

1447-2554 (On-line)

<https://museumsvictoria.com.au/collections-research/journals/memoirs-of-museum-victoria/>

DOI <https://doi.org/10.24199/j.mmv.2021.80.08>

The unusual genus *Ramiheithrus* (Trichoptera: Philorheithridae)

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Abstract

St Clair, R.M.. 2021. The unusual genus *Ramiheithrus* (Trichoptera: Philorheithridae). *Memoirs of Museum Victoria* 80: 159–168.

The descriptions of males of the two described species of *Ramiheithrus* (*R. virgatus* Neboiss and *R. kocinus* Neboiss) are expanded using additional material. The female, pupa and larva of *Ramiheithrus virgatus* are described for the first time for the genus. Preliminary genetic barcoding analysis suggests the presence of additional undescribed species that are only known as larvae. The unusual larval habitat is described and issues relating to this are discussed.

Keywords

Australia, groundwater habitat, COI gene, pectinate antennae

Introduction

The genus *Ramiheithrus* was created for two species, one each in Victoria and Tasmania, by Neboiss (1974) with only adult males known at the time. Information here brings the number of species in the genus to four but the two additional species are known only as larvae. The descriptions of males of the two described species are revised here, partly based on specimens from additional sites. Figures indicating differences from the original descriptions, or those showing additional information, are included for males. The adult female, pupa and larva of *Ramiheithrus virgatus* Neboiss are described. The larva of *R. virgatus* is compared with the larvae of two additional species.

The “rami” in *Ramiheithrus* refers to the distinctive pectinate antennae. Pectinate antennae are otherwise unknown in Trichoptera, except for three species of the Odontoceridae genus *Phraepsyche* from South East Asia (Oláh and Johanson, 2010) although the form of the pectination is apparently different. A fossil trichopteran with yet another form of pectination is discussed in Gao et al. (2016).

Few females of Philorheithridae are described and there is variation between species in genera where more than one species is known. *Philorheithrus* now has females of four species described, and these show considerable variation in shape of genitalia (Henderson and Ward, 2006; Mosely and Kimmins, 1954). This suggests that females of other *Ramiheithrus* species may differ from the description below. The vaginal apparatus of *R. virgatus* is illustrated below but there is no information on other Philorheithridae females to compare with.

Larvae of *Ramiheithrus* have very rarely been collected. The few available specimens provide information on variation in the larvae and the likely reason for this rarity.

DNA barcoding data is preliminary but has assisted taxonomic decisions.

Material and methods

The terminology here follows Henderson and Ward (2006) and Holzenthal et al. (2007) but not Neboiss (1974).

All specimens are lodged in the Museum of Victoria (NMV) and have been assigned registration (TRI) numbers. Reared specimens usually include the case with pupal closure membranes and larval sclerites, pupal skin and adult. The adult has not emerged from the pupal skin in some cases and wing venation cannot be discerned. Tissue samples sent to the Barcode of Life Data System (BOLD; <http://www.boldsystems.org>) for sequencing and analysis have been allocated LTUT numbers. Information on the methods used for sequencing and gene segment sequenced are available on the BOLD website. Data for specimens included in fig. 24 and Table 1 are available from the BOLD website. The neighbour joining tree (fig. 24) was produced on the BOLD website using a Kimura 2 parameter and BOLD aligner (amino acid–based HMM) and produced in Newick format using MEGA 7 on 26 October 2019.

Taxonomy

Ramiheithrus Neboiss

Type species: *Ramiheithrus virgatus* Neboiss

The information here uses and extends that given by Neboiss (1974).

Diagnosis. This genus is strikingly unique with the antennae of the adult pectinate on the basal half (fig. 1). In males, the superior appendages are fused to segment IX and in basal half to each other to form a hood over the genitalia (figs 2, 3, 22). This is like the hood in *Kosreithrus* and, to a lesser extent, some species of *Aphilorheithrus*. In *Ramiheithrus*, the superior appendages have

the ventral surfaces covered with moderately long spines in the distal third. The inferior appendages are short and very simple, most similar to those of *Kosrheithrus*. As with most Philorheithridae, the forewing has vein R1 joining R2 just before the wing margin in both sexes. However, this join is not present in some specimens in some populations of *R. virgatus* (fig. 5) and *R. kocinus* (fig. 23) and seems to be absent in at least some species in other genera. The forewing has 0 to 5 crossveins present between Sc and the costa proximal to the level of the r vein in both species, fewer than most *Aphilorheithrus* species but otherwise unusual in the family (fig. 5). The hind wing of both sexes with Sc and R1 merged for a short distance before separating and diverging again (fig. 5). Spiracles on pleura ventrally on segments 2 to 4 enlarged, elongate and variably fleshy, smaller and more rounded on segments 5 to 8, only lightly sclerotised around them (fig. 4).

In the larva, the anterior part of the frontoclypeal apotome is almost twice as wide as posterior to the constriction (figs 10, 24, 26). On the mid leg, the strong dark distal spine on the tibia remains despite the segment being fused with the tarsus (fig. 14). This spine is not present in other Australian Philorheithridae genera, although long and short pale setae are often present in this region. The larval case has the posterior opening almost completely closed by larger sand grains and large flat mineral particles but with gaps between these particles (fig. 17).

Generic diagnoses are not given for females or pupae because they are known from one species only.

Description. Head: pilifers absent; maxillary palps 5-segmented, long and slender in both sexes; labial palps short, about as long as maxillary palps segments one and two combined; antennae about as long as forewing, first segment stout, slightly longer than vertical diameter of eye, second segment very short, next 14 to 16 segments with long lateral branches, becoming shorter on the more distal segments, first 2 or 3 segments may have branched rami (fig. 1); a pair of small anterior setal warts near midline, pair of large, elongate, kidney-shaped posterior setal warts and pair of posterolateral setal warts encircling posterior half of eye. Thorax: 2 pairs of warts on pronotum, most medial pair elevated; 1 pair of rounded scutal warts, usually larger than scutellar warts; male forewing length 9 to 12 mm and lobe on anal margin strongly sclerotised, apex broad apically truncate, forks 1, 2, 3 and 5 present, male hind wing with forks 1, 2, 3 and 5, fork 3 footstalk of variable length. Abdomen: a small projection on sternite VII, variable in size.

Male genitalia: superior appendages moderately long, deeply excised, very broad and fused basally to segment IX and each other forming a hood, ventral surface of each with short pointed spines covering apical half; segment X (trilobed hood-like plate of Neboiss, 1974) trilobed, lateral lobes similar to each other but different to median lobe; inferior appendages a simple short broad plate, sometimes scooped out apically, almost bilobed in some specimens (figs 2, 3, 22). Phallus narrow, tapering apically.

Larva. Final instar about 9 to 12 mm long. Head: rounded and somewhat flattened dorsally; strong carinae present just above the eye from the anterior margin to about three quarters head length, forming the lateral margin of the head on each side; region between the carinae sloping ventrally near each

carina which are raised above the head surface, roughened by minute protuberances; antennae very short, close to front of head and below carinae; labrum with 3 pairs of setae at about 3/4 length and another 3 pairs of short stout setae on front margin, lateral two pairs curved along margin; ventral apotome about 1/15 length of ventral ecdysial line; head setation as in fig. 10, most setae on the dorsal surface (but not on the carina) are short and very pale usually bent over or lying flat. Thorax: metanotum with 3 pairs of sclerites, anteriomedial pair about 4 times as long as posterior pair (figs 11, 24); prosternum with a large posterior central sclerite (figs 12, 25); mesosternum with 2 pairs of closely aligned sclerites medially, smaller and paler than pronotal sclerite, posterior pair rectangular, anterior pair tapering laterally (fig. 12); forelegs each with cluster of more than 12 stout black setae centrally on anterior face of femur, all arising from same point (fig. 13), tibial spur about 1/3 tarsal length, tarsal claw about half as long as tarsus, apical spine of tarsal claw almost as long as tarsal claw and lying closely along its length; mid leg tarsal claw about 1/5 tibiarsus, basal spine of claw almost as long as tarsal claw and lying closely along its length; hind legs much longer than forelegs, tarsal claw about half tarsal length, 2 tibial spines on each tibiae about 1/3 tarsal length, 1 on anterior face, 1 ventral, a group of short spines apicoventrally (fig. 14). Abdomen: tergite IX short, not completely covering the segment, lightly sclerotised, with fringe of about 14 long setae and about 30 short or moderate length setae (fig. 15). The lightly sclerotised channel running ventrally from the base of anal claw towards the ventral junction of the prolegs is deeper and more obvious than in some other genera (fig. 16). This channel is darkly sclerotised at the deepest point near the base of the anal claw. Anal claws with tooth almost 1/2 length of claw.

***Ramiheithrus virgatus* Neboiss, 1974:**

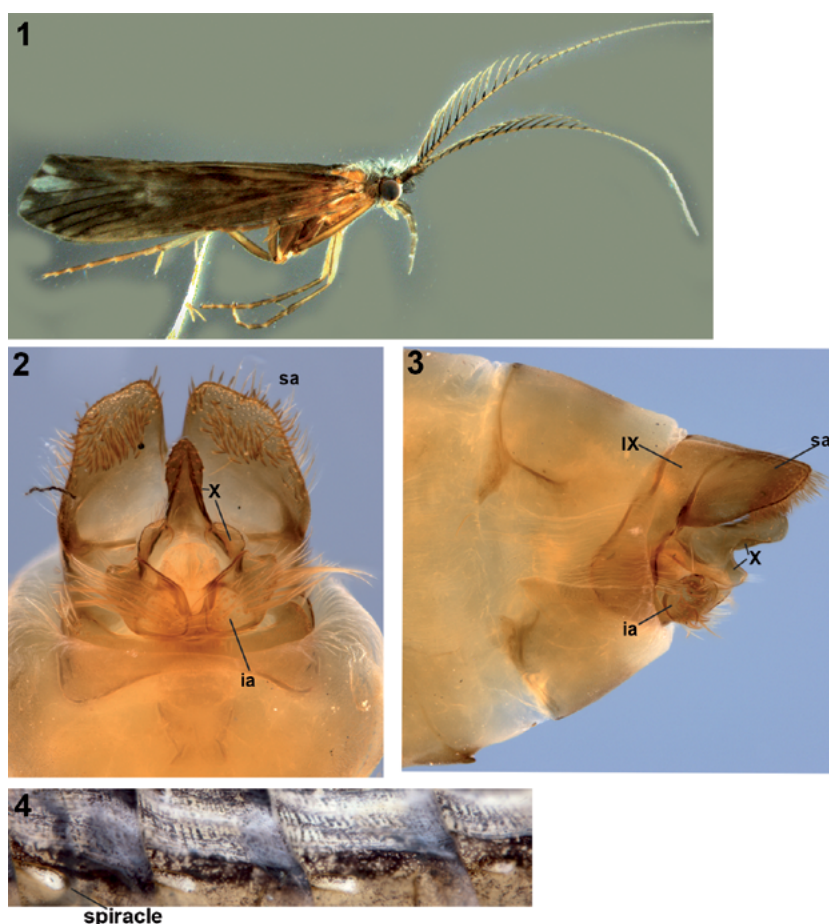
Figures 1–21

Type Material. Holotype male, Victoria. McKay Creek, Sassafras Gap, 36° 36' S 147° 47' E, 2 February 1974, A. Neboiss (NMV TRI-4558). Paratypes 8 males, collected with holotype (NMV TRI-4559 to 4566).

Other Material examined. New South Wales. Alpine Creek Kiandra, 35° 52' S 148° 29' E, 20 January 1966, 3 males 2 females (NMV TRI-29289), 16 January 1968, 1 male (NMV TRI-29288), 13 December 1974, 2 males, 3 females (NMV TRI-29290), E.F. Riek. Victoria. Roadside trickles 1.2 km N of Sassafras Gap, -36.6° E 147.79° S, 10 February 2010, 1 larva (LTUT720-12) (NMV TRI-54703), D. Cartwright and R. St Clair, 19 December 2013, 1 larva (NMV TRI-55169), D. Cartwright; Roadside seep 1.6 km N of Sassafras Gap, 10 February 2010, 1 larva (NMV TRI-55167), 11 February 2010, preserved assorted dates to 21 February 2010, 3 reared males, 1 reared female, 2 male pupae, 1 female pupa all with larval case and sclerites, 6 larvae (NMV TRI-54806 to 54815), D. Cartwright and R. St Clair; Whites Bridge Mt. Buller Road 5 km SE Mirimbah, 37° 08' S 146° 26' E, 22 Mar 1985, 1 male A. Neboiss (NMV TRI-29291), 1 male, 2 Jan 2011, M. Shackleton (LTUT723-12) (NMV TRI-54702).

Diagnosis. As for the generic diagnosis with the following additions. Male genitalia with segment X median lobe elongate, longer than lateral lobes (figs 2, 3).

Larva distinguishable from larvae of the two other species in the genus by the absence of small anterior sclerites on the



Figures 1–4. *Ramiheithrus virgatus* male: 1, Sassafras Gap body lateral; 2, Mt Buller genitalia ventral; 3, Kiandra genitalia lateral; 4, Mt Buller abdomen lateral showing elongate spiracles on segments. ia = inferior appendage, sa = superior appendage, IX = segment IX, X = segment X.

prosternum, the more strongly developed brush of small spines apically on the hind legs (fig. 14) and the reddish-brown head and thorax (fig. 11). Collection of additional larval specimens of the two undescribed species may show these larval characters to not be as diagnostic.

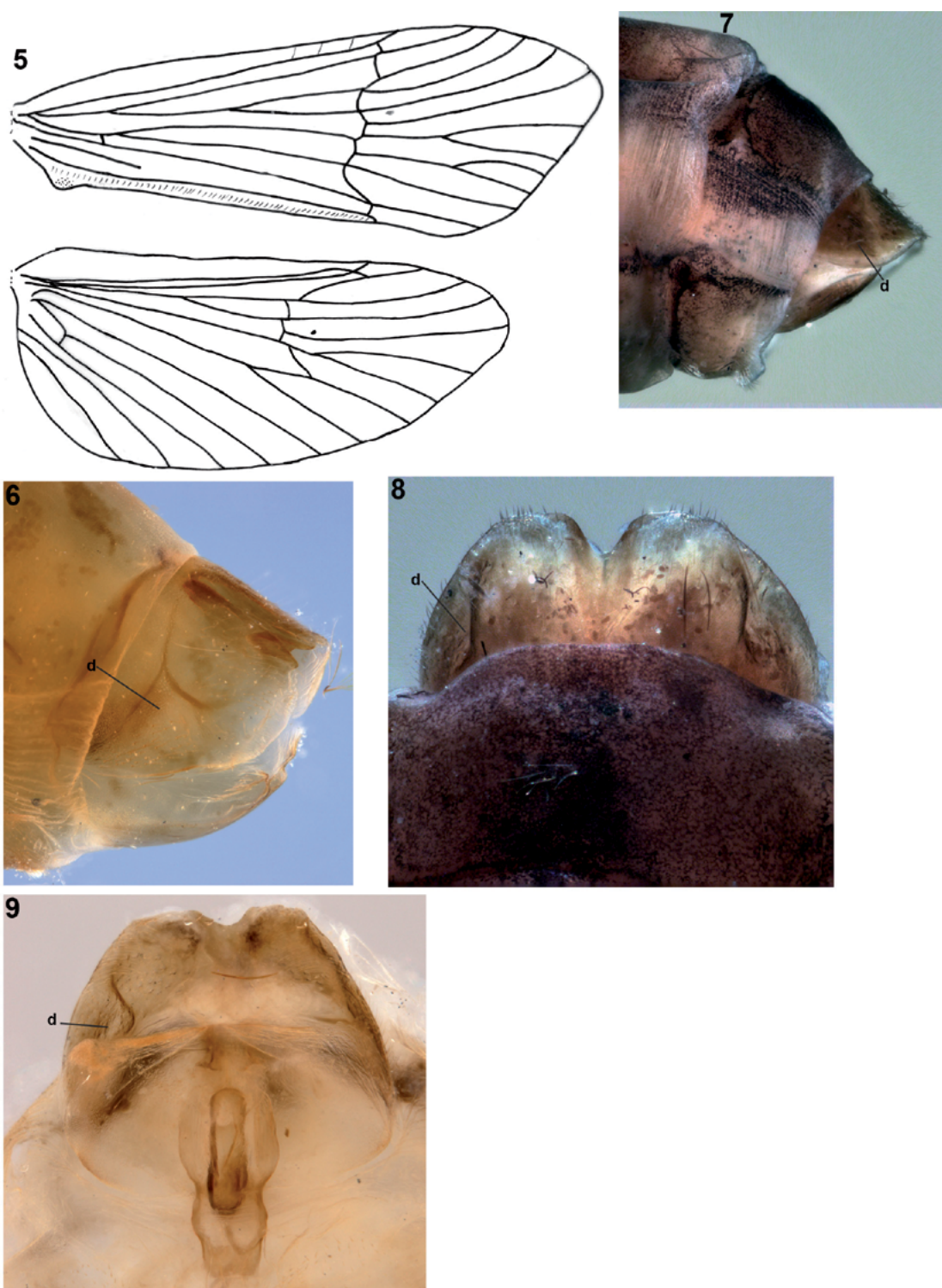
Description. Revised after Neboiss (1974). As for the generic description with the following. Antennae rami dark brown, antennal segments yellowish. Forewings irregularly mottled greyish brown, a distinct pale oval spot close to wing margin within fork 3, smaller and less conspicuous ones between the veins above and below it (fig. 1); R1 joins R2 in males and females although R1 joins the wing margin instead in most specimens collected near Kiandra (fig. 5) and a few from Sassafras Gap.

Male genitalia: segment X median lobe laterally compressed, lobe longer than lateral lobes; lateral lobes flat, somewhat truncate in lateral view; phallus comes to a point apically, curved downwards ventrally; inferior appendages somewhat truncate distally in ventral view, irregular or with a concavity (rarely 2) apically in some specimens (figs 2, 3).

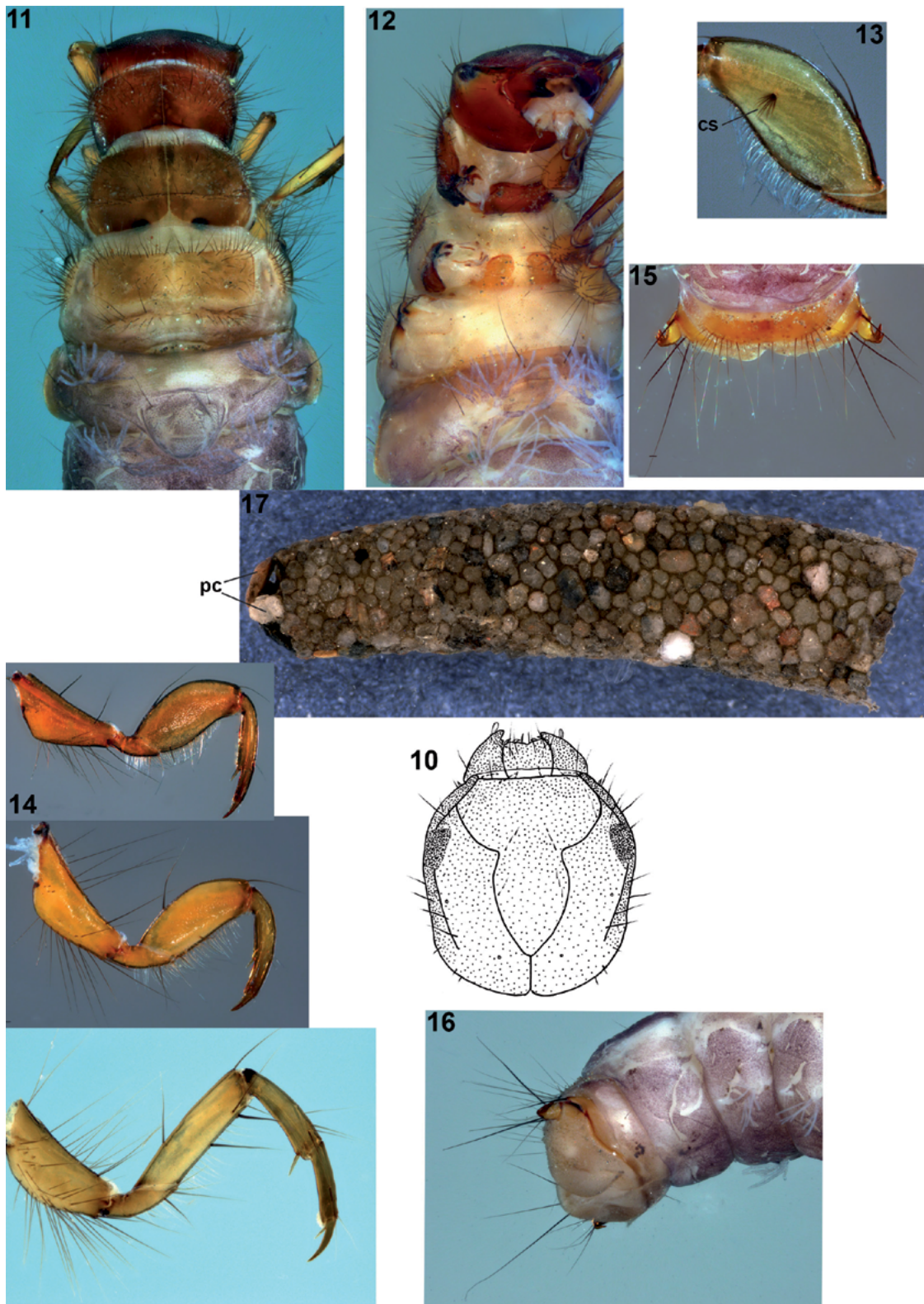
Female. Antennal rami on segments 3 to 11, 12, or 13, rami less than half the length of those in males. Forewings with

forks 1 to 5 present, forks 3 and 4 on long footstalks, hind wings similar to male (fig. 5). Sternite VIII with a band of short pale setae along posterior margin (figs 6, 7). Genitalia: tergite IX very broad and sclerotised dorsally, narrowly cleft mesally, medial notch on posterior margin. With a pair of depressions laterally on segment, with a weak carina along dorsal margin of each. Sternite IX with triangular sclerotised area on anterior half of segment, fleshy laterally and posteriorly (figs 6–9). Simple, apical lobes fused with segment X or lost.

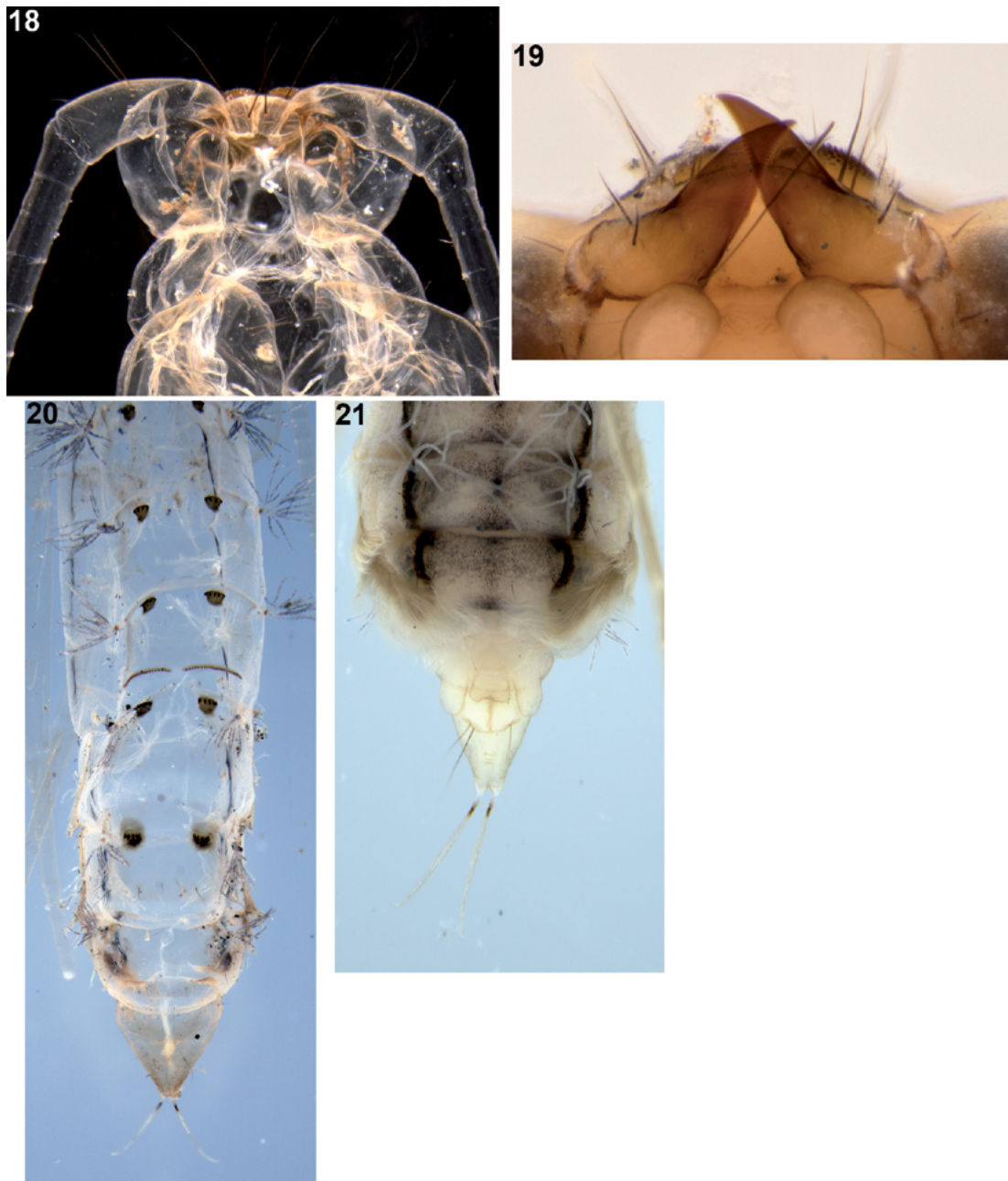
Pupa. Head with a pair of prominent rounded, tuberculate warts on the frons taking up most of the width of the frons which are similar to, but more prominent than, those of *Aphilorheithrus* and *Kosrheithrus*; a pair of long setae dorsally medially on the head between the antennae, a second pair just dorsal to the tuberculate warts (fig. 18), labrum with 3 pairs of setae in each dorsolateral corner; mandibles blade-like in apical half, narrowing to a point, straight and serrate on inner margin, rounded and smooth on outer margin, with a pair of medium length setae basally (fig. 19); each scape with medium length setae, 1 pair mesal dorsal and 1 pair distal ventral (fig. 18). Legs without swimming fringes, rarely a weak fringe on at least one mid leg. Abdomen with pairs of



Figures 5–9. *Ramiheithrus virgatus* female: 5, Kiandra right fore and hind wings; 6, Kiandra genitalia lateral; 7, Sassafras Gap genitalia lateral; 8, Sassafras Gap genitalia dorsal; 9 Sassafras Gap genitalia ventral. d = depression.



Figures 10–17. *Ramiheithrus virgatus* Sassafras Gap larva: 10, head dorsal; 11, head, thorax and first abdominal segment dorsal; 12, head, thorax and first abdominal segment ventral; 13, foreleg anterior face lateral; 14, legs posterior face lateral; 15, abdominal segment IX dorsal; 16, last abdominal segment ventrolateral; 17, case and posterior closure. cs = cluster of setae, pc = particles closing posterior opening.



Figures 18–21. *Ramiheithrus virgatus* Sassafras Gap pupa: 18, shed cuticle head and thorax dorsal; 19, anterior of head ventral; 20, shed cuticle abdomen dorsal; 21, last abdominal segment ventral.

anterior dorsal hook plates on segments 3 and 4 each with 2 to 4 teeth, segments 5, 6 and 7 each with 3 to 6 teeth; pair of posterior hook plates on segment 5 each composed of a row of 16 to 25 spines, many bifid (usually different number on each side) covering most of the width of the segment and with a short gap between each plate, spines as long as plates (fig. 20). Last abdominal segment elongate subtriangular, with 2 pairs of long setae mesally, ventrally (fig. 21). Anal processes as for the family: lightly sclerotised, long, very narrow and slightly

curved apically, each with a short dark pigment band close to the apex and without setae (figs. 20, 21). Larval case with anterior pupal closure a disc of silk without any openings holding on a capping stone.

Larva. As for diagnosis. Final instar larva head capsule width across eyes 1.3 to 1.4 mm.

Remarks. Note that the variation found in wing and genitalic structures of the male were found in all populations.

***Ramiheithrus kocinus* Neboiss, 1974.**

Figures 22–23

Type Material. Holotype male: Tasmania. Small creek in forest, Corinna. 5 November 1972. A. Neboiss and G. Kocins (NMV TRI-4567). 1 Paratype collected with holotype (NMV TRI-4568).

Other Material Examined. Tasmania. Creek crossing Gordon River Road. 42.887° S 146.379° E, 11 January 2012, 1 male (LTUT1103-12) (NMV TRI-54701) M. Shackleton and J. Mynott.

Diagnosis. As for the generic diagnosis with the following additions. Male genitalia with segment X median lobe broad, shorter than lateral lobes (fig. 22).

Description. Revised after Neboiss (1974). As for the generic description with the following. Type specimens blackish with faint paler irregular mottling on forewings of which the most conspicuous is a pale area covering cross veins s, r-m and m, and extending along back along M1 about half the length of the wing. Gordon River Road specimen greyish brown with indistinct white mottling and spots, forewing 9 to 9.5 mm long. R2 joining R3 in type specimens only, fork 1 with or without a short footstalk. Forewings of male with vein A2 long in type specimens, short in Gordon River Road specimen (fig. 23).

Male genitalia. Phallus broad apically; segment X lateral lobes with a few moderately long spines medially; inferior appendages come to a slight point laterally (fig. 22).

Female, pupa and larva unknown.

Remarks. This species is known from only two specimens from the type locality and one specimen from a site (Gordon River Road) about 170 km southeast of the type locality. DNA information is only available for the southern specimen. There is some variation in wing colour and venation and male genitalia but with so few specimens available they are considered conspecific for now.

Additional presumed species of *Ramiheithrus*

Larvae found at two localities in the Yarra Ranges National

Park are included here and are the only other species known in the larval stage. This enables preparation of the generic diagnosis and description. The distinctness of these two species is based on the genetic distance, as discussed in the preliminary genetic analysis below.

***Ramiheithrus* sp. 1**

Figures 24–25

As for the larval generic diagnosis and description with the following additions.

Description. Larval head capsule yellowish brown, width across eyes 1.4 mm, body length 12 mm. The only mid leg on the one specimen has the remnant tibial spine about 1/3 as long as that of *R. virgatus*; prosternum with pair of small sclerites immediately anterior to the large central sclerite (fig. 24). These characters may not enable separation from larvae of other species when variation within each species is known. *Philorheithrus* larvae also show almost no variation between the three species recognised (Henderson and Ward, 2006).

Material examined. Victoria: Alderman Creek at Track 32, Yarra Ranges National Park, 37.721° S 145.941° E, 5 November 2009, 1 larva, J. Dean and R. St Clair (LTUT721-12) (NMV TRI54435).

***Ramiheithrus* sp. 2**

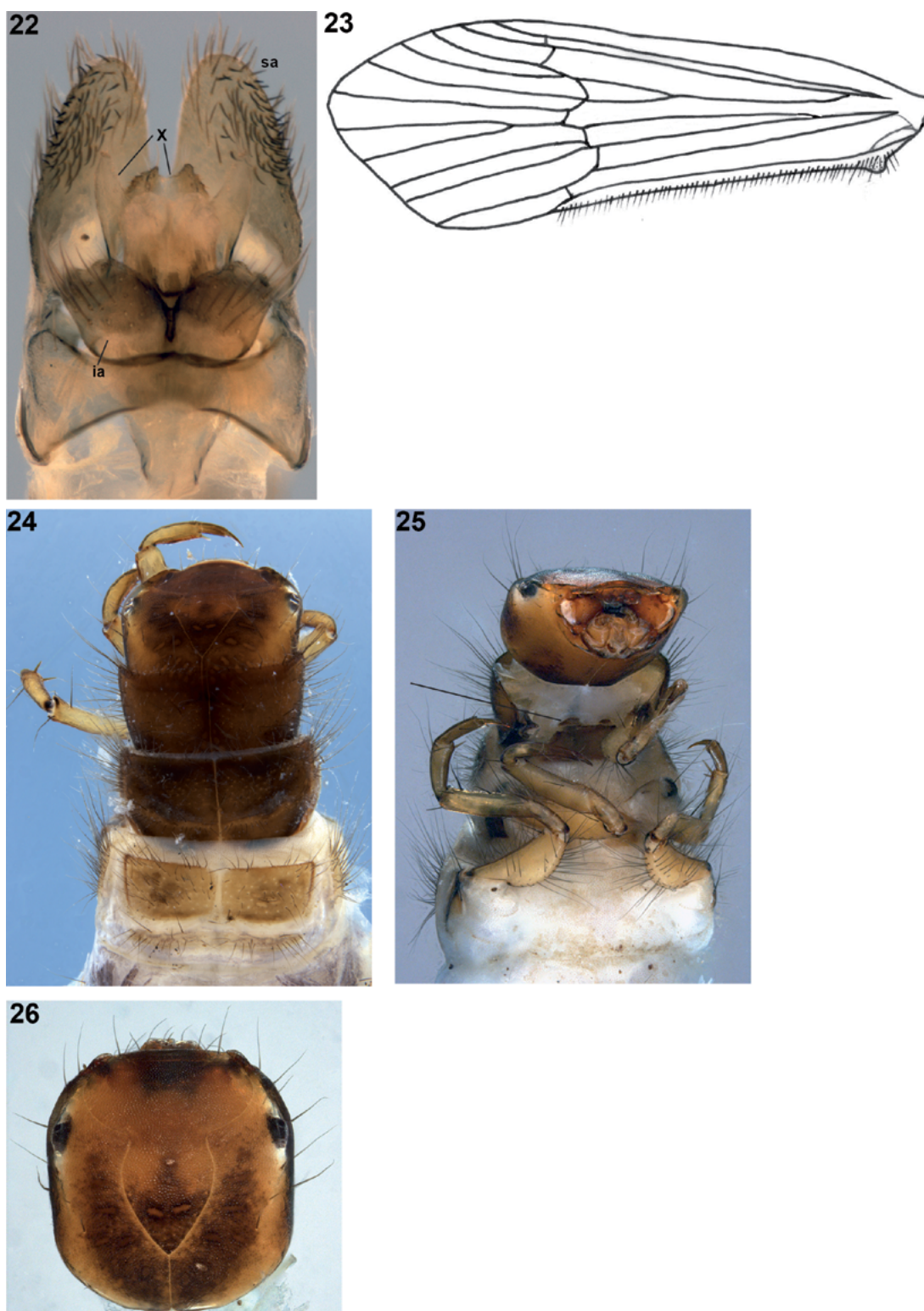
Figure 26

Description. Adult unknown. Larva. As for generic diagnosis and description. Head capsule yellowish brown, width across eyes 1.5 mm, body length 12 mm. Case posterior closure comparatively flat with attached particles smaller and sparser than in the other two species.

Material examined. Victoria: Contentment Creek site ETT4DS R5, CAPIM Black Spur Weir Project, 10 January 2013, 1 larva (LTUT1385-14) (NMV TRI-55176); Contentment Creek, CAPIM Black Spur Weir Project, 10 January 2015, 1 larva (NMV TRI-54816).

Table 1. Specimen details for sequenced *Ramiheithrus*

MV Registration No. (TRI)	BOLD Process ID	COI GenBank Accession No.	BOLD BIN No.	Accession No.	Life stage
54701	LTUT721-12	KX291310	ABV8839	JOS152	Larva
54702	LTUT1385-14	KX296550	ACM3556	EPAVT54	Larva
54437	LTUT720-12	KX296621	ABV9442	JOS151	Larva
54703	LTUT723-12	KX292939	ABV8840	JOS154	Adult male
54435	LTUT1103-12	KX293205	ACC8741	JOS316	Adult male



Figures 22 – 23. *Ramiheithrus kocinus* Gordon River Road male: 22, genitalia ventral; 23, left forewing (rotated 180° for comparison with other wings). Figures 24 – 26. *Ramiheithrus* sp. 1 larva: 24, head and thorax dorsal; 25, head and thorax ventral. *Ramiheithrus* sp. 2 larva: 26, head dorsal. ia = inferior appendage, sa = superior appendage, X = segment X.

Discussion of COI data

An abbreviated COI taxon identification tree is shown in fig. 27 and information on specimens is provided in Table 1. Only one specimen was barcoded from each of five localities. Usually, at least five specimens over the range of the species are required to confirm genetic distinctness. Thus, more sequencing is required to confirm the current interpretation.

The COI taxon identification tree indicates that the two larvae from the Yarra Catchment (LTUT721-12 and LTUT1385-14) are genetically very different from each other and from other specimens (fig. 27). Because there is no adult specimen available from either locality, these species are not being formally described.

The genetic distance between the specimen of *Ramiheithrus virgatus* from near the type locality (LTUT720-12) and the specimen from near Mt Buller (LTUT723-12) is close to 5%. This is greater than often considered necessary to indicate distinct species. However, no consistent morphological features were found to support recognition of

the Mt Buller population as a new species. The generally large distances between all specimens indicate that the commonly applied 2% or 3% threshold for species separation may not apply to this genus. As a result, all the Victorian (apart from the larvae from the Yarra catchment) and New South Wales specimens are here assigned to the one species.

Discussion of larval habitat and its implications

Larvae of *Ramiheithrus virgatus* have been collected for the first time. The first larva collected was in a roadside trickle that is only present after rain and which was dry the day following collection. The trickle appeared as a point where a narrow conduit of groundwater reached the surface, rather than just a point where a broad area of groundwater happened to reach the surface. Investigation found pupae and more larvae in saturated coarse gravel behind a large mound of moss. The seep was less than two square metres on a vertical area of road cutting. Water flowed into the roadside ditch from this area in rainy conditions. This locality was very close to

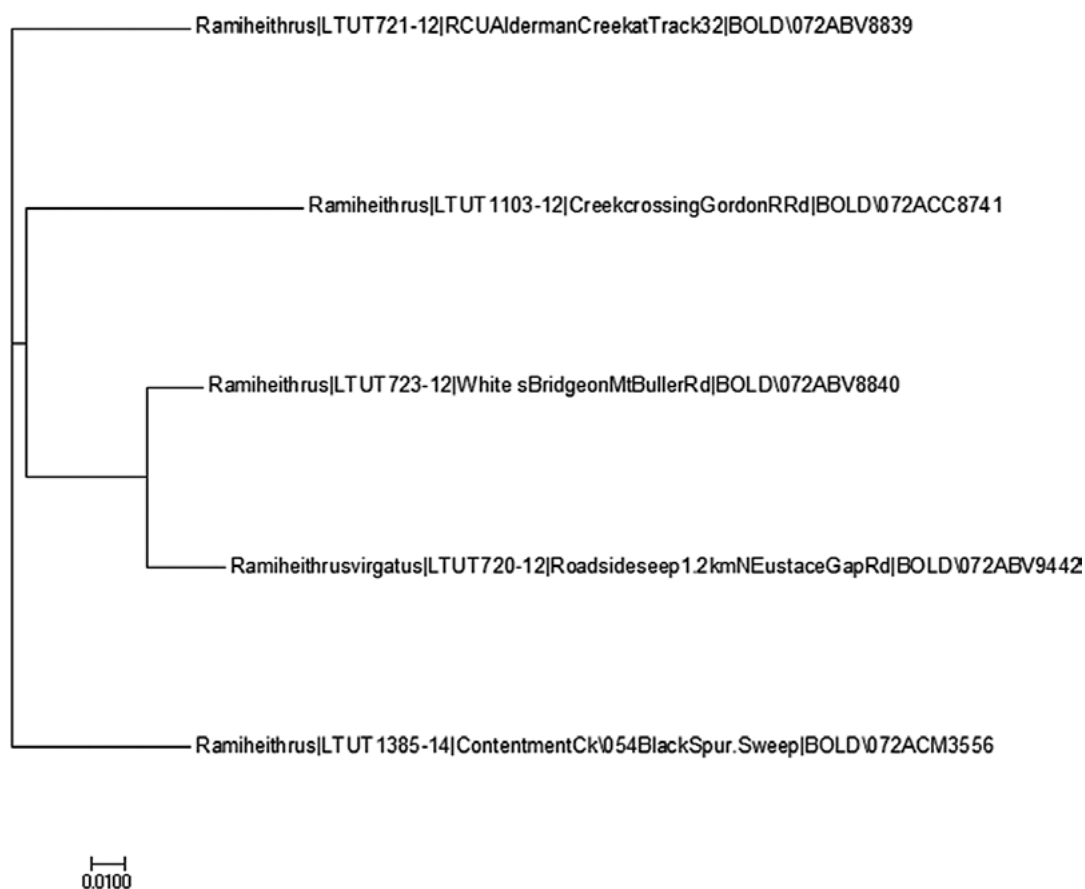


Figure 27. BOLD Taxon identification tree.

the type locality and an adult was collected at light at this new locality soon after finding the larvae and pupae. Some pupae were reared to the adult stage. No similar localities were found nearby, despite extensive searching.

After the habitat was recognised, attempts were made to find similar habitats on the Mt Buller Road, the only other locality in Victoria where adults have been collected. No such habitat or *Ramiheithrus* larvae were found. Areas where ground water was flowing into roadside ditches following rain were found but not associated with a particular moss and not appearing to lead to a gravelly conduit. Presumably, such areas are present but not on the roadside, at least at the time of searching. Being so small, such habitats would be extremely difficult to locate hidden within the vegetation.

The only specimen of *Ramiheithrus* sp. 1 was collected at a standard riverine habitat. However, as only one larva was collected, despite the site being sampled on numerous occasions by the Environment Protection Authority Victoria over a 20-year period, this clearly is not the preferred habitat of the species. This larva was presumably washed in from its usual habitat. A similar but smaller area of moss was noted on a road cutting about 100 metres uphill from the site but not investigated.

The specimens of *Ramiheithrus* sp. 2 were collected from very small trickles, a slightly different habitat to that of *Ramiheithrus virgatus*. Several specimens were collected; therefore, this appears to be their habitat.

The assumption is made that the habitat is similar for all species of *Ramiheithrus*. The fact that only a single larva has been found in a true riverine habitat supports the fact that the main habitat is unusual, and soaks like that where *R. virgatus* was collected near Sassafras Gap are considered the most likely.

Although found in alpine areas, this genus has not been found above 1500 metres. This also could be related to the habitat as it would occur lower down the mountain where ground water seeps out of conduits and associated with a particular type of moss.

The fact that collection of the larvae is so difficult means that species in this genus will be difficult to manage. *Ramiheithrus virgatus* is listed as vulnerable in Victoria and *R. kocinus* is listed as threatened in Tasmania. The adults are rarely found and in low numbers when they are collected. However, E. Riek collected adults at the one locality four times over 11 years. Adult specimens have been collected from the White Bridge locality on more than one occasion, although collecting on other occasions was unsuccessful. It is possible that adults usually remain near the larval habitat. Targeted collecting would be extremely difficult due to the very patchy nature of the habitat.

With the larvae and adults probably living in small areas of habitat spread over a wide area, could this be the reason

why adults have rami-bearing antennae. Presumably they have a sensory function. Gao et al. (2016) suggested rami enable greater sensory function for detection of pheromones or volatile chemicals from food. It is possible the rami in *Ramiheithrus* are used to locate other adults in highly localised habitat patches.

The absence of swimming fringes on the mid legs of the pupae may be an adaptation to the larval habitat as the pupae were not collected in free water. At this stage, the pupa of only one species is known so this may not be typical of all *Ramiheithrus* pupae. Examination of pupae from most Australian Philorheithridae genera found they have swimming fringes. One of the two species of Philorheithridae pupae described from New Zealand, *Philorheithrus agilis* is found in swift streams has swimming fringes while *Philorheithrus litoralis* has a very weak fringe of few pale setae and is found in small streams or seepages (Henderson and Ward, 2006).

Two females of *Ramiheithrus virgatus* reared from pupae had many well-developed eggs in the abdomen. This may be another adaptation to the unusual habitat, but because it is not known how many philorheithrid species are similarly advanced when emerging, this cannot be confirmed.

Acknowledgements

I am very grateful to David Cartwright for organising our collecting trip and finding the larval habitat of *Ramiheithrus virgatus*. John Dean is thanked for improving the manuscript and David Cartwright for very helpful reviewer comments.

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