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SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

Barbadocladius Cranston & Krosch, a New Genus of Orthocladiinae (Diptera: Chironomidae) from South America

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Abstract

Barbadocladius n. gen. is erected and described in larval, pupal and adult stages for two species: *B. andinus* sp. nov. and *B. limay* sp. nov., from Andean streams. The larva is distinctive by virtue of the very large ventromental 'beard' and the anterior parapods with a 'sleeve' of hooklets in addition to apical pectinate claws. The pupa has hooklets on some tergal and sternal intersegmental membranes. The adult, reported only in teneral specimens has hairy eyes, no antennal apical strong seta, no acrostichals, bare and unmarked wings, cylindrical 4th tarsomere subequal in length to the 5th, pulvilli about half the claw length, and hypopygium with anal point, lacking a virga. Molecular phylogenetic analysis eliminates relationships directly to the *Eukiefferiella* complex (which also have pupal hooklets), or to the *Cricotopus* group (adults also with hairy eyes), suggesting instead a sister group relationship to a suite of predominantly austral genera of Orthocladiinae.

Introduction

Thirty-five years ago the Catherwood expedition to Bolivia-Peru collected aquatic insects in the altiplano (Roback *et al* 1980). Many of the Chironomidae collected were treated by Roback & Coffman (1983) along with a smaller collection from Venezuela. The report emphasized the immature stages, many of which were coded and unable to be allocated with confidence to the described fauna even at generic level. At this time the classificatory framework for Chironomidae was based largely on North America and Europe (the Holarctic), and it was to be expected that exceptions and additions to this biota would be found when the southern continents were better surveyed and the biota compared with a modern understanding of northern hemisphere taxa. Progress continues in revealing details of the full life history, which are needed for valid taxonomic descriptions of these informally diagnosed taxa from the Altiplana collections.

Amongst the most distinctive of the orthoclad larvae is one with a very strongly developed 'beard' associated with the ventromentum, perhaps the strongest amongst the Orthocladiinae being dense and extending almost to the lateral margins of the head. This larval type, referred to as 'Genus 9 sp.' by Roback & Coffman (1983) was associated tentatively by co-occurrence with a pupal exuviae 'Genus 4 sp.'. Both were found only at one site in Bolivia (BP4: Tributary of Rio Palca, ca 55 km East La Paz on road to Palca, 16.vi.1977, 4200 masl; Roback & Coffman 1983). The first author of this paper found immature stages and pharate adults in the Patagonian Andes some 15 years ago, demonstrating that the proposed association was correct. The second author, in studying evolutionary relationships of putatively Gondwanan Orthocladiinae using molecular techniques, included this taxon from

trans-Andean sampling between 39° and 41°S up to 1100 m in elevation.

The knowledge of morphology from all stages and a molecular estimation of the phylogenetic relationships encourage us to describe the genus, note its natural history and distribution, and to discuss its evolutionary relationships.

Material and Methods

Collection methods involved interception of surface drift in running waters for pupae and their exuviae, and dislodge larvae from kicking rocks in the benthos. Nets with a 300 µm mesh were exposed immediately downstream of kick sites, or for longer duration to intersect flowing water surfaces for up to 24h. Association between larva, pupa and adult by rearing from live larvae was unsuccessful: pharate individuals form the basis of adult descriptions.

Microscope slide preparation involved clearing with 10% KOH when necessary, followed by neutralization with glacial acetic acid and dehydration continued with isopropanol. Final mounts were in Euparal (BioQuip, Rancho Dominguez, CA, USA); however, larval heads were mounted originally in Hoyer´s for optimal optical quality. Larval bodies were sacrificed for DNA extraction. Attempts were made to remove the genitalia of the pharate adults from exuviae with modest success. Some pupae were mounted laterally, allowing examination of both dorsal and ventral half surfaces of the pupal abdomen.

Morphological terminology follows Sæther (1980), except for numbering of the pupal conjunctives. Here each conjunctive is treated as belonging only to the preceding segment (e.g. conjunctive I, follows tergite 1) rather than specifying the number of the segment anterior and posterior to that conjunctive (e.g. conjunctive I-II).

Photographs were obtained using a Leica® DMRX compound microscope with Nomarski® interference optics. Photographs were taken with an Automontage™ system, allowing automated retention of focused parts of a sequence of exposures at different focal depths. All subsequent manipulations were made in Adobe® Photoshop™.

Material is deposited at Museo de la Plata, La Plata, Argentina (MLPA); Museo Nacional de Historia Natural, Santiago Chile (MNHN); Zoologisches Staatssammlung, Munich (ZSM), Australian National Insect Collection, Canberra (ANIC). Abbreviations used in the data presentation: L = larva, P = pupa, Pe = pupal exuviae, P♀ = pharate female pupa, P♂ = pharate male pupa. All measures are in µm if not otherwise stated.

The specimens described in here were also utilized for molecular analysis of the mtDNA COI and the nuclear 28S rDNA genes and of two regions (CAD1 – 753 bp; CAD3 – 735 bp) of the nuclear protein-coding CAD (rudimentary) gene. The sequences obtained were deposited in GenBank with accession numbers HQ872983-4 (COI), HQ872723-4 (28S), HQ872788 (CAD1), and HQ872840-1 (CAD3), respectively under taxon codes 'CH062M2 *Eukiefferiella* hooklets' and 'ARb23 *Eukiefferiella* hooklets' (Krosch *et al* 2011).

Results

Barbadocladius *gen. n.*

Etymology. From *barbado*, Spanish, 'bearded', and *-cladius* – Latin, 'a branch', widely used in the naming of Orthocladiinae.

Type species. *Barbadocladius andinus* Cranston & Krosch, sp. n. (here designated).

Genus description

Adult male

Small, pharate wing length 1.5-2.0 mm.

Head. Antenna with 13 flagellomeres, well-developed plume extending to simple or shallowly bifid apex lacking any strong apical/subapical seta. Antennal ratio 0.24-0.36. Head with hairy eyes, almost rounded with very slight angle representing dorsomedial extension. Temporal setation restricted to 1-2 outer postorbitals. Clypeus narrow, with few setae. Palp rather short, 5 segmented, consecutively longer from two to five; 3rd segment without sensilla chaetica.

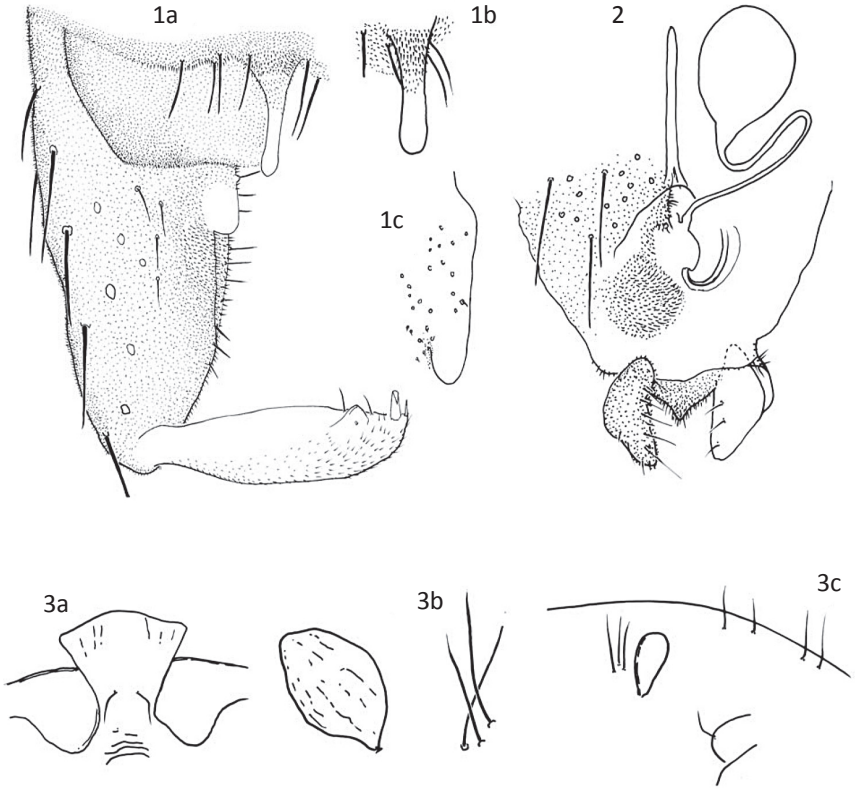
Thorax uniformly light brown. Antepronotum poorly developed, with narrow lobes, medially separated. No humeral pits visible. Thoracic setation: two lateral antepronotals; acrostichals absent; 2-3 uniserial dorsocentrals; 2-3 prealars; 4-7 scutellars.

Wing (all pharate) membrane with fine punctation, without macrotrichia. Apparently without setae on all veins. Squama uniserially setose.

Legs with slender fore-tibial spur, subequal to width of tibial apex, mid-tibia with two short spurs, subequal in length; hind tibia with one long spur, with strong comb; mid- and hind spurs weakly denticulate only basally; pseudospurs absent. Sensilla chaetica apparently absent. Tarsomere IV cylindrical, subequal in length to tarsomere V, pulvilli half length of claw, empodium strong. Claws apically microspatulate

Tergites with quite numerous long, thin setae more developed on anterior half of each segment. Tergite IX without median dorsal setae.

Hypopygium (Figs 1a-c). Anal point with microtrichia at its base, apically bare, hyaline and almost parallel-sided, 25-30 µm long, slightly swollen apically, TIX with



Figs 1-3. *Barbadocladius* spp., adults. 1a) hypopygium left side dorsal, *Barbadocladius andinus* sp. n.; 1b) anal point, IC. volsella, *Barbadocladius limay* sp. n.; 2) Female genitalia *B. andinus* sp. n., left side ventral, right side 'internal'; 3a) frontal area, 3b) thoracic horn and precorneals, 3c) thorax, anterior to right, all *B. andinus* sp. n.

2-4 strong setae on its posterior margin, on each side of anal point. Transverse sternapodeme and phallapodeme normal. Virga absent. Superior volsella absent; inferior volsella present as a weakly developed lobe. Gonostylus simple, apically tapering distally, with bifid megaseta and a moderately developed crista dorsalis.

Adult female as male, except:
Antenna five segmented, AR 0.5-0.7. Clypeus rectangular. Claws simple, pointed.
Genitalia (Fig 2). Tergite IX narrow and undifferentiated, without protruding gonocoxite IX lobes, and seemingly without strong setae. Gonapophysis VIII divided, with small anterior dorsomesal lobe and large rectangular ventrolateral lobe, with hyaline curved apodeme lobe. Two pigmented, subovoid spermathecae taper to recurved seminal ducts that end in separate subterminal bulbs opening into vagina. Notum hyaline, narrow, extending to near anterior of spermathecae. Labia hyaline. Tergite X narrow with posteriorly protruding corners and strong 'anal point'. Cerci squat, microtrichiose.

Pupa
Small, 1.5-2.8 mm long. Exuviae pale brown.

Cephalothorax. Cephalic tubercles and frontal warts absent. Short frontal setae on a weakly creased tubercle almost on prefrons (Fig 3a), or absent. Antennal sheath smooth. Ocular field with two postorbitals.

Antepronotum with one simple, median and one similar lateral antepronotal. Thoracic horn small, globular, hyaline (Fig 3b), or apparently absent, perhaps easily lost. Precorneals non-taeniate, arranged in triangle (Fig 3b). Dorsocentrals dc_1 near to dc_2, separated from dc_3, dc_3 near or at some distance from dc_4, almost aligned. One prealar present (Fig 3c). Anterior thorax weakly rugulose. Wing sheath bare, without pearls.

Abdomen (Fig 4a-d). Tergites either bare anterior to posterior transverse spine rows, in *B. andinus*, with anteromedian patch of short spinules on TII-VIII, more extensive posteriorly in *B. limay*. Tergite II without hookrow. Each successive tergite from TIII posteriorly with transverse uniserial spine row. Conjunctives of TII-IV (in *B. limay*) or TIII-IV (in *B. andinus*) with transverse uniserial row of anteriorly-directed hooklets. Sternite I bare, SII-VII with anteromedian patch of spinules, SVII with uniserial transverse band of spines. Conjunctives of SIV (sometimes), V and VI (always) with posterior transverse uniserial band of hooklets. Pedes spurii A and B absent.

Abdominal setation with three (TI), five (TII-VII) or one (TVIII) D setae, five fine and short V setae; L setae fine, all non-taeniate.

Anal lobe squat, bare, with three macrosetae each subequal in length to anal lobe (*B. andinus*), or two macrosetae with one longer, one fine and shorter (*B. limay*). Male genital sac rounded, extending posteriorly well beyond anal lobe, female squat.

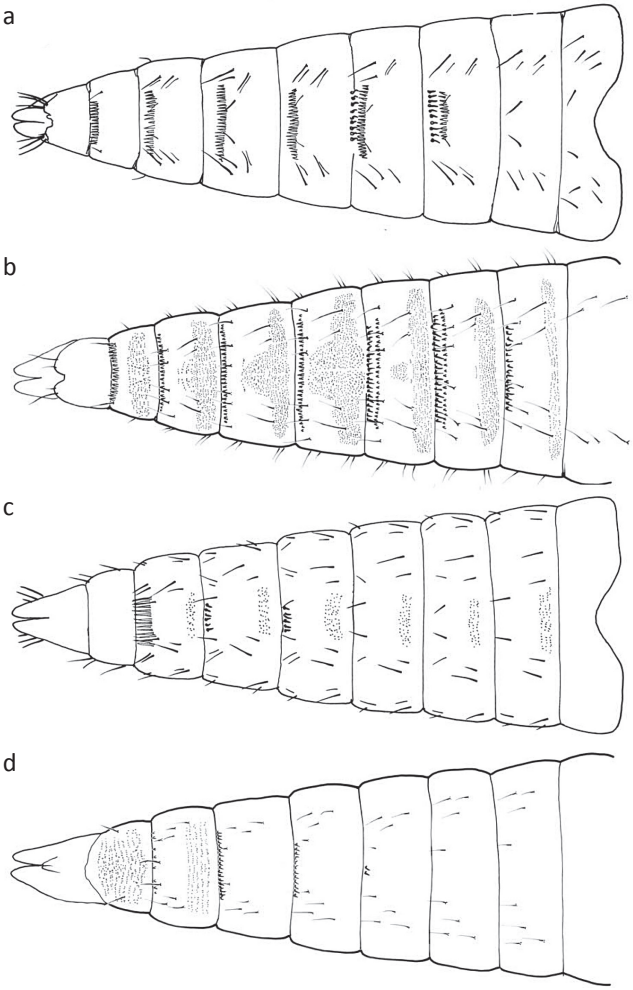


Fig 4 *Barbadocladius* spp., pupal abdomens. a) Tergites, *Barbadocladius andinus* sp. n.; b) Tergites, *Barbadocladius limay* sp. n.; c) Sternites, *B. andinus* sp. n.; d) Sternites, *B. limay* sp. n.

Larva

Body length 2.5-3.5 mm, Head capsule brown, mentum and mandibles darker brown, length c. 290-355 µm. Dorsal head with frontal apotome with S3 lying in parallel-sided anterior section (Fig 5a), immediately posterior to transverse suture, with single anterior smooth sclerite bearing S1 and S2.

Antenna (Figs 5b, 6a) with five segments; segments III-V subequal in length, no fine 6th. Antennal ratio about 1.0. Ring organ large, in basal quarter. Blade short extending to apex of 2nd segment, with short accessory blade. Lauterborn organs broad, extending to apex of segment III; style subequal to antennal segment III.

Labrum (Fig 6b) with SI bifid, SII simple, lanceolate, SIII simple, strong, S IV small. Labral lamella absent. Chaetae all simple, short. Pecten epipharyngis consisting of three simple triangular spines. One (or two) chaetula lateralis spatulate, pectinate; other chaetulae simple. Premandible with one large broad apical tooth, with short, sparse brush.

Mandible (Fig 6c) with apical tooth shorter than first of four inner teeth, subequal to next inner tooth. Innermost (4th) tooth well delimited from mola. Seta subdentalis a tapering spine. Seta interna a weak brush of four-five finely serrate branches.

Mentum (Figs 5c, 6d) with broad flat median tooth, with five pairs of lateral teeth decreasing on even slope. Ventromental plate seemingly absent, or obscured by very well developed beard of many setae extending at least to margin of head. Setae submenti (SSm) posterior to base of mentum, broad at base, tapering strongly. Cephalic setae S9 and S10 (genal) approximated, in near dorso-ventral alignment with large ventral (VP) and dorsal pit (DP) plus supraorbital S7 and frontal seta S5.

Maxilla (Fig 6e) squat, without pecten galearis. Lacinial chaetae simple, appendix differentiated, strongly plumose on one side only.

Body setae short, simple. Anterior parapods long (250-280 µm), with many golden hooklets on midsection grading into crown of highly serrate, brown claws (Fig 5d). Procercus absent, 4-5 setae (120-135µm long) a little longer than anal tubules, arise on posterior margin of segment, with two weak setae arising at a short distance. Posterior parapods simple, numerous, dark brown. Supra-anal setae absent. Anal tubules (80-100 µm) apparently subequal to posterior parapod in length.

Keys to species

Adult

1. Antenna cylindrical, Antennal ratio *ca*. 0.35. Inferior volsella rectangular (Fig 1a) ..... *B. andinus*
- Antenna apically bilobed. Antennal ratio *ca*. 0.25. Inferior volsella bulbous (Fig 1c) ..... *B. limay*

Pupa

1. Hooklets present only on conjunctives of TIII and IV, SV: TII and SIV lack hooklets (Figs 4a, c). Tergal spines longer, narrow (Fig 4a). Anal lobe macroseta III, strong (Fig 4a)..... *B. andinus*
- Hooklets present on conjunctives of TII-IV and SIV and V (Figs 4b, d). Tergal spines stout and coarse (Fig 4b). Anal lobe macrosetae II, weak (Fig 4b) ..... *B. limay*

The larvae cannot yet be distinguished (see below)

Barbadocladius andinus *Cranston & Krosch, sp. n. (Figs 1a, 2, 3a-c, 4a,c, 5a-e, 6a-d)*

Description (mostly mensural to complement generic diagnosis and key)

Adult ♂ (n = 2, largely teneral). Head with two postorbitals, 6-7 clypeals. Antennal segments length: 43-50, 20-25, 25, 35-38, 40-43, 45-47, 50, 47-48, 47-50, 42-50, 50, 50, 172-

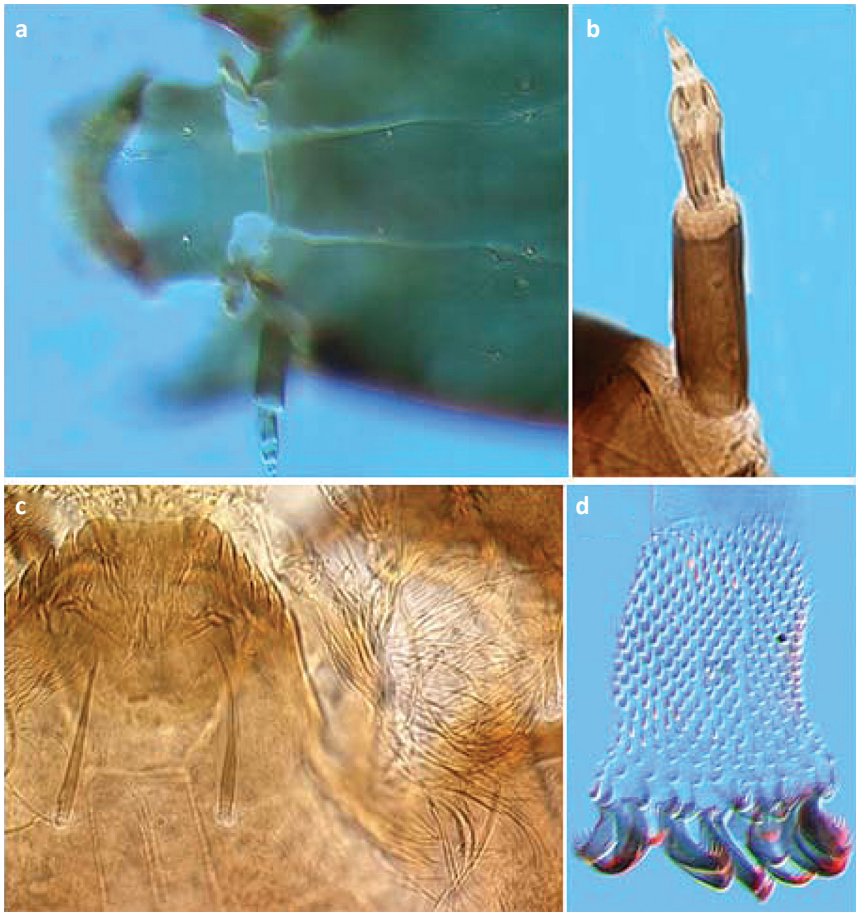


Fig 5 *Barbadocladius andinus* sp. n. larva, photographs. a) Dorsal head; b) Antenna; c) Mentum; d) Anterior parapod.

187. Antennal ratio 0.34-0.36. Palp (teneral, n = 1, segments II-V): 75, 45, 38, 50. Thorax with two antepronotals, three dorsocentrals, 2-3 prealars, four scutellars. Legs (teneral, n = 1): P1 LR (leg ratio) 70:105 – 0.66. Lengths of ta_4: ta_5 P1 25:27, P2 27-29, P3 33:33. All claws simple. Hypopygium with anal point 28-35 long, with 7-8 flanking setae (total). Lengths of gonocoxite 157-170, gonostylus 70-75.

Adult ♀ (n = 3, largely teneral). Head with one postorbital, four clypeals. Antenna segment lengths (in µm) 29-40, 13-15, 11-12. 20-21. Antennal ratio 0.5-0.7. Thorax with one antepronotal, four dorsocentrals (dc_4 retracted), three prealars, four scutellars. Legs (teneral): P1 LR (leg ratio) 80:150 – 0.53. Lengths of ta_4: ta_5: P1 21:27, P2 20:25, P3 21:28. All claws simple. Spermathecal dimensions 62-70 x 55-62; cerci dimensions 62-87 x 25-38.

Pupa (n = 10). Total length 2.0-2.8 mm. Tergite III with 22-30 spines, 7-17 hooklets; TIV with 19-36 spines, 8-16 hooklets; TV with 23-35 spines, TVI with 19-38 spines, TVII with 17-29 spines, TVIII with 10-18 spines. Sternite V with 0-6 hooklets, VI with 6-16 hooklets, SVII with 10-15 spines. Anal lobe MS 70-98 um.

Larva (n = 3 unless stated otherwise). Head length 295 (n = 1), postmentum 102-110, mandible length 76-85, mentum width 53-60, seta submentum 31-31.

Antennal segment lengths (in µm, n = 2) 32-33, 10-11, 5, 3, 3. Antennal ratio 1.50-1.52. Blade length 19-20, subsidiary blade 10. Ring organ 5-7 from base.

Type material. Holotype P♂. Slide mounted in Euparal: Chile: P.N. Puyehue, sector Anticura, Rio Gol Gol, 40°39'36"S 72°10'05" W, 348 m, 19-20.ii.2006, P.S. Cranston leg. (MNNC)

Paratypes (all leg. P.S. Cranston). 3Pe, as holotype (ZSM); Chile: IX Region: P♂, P♀, 6Pe (on two slides), P.N. Villarrica, Rio Palquin, 39°27'18"S 71°48'41"W, 826 m,14.ii.2006; L (P), 12Pe (1 slide) P.N. Puyehue, sector Aguas Calientes, Rio Chanleufu, 40°44'11"S 72°18'26" W, 450 m, 20.ii.2006, (MV-CH06-17M1) (ANIC). Argentina, Neuquen: 2 L(P), 2P♂, P♀, 18Pe (three slides) Arroyo Partida, 40°14'S 71°22'W, 1185 m, 8.x.2007; P♂, P♀, 8Pe, Puerto Blest, Arroyo Blest, 41°01'S 71°50'W, 2.i.1997; Pe, Puerto Alegre, Lago Frias, 41°02'S 71°49'W, 3.i.1997; Lago Nahuel Huapi, trib. N. shore, 41°01'S 71°50W, 1.i.1997.

Etymology. Named for the distribution of this species in

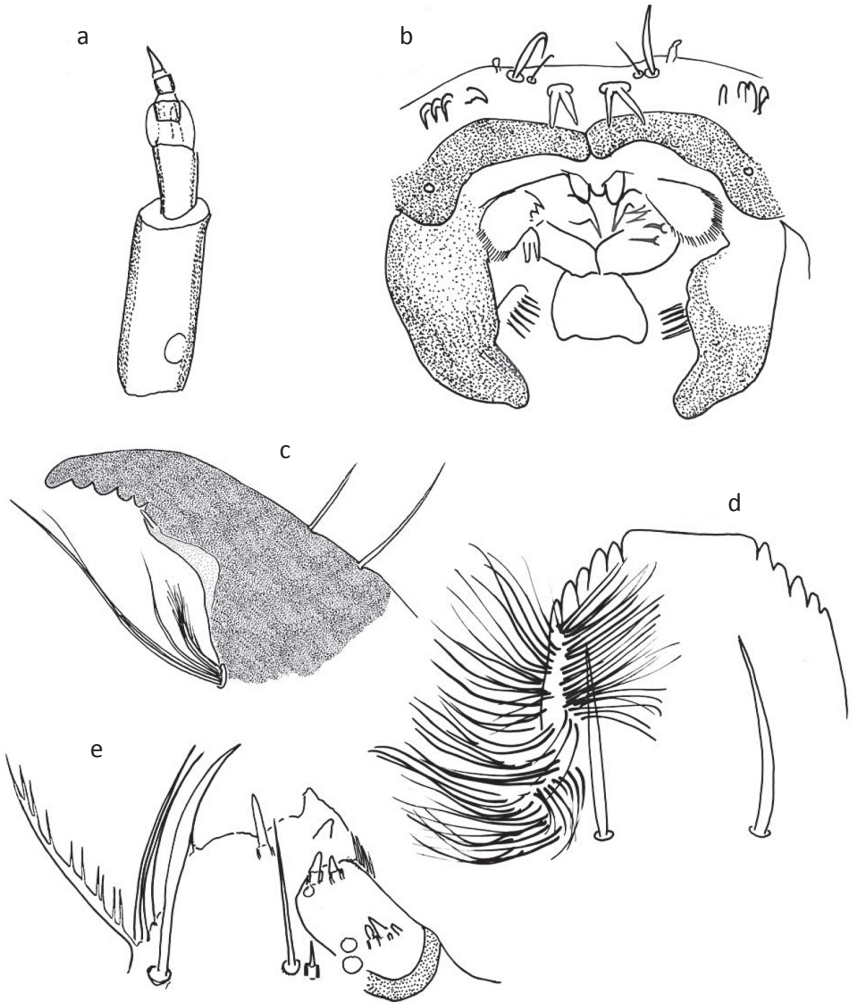


Fig 6 *Barbadocladius andinus* sp. n. larva. a) antenna; b) labrum; c) mandible; d) mentum (beard of left side only shown); e) maxilla.

the Andes [from Patagonia to Bolivia, (Roback & Coffman 1983)], more widespread than its congener.

Barbadocladius limay *Cranston & Krosch, sp. n.* *(Figs 1b,c, 4b,d)*

Description (mostly mensural to complement generic diagnosis and key)

Adult ♂ (n = 1). Head with one postorbitals, seven clypeals. Antennal segments length: 27; 18; 22; 35; 38; 38; 38; 38; 38; 32; 30; 30; 90. Antennal Ratio 0.24. Thorax with no antepronotal, 4-5 dorsocentrals (dc_4 retracted), three prealars, four scutellars. Hypopygium with anal point 28 long, with two flanking setae each side. Lengths of gonocoxite 87, gonostylus 47.

Pupa. Total length 1.5-1.9 mm. Tergite II with 18-20 hooklets, III with 19-28 spines, 17-22 hooklets; TIV with 20-32 spines, 15-21 hooklets; TV with 23-41 spines, TVI with 20-39 spines, TVII with 21-33 spines, TVIII with 14-18 spines. Sternite IV with 0-2 hooklets, SV with 10-20 hooklets, SVI with 18-23 hooklets, SVII with 3-4 spines.

Adult female and larva. Unknown

Type material. Holotype P♂ Chile: IX Region, P.N. Vicente Perez Rosales, Petrohue, S. shore, 41°08'38"S 72°24'07" W, 260-280 m,16.ii.2006, P.S. Cranston (#6) (MNNC).

Paratypes (all leg. P.S. Cranston). 2Pe, Chile IX Region, P.N. Vicente Perez Rosales, Peulla, above hotel, 41°05'12"S 72°01'16" W, 260-280 m,17-18.ii.2006; 2Pe, P.N. Villarrica, Rio Palquin, 39°27'18"S 71°48'41"W, 826 m,14.ii.2006; 2Pe, X Region, P.N. Alerce Andino, sector Correntoso, Rio Chamiza, 41°08'38"S 72°24'07" W, 114 m, 19.ii.2006. 2Pe, Argentina, Puerto Blest, Arroyo Blest, 41°01'S 71°50W, 2.i.1997.

Etymology. Named for the distribution of this species in pristine streams in the region of the Mapuche – *limay* meaning 'clear water / translucency / clarity' in Mapudungan (Mapuche).

Notes on larvae. Three larvae associated with the pupa of *B. andinus* are described above and illustrated. No larva could be associated unambiguously with *B. limay*. Sites at which *B. limay* pupae and unassociated larvae of species of *Barbadocladius* were present (Arroyo Blest; Rio Palquin), either had both species present as pupae or, if *B. limay* pupae were present alone (Peulla, above hotel; Rio Chamiza), no larvae of *Barbadocladius* were collected.

Although the pupal exuviae of *B. andinus* are larger than those of *B. limay*, measurements of larvae do not segregate into two size categories.

The two molecular vouchers are both unreared larvae (ARb23 - Arroyo Quilanlahue; CH062M2 – Rio Palquin). These larvae are larger than the associated larvae of *B. andinus* (head length 360-380 µm, postmentum length 125-126, mentum 61-65, mandible 85-88) and perhaps may be distinguished from each other by the lengths of the first antennal segment (ARb23 - 31µm, CH062M2 38-40 µm) although the AR does not differ (1.76-1.85). At present, which (if either) of these two vouchers represents *B. andinus* cannot be determined.

Distribution details of unallocated larvae: 3L (inc. (MV-CH06-2M2) Chile, IX Region, P.N. Villarrica, Rio Palquin, 39°27'18"S 71°48'41"W, 826 m,14.ii.2006 (MVCH06-2M2); 2L, Argentina, Neuquen, Puerto Blest, Arroyo Blest, 41°01'S 71°50'W, 2.i.1997; 6L, Arroyo Partida, 40°14'S 71°22'W, 1185 m, 8.x.2007; L, (MV-ARb23) Arroyo Quilanlahue 40°09'S 71°33'W 660 m asl.

Discussion

When first collected in the 1980s this larval taxon (as 'Genus 9 sp.') was said to be reminiscent of *Synorthocladius* (Thienemann) because of the dense beard (Roback & Coffman, 1983), but the authors dismissed this on other grounds. In supposing that the single pupal exuviae ('Genus 4 sp.') from the same site was associated, they argued it belonged to Cardiocladiini. However, they expressed doubts about the potential placement in suggesting also a connection to Orthocladiini. These tribes, which generally have not been adopted widely, were based on northern hemisphere taxa, and erected without rigorous analyses. Roback & Coffman's brief discussion does indicate that the larva and pupa present a chimera of features that precludes allocation on the basis of morphology to any extant genus, and with little indication of relationships.

In a project designed to examine the evolution of Gondwanan Orthocladinae – that is, taxa purported to show distributions largely among the southern continents (especially Australia, New Zealand and South America) - collections of larvae and pupae made on these landmasses were sorted and many sequenced for their DNA by the junior author. Only two specimens of this new taxon (termed 'Eukiefferiella hooklets' because of this pupal feature that initially resembled the genus *Eukiefferiella* Thienemann) were included in analyses. Although their abundance and distribution was too low to allow population genetic study, the genus could be located in a broader phylogenetic estimate of the Orthocladiinae (Krosch *et al* 2011)

The resultant molecular phylogenetic hypothesis placed *Barbadocladius* as sister to a large and diverse clade estimated to comprise taxa largely of 'Gondwanan' distribution, with moderately high statistical support (Bayesian (B) posterior probability 0.94 and maximum likelihood (ML) bootstrap support was 65.) (Krosch *et al* 2011). Taxa within this 'Gondwanan' sister clade include a speciose monophyletic Australian-S.American *Botryocladius* Cranston & Edward (1999) sister to a well supported New Zealand genus *Naonella* Boothroyd (1994), including *Tonnoirocladius* Cranston (2007). In turn this is related as sister to several clusters of varying taxonomic clarity from Chile, Australia and New Zealand. All show relationships and some morphological resemblance to the included Australian *Echinocladius* Cranston (2000) and recently described trans-Tasman *Anzacladius* Cranston (2009). This predominantly 'Gondwanan' clade does include *Limnophyes* Eaton, and perhaps some related taxa also found in the northern hemisphere. However the grouping, including *Barbadocladius*, clearly contains a substantial austral radiation, whose existence and diversity became recognized only relatively recently (Krosch *et al* 2011).

The sister group of this clade is postulated to be the '*Cardiocladius*' group of Saether & Halvorsen (1981) (of which *Cardiocladius* Kieffer, *Eukiefferiella* Thienemann and *Tvetenia* Kieffer were sampled for molecular data). In this clade hooklets are present in some taxa on some pupal conjunctives: our postulated relationships suggest that their presence in *Barbadocladius* either is symplesiomorphic at the node connecting the clades and lost in e.g. *Cardiocladius*, or acquired independently as a result of convergence. The presence of hooklets in pupae of the distantly related *Thienemannimyia* - *Corynoneura* clade suggests the feature indeed is somewhat homoplasious. However, we are confident that *Barbadocladius* is a new taxon that cannot be embedded in an existing group, including any of the '*Cardiocladius*' group.

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