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The higher phylogeny of Leptophlebiidae (Insecta: Ephemeroptera), with description of a new species of *Calliarcys* Eaton, 1881

Roman J. Godunko *,[1,2], Pavel Sroka[2], Tomáš Soldán[2] & Jindřiška Bojková[3]

[1] State Museum of Natural History, National Academy of Sciences of Ukraine, Teatralna 18, 79008 Lviv, Ukraine; Roman J. Godunko* [godunko@seznam.cz] — [2] Institute of Entomology, Biology Centre, Czech Academy of Sciences, Branišovská 31, 37005 České Budějovice, Czech Republic; Pavel Sroka [pavel.sroka@centrum.cz]; Tomáš Soldán [soldan@entu.cas.cz] — [3] Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 61137 Brno, Czech Republic; Jindřiška Bojková [bojkova@centrum.cz] — * Corresponding author

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Abstract

A detailed morphological description of the adults and larvae of *Calliarcys van* **sp.n.**, the second representative of the genus *Calliarcys* Eaton, 1881 is provided, including a differential diagnosis with regard to *Calliarcys humilis* Eaton, 1881. *C. van* **sp.n.** is distinguished in the adult mainly by the colouration of the upper part of male compound eyes, shape of costal process of hind wings, and genitalia; in larvae it is distinguished by the shape of hypopharynx and labium; and by comparatively densely arranged unbranched longitudinal ridges on the exochorion of the fusiform eggs. The generic diagnosis for *Calliarcys* is re-evaluated and specified. The genus had already previously been proposed to constitute the subfamily Calliarcyinae within Leptophlebiidae, a concept which has not been generally accepted. Now this classification is tested by phylogenetic analysis, for the first time using a set of 20 morphological characters. Ambiguous characters from previous studies are discussed. Leptophlebiinae is confirmed as sister group to all remaining Leptophlebiidae. *Calliarcys* is revealed as sistergroup of Habrophlebiinae + Atalophlebiinae s.l., thus justifying its classification as a subfamily Calliarcyinae.

Key words

Calliarcyinae, systematics, phylogeny, Asia Minor.

1. Introduction

The family Leptophlebiidae is among the most diverse groups of mayflies, with approximately 640 species and many genera (131 reported in 2005: Barber-James et al. 2008). The family is widely accepted as monophyletic (Ogden et al. 2009). The first attempt to establish a higher classification of Leptophlebiidae was published by Peters (1980), who defined two subfamilies, Leptophlebiinae and Atalophlebiinae. Kluge (1994) further divided Leptophlebiinae into Leptophlebiinae s.str. and Habrophlebiinae. The recent molecular analysis by O'Donnell & Jockusch (2008) did not support this classification; Habrophlebiinae and Leptophlebiinae were supported, whereas Atalophlebiinae was rendered paraphyletic. Nevertheless, O'Donnell & Jockusch (2008) used only two molecular markers (H3 and 28S) and did not even found Leptophlebiidae to be monophyletic, so the results should be taken with care. Furthermore, the monophyly of Atalophlebiinae is corroborated by a strong apomorphy among all insects, which is the presence of square ommatidia (Peters & Gillies 1995).

Kluge (2009) established 3 additional subfamilies (Terpidinae, Castanophlebiinae and Calliarcyinae) based on morphological characters. Terpidinae and Castanophlebiinae consist of only 15 species previously assigned to Atalophlebiinae. Here we refer to the complex Terpidinae + Castanophlebiinae + Atalophlebiinae s.str. as Atalophlebiinae s.l. (see Fig. 55). However, some authors retained Kluge's (1994) concept of three subfamilies (e.g. Bauernfeind & Soldán 2012) and did not reflect the classification of subfamilies proposed by Kluge (2009). In the concept of Bauernfeind & Soldán (2012), Terpidinae and Castanophlebiinae were included in Atalophlebiinae whereas Calliarcyinae (including only *Calliarcys*) remained within Leptophlebiinae. However, Bauernfeind & Soldán (2012) considered this placement of *Calliarcys* provisional, since *Calliarcys* shares some characters with Habrophlebiinae.

The proposed subfamily Calliarcyinae contains only *Calliarcys* Eaton, 1881. The first description of this genus with the type species *Calliarcys humilis* Eaton, 1881 was published based on males and females collected on the slopes of the Foia Mt., near Monchique town, Portugal (Eaton 1881: 21). Eaton (1884) considerably extended the description of males and females, and published drawings of the wings with venation details (including the structure of cubital field of forewings), genitalia and the proportions of legs for both sexes (Eaton 1884: 121 – 123, pl. XIV, fig. 23). Besides the material from the type locality, another collection site was mentioned "in the Estrella [*Serra da Estrela mountain range*], on a hill south of Sabugueiro [*town*]….; in streams [*within Alva river-basin*]", in the Distrito Guarda, Portugal (Eaton 1884). In a brief study on mayflies of Portugal, Eaton (1887: 5) again enumerated all collecting sites of adults of *C. humilis* known to him.

Further fragmentary information on the distribution of *C. humilis* and distinguishing imaginal characters was published early in the 20th century (Jakobson & Bianki 1905; Navás 1907; Ulmer 1920). The systematic position of *Calliarcys* was analyzed by Peters (1966, 1979, 1980) and Peters & Edmunds (1970). They presented a diagnosis of the genus based on adults, providing key distinguishing characters for separation from genera they considered as closely related. Kimmins (1960: 296, fig. 42) designated a lectotype for *C. humilis* (male imago) still housed in the collection of the Natural History Museum, London, UK; a few years later the lectotype was listed among the type specimens of this museum (Kimmins 1971: 315). The first description of larvae was published by Peters & DaTerra (1974), based on extensive material partly collected at A.E. Eaton's localities. It also include an analysis of phylogenetic relationships and detailed data on the biology of *C. humilis* and its distribution in Portugal. Further data on the distribution and ecology of the species in the Iberian Peninsula based on material from Catalonia, Castile and León, and Galicia were given by Alba-Tercedor (1981, 1983), Del Tánago (1984) and Palau & Puig (1992).

Finally, Bauernfeind & Soldán (2012) summarized available information on taxonomy, distribution and biology of *C. humilis*, figured and described the eggs for the first time and mentioned a paralectotype belonging to Eaton's type series and housed in the collection of the Museum of Comparative Zoology, Harvard University, Cambridge, USA. They also pointed out the existence of another undescribed species of *Calliarcys* occurring in NE Greece and W Turkey. In the present study, we provide the description of this new species, based on larvae and adults from Turkey.

The systematic position of the several fossil taxa presumably belonging to Leptophlebiidae is mostly unclear (cf. Kluge 2004). Demoulin (1965) established the genus *Oligophlebia* Demoulin, 1965 for two species from Eocene Baltic amber based on male adults and left another two specimens (fragments of male imago and subimaginal exuvia) unnamed. He pointed out the close relationships of *Oligophlebia* and *Calliarcys* due to similar forewing venation and genitalia. Demoulin (1970) synonymised *Oligophlebia* and *Paraleptophlebia*, comparing wing venation and genitalia of the type specimens of *O. calliarcys* Demoulin, 1965 and *P. prisca* Pictet-Baraban & Hagen, 1856. Peters (1979) discussed the possible position of *Oligophlebia* within "daughter line I", containing *Paraleptophlebia* and *Leptophlebia* (line IA1 according to Peters & Edmunds 1970), based on characters published by G. Demoulin, but without study of the type material of either fossil *Oligophlebia* species.

As mentioned above, the subfamily Calliarcyinae has so far not been generally accepted (Bauernfeind & Soldán 2012). Despite several Leptophlebiidae classifications, no formal cladistic analysis based on morphological data has been performed so far, and *Calliarcys* has not yet been included in molecular phylogenetic studies. In order to analyze the subfamilial phylogeny, we summarized the diagnostic characters of all previously proposed subfamilies (i.e. Atalophlebiinae s.str., Terpidinae, Castanophlebiinae, Habrophlebiinae, Calliarcyinae and Leptophlebiinae) from all relevant literature sources (Peters 1980, 1997; Peters & Gillies 1995, Kluge 1994, 2009; Bauernfeind & Soldán 2012). The monophyly of individual groups is tested using maximum parsimony (MP) and the character distribution within the tree is specified.

This phylogenetic analysis enables the discussion of the higher classification of Leptophlebiidae with emphasis on the systematic affinities of *Calliarcys* and the justifiability of a separate subfamily Calliarcyinae sensu Kluge (2009). Therefore, in this study we aimed to **(i)** describe the adults, larvae and eggs of *Calliarcys van* **sp.n.** and compare them with *C. humilis*; **(ii)** revise the generic diagnosis of *Calliarcys*; **(iii)** analyze the systematic position of the genus *Calliarcys*; and **(iv)** discuss the subfamilial diagnostic characters used for Leptophlebiidae and the justifiability of the subfamily Calliarcyinae within Leptophlebiidae. We do not discuss in detail the classification of the extremely species-rich Atalophlebiinae s.l., which will require a separate comprehensive treatment. This study also does not aim at corroborating the monophyly of Leptophlebiidae, which is widely accepted (Ogden et al. 2009; Bauernfeind & Soldán 2012). Thus, the apomorphies of all Leptophlebiidae as listed e.g. in Kluge (2004) or Bauernfeind & Soldán (2012) were not included in the dataset. Further, in agreement with Peters (1979), we found G. Demoulin's original description and drawings of *Oligophlebia* specimens not sufficient for a comparison with other genera of Leptophlebiidae and conclusions on its systematic position. Moreover, the set of characters used in this study includes a range of adult characters not available for *Oligophlebia*. Therefore, *Oligophlebia* was not included in the phylogenetic analysis in this study.

2. Material and methods

2.1. Material processing

Most specimens of *Calliarcys van* **sp.n.** were collected in the Kavuşşahap Dağları mountain range (Bitlis Province, E Turkey); other material was collected in W Turkey.

All specimens were preserved in 80% ethanol. Some specimens (paratypes) were mounted on slides with Liquide de Faure (soluble in water). Drawings were made using a stereomicroscope Olympus SZX7 with a camera lucida (Olympus SZX-DA) and a microscope Olympus BX41.

Photographs of adults and larvae were taken using a Leica Z16 APO Macroscope and processed with Leica Application Suite™ Version 3.1.8 to obtain combined photographs with suitable depth of field. Photographs were subsequently enhanced with Adobe Photoshop™ CS3. Photographic equipment was provided by the State Museum of Natural History, Stuttgart, Germany.

For scanning electron microscopy, samples were gradually transferred to acetone, critical point dried and coated with gold by sputtering using a Baltec SCD050 Sputter Coater. Observations were taken on the scanning microscope Jeol JSM 7401F at 4 kV in the Laboratory of Electron Microscopy, Institute of Parasitology, Biology Centre, Czech Academy of Sciences.

The following acronyms for scientific collections are used in this paper: **IE CAS** = Institute of Entomology, Biology Centre, Czech Academy of Sciences, České Budějovice; **SMNHL** = State Museum of Natural History, National Academy of Sciences of Ukraine, Lviv; **SMNS** = State Museum of Natural History, Stuttgart; **NMW** = Naturhistorisches Museum Wien.

2.2. Phylogenetic analysis

For the cladistic analysis a matrix of 20 morphological characters was assembled, including 14 larval and 6 adult characters (Table 1). The matrix was compiled according to Peters (1980, 1997), Kluge (1994, 2009), and Bauernfeind & Soldán (2012). Characters that were inconsistently defined in the literature are treated in the Discussion.

Apart from literature sources, adults and larvae of the following species were examined to corroborate character scorings: *Habroleptoides confusa* Sartori & Jacob, 1986 [Czech Republic, unnamed brook near Žloukovice village; 8.iv.2010, leg. T. Soldán]; *Habrophlebia lauta* Eaton, 1884 [Czech Republic, unnamed brook near Nuzice village, 21.v.2012, leg.T. Soldán]; *Leptophlebia marginata* (Linnaeus, 1767) [Czech Republic, pools near Vltava River near Pěkná village, 2.iv.2007, leg. T. Soldán]; *Paraleptophlebia submarginata* (Stephens, 1835) [Czech Republic, Senný potok brook near Drmoul village, 4.iv.2007, leg. T. Soldán]; *Castanophlebia* sp. [South Africa, Lundy Hill, Natal, leg. G.F. Edmunds, 1971]; *Terpides* sp. [Brazil, Pará State, Akahe brook, near Tiriyos Mission, near Brazil-Surinam border, 15.iii.1962, leg. E.J. Fittkau].

Binary characters were coded 0 and 1, multistate characters were assigned different numbers. Character no. 10 was treated as ordered, other characters were viewed as unordered. Cases of non-applicability of characters were scored as "–". Occurrence of several states in a terminal taxon was treated as polymorphic (i.e. no hypotheses on the groundplan states of taxa were made).

The data were analyzed using Paup* 4.0b10 (Swofford 2002) under maximum parsimony (MP). Tree searches were performed using the heuristic search option with tree bisection and reconnection (TBR) branch swapping. Branches collapsed (creating polytomies) if the maximum branch length was zero. A 50% majority rule consensus tree was constructed based on the most-parsimonious trees. Individual branch support was estimated calculating the bootstrap values with 1000 replicates.

The genus *Siphlonurus* (Ephemeroptera, Siphlonuridae) was used as outgroup taxon to root the tree. Autapomorphies with consistency index 1.0 were mapped on the cladogram (Fig. 55).

List of characters

1. Anteromedial emargination of labrum (larva): (0) without denticles (Figs. 50, 51); (1) with denticles (Figs. 52, 53).
2. First row of setae on labrum (larva): (0) present (Figs. 50, 51); (1) absent (Figs. 52, 53). (For a detailed discussion of characters 2–4 see section 4.1.1.)
3. Second row of setae on labrum (larva): (0) irregular, setae not aligned in single row (Figs. 50, 51); (1) regular, setae aligned in single row (Figs. 52, 53).
4. Third row of setae on labrum (larva): (0) absent (Figs. 50, 51, 52); (1) present (Fig. 53).
5. Apicolateral processes of lingua (larva): (0) absent (figs. 196, 197 in Peters & Edmunds 1970); (1) present (figs. 202, 203 in Peters & Edmunds 1970).
6. Apicolateral processes of lingua (larva): (0) not projected laterally (Fig. 22, 23); (1) projected laterally (figs. 202, 203 in Peters & Edmunds 1970).
7. Venter of lingua (larva): (0) with hairy patches (Fig. 23, arrow); (1) without hairy patches.
8. Lobes of superlinguae, shape of flank (larva): (0) not curved and expanded laterally (figs. 196, 197 in Peters & Edmunds 1970); (1) curved and expanded laterally (figs. 198, 199 in Peters & Edmunds 1970).
9. Vestiges of maxillary canines (larva): (0) present (fig. 7 in Kluge 1994); (1) absent (figs. 8, 9 in Kluge 1994).

Table 1. Character states for individual subfamilies of Leptophlebiidae.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Atalophlebiinae s.str.	01	1	01	01	1	1	1	1	1	01	1–	0	0	1	1	1	1	0–	01	01
Castanophlebiinae	1	1	1	0	0	–	1	1	1	1	1	0	0	1	1	1	1	0	0	0
Terpidinae	1	1	1	1	0	–	1	1	1	2	1	0	0	1	1	1	1	0	0	0
Habrophlebiinae	0	0	01	0	0	–	0	1	1	2	1	1	0	1	0	0	0	0	1	0
Calliarcyinae	0	0	0	0	1	0	0	0	1	2	0	1	1	0	1	0	1	0	0	0
Leptophlebiinae	0	0	0	0	0	–	01	0	0	2	0	0	0	0	0	0	1	01	1	0
Outgroup: *Siphlonurus*	0	0	1	0	0	–	1	0	0	3	0	0	0	0	1	0	1	0	0	1

10. Number of maxillary dentisetae (larva): (0) zero; (1) one (fig. 9 in Kluge 1994); (2) two (fig. 7, 8 in Kluge 1994); (3) three (fig. 3E in Kluge 2004).
11. Proximal dentiseta of maxilla (larva): (0) not comb-like (fig. 7 in Kluge 1994); (1) comb-like (figs. 8, 9 in Kluge 1994).
12. A pair of bulbous lobes ventrally on mentum (larva): (0) absent; (1) present (Fig. 26, arrow).
13. Dense long hair-like setae along foreleg tarsi: (0) absent; (1) present (Fig. 33).
14. Tibio-patellar suture on middle legs (larva): (0) present (Fig. 34, arrow); (1) absent.
15. Posterior margin of ventral forceps base (adult, male): (0) deeply concave medially (fig. 72 in Peters & Edmunds 1970); (1) entire (fig. 90 in Peters & Edmunds 1970).
16. Facets of compound eyes (adult, male): (0) hexagonal; (1) square.
17. Terminal and subterminal segments of forceps (adult, male): (0) subequal in length to first segment (fig. 72 in Peters & Edmunds 1970); (1) shorter than first segment (fig. 74 in Peters & Edmunds 1970).
18. Costal process of hind wing (adult): (0) present (Fig. 9, arrow); (1) absent.
19. Apical blade-like process of penis lobes (adult, male): (0) absent (fig. 89 in Peters & Edmunds 1970); (1) present (fig. 73 in Peters & Edmunds 1970).
20. Subanal plate (adult, female): (0) apically deeply cleft (fig. 131 in Peters & Edmunds 1970); (1) entire (fig. 140 in Peters & Edmunds 1970).

3. Taxonomy

3.1. *Calliarcys* Eaton, 1881

Calliarcys Eaton, 1881: *Entomol. Mon. Mag.* 18: 21

Type species: *Calliarcys humilis* Eaton, 1881; ibid.: 21 [original designation]

Included species: *Calliarcys humilis* Eaton, 1881; *Calliarcys van* **sp.n.**

***Calliarcys* Eaton, 1881:** Eaton 1883–1888: 1884, *Trans. Linn. Soc. London* 2(3): 121 [redescription]; Jakobson & Bianki 1905: *Priamokryl. Lozhno.Ros. Imp. Sopr. Stran*: 868 [adult characters; key]; Navás 1907: *Brotéria: Ser. Zool.*: 64, 68 [adult characters; key]; Lestage 1917: *Ann. Biol. Lacustr*. 8(3 – 4): 350 [listed]; Ulmer 1920: *Stettiner Entomol. Zeit*. 81: 114 [adult characters; key]; Edmund & Traver 1954: *Proc. Entomol. Soc. Wash*. 56(5): 238 [systematics; within Leptophlebiinae]; Peters 1966: *Ph.D. thesis*: 61 [adult characters]; Peters & Edmunds 1970: *Pacific Insects* 12(1): 184 [phylogeny, revision]; Peters & DaTerra 1974: *Ciên. Biol. (Portugal)* 1(3): 61 [nymphal characters, description]; Peters 1979: *Proc. Second Inter. Conf. Ephemer.*: 52, 54 [phylogeny]; Peters 1980: *Advan. Ephemer. Biol.*: 38 [systematics; within Leptophlebiinae]; Kluge 2009: *Russ. Entomol. Jour.* 18(4): 244 [systematics; new subfamily Calliarcyinae established]; Bauernfeind & Soldán 2012: *The Mayflies of Europe (Ephemeroptera)*: 419 [taxonomy, generic diagnosis].

Diagnosis. *Calliarcys* differs from other mayfly genera by the following combination of features: ***Adults***: **(a)** two pairs of connected intercalary veins in cubital field of forewing; **(b)** costal process of hind wing relatively developed, acute, or bluntly pointed apically; **(c)** penis lobes straight, simple and tubular, fused near their base; **(d)** tip of penis ± bent inwardly; **(e)** apical blade-like process of penis lobes absent; **(f)** terminal and subterminal segments of forceps shorter than first segment; **(g)** basal segment of forceps slightly tapering apically, without inner process or appendages; **(h)** posterior margin of ventral forceps base deeply concave medially, with V-shaped medial incision, and with two long submedian projections, apically rounded and directed caudally. ***Larvae***: **(i)** lingua of hypopharynx concave apically, with short and rounded apicolateral processes, not projected laterally; **(j)** superlinguae of hypopharynx with strongly curved, broadly rounded or acute outer margin; **(k)** pair of bulbous lobes covered with long hair-like bristles ventrally on mentum; **(l)** foreleg tarsi covered with dense long hair-like setae almost along their entire length; **(m)** gills 1 – 7 alike, slender, deeply forked to at least mid-length. ***Eggs***: **(n)** elongate, fusiform or oval; **(o)** chorionic surface with numerous unbranched and massive longitudinal ridges.

Figs. 1–3. *Calliarcys van* **sp.n.**: male and female imagines, paratypes. **1**: General view of male imago (laterally). **2**:Abdomen of male imago (dorsally). **3**: General view of female imago (laterally). Scale lines = 1 mm.

Comments on diagnostic characters. Distinguishing features in adults (most of them applying to the male imago) were published in the revision of Leptophlebiidae of the Eastern Hemisphere (Peters & Edmunds 1970: 185). The diagnosis of mature larva of *C. humilis* was published by Peters & DaTerra (1974: 61, 63). A set of diagnostic characteristics for *Calliarcys* was published also by Kluge (2004, 2009; 2015: http://www.insecta.bio.spbu.ru/) and Bauernfeind & Soldán (2012: 419). The new species described below enabled us to complete and clarify the generic diagnosis. Therefore, we summarize diagnostic characters and discuss their phylogenetic significance.

Several of the above-mentioned characters were previously considered autapomorphies of *Calliarcys*: character (i) according to Kluge (2015: http://www.insecta.bio.spbu.ru/) and characters (h), (i), (k), and (l) according to Bauernfeind & Soldán (2012).

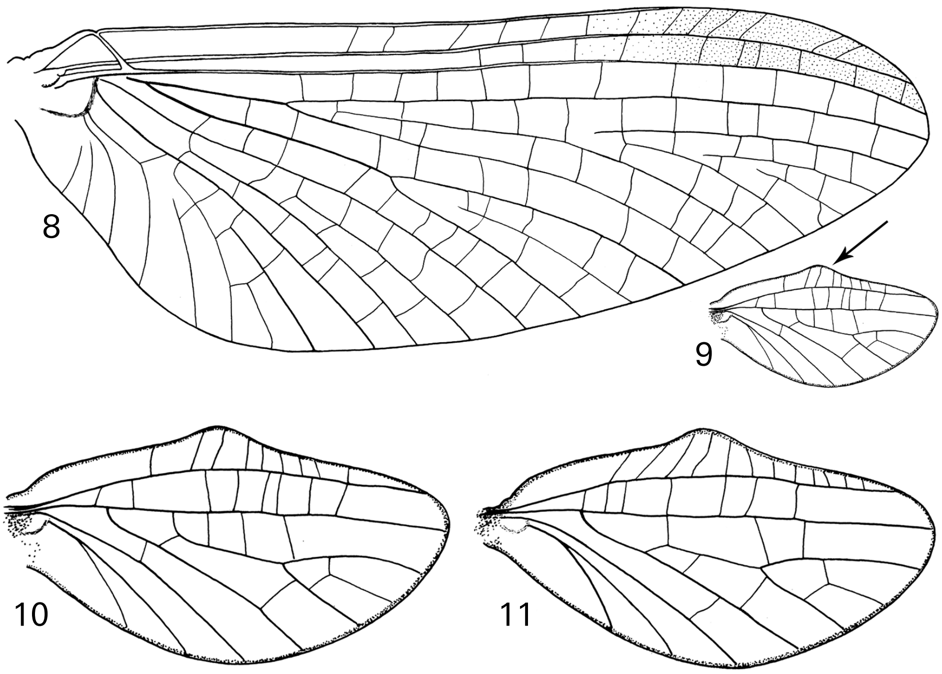
Figs. 4–7. *Calliarcys van* **sp.n.**: male imago, paratype. **4**: Head and anterior part of thorax (dorsally). **5**: Head and anterior part of thorax (laterally). **6**: Genitalia (ventrally). **7**: Tip of abdomen (laterally). Scale lines: 200 µm. [Plate with higher resolution in supplement.]

However, our data showed that only characters (i) and (l) are truly unique among Leptophlebiidae. Character (i), i.e. "lingua of hypopharynx concave apically, with short and rounded apicolateral processes", does not concern the presence of true apicolateral processes (such processes occur in all Atalophlebiinae s.str.), but their shape and position. Processes in *Calliarcys* are not extended laterally from the main body of lingua (as in Atalophlebiinae s.str.); but are short, rounded, and closely connected to the lingua instead. Character (l), i.e. "foreleg tarsi covered with dense long hair-like setae almost along their entire length" is also unique within Leptophlebiidae. The presence of long hair-like setae on forelegs usually represents an adaptation for filter feeding, acquired in several not related mayfly taxa (e.g. Caenidae genus *Barnardara* McCafferty & Provonsha, 1995).

An autapomorphic condition in *Calliarcys* is highly unlikely for characters (h) and (k). Regarding character (h), i.e. "forceps base deeply concave medially, V-shaped incised, with two rounded submedian processes of forceps base extending posteriorly", similar incision and processes of the forceps base occur in several Leptophlebiidae genera, e.g. *Paraleptophlebia* (see Bauernfeind & Soldán 2012: fig. 318a). Concerning character (k), i.e. "pair of bulbous lobes covered with long hairs on mentum ventrally", similar bulbous structures occur also in Habrophlebiinae (well visible e.g. in *Habroleptoides confusa*).

Distribution. Previously known as a monospecific genus endemic to the Iberian Peninsula. Bauernfeind & Soldán (2012: 419) considered it a Westpalaearctic genus. The finding and description of the new species from Turkey confirms the possibility of Paleomediterranean origin of the genus *Calliarcys*.

Biology. Information about the ecological requirements of the genus *Calliarcys* is still incomplete. Larvae inhabit



Figs. 8–11. *Calliarcys van* **sp.n.**: male imago, paratype. **8**: Right forewing (dorsally). **9**: Right hind wing, proportionally to Fig. 8 (dorsally). **10–11**: Left (10) and right (11) hind wings, details (dorsally).

streams and rivers with heterogeneous flow conditions and substratum dominated by stones and coarse and fine gravel. So far larvae and adults were collected between 150 and 1750 m a.s.l.

3.2. *Calliarcys van* sp.n. Godunko & Bauernfeind

Figs. 1–46

Diagnosis. ***Male imago***: upper part of compound eyes brick-red; costal process of hind wings smoothly rounded; penis lobes simple club-shaped, apex only slightly bent inwardly, with obliquely truncate inner margin. ***Larva***: superlinguae of hypopharynx with rounded outer margins; a pair of triangular processes on mentum dorsally; posterior margin of abdominal terga I–VI with a row of grouped spines. ***Egg***: elongate, fusiform, with equatorial area distinctly widest.

Description. ***Male imago***: Measurements: body length 7–9 mm; forewing length 6–8 mm; cercus length 8–9 mm. Maximum forewing width 0.4 × maximum length.

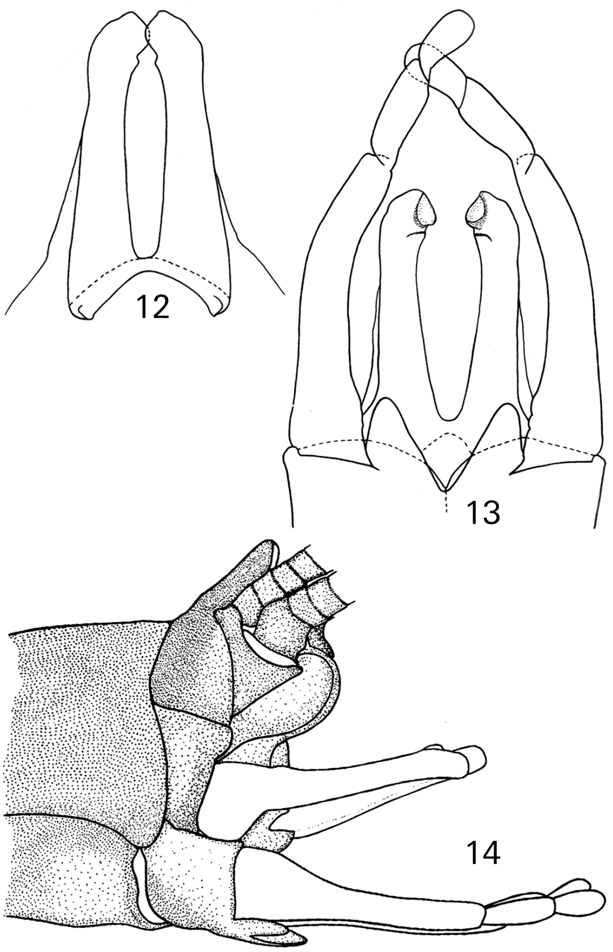
Head. General colour brown to dark brown. Clypeus and antennal bases paler, light brown. Antennae brown; flagellum paler than scape and pedicel. Ocelli whitish-grey to greyish-yellow apically, light brown to brownish-black basally. Upper portion of compound eyes brick-red (after two years of preservation greyish-brown to dirty brown); lower portion black (later: dark gray); facets of compound eyes hexagonal (Figs. 1, 4, 5). Compound eyes well separated, distance between eyes slightly less than base of central ocellus (Fig. 4).

Thorax. General colour dark, intensively brown to black (Figs. 4, 5). Blackish maculation near scutal protuberances. Pleurae intensively brown. Ventral side of thorax paler than dorsal side, brownish.

Forewings hyaline, translucent, slightly frosted-white (Fig. 1); longitudinal venation greyish-brown proximally, yellowish to whitish distally, hardly visible at least in distal 2/3; veins C and Sc slightly darker and visible over all their length. Rs forked near base; MA forked at less than 1/2 of their length, fork slightly asymmetrical; MP2 attached at the base to MP1 by cross veins (place of attachment of both veins is situated less than 1/5 of MP1 length); cubital field with four intercalaries connected by several cross veins; Cu–A angle smoothly curved and not well developed; pterostigma milky with 6–8 simple cross veins (Fig. 8).

Hind wings 3.8–4.0 × shorter than forewings. Costal process of hind wings smoothly rounded, not acute; apex of process situated almost in the middle of hind wing length; longitudinal and transversal veins whitish, hardly visible; cross veins not numerous, more or less evenly spaced throughout field between C and Sc (Figs. 9–11).

Ratio of foreleg segments: 0.82 : 1.00 : 0.03 : 0.37 : 0.40 : 0.37 : 0.13. Forefemora brown with dark brown maculation distally on both dorsal and ventral side; tibia yellowish brown proximally, brown distally. Tarsi pale, yellowish to whitish. Middle femora yellowish-brown to yellow, darker distally; tibia brownish basally, whitish apically; tarsi unicolorous whitish. Colouration of hind legs similar to middle legs. Pretarsal claws dissimilar



Figs. 12–14. *Calliarcys van* **sp.n.**: male imago, paratype. **12**: Penis lobes (dorsally). **13**: Genitalia (ventrally). **14**: Tip of abdomen (laterally).

(one pad-like, the other apically hooked). In the specimen from W Turkey fore legs are missing, middle and hind legs are slightly differently colored, with a distinct blackish band just above the middle of femora and tarsomeres are darker, purplish-grey; tibia and femur are yellowish-white.

Abdomen. Terga with contrasting pattern: tergum I intensively brown; tergum II pitch brown with a yellowish transversal band anteriorly and several pale spots centrally; terga III–VI with the same pattern consisting of a brownish posterolateral broad band with intensively brown lateral portions and a light brown to greyish area near posterior margin of tergum; central part of terga III–VI whitish-yellow, with more or less visible dirty brown maculation; tergum VII brown, with diffused longitudinal pattern and a lighter median line anteriorly; terga VIII–X unicoloured brown, with three more or less visible pale longitudinal strokes centrally (Figs. 1, 2). Abdominal sterna yellowish-white to brown or intensively brown: sterna I–II light brown; sterna III–VII unicoloured whitish to whitish-yellow; sternum VIII yellow; sternum IX with a brown band bordering segment margins and yellow central area; brown lateral spots on sterna I–VIII, size of these spots decreasing from sternum I to sternum VIII. Nerve ganglia greyish-brown, visible on segments II–VII. Cerci yellowish-white to white; terminal filament of the same colour, slightly longer than cerci.

Genitalia. Forceps base yellow to light brown; forceps yellowish-white. Forceps 3-segmented, slender; basal segment slightly tapering apically, slightly elongated, without inner process or appendages; segments II and III much shorter, approximately of equal length. Forceps base deeply concave medially, with V-shaped incision and two long, apically rounded projections directed caudally. Penis lobes simple, straight and tubular, somewhat club-shaped, fused basally; tip of penis only slightly bent inwards, without appendices or processes; inner margin of apex of each of penis lobe obliquely truncate (Figs. 6, 7, 12–14).

Female imago: Measurements: body length 9–10 mm; forewing length 8–9 mm; cercus length 9 mm (Fig. 3).

Head. Brown to yellowish-brown, with darker maculation around ocelli and clypeus. Antennae brown, slightly paler apically. Eyes widely separated, unicoloured black (after two years of preservation blackish-gray), surrounded by a narrow pale ring. Ocelli whitish apically, black basally.

Thorax. General colour brown to pitch brown, with dark pattern similar to male imago. Wing characters similar to male imago, except for venation colour, well visible, whitish to brown.

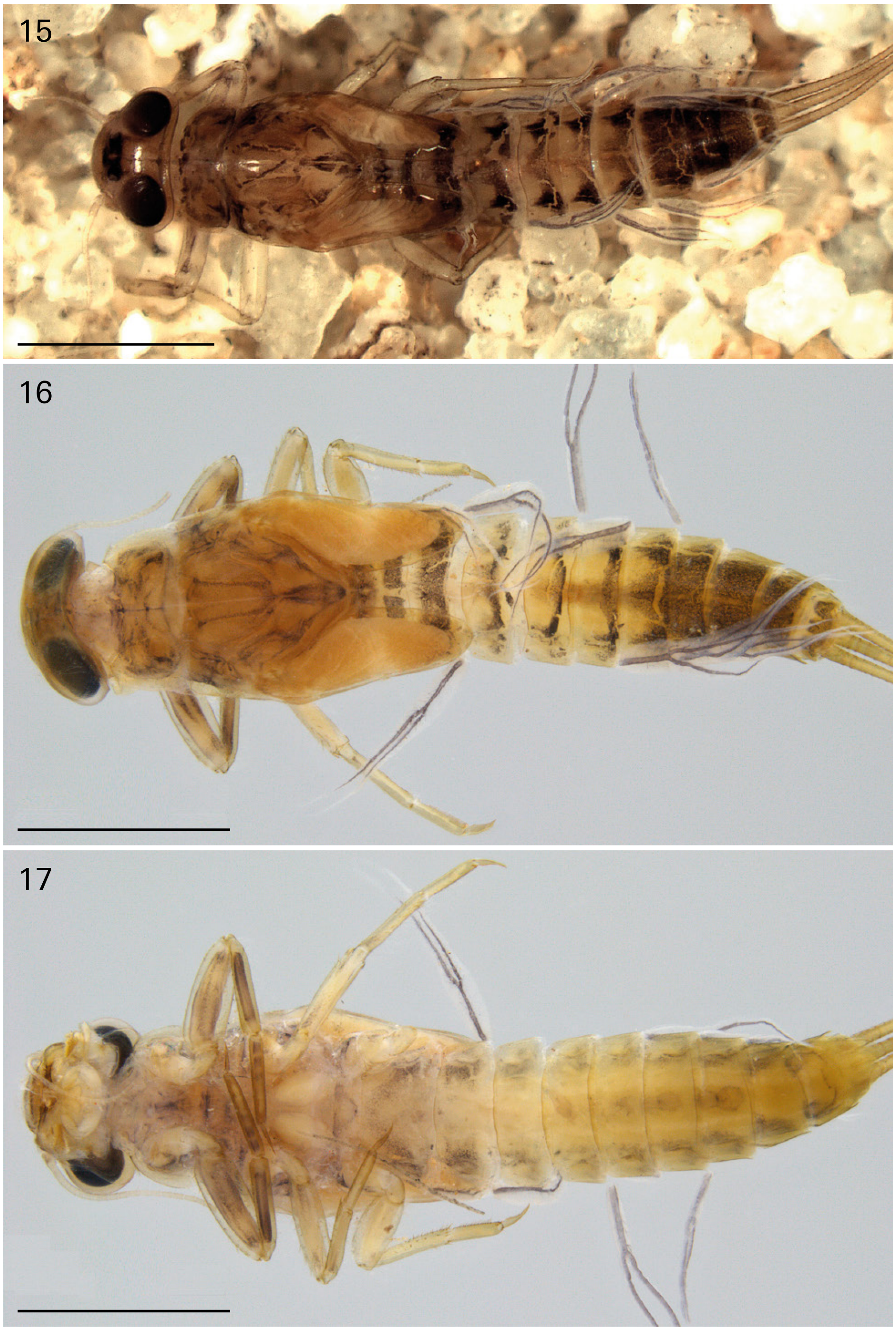
Forelegs darker than middle and hind legs; forefemora intensively brown, with diffused darker smudges centrally and apically; foretibiae dark brown, with yellowish transversal strip apically; foretarsi brown, with narrow yellowish ring apically on each segment. Middle legs with brown femora and proximal half of tibiae; distal half of tibiae and tarsi yellowish-white, except for brownish tarsomere V; small intensively brown maculation near distal end of middle femora. Hind legs lightest, yellow to yellowish-white; femora yellow with some brownish maculation distally; bases of tibiae and tarsomere V brownish, other segments yellowish-white.

Abdomen. All segments light brown to brown; segments I–III and VIII–X darker; occasionally the last three segments dirty brown. Tergum I brown; terga II–X with three pale strokes near central anterior margin; pleurae of terga with pale maculation. Sterna yellow to dirty brown, paler centrally; dark spots laterally on each segment. Subanal plate deeply cleft apically with V-shaped incision. Nerve ganglia reddish-brown. Cerci with 7–12 basal segments brownish, others whitish; terminal filament of the same colour, slightly longer than cerci.

Subimagines: Unknown.

Mature larva: Measurements: body length 4.7–8.5 mm [male], 5.5–9.0 mm [female]; cercus length 6.5–10.0 mm. (Figs. 15–17).

Cuticular coloration. Head relatively pale, yellowish-brown to brown, washed with gray (Figs. 15, 16); three well visible light spots touching outer margin of ocelli. Area between ocelli greyish to dirty brown; vertex with

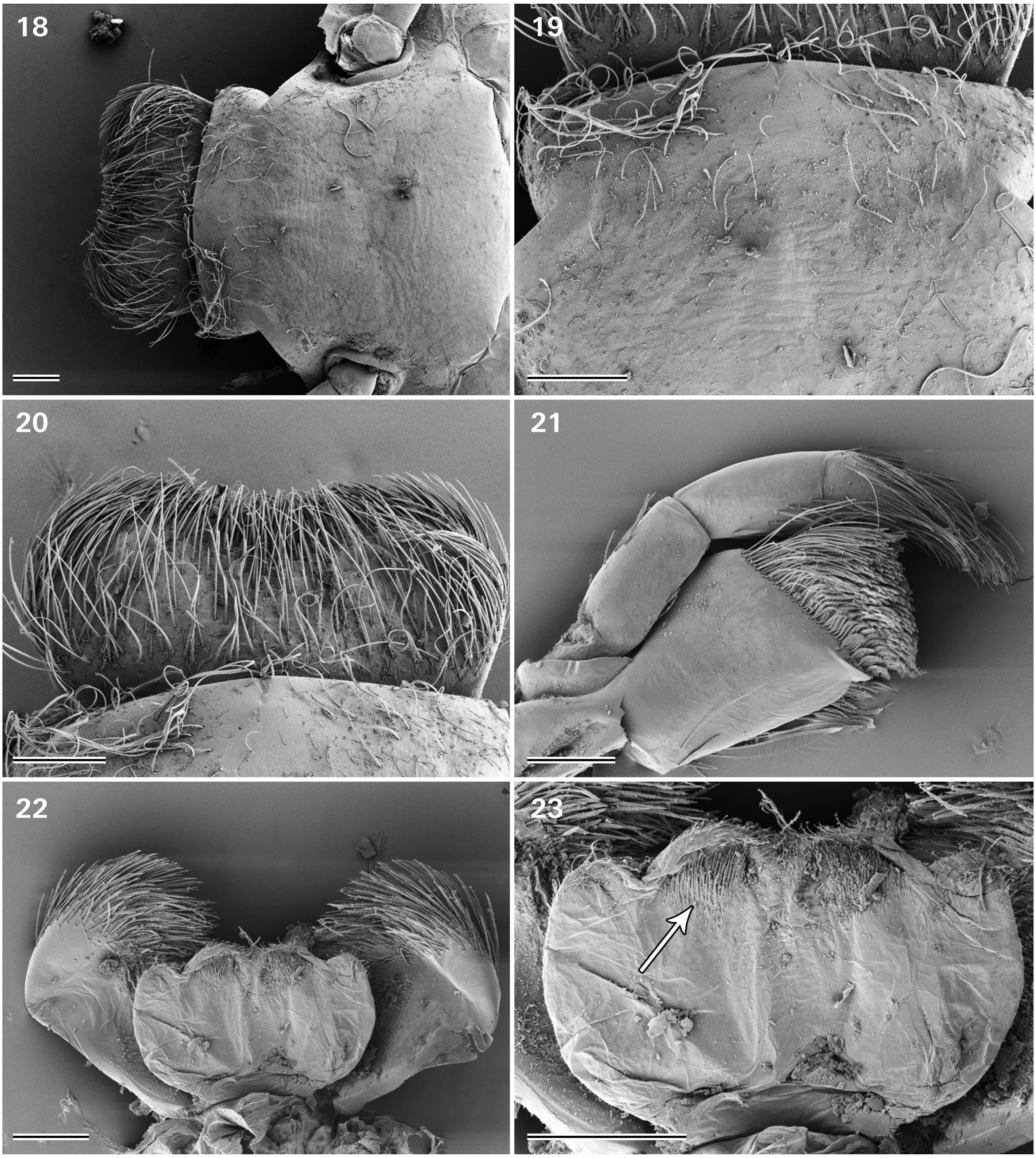


Figs. 15–17. *Calliarcys van* **sp.n.**: larvae, paratypes. **15–16**: General view (15, on natural background) (dorsally). **17**: General view (ventrally). Scale lines: 1 mm.

more or less distinct pale spot centrally, remaining part greyish to dirty brown; occiput pale, yellow to yellowish-brown; clypeus and genae light brown; antennae yellow, scape and pedicel darker than flagellum. Labrum brown; mandible light brown, slightly darker basally.

Thorax light brown to brown, with diffused dirty brown smudges over all surface (Figs. 15, 16); pleurae and sterna yellowish-brown; occasional several dark smudges and stripes on lateral sides; occasionally diffused brownish smudges near middle legs base. Pronotum with a median dark spot and several strokes laterally. Mesonotum yellowish-brown to brown (dark brown in last instar larvae); greyish-brown to dark brown stripes and spots around mesonotal sutures; wing pads brown. Metanotum with paired brownish spots centrally.

Legs yellowish-brown to intensively brown (in nymphs); occasionally forelegs distinctly darker. Coxae and trochanters with clear dark spots. Forefemora with brown a longitudinal spot centrally and a small brown smudge distally (Fig. 15); foretibiae brown or with two

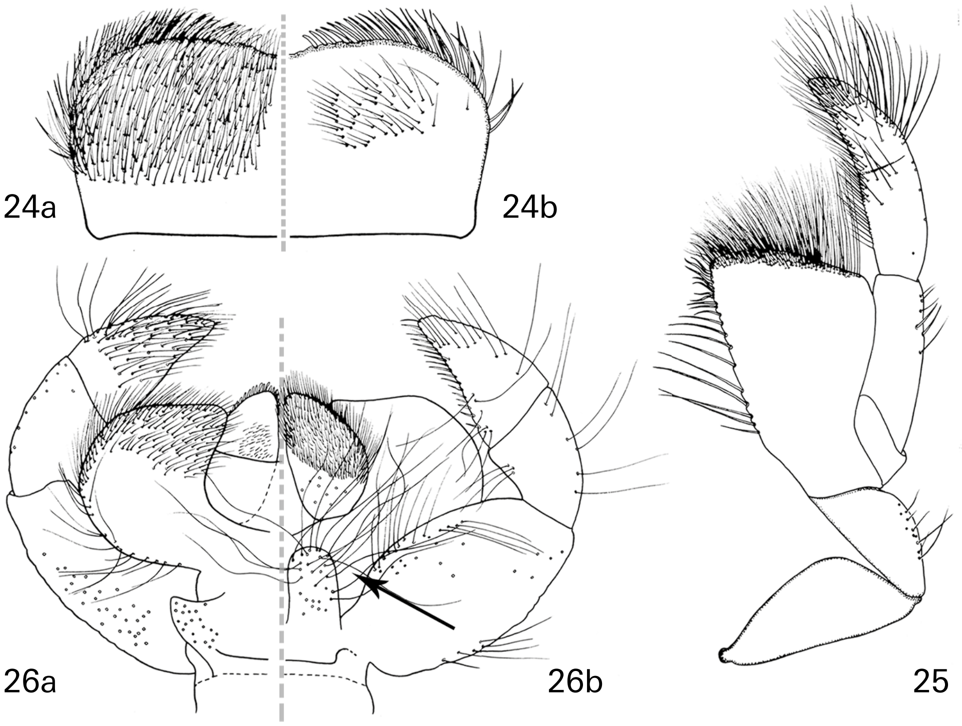


Figs. 18–23. *Calliarcys van* **sp.n.**: larvae. **18**: Head (dorsally). **19**: Clypeus (dorsally). **20**: Labrum (dorsally). **21**: Right maxilla (ventrally). **22**: Hypopharynx (ventrally). **23**: Lingua of hypopharynx (ventrally). Scale lines: 100 µm.

relatively wide brown proximal and distal transversal bands; foretarsi same colour as foretibiae or slightly darker proximally; pretarsal claws yellow to light brown. Middle and hind legs yellowish-brown; middle femora with a small diffuse brownish spot centrally. Several young larvae (males and females) with hardly visible femoral dark spots (especially on forelegs), generally unicolorous yellow.

Abdominal segments yellowish-brown to brown; sterna paler than terga. Dominant type of colour pattern of terga as in Figs. 15, 16; occasionally terga dirty brown to intensively brown, with a wide transversal yellowish band in the middle. Sterna laterally with diffuse greyish smudges; sterna I–VI paler; male larval genitalia darker, yellow to brown (Fig. 17). Cerci and terminal filament yellow to brown.

Hypodermal coloration. Head and thorax with greyish-brown to dirty brown maculation dorsally and laterally. Legs pale, yellow to yellowish-brown; small brownish spot near distal end of each of forefemora. Abdomi-



Figs. 24–26. *Calliarcys van* **sp.n.**: larvae, paratypes. **24**: Labrum (**a** – dorsally, **b** – ventrally). **25**: Right maxilla (dorsally). **26**: Labium (**a** – dorsally, **b** – ventrally).

nal terga yellowish-brown to brown; sterna unicolorous yellow with diffuse greyish smudges along each segment centrally and laterally. Gills dirty white, with unclear maculae; tracheae violet to dirty violet. Ventral ganglionic chain gray to dirty brown.

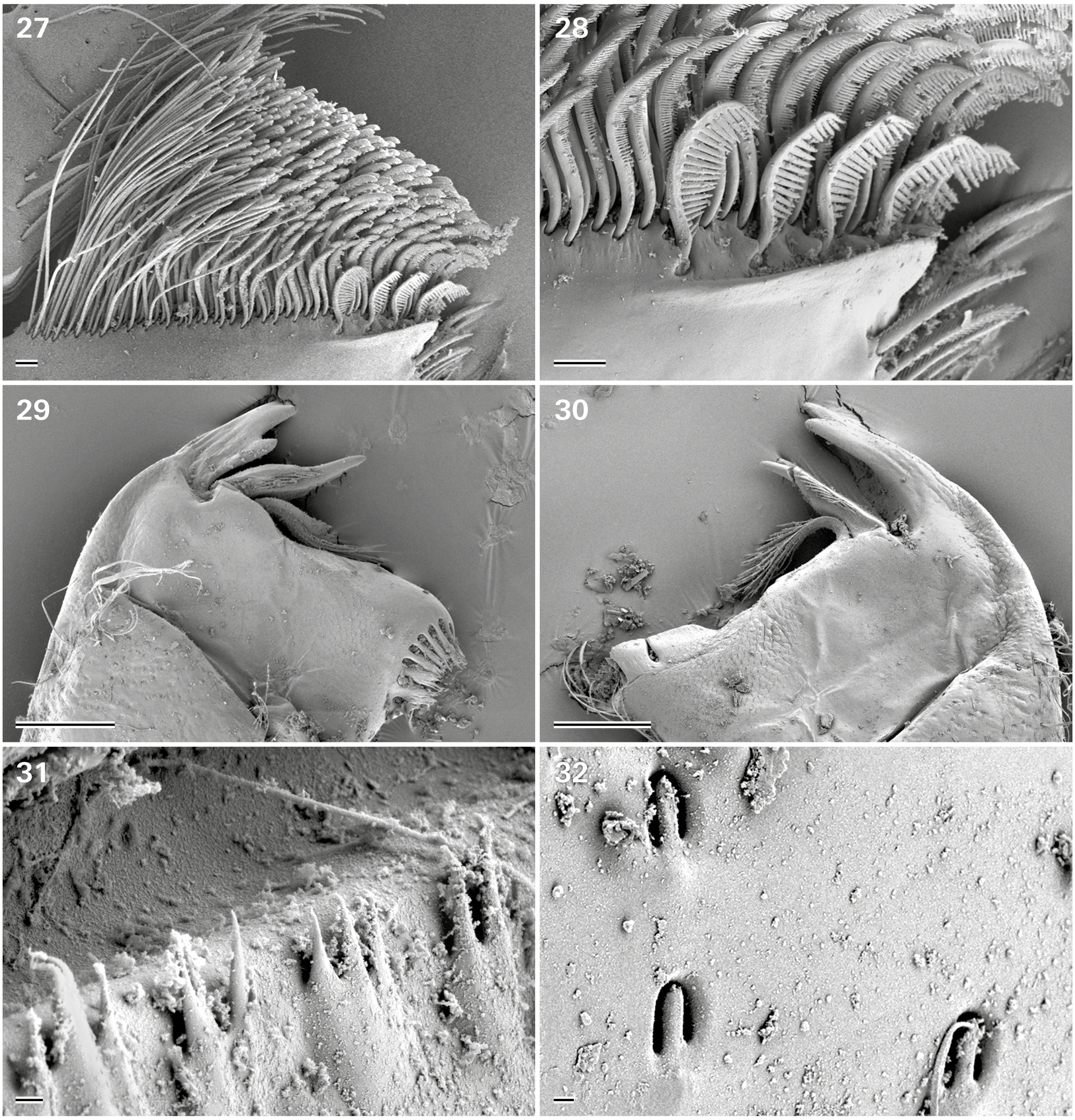
Head. Broadly rounded, triangular. Compound eyes and ocelli unicoloured greyish-black (in female); divided, with hepatic-brown upper, black lower part (in male). Clypeus without any processes; lateral margins slightly concave; the ratio of maximum width of labrum/clypeus = 1–1.2. Surface of head covered with tiny hair-like setae and stout bristles (most of them are concentrated on clypeus); several isolated setae near antennal base; small spatula-like spines (integral parts of the cuticle; terminology used according Gillies & Thorpe 1996) scattered on head surface (Figs. 19, 20; more detailed in Fig. 32). Antenna length 1.9–2.5 × maximal head length.

Mouthparts. Labrum without distinct anteromedial emargination bearing denticles; 25–30 stout bristles on anterior margin; numerous long stout bristles on dorsal surface; small setae on central part ventrally (Figs. 20, 24a, b). Outer margin of mandibles slightly curved, with 3–5 tiny setae; no long setae on dorsal surface; incisors brown to dark brown (Figs. 29, 30). Maxilla as in Figs. 21, 25; small tusk on inner apical end of galea-lacinia (Figs. 27, 28); vestiges of maxillary canines absent; maxilla with two dentisetae. Segment I of maxillary palp slightly longer than segment II; segment III subequal in length to segment II.

Hypopharynx as in Figs. 22, 23; lingua almost rectangular with a pair of rounded submedian processes, well separated from the pair of median processes by a deep V-shaped cleft; median processes broadly rounded apically, almost touching, separated at the tip by a shallow V-shaped cleft; numerous small hair-like setae on dorsal surface of median processes; a few setae on submedian processes apically. Superlinguae of hypopharynx with strongly curved, broadly rounded outer margin; numerous stout setae along outer apical margin of superlinguae (Fig. 22); ventral side with hairy patches (Fig. 23). Labium as in Fig. 26a,b; a pair of bulbous lobes covered with long hair-like setae on mentum ventrally; a pair of distinctive triangular processes on mentum dorsally; length ratio of labial segments I–III: 1 (I) : 0.60 (II) : 0.64 (III).

Thorax. Surface of thorax covered with short fine hair-like setae, their bases and small spatula-like spines.

Trochanters with stout, distally finely plumose bristles and fine short hair-like setae (Fig. 41); highest number of bristles on forelegs, lowest on hind legs. Femora relatively narrow. Outer margin of femora with an irregular row of a few stout bluntly pointed bristles (Fig. 40); distally situated bristles longer than proximal ones; distal half of femoral bristles finely plumose; scarcely any bristles on surface of femora; a few long hair-like setae and their bases present on femoral surface (Figs. 33–35). Inner margin of fore and middle legs with the same type of bristles, occasionally slightly longer as on outer margin (Figs. 38, 39); only several small pointed setae near distal end of hind femora (Fig. 35). Foretibiae with high concentration of numerous long bristles finely excised distally along inner margin; the same type of bristle arrangement on middle and hind tibiae, but with fewer bristles. An irregular sparse row of short pointed setae on outer margin of fore- and middle tibiae; occasionally several stout finely excised bristles along outer margin of hind legs. Foretarsi covered with dense long hair-like setae almost along their entire length (Figs. 33, 36, 37). Sparse setae of the same type arranged along outer and



Figs. 27–32. *Calliarcys van* **sp.n.**: larvae. **27**: Tip of right maxilla (ventrally). **28**: Right maxilla, details of structure of ventral pectinate setae and setae of ventro-median row (ventrally). **29–30**: Left (29) and (30) right mandibles (ventrally). **31**: Posterior margin of tergum V (dorsally). **32**: Surface of tergum V (dorsally). Scale lines: 10 µm (Figs. 27, 28); 100 µm (Figs. 29, 30); 1 µm (Figs. 31, 32).

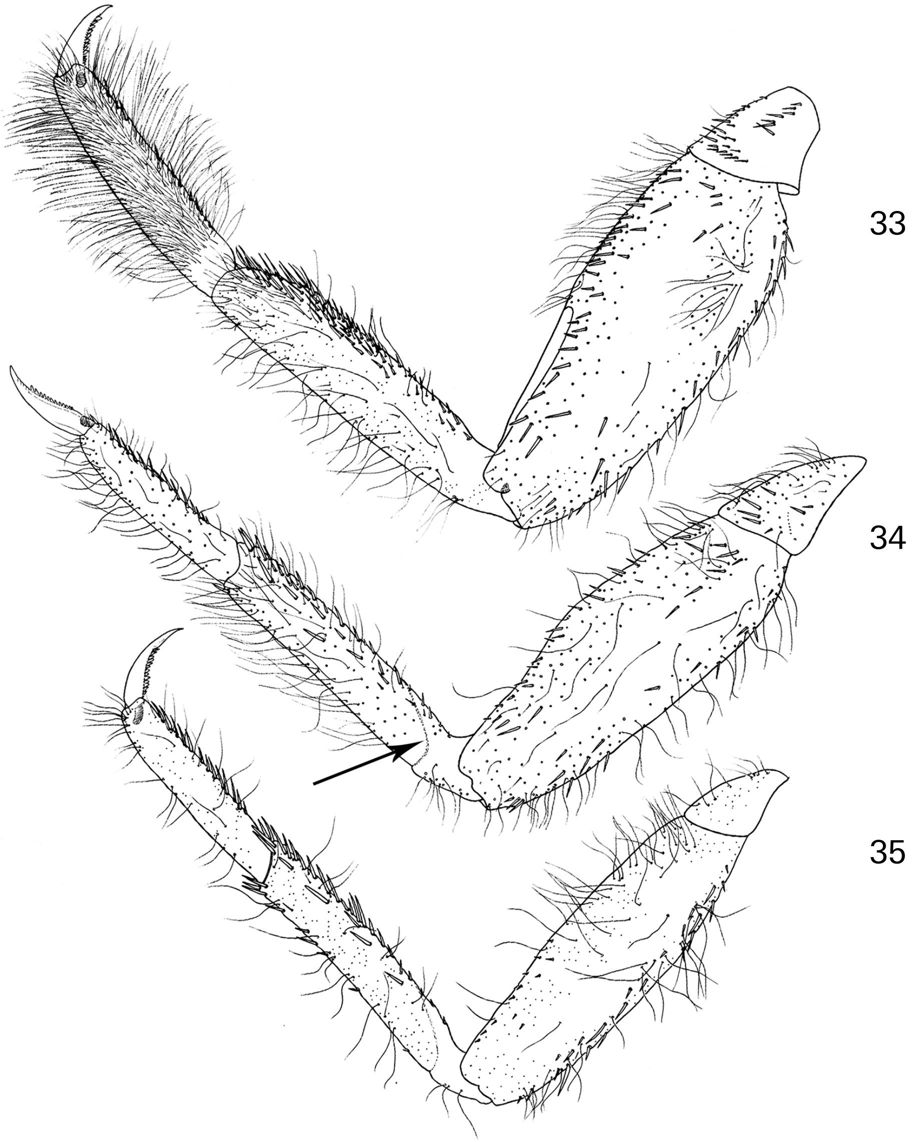
inner margins of middle and hind legs; inner margin of all tarsi with a regular row of pointed and bluntly pointed bristles, increasing in length towards apical end of tarsi; surface of all tarsi with dense long hair-like setae and their bases. Surface of all legs with a few characteristic small spatula-like spines (see Fig. 32). Pretarsal claws elongated, tip slightly hooked and narrow; all denticles equally sized (Figs. 36, 37).

Abdomen. Posterior margin of terga I–V (VI) with a row of small sharply pointed spines, in groups of 2–4; distance between these groups subequal to their width at base (as in Fig. 31). Posterior margin of terga VII–X with regular row of triangular stout spines. Small hair-like setae and spatula-like spines scattered over surface of terga (Fig. 32). Posterolateral spines on segments VIII and IX present. Ventral ganglionic chain well visible on segments IV–VII (VIII) (Fig. 17).

Gills I–VII alike, slender, deeply forked at least for half of their length; gill margins with long hair-like setae; shape of tracheal trunk as in Fig. 42.

Terminal filament slightly longer than cerci; long dense setae along entire length of caudal filaments.

Egg: Measurements: length 195–215 µm; width 70–85 µm; elongate, fusiform, distinctly widest in equatorial

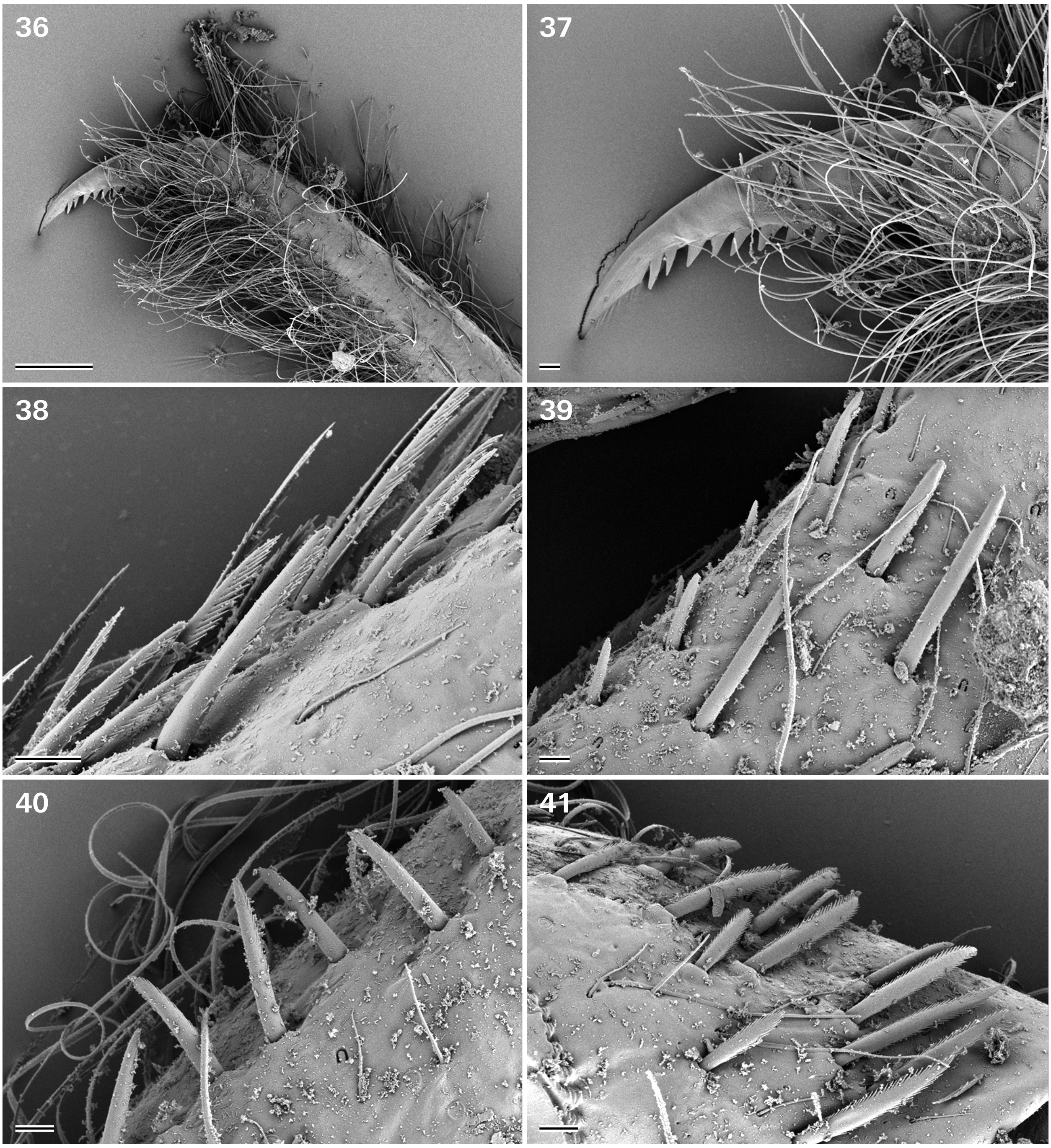


Figs. 33–35. *Calliarcys van* **sp.n.**: larvae, paratypes. **33**: Foreleg (dorsally). **34**: Middle leg (dorsally). **35**: Hind leg (dorsally).

area (Figs. 43, 44). Chorionic surface covered mainly by comparatively massive unbranched longitudinal ridges; distance between them 30–35 μm (Figs. 45, 46).

Derivatio nominis. The name of the new species refers to the region in Turkey where the type locality is situated, i.e. the environs of Van Lake [Van Gölü] in east Turkey.

Affinities. Both adult and larval stages of the new species are very similar to *C. humilis*. The colour pattern of male and female adults is very similar, except for some aspects of wing and abdominal colouration. The colour of the upper portion of male compound eyes is clearly different, brick-red in fresh specimens of *C. van* **sp.n.,** but creamy in *C. humilis* (Bauernfeind & Soldán 2012: 420). Both species are characterized by the presence of several simple cross veins in the pterostigma, but *C. humilis* has 8–12 cross veins, whereas *C. van* **sp.n.** has 6–8 cross veins in the pterostigmatic area of forewings (Fig. 8; cf. e.g. Peters 1966: 173, fig. 17; Peters & Edmunds 1970: 178, fig. 17; Bauernfeind & Soldán 2012: 420). A distinct difference between *C. van* **sp.n.** and *C. humilis* is found in the shape of the costal process of hind wings: smoothly rounded in *C. van* **sp.n.**, with the apex of the process situated nearly in a half of wing length (Figs. 9–11); in contrast the acute, step-like process in *C. humilis*, is situated slightly closer to the wing base (Peters 1966: 173, fig. 18; Peters & Edmunds 1970: 178, fig. 18). The male genitalia of *C. van* **sp.n.** differ markedly from *C. humilis* by the general shape of penis lobes, these being slender, club-shaped and only slightly bent inwards at the tip in *C. van* **sp.n.** (Figs. 6, 12, 13), in contrast to the widened and laterally stretched tips of penis lobes in *C. humilis* (see e.g. Peters 1966, fig. 76; Peters & Edmunds 1970: 186, fig. 76; Bauernfeind & Soldán 2012: 616, fig. 313). Some aspects of the shape of the submedian processes on the forceps base in the new species (large, broadly rounded apically), differ from those in *C. humilis*



Figs. 36−41. *Calliarcys van* **sp.n.**: larvae. **36**: Tip of foretarsus (dorsally). **37**: Pretarsal claw (dorsally). **38**: Bristles on inner margin of forefemur, proximally (dorsally); **39**: The same, distally (dorsally). **40**: Bristles on outer margin of forefemora, proximally (dorsally). **41**: Trochanters of foreleg (dorsally). Scale lines: 100 µm (Fig. 36); 10 µm (Figs. 37−41).

(rather slender, almost parallel-sided), described and depicted by Eaton (1884: pl. XIV, fig. 23 [genitalia]); Kimmins (1960: 298, fig. 42); Peters & Edmunds (1970: 185, 186, fig. 76); Bauernfeind & Soldán (2012: 419, 420, fig. 313 [genitalia]).

The larvae of *C. van* **sp.n.** differ from larvae of *C. humilis* by the combination of the following characters: **(i)** lack of long setae on the dorsal surface of mandibles (Fig. 29) in contract to *C. humilis* with a group of long setae on mandibles dorsally (cf. Peters & DaTerra 1974: 64, fig. 7); **(ii)** presence of median processes on hypopharyngeal lingua (Fig. 23), that are broadly rounded apically and separated by a shallow V-shaped cleft in contrast to the shallow U-shaped cleft in *C. humilis* (Peters & DaTerra 1974: 64, fig. 8); **(iii)** superlinguae of hypopharynx with strongly curved, broadly rounded outer margin (Fig. 22), in contrast to the acute outer margins of superlinguae in *C. humilis* (Peters & DaTerra 1974: 64, fig. 8); **(iv)** a pair of a distinct triangular processes on mentum dorsally (Fig. 26) in contrast to the small hump

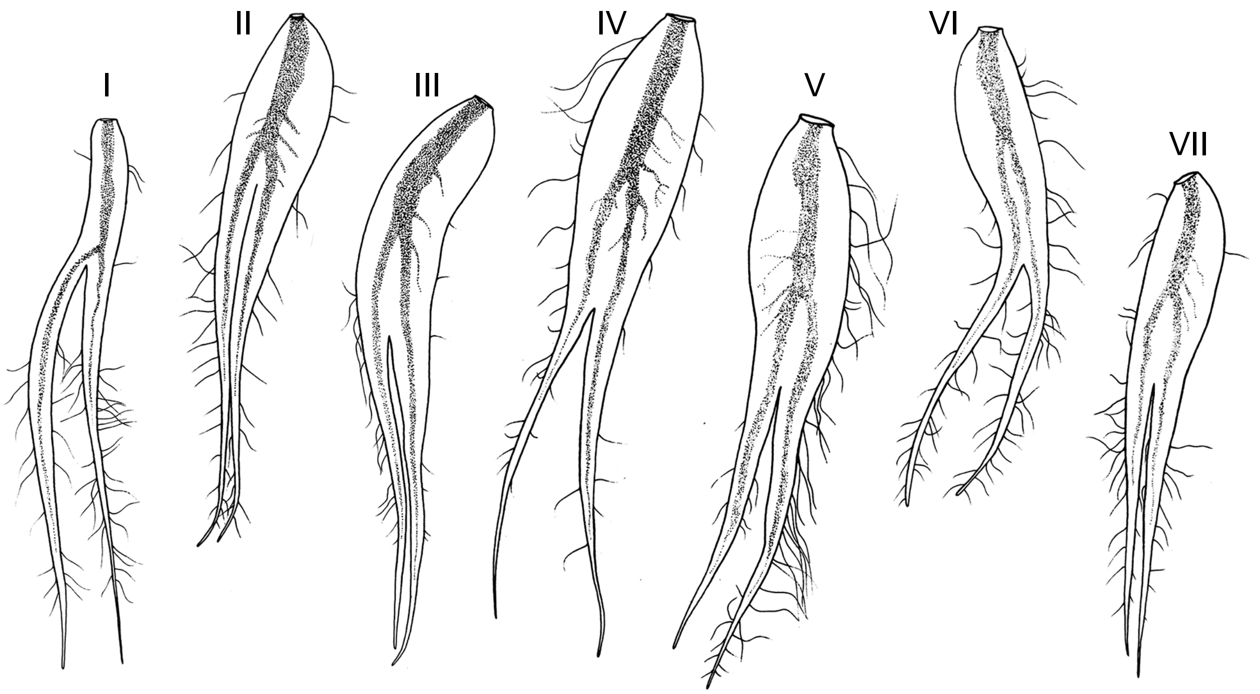


Fig. 42. *Calliarcys van* **sp.n.**: larvae, paratypes, gills I–VII (dorsally); roman numbers indicate respective abdominal segments.

on mentum in *C. humilis* (Peters & DaTerra 1974: 64, fig. 11); **(v)** posterior margin of abdominal terga I–VI with a row of small sharply pointed spines (Fig. 31) arranged in groups of 2–4 in contrast to the regular row of stout spines in the same terga in *C. humilis* (cf. Jacob 1984: 181, fig. 23).

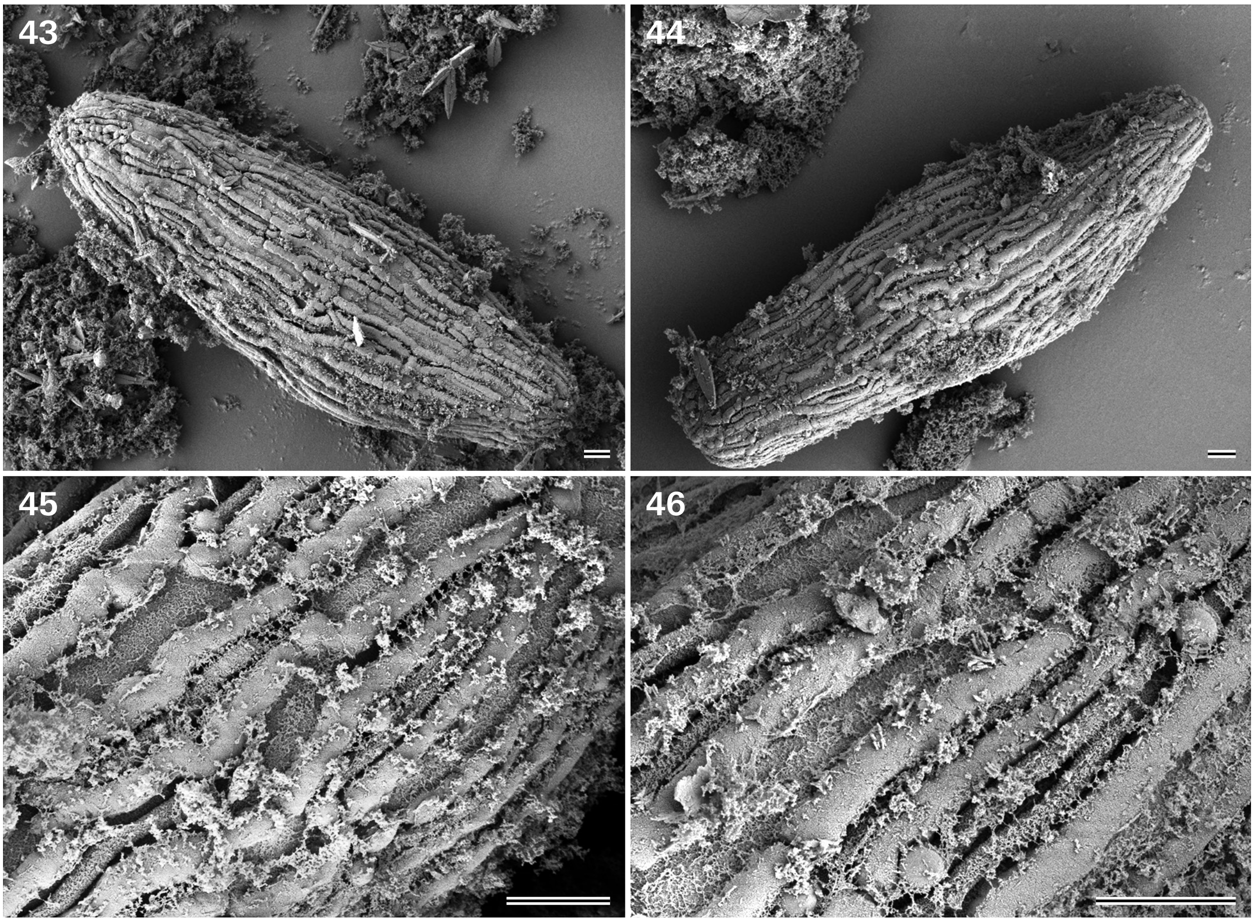
Additional differences between the new species and *C. humilis* can be found in the colour pattern of larvae, especially of abdominal terga. Bauernfeind & Soldán (2012) considered the larval colour pattern rather variable and therefore inadequate as a diagnostic character for *C. humilis*. However, in contrast to the Iberian species, *C. van* **sp.n.** could be characterized by the clear colour patter of abdominal terga in all collected larvae (Figs. 15, 16). Moreover, this pattern markedly differs from that depicted by Peters & DaTerra (1974: 64, fig. 5).

Both species also differ in the shape of eggs: elongate and fusiform in *C. van* **sp.n.** (see Figs. 43, 44) in contrast to the more regularly oval shaped eggs in *C. humilis*, cf. Bauernfeind & Soldán (2012: 633, fig. 313).

Type material. ***Holotype***: Male imago, E TURKEY, Bitlis Province, Kavuşşahap Dağları mountain range, Pınarca Çayı [river] and its small unnamed right tributary above Kuşlu village, 38°22′32″N 42°15′31″E, 1720 m a.s.l., about 20 km S of Tatvan town (western shore of the Van Lake), 14.vi.2011, J. Bojková & T. Soldán leg. — ***Paratypes***: 12 male imagines, 4 female imagines, 32 larvae, the same date and place as holotype; one male imago (genitalia on slide), W TURKEY, Province Izmir, unnamed brook, 7 km S Kamberler, 38°15′N 27°35′E, 430 m a.s.l., 21.v.1992, H. Malicky leg. — ***Type material deposition***: IE CAS: holotype (male imago), 36 paratypes (9 male imagines, 4 female imagines, 23 larvae); SMNHL: 7 paratypes: 2 male imagines (genitalia on slides), 6 larvae (3 of them on slides); NMW: 1 paratype: male imago; SMNS: 3 paratypes (male imago, 2 larvae).

Description of type locality. Pınarca Çayı at the type locality is a small mountain river (Figs. 47, 48), flowing approximately from north to south into Bitlis Çayı, a left tributary of the Tigris River (Dicle Nehri in Turkish). The type locality is situated at the relatively high altitude of 1720 m a.s.l., about 10 km from the river source on the slopes of the Şikirin Tepe Mt. (2658 m a.s.l.). The river stretch can be characterized as a wide and shallow channel which is branched and anastomosed into numerous lateral flows (Fig. 47). Stretches of swift, turbulent flow and slow, rather laminar flow irregularly alternate according to the floodplain morphology (cf. Figs. 47, 48). The channel is 8–15 m wide and 10–40 cm deep. The substrate is coarse, dominated by stones and coarse gravel in riffles; fine gravel, sand and silt occur at channel margins. The river stretch is characterized by heterogeneous flow conditions which vary from riffles with very fast current to nearly stagnant pools. Tufts of waterlogged sedges are abundant in the shallow marginal parts of the channel. Its right tributary (1–1.5 m wide and about 10 cm deep) is similar, only with riparian vegetation consisting mainly of willows (Fig. 49). Collecting places in the tributary were located at the mouth and up to about 20 m from the mouth. The river valley is treeless and streams are therefore not shaded.

Biology. Larvae were observed mostly in pools, sitting on roots and submerged parts of sedges. They inhabited the stony bottom too, but only in places with organic debris, never occurring at places with turbulent flow. They were very rare in the river but abundant in the mouth of the tributary. The taxocene of mayflies associated with *C. van* **sp.n.** was dominated by Heptageniidae (*Iron* spp.,



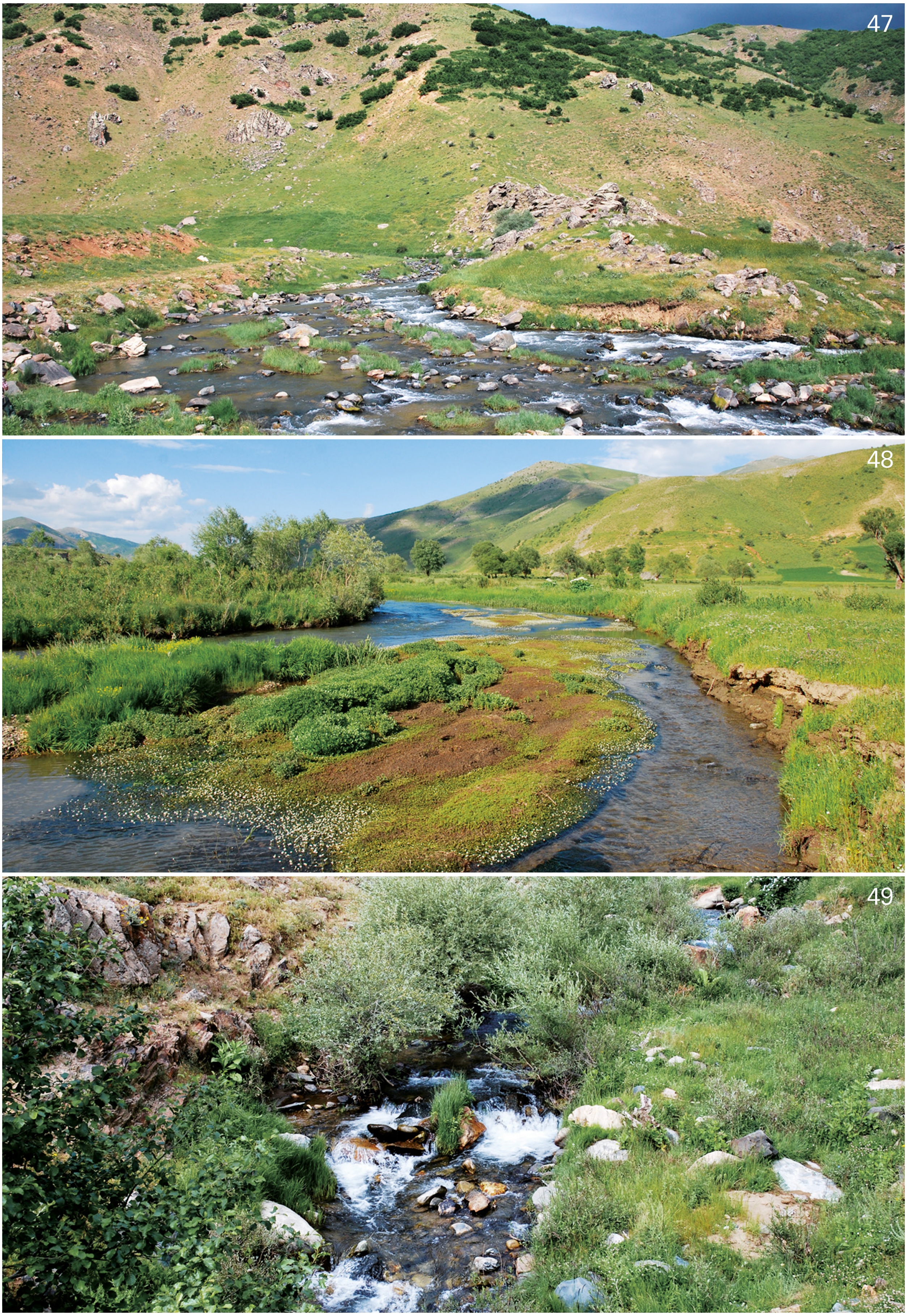
Figs. 43–46. *Calliarcys van* **sp.n.**: eggs. **43–44**: General view. **45–46**: Surface of exochorion. Scale lines: 10 µm.

Epeorus assimilis Eaton, 1885, *Ecdyonurus autumnalis* Braasch, 1980 and *Rhithrogena* spp.) and Baetidae (*Baetis* spp.). Places with slow flow and pools were inhabited by *Ephemera romantzovi* Kluge, 1988, *Baetis* (*Nigrobaetis*) sp. and *Caenis* sp.

Since no subimagines were collected (although some exuviae have been observed), timing of emergence remained unknown. Most probably last instar larvae moulted from vegetation. Imagines were observed flying in the afternoon (2:00–3:00 p.m. local time) showing the typical perpendicular fly of males. Flying imagines were observed solely above the mouth of the tributary. Some individuals were sitting on the riparian vegetation and were collected by sweeping. Weather conditions during collecting time might have affected flight activity by decreased atmospheric pressure and relatively higher temperature before a storm (Fig. 47) and heavy rain.

Notes on zoogeography. *Calliarcys van* **sp.n.** was found in two quite isolated areas of the Eastern Mediterranean, in W Turkey (biogeographic regions Marmara or Aegean) and in E Anatolia biogeographic regions. These two areas would indicate a somehow disjunctive area. Naturally, this is a hypothesis, but supported by the fact that the species has so far not been found elsewhere (cf. Kazanci 2001; Kazanci & Türkmen 2012). Despite our collecting effort (at more than 100 localities) in Central, Southeast and East Anatolia, the species has nowhere else been found by us.

The genus *Calliarcys* has been considered monotypic for a long time. The only species known was *C. humilis* described from Portugal and later found also in Spain (Alba-Tercedor & Jáimez-Cuéllar 2003; Bauernfeind & Soldán 2012) and thus usually considered to represent an Atlantomediterranean faunistic element. The present finding of another species of the genus *Calliarcys* indicates that the area of *Calliarcys* is actually much larger, but disjunctive and that the genus probably shows Paleomediterranean origin (Oosterbroek & Arntzen 1992). This type of area is very unusual in Ephemeroptera, but described for other groups of aquatic insects (see e.g. Malicky 1990; Sipahiler 2008). As far as we know, the only analogous distribution in Europe is represented by the genus *Thraulus* Eaton, 1881 (Leptophlebiidae: Atalophlebiinae). It includes two species: *T. bellus* Eaton, 1881 is distributed in Western Europe (Germany, Switzerland and France) and considered an Atlantomediterranean faunistic element, whereas *T. thraker* Jacob, 1988 is known from two localities in south eastern Bulgaria (Veleka and Ropotamo rivers) and from W Anatolia (Koruköy, 38°50′N 27°10′E, H. Malicky leg., NMW). However, further species of *Thraulus* are known from the Oriental region and the Far East.



Figs. 47–49. Type locality of *Calliarcys van* **sp.n.**: **47–48**: Pınarca Çayı [river], below Kuşlu village. **49**: Right unnamed tributary of Pınarca Çayı (photos J. Bojková).

4. Phylogeny and classification

4.1. Subfamilial diagnostic characters of Leptophlebiidae

In the analysis of the higher Leptophlebiidae phylogeny performed in this study, we used a set of 20 subfamilial diagnostic characters, compiled from the Leptophlebiidae classifications published so far (Peters 1980, 1997; Kluge 1994, 2009; Bauernfeind & Soldán 2012). Most characters were used without changes. However, some ambiguously defined characters were reformulated or omitted in the matrix, and some were newly added. We provide the reasons for the most substantial changes, as follows.

4.1.1. Setation on labrum

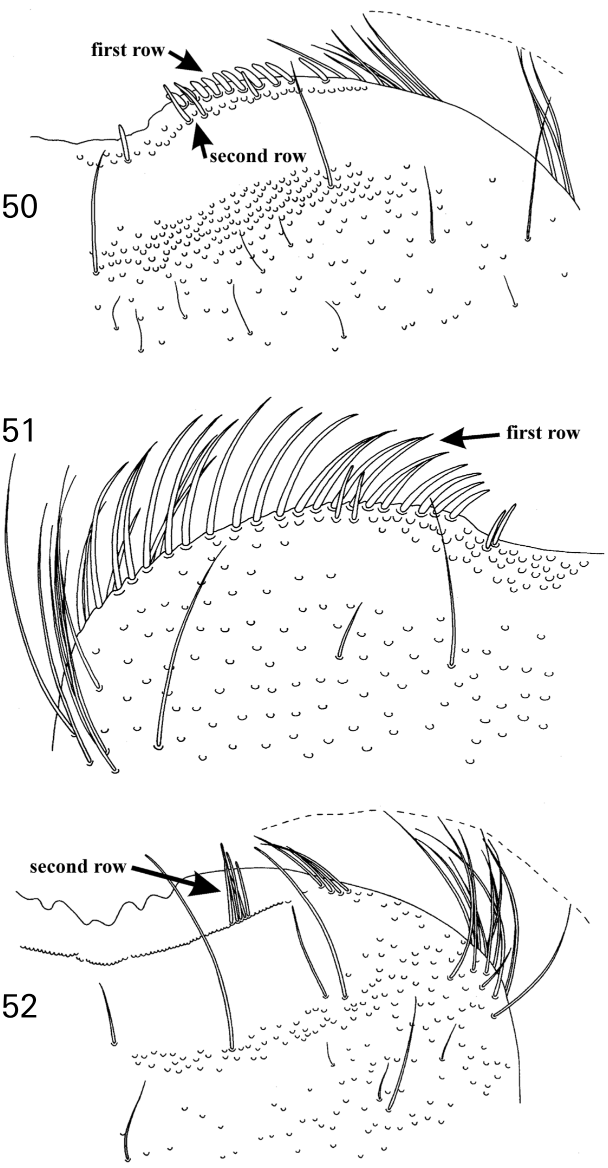
Maximally three rows of setae can occur on the dorsal surface of labrum (Figs. 50–53). In this study, we call them the first, the second and the third row. We consider it useful to clarify the terminology used by various authors in order to avoid confusion. Most anteriorly a first row of setae, called "stout bristles on anterior margin of labrum" by Kluge (1994), "heavy spines" by Peters (1997) and "lateral bristles of labrum" by Bauernfeind & Soldán (2012) is situated. These setae are stout, pointed to blade-like in Leptophlebiinae, Habrophlebiinae and Calliarcyinae; missing in Atalophlebiinae s.l.

One or two submarginal rows of setae can occur proximally from the first row of stout bristles. One or both of these submarginal rows can be disintegrated into fields of setae to a various degree. Therefore, we can observe three different rows of labral setae; presence/absence of each of these rows being a separate character.

Thus, the character given by Kluge (2009), presence of "two transverse rows of setae" actually means presence of second and third row with the first row absent. Accordingly, the "distal row of bristles" sensu Kluge (1994) is actually the second row and the "second transverse row of bristles proximad to the distal one" sensu Kluge (1994) is actually the third row (Kluge 1994: figs. 22, 23).

4.1.2. Setation on maxilla

According to Peters (1997) and Bauernfeind & Soldán (2012), anterior margin of maxilla bears bristles more or less evenly arranged in rows in Atalophlebiinae s.l. Remaining groups exhibit scattered or unevenly arranged setae. N.J. Kluge (viz., http://www.insecta.bio.spbu.ru/) considers these bristles arranged in rows in all Leptophlebiidae and disregards this character in subfamilial classification. According to our own investigation of the representatives of all subfamilies, we agree with N.J. Kluge's assumption. We also did not observe consistent differences in the arrangement of bristles for any of the proposed subfamilies (see Fig. 54). Therefore, we do not take this character into consideration when compiling the matrix for the analysis.

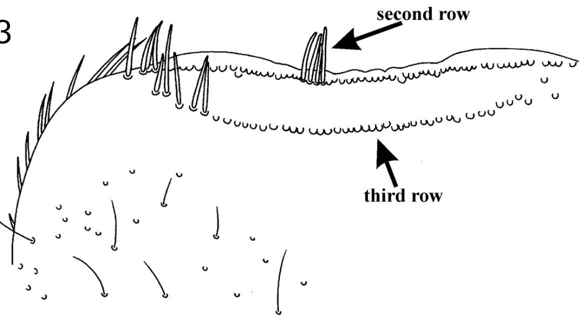


Figs. 50–52. Leptophlebiidae subfamilies, setation on the dorsal side of labrum. **50**: *Habroleptoides confusa* (Habrophlebiinae). **51**: *Paraleptophlebia submarginata* (Leptophlebiinae). **52**: *Castanophlebia* sp. (Castanophlebiinae).

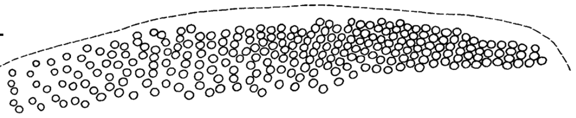
4.1.3. Male genitalia

The presence of the "prominent dorsal plate" was mentioned by Kluge (1994) as a synapomorphy of Habrophlebiinae and Atalophlebinae s.l. However, the character is highly variable and currently considered unreliable even by its original proponent (N.J. Kluge pers. comm). Therefore, we excluded it from the analysis. The character presented by Peters (1997) as a synapomorphy of *Terpides* lineage and *Castanophlebia*, i.e. presence/absence of ventral rod or ridge of penis was also not included, based on its rejection by Kluge (2009).

53



54



Figs. 53–54. Leptophlebiidae subfamilies. **53**: Setation on the dorsal side of labrum of *Terpides* sp. (Terpidinae). **54**: Part of setae bases on the apical part of maxilla of *Paraleptophlebia submarginata*.

4.1.4. Exochorion of eggs

Several chorionic structures, characteristic for individual genera and subfamilies were described by Bauernfeind & Soldán (2012). Analyzed exochorion structure in both species of *Calliarcys* is most similar to those in *Habrophlebia*. The chorionic surface with large longitudinal ridges was described for old and new world representative of *Habrophlebia* (e.g. Koss 1968: 708, 709, fig. 29; Gaino & Mazzini 1984: 195, fig. 1; Mazzini & Gaino 1988: 448, figs. 15, 16), and can be used to characterize the egg at genus level (Ubero-Pascal & Puig 2007: 333, 334, fig. 5f). On the other hand, the exochorion of *Calliarcys* eggs clearly differs from those in *Habrophlebia* by the presence of densely arranged unbranched longitudinal ridges, contiguous along their length in several points.

Nevertheless, regarding the relatively high variability within discussed subfamilies and still small number of species with known chorionic surface, we did not use this structure in the analysis.

Except of *Calliarcys* + *Habrophlebia* a similar structure of exochorion with presence of longitudinal ridges was described for *Brachycercus* Curtis, 1834 (Koss & Edmunds 1974: 341, pl. 20, figs. 217–219; Kluge 2004: 388) and *Acentrella* Bengtsson, 1912 (Ubero-Pascal & Puig 2007: 333, 334, fig. 5a), and specified as a clear generic character of eggs. At the same time, eggs of *Brachycercus* possess additionally a single polar cap.

4.2. Higher phylogeny of Leptophlebiidae and systematic placement of *Calliarcys*

Our phylogenetic analysis recovered 3 most parsimonious topologies of 35 steps and a 50% majority rule consensus tree was constructed (Fig. 55). The consistency and retention indices were 0.83 and 0.68, respectively. From 20 characters used, 14 were parsimony-informative.

The subfamily Leptophlebiinae was recovered as the sister lineage to all remaining Leptophlebiidae. This result supports Kluge (2009), who considers Leptophlebiinae as the sister lineage to the remaining subfamilies, which form the monophyletic group Atalophleboadentata sensu Kluge 2009. It also agrees with the molecular results of O'Donnell & Jockusch (2008). Nevertheless, the clade containing the remaining subfamilies except for Leptophlebiinae gained relatively low support (bootstrap value below 50). Within this clade, only Atalophlebiinae s.l. (including Terpidinae and Castanophlebiinae) formed a highly supported monophyletic cluster (bootstrap value 95). The branching pattern within Atalophlebiinae s.l. revealed a sister relationship of Atalophlebiinae s.str. + Castanophlebiinae (bootstrap support 69), which supports Kluge's (2009) monophyletic group Atalophlebomaxillata. Habrophlebiinae and Calliarcyinae are nested at the base of the non-Leptophlebiinae clade, Calliarcyinae forming the sister lineage to Habrophlebiinae + Atalophlebiinae s.l. Such a pattern also supports the concept of Kluge (2009), where Calliarcyinae originates from the most basal dichotomy in Atalophleboadentata. However, the support for these positions of Habrophlebiinae and Calliarcyinae was very weak in our analysis (bootstrap values below 50). With regard to the low support values, the position of Calliarcyinae as the sister group to Habrophlebiinae or Atalophlebiinae s.l. (or even Leptophlebiinae) can not be fully excluded. A more robust analysis containing also multiple molecular markers is needed to test this phylogeny of Leptophlebiidae.

As shown in Fig. 55, individual clades were characterized by autapomorphic states of several diagnostic characters. In some cases, these characters do not represent truly "unique" apomorphies of the groups in question, since they occur independently in other mayfly families (e.g. absence of costal process in hind wings occurs in most Leptophlebiinae but also in numerous unrelated mayfly taxa). However, if we compare the characters mapped on the cladogram on Fig. 55 across Leptophlebiidae and with unrelated outgroup taxa, in all probability they represent apomorphic states within Leptophlebiidae.

Calliarcyinae is characterized by the apomorphic ciliation of the foreleg tarsi. It also shows a unique shape of the apicolateral processes of the lingua, which are present (contrary to Leptophlebiinae), but not projected laterally (contrary to Atalophlebiinae s.l. + Habrophlebiinae). Calliarcyinae shares one apomorphy with Habrophlebiinae + Atalophlebiinae s.l. (absence of maxillary canines), but lacks four apomorphies of the Atalophlebiinae s.l. + Habrophlebiinae clade (apicolateral processes of lingua projected laterally, curved superlingua, comb-like proximal dentiseta and loss of tibiopatellar suture on middle legs). This rejects the attribution of Calliarcyinae within this clade and places the subfamily beside Atalophlebiinae s.l. + Habrophlebiinae.

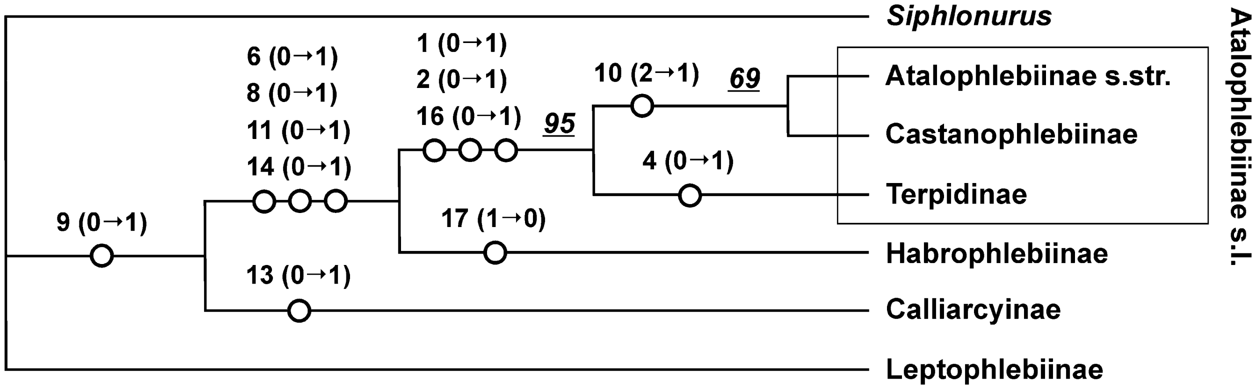


Fig. 55. Fifty percent majority rule consensus tree of 3 most-parsimonious topologies. The underlined numbers above branches are bootstrap support values. Unique morphological apomorphies with consistency index = 1.0 are mapped on the topology.

The apomorphies involved in this hypothesis represent well-defined character states and hence support the placement of *Calliarcys* in a separate subfamily Calliarcyinae of Leptophlebiidae.

5. Conclusion

Based on our study, the previously monotypic genus *Calliarcys* now includes two well-defined species and its distributional area is considerably extended. Based on the cladistic analysis of the updated set of morphological diagnostic characters, the phylogenetic position of *Calliarcys* is determined as the sister group to Habrophlebiinae + Atalophlebiinae s.l. According to the topology of the resulting phylogenetic tree, the genus can not be included in any other subfamily of Leptophlebiidae except for its own. Therefore, we consider the existence of a separate subfamily Calliarcyinae sensu Kluge (2009) as justified.

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