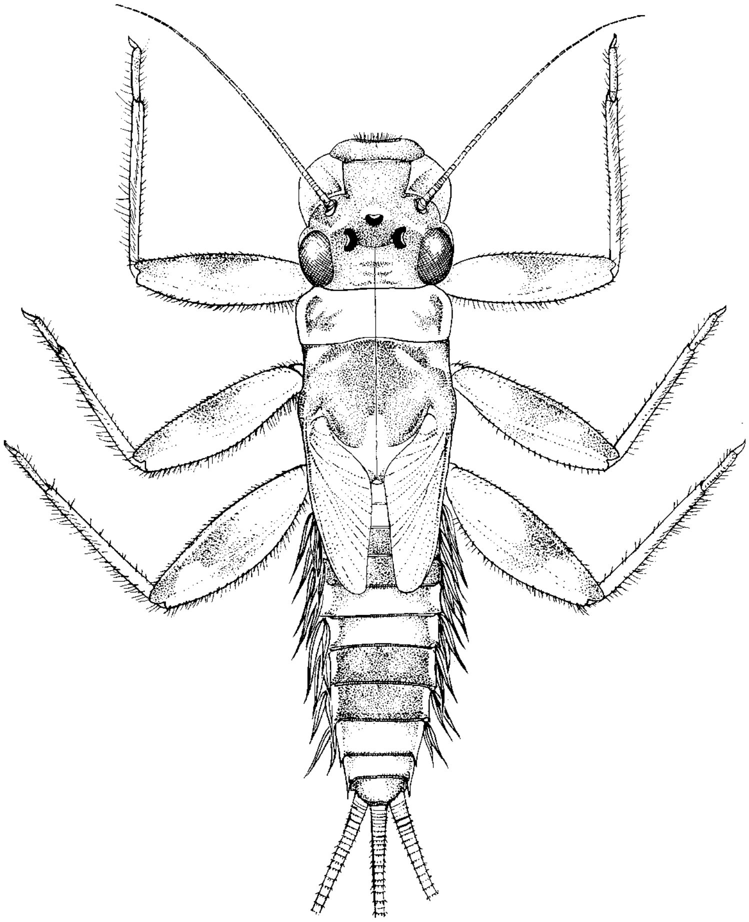
Systematics, Phylogeny and Ecology of Australian Leptophlebiidae (Ephemeroptera)



Thesis submitted by
Faye Christidis Bsc(Hons)
in May 2003

for the degree of Doctor of Philosophy
in Zoology and Tropical Ecology
within the School of Tropical Biology
James Cook University

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ABSTRACT

An understanding of the processes that govern patterns of distribution and abundance of organisms remains a central goal in ecology. Ecologists have traditionally focussed on proximate factors to explain patterns in community structure; however, the importance of historical factors in determining some present-day ecological patterns is increasingly being recognised. In this thesis I examine phylogenetic relationships among the Australian Leptophlebiidae (Ephemeroptera) and use the phylogenetic hypotheses obtained to investigate the role of evolutionary history in shaping selected ecological and morphological traits of these stream dwelling mayflies.

A cladistic parsimony analysis based on 43 morphological characters was used to explore phylogenetic relationships among the Leptophlebiidae genera from Australia, and selected genera from South America and New Zealand. The outcomes from this analysis were consistent with higher-level relationships previously proposed by Pescador and Peters (1980). The only point of conflict was the monophyly of the *Hapsiphlebia* lineage. Although the basal position of genera assigned to this lineage was confirmed, these genera did not form a monophyletic group in the present analysis. Clarification of the affinities of four Australian genera (*Neboissophlebia*, *Bibulmena*, *Loamaggalangta* and *Kaninga*) suggested that they belong to lineages not previously recognised among the Gondwanan Leptophlebiidae.

Three new species of *Austrophlebioides* were described from the Wet Tropics bioregion. Phylogenetic relationships among species of the genus *Austrophlebioides* were examined using cladistic analyses based on morphological characters of the nymph and the adult. The results from these analyses support the recognition of three monophyletic species groups within the genus, the "wet tropics", "*pusillus*" and "*marchanti*" clades. The "*marchanti*" clade was the most basal clade within the genus, and the "*pusillus*" clade was sister to the "wet tropics" clade. Congruence among phylogenies derived from different life-history stages and combined adult and nymph data, along with good bootstrap support, indicated that the proposed relationships within the genus were robust.

Minimal overlap was observed in the geographic distribution of the three *Austrophlebioides* clades. The "wet tropics" clade is confined to the Wet Tropics bioregion of north-eastern Queensland. The "*pusillus*" clade is restricted to eastern Queensland and northern New South Wales with the exception of *A. pusillus*, which extends 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), strongly suggesting that vicariance has been important in the differentiation of the group and in determining present-day distributions of species.

The phylogenetic hypothesis for the genus *Austrophlebiodes* was used to infer the pattern of evolution of labrum morphology in the genus, and evaluate the importance of flow regimes in the evolution of labrum shape. Reconstruction of ancestral character-states suggests that a broad labrum and use of high flow environments are ancestral features that have evolved only once, early in the evolutionary history of the genus. A narrow labrum and the use of slow flow habitats appear to have evolved secondarily on two independent occasions. Retention of a broad labrum in species that occur in fast flow, and evolutionary changes from a broad to a narrow labrum with shifts from fast to slow flow habitats, suggest that flow has been an important selective factor in the evolution of labrum morphology in *Austrophlebioides*. However, high levels of conservatism were observed within individual clades, indicating that phylogenetic history is also an important determinant of labrum morphology and habitat use in this genus.

The incorporation of phylogenetic information into the ecological study of leptophlebiid mayfly assemblages of four streams in the Wet Tropics revealed that substrate and habitat use among species often reflected phylogenetic relationships. The high levels of segregation observed on the basis of substrate type, with stones and leaf litter supporting distinct assemblages, appears to be the result of historical differences among lineages in the use of substrate type. The leaf litter fauna was dominated by species of the *Nousia* lineage, whereas the stone fauna was dominated by species of the *Meridialaris* lineage. Similarly, the association of species within some lineages to certain habitat types contributed to the distinctiveness of mayfly assemblages of pools, runs, and riffles. These findings suggest that phylogenetic history is important in

determining some ecological traits of leptophlebiid species and their present-day distributions among substrate and habitat types. However, patterns of habitat and substrate use did not always reflect phylogenetic relationships. Within the *Meridialaris* lineage, coexisting species often differed in their use of pool, run or riffle, and tended to separate along a velocity gradient. Similar trends in habitat segregation were observed among the *Nousia* species (*Nousia* lineage). In such instances present-day ecological factors may play an important role in determining the distributions of species.

Overall, the present-day structure of tropical mayfly assemblages in the four study streams appears to be the outcome of a combination of historic (phylogenetic) and contemporary ecological processes. Without knowledge of phylogenetic relationships among species, the historical basis for some present-day patterns of substrate and habitat use would have gone unrecognised. The integration of phylogenetic and ecological approaches has provided a greater understanding of the processes that shape contemporary ecological patterns in Leptophlebiidae mayfly assemblages.

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Front cover: *Austrophlebioides* nymph (Illustrator: Sybil Monteith)

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STATEMENT OF SOURCES
DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references given.

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Faye Christidis 21-5-03

Chapter 1
General introduction

1.1 Phylogeny and ecology

A central goal in ecology is to elucidate the factors that determine the distribution and abundance of organisms, and the structure of communities. Traditionally, ecologists have focused on proximate ecological factors (e.g. competition, predation and disturbance) to explain patterns in community structure (Schoener 1974; Cody and Diamond 1975; Strong *et al*. 1984; Morin 1999). Similarly, the morphological and behavioural attributes of organisms frequently are interpreted as adaptations to present-day selective regimes (Wanntorp *et al*. 1990). However, these traditional views of communities being dictated by contemporary influences often have neglected the influences of evolutionary factors (Ricklefs 1987; Ricklefs and Schluter 1993). For example, variations in local community diversity often have been attributed to the influences of contemporary biotic and abiotic factors operating at local levels, yet the failure of diversity to converge under similar physical conditions led Ricklefs (1987) to suggest that regional and historical processes profoundly influenced local community structure. As Ricklefs and Schluter (1993, p. 241) note, the present properties of communities "reflect the phylogenetic origins of the taxa they contain, as well as the unique events and geographic circumstances that occurred during their formation".

Historical processes may influence community structure in a number of ways. The phylogenetic history of lineages that comprise communities may impose constraints that limit the ecological roles of lineage members (Cadle and Greene 1993). For example, past evolutionary modifications in morphology may limit the extent and direction of future morphological change, thus restricting the ecological scope of taxa (Cadle and Greene 1993; Gittleman *et al*. 1998). The biogeographic history of a region influences the composition of the species present and thus the pool of species which can interact within communities. Both the phylogenetic history of component taxa and biogeographic processes limit the degree to which the composition and organisation of present-day communities can be explained solely by present-day ecological factors (Ricklefs 1987; Cadle and Green 1993).

Phylogenetic approaches to the study of community ecology have been discussed widely in the recent literature (Wanntorp *et al.* 1990; Brooks and McLennan 1991; Harvey and Pagel 1991; Gorman 1992; Miles and Dunham 1993; Ricklefs and Schluter 1993; Eggleton and Vane-Wright 1994; Losos 1996; McPeek and Miller 1996; Grandcolas 1998; Gittleman *et al*. 1998; Webb *et al*. 2002). This increased awareness of the importance of historical factors on the organisational properties of contemporary communities has resulted in a greater number of ecological studies now incorporating phylogenetic information (e.g. Cadle and Greene 1993; Farell and Mitter 1993; Zimmerman and Simberloff 1996; Vitt *et al*. 1999; Forstmeier *et al*. 2001). Such studies have revealed that ecological traits of taxa (e.g. habitat use and diet) can often be strongly associated with phylogenetic history, and that some aspects of community organization (e.g. resource partitioning) may be the result of evolutionary difference among lineages that comprise communities rather than the outcome of more proximate causes. For example, in a study of Amazonian lizard assemblages, Vitt *et al.* (1999) noted that species tended to separate on the basis of microhabitat use and prey type. Using a phylogenetic approach they found a strong association between diet and evolutionary history (i.e. closely related species had similar dietary preferences). Their results support the hypothesis that food preferences among species have an historical basis and reduce the strength of argument for the alternative hypothesis, that present-day competitive interactions cause dietary partitioning among these lizard species.

1.2 Benthic stream communities

The relative importance of historical processes in structuring benthic communities of streams has rarely been addressed by stream ecologists. Stream studies generally have related the distribution and abundance of benthic organisms to contemporary abiotic factors (e.g. current velocity, substrate, temperature and food resources), and emphasized the importance of such factors in structuring benthic stream communities (Hynes 1970; Rabeni and Minshall 1977; Statzner and Higler 1986; Hildrew and Townsend 1986; Growns and Davis 1994; Allan 1995). Species interactions (e.g.

competition and predation) have been shown to influence the distribution and abundance of some benthic organisms (Kohler 1992; Kohler and Wiley 1997) but the outcomes of such interactions are often determined by abiotic factors (Hemphill and Cooper 1983; McAuliffe 1984; Hansen *et al*. 1991; Hart and Merz 1998).

1.2.1 Flow regimes

Water flow is viewed as one of the most important abiotic factors affecting benthic stream organisms (Allan 1995; Hart and Finelli 1999). Flow exerts direct physical forces (e.g. shear stress) on organisms, which can greatly affect rate of movement and foraging abilities (Poff and Ward 1992, 1995), and may result in the dislodgement of organisms from the stream bed. In addition, flow can affect the stream biota indirectly, through its influence on the physical structure of the stream habitat (e.g. substrate particle size), oxygen availability, and the distribution of food resources (e.g. leaf litter and other suspended particulate matter) (Allan 1995).

Within streams there is a great deal of spatial variability in flow conditions (Davis and Barmuta 1989; Hart and Finelli 1999). At the larger spatial scale of stream habitat type, riffle habitats tend to have higher current velocities than pools. As a consequence, organisms residing in riffles are likely to be exposed to faster flows and higher shear stress than those inhabiting pools (Davis and Barmuta 1989; Giller and Malmqvist 1998). However, even within fast-flowing riffles there are areas of reduced flow (e.g. the underside of stones and spaces between stones). At even finer spatial scales, invertebrates separated by only a few centimetres on the surface of a single stone can experience substantially different current velocities (Hart *et al*. 1996). Thus the type of near-bed flow conditions experienced by benthic organisms depends upon their precise location within the stream.

Benthic invertebrates vary in their response to flow. Some species appear to tolerate a wide range of flow conditions whereas others show a distinct preference for either the fast flow of riffles or the slow flow of pools (Edington 1968; Hynes 1970; Barmuta 1990; Hearnden and Pearson 1991; Growns and Davis 1994). The association of species with other abiotic factors such as substrate type similarly varies in a species-specific manner. Such preferences often result in distinct assemblages associated with pool and riffle habitats within streams (Hynes 1970; Barmuta 1990; Giller and Malmqvist 1998), as well as with stone and leaf litter substrates (Angradi 1996; Murphy and Giller 2000). However, the questions of whether species-specific habitat preferences reflect phylogenetic relationships, and whether evolutionary trends within lineages affect the structure of stream invertebrate assemblages, have rarely been addressed.

1.2.2 Morphological adaptation to flow

Water flow is believed to have been an important selective factor in the evolution of several morphological traits of stream invertebrates (Hynes 1970; Ward 1992; Allan 1995; Chapter 5). In the Simuliidae (Diptera), for example, the size and structure of the labral feeding fans have been shown to vary among species and individuals that occupy different flow regimes: species that occur in fast-flowing water have shorter and stouter fans whereas those that are found in slow-flowing water have more elongate and slender fans (Zhang and Malmqvist 1996, 1997; Palmer and Craig 2000). Observed differences in fan morphology are believed to be adaptive, and it has been proposed that flow has played an important role in the evolution of simuliid fan structure (Zhang and Malmqvist 1996; Palmer and Craig 2000; Craig *et al*. 2001).

In several mayfly (Ephemeroptera) genera the shape of the labrum of the nymph also appears to be related to habitat flow regimes. Species found in fast flow have a broad, laterally expanded labrum, whereas those species that occur in slow flow have a narrower labrum. A broad labrum, by protecting the preoral cavity and mouthparts from strong currents, may enable mayfly nymphs to feed more effectively on the upper surface of stones in fast-flowing waters (Hynes 1970; Arens 1989; Ward 1992).

Although some morphological features of stream insects appear to be associated with flow regimes and may have evolved in response to contemporary flow environments, closely related species may share traits due to common ancestry (Felsenstein 1985a; Harvey and Pagel 1991). As noted by Allan (1995, p 50-51), “the principle features of any organism are inherited within a taxonomic lineage and subject to multiple selective forces”. As such “we should be careful not to make current [velocity] the explanation for every behavioural and morphological feature of biota of flowing water”. Knowledge of evolutionary relationships among taxa is required to adequately assess the importance of flow regimes in the evolution of morphological traits of stream organisms. To date, this has been difficult due to the absence of robust phylogenies for many stream insects.

1.3 Study organisms

In this thesis I investigate the role of phylogenetic history in shaping selected ecological and morphological traits of leptophlebiid mayflies (Ephemeroptera: Leptophlebiidae).

The family Leptophlebiidae is widely distributed, with the greatest diversity of genera and species in the southern hemisphere (Edmunds *et al*. 1976; Pescador and Peters 1980). Over 100 extant genera have been described (Hubbard 1990), although it is estimated that as many as 300 genera may exist (Peters 1980). Two extant subfamilies are presently recognised within the Leptophlebiidae, the Leptophlebiinae and the Atalophlebiinae (Peters 1980). The monophyly of the Atalophlebiinae is strongly supported by the following synapomorphies: upper portion of compound eyes of adult male with square facets; styliger plate of adult male fused; anterior maxillary brush of nymph with hairs or spines evenly arranges in rows; labrum of nymph with denticles (or cleft) on anterior margin (Peters 1980, 1997). Synapomorphies given in support for the monophyly of Leptophlebiinae are, in the nymph: anterior margin of labrum with heavy spines; venter of lingua of hypopharynx with hair patches (except for the genus *Paraleptophlebia*) (Peters 1980, 1997).

The divergence between the Leptophlebiinae and Atalophlebiinae is believed to have occurred prior to the fragmentation of the ancient continents of Laurasia and Gondwana, more than 120 million years before present (Peters 1980; Towns and Peters 1996; Peters 1997). The Leptophlebiinae is presently restricted to the northern hemisphere and is believed to be of Laurasian origin (Peters 1980; 1988). The Atalophlebiinae occurs in both the northern and southern hemispheres but is most diverse in the southern hemisphere, and is believed to be of Gondwanan origin (Peters 1980, 1988; Savage 1987; Towns and Peters 1996).

The Australian Leptophlebiidae all belong to the subfamily Atalophlebiinae, and presently comprise 68 described species in 17 genera. Except for the genus *Nousia* which occurs in Australia and South America, all of the other genera are endemic to Australia. Early taxonomic publications on the Australian Leptophlebiidae were mostly isolated descriptions of new genera and species (Eaton 1871, Ulmer 1916, 1919). These were followed by more detailed taxonomic studies by Tillyard (1934; 1936), Harker (1950, 1954, 1957) and Riek (1970). Recent studies (Suter 1986; Dean 1987, 1988, 1997, 1999, 2000; Campbell and Suter 1988; Campbell and Peters 1986, 1993; Peters and Campbell 1991; Campbell 1993) have greatly increased our knowledge of the alpha-taxonomy of the group. However, the fauna of several regions of Australia remains poorly known with many new genera and species still to be formally described (Dean 1999).

The phylogenetic affinities of the Australian Leptophlebiidae genera are believed to be with leptophlebiid genera present on other southern landmasses, particularly southern South America and New Zealand, which were once part of the ancient continent of Gondwana (Peters and Edmunds 1972; Tsui and Peters 1975; Pescador and Peters 1980; Towns and Peters 1980, 1996). A higher-level phylogeny for these Gondwanan genera was proposed by Pescador and Peters (1980), based on morphological characters and constructed using traditional Hennigian methods (Peters 1980). Five monophlyletic lineages were recognised: the *Hapsiphlebia, Penaphlebia*, *Nousia, Dactylophlebia* and *Meridialaris* lineages. Presently, all but the *Dactylophlebia* lineage are represented in Australia. A reconstruction of phylogenetic relationships among all described Leptophlebiidae genera from Australia has not been attempted, nor have the higher-level relationships proposed by Pescador and Peters (1980) been tested using explicit cladistic parsimony analysis.

Leptophlebiid adults are aerial/terrestrial and most live for only one or two days. The nymphs are aquatic and inhabit a wide variety of stream habitats including pools, runs, riffles and even the rock faces of boulders and waterfalls (Peters and Campbell 1991; Towns and Peters 1996). A few species are also found in standing waters (Peters and Campbell 1991). Most leptophlebiid nymphs graze on detritus and periphyton (Peters and Campbell 1991). Although the family has diversified into a wide range of habitats, the same diversification does not appear to have occurred within individual lineages. For example, the nymphs of the *Hapsiphlebia* lineage are mostly found in slow-flowing and standing waters, among leaf litter or burrowed into the sediment (Pescador and

Peters 1980; Peters and Campbell 1991), whereas the nymphs of the *Meridialaris* lineage are commonly found clinging to stones in moderate and fast-flowing sections of streams (Peters and Campbell 1991; Towns and Peters 1996). It thus appears that several aspects of the ecology of leptophlebiid mayflies may be strongly influenced by phylogenetic history.

1.4 Project aims and thesis outline

The two major aims of this project were: (1) to reconstruct the phylogenetic relationships among the Australian Leptophlebiidae genera, and to use the phylogenetic hypothesis obtained to assess the role of evolutionary history in shaping patterns of substrate and habitat use among leptophlebiid mayfly assemblages; and (2) to reconstruct the phylogenetic relationships among species of the genus *Austrophlebioides*, and to use the phylogenetic hypothesis derived to infer the pattern of evolution of labrum morphology and to evaluate the importance of flow regimes in the evolution of labrum shape. To this end I investigated various aspects of the taxonomy, systematics and ecology of the Australian Leptophlebiidae as follows.

Chapter 2 investigates the phylogenetic relationships among the Australian Leptophlebiidae genera and selected genera from South America and New Zealand using morphological characters and cladistic parsimony analysis. The outcomes of this analysis were compared with the higher-level relationships proposed by Pescador and Peters (1980). The phylogenetic hypothesis generated in this chapter was used to investigate the influence of evolutionary history on patterns of substrate and habitat use within the Leptophlebiidae (Chapter 6), and provides a basis for the study of the Australian genus *Austrophlebioides* (Chapter 2, 3, 4).

Chapter 3 addresses the paucity of taxonomic knowledge of the Leptophlebiidae fauna of the Wet Tropics bioregion of north-eastern Australia with the description of three new species of *Austrophlebioides* from this region.

Chapter 4 investigates the phylogenetic relationships among species of the genus *Austrophlebioides* using morphological characters of the nymph and adult, and proposes the first phylogenetic hypothesis for the genus. This chapter also documents current knowledge of the geographic distribution of species and investigates biogeographic patterns within the genus in light of the proposed phylogeny.

Chapter 5 uses the phylogenetic hypothesis for the genus *Austrophlebioides* generated in Chapter 4 to infer evolutionary changes in labrum morphology and assess the role of water flow regimes in the evolution of labrum shape.

Chapter 6 documents the mayfly fauna associated with leaf litter and stones in pools, runs and riffles of four streams to determine patterns of substrate and habitat use among species. Secondly, the phylogenetic hypothesis generated in Chapter 2 is used to determine if observed patterns in the use of substrate and habitat type among species reflect phylogenetic relationships.

Chapter 7 presents an overview and general conclusions derived from the previous chapters and outlines future research arising from this project.

Appendix 1 comprises taxonomic identification keys to nymphs of genera and species of Leptophlebiidae from the Wet Tropics bioregion of north-eastern Australia. The development of these keys was necessary to provide a solid taxonomic basis for the study of substrate and habitat use of leptophlebiid species (Chapter 6). These keys help overcome the taxonomic impediments previously faced by researchers studying the ecology, systematics and biogeography of the Leptophlebiidae.

CHAPTER 2

Phylogenetic relationships of the Australian Leptophlebiidae

2.1 Introduction

The Leptophlebiidae is the most speciose mayfly family in Australia comprising 68 described species in 17 genera. All Australian genera belong to the subfamily Atalophlebiinae and are believed to be members of a Gondwanan group, which is distributed throughout the southern hemisphere (Pescador and Peters 1980). A phylogeny was proposed by Pescador and Peters (1980) for this Gondwanan group of genera, in which they recognised five monophyletic lineages–the *Hapsiphlebia, Penaphlebia*, *Nousia*, *Dactylophlebia* and *Meridialaris* lineages (Fig. 2.1). Their phylogeny was based on morphological characters of nymphs and adults and was constructed using traditional Hennigian methods. Character polarity was inferred by reference to a hypothetical ancestor, and the possession of shared derived character states (synapomorphies) was used to define each monophyletic lineage. The majority of taxa in the analysis were from South America with additional genera (known at the time) from Australia, New Zealand and Africa. Phylogenetic relationships among the New Zealand genera were investigated by Towns and Peters (1980) and again more recently by Towns and Peters (1996).

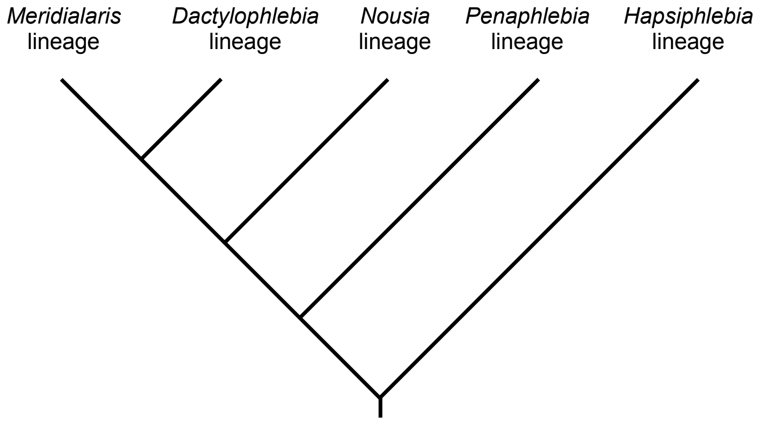


Figure 2.1. Phylogeny proposed by Pescador and Peters (1980).

Derived characters states identified by Pescador and Peters (1980) and Towns and Peters (1980, 1996) for each of the five lineages have been used to determine the phylogenetic affinities of the Australian genera. The genera *Atalophlebia*, *Kalbaybaria,* *Ulmerophlebia*, *Jappa* and *Atalomicria* are currently placed in the *Hapsiphlebia* lineage (Pescador and Peters 1980; Campbell 1993). *Garinjuga* is presently the only Australian member of the *Penaphlebia* lineage (Campbell and Suter 1988). *Nousia*, *Koorrnonga* and *Nyungara* have been placed in the *Nousia* lineage (Pescador and Peters 1980; Dean 1987; Campbell and Suter 1988). *Austrophlebioides*, *Kirrara*, *Tillyardophlebia* and several undescribed genera are believed to belong to the *Meridialaris* lineage (Pescador and Peters 1980; Campbell and Peters 1986; Campbell and Suter 1988; Dean 1997). The *Dactylophlebia* lineage is not known from Australia. Although most Australian genera can be assigned to one of the five lineages, the affinities of several genera, including *Neboissophlebia*, *Bibulmena*, *Loamaggalangta* and *Kaninga* are unclear (Dean 1987, 1988, 2000; Dean *et al*. 1999). These genera cannot be placed in any of the five lineages as presently defined. As more new genera are described from Australia it has become increasingly apparent that some of the derived character states used by Pescador and Peters (1980) to define the lineages require re-assessment.

A phylogenetic analysis of all described genera from Australia has not been attempted previously, nor have the higher-level relationships proposed by Pescador and Peters (1980) been tested using explicit cladistic parsimony analysis. Relationships among genera within the individual lineages are also unknown. The aim of this study was to investigate the phylogenetic relationships among the Australian genera and selected genera from South America and New Zealand using parsimony analysis, and compare the outcomes from this analysis with the higher-level relationships proposed by Pescador and Peters (1980). Relationships among the genera of the *Meridialaris* lineage were also examined and the monophyly of this lineage tested to provide a basis for a study of the Australian genus *Austrophlebioides*.

2.2 Methods

2.2.1 Taxa

The taxa used in the analysis are listed in Table 2.1. Representative species of sixteen of the seventeen genera described from Australia were included, along with two undescribed species, WT sp. 1 and WT sp. 2, which are believed to belong to two new genera. The Australian monotypic genus *Thaulophlebia* was omitted from the present study as no specimens of the type species, *Thaulophlebia lucida* (Ulmer), could be obtained. Selected genera from South America and New Zealand were also included in the analysis to test the higher-level relationships within the group and the monophyly of the *Meridialaris* lineage.

Table 2.1. Leptophlebiid taxa (ingroup) included in the cladistic analysis. Origin of non-Australian taxa are indicated in parentheses.

Hapsiphlebia lineage:	*Atalophlebia* sp. AV13 *Kalbaybaria doantrangae* *Ulmerophlebia* sp. AV3 *Jappa edmundsi* *Atalomicria sexfasciata*
Penaphlebia lineage:	*Garinjuga maryannae*
Nousia lineage:	*Nousia fuscula* *Koorrnonga inconspicua* *Nyungara bunni*
Dactylophlebia lineage:	*Austroclima sepia* (New Zealand) *Mauiulus luma* (New Zealand)
Meridialaris lineage:	*Austrophlebioides pusillus* *Kirrara procera* *Tillyardophlebia rufosa* WT sp. 1 WT sp. 2 *Deleatidium lillii* (New Zealand) *Atalophlebioides cromwelli* (New Zealand) *Meridialaris chiloeense* (South America)
Affinities uncertain:	*Bibulmena kadjina* *Neboissophlebia hamulata* *Kaninga gwabbalitcha* *Loamaggalangta* sp.

2.2.2 Morphological characters

Morphological characters were obtained from examination of nymphal and adult material, and from the literature (Towns and Peters 1978, 1979, 1996; Suter 1986; Pescador and Peters 1987; Dean 1987, 1988, 1997, 2000; Campbell and Suter 1988; Campbell 1993; Campbell and Peters 1993; Dean *et al*. 1999). Nymphal characters were scored from all body regions including the mouthparts, thorax, abdomen and legs. Adult characters were scored from the hind wings, male genitalia and female abdomen. A total of 43 characters were obtained, 27 of which were binary and 16 were multistate. Characters and character states used in the analysis are listed below. The data matrix is given in Table 2.2. The terminology used in this study follows that of Edmunds *et al*. (1976). Morphological characters identified by other systematists as phylogenetically informative (particularly by Pescador and Peters 1980; and Towns and Peters 1980, 1996), were included in the matrix. Adults and nymphs of undescribed species were associated by rearing.

List of characters used in the cladistic analysis

Characters identified by Pescador and Peters (1980) and Towns and Peters (1980; 1996) as phylogenetically informative are indicated by an asterisk (*).

Nymph

Labrum/Clypeus

1.* Labrum width/clypeus width: (0) labrum narrower than clypeus; (1) labrum subequal or broader than clypeus.

Clypeus

2.* Lateral margins: (0) sub-parallel or convergent apically; (1) slightly divergent apically; (2) divergent apically.

Labrum

3.* Anterior margin: (0) straight or with broad emargination; (1) with narrow U or V-shaped emargination.

4.* Denticles on anterior margin: (0) absent; (1) prominent; (2) greatly reduced.

5. Denticles: (0) of similar length; (1) median denticle much longer.

6.* Median hood on anterior margin: (0) absent; (1) present.

7. Setae on dorsal surface of labrum: (0) scattered; (1) in 3 rows, apical, median and basal; (2) in 2 rows, apical and median; (3) in 1 or 2 rows, apical only or apical plus subapical.
8. Subapical (or median) setal row on dorsal surface of labrum: (0) from three quarters to as wide as apical row; (1) up to half width of apical row; (2) wider than apical row.

Mandible

9.* Right outer incisor: (0) slender, parallel-sided; (1) robust, triangular, with subapical denticles.
10.* Serrations on apex of outer incisors: (0) absent; (1) present.
11. Tuft of setae midway along outer margin of mandible: (0) absent; (1) present.
12. Series of long setae on basal 2/3 of outer margin: (0) absent; (1) present.
13. Fine setae along apical 1/3 of outer margin between setal tuft and outer incisor: (0) absent; (1) present.
14.* Prosthecal tuft: (0) well developed; (1) greatly reduced.
15.* Prosthecal tuft: (0) broad and fleshy at base; (1) slender and elongated or very reduced.

Hypopharynx

16. Lingua: (0) without well developed lateral processes; (1) with well developed lateral processes.

Maxillae

17.* Galea-lacinia: (0) narrow; (1) broad.
18.* Galea-lacinia: (0) subapical pectinate setae absent; (1) with up to 15 subapical pectinate setae; (2) with 17 to 21 subapical pectinate setae; (3) usually with more than 22 subapical pectinate setae.
19. Outer margin of cardo fringed with: (0) long or moderately long spines or setae; (1) short spines.

Labium

20.* Glossae: (0) turned under ventrally, not on the same plane as paraglossae; (1) not turned under ventrally, on about the same plane as paraglossae.
21.* Submentum: (0) with long spines along lateral margins; (1) bare or with few spines at base of lateral margins.
22.* Segment 3 of labial palp: (0) 0.6 to 1.0 times length of segment 2; (1) less than 0.6 times length of segment 2; (2) greater than 2 times length of segment 2.

23. Labial palp length/width ratio of segment 3: (0) < 1.5; (1) 1.6 to 2.1 (2) > 2.1.

24. Inner margin of segment 3 of labial palp: (0) with stout spines; (1) with small denticles; (2) without spines or denticles.

Thorax

25. Long setae on lateral margins of pronotum: (0) present; (1) absent.

Legs

26. Tarsal claws: (0) with teeth; (1) with fine denticles; (2) smooth.

27. Spines on ventral margin of tarsi: (0) all of similar length; (1) one or more apical spines greatly elongated on tarsi of all legs or on tarsi of mid and hind legs only.

Abdomen

28. Posterolateral projections on segments: (0) 2 to 9; (1) 4, 5 or 6 to 9; (2) 7 or 8 to 9.

29*. Lateral margin of abdominal segments: (0) bare; (1) fringed with fine setae; (2) fringed with spines and/or thick setae.

30. Row of setae on mid-dorsal region of segments: (0) absent; (1) present.

Gills

31. Gill shape: (0) plate-like to broadly ovate, apex rounded or with small point; (1) narrow to broadly ovate with single apical filament or lanceolate; (2) divided into 3 or more filaments.

32. Outer margin of gill: (0) without dense fringe of setae; (1) with dense fringe of setae.

Caudal filaments

33. Whorl of spines on segments : (0) present; (1) absent.

34. Setae on segments: (0) long; (1) short or of moderate length.

Adult

Hind wing

35. Length of subcostal vein: (0) 0.9 or more of wing length; (1) less than 0.9 of wing length.

36. Total number of cross veins in hind wing: (0) more than 18; (1) less than 15.

Legs

37.* Tarsal claws: (0) similar, both hooked; (1) dissimilar, pad-like claw not hooked; (2) dissimilar, pad-like claw with small apical hook.

Female abdomen

38.* 9th sternum with: (0) deep to moderate cleft; (1) shallow cleft or entire.

Male eye

39.* Shape of facets of compound eye of adult male (as well as male subimago and male nymph): (0) hexagonal; (1) square on upper portion of eye and hexagonal on lower portion.

Male genitalia

40.* Styliger plate: (0) deeply cleft ventrally; (1) not deeply cleft ventrally, posterior ventral margin entire or with small U-shaped notch.

41. Penes: (0) separated except at base; (1) separated in apical $^1/_2$ to $^1/_5$; (2) fused or separated only at very apex.

42. Apical half of penes with: (0) spines absent or minute; (1) ventral spine or ventrally directed apical spine; (2) apical spine, spine directed apically; (3) dorsal spine, spine on accessory lobe; (4) dorsal spine, spine not on accessory lobe.

43. Midventral subapical appendage: (0) absent; (1) present, appendage round and knob-like in ventral view; (2) present, appendage not round and knob-like in ventral view.

Table 2.2 Data matrix used in cladistic analysis.

	1111111111222222222233333333334444
	1234567890123456789012345678901234567890123
L. cupida	0000?00?000000000110001000022010000002000010
L. marginata	0000?00?000000000110001000020010000002000010
Atalophlebia	0001002010010001010000000100202000100011200
Atalomicria	0001002010110001010000000202201000110011210
Jappa	0001101010010001020000110102211110101011100
Ulmerophlebia	0001101010010001020000110101211110101011100
Neboissophlebia	1101001000100011020000110201001000102011010
Kalbaybaria	0000?00?10?000010000022011022020000000011200
Bibulmena	1101003000100011020000211201001000000011200
Kaninga	1101003000100011031000211101001001100011000
Loamaggalangta	1101003000100011021000211202001000100011200
Garinjuga	1101003000110011020100221002001001000011040
Nyungara	1101003?00100011011100221001001001110011140
Nousia	1101003000100011011100221001001001110011100
Koorrnonga	1101003100100011011100221001001001110011100
Kirrara	1200?03?01100011121111221010010001002111001
Tillyardophlebia	1212013200101011131111221000001001002111000
WT sp.1	1112013200101011131111221000001001102111110
WT sp.2	1112013201101011131111221000001001002111140
Austrophlebioides	1212013201101011131111221010101001002111140
Meridialaris	1212013200101011131111121010101001002111220
Mauiulus	1112003000100011011100221002001001102111130
Austroclima	1112003000100011011100221002001001102111130
Deleatidium	1212013?01100111131111221010000001001011202
Atalophlebioides	1212013?01100111131111221 0?0001001?01111202

? = missing data or inapplicable character.

2.2.3 Phylogenetic analyses

Cladistic analyses were performed using PAUP* version 4.0b10 (Swofford 2002). The branch-and-bound search option was used, with characters treated as unordered and equally weighted. Two representative species from the subfamily Leptophlebiinae–*Leptophlebia cupida* (Say), and *L. marginata* (Linn.)–were used as the outgroup. Bootstrap analysis (1000 randomizations, full heuristic search, simple addition, tree bisection reconnection (TBR) branch swapping) was used to assess support for each node of the tree (Felsenstein 1985b; Hillis and Bull 1993).

2.3 Results

Cladistic analysis resulted in two most parsimonious trees, each with a length of 113 steps, a consistency index (CI) of 0.5575, and a retention index (RI) of 0.8031. The two trees shown in Fig. 2.2 differ only in the placement of *Austrophlebioides, Meridialaris* and *Tillyardophlebia*. In the strict consensus tree (Fig. 2.3) relationships among *Austrophlebioides, Tillyardophlebia, Meridialaris* and the clade containing *Kirrara*, *Deleatidium* and *Atalophlebioides* are unresolved.

Kalbaybaria, Atalophlebia, *Atalomicria*, *Jappa* and *Ulmerophlebia,* all members of the *Hapsiphlebia* lineage, occupy a basal position in both trees. The grouping of *Atalophlebia* and *Atalomicria* as sister taxa was poorly supported, whereas a sister group relationship between *Jappa* and *Ulmerophlebia* was strongly supported with a bootstrap value of 99%.

All remaining ingroup taxa were placed in a separate clade (Figs 2.2, 2.3), which had good bootstrap support of 84%. Within this larger clade *Neboissophlebia*, *Bibulmena*, *Loamaggalangta* and *Kaninga* were the most basal genera. *Garinjuga* (*Penaphlebia* lineage) was next to branch off followed by the clade containing *Nousia*, *Koorrnonga* and *Nyungara* (*Nousia* lineage). *Mauiulus* and *Austroclima* (*Dactylophlebia* lineage) were the sister group to the clade comprising the genera of the *Meridialaris* lineage, WT sp. 1, WT sp. 2, *Austrophlebioides*, *Meridialaris*, *Tillyardophlebia*, *Kirrara*, *Deleatidium* and *Atalophlebioides*. The monophyly of the *Meridialaris* clade was strongly supported, with a bootstrap value of 97%.

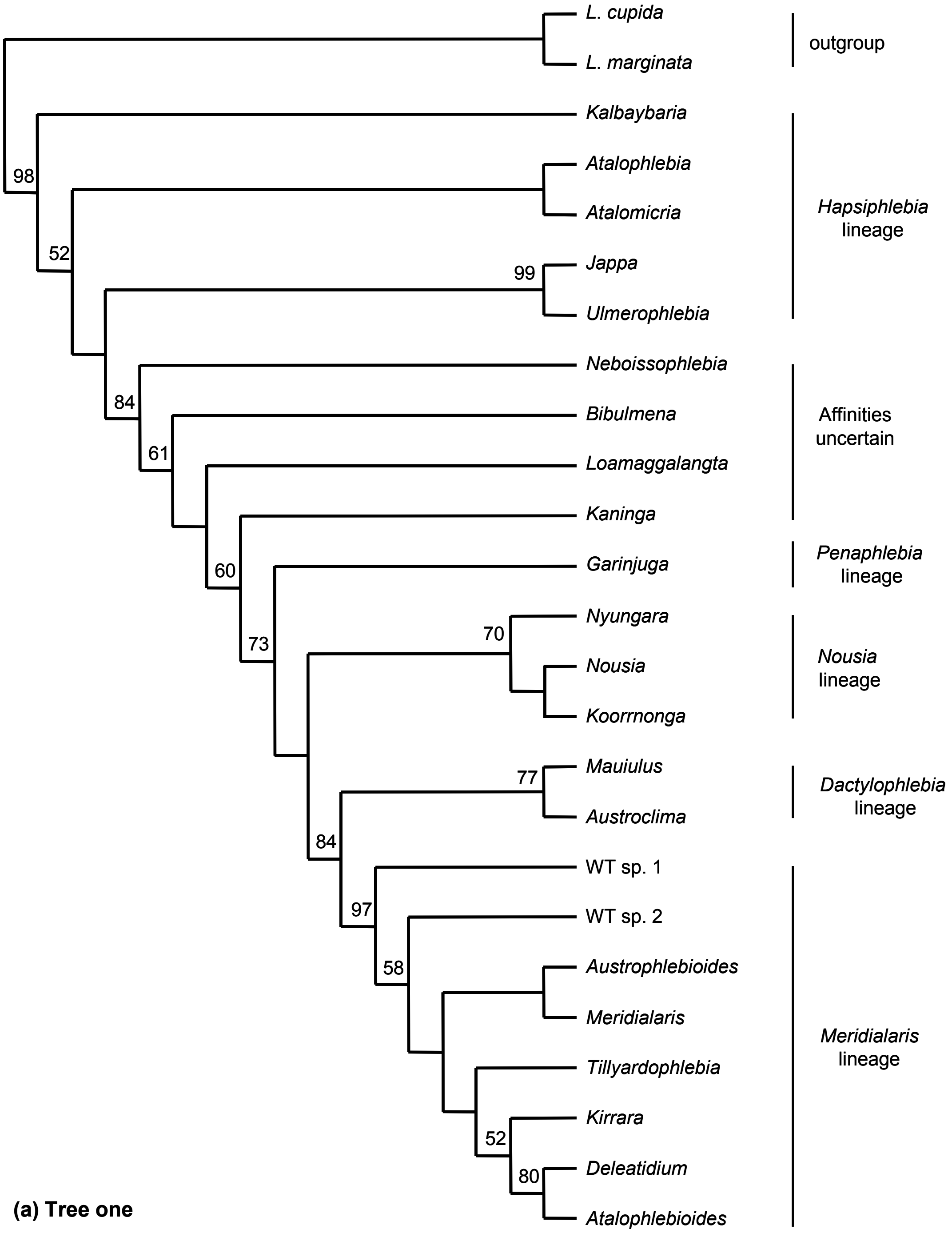


Figure 2.2. Two most parsimonious trees obtained from a branch-and-bound search: (a) tree one, (b) tree two. Bootstrap values are shown above the nodes for those groups with >50% support.

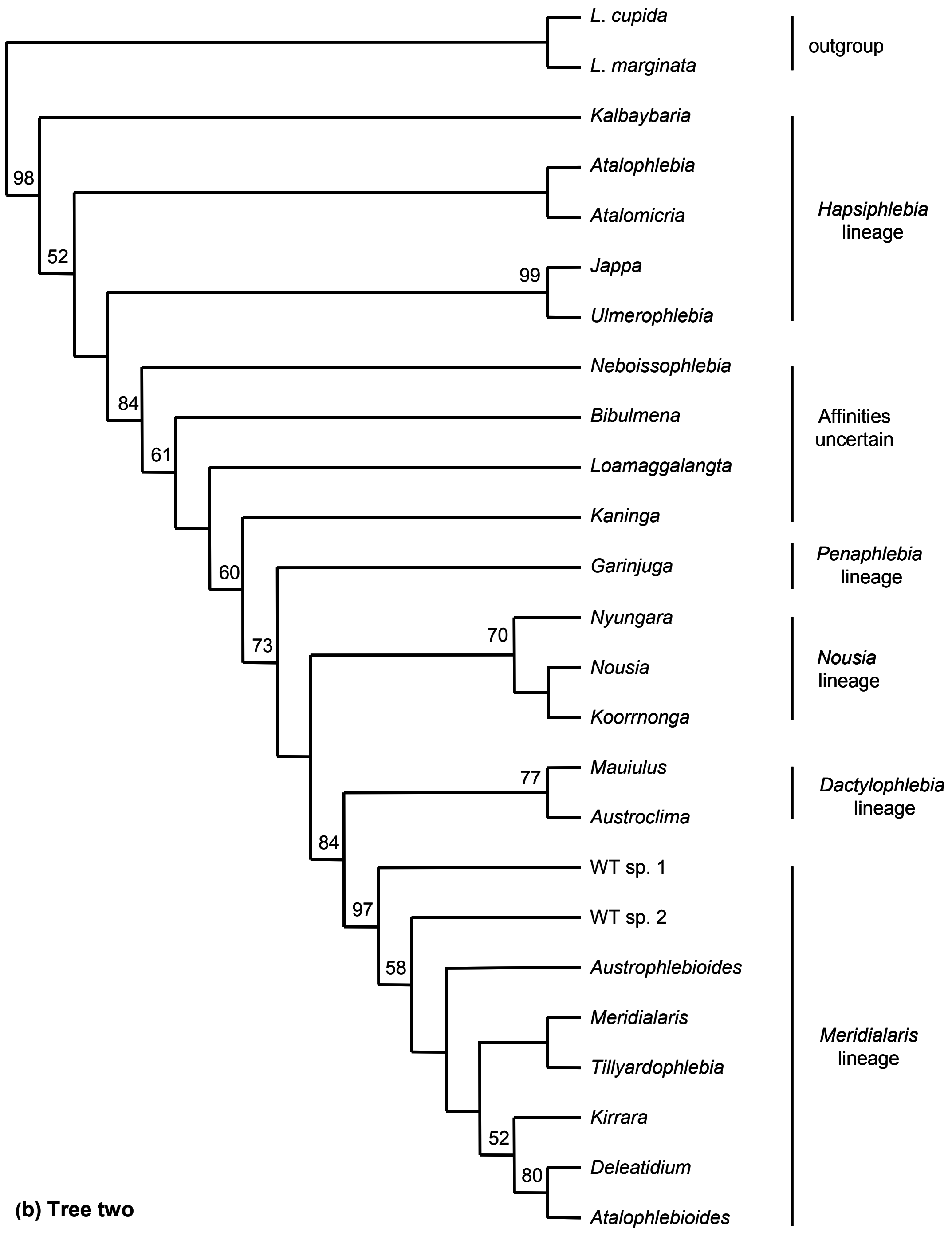


Figure 2.2. (cont.).

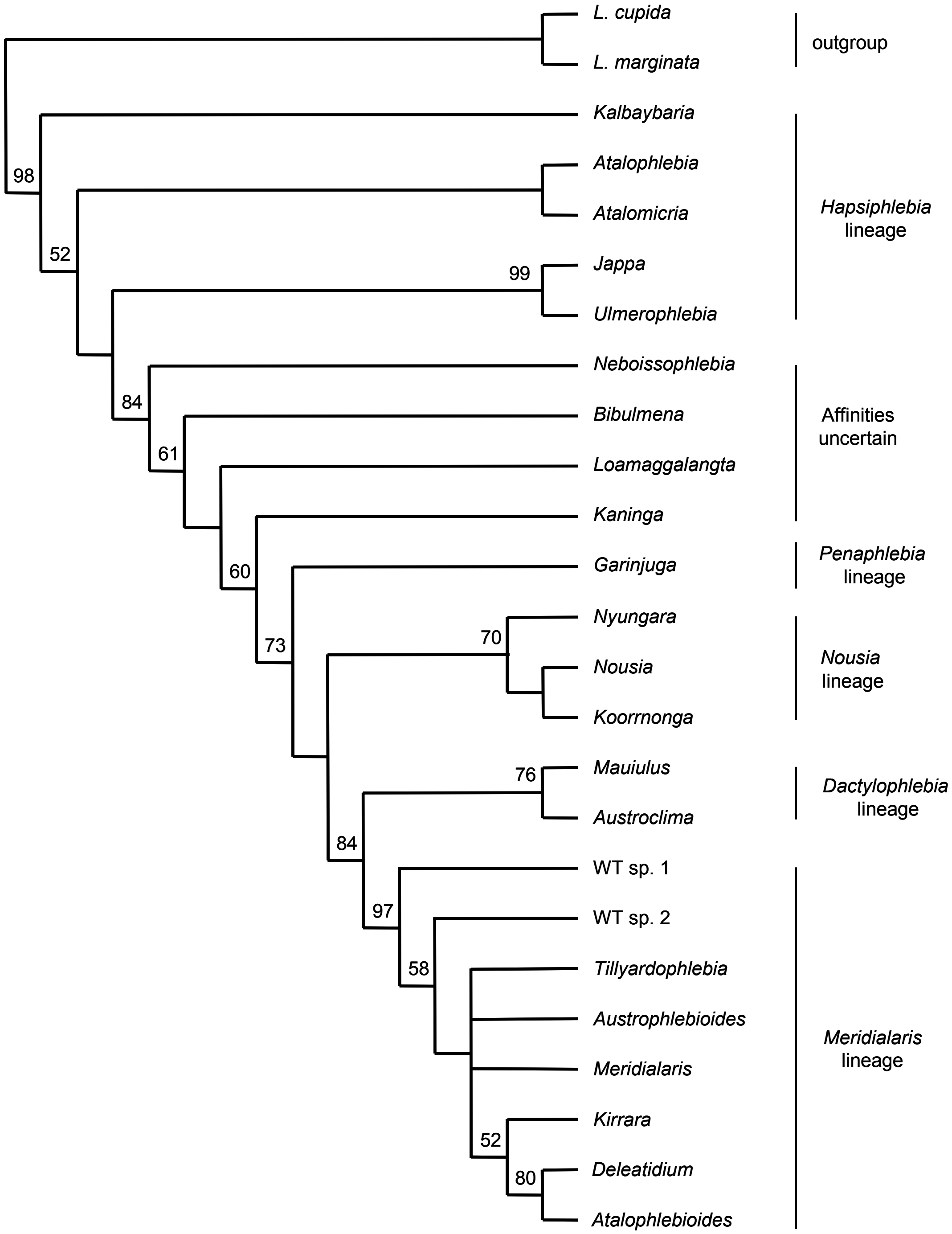


Figure 2.3. Strict consensus tree of two most parsimonious trees obtained from a branch-and-bound search. Bootstrap values are shown above the nodes for those groups with >50% support.

2.4 Discussion

The outcomes from the parsimony analysis are consistent with the higher-level relationships proposed by Pescador and Peters (1980). Genera included in the present analysis were placed within the lineages to which they were previously assigned. As proposed by Pescador and Peters (1980), the *Hapsiphlebia* lineage is the most basal, followed by the *Penaphlebia*, *Nousia*, *Dactylophlebia* and *Meridialaris* lineages. *Neboissophlebia*, *Bibulmena*, *Loamaggalangta* and *Kaninga* were excluded from all lineages presently recognised and placed between the genera of the *Hapsiphlebia* lineage and *Garinjuga* of the *Penaphlebia* lineage.

The basal position of the genera of the *Hapsiphlebia* lineage–*Kalbaybaria*, *Atalophlebia*, *Atalomicria*, *Ulmerophlebia* and *Jappa*–was supported; however, it is uncertain whether these genera represent a monophyletic group. In the present study these genera were not placed into a single clade. All possess subapical denticles on the outer incisor of the right mandible of the nymph, one of the derived character states given by Pescador and Peters (1980) in support for the monophyly of this group. The other derived character state is the presence of prominent lateral setae on the nymphal abdominal terga. Although long setae are present on the lateral margins of the abdominal segments of *Ulmerophlebia*, *Jappa* and *Atalophlebia*, they are very sparse or absent in *Atalomicria*, and in *Kalbaybaria* they are short and spine-like. Given the variability of this character its usefulness in defining the lineage is doubtful.

In the genera of the *Hapsiphlebia* lineage, ventral teeth on the tarsal claw of the nymph are reduced or absent. This appears to be a derived feature but is not unique to this group, having also occurred in *Neboissophlebia*, *Bibulmena*, *Loamaggalangta*, and *Kaninga*. The nymphs of these genera are commonly found in slow-flowing habitats and a reduction or loss of ventral teeth maybe an adaptation to such environments (Dean 1999) and could have evolved on a number of occasions. The genera of the *Hapsiphlebia* lineage share many additional morphological characters distinguishing them from genera of other lineages, but these appear to be ancestral (Pescador and Peters 1980; Towns and Peters 1980).

Relationships among the genera of the *Hapsiphlebia* lineage were poorly supported, except for the sister group relationship between *Jappa* and *Ulmerophlebia*. These two genera are very closely related and morphologically very similar. The major difference between the nymphs of these genera is the presence of large frontal horns in *Jappa*. The many similarities of the nymphs and adults of the two genera have previously been discussed by Tsui and Peters (1975) and Suter (1986). Tsui and Peters (1975) also speculate that *Jappa* may have evolved from an *Ulmerophlebia*-like ancestor.

Kalbaybaria, placed at the very base of the tree, has many characters not shared by any other member of the *Hapsiphlebia* lineage. The anterior margin of the labrum lacks denticles and the setae on the dorsal surface are scattered rather then organised into distinct rows. Subapical pectinate setae are absent from the galea-lacinia of the maxilla, the second segment of the labial palp is elbow shaped, and the third segment bears elongated setae. Campbell (1993) commented on the similarity of the nymphal gills and shape of the penes of *Kalbaybaria* to those of some species of *Atalophlebia*; however, these genera did not group together in the present study.

The placement of *Neboissophlebia*, *Bibulmena*, *Loamaggalangta* and *Kaninga* between the genera of the *Hapsiphlebia* lineage and *Garinjuga* of the *Penaphlebia* lineage is in agreement with Dean (1987, 1988, 2000) and Dean *et al*. (1999). Their inclusion at the base of a larger clade containing all other genera, except those of the *Hapsiphlebia* lineage, was strongly supported. *Neboissophlebia* was the most basal of the four, followed by *Bibulmena*, *Loamaggalangta* and *Kaninga*. These genera appear to have close affinities to each other but may not all belong to a single monophyletic group. Interestingly, all four genera are found in south-western Australia, and *Bibulmena* and *Kaninga* are endemic to this region. These four genera appear to belong to lineages not recognised previously.

Neboissophlebia, *Bibulmena*, *Loamaggalangta* and *Kaninga* differ from the genera of the *Hapsiphlebia* lineage in the morphology of the mouthparts of the nymph, especially the shape of the mandible. The nymphal mouthparts of these genera also display several features that are believed to be more derived than those found in the *Hapsiphlebia* lineage. The labrum is subequal or broader than the clypeus, a median setal tuft is present along the outer margin of the mandible, the prosthecal tuft is elongated, and the inner margin of segment 3 of the labial palp has small denticles rather then stout spines. As discussed by Dean (1987, 1988, 2000) and Dean *et al*. (1999) these genera retain several ancestral character states which exclude them from other lineages, particularly the ventrally curved glossae of the labium and the presence of denticles along the inner margin of segment 3 of the labial palp. They cannot be placed into the *Penaphlebia* lineage because they lack the defining pectinate setae on the second segment of the maxillary palp. As previously mentioned, the nymphs of *Neboissophlebia and Loamaggalangta* have smooth tarsal claws and those of *Bibulmena* and *Kaninga* have tarsal claws with small denticles, in which feature they resemble genera of the *Haspiphlebia* lineage.

Garinjuga is the only known Australian genus in the *Penaphlebia* lineage, which also includes *Penaphlebia* and *Massartella* from South America (Pescador and Peters 1980). It was placed in the *Penaphlebia* lineage by Campbell and Suter (1988) on the presence of several derived character states, including pectinate setae on the second segment of the maxillary palp. In the present study, *Garinjuga* was placed at the base of a clade comprising genera of the *Nousia*, *Dactylophlebia* and *Meridialaris* lineages. Although there is good support for this clade, the basal position of *Garinjuga* within the clade was poorly supported.

The grouping of *Nyungara*, *Nousia* and *Koorrnonga* into a monophyletic clade was well supported and is consistent with the current placement of these genera (Pescador and Peters 1980; Dean 1987; Campbell and Suter 1988). Derived character states given by Pescador and Peters (1980) for the lineage include the anterior margin of the nymphal labrum having a broad emargination with prominent denticles. In the present study, a synapomorphic reduction in the number of cross veins of the hind wing was observed in all genera and the length of the Sc vein ranged from 0.6 to 0.8 times the length of the hind wing. Relationships among *Nyungara*, *Nousia* and *Koorrnonga*, although resolved, were poorly supported. Additional characters will be required to determine sister group relationships within the clade. In Australia, *Nousia* and *Koorrnonga* both occur in eastern Australia including Tasmania, but *Nousia* is also found in south-western Australia (Dean 1999). *Nyungara* is presently restricted to south-western Australia (Dean 1987, 1999).

The clade comprising the two New Zealand genera of the *Dactylophlebia* lineage, *Mauiulus* and *Austroclima*, was well supported. As proposed by Pescador and Peters (1980) the *Dactylophlebia* lineage appears to be the sister clade to the *Meridialaris* lineage. Features shared by the genera of these two groups include: labrum of the nymph with narrow emargination along anterior margin and greatly reduced denticles (Pescador and Peters 1980); ninth abdominal sternum of adult female entire or with a shallow apical cleft; and dissimilar tarsal claws in the adult. The *Dactylophlebia* lineage has representative genera in southern South America and New Zealand but is not recorded in Australia.

There was strong support for the monophyly of the *Meridialaris* lineage, comprising *Austrophlebioides*, *Tillyardophlebia*, *Kirrara*, WT sp. 1, and WT sp. 2 from Australia, *Meridialaris* from South America, and *Deleatidium* and *Atalophlebioides* from New Zealand. These genera share several derived character states, all of which have been identified previously by Pescador and Peters (1980), as follows. In the nymph, the lateral margins of the submentum are bare or have only a few basal spines. The galea-lacinia of the maxilla is broad at the apex and usually has more than 22 subapical pectinate setae, slightly fewer in *Kirrara*. The anterior margin of the labrum has a narrow U- or V-shaped emargination that is hooded dorsally, in all genera except in *Kirrara*. It appears that this emargination may have been secondarily lost in *Kirrara* (Campbell and Peters 1986). In the adult, the tarsal claws are dissimilar, and the ninth abdominal sternum of the female is entire or has a very shallow cleft, except in some species of *Deleatidium* which have a more pronounced cleft (Towns and Peters 1996). Dissimilar claws are not unique to this group but appear to have evolved independently several times within the Leptophlebiidae, including *Jappa*, *Ulmerophlebia* and *Neboissophlebia*.

Two additional derived character states given by Pescador and Peters (1980) for the *Meridialaris* lineage are: (i) the outer margin of the mandible distinctly angular, and (ii) labrum with a length width ratio of $^{1}/_{3}$ to $^{1}/_{2}$. The outer margin of the mandible is very angular in *Kirrara* and *Deleatidium*, but is variable among the other genera. The shape of the outer margin was a difficult character to code into discrete states and was not included in this study. Similarly, labrum shape was variable within this lineage and a labrum with a length/width ratio of $^{1}/_{3}$ to $^{1}/_{2}$ was not unique to this group.

The two undescribed taxa, WT sp. 1 and WT sp. 2, clearly belong to the *Meridialaris* lineage. Both occupy basal positions within the lineage but support for these nodes was low. The nymphs of these two taxa share some similarities with those of *Tillyardophlebia* but the genitalia of the adult males differ in shape. The penes of the two undescribed taxa lack the diagnostic pair of ventral spines present at the base of the penes of *Tillyardophlebia* and are not divided but fused along most of their length. It is likely that these taxa represent two new genera.

Kirrara appeared to have a closer relationship to *Deleatidium* and *Atalophlebioides* of New Zealand than to the other Australian genera within this lineage, although support for this clade was low. The grouping of *Deleatidium* and *Atalophlebioides* is consistent with Towns and Peters' (1978, 1996) suggestion that *Atalophlebioides* is most closely related to *Deleatidium*. The penes of these two genera are both fused and have a midventral subapical appendage. The tarsal claws of the adult are dissimilar, as in other genera of the lineage; however, in *Deleatidium* and *Atalophlebioides* the pad-like claw has a small apical hook, not found in the other genera. In the nymph, the prosthecal tuft of the mandible is greatly reduced in both genera.

Relationships among *Tillyardophlebia*, *Austrophlebioides, Meridialaris* and the clade containing *Kirrara*, *Deleatidium* and *Atalophlebioides* could not be resolved in the strict consensus tree. This was partly due to homoplasy among several characters. For example, the outer incisors of the mandibles are serrated in *Deleatidium*, *Atalophlebioides, Kirrara* and *Austrophlebioides*, but not serrated in *Meridialaris* and *Tillyardophlebia*. The outer incisors of WT sp. 2 are serrated but those of WT sp. 1 are not. This implies that serrations may have evolved independently on several occasions or have been secondarily lost in some genera. Similarly, fine setae along the outer margin of the mandible between the outer incisor and the median setal tuft occur in all genera except for *Kirrara, Deleatidium* and *Atalophlebioides*. The nymphs of many of the genera of the *Meridiararis* lineage have one or two elongated spines along the inner margin of the tarsi, exceptions being *Tillyardophlebia*, WT sp. 1 and WT sp. 2. Again these elongated spines may have evolved on more than one occasion or may have been secondarily lost. Clearly additional characters will be required to resolve relationships within the *Meridialaris* group.

CHAPTER 3

Three new species of *Austrophlebioides* Campbell and Suter (Ephemeroptera: Leptophlebiidae: Atalophlebiinae) from the Wet Tropics bioregion of north-eastern Australia

3.1 Introduction

The genus *Austrophlebioides* was established by Campbell and Suter (1988) to resolve some of the confusion that resulted from placement of several Australian species (Harker 1950, 1954) into the genera *Deleatidium* Eaton and *Atalophlebioides* Phillips, both of which were subsequently recognized as endemic to New Zealand (Towns and Peters 1978; 1996). *Deleatidium pusillum* Harker was redescribed by Campbell and Suter (1988) from material collected from the type locality, and designated the type species of a new genus, *Austrophlebioides*. At that time *Deleatidium unguiculare* (Ulmer) was also transferred by Campbell and Suter (1988) to *Austrophlebioides*. Two additional species of *Austrophlebioides*, *A. marchanti* and *A. booloumbi,* were described by Parnrong and Campbell (1997) and the generic diagnosis modified to accommodate *A. marchanti*. The original diagnosis of the genus included the presence of a series of fine ventral spines on the penes. These are absent in *A. marchanti*, and Parnrong and Campbell (1997*)* regard this character as species-specific rather than characteristic of the entire genus. On the basis of the morphology of the male genitalia, Parnrong and Campbell (1997*)* also transferred *Deleatidium decipiens* Harker into *Austrophlebioides*. The genus presently comprises five described species, *A. pusillus*, *A. unguicularis*, *A. marchanti*, *A. booloumbi* and *A. decipiens*, all of which are endemic to Australia.

Several undescribed species of *Austrophlebioides* are also known. Dean (1999) has recognised and provided a key to nymphs of eleven species of *Austrophlebioides*. Included in the key were *A. pusillus* and *A. marchanti*, whose nymphs are known, and nine other undescribed species. *A. unguicularis, A. booloumbi* and *A. decipiens* are known only from adult material and thus it is possible that some of the undescribed species recognised by Dean (1999) are the nymphs of these species.

The nymphs of *Austrophlebioides* are typically found on stones in cool forest streams. The adults are aerial/terrestrial and live for only a few days. The genus is widely distributed in Australia (Dean 1999; Chapter 4). Until recently, taxonomic studies on the genus have centered around New South Wales and Victoria, with the fauna of northern and north-eastern Australia receiving little attention. In this study three new species of *Austrophlebioides* are described from the Wet Tropics bioregion of north-eastern Australia and the generic diagnosis modified. Evolutionary relationships within the genus are briefly discussed, and are explored further in Chapter 4.

3.2 Materials and methods

3.2.1 Collection

Imagos (adults) and subimagos (subadults) were captured using a light trap and a white sheet. A black UV fluorescent light powered by a 12V battery was used to attract mayflies. Light traps were set up alongside streams, and operated from 1 hour before sunset until 1 to 2 hours after sunrise. Subimagos and female imagos were collected successfully using this method; however, few male imagos were attracted. A white sheet illuminated by a black light was also used to collect emergent mayflies. Live subimago males were kept in holding containers until they moulted into the imago stage or were placed directly into 70% ethanol. Nymphs were carefully removed from stones in streams using forceps. They were either preserved in 70% ethanol or kept alive in aerated containers for rearing.

3.2.2 Rearing

The association of nymphs and imagos was made through rearing. Mature nymphs were placed singly in rearing chambers similar to those recommended by Edmunds *et al*. (1976). The chamber consisted of a plastic cup with side panels removed and replaced by nylon mesh to allow circulation of water. The chamber was capped with a piece of mesh secured by a rubber band and placed in a small aquarium tank filled with water collected from the same stream locality as the nymph. The tanks were aerated via a pump and small air stone. Nymphs were reared at temperatures ranging from 18° to 24°C, which were typical of the water temperatures from which the animals were collected. No food was provided as all nymphs used for rearing were mature final instar nymphs. On emergence the subimago was carefully transferred to a dry chamber, where it was allowed to moult to the imago stage. The nymphal cast was removed from the rearing chamber and placed in 70% ethanol.

3.2.3 Examination of specimens and slide preparation

All material examined was preserved in 70% ethanol. Imago wings were dissected and dry mounted on slides as recommended by Edmunds *et al*. (1976). The dissected wings were floated on top of several drops of 70% ethanol placed on a slide. They were then arranged on the slide and a cover slip was carefully placed over the wings. When the ethanol evaporated the cover slip was secured with two thin strips of white gummed paper. The male genitalia were placed in 10% potassium hydroxide overnight at room temperature to clear; the next day they were rinsed in water, placed back into 70% ethanol, transferred to isopropanol and mounted in Euparal. Water rather than glacial acetic acid was used to stop the clearing process because the use of glacial acetic acid caused the claspers of the male imago to collapse. The mouthparts, fore and hind legs and some abdominal gills of nymphs were also mounted in Euparal.

3.2.4 Scanning electron microscopy

Specimens for scanning electron microscopy were dehydrated in a graded series of ethanol up to 100%, and critical point dried. They were then mounted on to stubs, sputter coated with gold or platinum, and viewed with a Phillips XL 20 or JEOL 5410 LV scanning electron microscope.

3.2.5 Terminology and measurements

The terminology used in this study follows Edmunds *et al*. (1976). All measurements are in millimetres and were made using a stereo dissecting or compound microscope and an ocular micrometer. The relative lengths of the segments of the male foreleg are given as a ratio of the length of each segment to the length of the tibia. The actual length of the tibia is presented in parentheses. All drawings were prepared using a micro-projector or a camera lucida attached to either a Leica MZ6 stereo microscope or an Olympus BH-2 compound microscope. Specimens are lodged with the Queensland Museum (QM), Brisbane, Australia. All specimens were collected by the author, F. Christidis.

3.3 Description of new species

Genus *Austrophlebioides* Campbell and Suter 1988

Type species. *Austrophlebioides pusillus* (Harker 1954) (originally placed in *Deleatidium*).

Austrophlebioides wooroonooran sp. nov.

(Figures 3.1-3.4)

"Henrietta" Christidis (2001)

Type material. Queensland. Holotype, male imago reared from nymph, Henrietta Ck, Wooroonooran National Park (17°33.9'S, 145°45.4'E), collected 5 June 1999, F. Christidis, in QM. Paratypes, type locality: 1 reared male imago, 18 June 1998; 5 reared male imagos and 2 reared female imagos, 30 May 1999; 1 reared male imago and 1 reared female imago, 5 June 1999. All collected by F. Christidis, in QM.

Other material examined. Queensland. Type locality: 2 reared male subimagos, 30 May 1999; 2 reared male subimagos, 5 June 1999; 5 male imagos, 29 May 1999; 1 nymph, 31 December 1997; 8 nymphs, 24 April 1998; 2 nymphs, 30 May 1999; 2 nymphs 5 June 1999. Charmillan Ck (17°42.1'S, 145°31.3'E): 1 reared male imago, 3 reared female imagos, 1 reared female subimago and 12 nymphs, 1 June 1999. All collected by F. Christidis, in QM.

Etymology. Named for the type locality, Wooroonooran National Park. To be used as a noun in apposition.

Male Imago. Length: body 7.1-8.3 mm, forewings 8.0-8.7 mm. Head pale brown. Eyes touching dorsally. Upper portion of eye brownish-red, lower portion black. Antennae pale brown. Thorax mostly pale brown. Wings hyaline (Fig. 3.1a,b), costal and subcostal cells in apical third of forewing whitish, longitudinal veins and cross veins yellowish brown. Forewing with 16-20 costal cross veins; MA forked at about 0.5 distance from base of vein to margin of wing, fork symmetrical; MP_2 attached to MP_1 at about 0.2 length of MP_1; ICu_1 usually attached at base to CuA-CuP cross vein. Hind wing 0.23-0.24 times length of forewing, hind wing width 0.58-0.64 of length. Hind wing with 6-8 costal cross veins and 5-7 subcostal cross veins; costal margin with concavity at about midlength; vein Sc 0.95-0.96 length of hind wing (Fig. 3.1b). Foreleg with femur golden brown, remainder of leg pale yellowish-brown. Middle and hind legs pale yellowish-brown. Length ratios of segments in foreleg 0.82-0.90: 1.00 (2.5- 2.8 mm): 0.04-0.05: 0.35-0.42: 0.31-0.38: 0.23-30: 0.10-0.11. Claws dissimilar, one with an apical hook the other obtuse, pad-like (Fig. 3.1f). Abdomen as in Fig. 3.1c. Terga 1-6 translucent whitish in appearance, terga 7-10 opaque reddish-brown. Anterior of terga 7-8 with paired pale submedian maculae. Posterior margin of all terga with a transverse black band (Fig. 3.1c). Sternite 1 brown along lateral margins, sterna 2-6 translucent, and sterna 7-10 opaque pale brown with a pink hue. Genitalia pale brown to cream. Claspers 3 segmented, segment 1 narrowing at about 0.3 of length, segment 3 slightly shorter than segment 2 and about 0.2 length of segment 1 (Fig. 3.1d). Penes fused in basal 0.8 of length, widest at base tapering slightly towards apex (Figs 3.1d, 3.2a,b). Each penis lobe with a large subapical dorsal spine, most of which is embedded within lobe with only tip of spine showing at surface (Fig. 3.1e). Prominent ventral projection present on each lobe of penes (Fig. 3.2c,d). Caudal filaments pale brown with reddish-brown bands at annulations, terminal filament slightly longer than cerci.

Female Imago. Length: body 7.2-8.5 mm, forewings 9.2-10.0 mm. Similar to male except as follows. Eyes black, separated on meson of head by a distance of 2.5 times the maximum width of an eye. Fore wing with 19-22 costal cross veins. Hind wing with 7-9 costal cross veins and 7-9 subcostal cross veins. Width of hind wing 0.56-0.57 of length. Hind wing length 0.22-0.24 length of forewing. Pronotum of thorax pale brown with reddish-brown pigmentation. Abdomen reddish-brown with paired pale submedian maculae present at anterior margin of all terga. Sterna pale brown to cream with limited dark reddish-brown pigmentation. Ninth sternum entire, pointed apically (Fig. 3.1g).

Subimago. Similar in appearance to imago except as follows. Wings pale brown. Abdominal terga 1-6 of male subimago opaque pale brown, submedian maculae visible at anterior of terga 2-6 as well as on terga 7-8; sterna opaque pale brown, sterna 1-2 with some darker brown markings.

Nymph. Body length: male 7.0-8.9 mm, female 7.5-9.3 mm. Head prognathus, brown. Antennae approximately two times length of head. Mouthparts as in Figs 3.3a-f. Maximum width of labrum 1.19 to 1.28 times maximum width of clypeus. Lateral margins of clypeus diverging apically (Fig. 3.3a). Anterior margin of labrum with narrow median notch, hooded dorsally; lateral margins rounded; maximum width of labrum 2.50 to 2.75 times maximum length (Fig. 3.3a). Outer margin of mandible smoothly rounded with small median setal tuft, fine setae absent on outer margin between tuft and outer incisor (Fig. 3.3b). Incisors slender with serrated apical teeth. Prostheca well developed. Ligula of hypopharynx with well developed lateral processes, anterior margin cleft (Fig. 3.3c). Maxillae broad apically (Fig. 3.3d), with 26-28 subapical pectinate setae. Segments 1 and 2 of maxillary palp of similar length, segment 3 0.61-0.68 length of segment 2. Labial palp with segment 1 and 2 subequal in length, segment 3 0.41-0.45 length of segment 2. Glossae on about the same plane as paraglossae (Fig. 3.3e). Lateral margins of submentum without hairs (Fig. 3.3e). Thorax brown. Pronotum with small spines on anterolateral margin. Forelegs pale brown with dark brown markings (Fig. 3.4a). Middle and hind femur with small round pale area near base (Fig. 3.4b). Inner margin of middle and hind tarsi with 1 or 2 elongated spines in apical third. Tarsal claws with ventral teeth, teeth increasing in size apically. Abdomen with posterolateral spines present on segments 2-9. Lateral margins of abdominal segments fringed with fine setae. Abdomen brown with some lighter and darker areas. Gills lanceolate, present on segments 1-7; lower and upper lamella about the same size; lateral tracheae well developed (Fig. 3.4c). Gill lamellae translucent, cream coloured; tracheae black. Caudal filaments brown, up to two and a half times length of body, each segment with whorls of spines and short setae.

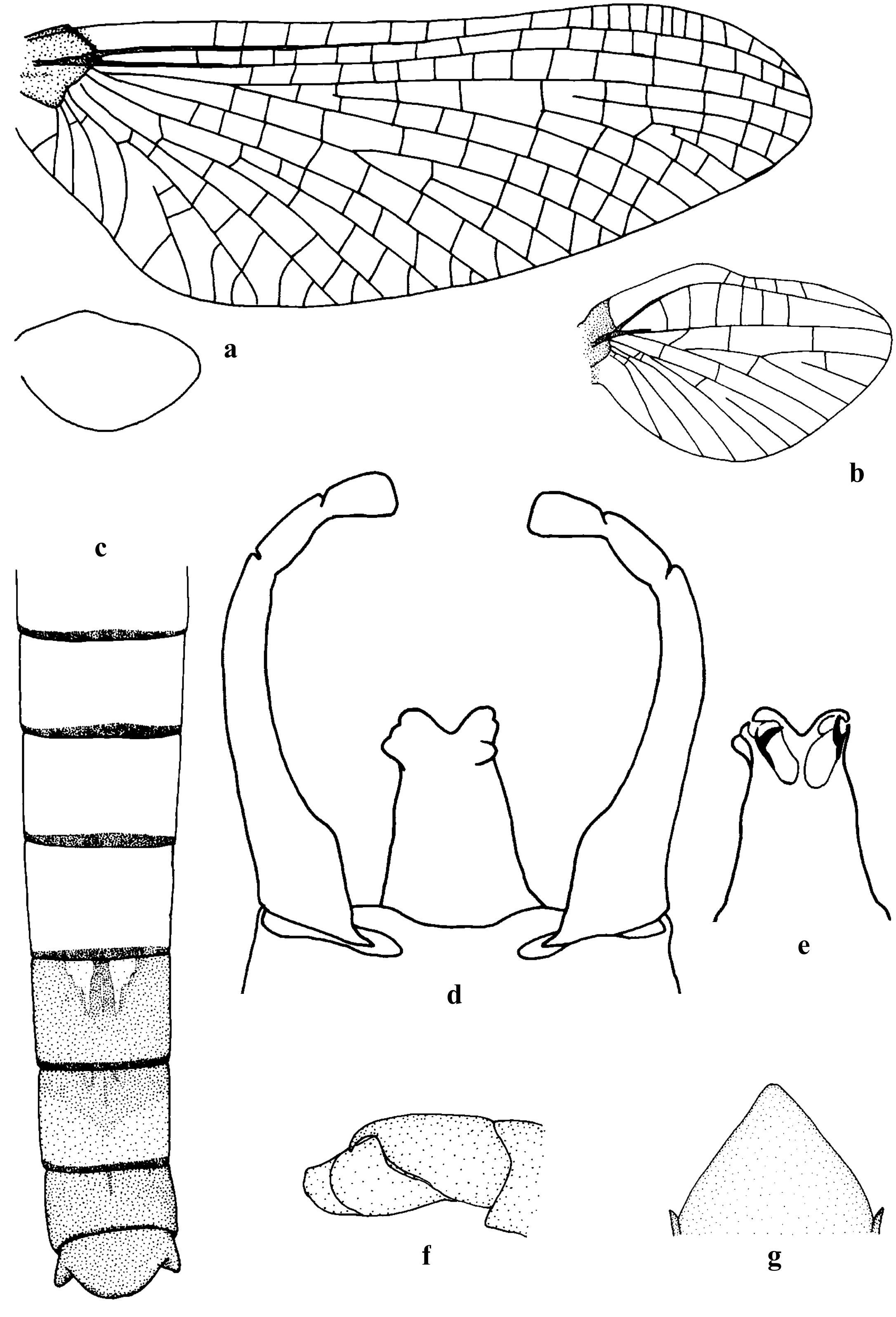


Figure 3.1. *Austrophlebioides wooroonooran*. Male imago: (a) wings; (b) hind wing; (c) abdominal terga 3-10; (d) penes, ventral view; (e) penes, dorsal view; (f) tarsal claw. Female imago: (g) ninth abdominal sternum.

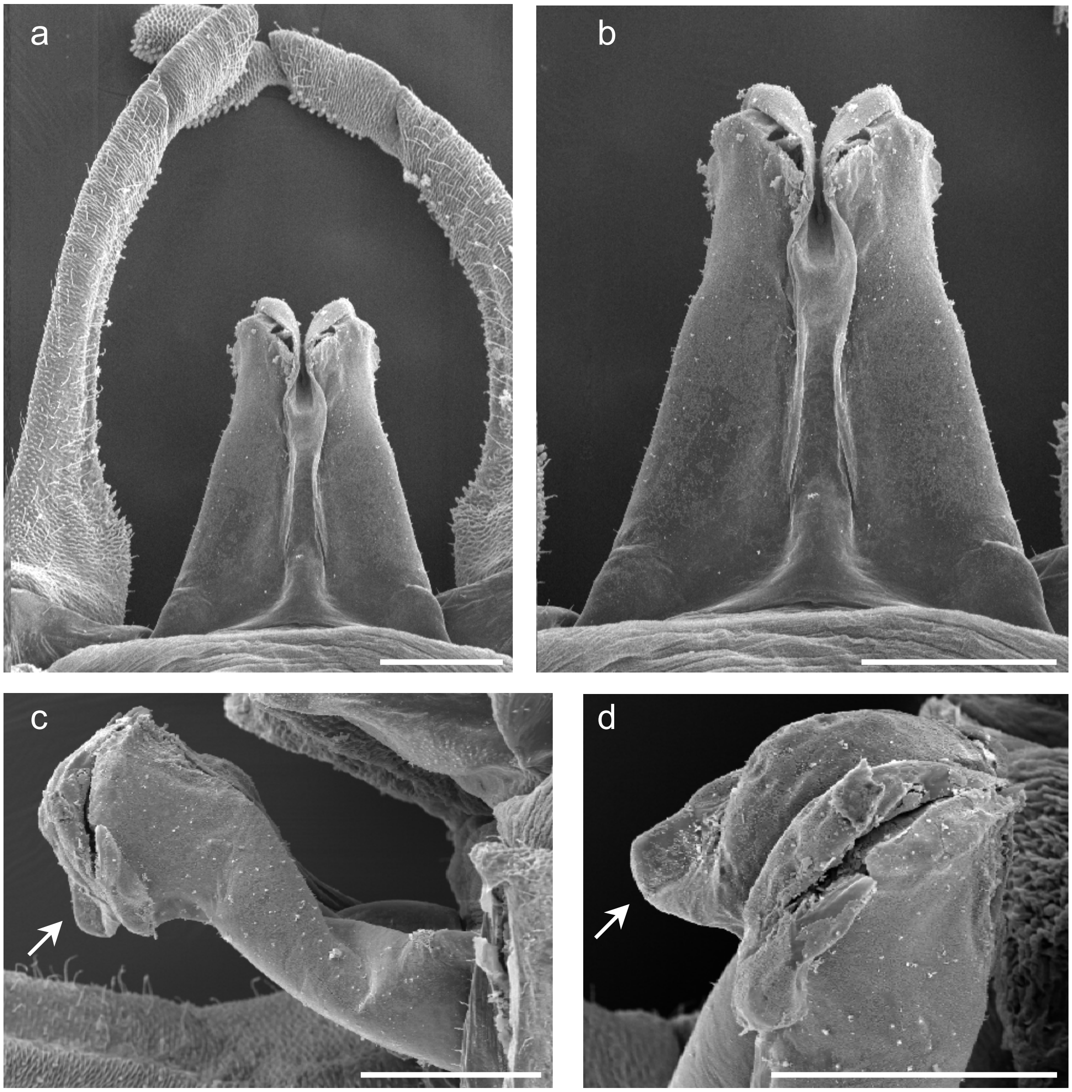


Figure 3.2. *Austrophlebioides wooroonooran*, scanning electron micrographs of genitalia of male imago: (a) penes and claspers, dorsal view; (b) penes, dorsal view; (c) penes, lateral view; (d) apex of penes. Arrow indicates ventral projection of penes lobe. Scale bar = 100 µm.

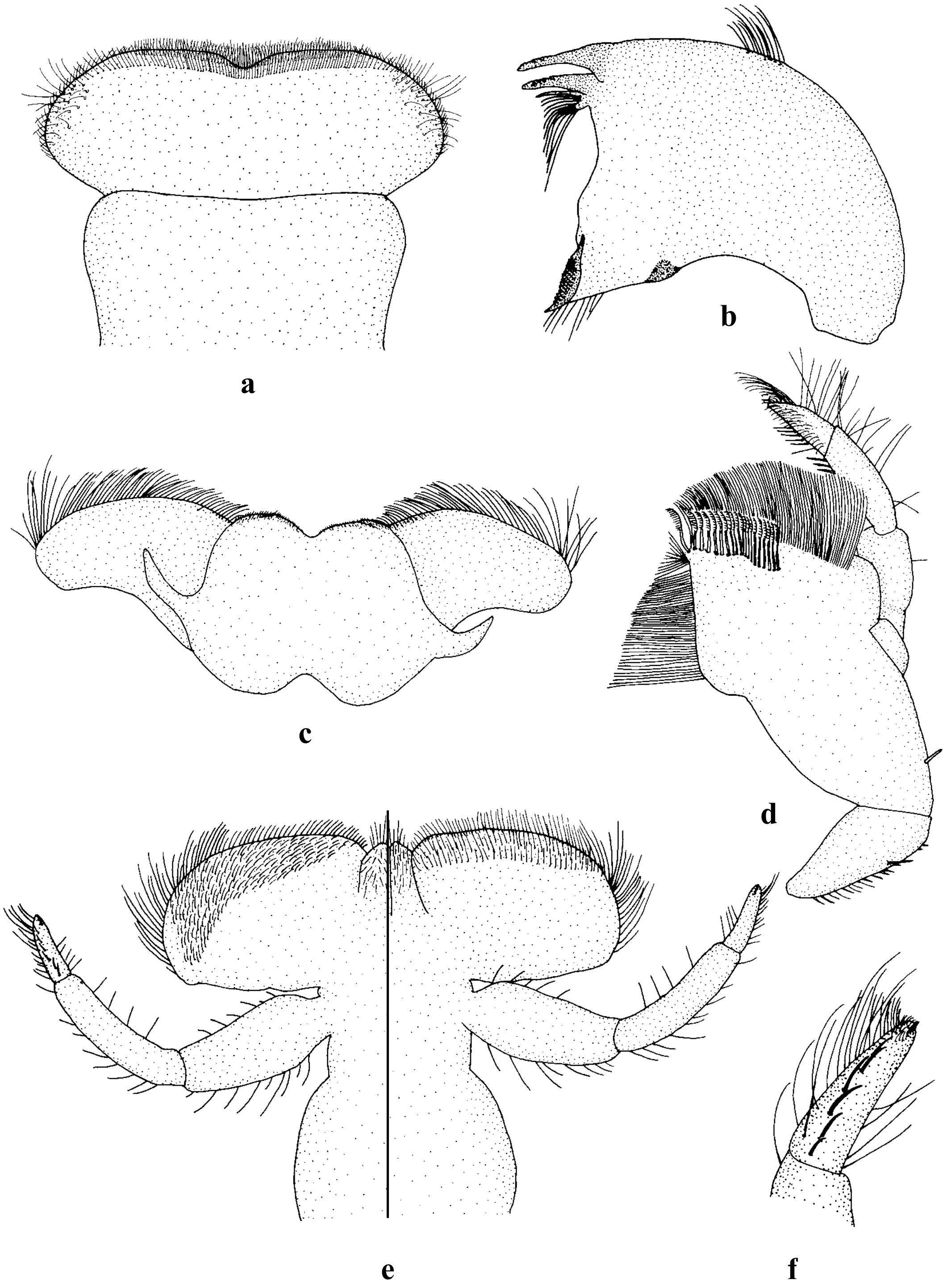


Figure 3.3. *Austrophlebioides wooroonooran*. Nymph: (a) labrum and clypeus; (b) right mandible; (c) hypopharynx; (d) maxilla; (e) labium, dorsal view left of line and ventral view right of line; (f) segment 3 of labial palp, dorsal view.

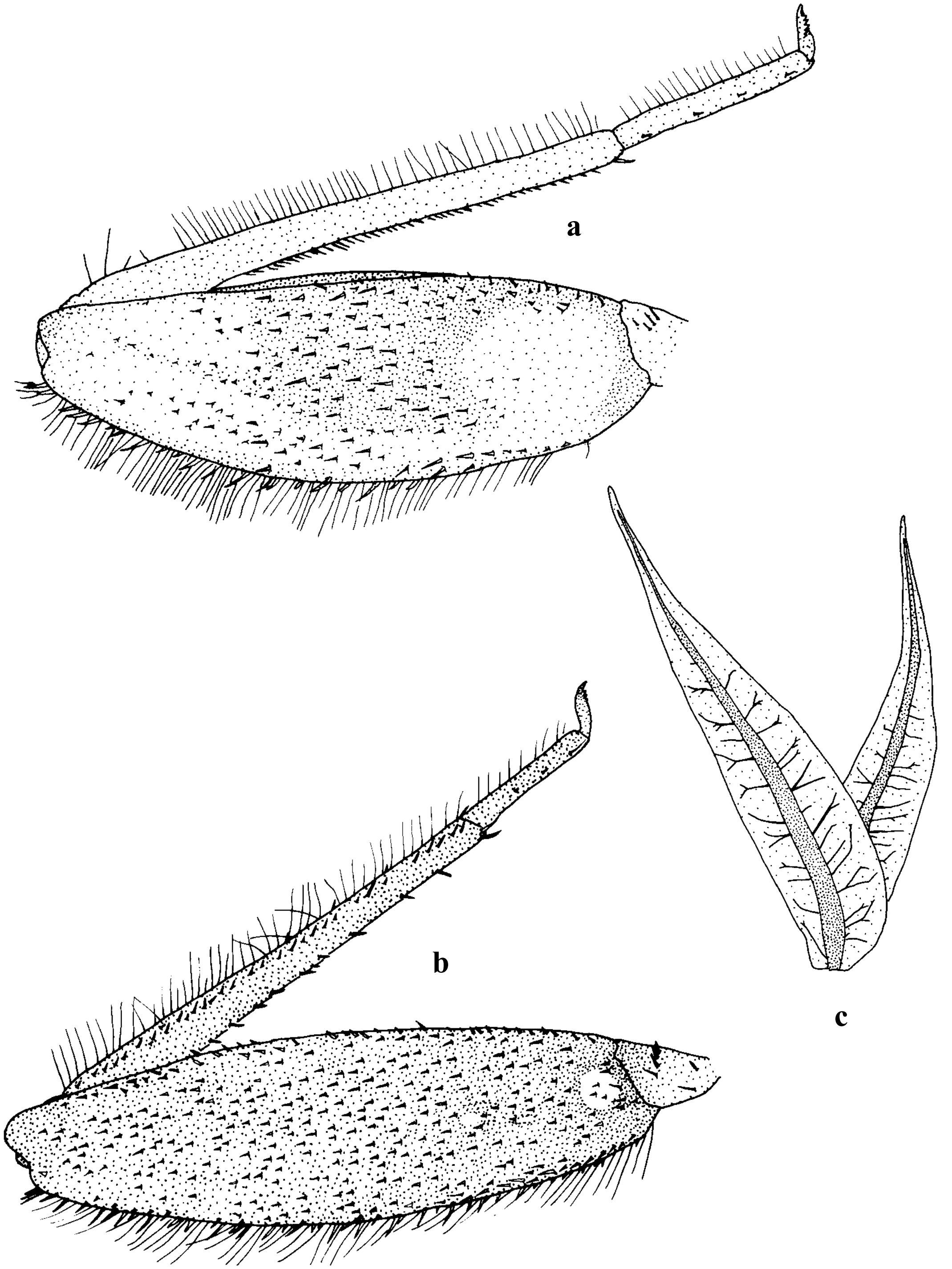


Figure 3.4. *Austrophlebioides wooroonooran*. Nymph: (a) foreleg; (b) hind leg; (c) abdominal gill.

Remarks. *Austrophlebioides wooronooran* can be distinguished from all other species of *Austrophlebioides* by the following combination of characters. In the male imago by: (1) each lobe of penes with ventral projection; (2) penes without ventral spines; (3) segment1 of claspers narrowing at 1/3 of length, (4) penes reaching beyond 0.55 length of segment 1 of clasper; (5) base of penes not overly broad, penes tapering slightly towards apex; (6) abdominal terga with prominent transverse black band along posterior margin; (7) longitudinal veins and cross veins of forewing distinct. In the nymph by: (1) the absence of fine hairs along the outer margin of the mandible between the outer incisor and the median setal tuft; (2) gills cream coloured; (3) lateral margins of labrum rounded; (4) femur of mid and hind legs with small round pale area near base.

Habitat. The nymphs of *A*. *wooronooran* are found on stones within streams in areas of slow to moderate flow, such as rock pools and runs.

Austrophlebioides rieki **sp. nov.**

(Figures 3.5-3.7)
“Daintree” Christidis (2001)

Type material. Queensland. Holotype, male imago reared from nymph, Oliver Ck, Daintree National Park (16^{0}8.3’S, 145^{0}26.4’E), collected 23 August 1999, F. Christidis, in QM. Paratypes: 6 reared male imagos and 5 reared female imagos, same locality and collection date as holotype, F. Christidis, in QM.

Other material examined. Queensland. Type locality: 12 nymphs, 22 August 1999; 1 reared male subimago and 1 reared female subimago, 23 August 1999; 1 nymph, 28 December 1997. Emmagen Ck (16^{0}2.5’S, 145^{0}27.3’E): 3 reared male imagos and 1 reared female imago, 19 June 1998. All collected by F. Christidis, in QM.

Etymology. This species is named for Edgar Riek in recognition of his contribution to mayfly research.

Male Imago. Length: body 5.9-7.2 mm, forewings 6.0-7.3 mm. Head pale brown to pale reddish-brown. Eyes touching dorsally. Upper portion of eye brownish-red, lower portion black. Ocelli whitish with black bases. Antennae pale reddish-brown. Thorax brown. Wings hyaline (Fig. 3.5a,b), costal and subcostal cells in apical third of forewing whitish, longitudinal and cross veins pale yellowish-brown. Forewing with 14-18 costal cross veins; MA forked at about 0.5 distance from base of vein to margin of wing, fork symmetrical; MP_2 attached to MP_1 at about 0.2 length of MP_1; ICu_1 attached at base to CuA-CuP cross vein. Hind wing 0.22-0.24 times length of forewing, hind wing width 0.63-0.65 of length. Hind wing with 6-8 costal cross veins and 5-6 subcostal cross veins; costal margin with concavity at about midlength; vein Sc 0.94-0.96 length of hind wing (Fig. 3.5b). Foreleg with femur golden brown, remainder of leg pale yellowish-brown. Middle and hind legs pale yellowish-brown. Length ratios of segments in foreleg 0.83-0.92: 1.00 (1.7-2.3 mm): 0.04: 0.38-0.46: 0.38-0.42: 0.27-0.31: 0.11-0.12. Claws dissimilar, one with an apical hook the other obtuse, pad-like (Fig. 3.5f). Abdomen as in Fig. 3.5c. Terga 1-6 translucent whitish in appearance, terga 7-10 reddish-brown. Terga 7-8 with paired pale submedian maculae along anterior margin. Posterior margin of all terga with dark brown to black band (Fig. 3.5c). Sternite 1 brown, sterna 2-6 translucent, and sterna 7-10 opaque pale brown. Genitalia pale brown. Claspers 3 segmented; segment 1 narrowing at 0.3 of length; segment 3 slightly shorter than segment 2 and about 0.2 length of segment 1 (Fig. 3.5d). Penes fused along basal 0.85 of length, widest at base and tapering towards apex (Figs 3.5d, 3.6a,b). Each penis lobe with large subapical dorsal spine, most of spine embedded within lobe with only the tip of the spine showing at surface (Fig. 3.5e). A prominent ventral projection is present on each lobe of penes (Fig. 3.6c,d). Caudal filaments whitish with reddish-brown bands at annulations, terminal filament slightly longer than cerci.

Female Imago. Length: body 5.9-7.0 mm, forewings 6.6-8.0 mm. Similar to male except as follows. Eyes black, separated of meson on head by a distance of approximately two times the maximum width of an eye. Thorax pale brown. Fore wing with 17-19 costal cross veins. Hind wing with 5-7 costal cross veins and 6-7 subcostal cross veins. Hind wing width 0.57-0.58 of length. Hind wing length 0.19-0.22 length of forewing. Abdomen reddish-brown; paired pale submedian maculae present at anterior margin of all terga, most clearly visible on terga 2-8. Sterna pale brown, sterna 1-2 with reddish brown markings. Ninth sternum entire, pointed apically (Fig. 3.5g).

Subimago. Similar in appearance to imago except wings pale brown and abdominal terga 1-6 of male subimago opaque pale brown.

Nymph. Body length: male 6.6–7.4 mm, female 6.1–7.7 mm. Head prognathus, brown. Antennae about twice the length of head. Mouthparts as in Figs 3.7a-f. Maximum width of labrum 1.28 to 1.38 times maximum width of clypeus. Lateral margins of clypeus diverging apically (Fig. 3.7a). Anterior margin of labrum with narrow median notch, hooded dorsally; lateral margins angular; maximum width of labrum 2.86 to 3.03 times maximum length (Fig. 3.7a). Outer margin of mandible smoothly rounded with small median setal tuft; fine setae absent on outer margin between tuft and outer incisor (Fig. 3.7b). Incisors slender with serrated apical teeth. Prostheca well developed. Ligula of hypopharynx with well developed lateral processes, anterior margin cleft (Fig. 3.7c). Maxillae broad apically (Fig. 3.7d), with 26-28 subapical pectinate setae. Segments 1 and 2 of maxillary palp of similar length, segment 3 0.60-0.71 times length of segment 2. Labial palp with segment 1 and 2 subequal in length, segment 3 0.40-0.43 times length of segment 2. Glossae on about the same plane as paraglossae (Fig. 3.7f). Lateral margins of submentum without hairs (Fig. 3.7f). Thorax brown. Pronotum with small spines on anterolateral margin. Legs pale brown with darker markings (Figs 3.8b,d). Middle and hind femurs with a pale area in basal half in the form of an elongated streak (Fig 3.8d). Inner margin of middle and hind tarsi with 1 or 2 elongated spines in apical third (Fig 3.8c). Tarsal claws with ventral teeth, teeth increasing in size apically. Abdomen with posterolateral spines present on segments 2-9. Lateral margins of abdominal segments fringed with fine setae. Abdominal terga brown with some lighter and darker areas. Abdominal sterna pale brown. Gills lanceolate, present on segments 1-7; lower and upper lamella about the same size; lateral tracheae moderately developed (Fig. 3.8a). Gill lamellae translucent, creamed coloured, tracheae black. Caudal filaments 1.5 to 2.0 times length of body, each segment with whorls of spines and short setae.

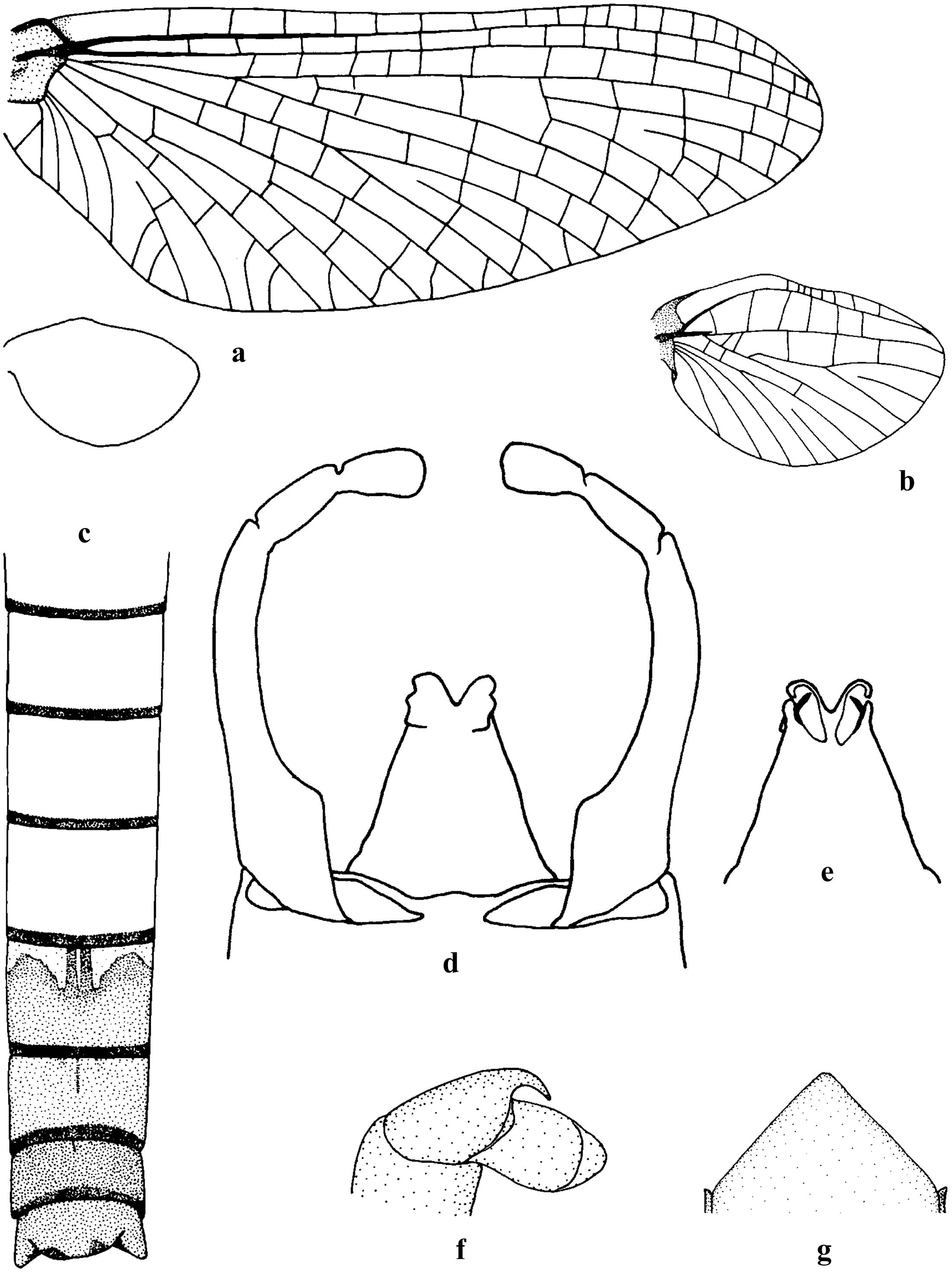


Figure 3.5. *Austrophlebioides rieki*. Male imago: (a) wings; (b) hind wing; (c) abdominal terga 3-10; (d) penes, ventral view; (e) penes, dorsal view; (f) tarsal claw. Female imago: (g) ninth abdominal sternum.

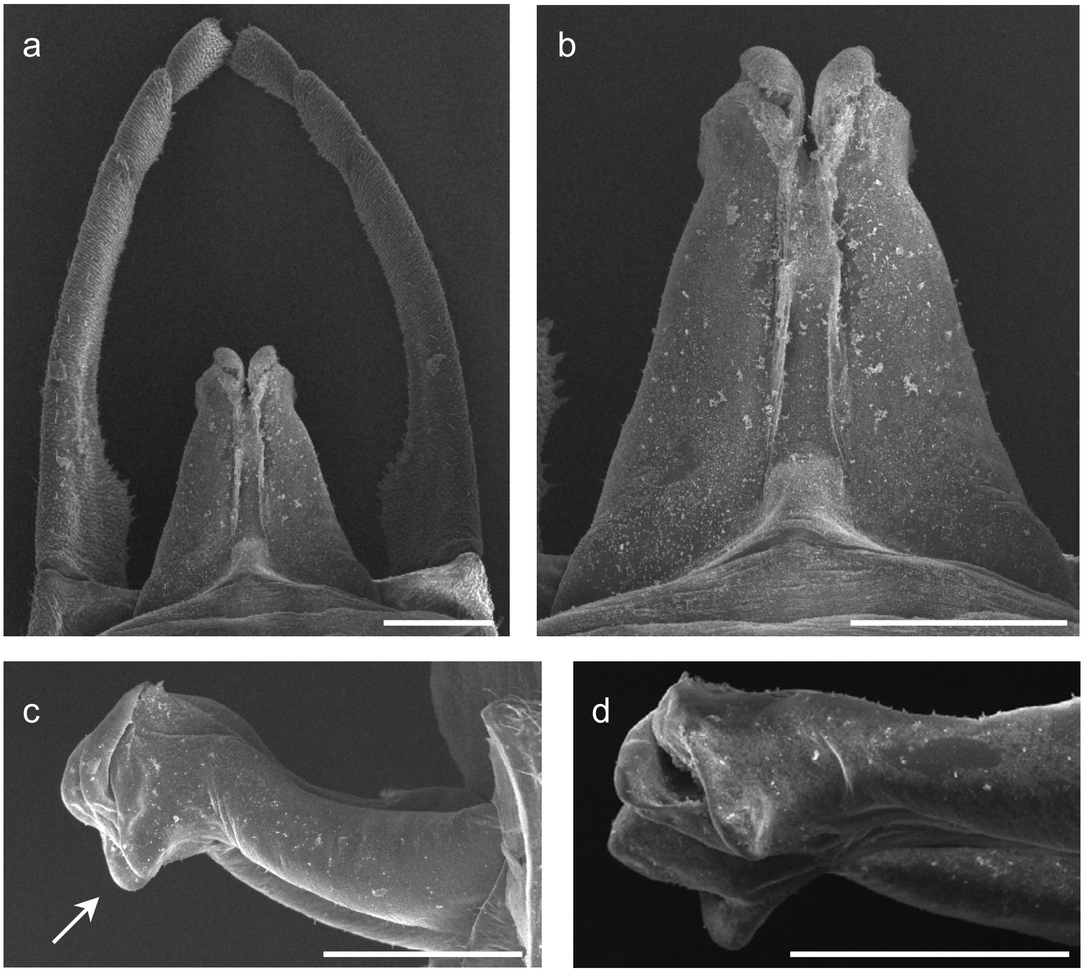


Figure 3.6. *Austrophlebioides rieki*, scanning electron micrographs of genitalia of male imago: (a) penes and claspers, dorsal view; (b) penes, dorsa view; (c) penes, lateral view; (d) penes, ventral-lateral view. Arrow indicates ventral projection of penes lobe. Scale bar = 100 μm.

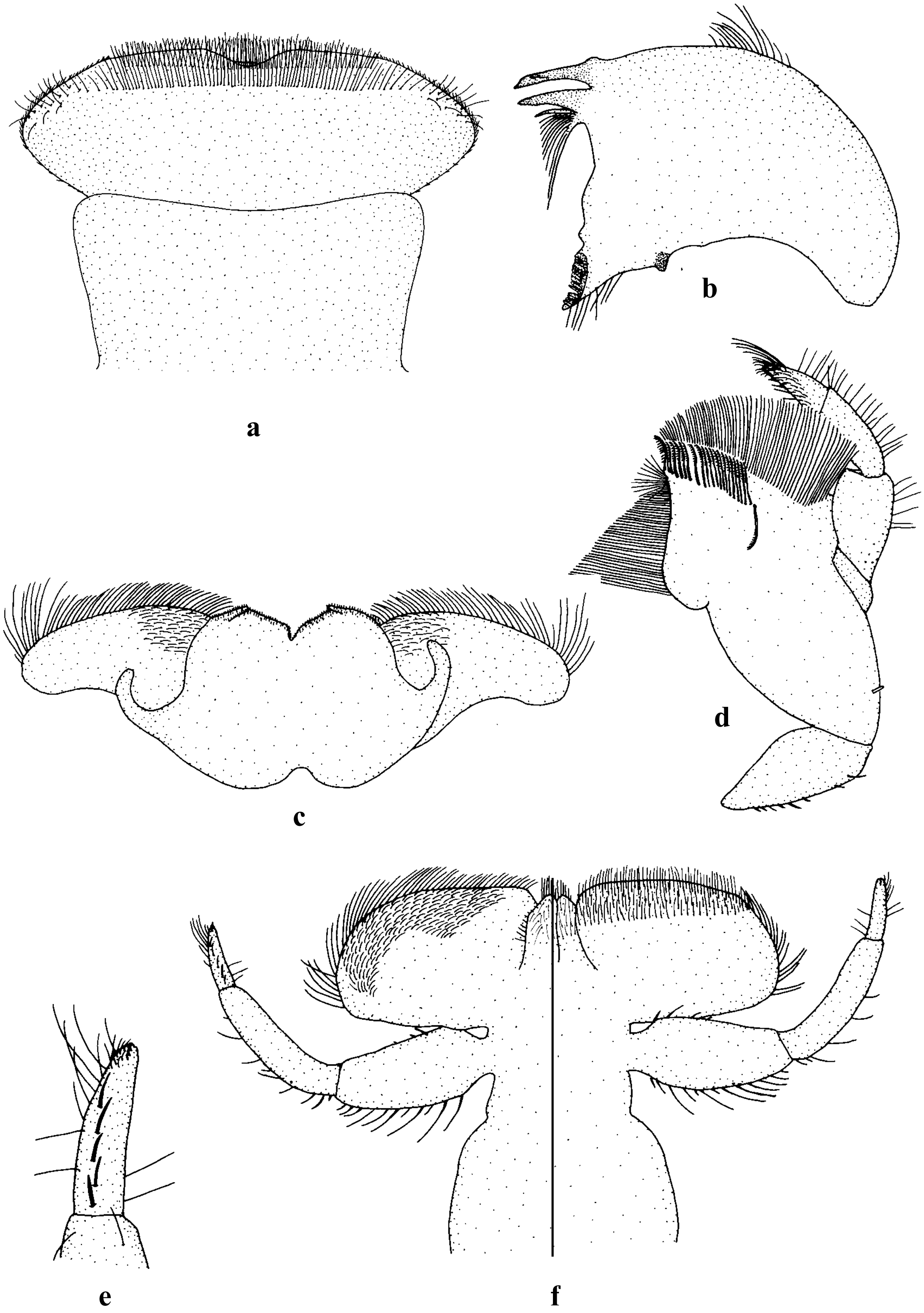


Figure 3.7. *Austrophlebioides rieki*. Nymph: (a) labrum and clypeus; (b) right mandible; (c) hypopharynx; (d) maxilla; (e) segment 3 of labial palp, dorsal view; (f) labium, dorsal view left of line and ventral view right of line.

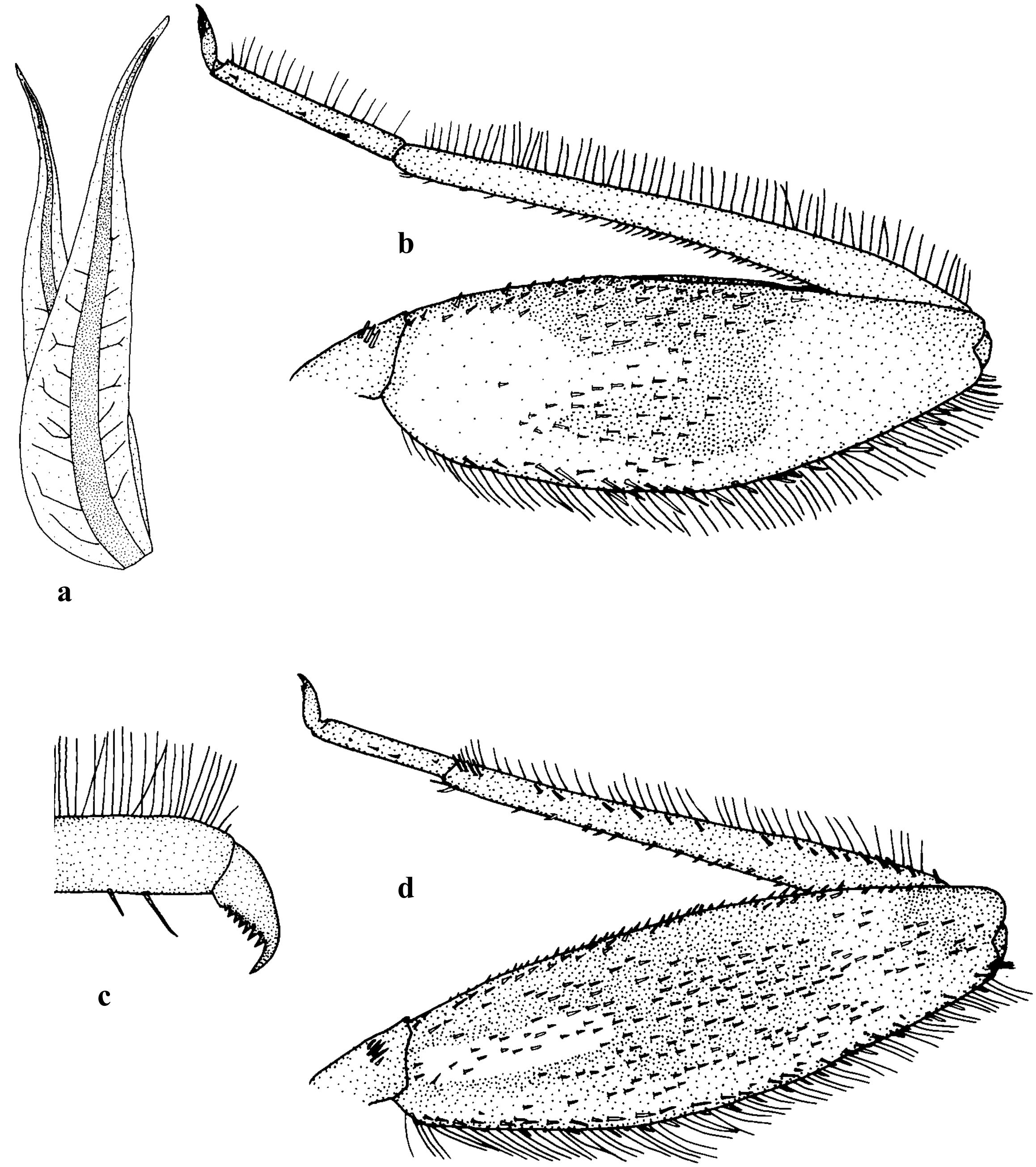


Figure 3.8. *Austrophlebioides rieki*. Nymph: (a) abdominal gill; (b) foreleg; (c) tarsus and tarsal claw of hind leg; (d) hind leg.

Remarks. *Austrophlebioides rieki* can be distinguished from all other species of *Austrophlebioides* by the following combination of characters. In the male imago by: (1) each lobe of penes with ventral projection; (2) penes without ventral spines; (3) segment 1 of claspers narrowing at 1/3 of length; (4) penes reaching beyond 0.55 length of segment 1 of clasper; (5) penes broad at base and tapering noticeably towards apex; (6) abdominal terga with prominent transverse black band along posterior margin; (7) longitudinal veins and cross veins of forewing very pale. In the nymph by: (1) absence of fine hairs along the outer margin of the mandible between the outer incisor and the median setal tuft; (2) gills cream coloured; (3) lateral margins of labrum angled; (4) pale area at base of femur of mid and hind legs in form of an elongated streak.

Habitat. Nymphs of *A. rieki* are found commonly on stones in fast flowing sections of streams.

***Austrophlebioides porphyrobranchus* sp. nov.**

(Figures 3.9-3.11)

Type material. Queensland. Holotype, male imago reared from nymph, Charmillan Ck (17°42.1'S, 145°31.3'E), collected 26 August 2000, F. Christidis, in QM. Paratypes: 3 reared male imagos and 1 reared female imago, same locality and collection date as holotype, F. Christidis, in QM.

Other material examined. Queensland. Type locality: 1 male imago, 1 June 1999; 2 nymphs, 2 June 1999; 1 reared female subimago, 1 reared male subimago and 12 nymphs, 26 August 2000. Goolagan Ck (17° 36.4'S, 145° 45.6'E): 5 nymphs, 25 August 2000. Silver Ck below falls (17° 35.5'S, 145° 44.9'E): 1 reared female and 2 nymphs, 25 August 2000. All collected by F. Christidis, in QM.

Etymology. Named after the purple gills of the nymph. From the Greek *porphyra* for purple and the Greek *branchos* for gill.

Male Imago. Length: body 9.0-10.5 mm, forewings 9.9-11.3 mm. Head brown. Eyes touching dorsally. Upper portion of eye brownish-red, lower portion black. Antennae brown. Thorax dark brown. Wings hyaline (Fig. 3.9a,b), costal and subcostal cells in apical third of forewing whitish, longitudinal veins and cross veins yellowish brown. Forewing with 17-20 costal cross veins; MA forked at about 0.5 distance from base of vein to margin of wing, fork symmetrical; MP_2 attached to MP_1 at about 0.2 length of MP_1; ICu_1 attached at base to CuA-CuP cross vein. Hind wing 0.26 times length of forewing; hind wing width 0.61 of length. Hind wing with 11-13 costal cross veins and 8-10 subcostal cross veins; costal margin with concavity at about midlength; vein Sc 0.95-0.97 length of hind wing (Fig. 3.9b). Foreleg with femur brown, remainder of leg yellowish brown. Middle and hind legs pale yellowish brown. Length ratios of segments of male foreleg 0.75-0.83: 1.00 (2.3-2.6 mm): 0.03-0.04: 0.40-0.48: 0.36-0.45: 0.25-0.31: 0.08-0.10. Claws dissimilar, one with an apical hook the other obtuse, pad-like (Fig. 3.9f). Abdomen as in Fig. 3.9c. Terga 1-6 translucent, cream to whitish in appearance; terga 3-6 with small elongated dark marks near lateral margins. Terga 7-10 reddish, terga 7-8 with paired pale submedian maculae along anterior margin. Faint and very narrow black band visible along posterior margin of most terga, but particularly terga 5-9 (Fig. 3.9c). Sternite 1 brown, sterna 2-6 cream to whitish and sterna 7-10 pale brown with a pinkish hue. Genitalia pale brown. Claspers 3 segmented; segment 1 narrowing at about 0.30 of length; segment 3 0.75 length of segment 2 and about 0.20 length of segment 1 (Fig. 3.9d). Penes relatively short, only reaching up to 0.55 length of segment 1 of claspers (Fig. 3.9d). Penes fused in basal 0.76 of length, widest at base tapering gradually towards apex (Fig. 3.9d). Each penis lobe with a large subapical dorsal spine, most of which is embedded within the lobe (Fig. 3.9e). A prominent ventral projection is present on each lobe of penes (Fig. 3.9d). Caudal filaments pale brown with reddish brown bands at annulations, terminal filament slightly longer than cerci.

Female Imago. Length: body 9.0-12.0 mm, forewings 10.9-15.3 mm. Similar to male except as follows. Eyes black, separated on meson of head by a distance of approximately 2.5 times the maximum width of an eye. Thorax pale brown, not as dark as in male imago. Forewing with 21-22 costal cross veins. Hind wing width 0.57-0.58 of length. Hind wing length 0.23 length of forewing. Abdomen dark red, pale submedian maculae present at anterior margin of all abdominal terga, most clearly visible on terga 2-8. Sternum 1 predominantly dark red, sterna 2-7 pink with dark makings, sterna 8-9 pale pink to cream. Ninth sternum entire, pointed apically (Fig. 3.9g).

Subimago. Similar in appearance to imago except wings brown. Thorax of male subimago lighter than male imago and abdominal terga 1-6 opaque, pale brown. Thorax of female subimago darker than female imago.

Nymph. Body length: male 9.0-11.1 mm, female 9.5-13.1 mm. Head prognathus, brown. Antennae about 2.2 times the length of head. Mouthparts as in Figs 3.10a-f. Maximum width of labrum 1.30 to 1.41 times maximum width of clypeus. Lateral margins of clypeus diverging apically (Fig. 3.10a). Anterior margin of labrum with narrow median notch, hooded dorsally; lateral margins angular; maximum width of labrum 2.94 to 3.10 times maximum length (Fig. 3.10a). Outer margin of mandible smoothly rounded with small median setal tuft; fine setae absent on outer margin between tuft and outer incisor (Fig. 3.10b). Incisors slender with serrated apical teeth. Prostheca well developed. Ligula of hypopharynx with well developed lateral processes, anterior margin cleft (Fig. 3.10c). Maxillae broad apically (Fig. 3.10d), with 26-28 subapical pectinate setae. Segments 1 and 2 of maxillary palp of similar length, segment 3 0.66-0.71 times length of segment 2. Labial palp with segment 1 and 2 subequal in length, segment 3 0.37-0.42 times length of segment 2. Glossae on about the same plane as paraglossae (Fig. 3.10e). Lateral margins of submentum without hairs (Fig. 3.10e). Thorax dark brown. Pronotum with small spines on anterolateral margin. Legs brown with darker markings. Fore and hind legs as in Figs 3.11b,d. Middle and hind femurs with small round pale area near base (Fig. 3.11d). Tarsi of all legs with 1 or 2 elongated ventral spines in apical third (Fig. 3.11a). Tarsal claws with ventral teeth, teeth increasing in size apically. Abdomen with posterolateral spines present on segments 2-9. Lateral margins of abdominal segments fringed with fine setae. Abdomen medium to dark brown, venter pale brown. Gills lanceolate, present on segments 1-7; lower and upper lamella about the same size; lateral tracheae well developed (Fig. 3.11c). Gill lamellae purple, tracheae black. Caudal filaments brown; up to one and a half times length of body, each segment with whorls of spines and short setae.

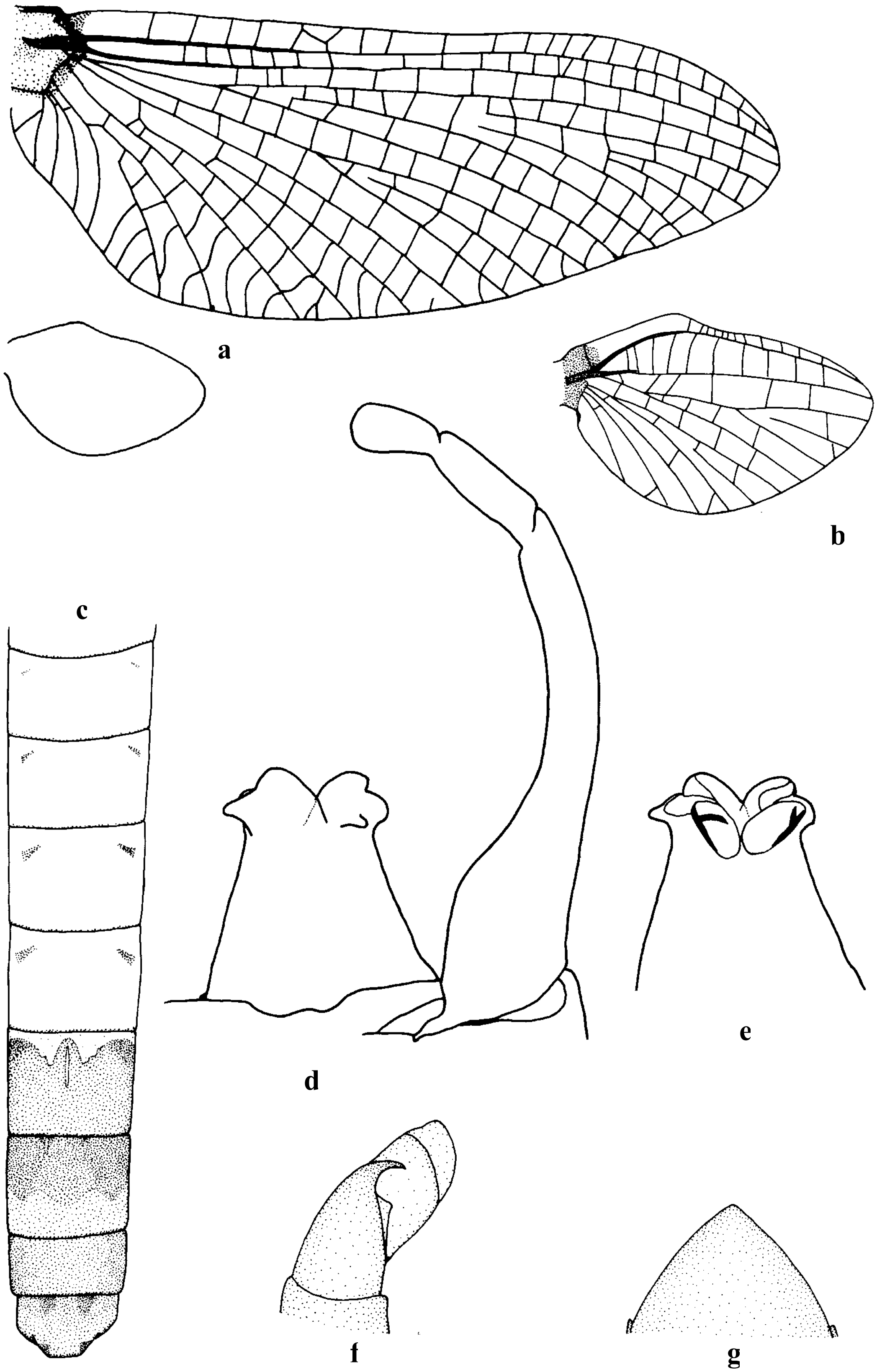


Figure 3.9. *Austrophlebioides porphyrobranchus*. Male imago: (a) wings; (b) hind wing; (c) abdominal terga 2-10; (d) penes, ventral view; (e) penes, dorsal view; (f) tarsal claw. Female imago: (g) ninth abdominal sternum.

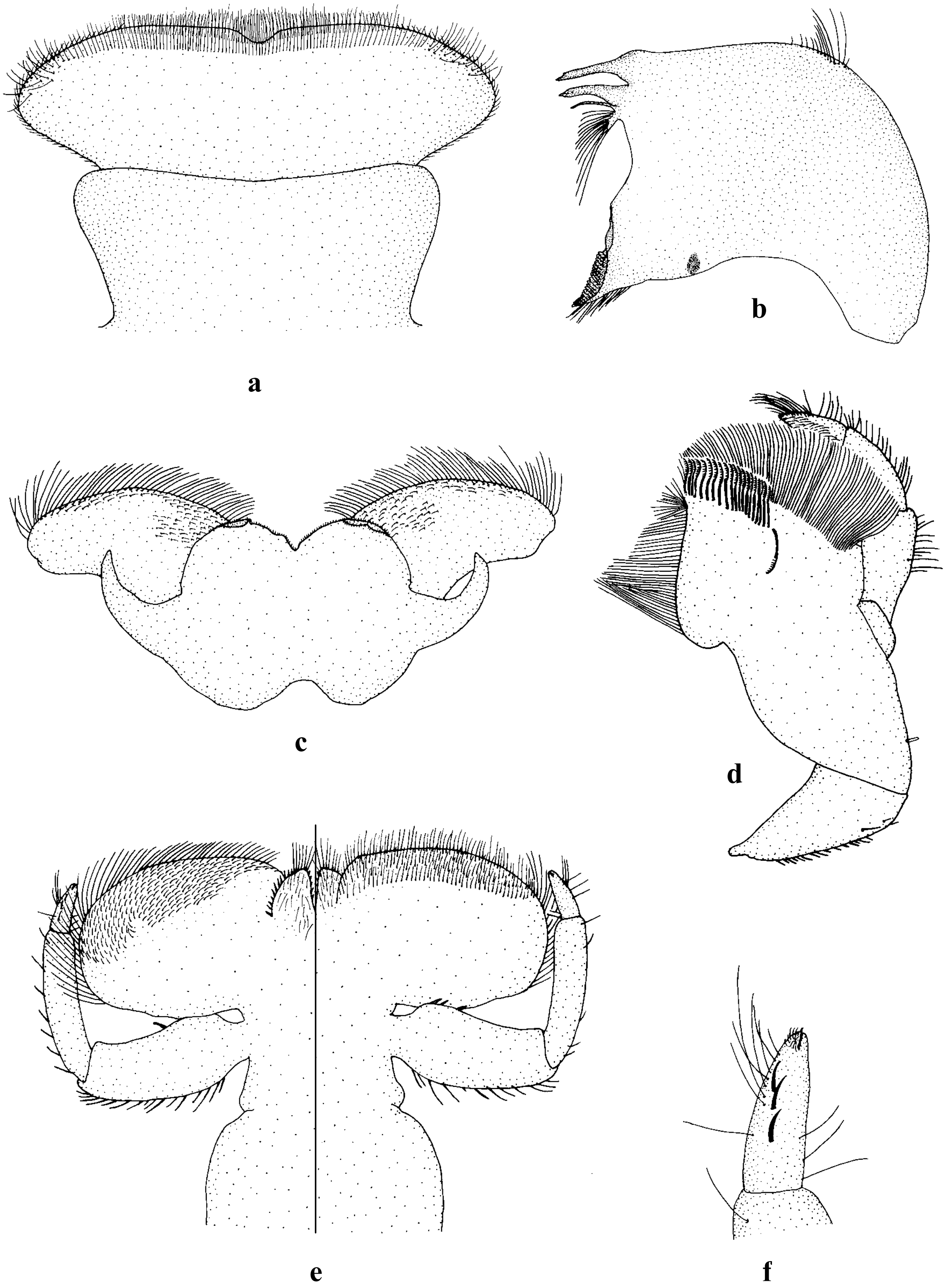


Figure 3.10. *Austrophlebioides porphyrobranchus*. Nymph: (a) labrum and clypeus; (b) right mandible; (c) hypopharynx; (d) maxilla; (e) labium, dorsal view left of line and ventral view right of line; (f) segment 3 of labial palp, dorsal view.

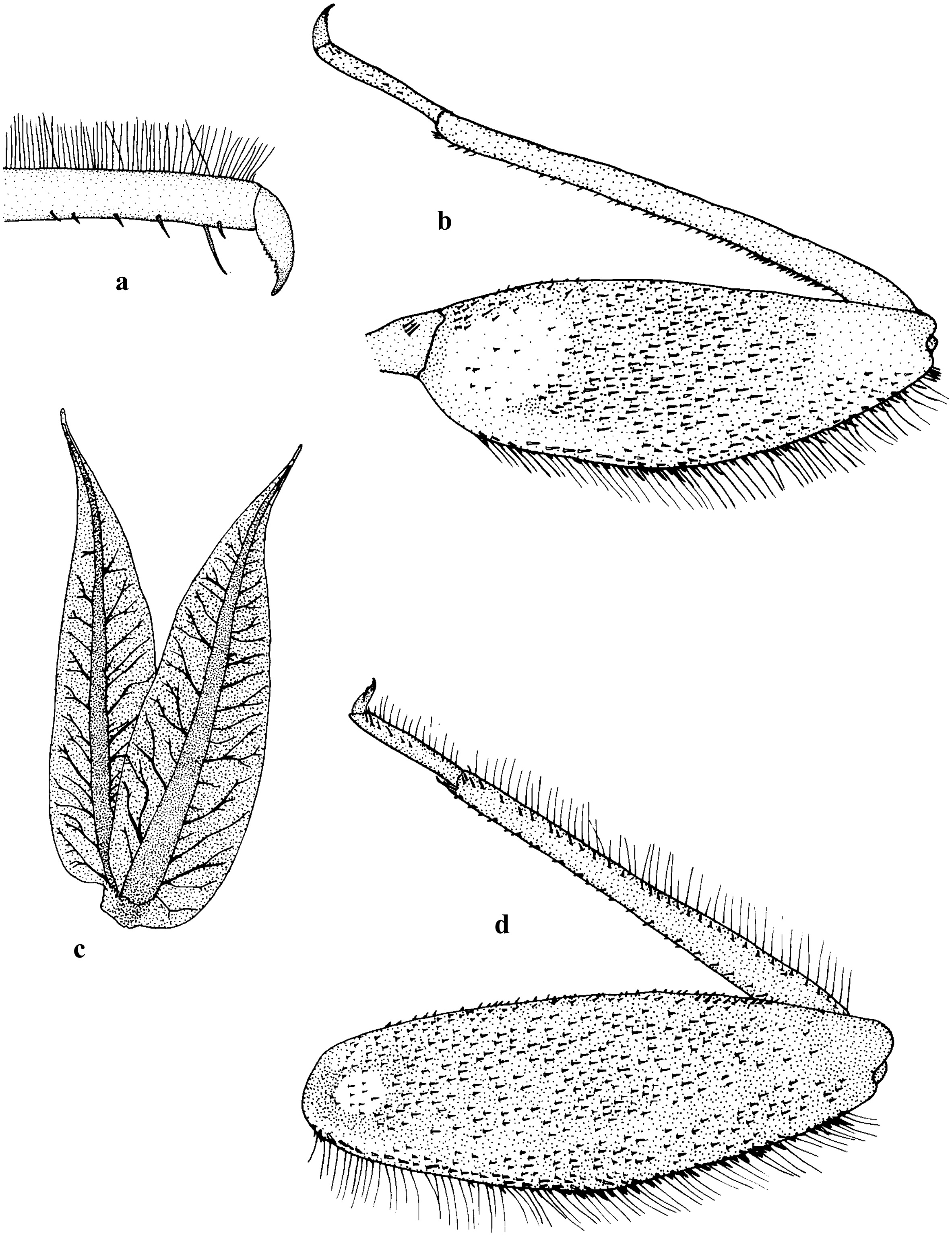


Figure 3.11. *Austrophlebioides porphyrobranchus*. Nymph: (a) tarsus and tarsal claw of foreleg; (b) foreleg; (c) abdominal gill; (d) hind leg.

Remarks. *Austrophlebioides porphyrobranchus* can be distinguished from all other species of *Austrophlebioides* by the following combination of characters. In the male imago by: (1) each lobe of penes with ventral projection; (2) penes without ventral spines; (3) segment 1 of claspers narrowing at 1/3 of length, (4) penes relatively short, not reaching beyond 0.5 length of segment 1 of clasper; (5) base of penes broad, penes tapering markedly towards apex; (6) abdominal terga with only faint transverse black band along posterior margin; (7) longitudinal veins and cross veins of forewing distinct. In the nymph by: (1) the absence of fine hairs along the outer margin of the mandible between the outer incisor and the median setal tuft; (2) purple pigmentation of the gills; (3) lateral margins of labrum angled; (4) femur of mid and hind legs with small round pale area near base.

Habitat. The nymphs of *A. porphyrobranchus* are found on stones in fast flowing areas of streams.

3.4 Discussion

The three species of *Austrophlebioides* from the Wet Tropics, *A. rieki*, *A. wooroonooran* and *A. porphyrobranchus*, are closely related and form a distinct species group within the genus. They can be distinguished from all other species of *Austrophlebioides* by the following combination of characters. In the male imago: (1) each lobe of penes with prominent ventral projection; (2) subapical dorsal spine of penes embedded within lobe with only tip of spine showing at surface; (3) penes fused in basal 0.7 to 0.8 of length; (4) segment 1 of claspers narrowing at 0.3 of length; (5) penes without ventral spines. In the nymph: by the absence of fine setae along the outer margin of the mandible between the outer incisor and the median setal tuft.

The inclusion of *A. rieki*, *A. wooroonooran* and *A. porphyrobranchus* into *Austrophlebioides* requires some modifications to the generic diagnosis. Diagnosis of the nymph follows Campbell and Suter (1988), except: mandible with or without fine setae along outer margin between medial setal tuft and outer incisor, and one to two elongated ventral spines in apical third of all tarsi or middle and hind tarsi only.

Based on the male genitalia it is possible to identify three species groups within the genus: (i) a group comprising *A. pusillus*, *A. decipiens*, *A. booloumbi* and *A. unguicularis*, characterised by the presence of a series of fine ventral spines on the penes; (ii) a group consisting of *A. marchanti*, characterised by the penes being fused basally and widely separated apically; and (iii) a group comprising *A. rieki*, *A. wooroonooran* and *A. porphyrobranchus*, characterised the presence of a prominent ventral projection on each lobe of the penes. Phylogenetic relationships among species of *Austrophlebioides* are further investigated in Chapter 4.

CHAPTER 4

Phylogeny and distribution of the genus *Austrophlebioides* Campbell and Suter (Ephemeroptera: Leptophlebiidae: Atalophlebiinae)

4.1 Introduction

The genus *Austrophlebioides*, as discussed in Chapter 3, was established by Campbell and Suter (1988) and comprises five described species: *A. unguicularis, A. marchanti*, *A. booloumbi, A. decipiens* and *A. pusillus*. Of these, only *A. marchanti* and *A. pusillus* are known from both the adult and nymph stages. The remaining species have been described from adult material only. The present study has revealed three new species, *A. rieki*, *A. wooroonooran* and *A. porphyrobranchus*, from the Wet Tropics biogeographic region of north-eastern Queensland, and these were described in Chapter 3. With the inclusion of these three species, the total number of described species placed in the genus is now eight. Several undescribed species believed to belong to the genus *Austrophlebioides* have also been recognised by Dean (1999) but these are known from the nymphal stage only. J. Dean (pers. comm.) also proposes that the species *Deleatidium (Atalophlebioides) bundutum* described by Harker (1954) be transferred to the genus *Austrophlebioides*.

Although knowledge of the alpha taxonomy of *Austrophlebioides* has increased considerably in recent years (Parnrong and Campbell 1997; Dean 1999), there have been no phylogenetic studies on the genus and evolutionary relationships among species remain poorly understood. Based on features of the male genitalia, I proposed three species groups within *Austrophlebioides* (Chapter 3): a group comprising of *A. pusillus*, *A. decipiens*, *A. booloumbi* and *A. unguicularis*; a second group, consisting of *A. marchanti*, and a final group comprising *A. rieki*, *A. wooroonooran* and *A. porphyrobranchus*. Support for the monophyly of these three species groups, however, awaits a species-level cladistic analysis of the genus.

The genus *Austrophlebioides* is endemic to Australia and is widely distributed in eastern Australia and Tasmania (Dean 1999). *Austrophlebioides* is also believed to occur in north-western Australia and the Northern Territory (Dean 1999). The genus has not been collected from the south west of Western Australia or from South Australia. The majority of *Austrophlebioides* species appear to have fairly restricted geographic distributions (Dean 1999). 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 chapter is 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 of this chapter is to document current knowledge on the geographic distribution of species and investigate biogeographic patterns within the genus in light of the proposed phylogeny.

4.2 Methods

4.2.1 Phylogenetic analyses

As not all species of *Austrophlebioides* are presently known from all 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 4.1). They were: *A. pusillus*, *A. marchanti*, *Austrophlebioides* sp. AV2; *A. rieki*, *A. wooroonooran*, *A. porphyrobranchus* and *A. bundutus*. 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 4.2). 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. Excluded from the analysis due to insufficient material were four undescribed species (*Austrophlebioides* sp. AV6, *Austrophlebioides* sp. AV9, *Austrophlebioides* sp. AV10, *Austrophlebioides* sp. AV11), *A. unguicularis* and *A.*

decipiens. These last two species are only known 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 ascertain the affinities of these two species to other members within the genus.

Characters were obtained from examination of specimens and from published descriptions (Dean 1997; Parnrong and Campbell 1997). For *A. booloumbi* the type series could not be located and all characters were coded from the species description in Parnrong and Campbell (1997). Nymphal characters were scored from various body regions including 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 relative 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 to 2.6; (1) 2.7 to 3.2) is due to this one species. Characters and character states used in the analyses are listed below, and the data matrices are given in Table 4.1 and Table 4.2. All characters were equally weighted and multi-state characters were treated as unordered.

Cladistic analyses were performed using PAUP* version 4.0b10 (Swofford 2002). The branch-and-bound search option was used in all analyses. The Australian genus *Tillyardophlebia* was selected as the outgroup because of its close affinity to *Austrophlebioides* (Chapter 2). Both described species of *Tillyardophlebia*, *T. rufosa* and *T. alpina*, were included as outgroups in the analyses. Bootstrap analysis (1000 randomizations, full heuristic search, simple addition, tree bisection reconnection (TBR) branch swapping) was used to assess support for each node of the tree (Felsenstein 1985b; Hillis and Bull 1993).

List of morphological characters used in first series of analyses.

Nymph Characters

1. Subapical setal fringe on dorsal surface of labrum: (0) arranged in straight row, 1 to 2 setae deep and up to 3 setae deep in middle; (1) arranged in straight row, 2 to 3 setae deep and 4 to 5 setae deep in middle; (2) merged with apical setal fringe, setae arranged into an elliptical or round "sucker".
2. Median notch on anterior margin of labrum: (0) broad U-shaped; (1) V-shaped or narrow U-shaped; (2) greatly reduced; (3) absent.
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.
5. Length / width ratio of segment 3 of labial palp: (0) 2.2 to 2.6; (1) 2.7 to 3.2.
6. 1 or 2 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 to 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.
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.

Adult Characters

11. Segment 1 of claspers narrowing at: (0) 0.40 to 0.50 length of segment; (1) at 0.25 to 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 ½ 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.

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

Nymphal Characters

20. Lateral margins of submentum: (0) with short spines along basal $^{2}/_{3}$ of margins; (1) bare, or with few spines at base.
21. Femur, dark pigmentation near ventral margin: (0) absent; (1) present.

Table 4.1 Data matrix used in first series of analyses.

	1111111111
	1234567890123456789
T. rufosa	0101000100000001000
T. alpina	0101000000000001000
A. pusillus	0011110111010220101
A. bundutus	0011110111010210101
A. marchanti	0111011122001110002
A. sp. AV2	0111011222001110001
A. wooroonooran	1110120111110210011
A. rieki	1110120021110210011
A. porphyrobranchus	1110111111110210012

Table 4.2 Data matrix used in second series of analyses.

	111111111122 123456789012345678901
T. rufosa	010100010000000100010
T. alpina	010100000000000100010
A. pusillus	001111011101022010110
A. bundutus	001111011101021010110
A. marchanti	011101112200111000210
A. sp. AV2	011101122200111000111
A. wooroonooran	111012011111021001110
A. rieki	111012002111021001110
A. porphyrobranchus	111011111111021001210
A. booloumbi	??????????010220101??
*A. "*eungella*"*	0011110011?????????10
A. sp. AV4	0111011122?????????10
A. sp. AV5	2201011022?????????01
A. sp. AV7	2301011022?????????01

4.2.2 Distribution

Distributional data for species was compiled from localities given in published species descriptions (Harker 1954; Campbell and Suter 1988; Parnrong and Campbell 1997), distributions given in Dean (1999), and from unpublished data provided by J. Dean. For species from the Wet Tropics and Eungella National Park, data were obtained from material collected during this study as well as from examination of existing collections held by the Australian Centre for Tropical Freshwater Research, James Cook University, Townsville.

4.3 Results

4.3.1 Phylogeny

First series of analyses

(a) Combined analysis of adult and nymph

The combined analysis of the adult and nymph data resulted in a single parsimonious tree (Figure 4.1a) with a length of 30 steps, a consistency index (CI) of 0.866, and a retention index (RI) of 0.88. Three monophyletic species groups were identified in the analysis and all three were supported by high bootstrap values. The first comprised *A. marchanti* and *Austrophlebioides* sp. AV2 ("*marchanti*" clade), the second *A. pusillus* and *A. bundutus* ("*pusillus*" clade), and the third, *A. rieki*, *A. wooroonooran* and *A. porphyrobranchus* ("wet tropics" clade). The "*pusillus*" clade was the sister group to the "wet tropics" clade, and the clade comprising these two groups was sister to the "*marchanti*" clade.

(b) Adult only analysis

Analysis of adult characters resulted in a single parsimonious tree (Fig. 4.1b) with a length of 13 steps, a CI of 0.923, and a RI of 0.933. The tree resulting from the adult data was identical to the combined adult and nymph tree (Fig. 4.1a), except that relationships among the three species of the "wet tropics" clade, *A. rieki*, *A. wooroonooran* and *A. porphyrobranchus*, were unresolved.

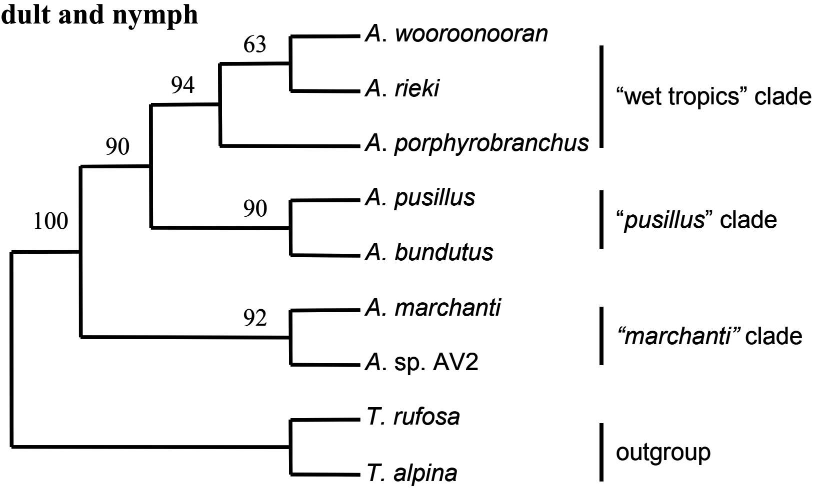
(c) Nymph only analysis

Analysis of nymphal characters resulted in a single most parsimonious trees (Fig. 4.1c) with a length of 17 steps, a CI of 0.823, and a RI of 0.842. This tree was identical to the combined adult and nymph tree (Fig. 4.1a).

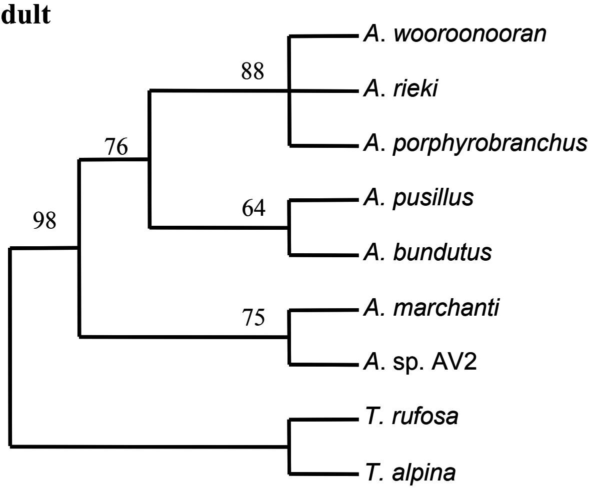
Second series of analyses

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, a CI of 0.815, and a RI of 0.875. One of these trees is shown in Figure 4.2a and a strict consensus tree in Figure 4.2b. The fifteen trees differed only in the placement of

(a) adult and nymph



(b) adult



(c) nymph

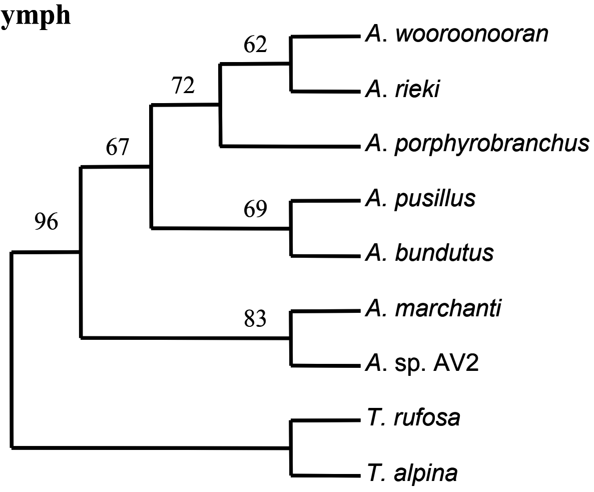


Figure 4.1. First series of analyses: (a) single most parsimonious tree from analysis of combined nymph and adult data; (b) single most parsimonious tree resulting from analysis of adult data only; (c) single most parsimonious tree from analysis of nymph data only. Bootstrap values are shown above nodes for groups with >50% support.

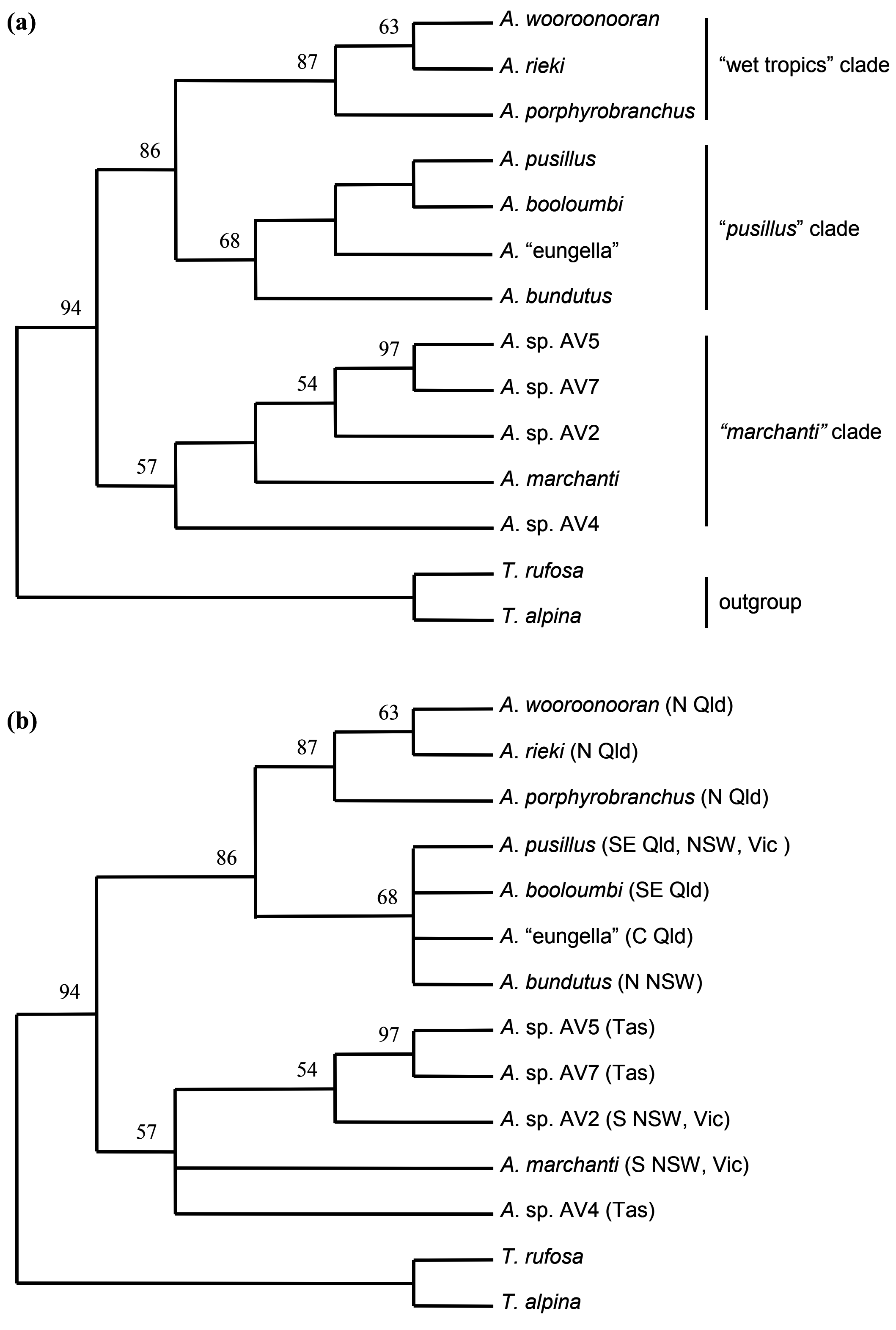


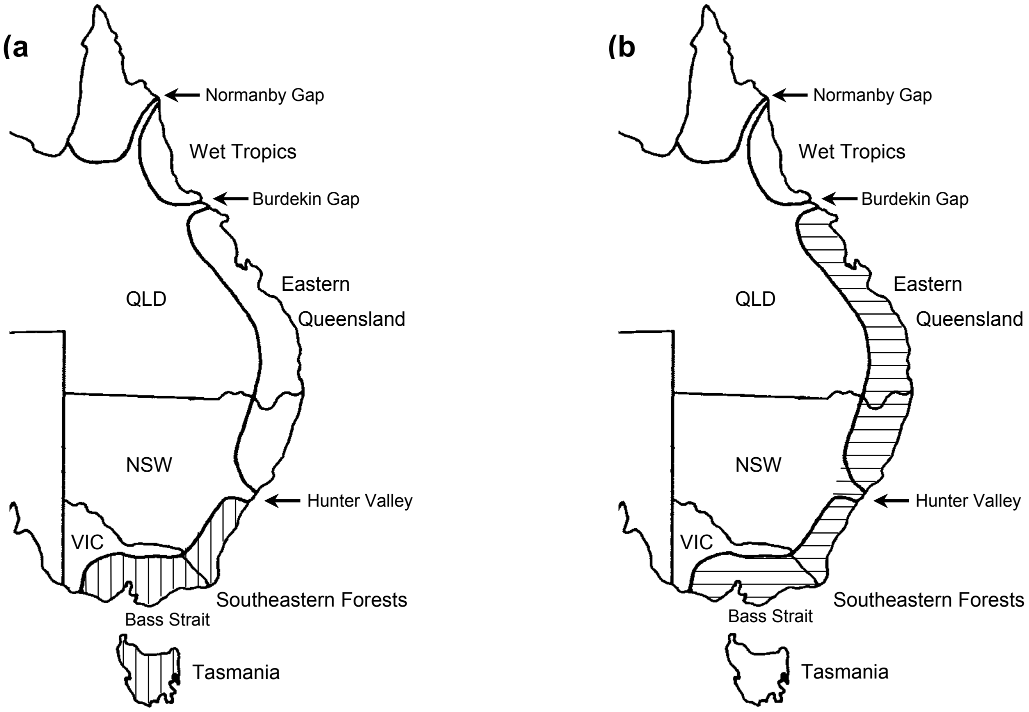
Figure 4.2. Second series of analyses: (a) one of fifteen most parsimonious trees resulting from analysis of data matrix in Table 4.2; (b) strict consensus tree of fifteen most parsimonious trees. Bootstrap values are shown above nodes for groups with >50% support.

Austrophlebioides 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. 4.1a), with the same three major clades identified. *A. booloumbi* and *A.* "eungella" grouped with *A. pusillus* and *A. bundutus* of the "*pusillus*" clade, and the three Tasmanian species, *Austrophlebioides* sp. AV4, *Austrophlebioides* sp. AV5, and *Austrophlebioides* sp. AV7, grouped with *A. marchanti* and *Austrophlebioides* 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.

4.3.2 Distribution

The distribution of each species is superimposed on to the strict consensus tree in Figure 4.2b, and broad areas of distribution of the three major clades are shown in Figure 4.3. The "*marchanti*" clade is present in southern New South Wales and Victoria (Southeastern Forests), and is the only clade found in Tasmania (Fig. 4.3a, Fig. 4.2b). The Tasmanian species *Austrophlebioides* sp. AV5 and *Austrophlebioides*. sp. AV7 occur in alpine areas of the Cradle Mountain and Lake St Clair National Park, whereas *Austrophlebioides* sp. AV4 appears to be more widespread within Tasmania (J. Dean, pers. comm.). All three species appear to be endemic to Tasmania. No mainland species of *Austrophlebioides* has been recorded from Tasmania. The mainland species of the "*marchanti*" clade, *A. marchanti* and *Austrophlebioides* sp. AV2, occur in Victoria and southern New South Wales (Fig. 4.3a, Fig. 4.2b). 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 (Southeastern Forests) (Fig. 4.3b). *A. pusillus* is widely distributed and has been recorded from south-eastern Queensland, northern and southern New South Wales, and Victoria (Fig. 4.2b). Other species of this clade appear to have far more restricted distributions. *A.* "eungella" is known from the Eungella National Park in central-eastern Queensland, *A. booloumbi* is presently only known from the Conondale Ranges in south-eastern Queensland and *A. bundutus* has only been



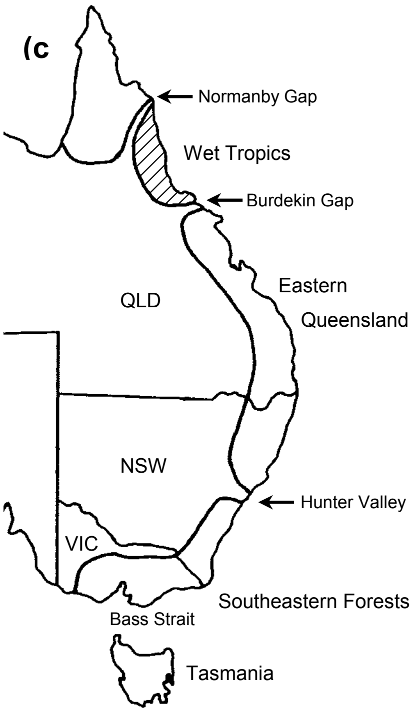


Figure 4.3. Broad areas of distribution of the three clades of the genus *Austrophlebioides*: (a)"*marchanti*" clade, indicated by vertical hatching; (b) "*pusillus*" clade, indicated by horizontal hatching; (c) "wet tropics" clade, indicated by diagonal hatching. Maps also show the major biogeographic regions of eastern Australia and geographic barriers (arrows) (modified from Ford (1987) and Cracraft (1991)).

collected from northern New South Wales, north of the Hunter Valley. The distribution of the "*pusillus*" clade partly overlaps with that of the "*marchanti*" clade, with both clades present in New South Wales and Victoria (Southeastern Forests). This overlap is due only to the widespread presence of *A*. *pusillus* in southern New South Wales and Victoria.

The "wet tropics" clade, which comprises *A*. *rieki*, *A*. *wooroonooran* and *A*. *porphyrobranchus*, is distributed from the Paluma Range near Townsville in the south to the Annan River near Cooktown in the north (Fig. 4.3c), and is endemic to this region. Presently, *A*. *wooroonooran* and *A*. *porphyrobranchus* have only been collected from the Atherton subregion of the Wet Tropics. *A*. *rieki* was described from material collected from the Daintree subregion. However, it is possible that the distribution of this species extends further south, as nymphs and adults belonging to this or a morphologically very similar species have been collected from the Atherton and Paluma subregions. On morphological grounds alone it is difficult to establish whether these specimens belong to a single widespread species or several very closely related species.

4.4 Discussion

4.4.1 Phylogeny

The cladistic analyses support the recognition of three monophyletic species groups within the genus *Austrophlebioides*. These three groups, the "*marchanti*", "*pusillus*" and "wet tropics" 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 "*marchanti*" clade was the most basal clade within the genus, and the "*pusillus*" clade was sister to the "wet tropics" clade. Congruence among the phylogenies derived from the different life history stages and the combined data, along with bootstrap support values, suggest that these relationships are robust. Similar patterns were obtained from the second series of analyses which included additional species known from one life history stage only.

The "*marchanti*" clade comprises *A*. *marchanti* and *Austrophlebioides* 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, *Austrophlebioides* sp. AV4, *Austrophlebioides* sp. AV5 and *Austrophlebioides* 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 *Austrophlebioides* sp. AV5, *Austrophlebioides* sp. AV7 and *Austrophlebioides* sp. AV2 possess the following synapomorphic character: 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 *Austrophlebioides* sp. AV4. Although there is some support for placement of the Tasmanian species into the "*marchanti*" clade, information from adult males is required to confirm this placement.

The nymphs of two of the Tasmanian species, *Austrophlebioides* sp. AV5 and *Austrophlebioides* 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 *Austrophlebioides*, and small spines are present along the basal $^{2}/_{3}$ of the lateral margins of the submentum. This maybe 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 very derived. The setae on the dorsal surface of the labrum are arranged into a broad elliptical disc in *Austrophlebioides* sp. AV5, and into a round disc in *Austrophlebioides* sp. AV7. The two species appear to be very closely related.

The "*pusillus*" clade is defined by the synapomorphic character: presence of a series of fine ventral spines in 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. bundutus*, *A. booloumbi* 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 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.

A. 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. They are as follows. In the adult male, a prominent pointed ventral projection is present on each lobe of the penes (character 18), and segment one of the claspers narrows at about 0.25 to 0.30 of length (character 11). In the nymph, fine hairs are absent from the outer margin of the mandible, between the outer incisor and the median setal tuft (character 4). These are present in all other *Austrophlebioides* species and have presumably been secondarily lost in the Wet Tropics species. Similarly, the nymphs of all known *Austrophlebioides* species have 1 to 2 elongated ventral spines in apical third of tarsi (character 6). However, in *A. rieki* and *A. wooroonooran* these spines appear to have been secondarily 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 were insufficient data in the adult-only phylogeny to resolve relationships among these species.

4.4.2 Distribution

Distributional patterns within the genus *Austrophlebioides* correspond well with previously established biogeographic regions for Australia, based on areas of endemism of birds (Cracraft 1991) and plants (Crisp *et al*. 1995, 2001). There was little overlap in the distribution of the three *Austrophlebioides* clades, with closely related species generally found within the same biogeographic region.

The "wet tropics" clade was confined to the Wet Tropics bioregion of north-eastern Queensland and was 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 insects (Mackerras 1962; Watson and Theischinger 1984; Wang *et al*. 1996), birds (Keast 1961; Ford 1987; Cracraft 1982, 1986, 1991; Joseph *et al*. 1993), and frogs (James and Moritz 2000). 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 wet tropics and "*pusillus*" clades suggests that the Burdekin Gap is a significant barrier for these mayflies and has been for some considerable time. Time of divergence of the two clades is difficult to establish, as molecular data is currently unavailable for *Austrophlebioides*. However, interspecific genetic analyses of vertebrate taxa indicate that speciation events in the Wet Tropics bioregion date to the Miocene or Pliocene age (Moritz *et al*. 1997; Schneider *et al*. 1998).

Species of the "*pusillus*" clade, with the exception of *A*. *pusillus*, are restricted to the Eastern Queensland biogeographic region (Cracraft 1991), which includes northern New South Wales, south to the Hunter Valley. The distribution of these species within Eastern Queensland appears to correspond to recognised subregions (Crisp *et al*. 1995; James and Moritz 2000). *Austrophlebioides* "eungella" occurs in central-eastern Queensland, *A. booloumbi* in south-eastern Queensland, and *A*. *bundutus* 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 region, and is consistent with the findings of Joseph *et al*. (1993) for birds, and Hugall *et al*. (in press) 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 Southeastern Forests. It is unclear why *A*. *pusillus* is so widely distributed when other species within the "*pusillus*" clade are confined to Eastern Queensland. However, some aspects of the ecology of *A*. *pusillus*, such as the ability to survive in lowland streams with reduced current and elevated water temperatures (J. Dean pers. comm.), may account for the wider distribution. Although *A*. *pusillus* is distributed north and south of the Hunter Valley, some morphological differences among populations have been observed. Adult males of *A*. *pusillus* collected from northern New South Wales differ in their markings from individuals found south of the Hunter Valley in southern New South Wales and Victoria (Dean unpublished data). Additional morphological and molecular studies will be required to determine whether *A*. *pusillus* represents a single widely distributed species or several closely related species with more limited distributions. If the former is true, the presence of *A*. *pusillus* in Eastern Queensland and the Southeastern 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 were restricted to the

Southeastern Forests (southern New South Wales and Victoria), 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* (Oligoneuriidae) (Campbell 1981). The discontinuity between northern New South Wales and southern New South Wales was also noted by Watson and Theischinger (1984) in relation to Odonata, with several species having their distributional limits at or near the Hunter Valley.

The three species of *Austrophlebiodes* presently known from Tasmania belong to the "*marchanti*" clade and all three are endemic to Tasmania. High levels of endemism in Tasmania have also 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 the 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.

CHAPTER 5

Habitat use and the evolution of labrum shape in the genus *Austrophlebioides* (Leptophlebiidae: Atalophlebiinae)

5.1 Introduction

Current velocity is one of the most important physical factors affecting organisms living in running waters and several morphological and behavioural traits of stream insects are believed to be adaptations to fast flow (Hynes 1970; Allan 1995; Giller and Malmqvist 1998). The extent to which organisms are exposed to rapid current varies greatly. Many species show distinct preferences for particular water velocities with some preferring fast-flowing areas such as riffles, whereas others inhabit regions with reduced current, such as pools (Edington 1968; Hynes 1970; Hearnden and Pearson 1991). Animals that reside in spaces among stones or in leaf litter, or that burrow into the sediment are exposed only to slow currents. Those that occur on the surface of stones in fast-flowing regions of streams experience greater current velocities. Insects probably endure high flow conditions to feed on the periphyton and biofilm found on the upper surfaces of stones (Smith and Dartnall 1980; Ward 1992; Giller and Malmqvist 1998).

Several morphological features of insects that occur in fast flow have been suggested to be adaptive, enabling these organisms to maintain their position and move about on the surface of the substrate without being dislodged. They include body shapes that are dorsoventrally flattened or streamlined, attachment structures such as well developed tarsal claws, hooks, suckers and silks, as well as additional modifications that increase contact with the substrate (see reviews by Hynes 1970; Ward 1992; Allan 1995; Giller and Malmqvist 1998). The form of several other morphological structures also appear to be related to current velocity. For example, in simuliids, differences in the morphology of the labral feeding fans have been observed among species inhabiting different flow regimes (Zhang and Malmqvist 1996, 1997; Palmer and Craig 2000). Likewise, in several mayfly taxa, including the Australian leptophlebiid genus *Austrophlebioides*, the nymphal labrum is laterally expanded in species that inhabit fast-flowing waters (Campbell and Peters 1986; Dean 1999). It has been suggested that a broad labrum may protect the preoral cavity from strong currents, thus enabling mayfly nymphs to feed on the surface of stones in fast flow (Hynes 1970; Arens 1989; Ward 1992).

Previous studies and discussions on morphological adaptations of stream insects to flow have seldom considered evolutionary relationships among species (Allan 1995). This omission greatly limits the conclusions that can be drawn from such studies, as related species may simply share a trait due to common ancestry, rather than as a result of independent evolution of the trait in response to similar selection pressures. It is now recognised that comparative studies must take into account evolutionary relationships among taxa (Felsenstein 1985a; Harvey and Pagel 1991), but the lack of robust phylogenies for many freshwater insects has hampered use of such an approach. In this study I use the phylogenetic hypothesis for the genus *Austrophlebioides* proposed in Chapter 4 to investigate the evolution of both labrum morphology and habitat use in this genus.

The genus *Austrophlebioides* is found predominantly on stones in a variety of flow environments ranging from riffles with very fast flow to rock pools with slow flow. Individual species vary in their preference for fast flow or moderate to slow flow conditions. The morphology of the labrum also varies within the genus, with those species found in fast-flow habitats tending to have a broader labrum whereas those that occur in moderate to slow flow have a narrower labrum. In addition, in some species that occur in fast flow, the labrum is highly modified with the presence of a sucker-like setal disc at the anterior margin (Dean 1999). Although labrum shape appears to be associated with habitat flow regimes, it is unclear what role flow may have played in the evolution of the morphology of the labrum in this genus.

The aim of this chapter is to infer the pattern of evolution of labrum morphology and habitat use in *Austrophlebioides* to address the following questions: (i) what has been the direction of evolutionary change in the morphology of the labrum; (ii) how often has a broad labrum evolved within the genus; and (iii) are changes in labrum morphology associated with evolutionary shifts in habitat use? If flow has been an important selective force in the evolution of labrum shape we would expect evolutionary shifts in habitat use (flow regime) to be accompanied by changes in the morphology of the labrum.

5.2 Methods

5.2.1 Labrum morphology

Nymphs of all *Austrophlebioides* species included in this study were examined, except for the nymph of *A. booloumbi* which is unknown at the present time. Data on labrum morphology were gathered also from the literature (Campbell and Suter 1988; Parnrong and Campbell 1997; Dean 1999). Nymphs were placed in 70% ethanol and the head was viewed under a Leica MZ6 dissecting microscope. For a more detailed examination, the labrum and clypeus were dissected and mounted on to slides in Euparal following procedures outlined in Chapter 3. Mounted specimens were then examined under a compound microscope. Selected specimens were also prepared for viewing and photography with a BIO-RAD Radiance 2000 confocal microscope. The dissected labrum and clypeus were stained with Young's Eosin-Erythrosin, a fluorescent dye, for five to seven hours, rinsed in ethanol, passed through several changes of xylene and mounted in DPX. Specimens for scanning electron microscopy were prepared as described in Chapter 3.

Observations were made on the general shape of the labrum in dorsal view, its breadth, and the arrangement and density of setae on the apical and dorsal surfaces. Measurements of the maximum breadth and the maximum length of the labrum were taken from slide-mounted material, and a ratio of maximum breadth to length was calculated for each species. This ratio is reported routinely in species descriptions as it is a useful taxonomic character that appears to be consistent within species. In this study the ratio was used only as a general descriptor of labrum shape and as a means of categorising the overall shape of the labrum for character-state reconstruction. The number of specimens measured ranged from 2-10 for each *Austrophlebioides* species. Where only two specimens were available for measurement additional data were obtained from the figures in Dean (1999).

5.2.2 Habitat use

Data on habitat use of nymphs of *Austrophlebioides* species were obtained from direct observation of the stream habitat from which the nymphs were collected. Stream habitats were recorded as riffles (fast turbulent flow), runs (moderate non-turbulent flow) and rock pools (slow to negligible flow). Habitat use data for *A. wooroonooran*, *A. rieki*, *A. porphyrobranchus*, *A.* "eungella", *Austrophlebioides* sp. AV5, and *Austrophlebioides* sp. AV7, were collected during the present study. Information on habitat use of nymphs of *A. pusillus*, *A. bundutus*, *A. marchanti*, *Austrophlebioides* sp. AV2, and *Austrophlebioides* sp. AV4, as well as additional data for *Austrophlebioides* sp. AV5, and *Austrophlebioides* sp. AV7, were provided by J. Dean (per. com.). Information on the habitat use of *A. pusillus* was also obtained from Campbell and Suter (1988) and for *A. marchanti* from Parnrong and Campbell (1997).

5.2.3 Character mapping

Character-state reconstruction based on parsimony in MacClade 4.02 (Maddison and Maddison 2001) was used to investigate the pattern of evolutionary change in labrum morphology and habitat use in the genus *Austrophlebioides*. One of the fifteen most parsimonious trees generated in Chapter 4 (Fig. 4.2a) was used for reconstruction of ancestral character states. Habitat use was scored as either: (1) fast flow (riffles), or (0) slow to moderate flow (pools and runs), and mapped on to the phylogeny. The shape of the labrum was categorised as follows: (0) narrow (breadth/length ratio of 2.5 to 2.75), or (1) broad (breadth/length ratio of 2.90 to 3.30), and mapped on to the phylogeny. The distribution of these two traits on the phylogeny was compared to assess whether character state changes in labrum shape coincided with evolutionary shifts in habitat use. It should be noted that labrum shape was not included as a character in the cladistic analysis that generated the phylogeny for the genus (Chapter 4). The arrangement of setae on the dorsal surface of the labrum (character 1) was included but its exclusion had no effect on tree topology. As the nymph of *A. booloumbi* is unknown this species was coded as missing (?) in all reconstructions.

5.3 Results

5.3.1 Labrum morphology

The shape of the labrum, although variable within *Austrophlebioides*, appears to be fairly conserved within individual clades with the exception of the "wet tropics" clade (Figs 5.1-5.4). Species of the "*marchanti*" clade all have a very broad labrum with a breadth-to-length ratio of 3.00 to 3.30 (Fig. 5.4a-d). In contrast, species of the *pusillus* clade all possess a narrower labrum with a breadth-to-length ratio of 2.50 to 2.75 (Fig. 5.3a-c). Within the "wet tropics" clade, *A. wooroonooran* has a relatively narrow labrum with a breadth to length ratio of 2.50 to 2.75 (Fig. 5.2a), whereas *A. porphyrobranchus* and *A. rieki* both have a broad labrum with breadth-to-length ratios of 2.90 to 3.03 (Fig. 5.2b,c). Reconstruction of ancestral character states suggests that a broad labrum is the ancestral state within the genus and appears to have been retained in all species of the "*marchanti*" clade, and *A. porphyrobranchus* and *A. rieki* of the "wet tropics" clade (Fig. 5.1a). A narrower labrum appears to have evolved independently on two occasions, at the base of the "*pusillus*" clade and on the branch leading to *A. wooroonooran* of the "wet tropics" clade (Fig. 5.1a).

Austrophlebioides species typically have two distinct transverse rows of setae on the dorsal surface of the labrum, the apical and subapical setal fringes (Figs 5.2, 5.3, 5.4c,d). In *Austrophlebioides* sp. AV5 and *Austrophlebioides* sp. AV7 these setal fringes cannot be distinguished as two separate rows of setae, but appear to be incorporated into a novel structure at the apex of the labrum, which I refer to as a setal disc (Figs 5.4a,b 5.6). Both these species have much higher densities of setae on the apical surface of the labrum than other *Austrophlebioides* species. In *Austrophlebioides* sp. AV5 the apical setae are arranged into a fairly loosely formed elliptical disc, which extends across most of the anterior margin of the labrum (Fig. 5.5a). In *Austrophlebioides* sp. AV7 the apical setae are organised into a well formed round disc, which protrudes forward of the labrum (Figs 5.5b, 5.6b,c). An additional modification to the labrum of these two species is the reduction in size of the median notch at the anterior margin of the labrum in *Austrophlebioides* sp. AV5, and absence of a median notch on the labrum in *Austrophlebioides* sp. AV7.

5.3.2 Habitat use

Habitat use was generally conservative within the three *Austrophlebioides* clades (Fig. 5.1b). Species of the "*pusillus*" clade, *A*. *pusillus*, *A*. "eungella" and *A*. *bundutus,* are all found in moderate to slow-flow habitats such as runs and pools. All species of the "*marchanti*" clade, *A*. *marchanti*, *Austrophlebioide*s sp. AV2, *Austrophlebioides* sp. AV4, *Austrophlebioides* sp. AV5, and *Austrophlebioides* sp. AV7, occur in fast-flowing riffle habitats. Within the "wet tropics" clade *A*. *rieki* and *A*. *porphyrobranchus* are found in riffles with fast flow, whereas *A*. *wooroonooran* occurs mostly in pools and runs in which flow conditions range from slow to moderate. Reconstruction of ancestral character states suggests that the ancestral habitat for the genus *Austrophlebioides* was one with fast flow (Fig. 5.1b). The character-state reconstruction also implies that there have been two independent evolutionary shifts in habitat use within the genus and on both occasions these shifts were from fast to slow flow habitats. The first occurred on the branch leading to the species of the "*pusillus*" clade, and the second leading to *A*. *wooroonooran* within the "wet tropics" clade (Fig. 5.1b).

5.3.3 Association between labrum morphology and habitat use.

When the distribution of habitat use and labrum shape are compared on the phylogeny, it is evident that there is a correspondence between the morphology of the labrum and the type of habitat occupied by the nymph (Fig. 5.1a,b). Species that occur in fast flow have a broad labrum whereas species that occur in moderate to slow flow have a narrower labrum. On the two occasions where there has apparently been an evolutionary shift in habitat use from fast to slow-moderate flow, a change in the shape of the labrum is postulated from broad to narrow (Fig. 5.1a,b). With so few evolutionary changes in both habitat use and labrum shape within the genus, testing the statistical significance of this association using phylogenetic independent contrasts (Harvey and Pagel 1991) was not possible. Maddison's (1990) concentrated-changes test for correlated character evolution could be performed on the data; however, it has been demonstrated that this test may fail to detect a significant association when the number of species involved is fewer than 25 (Lorch and Eadie 1999).

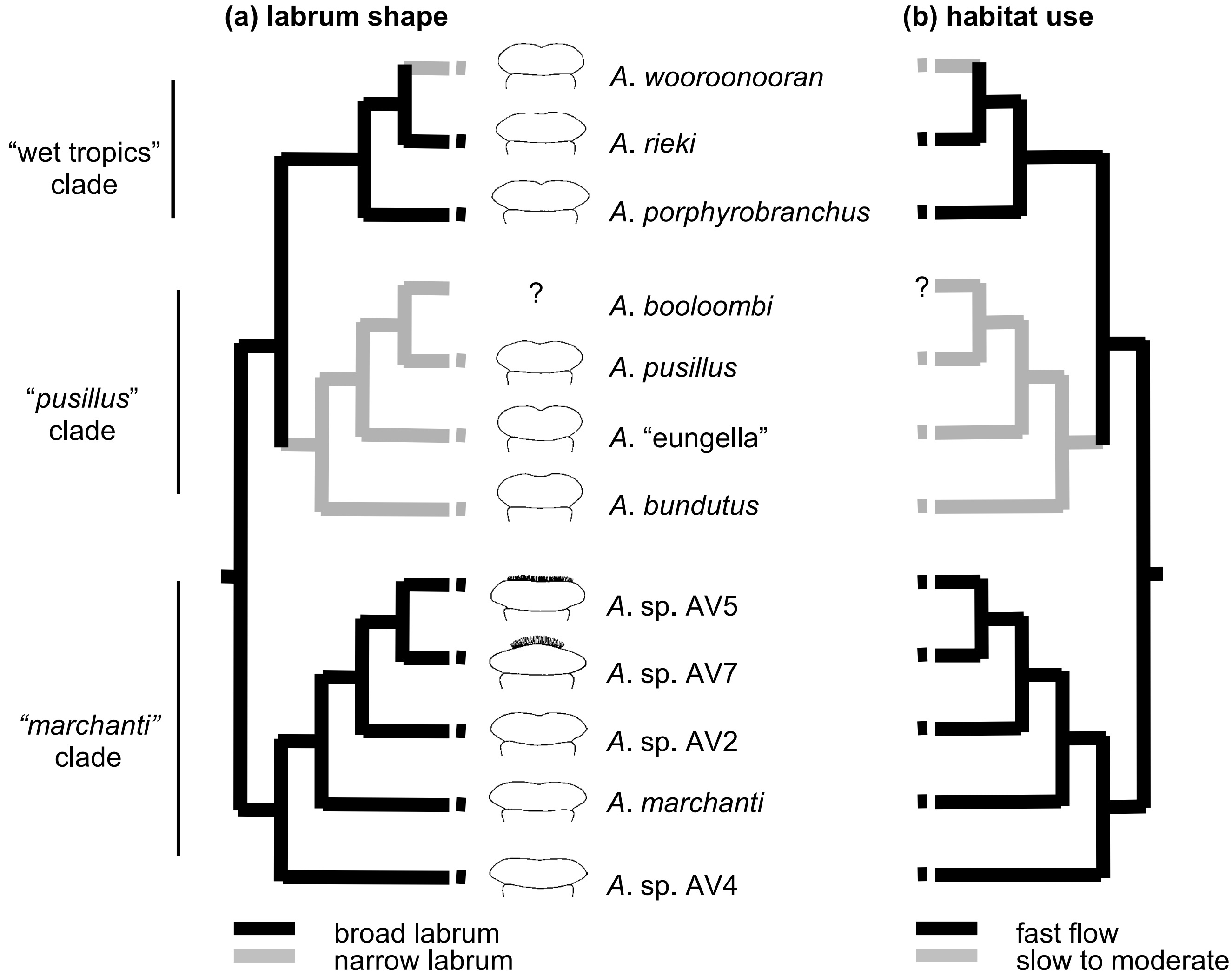


Figure 5.1. Phylogenetic hypothesis for the genus *Austrophlebioides* showing reconstruction of ancestral character states for: (a) labrum shape, and (b) habitat use

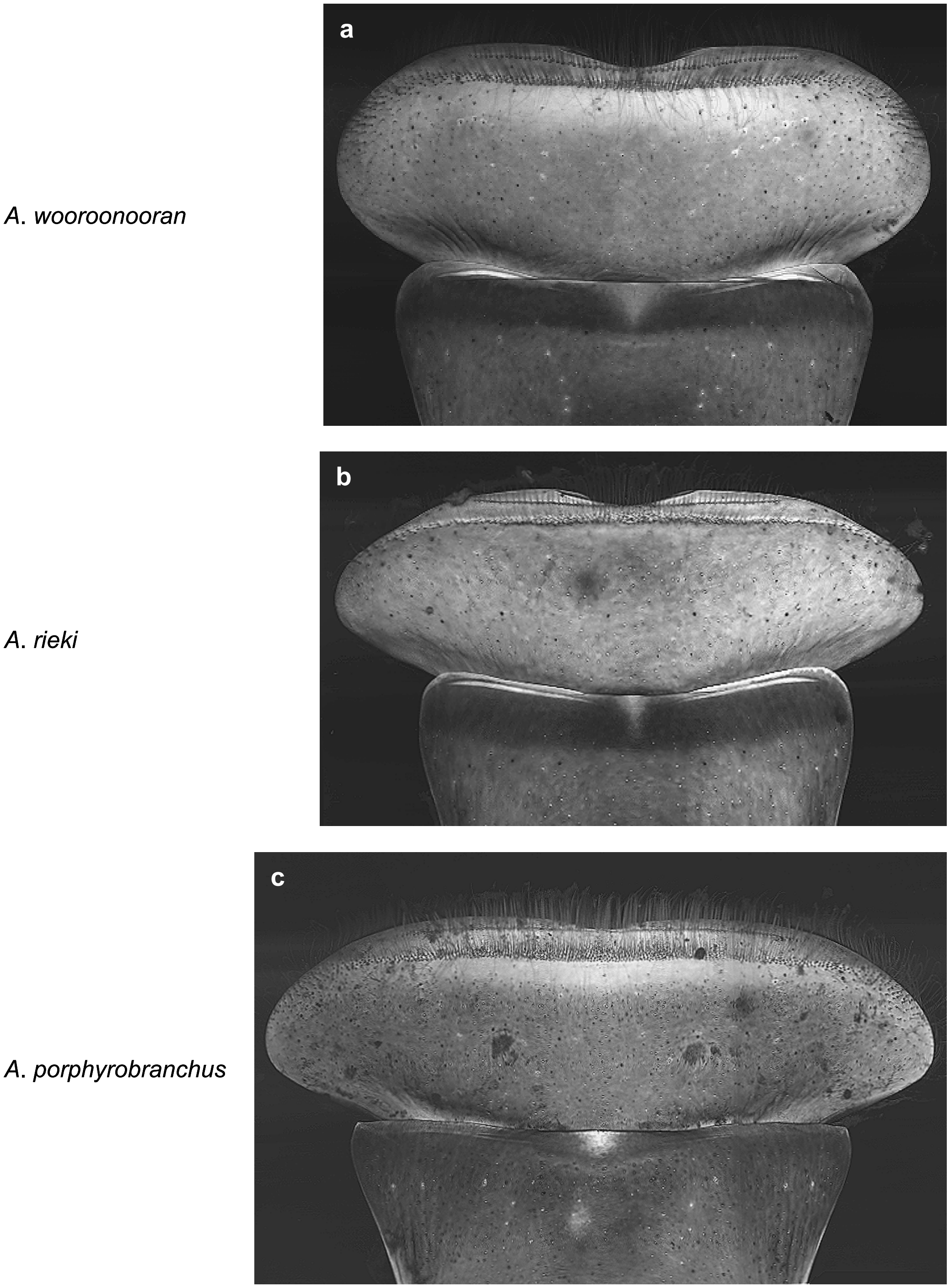


Figure 5.2. Labrum and clypeus (dorsal view). Species of the “wet tropics” clade: (a) *A. wooroonooran*; (b) *A. rieki*; (c) *A. porphyrobranchus*.

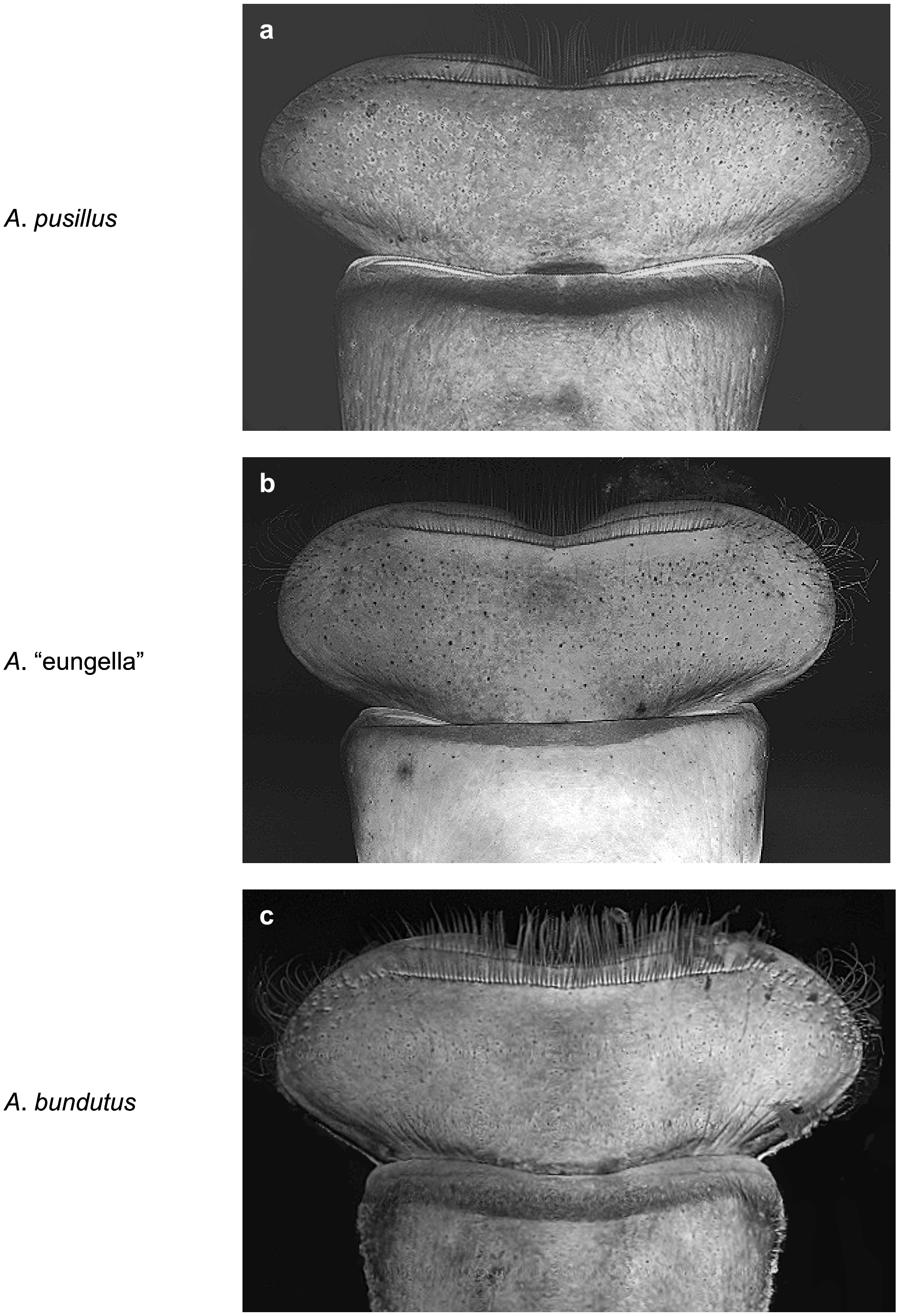


Figure 5.3. Labrum and clypeus (dorsal view). Species of the "*pusillus*" clade: (a) *A*. *pusillus*; (b) *A*. "eungella"; (c) *A*. *bundutus*.

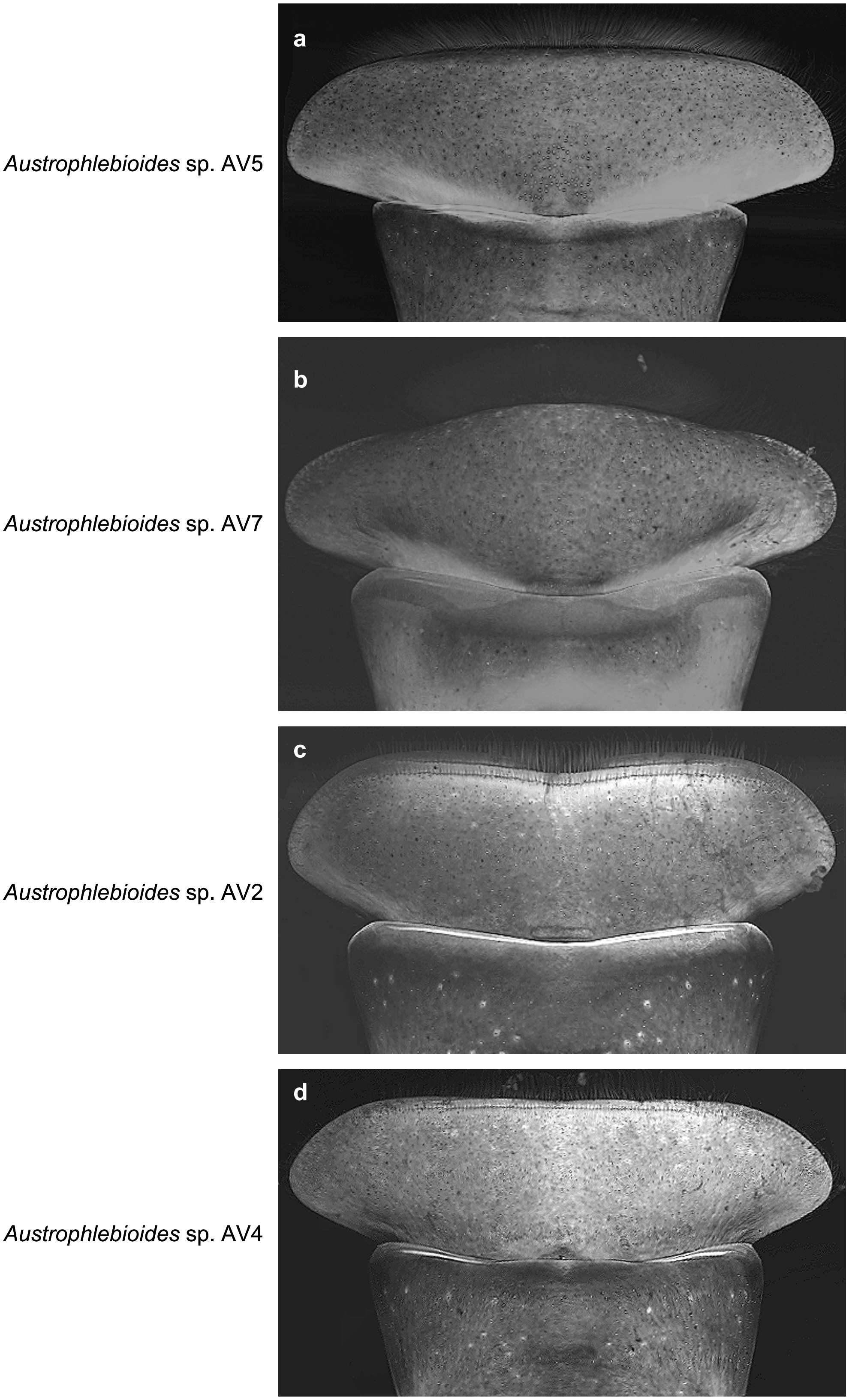


Figure 5.4. Labrum and clypeus (dorsal view). Species of the "*marchanti*" clade: (a) *Austrophlebioides* sp AV5; (b) *Austrophlebioides* sp AV7; (c) *Austrophlebioides* sp AV2; (d) *Austrophlebioides* sp AV4.

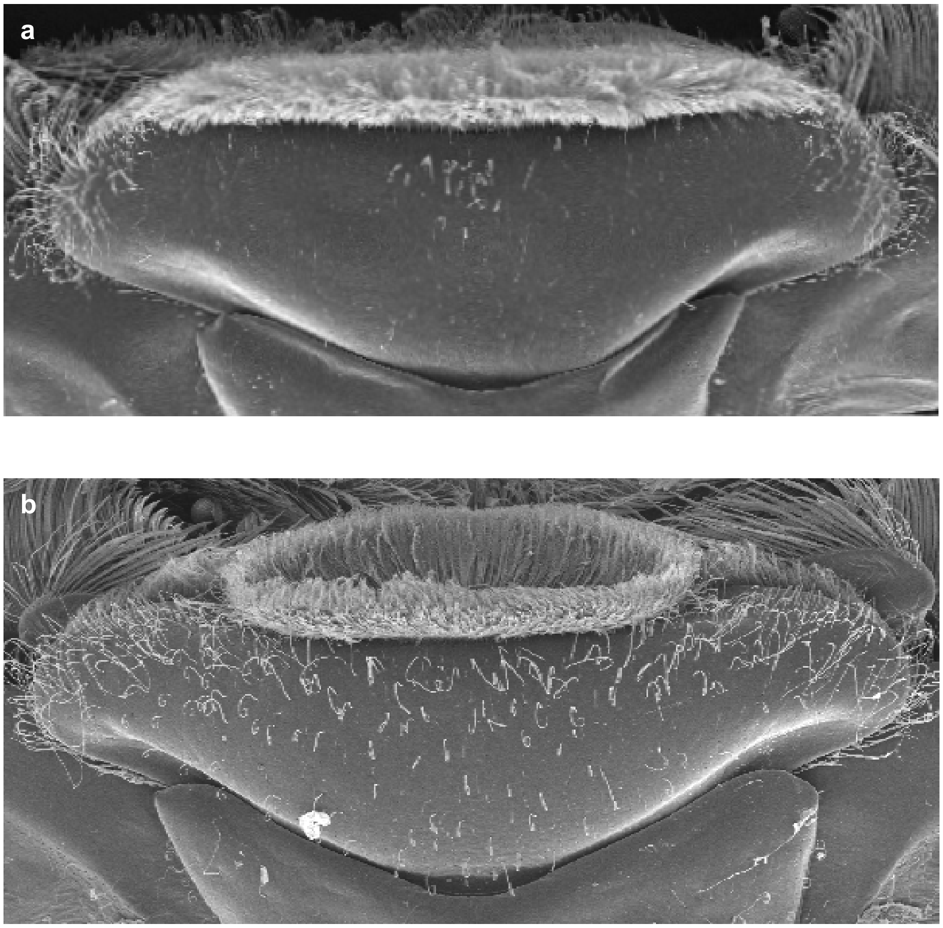


Figure 5.5. Scanning electron micrographs of labrum showing arrangement of setae (dorsal view): (a) *Austrophlebioides* sp. AV5; (b) *Austrophlebioides* sp. AV7.

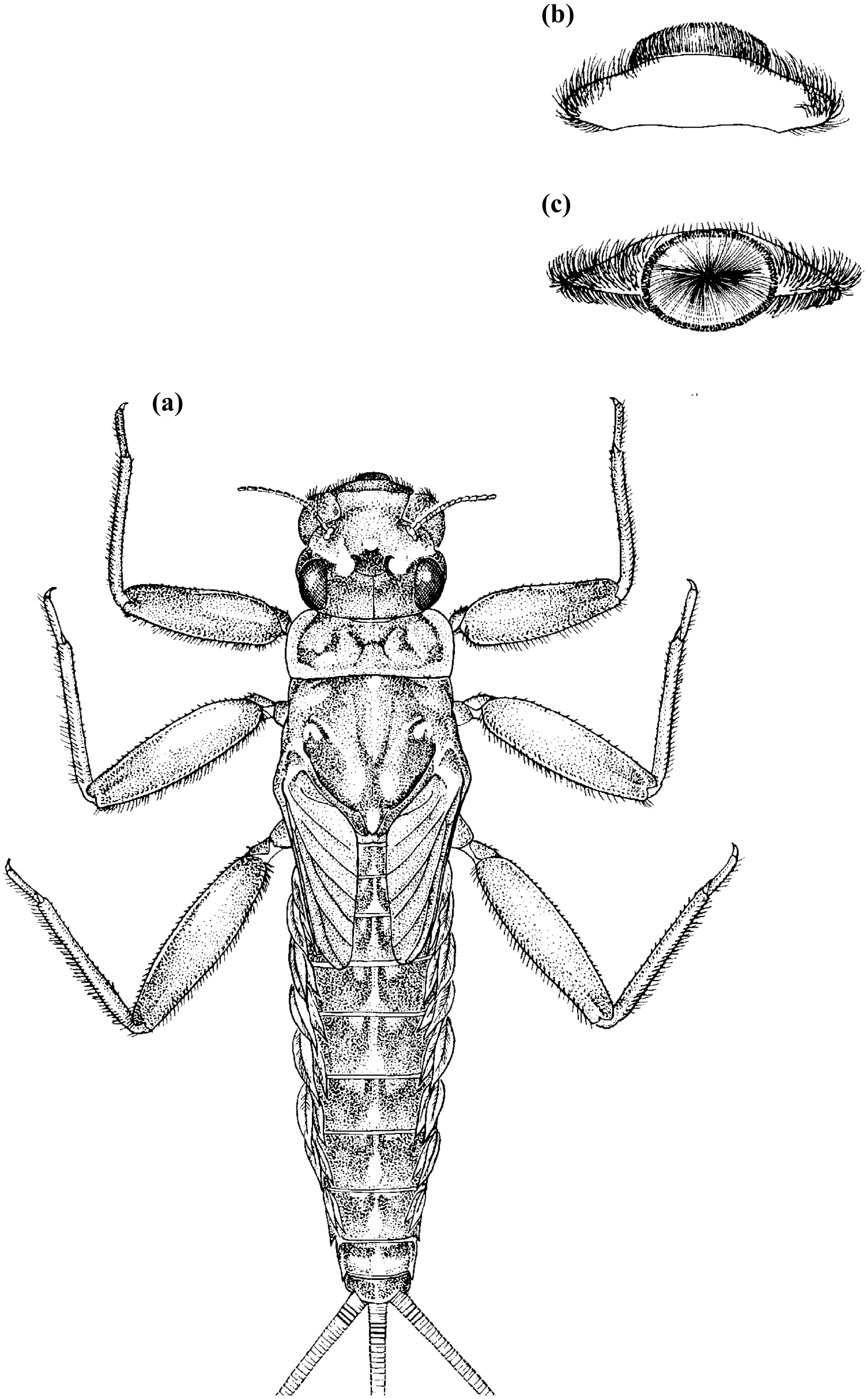


Figure 5.6. *Austrophlebioides* sp. AV7: (a) whole nymph; (b) labrum, dorsal view; (c) labrum, apical view. Illustration by Sybil Monteith.

5.4 Discussion

The goal of this study was to investigate the evolution of the morphology of the labrum in the genus *Austrophlebioides*, to allow inferences to be made on the role that flow regimes have played in shaping labrum morphology. Reconstruction of ancestral character states suggests that a broad labrum and use of high-flow environments are ancestral features which have evolved only once, early in the evolutionary history of the genus. The present-day use of habitats with slow to moderate flow by several species of *Austrophlebioides* appear to be reversals or shifts from fast-to slow-flow environments, and appear to have occurred independently twice within the genus. On each occasion an evolutionary shift in habitat use from fast to slow-moderate flow was accompanied by a change in the shape of the labrum from broad to narrow, suggesting that flow has been an important selective factor in the evolution of the morphology of the labrum in *Austrophlebioides*.

Evolutionary changes in labrum morphology in the genus *Austrophlebioides* appear to have occurred in two divergent directions: (1) a reversal from a broad to narrower labrum, and (2) retention of a broad labrum but with an increase in the density of setae at the anterior margin. Species with a relatively narrow labrum are not basal in the *Austrophlebioides* phylogeny as would be expected if changes in labrum shape had proceeded from narrow to broad. This contrasts with a general observation in the Leptophlebiidae, in which a broad labrum is believed to be the derived character state, and a narrower labrum the ancestral condition (Pescador and Peters 1980; Towns and Peters 1980). It was expected that changes in the shape of the labrum in *Austrophlebioides* would follow this general pattern but this was not the case. In *Austrophlebioides* a narrower labrum appears to have evolved secondarily on two independent occasions.

An extreme and novel modification to the labrum is seen in the two closely related species, *Austrophlebioides* sp. AV5 and *Austrophlebioides* sp. AV7, of the "*marchanti*" clade. Both have retained a broad labrum but with a marked increase in the density of setae at the anterior margin. The subapical and apical setal fringes on the dorsal surface of the labrum are no longer distinct as in other *Austrophlebioides* species. In *Austrophlebioides* sp. AV5 apical setae are arranged loosely into an elliptical disc, and in *Austrophlebioides* sp. AV7 these setae are organised more tightly into a round setal disc. As these species appear to be sister taxa it suggests that they have evolved from an ancestor that possessed a dense band of setae at the apex of the labrum. An increase in the density of setae, followed by the arrangement of these setae into a loosely formed disc, may precede the evolution of a more well-formed setal disc. Along with the evolution of a setal disc, the median notch at the anterior margin of the labrum appears to have been lost secondarily in *Austrophlebioides* sp. AV7 and greatly reduced in *Austrophlebioides* sp. AV5. Development of a setal disc and changes to the median notch represent further evolutionary modifications to the labrum and may confer some selective advantage to these species.

The presence of dense setal fringes along anterior and ventral margins of the labrum has been suggested to assist in the shielding of the mouthparts from strong current (Arens 1989). However, dense setae at the apical margin of the labrum may also function to increase contact with the substrate, enabling a better hold and the avoidance of dislodgment in fast flow. The arrangement of dense setae at the apex of the labrum into a sucker-like setal disc in *Austrophlebioides* sp. AV7 suggests such a function.

The retention of a broad labrum in all *Austrophlebioides* species that occur in fast flowing habitats tends to support the suggestions that a broad labrum might help prevent dislodged food particles from being washed away, by shielding the preoral cavity and mouthparts from strong current (Hynes 1970; Arens 1989; Ward 1992). A broad labrum may thus enable species to access food resources unavailable to other species lacking this feature. Given that a broad labrum has not been retained when there has been an evolutionary shift into habitats with slow flow, it suggests that this feature may not be as beneficial in slow currents. At present it is unclear what selective advantages a narrower labrum might confer on species living in slow-flow habitats.

Modifications to the labrum, similar to those seen in *Austrophlebioides*, have also been observed in the two species of the Australian genus *Kirrara* (Campbell and Peters 1986; Dean 1999). The labrum of the two *Kirrara* species is even more laterally expanded than in the species of *Austrophlebioides*. In *K. procera* the setae at the apex of the labrum are very dense, as in *Austrophlebioides* sp. AV5, and in *Kirrara* sp. AV1 the setae are arranged in a round disc, similar to that observed in *Austrophlebioides* sp.

AV7 (Appendix 1). A median notch is also absent in the two *Kirrara* species, and is believed to have been secondarily lost (Campbell and Peters 1986). The nymphs of *K. procera* occur on boulders in rapid flowing sections of large streams and rivers (Peters and Campbell 1991), and those of *Kirrara* sp. AV1 are found on rock-faces of waterfalls and on stones in riffles of nearby streams (Clayton 1995; pers. obs.). Whether *Austrophlebioides* sp. AV7, which also possesses a well-formed setal disc on the labrum, occurs in areas of torrential flow is unknown. The nymphs of *Austrophlebioides* sp. AV5 and *Austrophlebioides* sp. AV7 have been collected from riffle sections of streams in close proximity to waterfalls within the Cradle Mountain National Park in Tasmania, but the waterfalls were not sampled (J. Dean pers. comm.; pers. obs.). The secondary loss of a median notch in these two *Austrophlebioides* species and in the two species of *Kirrara* may be a further modification to restrict water flow underneath the labrum and protect the preoral cavity from the current.

It appears that labral setal discs have evolved independently on least two occasions within the Australian Leptophlebiidae, once in the genus *Kirrara* and once in *Austrophlebioides*. It is noteworthy that setal discs have so far only been observed in species that possess a broad labrum. This suggests that a broad labrum may precede the development of more elaborate structures such as setal discs which may allow access to habitats with very fast to torrential flow.

In conclusion, flow does appear to have played an important role in the evolution of the morphology of the labrum in *Austrophlebioides*. However, both the shape of the labrum and the type of habitat occupied by the nymph are fairly conservative within the three *Austrophlebioides* clades, indicating that evolutionary history is also an important determinant of labrum morphology and habitat use in this genus. Future studies could be conducted to investigate performance and fitness advantage associated with the different labrum morphologies in the variety of flow regimes within streams.

CHAPTER 6

A phylogenetic perspective on patterns of substrate and habitat use among leptophlebiid mayflies (Ephemeroptera: Leptophlebiidae)

6.1 Introduction

Ecologists have traditionally focused on the role of contemporary ecological factors (e.g. competition, predation, disturbance) in determining the distribution and abundance of organisms and the structure of communities (Schoener 1974; Cody and Diamond 1975; Strong *et al*. 1984; Morin 1999). More recently, the importance of historical influences on the ecological characteristics of organisms and on community structure has been recognised (Ricklefs 1987; Wanntorp *et al*. 1990; Ricklefs and Schluter 1993). The evolutionary history of lineages that comprise communities may influence community structure by limiting the ecological roles of lineage members (Cadle and Greene 1993). Recent studies on a diverse range of organisms including snakes (Cadle and Greene 1993), herbivorous insects (Farell and Mitter 1993), frogs (Zimmerman and Simberloff 1996), lizards (Vitt *et al*. 1999) and birds (Forstmeier *et al*. 2001) have shown that some aspects of community organisation (e.g. patterns of resource use) may be the result of evolutionary differences among lineages that comprise communities, rather than the outcome of proximate causes.

Studies on stream fauna generally have emphasised the importance of abiotic factors (e.g. current velocity, substrate type, presence of organic matter) in structuring benthic invertebrate communities and affecting the distribution of species among habitats (Cummins and Lauf 1969; Hynes 1970, Rabeni and Minshall 1977; Statzner and Higler 1986; Allan 1995; Hart and Finelli 1999). Physical factors are believed also to mediate the effects of species interactions such as competition (Hemphill and Cooper 1983; McAuliffe 1984) and predation (Peckarsky *et al*. 1990; Hansen *et al*. 1991; Hart and Merz 1998). Individual species vary in their response to abiotic factors in a species-specific manner, and many show a preference for either riffle (fast flow) or pool (low flow) habitats (Edington 1968; Hynes 1970; Barmuta 1990; Hearnden and Pearson 1991; Growns and Davis 1994). Similarly, different species may vary in their degree of association with substrate type (Hynes 1970; Minshall and Minshall 1977; Reice 1980; Minshall 1984; Holomuzki and Messier 1993). Whether such distributional patterns are influenced by historical processes has rarely been addressed by stream ecologists (Vinson and Hawkins 1998). In this chapter I assess the role of phylogenetic history in shaping patterns of habitat and substrate use among leptophlebiid mayfly assemblages of four tropical Australian streams.

The Leptophlebiidae is the most speciose mayfly family in Australia (Dean 1999), and in many Australian streams the nymphs form an important component of the benthic fauna (Pearson *et al*. 1986; Peters and Campbell 1991). Leptophlebiid nymphs occur on a range of substrate types such as stones and organic matter, and in a variety of stream habitats including pools, runs, riffles, and even the rock faces of boulders and waterfalls (Peters and Campbell 1991; Towns and Peters 1996). Although the family has diversified into a wide range of habitats, the same diversification appears not to be true of genera and species within individual lineages (Pescador and Peters 1980). For example, the nymphs of some lineages are found predominantly on stony substrates whereas others are associated more commonly with leaf litter accumulations. Some lineages appear to be restricted to slow flow environments whereas others are also common in fast flow. Thus, several aspects of the ecology of leptophlebiid mayflies appear to be strongly influenced by phylogenetic history.

In this study I firstly document the mayfly fauna of leaf litter and stones in pools, runs and riffles of four streams, to determine patterns of substrate and habitat use among species. Specifically, I ask if stones and leaf litter, and pools, runs and riffles, support similar mayfly assemblages, and if individual species are strongly associated with either stones or leaf litter, and/or fast or low flow. Secondly, I investigate whether patterns of substrate and habitat use among species reflect phylogenetic relationships. If phylogeny is important, closely related species are expected to be similar in their use of substrate and habitat type. Alternatively, if closely related species differ markedly in substrate or habitat use then factors other then phylogenetic history may be responsible for observed patterns.

This approach, incorporating phylogenetic information into an ecological study, has rarely been attempted for stream fauna and is particularly interesting because of the known, strong influences of physical factors on the distributions of stream organisms.

6.2 Methods

6.2.1 Study sites

The four rainforest streams selected for this study are located within the Wet Tropics bioregion of north-eastern Australia (Fig. 6.1). All are pristine, third or fourth order streams and the surrounding vegetation is vine forest (Webb and Tracey 1981). Cloudy Ck (19^o 00′S 146^o 12′E) is located at the southern end of the Wet Tropics, on the Paluma Range at an elevation of 850 m. Goolagan Ck (17^o 36.5′S 145^o 45.5′E) and Charmillan Ck (17^o 42.1′S 145^o 31.3′E) are situated on the central Atherton region of the Wet Tropics, at elevations of 380 m and 900 m respectively. Oliver Ck (16^o 08.3′S 145^o 26.4′E) is located at the northern end of the Wet Tropics, at an elevation of 20 m. Sampling was conducted during the dry season, from June to August 1999, when water levels were low and less variable than during the rainy season (December - March).

At each stream, two sites were chosen and at each site, one pool, one run and one riffle was selected. The three habitat types were clearly distinguished by current velocity, depth and flow characteristics (Tables 6.1 and 6.2). Riffles had higher current velocities, were relatively shallow, and water flow was turbulent and broken at the surface. Pools had much lower current velocity, were deeper and flow at the surface was smooth. Runs had current velocities and depths intermediate to those of pools and riffles, water flow at the surface was not broken but was not as smooth as in pools.

6.2.2 Invertebrate sampling and identification

Two replicate samples of stones and of leaf litter were taken from the pool, run and riffle, at each of the two sites, within each stream. The mayfly fauna of stones was sampled using a 50 x 50 cm quadrat (0.25 m^2). A net was positioned downstream from the quadrat and a small hand net was placed below each stone as it was removed from

Figure 6.1. Location of the four study streams within the Wet Tropics bioregion of north-eastern Australia.

the stream. The stones were gently washed in a bucket of water and any remaining mayflies were picked from stones using forceps, and nets checked for dislodged mayflies. The stone samples were taken from areas with minimal leaf litter. The leaf litter was sampled by filling a 2000 ml bag with leaves from leaf litter accumulations within the stream. Once again, as for stone samples, a small hand net was placed below the leaf litter as it was brought to the surface. Care was taken in collecting leaf samples to not disturb adjacent stones, but some stone fauna may have been collected accidentally. All samples were preserved in 70% alcohol and sorted in the laboratory.

All leptophlebiid mayflies were identified to species using keys developed for this study (Appendix 1). The majority of species are undescribed, and four appear to belong to two new genera. The species WT sp. 2 and WT sp. 4 belong to one new genus, and WT sp. 1 and WT sp. 3 to a second new genus. Undescribed species were allocated a voucher number or an informal name for use in this study. Most species are easily distinguished using the keys, except for the nymphs of *Austrophlebioides* ‘paluma’ collected from Cloudy Ck and *Austrophlebioides rieki* collected from Oliver Ck. On morphological grounds alone it is difficult to establish whether these specimens belong to a single widespread species or to two very closely related species. In the present study they are considered as separate species because they differ in their pattern of habitat use. Individuals of other families–Baetidae, Caenidae and Ameletopsidae–were counted but were not identified beyond family level. All abundance values are expressed as number of individuals per sample.

6.2.3 Physical measurements

At each sampling point, current velocity just below the surface was measured using a Swoffer 3000 current meter and the depth of water was recorded. The water temperature at each stream was also measured.

6.2.4 Analyses

The similarity of the mayfly assemblages of stones and leaf litter in pools, runs and riffles at the four streams was examined using the multivariate ordination technique of semi-strong hybrid multidimensional scaling (SSH) in the PATN package (Belbin 1995). Analyses were performed on combined stone and leaf litter data, and on stone and leaf litter data separately. As sampling of stones and leaves was not comparable, a range standardisation by row (Belbin 1995) was applied to the mean abundance data prior to ordination of combined stone and leaf litter samples. This had the effect of placing all abundance values in rows on a scale of zero to one (Belbin 1995). For the separate analyses of the stone and leaf data, mean abundances of individual species at each site were $\log_{10}(x+1)$ transformed to reduce the influence of highly abundant species. In all ordinations, the Bray-Curtis association measure was used as a measure of similarity, and three dimensions were required to achieve an acceptable level of stress from 500 random starts. The Principal Axis Correlation (PCC) procedure in PATN was used to calculate maximum linear correlations between individual taxa and ordinational space. The statistical significance of the correlation coefficients generated by PCC was determined by performing Monte-Carlo tests using 100 randomizations.

Analysis of variance was used to test for differences in abundance of common species among the three habitat types (pools, runs, riffles) and four streams. Habitat type was treated as a fixed factor and stream and site as random factors, with site nested within stream. Leaf litter and stone data were analysed separately. When significant differences were found, a Tukey's Honest Significance Difference test (alpha level of 0.05) was used to compare means. Abundance data (number of individuals per sample) were $\log_{10}(x+1)$ transformed to improve normality and homogeneity of variance. ANOVAs were not performed on species found in only one habitat type. Streams with fewer than 10 individuals of a given species or taxon were not included in the analyses. All analyses were performed using SPSS version 10.

To investigate the influence of evolutionary history on substrate and habitat use, I used the strict consensus tree generated in Chapter 2 (Fig. 2.3) as an estimate of phylogenenetic relationships among the Australian Leptophlebiidae genera (Fig. 6.2). As the *Hapsiphlebia* lineage does not appear to be monophyletic, the term "grade" is used to refer to the group of basal genera previously assigned to this lineage.

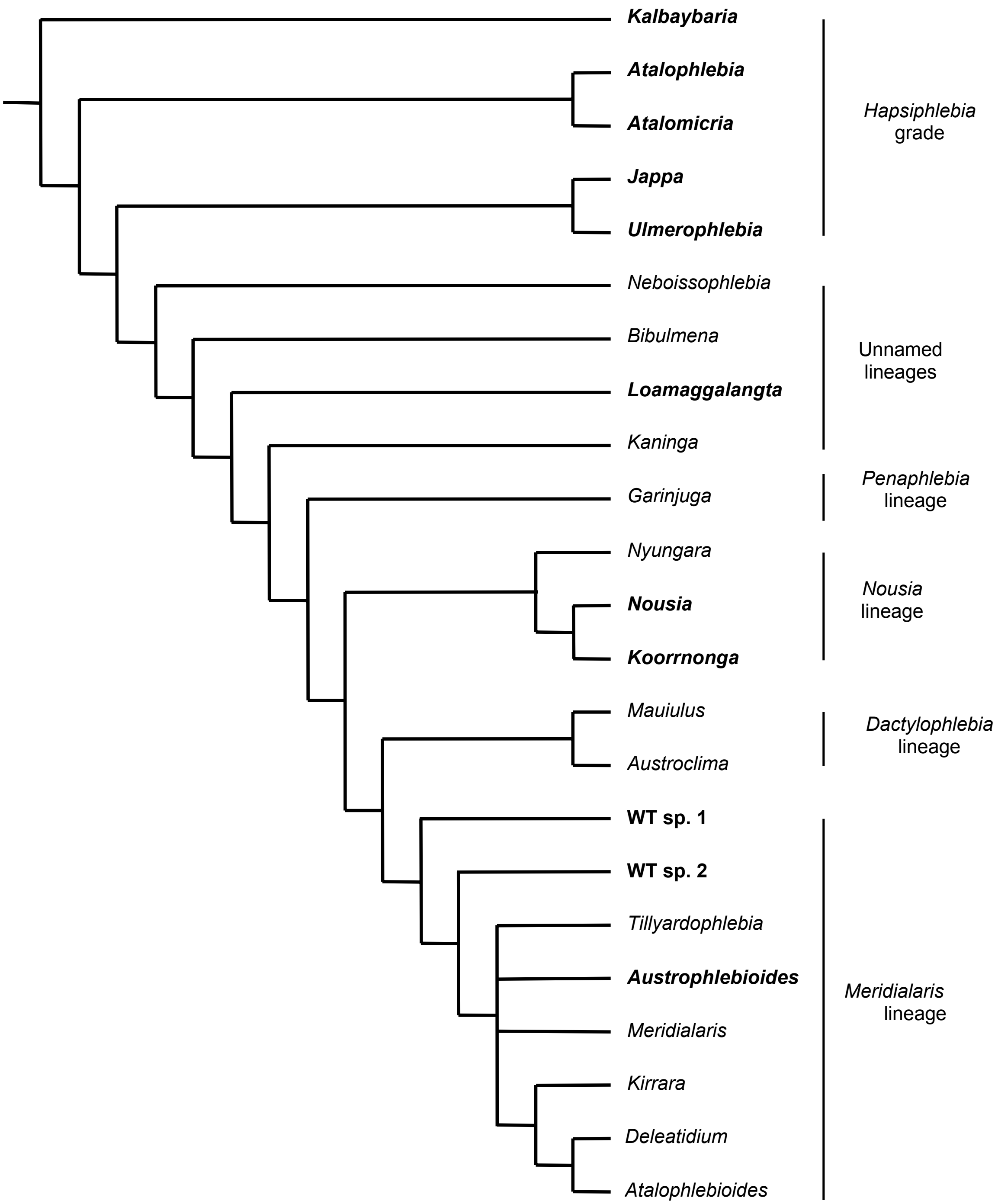


Figure 6.2. Proposed phylogenetic relationships among Australian Leptophlebiidae genera (and selected genera from New Zealand and southern South America), based on strict consensus tree generated in Chapter 2. Genera recorded in present study in bold.

6.3 Results

6.3.1 Physical characteristics

Current velocities differed markedly between the three habitat types (Tables 6.1 and 6.2). Mean current velocity of pools generally was less than 4.0 cm/sec, slightly higher at Charmillan Ck. Runs had mean velocities ranging from 5.4 to 17.7 cm/sec; and riffles ranged from 25.8 to 60.6 cm/sec. Within each habitat type, leaf litter was generally collected from areas of lower current velocities than stones (Tables 6.1 and 6.2). Riffles at all four streams were shallow, with mean depths of 7.5 to 10.5 cm. Pools were deeper with mean depths of 23.2 to 40.0 cm. Runs were intermediate in depth between the riffles and pools. Water temperature was 12°C at Cloudy Ck, and 18 to 19°C at Henrietta Ck, Charmillan Ck and Oliver Ck.

6.3.2 Species richness and distribution

A total of nineteen species of Leptophlebiidae were recorded from the four streams (Table 6.3). Species richness across the four streams was comparable, with Goolagan Ck having a slightly higher number of species. Several species, including *Atalomicria sexfasciata*, *Nousia* sp. NQ1 and *Nousia* sp. NQ2, were found at all four streams, whereas others had more limited distributions. Two genera, *Austrophlebioides* and *Koorrnonga*, occurred in all streams but were represented by different species.

6.3.3 Assemblage structure

Ordination of combined data by SSH revealed a major separation between the mayfly assemblages of leaf litter and stones (Fig. 6.3). Species belonging to the *Meridialaris* lineage (including WT sp. 1, WT sp. 2, WT sp. 3, WT sp. 4, *Austrophlebioides* species) and the Baetidae, were associated with stones (Fig. 6.3, Table 6.4). Species associated with leaf litter included members of the *Nousia* lineage (*Koorrnonga* sp. AV4, *Koorrnonga* sp. AV6, *Nousia* sp. NQ1 and *Nousia* sp. NQ2) and *Atalophlebia* sp. AV13 (*Hapsiphlebia* grade) (Fig. 6.3, Table 6.4). Given the strong separation by substrate type, separate ordination analyses were performed on the stone and leaf litter samples.

Table 6.1. Mean velocity and mean depth (plus standard errors) of stone samples.

	Mean Velocity (cm/second)			Mean Depth (cm)		
	Pool	Run	Riffle	Pool	Run	Riffle
Cloudy Ck	1.97 (0.74)	9.92 (2.00)	39.22 (1.96)	23.25 (2.88)	12.62 (1.55)	9.25 (0.99)
Goolagan Ck	2.77 (0.74)	15.35 (1.08)	45.07 (6.97)	25.50 (2.23)	15.00 (2.21)	10.50 (1.53)
Charmillan Ck	6.30 (1.34)	13.60 (2.93)	60.52 (8.21)	40.00 (6.46)	15.25 (1.59)	8.12 (1.80)
Oliver Ck	2.10 (0.63)	17.70 (6.11)	60.65 (3.34)	30.12 (6.76)	17.50 (3.45)	10.25 (0.99)

Table 6.2. Mean velocity and mean depth (plus standard errors) of leaf litter samples.

	Mean Velocity (cm/second)			Mean Depth (cm)		
	Pool	Run	Riffle	Pool	Run	Riffle
Cloudy Ck	0.50 (0.00)	10.28 (1.12)	35.40 (5.42)	26.50 (11.04)	13.00 (5.94)	8.25 (1.36)
Goolagan Ck	3.88 (0.06)	6.25 (1.68)	25.83 (2.74)	34.50 (0.33)	10.50 (2.43)	7.75 (1.19)
Charmillan Ck	0.05 (0.03)	5.43 (0.89)	29.93 (6.22)	37.50 (5.00)	18.50 (2.33)	7.50 (1.20)
Oliver Ck	0.05 (0.00)	10.23 (1.57)	31.53 (3.62)	30.25 (12.11)	8.75 (1.44)	8.25 (1.36)

Table 6.3. List of Leptophlebiidae species recorded from the four study streams.

Species	Stream Cloudy Ck	 Goolagan Ck	 Charmillan Ck	 Oliver Ck
Hapsiphlebia grade				
Atalophlebia sp. AV13	+	+	+	
Ulmerophlebia sp. AV3	+	+	+	+
Jappa serrata	+			+
Jappa edmundsi		+	+	+
Atalomicria sexfasciata	+	+	+	+
Kalbaybaria sp.		+		
Unnamed lineage				
Loamaggalangta sp. AV2	+	+	+	
Nousia lineage				
Koorrnonga sp. AV4	+	+	+	
Koorrnonga sp. AV6				+
Nousia sp. NQ1	+	+	+	+
Nousia sp. NQ2	+	+	+	+
Meridialaris lineage				
WT sp. 1	+			
WT sp. 3		+		
WT sp. 2				+
WT sp. 4				+
Austrophlebioides porphyrobranchus		+	+	
Austrophlebioides wooroonooran		+	+	
Austrophlebioides 'paluma'	+			
Austrophlebioides rieki				+
Total number of species	10	12	10	10

The ordination of samples from leaf litter indicated that the three habitat types (pools, runs, riffles) supported different mayfly assemblages (Fig. 6.4, Table 6.5). Samples grouped predominantly by habitat type and not by stream, except for pool and run samples from Oliver Ck. These habitats were characterised by the presence of *Koorrnonga* sp. AV6, which was not recorded from other streams. The Oliver Ck riffle samples grouped with riffle samples from other creeks (Fig. 6.4). *Nousia* sp. NQ2 was significantly associated with run and riffle samples, and *Nousia* sp. NQ1 with riffle samples at all four creeks (Fig. 6.4, Table 6.5). In contrast, *Atalophlebia* sp. AV13, *Ulmerophlebia* sp. AV3, *Koorrnonga* sp. AV4, *Koorrnonga* sp. AV6, and Caenidae were significantly associated with pool samples at Cloudy Ck, Charmillan Ck and Goolagan Ck (Fig. 6.4, Table 6.5).

Ordination of the stone fauna revealed differences among assemblages in pools, runs and riffles (Fig. 6.5), although there were considerable differences in the composition of the stone fauna between streams. The separation of streams by location was due primarily to the occurrence of several species with restricted distributions. The genus *Austrophlebioides*, although present in all streams, was represented by different species: *A*. 'paluma' at Cloudy Ck, *A. rieki* at Oliver Ck, and *A. wooroonooran* and *A. porphyrobranchus* at Goolagan Ck and Charmillan Ck (Table 6.3). Several additional species were only recorded from one locality. For example, WT sp. 2 and WT sp. 4 were recorded only at Oliver Ck and WT sp. 1 was found only at Cloudy Ck (Table 6.3). All these species were correlated significantly with their respective streams in the ordination of the stone data (Fig. 6.5, Table 6.6). Within each of the four streams the stone samples grouped according to habitat type with runs occupying an intermediate position between pools and riffles (Fig. 6.5).

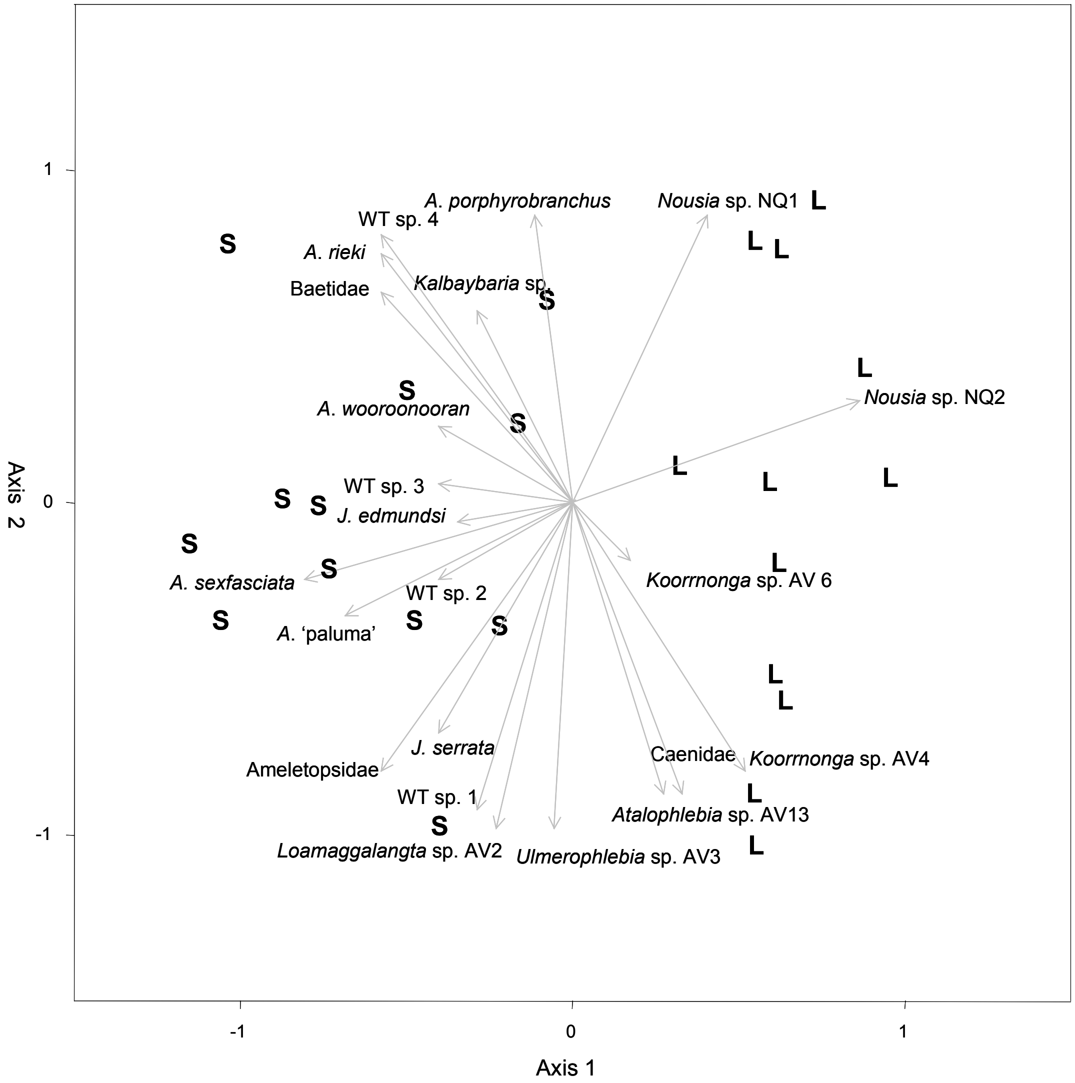


Figure 6.3. SSH ordination of combined stone (**S**) and leaf litter (**L**) data, dimension 1 vs dimension 2, with vectors of maximum correlation for individual taxa (all mayfly taxa plotted; correlation coefficients and Monte-Carlo significance levels are given in Table 6.4).

Table 6.4. Maximum correlation coefficients for mayfly taxa with ordination of combined stone and leaf litter data. Significance levels derived from 100 Monte-Carlo simulations.

Taxon	*r*
Austrophlebioides wooroonooran	0.81**
Atalophlebia sp. AV13	0.88**
WT sp. 2	0.82**
Koorrnonga sp. AV4	0.80**
Nousia sp. NQ1	0.80**
Nousia sp. NQ2	0.68*
Baetidae	0.65*
Caenidae	0.64**
Jappa serrata	0.59ns
Ulmerophlebia sp. AV3	0.55*
Koorrnonga sp. AV6	0.55ns
Jappa edmundsi	0.50ns
Atalomicria sexfasciata	0.48ns
Austrophlebioides rieki	0.46ns
Loamaggalangta sp. AV2	0.44ns
WT sp. 4	0.43ns
WT sp. 1	0.41ns
Mirawara sp.	0.38ns
WT sp. 3	0.34ns
Kalbaybaria sp.	0.30ns
Austrophlebioides porphyrobranchus	0.28ns
Austrophlebioides ‘paluma’	0.10ns

*$P \leq 0.05$; ** $P \leq 0.01$; ns not significant.

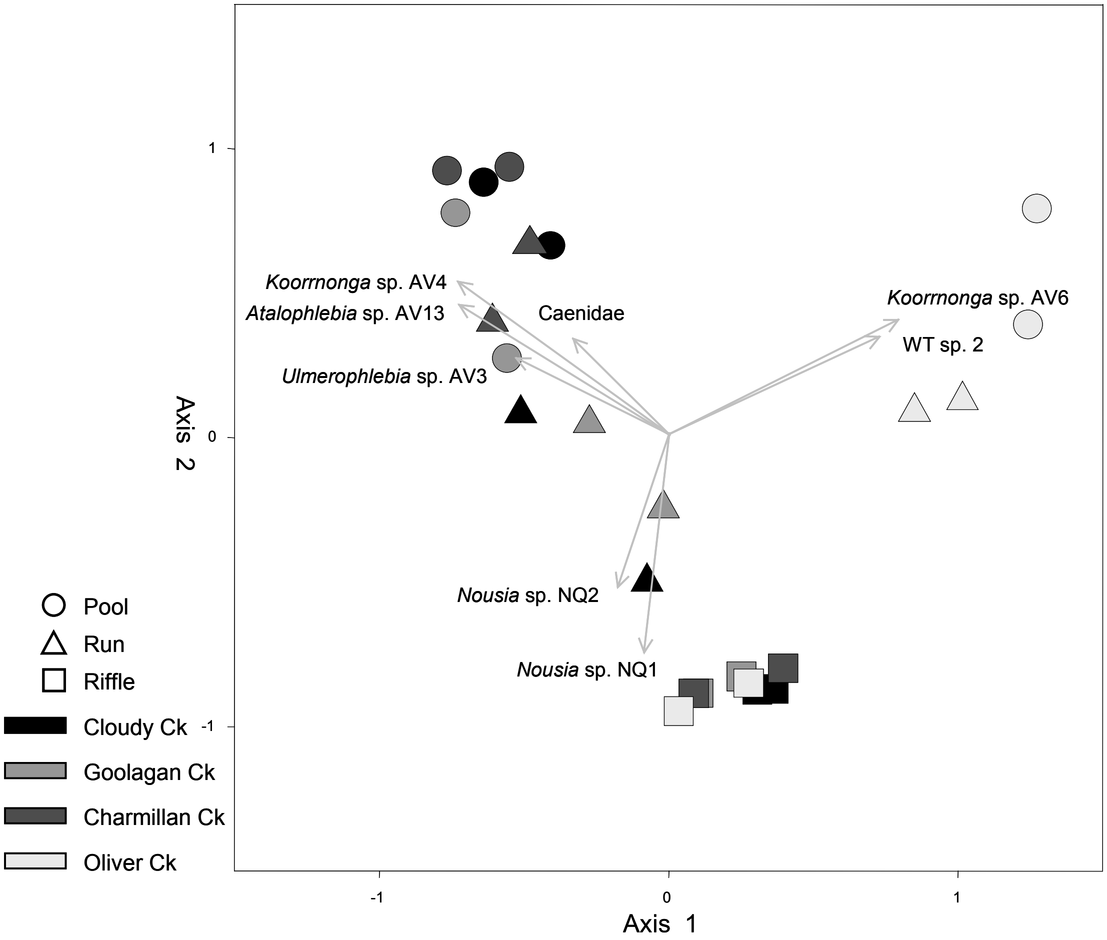


Figure 6.4. SSH ordination of leaf litter data (dimension 2 vs dimension 1), with vectors of significant maximum correlation of individual taxa (correlation coefficients and Monte-carlo significance levels are given in Table 6.5)

Table 6.5. Maximum correlation coefficients for mayfly taxa with SSH ordination of leaf litter data. Significance levels derived from 100 Monte-Carlo simulations.

Taxon	*r*
Koorrnonga sp. AV4	0.99**
Nousia sp. NQ1	0.98**
Atalophlebia sp. AV13	0.96**
Koorrnonga sp. AV6	0.92**
Nousia sp. NQ2	0.90**
Caenidae	0.83**
Ulmerophlebia sp. AV3	0.77**
WT sp. 2	0.68*
Baetidae spp.	0.48ns
Austrophlebioides ‘paluma’	0.48ns
Austrophlebioides wooroonooran	0.45ns
WT sp. 4	0.37ns
Austrophlebioides rieki	0.37ns
Loamaggalangta sp. AV2	0.35ns

* $P \leq 0.05$; ** $P \leq 0.01$; ns not significant.

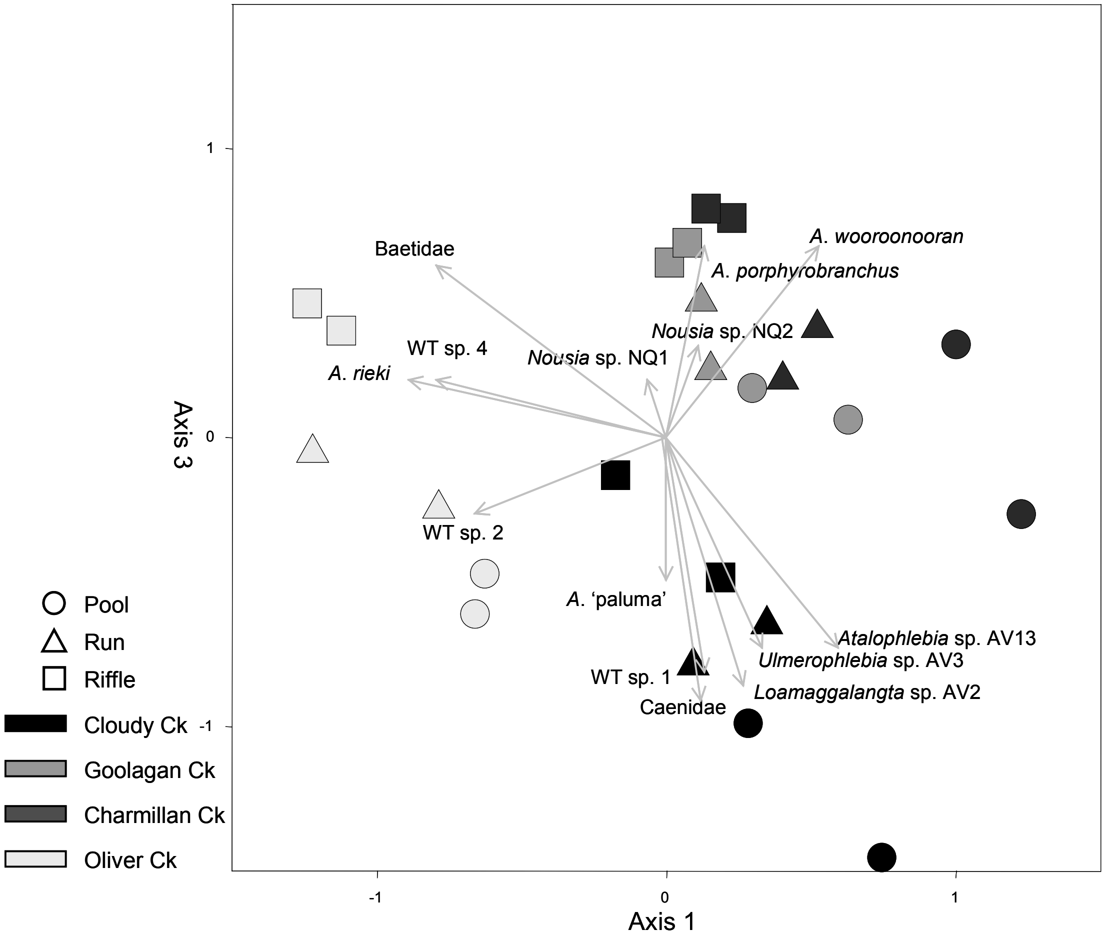


Figure 6.5. SSH ordination of stone data (dimension 3 vs dimension 1), with vectors of significant maximum correlation of individual taxa (correlation coefficients and Monte-carlo significance levels are given in Table 6.6).

Table 6.6. Maximum correlation coefficients for mayfly taxa with ordination of stone data. Significance levels derived from 100 Monte-Carlo simulations.

Taxon	r
Austrophlebioides wooroonooran	0.89**
Atalophlebia sp. AV13	0.89**
WT sp. 1	0.84**
WT sp. 2	0.83**
Loamaggalangta sp. AV2	0.78**
Nousia sp. NQ1	0.77**
Austrophlebioides 'paluma'	0.74*
Baetidae	0.74**
Austrophlebioides rieki	0.71**
Jappa serrata	0.69*
Nousia sp. NQ2	0.65*
Ulmerophlebia sp. AV3	0.65*
Caenidae	0.64**
Austrophlebioides porphyrobranchus	0.63*
WT sp. 4	0.63**
Mirawara sp.	0.49ns
WT sp. 3	0.47ns
Jappa edmundsi	0.47ns
Koorrnonga sp. AV4	0.44ns
Kalbaybaria sp.	0.35ns
Atalomicria sexfasciata	0.30ns

* $P \leq 0.05$; ** $P \leq 0.01$; ns not significant.

6.3.4 Habitat and substrate use of individual taxa

Species belonging to the *Meridialaris* lineage were found almost always on stones and collected only rarely in leaf litter (Fig. 6.6, Table 6.9). Coexisting species from this lineage often differed in habitat use. At Cloudy Ck, numbers of *A*. “paluma” were significantly higher in runs and riffles (Fig. 6.7, Table 6.7), whereas WT sp. 1 was significantly more abundant in pools and runs (Fig. 6.7, Table 6.7). Likewise, at Oliver Ck, *A*. *rieki*, and WT sp. 4 were significantly more abundant in riffles (Fig. 6.7, Table 6.7), whereas WT sp. 2 was most abundant in runs and pools, with numbers dropping significantly in riffles (Fig. 6.7, Table 6.7). At Goolagan Ck and Charmillan Ck (Fig. 6.7) *A*. *wooroonooran* occurred across all three habitat types but was most abundant in runs. However, differences were not statistically significant because of the high variability among samples (Table 6.7). At these two streams *A*. *porphyrobranchus* was collected only in riffles (Fig. 6.7).

Species of the *Nousia* lineage dominated the leaf litter fauna (Fig. 6.6, Fig. 6.8). *Nousia* sp. NQ1 and *Nousia* sp. NQ2 also were consistently found on stones in low numbers (Fig. 6.7), whereas the *Koorrnonga* species were rarely found on stones (in total, only seven individuals were recorded on stones). *Koornonga* sp. AV4 was significantly more abundant among leaf litter in pools than in runs, and was absent from riffles (Fig. 6.8, Table 6.8). *Koornonga* sp. AV6 was equally abundant in pools and runs but was absent from riffles (Fig. 6.8, Table 6.8). *Nousia* sp. NQ2 occurred in all habitat types in leaf litter, with abundances significantly higher in runs and riffles than pools (Fig. 6.8, Table 6.8). *Nousia* sp. NQ2 was not recorded from stones in pools (Fig. 6.7, Table 6.7). *Nousia* sp. NQ1 was significantly more abundant in riffles at all four streams in the leaf litter (Fig. 6.8, Table 6.8), and on stones was recorded only from riffles (Fig. 6.7, Table 6.7).

The species of the *Hapsiphlebia* grade were found on stones and in leaf litter (Fig. 6.6, Table 6.9). Several species only occurred on stones (*Atalomicria sexfasciata*, *Jappa serrata* and *Jappa edmundsi*), whereas others (*Atalophlebia* sp. AV13 and *Ulmerophlebia* sp. AV3*)* were found on both leaf litter and stones (Fig. 6.7, Fig. 6.8, Table 6.9). *Atalophlebia* sp. AV13 and *Ulmerophlebia* sp. AV3 were significantly more abundant in pools and runs and were not recorded in leaf litter samples in riffles (Fig. 6.8, Table 6.8). A similar pattern of habitat use for these two species was observed on stones, although a few individuals were recorded in riffles (Fig. 6.7, Table 6.7). *Atalomicria sexfasciata* occurred in all three habitats on stones and numbers were not significantly different (Fig. 6.7, Table 6.7). *Jappa serrata* and *Jappa edmundsi* were found in low numbers (14 individuals in total) in pools and runs. The few individuals of *Kalbaybaria* sp. recorded from Goolagan Ck were from stones in riffles, although the species has been recorded previously from leaf litter (pers. obs.).

Loamaggalangta sp. AV2, which belongs to an unnamed lineage, showed similar patterns of habitat use to some species of the *Haspiphlebia* grade (Table 6.9). It occurred on both stones and leaf litter, and at Cloudy Ck was more abundant on stones in pools and runs than riffles (Fig. 6.7). However, numbers were variable between sites and these differences were not significant (Table 6.7). Of the three other mayfly families recorded, the Caenidae and Ameletopsidae were found in low numbers (Fig. 6.6), and Baetidae were common and were significantly more abundant on stones in riffles (Fig. 6.7, Table 6.7).

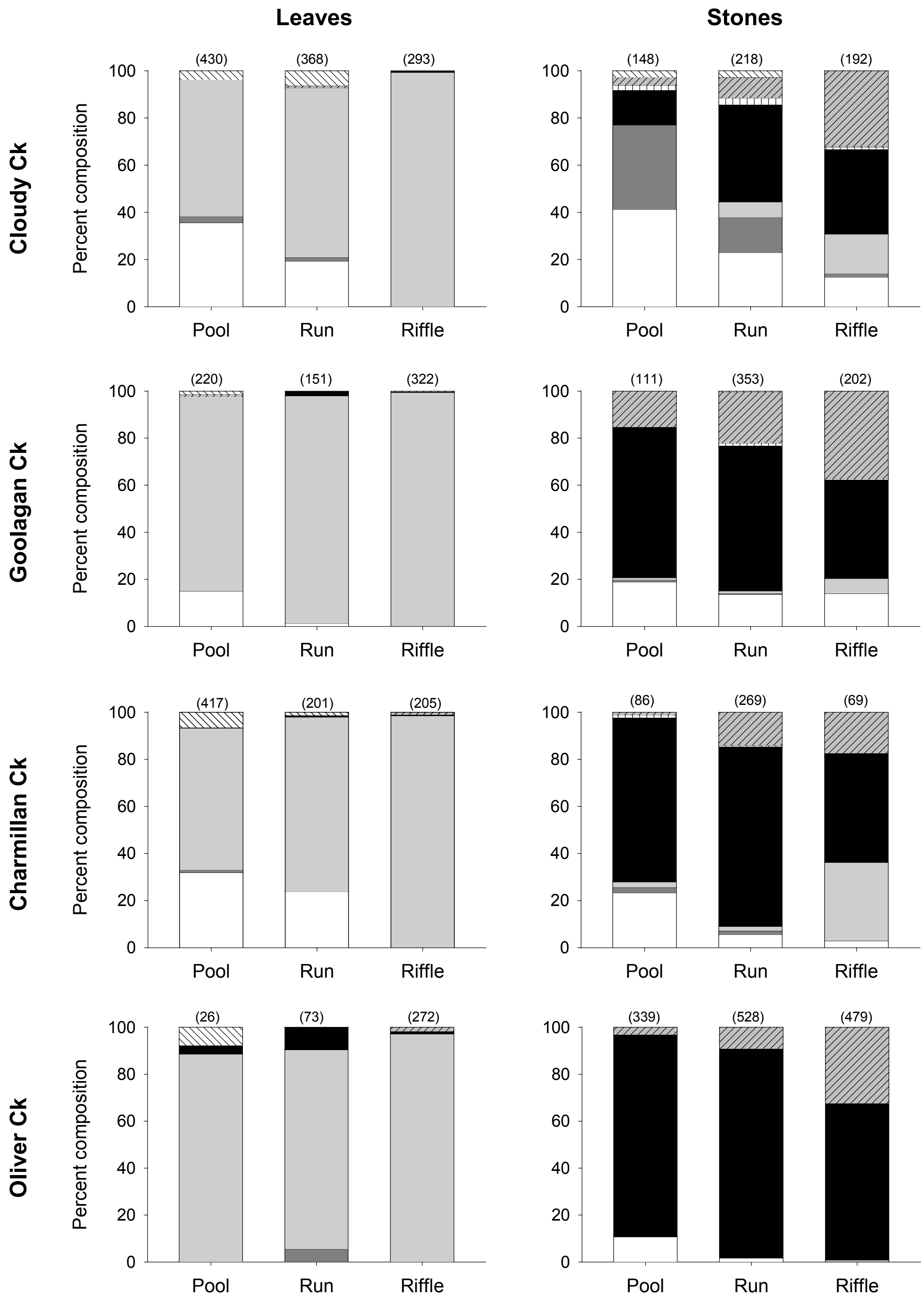


Figure 6.6. Percent composition of Leptophlebiidae lineages and other mayfly families on stones and leaves across four streams and three habitat types. Total numbers of individuals collected in each stream habitat are shown in parentheses.

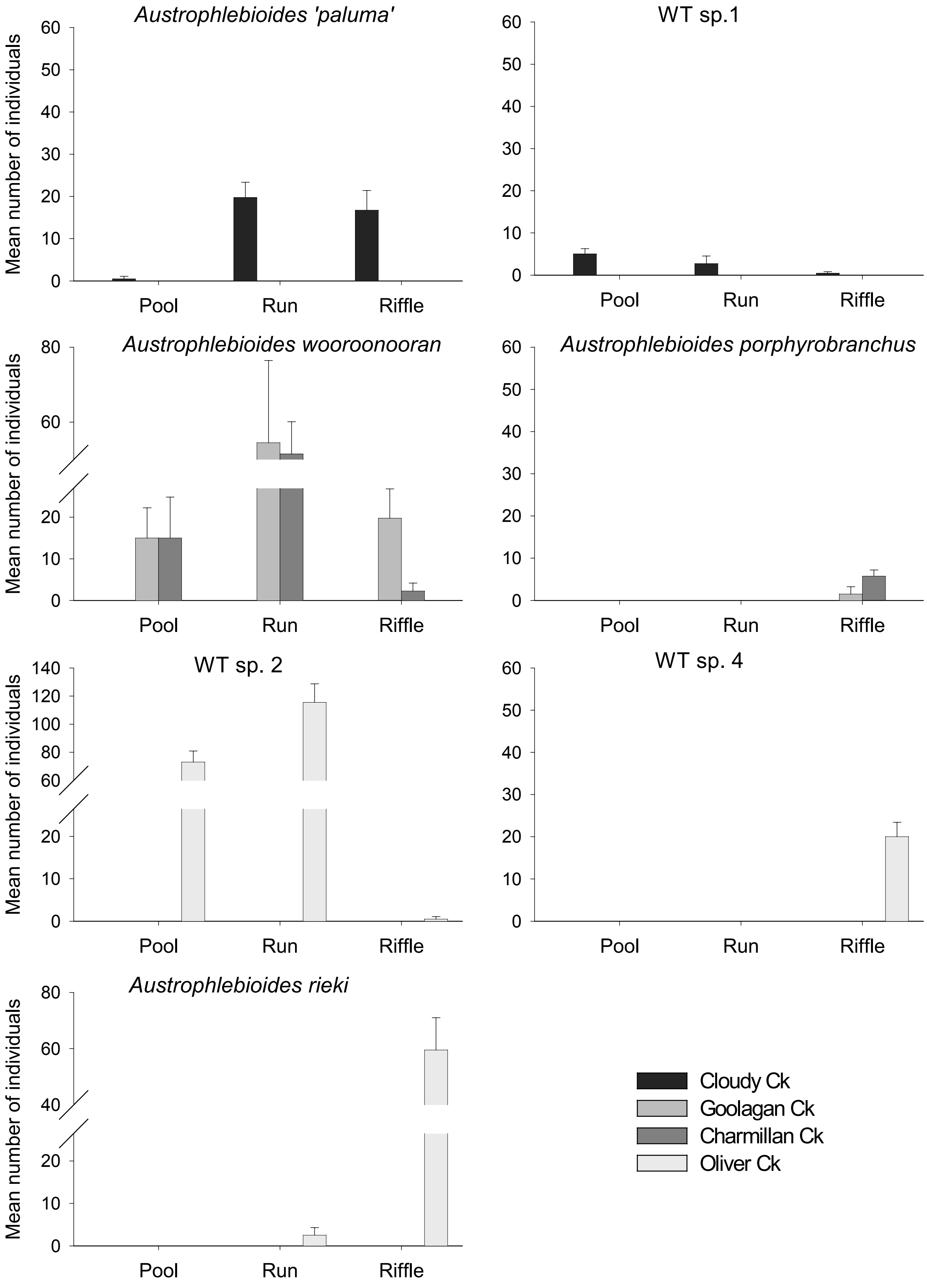


Figure 6.7. Mean number of individuals per sample (± one standard error) of common stone mayfly taxa across three habitat types and four streams.

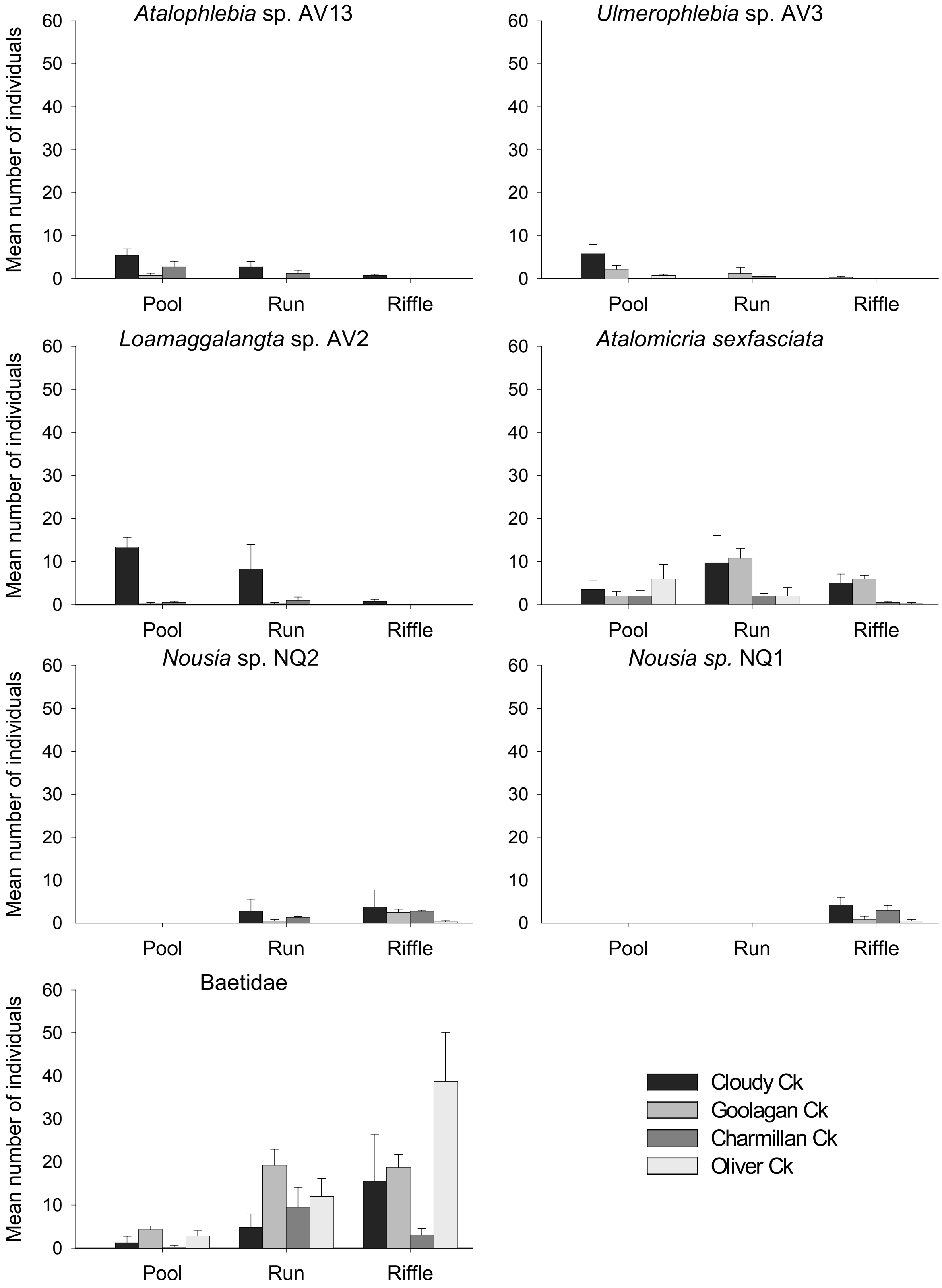


Figure 6.7 (cont'd). Mean number of individuals per sample (± one standard error) of common stone mayfly taxa across three habitat types and four streams.

Table 6.7. ANOVA results for common taxa occurring on stones, comparing abundances (log (x+1)-transformed) among habitat types (pool, run, riffle) and streams.

Source	**df**	**MS**	**F**	**P**
***Austrophlebioides* 'paluma'**				
Habitat	2	1.711	29.979	0.010*
Site (Habitat)	3	0.057	1.174	0.395
Error	6	0.048		
WT sp. 1				
Habitat	2	0.369	23.828	0.014*
Site (Habitat)	3	0.155	0.165	0.916
Error	6	0.093		
Austrophlebioides rieki				
Habitat	2	6.356	53.766	0.011*
Site (Habitat)	3	3.392	28.693	0.043*
Error	6	0.023		
WT sp. 2				
Habitat	2	4.562	234.694	0.001**
Site (Habitat)	3	0.019	0.754	0.559
Error	6	0.025		
Austrophlebioides wooroonooran				
Habitat	2	1.687	3.094	0.244
Stream	1	0.588	1.087	0.407
Habitat*Stream	2	0.541	3.090	0.120
Site (Habitat*Stream)	6	0.175	1.436	0.279
Error	12	0.122		
***Nousia* sp. NQ2**				
Habitat	2	0.591	8.836	0.016*
Stream	3	0.174	2.599	0.147
Habitat*Stream	6	0.066	0.916	0.516
Site (Habitat*Stream)	12	0.072	1.654	0.142
Error	24	0.044		
***Loamaggalangta* sp. AV2**				
Habitat	2	0.896	2.091	0.270
Site (Habitat)	3	0.429	20.402	0.002**
Error	6	0.021		

Table 6.7. (continued).

Source	**df**	**MS**	**F**	**P**
***Atalophlebia* sp. AV13**				
Habitat	2	0.582	317.857	0.003**
Stream	2	0.352	192.631	0.005**
Habitat*Stream	4	0.001	0.098	0.908
Site (Habitat*Stream)	9	0.018	0.385	0.875
Error	18			
***Ulmerophlebia* sp. AV3**				
Habitat	2	0.838	6.849	0.127
Stream	3	0.021	0.176	0.715
Habitat*Stream	6	0.122	1.683	0.263
Site (Habitat*Stream)	12	0.072	2.156	0.121
Error	24			
Atalomicria sexfasciata				
Habitat	2	0.234	0.891	0.458
Stream	3	0.627	2.385	0.168
Habitat*Stream	6	0.263	2.581	0.077
Site (Habitat*Stream)	12	0.102	0.991	0.485
Error	24	0.103		
Baetidae				
Habitat	2	2.247	11.227	0.009**
Stream	3	1.124	5.617	0.035*
Habitat*Stream	6	0.200	1.155	0.390
Site (Habitat*Stream)	12	0.173	1.744	0.119
Error	24	0.099		

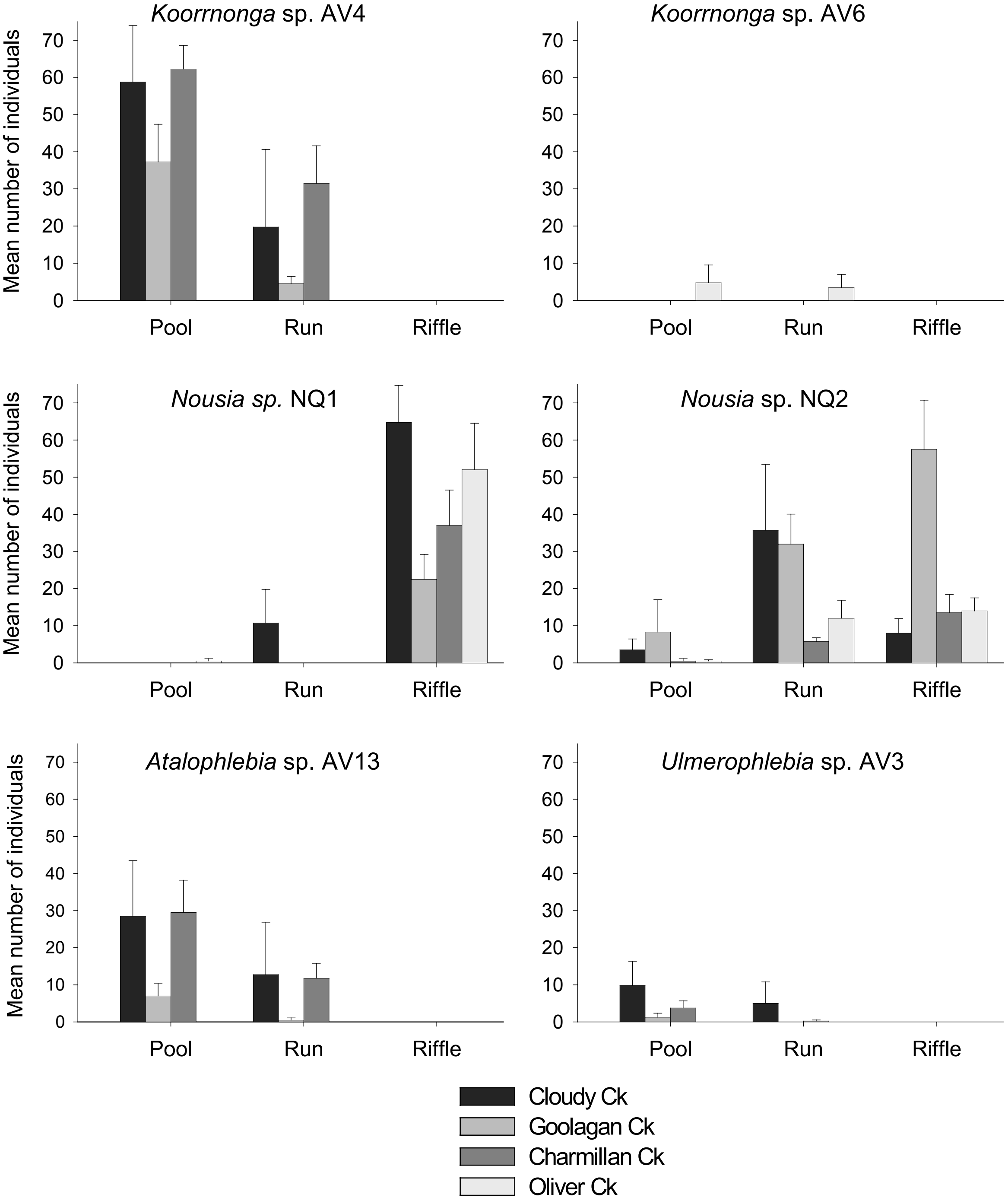


Figure 6.8. Mean number of individuals per sample (± one standard error) of the most common leaf litter mayfly species across three habitat types and four streams.

Table 6.8. ANOVA results for common taxa occurring on leaf litter, comparing abundances (log (x+1)-transformed) among habitat types (pool, run, riffle) and streams.

Source	**df**	**MS**	**F**	**P**
***Koorrnonga* sp. AV4**				
Habitat	2	8.648	38.891	0.002**
Stream	2	0.398	1.789	0.279
Habitat*Stream	4	0.222	1.244	0.359
Site (Habitat*Stream)	9	0.179	2.189	0.075
Error	18	0.081		
***Koorrnonga* sp. AV6**				
Habitat	2	0.616	11.308	0.040*
Site (Habitat)	3	0.054	6.400	0.027*
Error	6	0.085		
***Nousia* sp. NQ1**				
Habitat	2	11.821	63.984	<0.001**
Stream	3	0.384	2.081	0.204
Habitat*Stream	6	0.185	11.509	<0.001**
Site (Habitat*Stream)	12	0.016	0.234	0.994
Error	24	0.068		
***Nousia* sp. NQ2**				
Habitat	2	4.007	26.772	0.001**
Stream	3	0.754	5.034	0.045*
Habitat*Stream	6	0.150	1.059	0.437
Site (Habitat*Stream)	12	0.141	0.767	0.677
Error	24	0.184		
***Atalophlebia* sp. AV13**				
Habitat	2	4.050	15.797	0.013*
Stream	2	0.903	3.522	0.131
Habitat*Stream	4	0.256	1.402	0.308
Site (Habitat*Stream)	9	0.183	1.600	0.189
Error	18	0.114		
***Ulmerophlebia* sp. AV3**				
Habitat	2	0.758	12.857	0.018*
Stream	2	0.180	3.055	0.157
Habitat*Stream	4	0.052	0.232	0.913
Site (Habitat*Stream)	9	0.254	2.747	0.033*
Error	18	0.092		

Table 6.9. Summary of substrate and habitat use for Leptophlebiidae species.

Species	Substrate type	Habitat type
Hapsiphlebia grade		
Atalophlebia sp. AV13	Leaf litter, Stones	Pool, Run
Ulmerophlebia sp. AV3	Leaf litter, Stones	Pool, Run
Jappa serrata	Stones	Pool, Run
Jappa edmundsi	Stones	Pool
Atalomicria sexfasciata	Stones	Pool, Run, Riffle
Kalbaybaria sp.	Leaf litter, Stones	Riffle
Unnamed lineage		
Loamaggalangta sp. AV2	Leaf litter, Stones	Pool, Run
Nousia lineage		
Koorrnonga sp. AV4	Leaf litter	Pool, Run
Koorrnonga sp. AV6	Leaf litter	Pool, Run
Nousia sp. NQ1	Leaf litter, Stones	Run, Riffle
Nousia sp. NQ2	Leaf litter, Stones	Pool, Run, Riffle
Meridialaris lineage		
WT sp. 1	Stones	Pool, Run
WT sp. 3	Stones	Pool
WT sp. 2	Stones	Pool, Run
WT sp. 4	Stones	Riffle
Austrophlebioides ‘paluma’	Stones	Run, Riffle
Austrophlebioides rieki	Stones	Run, Riffle
Austrophlebioides wooroonooran	Stones	Pool, Run, Riffle
Austrophlebioides porphyrobranchus	Stones	Riffle

6.4 Discussion

Patterns of substrate and habitat use varied among the leptophlebiid mayflies and often reflected phylogenetic relationships, with closely related species tending to occur on similar substrates (e.g. *Austrophlebioides* species), and in some instances in similar habitat types (e.g. *Koorrnonga* species). However, not all aspects of substrate and habitat use had a phylogenetic basis. In some lineages, use of substrate (stone, leaf litter) was highly conservative but the use of habitat type (pool, run, riffle) was not (e.g. *Meridialaris* lineage); in others, the opposite appeared to be true, with habitat type being phylogenetically conservative (e.g. *Hapsiphlebia* grade).

6.4.1 Substrate use

The almost exclusive use of stones by species of the *Meridialaris* lineage suggests that phylogeny is an important determinant of the type of substrate used by these species. Species of the *Meridialaris* lineage were the numerically dominant component of the leptophlebiid fauna on stones in all four streams, and they were rarely found in leaf litter. Other genera of this lineage from Australia, New Zealand, and South America also predominantly occur on stony substrates (Pescador and Peters 1987; Towns and Peters 1996; Dean 1997). The high level of conservatism in substrate use within the lineage implies that the present-day use of stones by species of this lineage is a consequence of their evolutionary history.

The association between species of the *Nousia* lineage and leaf litter appears also to have a phylogenetic basis. Species of the *Nousia* lineage were the most common species in leaf litter in all habitat types. Leaf litter appears to be the preferred substrate type particularly in the genus *Koorrnonga*, which rarely occurred on stones. *Nousia* species were consistently found on stones, albeit in low numbers, and appeared less restricted in their substrate use. Similar patterns have been observed for southern species of these genera, with *Koorrnonga* species mostly associated with leaf litter whereas species of *Nousia* are also commonly found on stones (J. Dean pers. com.)

Overall, substrate use was not a conservative trait among the *Hapsiphlebia* grade with many species found on both stones and leaf litter, although some species of this grade appeared to be restricted to stones. The presence of species from this basal grade on both substrate types suggests that the ability to use leaves and stones may be the ancestral condition within the Atalophlebiinae, and that the predominant use of either stones or leaf litter is more derived. Interestingly, the majority of species that were common on leaf litter were able to also use stones, but the reverse did not appear to be so: species found commonly on stones were found very rarely in leaf litter.

Patterns of substrate use within the leptophlebiid lineages clearly had an effect on assemblage structure. The high level of segregation among the leptophlebiid fauna, with stones and leaf litter supporting distinct mayfly assemblages, appears to be the result of evolutionary differences among lineages in the use of the two substrate types. In all four streams the leaf litter was dominated by species of the *Nousia* lineage whereas the stone fauna was dominated by species of the *Meridialaris* lineage. It thus appears that phylogenetic history has had a strong influence on the present-day structure of these mayfly assemblages. Several studies investigating the effects of phylogenetic history on the community organisation of a range of taxa, including snakes (Cadle and Greene 1993), frogs (Zimmerman and Simberloff 1996) and lizards (Vitt *et al.* 1999), similarly found that evolutionary trends within lineages had influenced the organisational properties of contemporary communities.

The observed differences in the composition of mayfly fauna associated with stones and leaf litter are consistent with previous studies that have demonstrated the importance of substrate type in influencing the distribution of mayflies (Corkum *et al*. 1977; Towns 1987; Holomuzki and Messier 1993; Holomuzki 1996) as well as other aquatic insects (Rabeni and Minshall 1977; Reice 1980; Minshall 1984; Angradi 1996; Murphy and Giller 2000). However, unlike previous studies, this study demonstrates that use of substrate type (stones vs. leaves) can have an historical basis.

The extent to which patterns of substrate use among leptophlebiid mayflies may be maintained by present-day interactions between species is unclear but such interactions appear unlikely in the *Meridialaris* lineage. In some instances, for example in the pools at Oliver Ck, where numbers of leaf litter fauna were low and stone fauna high, there was little change in the patterns of habitat use among the stone species. Species interactions cannot be excluded as a possible cause for the observed distribution of *Nousia* species (*Nousia* lineage). *Nousia* species occurred mostly in leaf litter but were also consistently found on stones in low numbers. It is possible that these species may be limited in their use of stones by the presence of species of the *Meridialaris* lineage. Future studies could determine whether *Nousia* species alter their pattern of substrate use in the absence of individuals from the other lineages.

The preferential use of stone or leaf litter by many of the mayfly taxa may be partly related to diet. The importance of leaf litter as a food source rather than a structural habitat for many stream invertebrates has been demonstrated clearly by several studies (Richardson 1992; Rowe and Richardson 2001). In the present study, the strong association between leaf litter and species of the *Nousia* lineage (*Koorrnonga* sp. AV4, *Koorrnonga* sp. AV6, *Nousia* sp. A, *Nousia* sp. B) and *Atalophlebia* sp. AV13 of the *Hapsiphlebia* grade, may be due to the use of leaves as a food source by these species. A dietary study by Chessman (1986) reported that southern species of *Atalophlebia* are capable of processing leaves and wood, and that species of *Nousia* and *Koorrnonga* can also feed on leaves.

For species found predominantly on stones, periphyton and other particles attached to the surface of stones (epilithic biofilm) may be an important source of food. The general absence of these species from leaf litter suggests they make little use of litter as a direct food source. *Austrophlebioides* nymphs are known to feed on periphyton on the tops of stones, their gut contents often containing substantial quantities of algae, particularly diatoms (Chessman 1986). The use of stones rather than leaf litter by members of the *Meridialaris* lineage may be related to food preference. If this is the case, dietary preference (e.g. use of epilithic periphyton or biofilm) also may be a conservative trait within the lineage.

Dietary studies on mayflies of the Wet Tropics using gut content analysis have found species to have similar diets (Hearnden and Pearson 1990; Cheshire 1997). Gut contents of most species reported consisted of fine and coarse particulate matter, inorganic material, and varying amounts of algae. Such studies generally conclude that species are unselective in their diets. However, it is difficult to determine purely from gut contents which items are selected and which are ingested incidentally, or what portion of the gut contents is utilised. The use of stable carbon isotope analysis has revealed that some macroinvetebrates can assimilate carbon from algae and detritus differentially (Rounick *et al*. 1982). Insect growth has also been shown to vary significantly with food type (Mihuc and Minshall 1995; Ledger and Winterbourn 2000). Such studies have demonstrated differences in the abilities of species to consume and assimilate various food items. If the leptophlebiid species in the present study are selecting substrate type based on food preferences then diet may also reflect phylogeny with closely related species having similar dietary preferences or abilities.

6.4.2 Habitat use

Among the *Hapsiphlebia* grade, use of slow flow habitats appear to be a conservative trait. Species belonging to this grade were found predominantly in pools and runs, with only some species (e.g. *Atalomicria sexfasciata*, *Kalbaybaria* sp.) able to persist in riffles, possibly by residing on the underside of stones or in leaf litter, where current velocity is reduced. The observed patterns of habitat use are consistent with previous recorded habitats for these and other species of the *Hapsiphlebia* grade (Tillyard 1934, 1936; Riek 1970; Peters and Campbell 1991), suggesting that use of slow flow conditions is an ancestral trait within this grade.

Genera within the *Nousia* lineage displayed different degrees of habitat conservatism. Habitat use appeared to be conservative in the genus *Koorrnonga* but not in the genus *Nousia*. The two *Koorrnonga* species had very similar patterns of habitat use, with both species occurring among leaf litter in pools, and to a lesser extent in runs. Other *Koorrnonga* species are also known to occur in similar habitats (J. Dean pers. com.) and the use of leaf litter and low flow habitats (pools and runs) may be a conservative trait in this genus. Whereas *Koorrnonga* species were primarily found in pools, the *Nousia* species were predominantly found in runs and riffles, with *Nousia* sp. NQ2 occurring across all habitat types and *Nousia* sp. NQ1 found mostly in riffles. These patterns in habitat use were consistent at all four streams even though the species composition of the streams varied. This consistency suggests that patterns of habitat use may be due to ecological requirements of these species rather than species interactions. This explanation may be particularly so for *Nousia* sp. NQ1 which generally was absent from pools.

The use of pool, run or riffle habitats by species of the *Meridialaris* lineage appeared to have no phylogenetic basis. Closely related species did not necessarily occur in similar habitats (flow regimes). On the contrary, coexisting species often differed in their preference for pool, run or riffle, and tended to separate along a velocity gradient. This pattern was observed in all four streams with different combinations of species. At Oliver Ck in particular, which had the highest densities of mayflies, very little overlap was observed in the distribution of the stone species. WT sp. 2 occurred in pools and runs whereas a closely related species, WT sp. 4, and *Austrophlebioides rieki* occurred in riffles. Such patterns of habitat use may reflect different physiological and/or ecological requirements of individual species. Alternatively, the observed habitat segregation may be due to present day ecological factors (e.g. competition).

Interestingly, WT sp. 2 and WT sp. 4 have very similar morphologies but occupy different habitats at Oliver Ck, suggesting that species interactions may be important in their distribution. *Austrophlebioides rieki* and *Austrophlebioides* 'paluma' are also morphologically very similar but differ in habitat use. *A. rieki* was restricted mostly to riffles at Oliver Ck, whereas *A.* 'paluma' occurred in riffles and runs at Cloudy Ck. The broader habitat distribution of *A.* 'paluma' may be due to the absence of an ecological equivalent of WT sp. 2 at Cloudy Ck. At Oliver Ck *A. rieki* may be limited in its distribution to riffles by the competitive presence of WT sp. 2 in the pools and runs. Whether current competition is responsible for the observed pattern of habitat segregation remains to be investigated. It is possible, however, that divergence in habitat use is due to past competition and that coexisting species are no longer competing–"the ghost of competition past" (Connell 1980).

All leptophlebiid lineages had representative species in pools and the majority of species from the basal *Hapsiphlebia* grade were more common in pools than in riffles. This suggests that slow flow environments may be the ancestral habitat for the

Gondwanan Atalophlebiinae and that the ability to use fast flow habitats is more derived. In fast flow environments species of the *Meridialaris* lineage predominated on stones, and species of the *Nousia* lineage in leaf litter. The riffle habitat may be a challenging environment for leptophlebiid mayflies, particularly the use of the upper surface of stones in fast flowing riffles. It is members of the *Meridialaris* lineage that appear to be adapted to such fast flow environments, and often show morphological adaptation to life in fast flow (Chapter 5).

That species display specific preferences for particular flow regimes within streams is very well established (Edington 1968; Hynes 1970; Hearnden and Pearson 1991; Growns and Davis 1994; Wellnitz *et al*. 2001). This study has revealed that for some leptophlebiid mayflies such flow preferences appear to have an historical basis (e.g. species of *Koorrnonga*; *Hapsiphlebia* grade). For others, however, (*Meridialaris* lineage; species of *Nousia*) the use of slow or fast flow habitats does not appear to reflect phylogeny, closely related species often differing markedly in habitat use. In such instances present-day ecological factors may play an important role in determining the distributions of species.

CHAPTER 7

General discussion

7.1 Overview

In this thesis I have taken a phylogenetic approach to investigate the role of evolutionary history in shaping selected ecological and morphological traits of Leptophlebiidae mayflies. The two main goals of this study were to: 1) reconstruct the phylogenetic relationships among the Australian Leptophlebiidae genera, and use the phylogenetic hypothesis obtained to determine if patterns of substrate and habitat use among Leptophlebiidae species reflected phylogenetic relationships; 2) reconstruct the phylogenetic relationships among species of the genus *Austrophlebioides*, and use the phylogenetic hypothesis derived to infer the pattern of evolution of labrum morphology and habitat use, and to evaluate the importance of flow regimes in the evolution of labrum shape. Distributional patterns in the genus *Austrophlebioides* were also assessed in light of the proposed phylogeny. The major findings resulting from this research are summarised and discussed below.

7.1.2 Phylogeny and ecology of the family Leptophlebiidae

The outcomes from a cladistic parsimony analysis of Australian Leptophlebiidae genera and selected genera from South America and New Zealand, using morphological characters (Chapter 2), were consistent with the higher-level relationships previously proposed by Pescador and Peters (1980). This consistency is remarkable given the differences in the genera and characters used in the two analyses, and attests to the robustness of the phylogeny proposed by Pescador and Peters (1980). The only point of conflict between their phylogeny and findings of the present analysis is the monophyly of the *Hapsiphlebia* lineage. In the cladistic parsimony analysis, the basal position of genera assigned to the *Hapsiphlebia* lineage (*Kalbaybaria, Atalophlebia*, *Atalomicria*, *Ulmerophlebia* and *Jappa*) was confirmed; however, these genera did not form a monophyletic group as proposed by Pescador and Peters (1980). The phylogenetic hypothesis obtained in Chapter 2 has clarified the affinities of *Neboissophlebia*, *Bibulmena*, *Loamaggalangta* and *Kaninga*. These four genera appear to belong to lineages not recognised previously among the Gondwanan Leptophlebiidae.

The incorporation of phylogenetic information into the ecological study of leptophlebiid mayflies of tropical streams revealed that substrate and habitat use among species often reflected phylogenetic relationships (Chapter 6), and that some major aspects of present-day assemblage structure appear to have a historical (phylogenetic) basis. For example, the high level of segregation on the basis of substrate type among the leptophlebiid fauna, with stones and leaf litter supporting distinct assemblages, appears to be the result of historical differences among lineages in the use of substrate type. The leaf litter fauna was dominated by species of the *Nousia* lineage, whereas the stone fauna was dominated by species of the *Meridialaris* lineage. In addition, differences in assemblage structure among pool, run, and riffle habitats appear partly due to evolutionary trends in habitat use among lineages. For example, the strong association of species of the *Hapsiphlebia* grade and species of the genus *Koorrnonga* (*Nousia* lineage) with pools and runs has contributed to the distinctiveness of mayfly assemblages among the three habitat types. These findings suggest that phylogenetic history is important in determining the ecological traits of leptophebiid species and the present-day distributions of species among substrate and habitat types.

However, patterns of habitat use did not always reflect phylogenetic relationships and some present-day patterns of assemblage structure do not appear to be influenced by phylogenetic history. Thus within the *Meridialaris* lineage closely related species did not necessarily occur in similar habitat types (e.g. species of *Austrophlebioides*; WT sp. 2 and WT sp. 4). On the contrary, species coexisting in a stream reach often differed markedly in habitat use, thereby contributing to the distinctiveness of the pool, run and riffle assemblages. Similar trends in habitat segregation were observed among the *Nousia* species (*Nousia* lineage). In such instances present-day ecological factors (e.g. competition) may play an important role in determining the distributions of species.

Overall, the present-day structure of tropical mayfly assemblages in the four study streams appears to be the outcome of a combination of historic (phylogenetic) and contemporary ecological processes. Without knowledge of phylogenetic relationships among species, the historical basis for some present-day patterns of substrate and habitat use would have gone unrecognised. The findings of this study are consistent with those of several other studies that have shown that evolutionary trends within lineages may force organisational properties on contemporary communities (Cadle and Greene 1993; Zimmerman and Simberloff 1996; Vitt *et al*. 1999).

7.2.2 Phylogeny, habitat use, and evolution of labrum shape in the genus *Austrophlebioides*

Three new species of *Austrophlebioides* from the Wet Tropics bioregion were recognised during the present study (Chapter 3). Examination of adults and nymphs of these species indicated that they formed a distinct group within the genus *Austrophlebioides*. Phylogenetic analyses of *Austrophlebioides* species based on morphological characters (Chapter 4) support the recognition of three monophyletic species groups within the genus: the "wet tropics" clade, the "*pusillus*" clade and the "*marchanti*" clade. Congruence among the phylogenies derived from the different life history stages and the combined adult and nymph data, along with high bootstrap support values, indicate that the proposed relationships within the genus are robust.

Reconstruction of ancestral character states suggests that a broad labrum has not evolved independently on numerous occasions within the genus *Austrophlebioides* in response to fast flow (Chapter 5). Rather, a broad labrum and the use of high flow environments appear to be ancestral features that evolved only once, early in the evolutionary history of the genus. A narrow labrum and the use of slow flow habitats appear to have evolved secondarily within the genus, on two independent occasions. Retention of a broad labrum in species that occur in fast flow, and evolutionary changes from a broad to a narrow labrum with shifts from fast to slow flow habitats, suggests that flow has been an important selective factor in the evolution of labrum morphology in *Austrophlebioides*. However, the high level of phylogenetic conservatism within the *Austrophlebioides* clades indicates that phylogenetic history is also an important determinant of labrum morphology and habitat use in this genus (Chapter 5). For example, all species of the "*marchanti*" clade have a broad labrum and occupy fast flow habitats. Species of the "*pusillus*" clade all have a narrow labrum and occur in slow flow. Only within the "wet tropics" clade were there evolutionary changes in both labrum shape and habitat use.

There was minimal overlap in the distribution of the three *Austrophlebioides* clades, with distributional patterns corresponding to previously established biogeographic regions (Chapter 4; Ford 1987; Cracraft 1986). The "wet tropics" clade is confined to the Wet Tropics bioregion of north-eastern Queensland. The "*pusillus*" clade is restricted to the Eastern Queensland biogeographic region, with the exception of *A. pusillus*, which extends to the Southern Forests biogeographic region. The "*marchanti*" clade has been recorded only from the Southern Forests biogeographic region and Tasmania (Chapter 4). Distributional limits of the three clades correspond with the presence of recognised biogeographic barriers (Burdekin Gap, Hunter Valley and Bass Strait), strongly suggesting that vicariance has been important in the differentiation of the group and in determining present-day distributions of species.

7.2 Future research directions

In this thesis I have addressed several key issues relating to the phylogeny, ecology and morphology of Leptophlebiidae mayflies. The findings of this research have raised a number of new issues that could be explored further, as indicated below.

The phylogenetic hypothesis obtained in Chapter 2 has contributed significantly to our understanding of evolutionary relationships among the Australian Leptophlebiidae genera, and provides a useful framework for further phylogenetic studies on the group. The addition of molecular approaches to future phylogenetic analyses would assist in clarifying relationships among genera within the *Meridialaris* clade, and may provide additional support for nodes presently supported by low bootstrap values. As well as future studies investigating phylogenetic relationships among the Australian Leptophlebiidae, it is important that the alpha taxonomy of the group is advanced. Much work still remains in describing formally the many new genera and species that are believed to exist in Australia (Dean 1999).

In the reconstruction of phylogenetic relationships among species of the genus *Austrophlebioides* (Chapter 4), data were unavailable for either the adult or nymph stage of several species. Additional collections and rearing to associate adults and nymphs will be required to obtain such data. The inclusion of data from both life history stages for all *Austrophlebioides* species would improve the analysis and test relationships presently proposed based on information from one life history stage only. It would be particularly useful to obtain the adults of the three Tasmanian *Austrophlebioides* species to test their hypothesised placement within the "*marchanti*" clade. Similarly, the collection of adults of *A*. *"eungella*" and nymphs of *A*. *boolombi* would assist in resolving relationships among species of the "*pusillus*" clade. Molecular data, preferably from both mitochondrial and nuclear markers, could be used to verify proposed relationships within the genus, and may further clarify relationship among species of the "*pusillus*" and "*marchanti*" clades.

The distribution of species within the genus *Austrophlebioides* appears to be strongly influenced by vicariance, with most species limited to well-known Australian areas of endemism (Chapter 4). A better resolved phylogeny for the genus as well as additional collections to verify the distributions of some species could be used to test existing hypotheses on relationships among areas of endemism and the historical sequence of vicariance events (Cracraft 1991, Crisp *et al*. 1995).

The hypothesis that water flow has been an important selective factor in the evolution of labrum morphology in the genus *Austrophlebioides* was supported by findings of this study (Chapter 5). Future studies could investigate the performance and fitness advantages associated with different labrum morphologies under the range of flow conditions that occur in streams. Specifically, such studies could examine if species with a broader labrum feed more effectively at faster current velocities and conversely whether species with a narrow labrum are better able to feed in slower flow. Such flow chamber studies may be technically difficult to establish, but if successful would provide additional support for the adaptive significance of labrum shape by linking morphology to performance and fitness.

The extent to which observed patterns of habitat segregation among coexisting species of the *Meridialaris* lineage (Chapter 6) are due to present-day species interactions requires further investigation. Habitat selection experiments would enable the habitat preferences (pool, run, riffle) of each species to be determined in the absence and presence of other species. However, these types of manipulative studies may require considerable time and effort to set up and run effectively.

In Chapter 6 it was suggested that the preferential use of stone or leaf litter by leptophlebiid species might be partly related to diet. Feeding trials could be conducted to assess the abilities of different species to utilise a range of food types (e.g. ability to process leaves and to remove periphyton from the surface of stones). In addition, experiments to measure growth of each species with different food resources could determine if species are able to grow well on a range of food types or if maximum growth is attained only on specific food resources. Such studies would establish the range of dietary preferences in leptophlebiid mayflies and assist in the understanding of ecological patterns. It would also be of interest to investigate whether dietary preferences have a phylogenetic basis.

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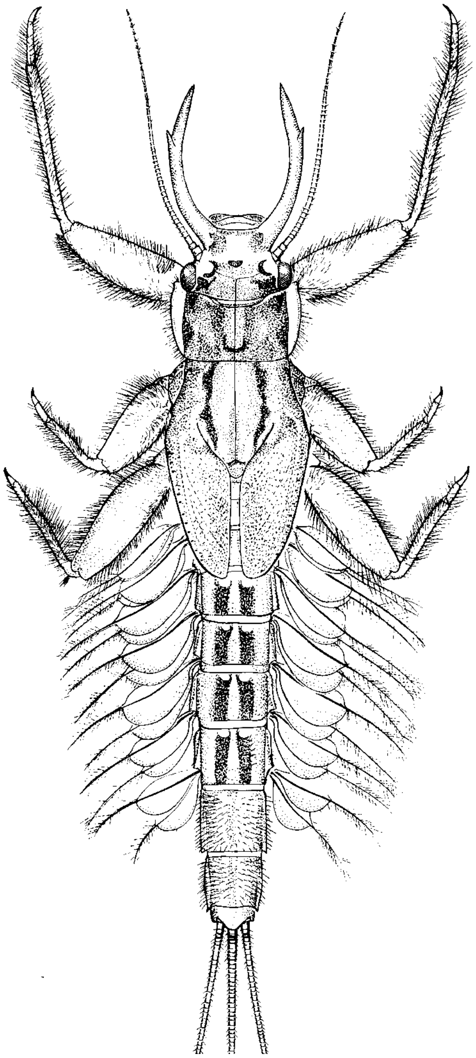
APPENDIX 1

A guide to the nymphs of the mayfly family Leptophlebiidae from the Wet Tropics bioregion of north-eastern Queensland



Faye Christidis[1] and John Dean[2]

Illustrations by Sybil Monteith[3] and Faye Christidis[1]

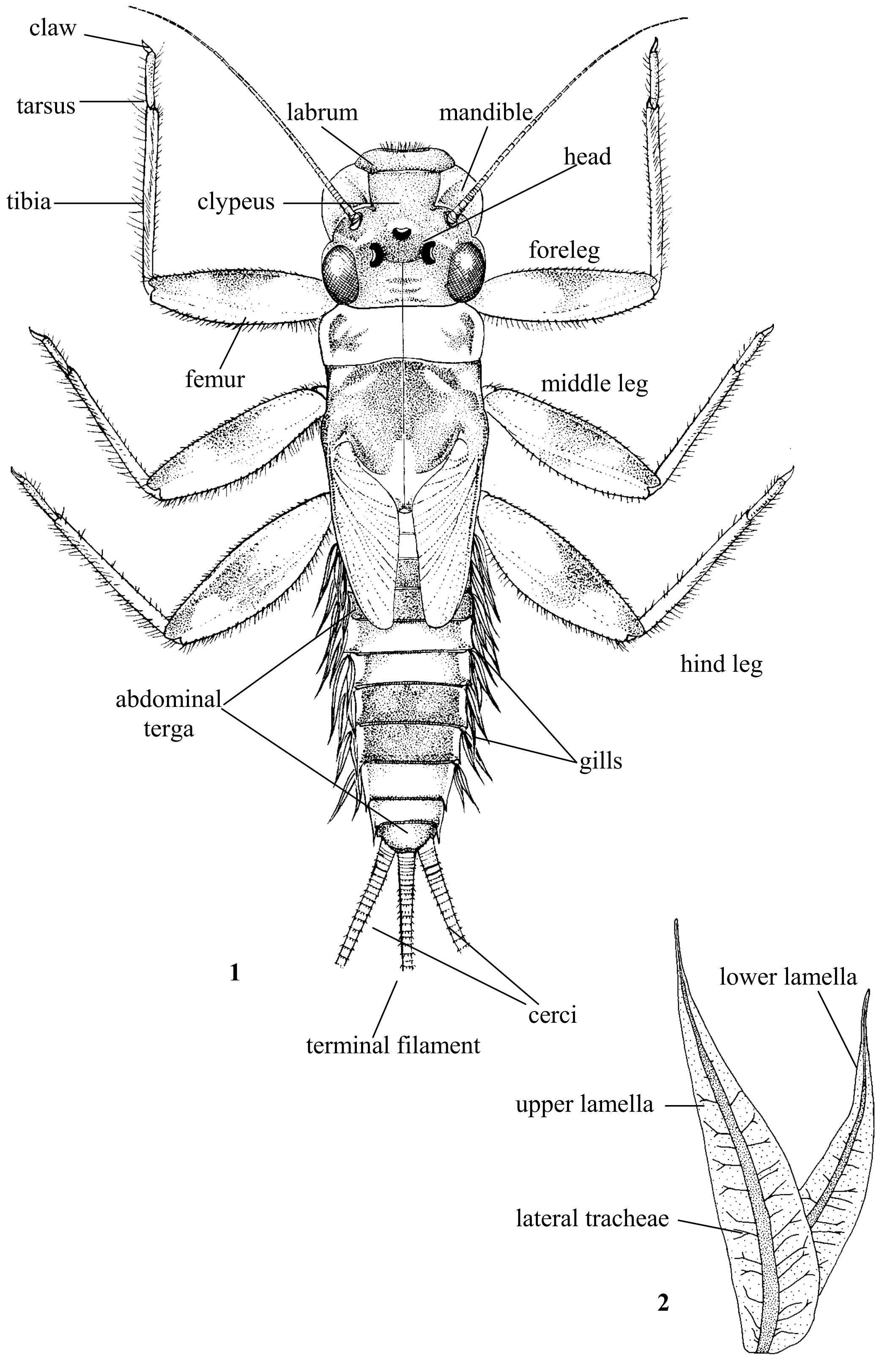
[1] School of Tropical Biology, James Cook University, Townsville, Queensland.
[2] Freshwater Sciences, Environmental Protection Authority, Melbourne, Victoria.
[3] Queensland Museum, Brisbane, Queensland.

Introduction

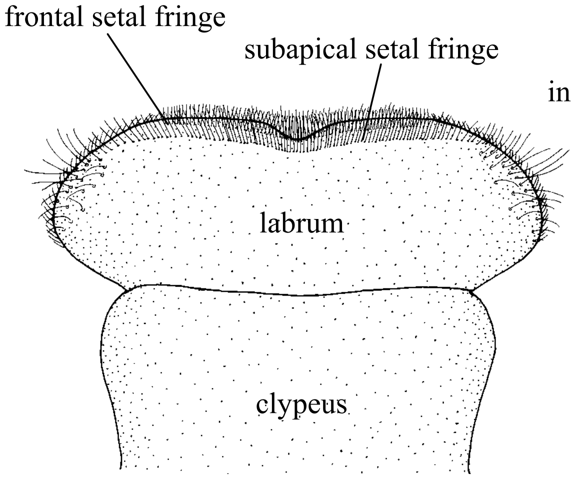
In this guide we present keys to the late instar nymphs of the Leptophlebiidae genera and species recorded from the Wet Tropics, a biogeographic region which extends from Townsville in the south to Cooktown in the north, and up to 85 km inland from the coast. We also provide a summary of the main diagnostic characters of each genus. The key to genera is based on Dean's (1999) key to the Australian Leptophlebiidae genera. We stress that the keys are for late instar nymphs, and additional undescribed species not covered by this guide may be encountered. The leptophlebiid fauna of the Wet Tropics is relatively diverse with 27 recorded species, the majority of which are presently undescribed.

Nymph morphology

The morphological characters used in the keys and generic diagnoses are illustrated in Figures 1 to 8. Characters of particular importance are the abdominal gills and mouthparts. Gills are present on abdominal segments one to seven, with each gill consisting of an upper and lower lamella (Figs 1, 2). The lower lamella can be greatly reduced, as in the genus *Kirrara*. The shape of the gill and the degree of development of the lateral tracheae vary among species. The mouthparts (Figs 3-8) are good diagnostic characters at the generic and species level. Dissection and the preparation of slides are necessary for the detailed examination of mouthparts. This is most easily achieved by first severing the head from the body. The head is then orientated ventral side up. With a pair of fine forceps and fine dissecting needles, the labium is gently removed, followed by the maxillae, hypopharynx, and mandibles. Finally, the clypeus and labrum are dissected from the remainder of the head with a blade. For temporary slides, the mouthparts are transferred from 80% ethanol directly on to a drop of Hoyers' mountant and covered with a cover slip. For permanent slides, the mouthparts are transferred from 80% ethanol into two changes of isopropanol for five minutes each, and then mounted in Euparal. The terminology used in this guide follows Dean (1999).



Figures 1-2. Nymph morphology: (1) nymph, (2) abdominal gill.



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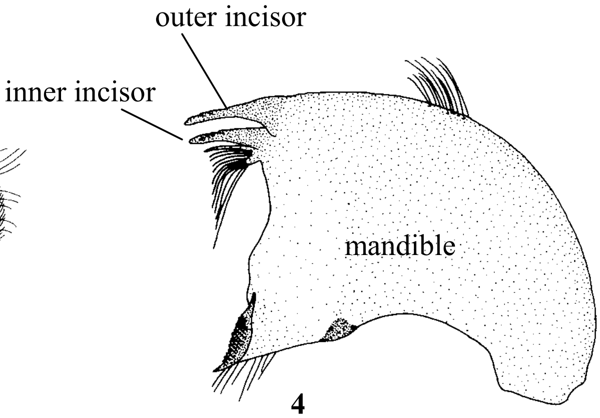

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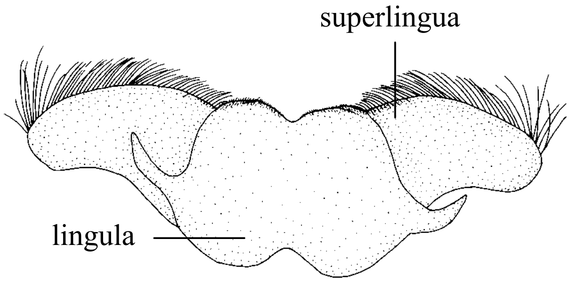
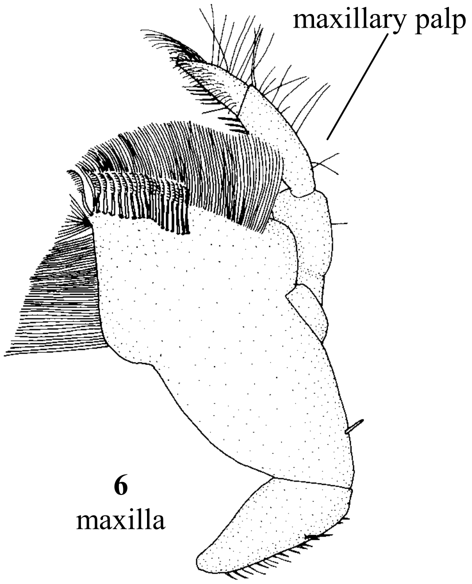
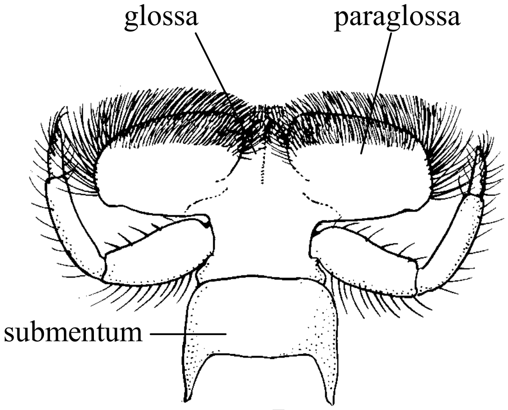


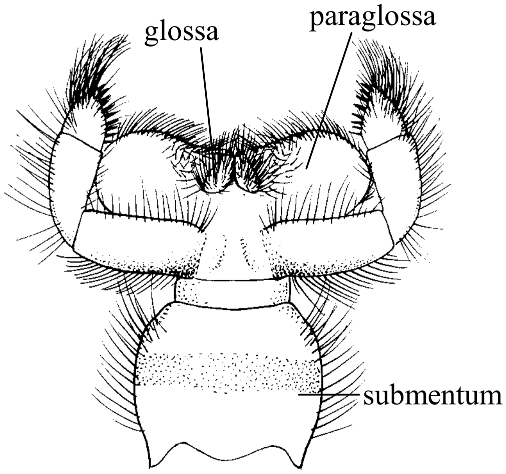
5
hypopharynx



6
maxilla



7
labium
(glossae and paraglossae on the same plane)



8
labium
(glossae turned under ventrally)
(not on the same plane as paraglossae)

Figures 3-8. Mouthparts: (3) labrum and clypeus, (4) mandible, (5) hypopharynx, (6) maxilla, (7) and (8) labium.

Checklist of Leptophlebiidae recorded from the Wet Tropics bioregion.

The list of species is based on specimens collected during this study, material held at the Australian Centre for Freshwater Research at James Cook University, and Dean (1999). Undescribed species were allocated a voucher number or an informal name (AV = Australian voucher; NQ = North Queensland voucher; WT = undescribed species not assigned to genus). *Names not to be used until formal taxonomic publication.

Atalomicria sexfasciata
Atalomicria sp. NQ1
Atalophlebia sp. AV13
Atalophlebia sp. AV21
Austrophlebioides ‘paluma’
*Austrophlebioides porphyrobranchus**
*Austrophlebioides rieki**
*Austrophlebioides wooroonooran**
Jappa edmundsi
Jappa kutera
Jappa serrata
Kalbaybaria doantrangae
Kirrara sp. AV1
Koorrnonga sp. AV2
Koorrnonga sp. AV4
Koorrnonga sp. AV6
Loamaggalangta sp. AV2
Neboissophlebia sp. NQ1
Nousia sp. NQ1
Nousia sp. NQ2
Nousia sp. NQ3
Ulmerophlebia sp. AV3
Ulmerophlebia sp. AV6
WT sp. 1
WT sp. 2
WT sp. 3
WT sp. 4

Key to genera of Leptophlebiidae of the Wet Tropics bioregion

Based on Dean (1999).

1 Gills divided into three or more digits, first gill with both upper and lower lamella digitate (Figs 16, 20)….……….…………..………………………….....2

\- Gills entire (Figs 23, 43, 62, 69) or with single apical filament (Figs 10, 36)…..4

2(1) Mandible with large tusk-like process (Figs 39, 42)…..……….….***Kalbaybaria***

\- Mandible without large tusk-like process….……….……………….***Atalophlebia***

3(1) Maxillary palps greatly elongated (Figs 13, 15)………..…..…….....***Atalomicria***

\- Maxillary palps not greatly elongated…………..……………………………..4

4(3) Gills ovate with a single apical filament, gill margin with dense fringe of fine setae in apical half (Figs 36, 66, 67); nymphs with dense setae on legs and pronotum (Figs 34, 65)…...…………………………………………...……....5

\- Not as above……………..…………………………………...……..…...……6

5(4) Head with frontal horns (Fig. 34)……………………………………..….***Jappa***

\- Head without frontal horns (Fig. 65)………...…………....…….***Ulmerophlebia***

6(4) Tarsal claws smooth (Figs 53, 57)…………………..……………………....…7

\- Tarsal claws with prominent ventral teeth (Fig. 75)……………..…..………....8

7(6) Legs very slender (Fig. 52); gills narrow, with or without lateral tracheae (Fig. 54); abdomen with postero-lateral spines on segments 7 or 8 to 9…………………………………………………………......***Loamaggalangta***

\- Legs robust, not slender; gills narrow, without lateral tracheae (Fig. 58); abdomen with postero-lateral spines on segments 5 to 9……….***Neboissophlebia***

8(6) Gills ovate, lower lamella very small or absent (Fig. 43)……….……….…***Kirrara***

\- Gills lanceolate, upper and lower lamella sub-equal in size (Figs 23, 28, 49, 59, 62, 69, 74)..……………………...………………….……….………….…9

9(8) Anterior margin of labrum entire or with a broad shallow concavity (Figs 47, 48, 51, 60, 61, 64); lateral margins of submentum fringed with setae………...…..10

\- Anterior margin of labrum with narrow U-shaped notch (Figs 22, 25, 29, 32, 68, 71, 76); lateral margins of submentum bare (Fig. 7) or with a few basal setae …………………………………………………………………………….11

10(9) Labrum with subapical setal fringe less than 0.6 times the width of the apical setal fringe (Figs 47, 48, 51)..………..………………..……………..***Koorrnonga***

\- Labrum with subapical setal fringe greater than 0.7 times the width of the apical setal fringe (Figs 61, 64) or labrum without subapical fringe but with broad band of setae close to anterior margin (Fig. 60)...…………..…………………..***Nousia***

11(9) Gills narrow without lateral tracheae (Fig. 74); tarsus of foreleg with more than 50 ventral spines (Fig. 75)………………..…………………………..……….12

\- Gills moderately broad with lateral tracheae (Figs 23, 28, 30, 33, 69, 70); tarsus of foreleg with less than 10 ventral spines…...……………….……………….…13

12(11) Abdominal terga without transverse black band along posterior margin (Fig. 73) …………………………………………………………………….....**WT sp. 2***

- Abdominal terga with prominent transverse black band along posterior margin …………………………………………………………..……..…...**WT sp. 4***

13(11) Maximum width of labrum more than 2.5 times maximum length (Figs 22, 25, 29, 32); lateral margins of abdomen usually fringed with fine setae …………………………………………………………...***Austrophlebioides***

- Maximum width of labrum less than 2.3 times maximum length (Figs 68, 71); lateral margins of abdomen without fringe of fine setae….……….…….……...14

14(13) Legs with conspicuous banding (Fig. 72)…………....…………………**WT sp. 3***

- Legs without conspicuous banding……………………..…...…...…**WT sp. 1***

* undescribed species believed to belong to new genera. The species WT sp. 2 and WT sp. 4 appear to belong to one new genus, and WT sp. 1 and WT sp. 3 to a second new genus.

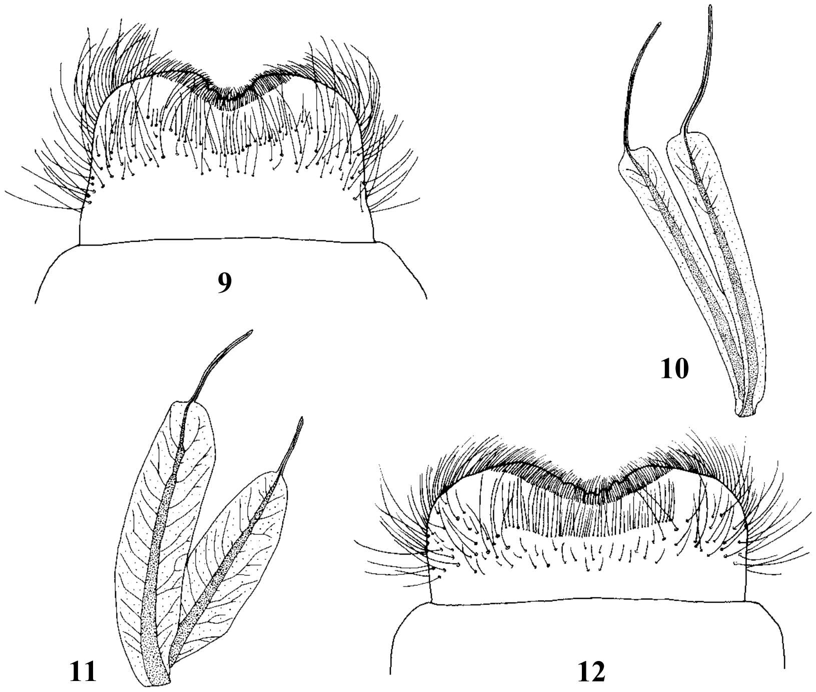
Genus *Atalomicria*

Diagnostic characters: maxillary palps greatly elongated (Fig. 15); labrum narrower than clypeus (Fig. 9); outer incisor of mandibles triangular; glossae of labium turned under ventrally, not lying in the same plane as paraglossae; lateral margins of submentum fringed with setae; tarsal claws smooth (Fig. 14); abdomen with postero-lateral spines on segments 8 to 9 or 9 only; gill lamellae with apical filament (Fig. 10).

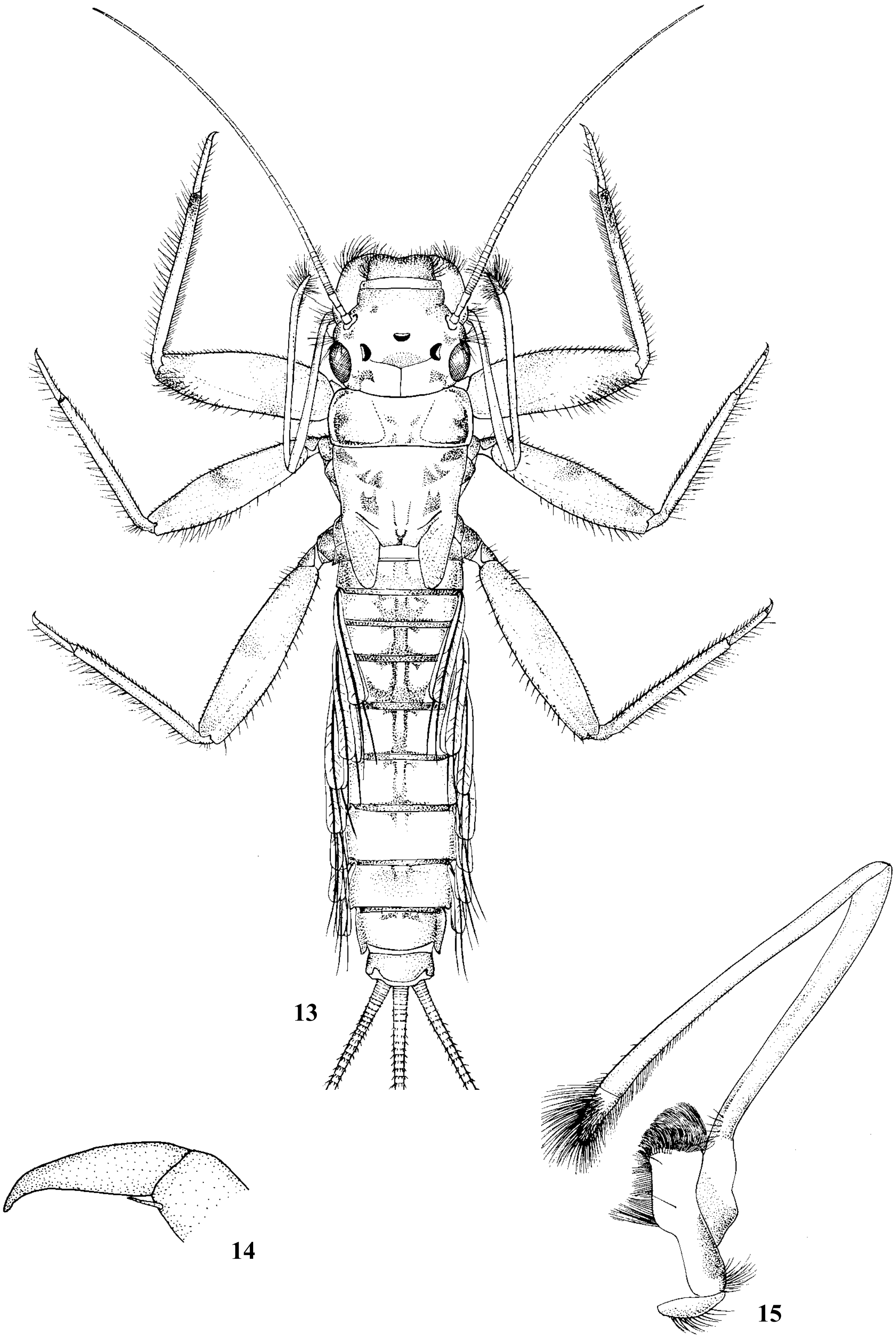
Key to Wet Tropics species

1 Gills relatively narrow, lateral tracheae weakly developed (Fig. 10); pigmentation on abdominal terga restricted mostly to segments 4, 5 and 8 (Fig. 13); abdominal segment 8 without postero-lateral spine; labrum as in Fig. 9. …………………………………………………….....***Atalomicria sexfasciata***

\- Gills moderately broad with prominent lateral tracheae (Fig. 11); abdominal terga with extensive dark pigmentation; abdominal segment 8 with strongly developed postero-lateral spine; labrum as in Fig. 12………….……….***Atalomicria* sp. NQ1**



Figures 9-12. *Atalomicria sexfasciata*: (9) labrum, (10) fourth gill. *Atalomicria* sp. NQ1: (11) fourth gill, (12) labrum.



Figures 13-15. *Atalomicria sexfasciata*: (13) nymph, (14) tarsal claw, (15) maxilla.

Genus *Atalophlebia*

Diagnostic characters: labrum narrower than clypeus (Fig. 17); outer incisor of right mandible triangular (Fig. 19); glossae of labium turned under ventrally, not lying in the same plane as paraglossae; lateral margins of submentum fringed with setae; tarsal claws with small denticles (Fig. 18); abdomen with postero-lateral spines on segments 2 to 9; each gill lamella with three or more digits (Figs 16, 20).

Key to Wet Tropics species

1 Gills with three digits (Fig. 16)…………………….………...***Atalophlebia* sp. AV21**

\- Gills with more than three digits (Fig. 20).….….….….…. ***Atalophlebia* sp. AV13**

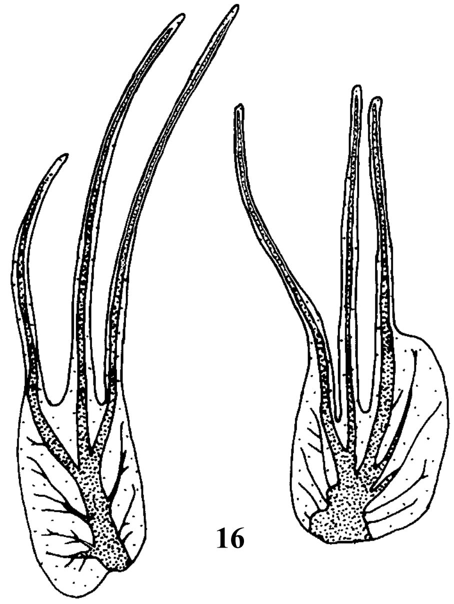
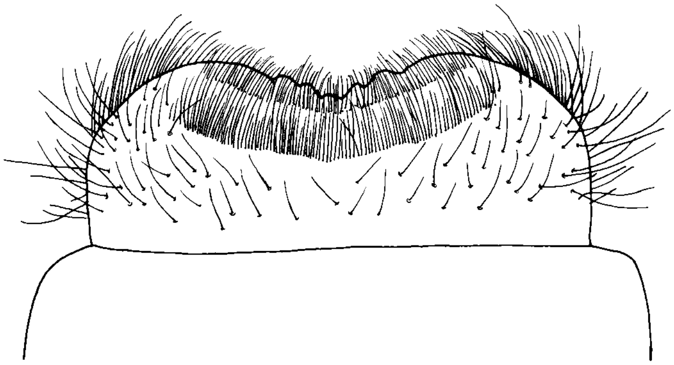


Figure 16. *Atalophlebia* sp. AV21: (16) upper and lower lamellae of fourth gill.



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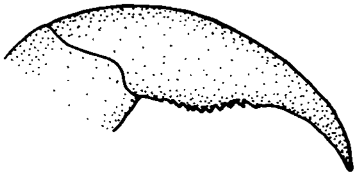

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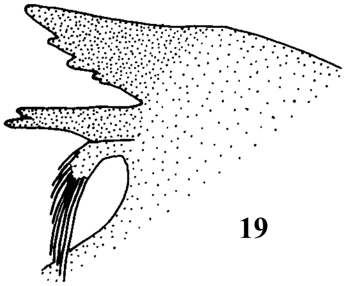

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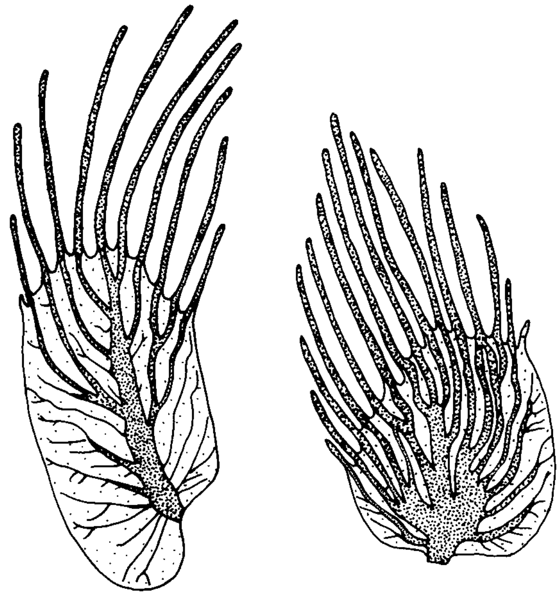

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Figures 17-20. *Atalophlebia* sp. AV13: (17) labrum, (18) tarsal claw, (19) incisor of right mandible, (20) upper and lower lamellae of fourth gill.

Genus *Austrophlebioides*

Diagnostic characters: labrum broader than clypeus (Fig. 22); lateral margins of clypeus apically divergent; anterior margin of labrum with narrow U-shaped notch, hooded dorsally (Fig. 22); outer incisor of mandibles slender parallel-sided (Fig. 26), apex of incisor serrated; glossae of labium lying in the same plane as paraglossae; lateral margins of submentum bare or with few setae near base; tarsal claws with ventral teeth (Fig. 24); abdomen with postero-lateral spines on segments 2 to 9; lateral margins of abdomen usually fringed with fine setae; gills lanceolate (Figs 21, 23, 30, 33).

Key to Wet Tropics species

1 Gill lamellae (Fig. 23) purple in colour; tarsus of foreleg with elongated apical spine (Fig. 24)…...………………………......***Austrophlebioides porphyrobranchus***

\- Gill lamellae whitish or cream in colour; tarsus of foreleg without elongated apical spine…………………..…………………………………...……………..2

2(1) Lateral margins of labrum rounded (Fig. 25); middle and hind femora with small pale round area near base (Fig. 27)…………....***Austrophlebioides wooroonooran***

\- Lateral margins of labrum angular (Figs 29, 32); middle and hind femora with either an extensive pale region near base or an elongated pale streak (Fig. 31) ……………………………………………………………...………………...3

3(2) Distribution: Paluma…………….………………….***..Austrophlebioides* ‘paluma’**

\- Distribution: Daintree…….….……..…………….……***Austrophlebioides rieki***

Note Names not to be used until formal taxonomic publication. Based on morphology alone, it is difficult to establish whether *A*. *rieki* and *A*. ‘paluma’ belong to a single widely distributed species or two closely related species.

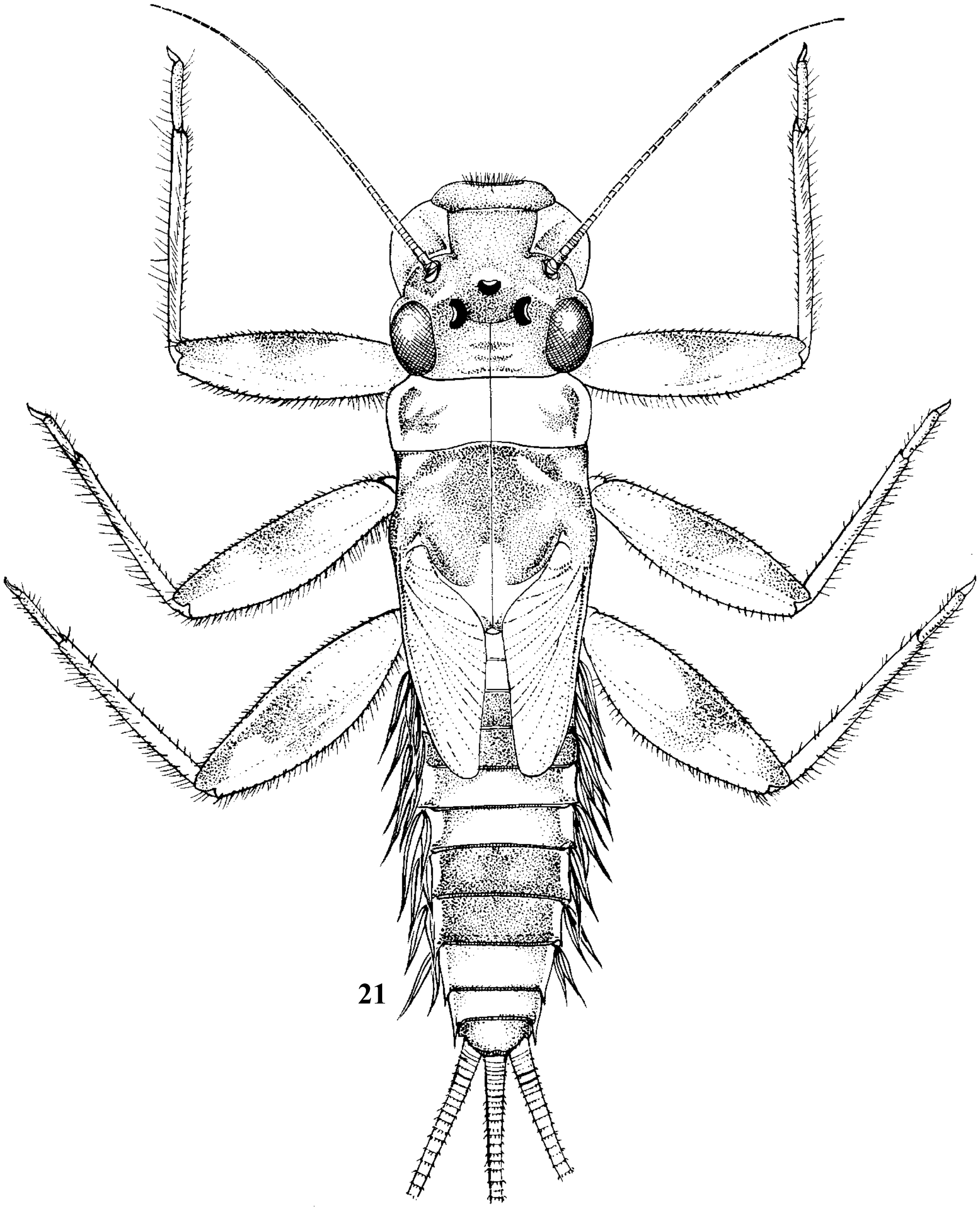

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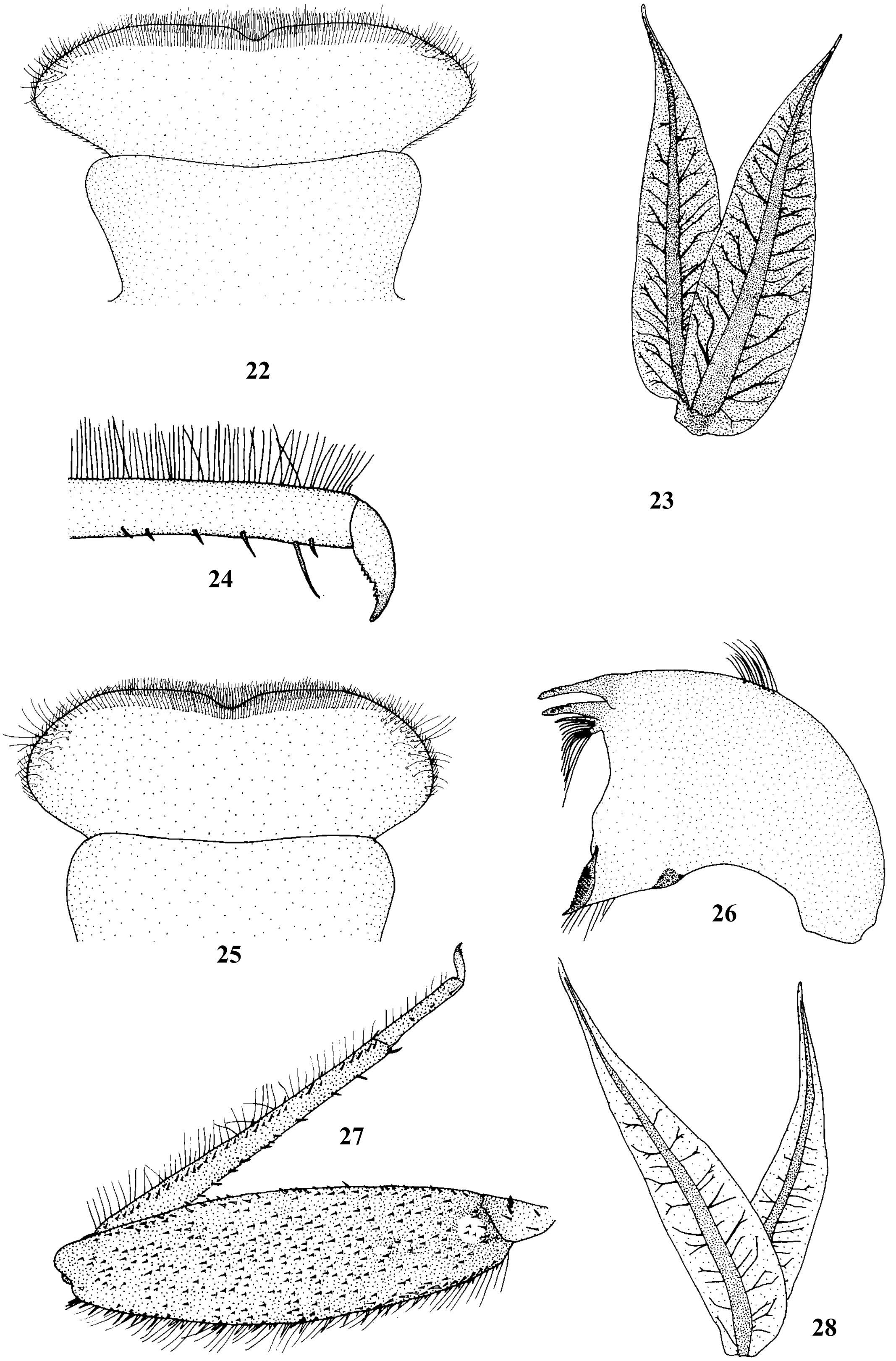
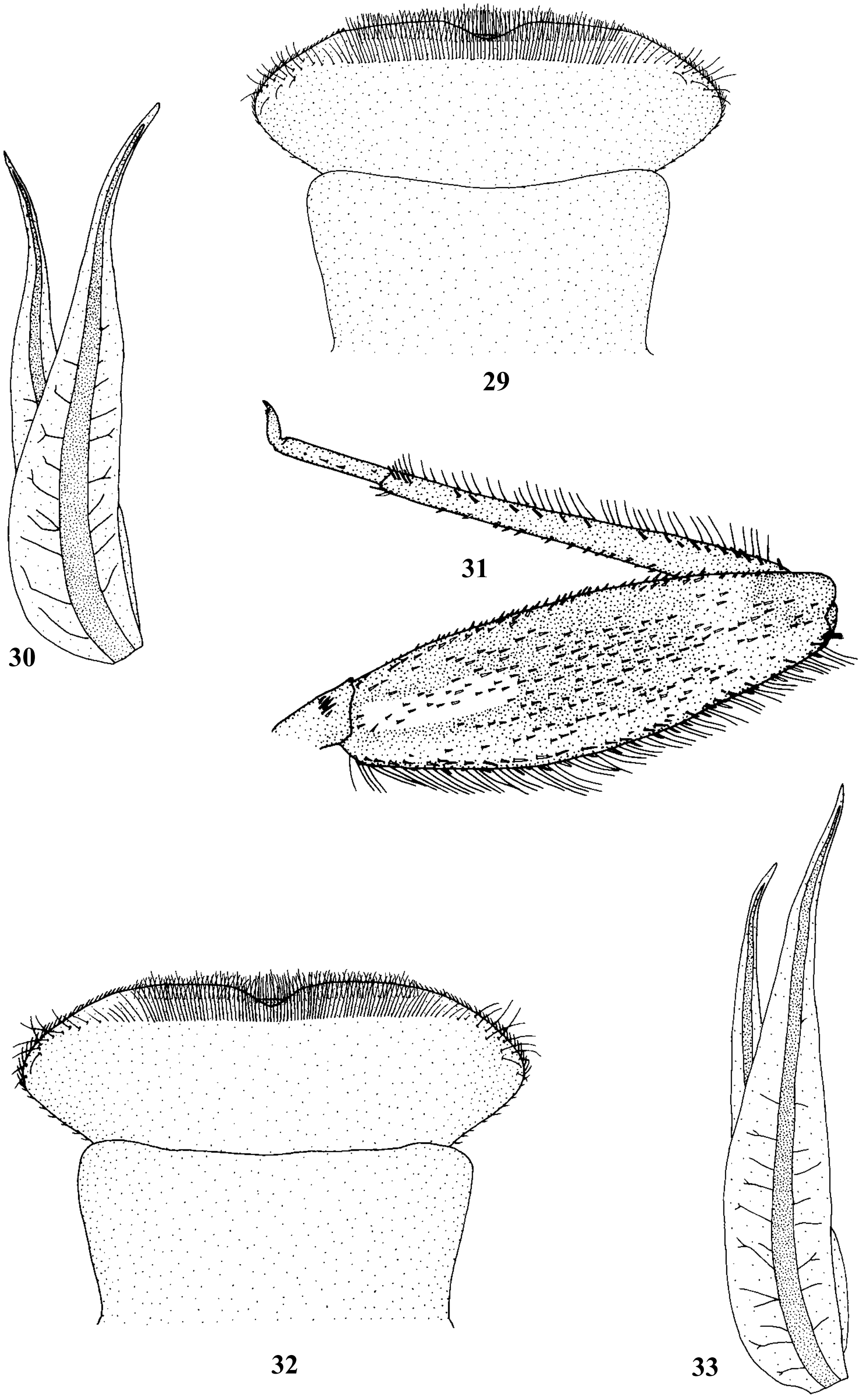
Figure 21. *Austrophlebioides* sp.: nymph.



Figures 22-28. *Austrophlebioides porphyrobranchus*: (22) labrum, (23) third gill, (24) tarsus and claw of fore leg. *Austrophlebioides wooroonooran*: (25) labrum, (26) mandible, (27) hind leg, (28) third gill.



Figures 29-33. *Austrophlebioides rieki*: (29) labrum, (30) third gill, (31) hind leg. *Austrophlebioides* ‘paluma’: (32) labrum, (33) third gill.

Genus *Jappa*

Diagnostic characters: frontal horns present on head (Fig. 34); labrum narrower than clypeus; outer incisor of mandibles triangular; glossae of labium turned under ventrally, not lying in the same plane as paraglossae; lateral margins of submentum fringed with setae; tarsal claws with small denticles (Fig. 35); abdomen with postero-lateral spines on segments 8 to 9; gills ovate with single apical filament; apical half of gill fringed with setae (Fig. 36).

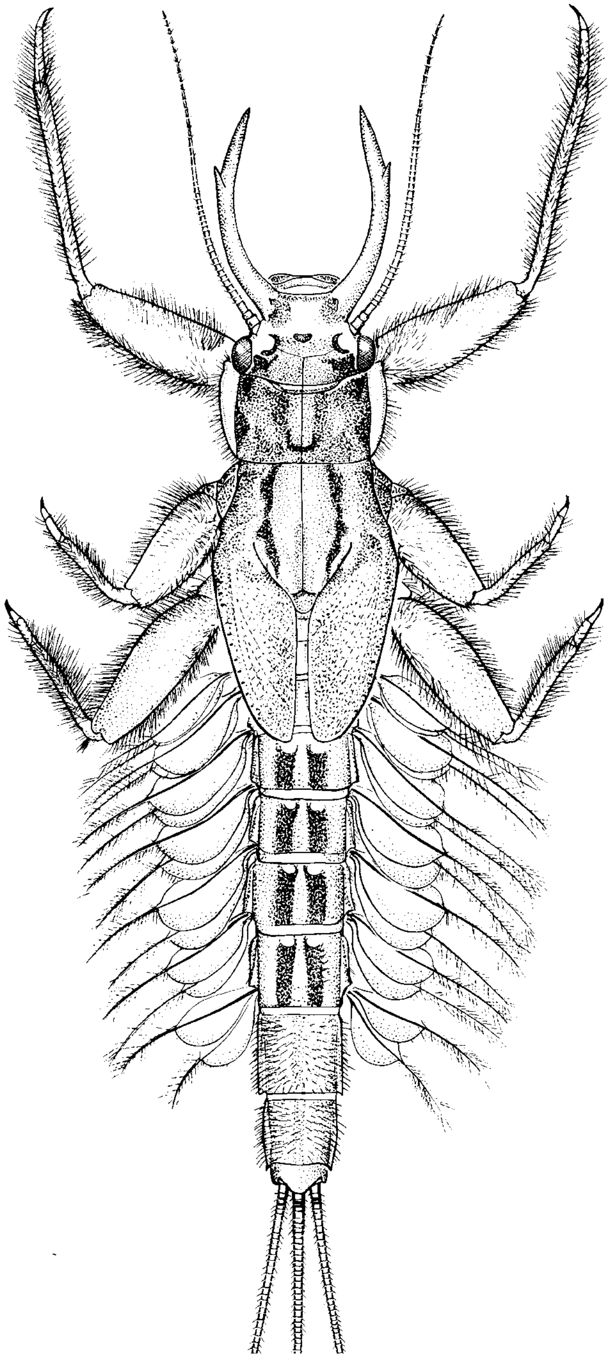
Key to Wet Tropics species

1 Frontal horns smooth without spines except for a dorsal protuberance near base ………………………………………………………………….……..***Jappa kutera***

- Frontal horns with spines in addition to a dorsal protuberance near base (Figs 34, 37) ……………………………………………………………..…………….2

2(1) Frontal horns with a single large dorsal spine (Fig. 34)…………..***Jappa edmundsi***

- Frontal horns with numerous spines and teeth on dorsal surface (Fig. 37) ……………………………………………………………......***Jappa serrata***



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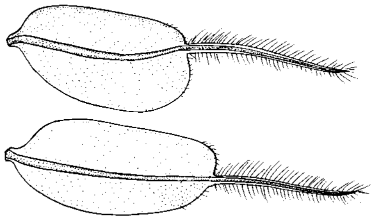

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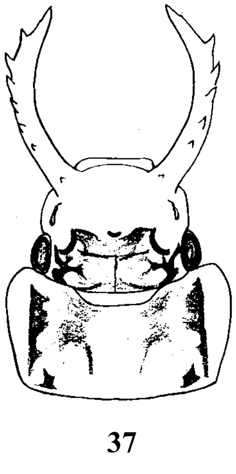

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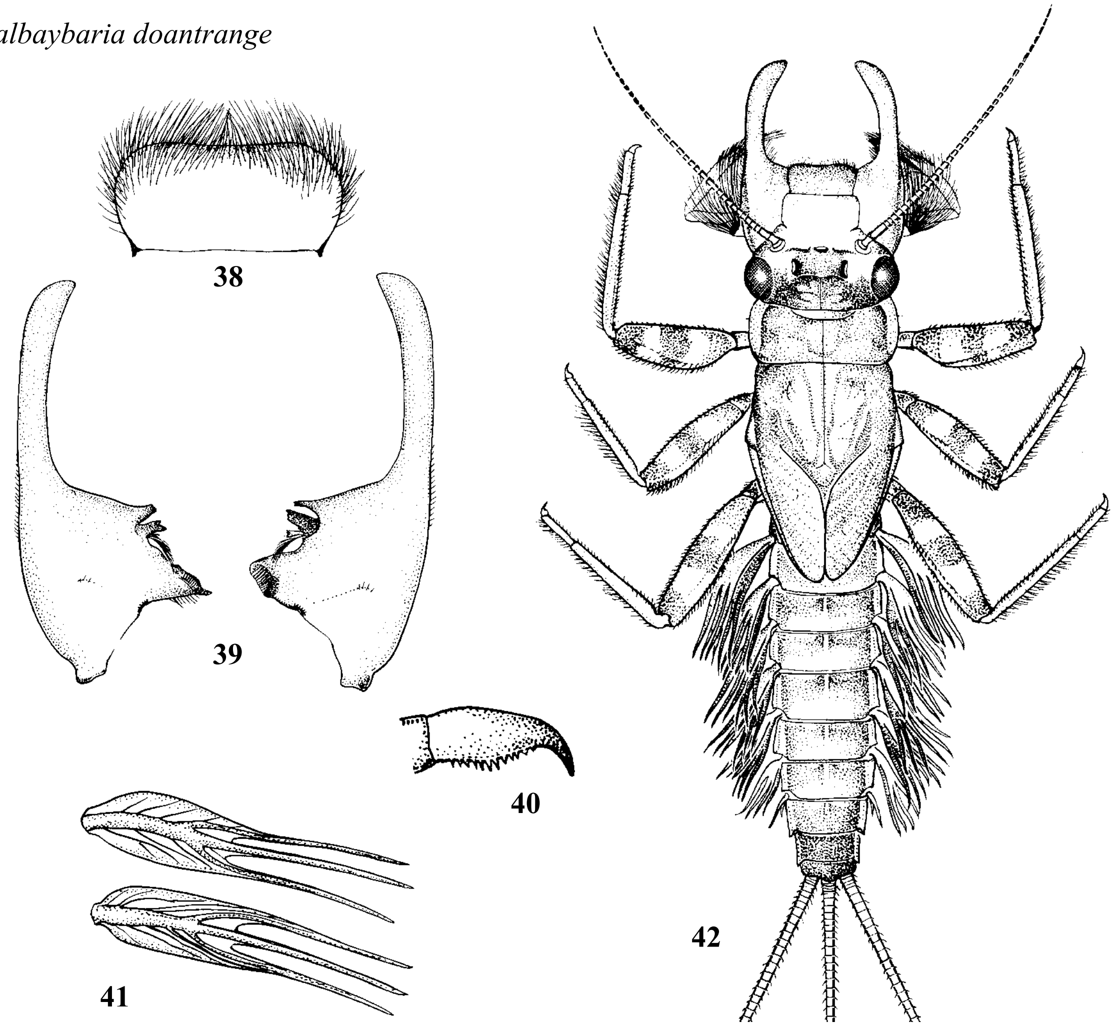
Figures 35-39. *Jappa edmundsi*: (34) nymph, (35) tarsal claw, (36) gill. *Jappa serrata*: (37) head.

Genus *Kalbaybaria*

Diagnostic characters: mandibles with a large flattened tusk-like process (Figs 39, 42); labrum narrower than clypeus (Fig. 42); setae on dorsal surface of labrum scattered, not organised into distinct rows (Fig. 38); glossae of labium turned under ventrally, not lying in the same plane as paraglossae; lateral margins of submentum fringed with setae; segment 2 and 3 of labial palps with numerous long setae; tarsal claws with small denticles (Fig. 40); abdomen with postero-lateral spines on segments 8 to 9; gills digitate, each lamella divided into three digits (Fig. 41).

Species recorded from the Wet Tropics

Kalbaybaria doantrange



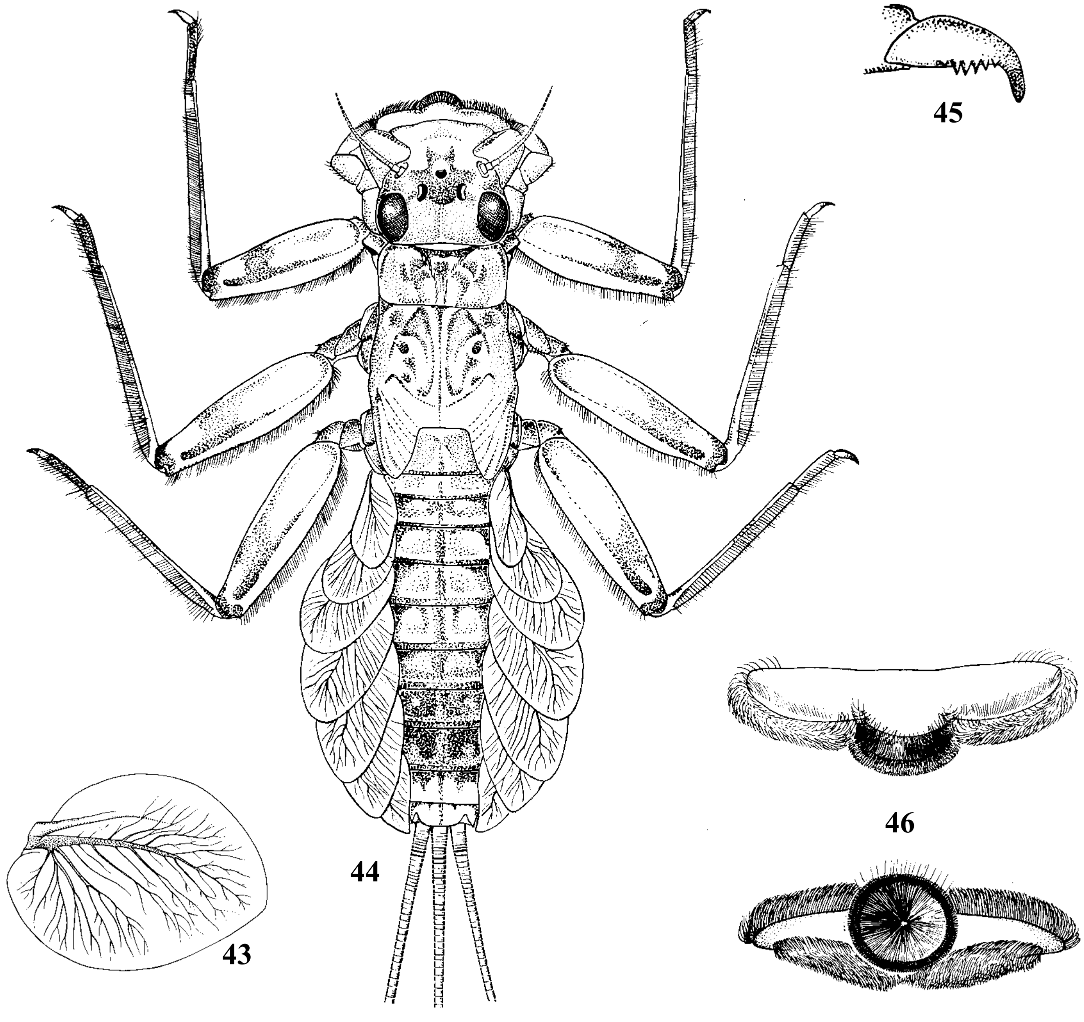
Figures 38-42. *Kalbaybaria doantrangae*: (38) labrum, (39) mandibles, (40) tarsal claw, (41) third gill, (42) nymph.

Genus *Kirrara*

Diagnostic characters: labrum considerably broader than clypeus, anterior margin straight or with setal suction disc (Fig. 46); lateral margins of clypeus apically divergent (Fig. 44); outer margin of mandible right-angled; incisors slender, parallel sided; apex of incisors serrated; glossae of labium lying in the same plane as paraglossae; lateral margins of submentum bare, without setae; tarsal claws with ventral teeth (Fig. 45); abdomen with postero-lateral spines on segments 2 to 9; gills with upper lamella large and ovate, lower lamella small or absent (Fig. 43).

Species recorded from the Wet Tropics

Kirrara sp. AV1



Figures 43-46. *Kirrara* sp. AV1: (43) gill; (44) nymph; (45) tarsal claw; (46) labrum.

Genus *Koornonga*

Diagnostic characters: labrum subequal to or slightly wider than clypeus (Figs 48, 51), subapical setal fringe less than 0.6 times the width of apical setal fringe (Fig. 48); incisors of mandibles slender; glossae of labium lying in about the same plane as paraglossae; lateral margins of submentum fringed with setae; tarsal claws with ventral teeth; abdomen with postero-lateral spines on segments 6-9; gills broadly lanceolate, lateral tracheae well developed (Fig. 49).

Key to Wet Tropics species

1 Labrum broadest in apical half (Fig. 47)……….………..….***Koorrnonga* sp. AV2**

\- Labrum broadest at about mid-length, (Figs 48, 51)..……………………………2

2(1) Both upper and lower gill lamellae tapering evenly in apical third, without constriction; inner margin of upper lamella without small recess (Fig. 49); subapical fringe with approximately 20 setae (Fig. 48)……..***Koorrnonga* sp. AV4**

\- Upper lamella of gill with constriction at 2/3 length of gill, inner margin with small recess (Fig. 50); subapical fringe with 10-15 setae (Fig. 51) ……………………………………………...……………..***Koorrnonga* sp. AV6**

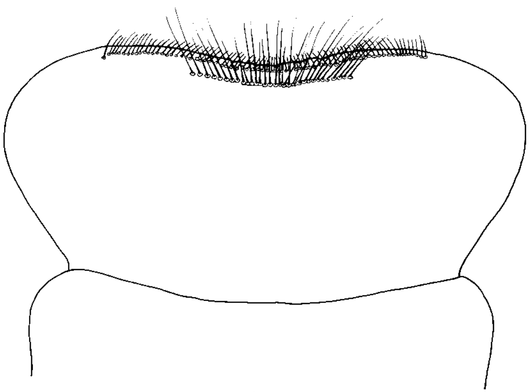

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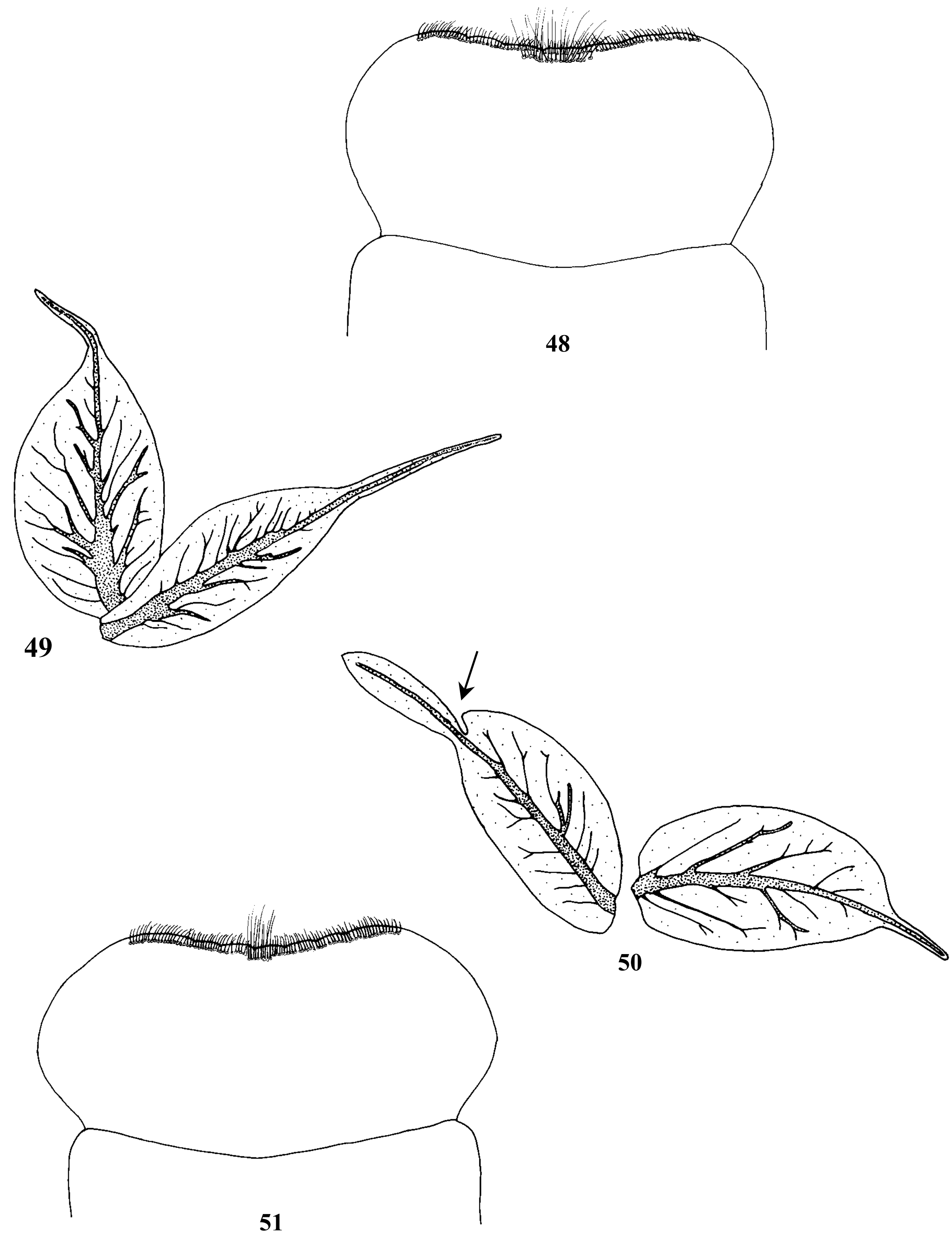
Figure 47. *Koorrnonga* sp. AV2: (47) labrum.



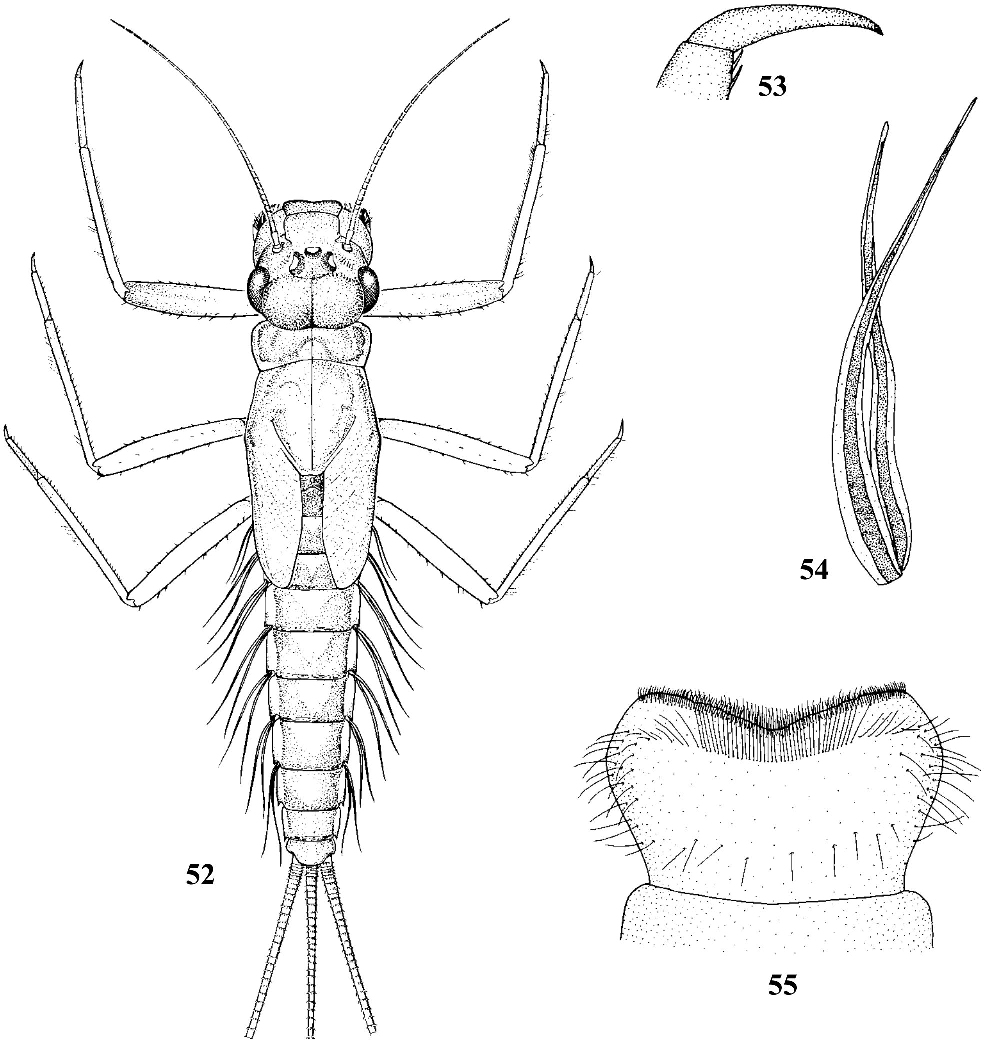
Figures 48-51. *Koorrnonga* sp. AV4: (48) labrum, (49) fourth gill. *Koorrnonga* sp. AV6: (50) fourth gill, (51) labrum.

Genus *Loamaggalangta*

Diagnostic characters: labrum of similar width to clypeus, subapical setal fringe set back from apical setal fringe (Fig. 55); incisors of mandibles slender; glossae of labium turned under ventrally, not lying in the same plane as paraglossae; lateral margins of submentum fringed with setae; tarsal claws smooth (Fig. 53); legs very slender (Fig. 52); abdomen with postero-lateral spines on segments 8 to 9; gills narrow (Fig. 54).

Species recorded from the Wet Tropics

Loamaggalangta sp. AV2



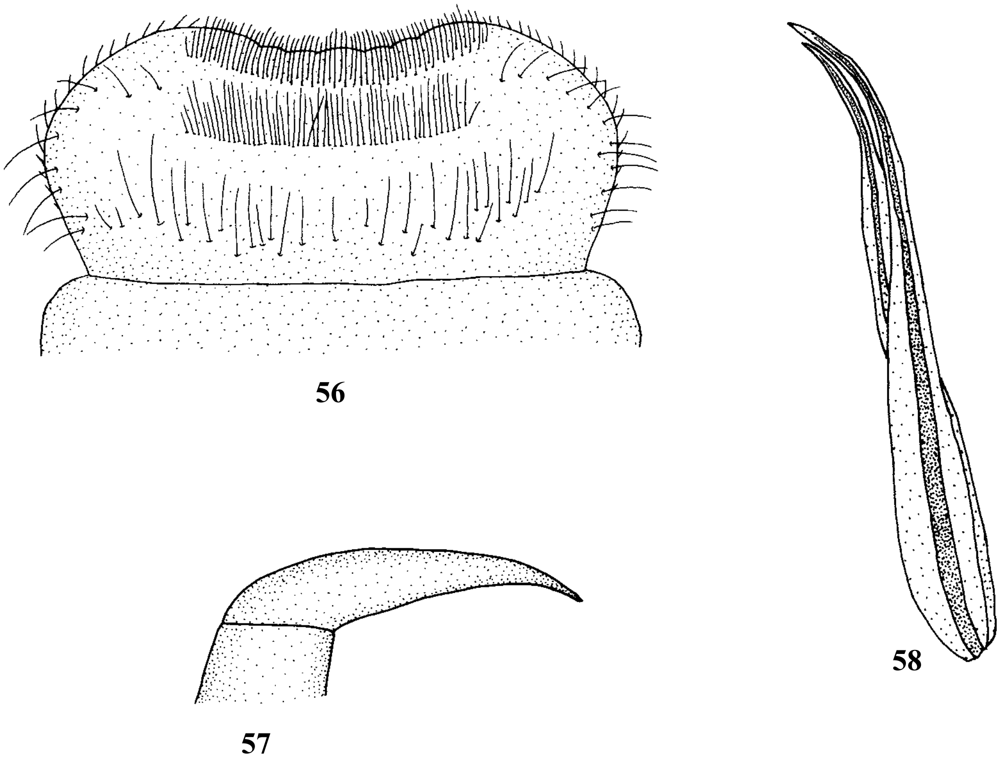
Figures 52-55. *Loamaggalangta* sp. AV2: (52) nymph, (53) tarsal claw, (54) fourth gill, (55) labrum.

Genus *Neboissophlebia*

Diagnostic characters: labrum subequal to or slightly broader than clypeus; subapical setal fringe set back from anterior margins of labrum (Fig. 56); incisors of mandibles slender; glossae of labium turned under ventrally, not lying on the same plane as paraglossae; lateral margins of submentum fringed with setae; tarsal claws smooth (Fig. 57); abdomen with postero-lateral spines on segments 5 to 9; gills narrow, lateral tracheae absent (Fig. 58).

Species recorded from the Wet Tropics

Neboissophlebia sp. NQ1



Figures 56-58. *Neboissophlebia* sp. NQ1: (56) labrum, (57) tarsal claw, (58) fourth gill.

Genus *Nousia*

Diagnostic characters: labrum subequal to or wider than clypeus (Figs 60, 61, 64); incisors of mandibles slender; glossae of labium lying on the same plane as paraglossae; lateral margins of submentum fringed with setae; tarsal claws with ventral teeth; abdomen with postero-lateral spines on segments 3 to 9 or 6 to 9; gills lanceolate, development of lateral tracheae variable (Figs 59, 62, 63).

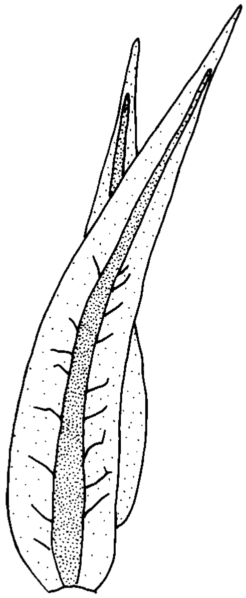
Key to Wet Tropics species

1 Labrum with two distinct rows of setae, an apical and a subapical setal fringe (Figs 61, 64) ….………...…………………………….……...……………....2

- Labrum with single dense row of setae near apical margin (Fig. 60) ………………………………………………………….…….…...***Nousia* sp. NQ1**

2(1) Gills with weakly developed lateral tracheae (Fig. 62); labrum with subapical setal fringe close to apical setal fringe (Fig. 61); abdomen with postero-lateral spines on segments 6 to 9……...…………...…………….…………….....***Nousia* sp. NQ2**

- Gills with well developed lateral tracheae (Fig. 63); labrum with subapical setal fringe set back from apical setal fringe (Fig. 64); abdomen with postero-lateral spines on segments 3 to 9…………………………...……...….***Nousia* sp. NQ3**



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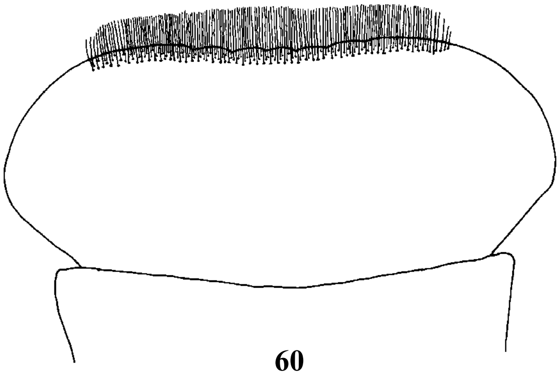

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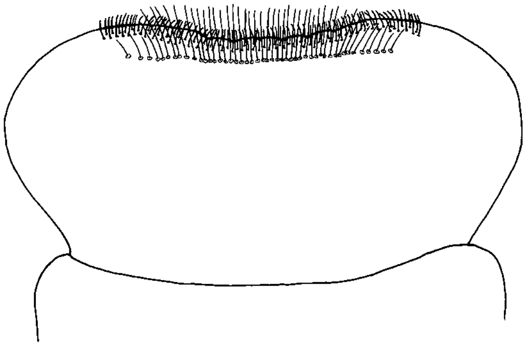

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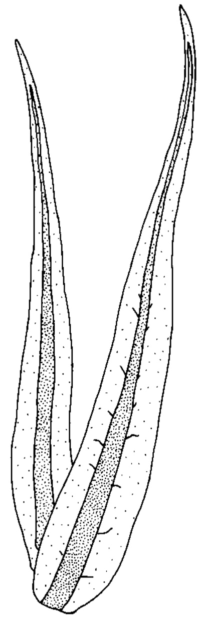

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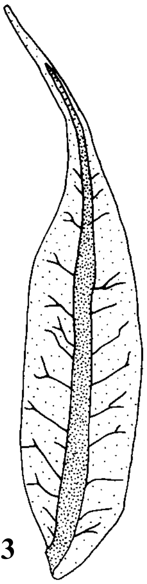

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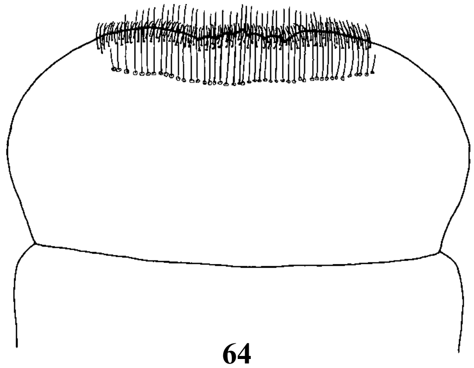

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Figures 59-64. *Nousia* sp. NQ1: (59) fourth gill, (60) labrum. *Nousia* sp. NQ2: (61) labrum, (62) fourth gill. *Nousia* sp. NQ3: (63) fourth gill, (64) labrum.

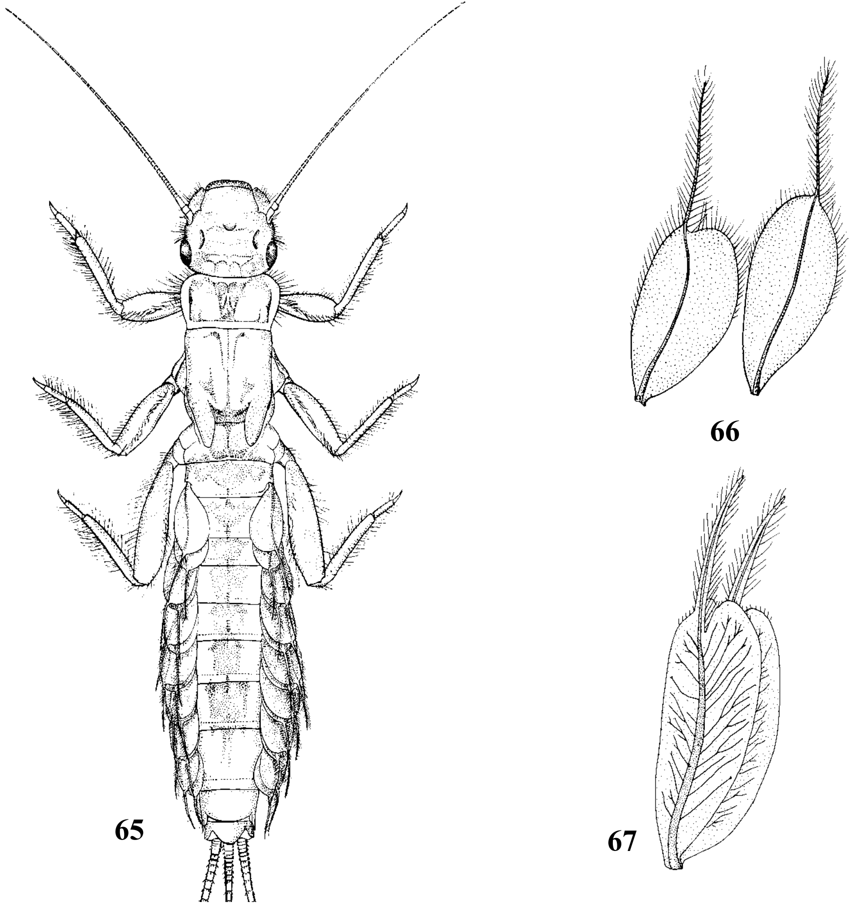
Genus *Ulmerophlebia*

Diagnostic characters: labrum narrower than clypeus; outer incisor of right mandible triangular; glossae of labium turned under ventrally, not lying in the same plane as paraglossae; lateral margins of submentum fringed with setae; tarsal claws with small denticles; abdomen with postero-lateral spines on segments to 7 to 9; gills broad with single apical filament (Figs 65, 66); apical half of gill fringed with setae (Figs 66, 67).

Key to Wet Tropics species

1 Gills with lateral tracheae inconspicuous, paler than gill membrane (Fig. 66) …………………………………………………………..***Ulmerophlebia* sp. AV3**

\- Gills with lateral tracheae conspicuous, darker than gill membrane (Fig. 67) …………………………………………………………..***Ulmerophlebia* sp. AV6**

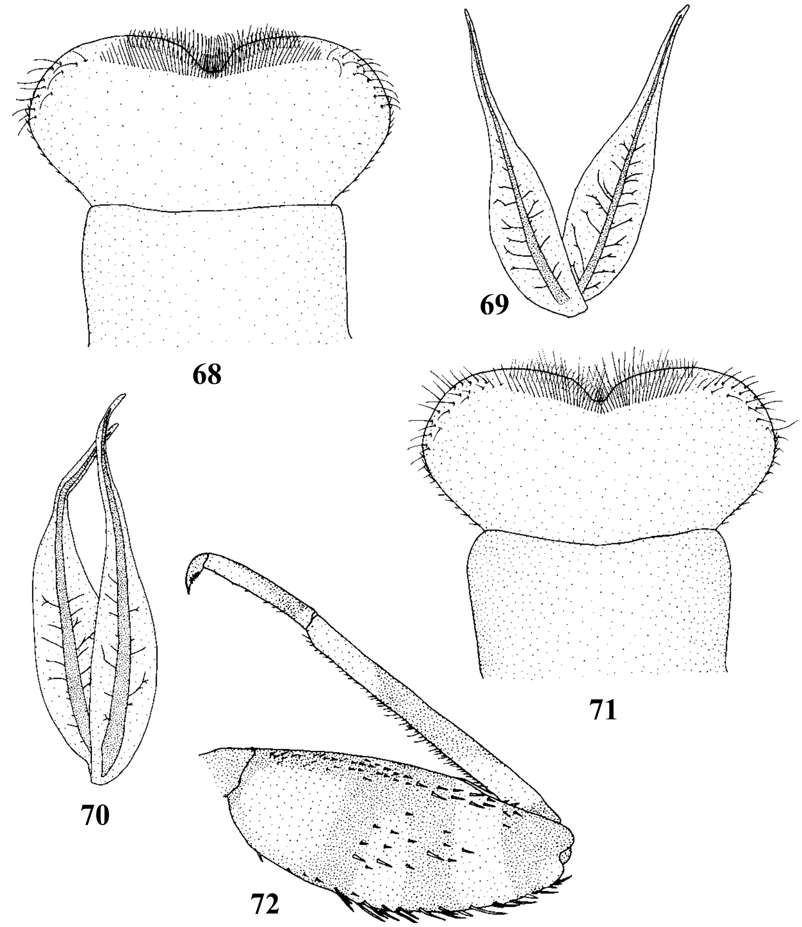


Figures 67-69. *Ulmerophlebia* sp. AV3: (65) nymph, (66) gill. *Ulmerophlebia* sp. AV6: (67) gill.

WT sp. 1 and WT sp. 3

Diagnostic characters: labrum broader than clypeus, anterior margin with U-shaped notch, hooded dorsally (Figs 68, 71); length of labrum greater than length of clypeus (Figs 68, 71); incisors of mandibles slender; lateral margins of submentum with a few setae near base; glossae in the same plane as paraglossae; tarsal claws with ventral teeth (Fig. 72); abdomen with postero-lateral spines on segments 2 to 9; lateral margins of abdomen bare; gills lanceolate, lateral tracheae moderately developed (Figs 69, 70).

Note WT sp. 1 was included as *Tillyardophlebia* sp. AV7 in Dean (1999); however, examination of reared adult material suggests that WT sp. 1 belongs to a new genus. WT sp. 3 appears to be closely related to WT sp. 1, and can be distinguished from WT sp. 1 by the conspicuous banding on the foreleg (Fig. 72).

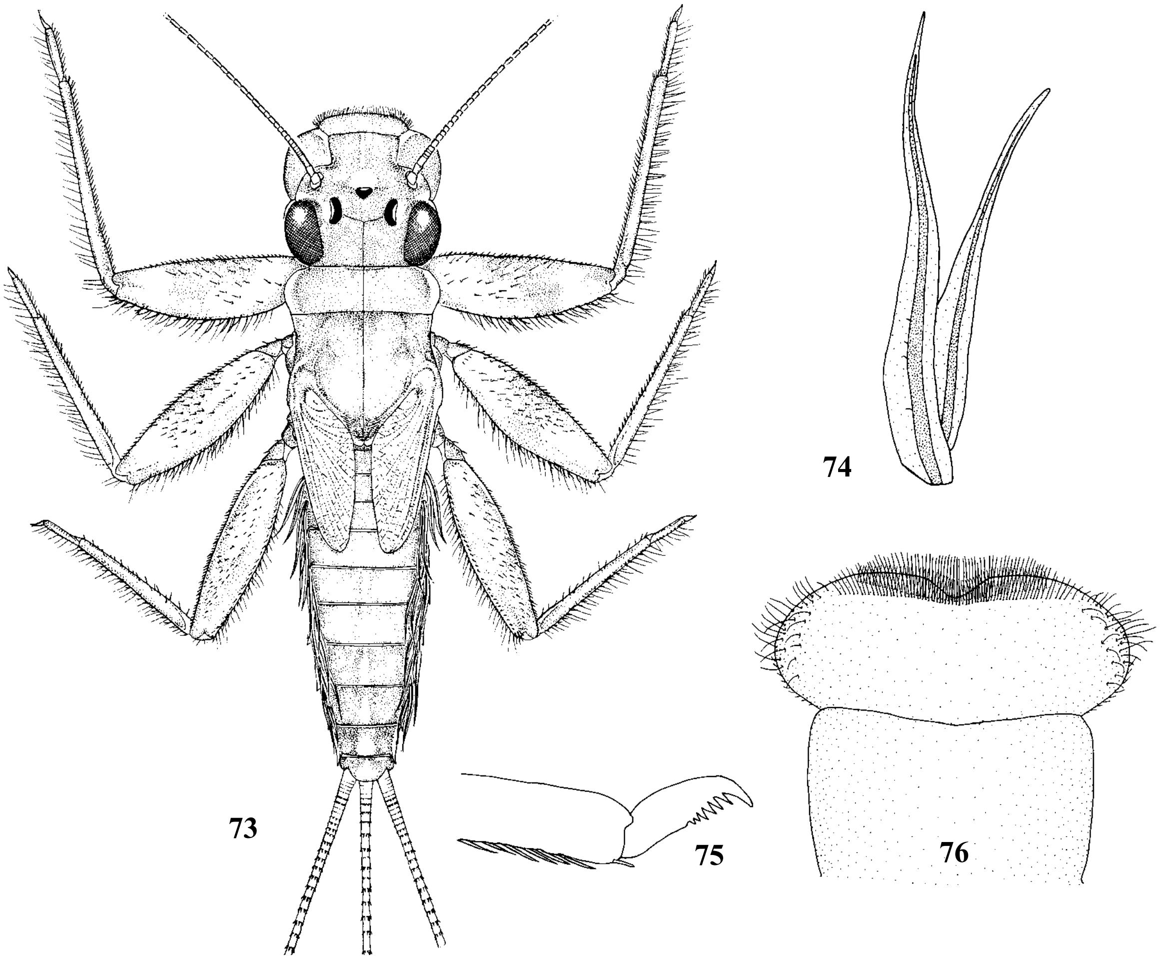


Figures 70-74. WT sp. 1: (75) labrum, (76) fourth gill. WT sp. 3: (77) third gill, (78) labrum, (79) foreleg.

WT sp. 2 and WT sp. 4

Diagnostic characters: labrum broader than clypeus (Fig. 76), anterior margin with U-shaped notch, hooded dorsally; incisors of mandibles slender; lateral margins of submentum bare; glossae in the same plane as paraglossae; tarsal claws with ventral teeth (Fig. 75); abdomen with postero-lateral spines on segments 2 to 9; lateral margins of abdomen bare; gills lanceolate, lateral tracheae absent (Fig. 74); inner margin of fore tarsus with numerous spines (Fig. 75)

Note The nymphs of WT sp. 2 can be distinguished from those of WT sp. 4 by the absence of a prominent transverse black band along the posterior margin of the abdominal terga (Fig. 73).



Figures 73-76. WT sp. 2: (73) nymph, (74) fourth gill, (75) tarsus and claw of foreleg, (76) labrum.

Illustrators

Sybil Monteith illustrated all of the whole nymph drawings (Figs 1, 13, 21, 34, 42, 44, 52, 65, 73) as well as Figures 7, 8, 15, 35-41, 43, 45, 46, 66. All remaining figures were illustrated by Faye Christidis (Figs 2-6, 9-12, 14, 16-20, 22-33, 47-51, 53-64, 67-72, 74-76).

References

Dean, J.C. (1999) *Preliminary keys for the identification of Australian mayfly nymphs of the family Leptophlebiidae*. Co-operative Research Centre for Freshwater Ecology, Identification Guide No. 20.