



Testing the monophyly of Chaetarthriinae (Coleoptera, Hydrophilidae) and the phylogenetic position of *Guyanobius* with larval characters

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Abstract

The subfamily Chaetarthriinae includes morphologically distinct larvae that are adapted to a diversity of environments. Based on larval characters, cladistic analyses (maximum parsimony (MP) and Bayesian inference (BI) with homoplasy as a partitioning scheme) were performed to test the monophyly of the subfamily and the relationships of the two tribes included in it: Chaetarthriini and Anacaenini. The chaetotaxy of a third instar larva *Guyanobius adocetus* is described and illustrated in detail, including morphometric characters. This larva is compared to those of the known larvae of the tribe Chaetarthriini belonging to the genus *Chaetarthria*, and *Pseudorygmodus*, *Crenitis*, and *Crenitulus* from Anacaenini. None of the unconstrained analyses recover Chaetarthriinae as monophyletic. *Chaetarthria* diverges in an early branch, probably due to a series of unique morphological modifications associated with a riparian lifestyle whereas *Guyanobius* appears closely related to Anacaenini. Two alternative positions of *Guyanobius* are revealed: (1) as sister of all Anacaenini (unconstrained MP) or (2) nested within Anacaenini as sister of *Crenitis* + *Crenitulus* (constrained MP and unconstrained BI). The genera *Paracymus* and *Tormus* (tribe Laccobiini) diverge as two successive branches subordinate to Chaetarthriinae (excluding *Chaetarthria*) in the unconstrained MP analysis. However, the support is rather weak, and the position of *Paracymus* and *Tormus* is an artifact produced by some homoplastic characters. In this regard, homoplasy partitioning resulted a useful technique to solve some artifacts generated by convergent morphologies.

Key words

Hydrophiloidea, larvae, morphology, phylogeny, homoplasy, adaptive convergence, parsimony, Bayesian inference, water scavenger beetles.

1. Introduction

The Chaetarthriinae are a small group of morphological unique water scavenger beetles. This subfamily was erected by Short and Fikáček (2013) to include members of the tribe Chaetarthriini (except *Amphiops*) and Anacaenini *sensu* Hansen (1991) based on molecular evidence. Subsequently, Fikáček and Vondráček (2014) transferred *Pseudorygmodus* Hansen, 1999 to the tribe Anacaenini and restored the genus *Crenitulus* Winters, 1926 to include the species of *Anacaena suturalis*-group. Currently, the subfamily has more than 233 described species distributed in two tribes (Chaetarthriini, Anacaenini) and 14 genera.

The genus *Guyanobius* Spangler, 1986 belongs to the tribe Chaetarthriini (Short and Fikáček 2013), which includes six genera: *Apurebium* García, 2002, *Chaetarthria* Stephens, 1835, *Guyanobius*, *Hemisphaera* Pandellé, 1876, *Thysanarthria* Orchymont, 1926 and *Venezuelobium* García, 2002. *Chaetarthria* has a worldwide distribution, *Thysanarthria* and *Hemisphaera* are restricted to the Ethiopian, Oriental and Palearctic regions (Hansen 1999; Archangelsky et al. 2016; Fikáček and Liu 2019) and *Apurebium*, *Guyanobius* and *Venezuelobium* are restricted to the Neotropical region (Spangler 1986; García 2002; Gustafson and Short 2010). However, Short (2009) suggested that the genera *Apurebium* and *Venezuelobium* include species that seem to be variants of *Chaetarthria*, which would leave only two genera present in the Neotropical region: *Chaetarthria* and *Guyanobius* (Clarkson et al. 2018). Adults of this tribe are easily recognized by the characteristic fringe of long setae arising at the anterior margin of the first abdominal ventrite which covers a large depression usually filled with a hyaline substance.

Guyanobius, which includes the larger members within the tribe, comprises four neotropical species restricted to northern South America. The genus was erected by Spangler (1986) for a single species found in Guyana, *G. adocetus* Spangler, 1986. A few years later Spangler (1990) described a second species, *G. simmonsorum* Spangler, 1990 from Brazil. More recently Gustafson and Short (2010) revised the genus describing two new species, *G. lacuniventris* Gustafson and Short, 2010 from Venezuela and *G. queneyi* Gustafson and Short, 2010 from Guyana and Suriname; they also extended the distribution of *G. adocetus* to Venezuela.

Larvae of *Guyanobius adocetus* have been described by Spangler (1986) and Archangelsky (1997). Nonetheless, both descriptions focused on the basic morphology of the larvae, and did not include chaetotaxic or morphometric features. In this contribution, we describe in detail the chaetotaxic and morphometric characters of a third instar larva of *G. adocetus*. We compare the chaetotaxy of *G. adocetus* with that of two species of the genus *Chaetarthria*, *C. seminulum* (Herbst, 1797) and *C. bruchi* Balfour-Browne, 1939 (Fikáček 2006; Archangelsky 2021), and to that of other available Anacaenini larvae, *Pseudorygmodus flintispangleri* Moroni, 1985, *Crenitis morata* Horn, 1890 and *Crenitulus suturalis* LeConte, 1866. Lar-

val chaetotaxy of the remaining genera of Chaetarthriinae is not yet known.

Although relationships among Chaetarthriinae have been assessed with molecular data (Short and Fikáček 2013; Fikáček and Vondráček 2014; Clarkson et al. 2019), little is known about the morphological characters that support these phylogenetic patterns. Morphological data are essential for studying eco-morphological adaptations and reconstructing mechanisms of evolutionary pathways. Therefore, our goal is to conduct larval-based cladistic analyses to test whether immature morphology corroborates the molecular phylogeny of Chaetarthriinae, namely the monophyly of the subfamily and its tribes. Additionally, we discuss the most relevant characters and possible convergences given by the environment.

2. Material and Methods

2.1. Source of material

One third-instar larva of *G. adocetus* was studied. Guyana, Mazaruni-Potaro district, Takutu Mountains, 6°15' N, 59°5' W, 3–10.xii.1983, P. J. Spangler, R.A. Faitoute and P.D. Perkins leg.

For comparative purposes, larvae of *Chaetarthria bruchi* and unidentified larvae of *Chaetarthria* from Montana (USA) were examined. Information on *C. seminulum* comes from the literature (Fikáček 2006). Anacaenini larvae examined: *Pseudorygmodus flintispangleri*, one pharate third instar larva (the chaetotaxy therefore corresponds to that of the second instar larva), Chubut province (Argentina); *Crenitulus suturalis*, one second instar larva, La Rioja province (Argentina); *Crenitis morata*, two third instar larvae, Massachusetts (USA). The material studied is kept in the larval collection of the author and will be deposited in the larval collection of the Laboratory of Entomology, Buenos Aires University, Argentina.

2.2. Methods

Larval specimens were cleared in warm lactic acid, dissected, and mounted on glass slides with Hoyer's medium. Observations (up to 1.000 ×), photographs and drawings were made with a Leica S6D dissecting microscope and a Leica DMLB compound microscope, both with a camera lucida and a photographic camera attached.

2.3. Morphometry

Different measurements of the head capsule and head appendages were taken with a micrometer. Measurements were used to calculate ratios, which are useful for characterizing shapes. Measured structures were adjusted as parallel as possible to the plane of the objective. The following measurements were taken. TL: total body length;

MW: maximum body width, measured at level of prothorax; HL: head length, measured medially along epicranial stem from anterior margin of frontoclypeus to occipital foramen; HW: maximum head width; AL: length of antenna, derived by adding the lengths of the first (A1L), second (A2L) and third (A3L) antennomeres; SeL: length of antennal sensorium; SL: length of stipes; MPL: length of maxillary palpus, obtained by adding the lengths of the first (MP1L), second (MP2L), third (MP3L) and fourth (MP4L) palpomeres; ML: length of maxilla, derived by adding SL and MPL, cardo omitted; LPL: length of labial palpus, obtained by adding the lengths of the first (LP1L) and second (LP2L) palpomeres; LigL: length of ligula; MtW: maximum width of mentum; PrmtL: length of prementum, measured from its base to the base of LP1; PrmtW: maximum width of prementum.

2.4. Chaetotaxy

Primary (present in first-instar larva) and secondary (arising in later instars) setae and pores were identified in the cephalic capsule and head appendages. Since only a third instar larva was studied, the primary chaetotaxy was interpreted and coded by comparison with *Chaetarthria* larvae and also with other hydrophilid genera for which the chaetotaxy is well known (e.g., *Enochrus*, *Hydramara*, *Tropisternus*, etc.) (Fikáček 2006; Fikáček et al. 2008, 2018; Byttebier and Torres 2009; Minoshima and Hayashi 2011, 2012, 2015; Torres et al. 2014; Minoshima et al. 2015; Rodriguez et al. 2015, 2018, 2020; Archangelsky 2016; Archangelsky et al. 2016, 2018; Minoshima 2019). Homologies were established using the criterion of similarity of position (Wiley 1981). Sensilla were coded with a number and two capital letters, usually corresponding to the first two letters of the name of the structure on which they are located. The following abbreviations were used. AN: antenna; FR: frontale; LA: labium; MN: mandible; MX: maxilla; PA: parietale; gAN: group of antennal sensilla; gAPP: group of sensilla on the inner appendage of the maxilla; gFR1, gFR2: group of sensilla on the frontale; gLA: group of sensilla on the labial palp; gMX: group of sensilla on the maxillary palp. To standardize some homology interpretations with regard to setae FR9–10 and FR5–6, we considered FR9 to be the most distal seta closest to FR14 and FR13; FR10 to be the most basal, closest to FR4; FR6 to be more anterior and mesal, closer to FR4 and FR7; and FR5 to be more posterior and closer to FR1–2.

2.5. Phylogenetic analysis

For the analysis 27 taxa, belonging to 14 tribes of Hydrophilidae were included; *Helophorus liguricus* Angus 1970 (Helophoridae) was used as the outgroup to root the trees (Supplementary file 1). The resulting matrix had 128 larval characters (Supplementary file 2). The data matrix was built with Mesquite (Maddison & Maddison, 2019), and analyzed with TNT (Goloboff and Catalano

2016) under maximum parsimony (MP) (Supplementary file 3). Traditional morphological characters follow Archangelsky (2004) and Archangelsky et al. (2021); chaetotaxic characters were newly coded for all taxa included. The analysis treated all characters as unordered and equally weighted. Heuristic searches were implemented using ‘tree bisection reconnection’ as algorithm, with 200 replicates and saving 300 trees per replication (previously setting ‘hold 60.000’). Node support was evaluated with Bremer support (Bremer 1994) and bootstrap resampling with 1.000 replications. Optimizations were performed using fast and slow algorithms with WINCLADA-ASADO 1.62 (Nixon 2002). All character state changes supporting the nodes were mapped on the most parsimonious tree.

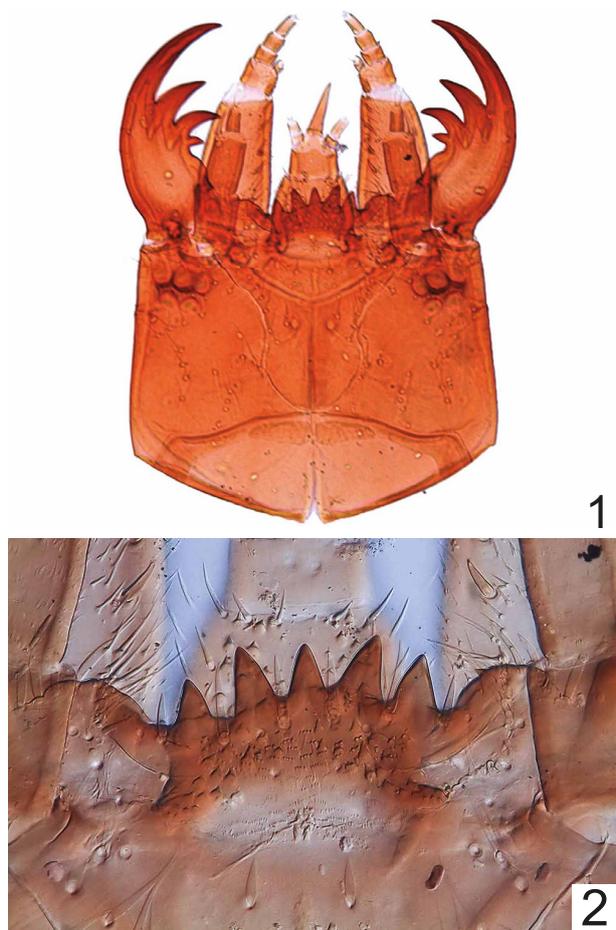
Since larvae of *Paracymus*-group appeared nested within Chaetarthriinae in our analyses and considering that based on molecular evidence they are not closely related, we performed a second analysis forcing the monophyly of Chaetarthriinae to examine the effect of *Tormus* + *Paracymus* on the topology. The analysis was implemented with the TNT *define constraints* option, and the constrained searches were carried out using the unconstrained settings.

In addition, we performed a Bayesian inference (BI) analysis using homoplasy as a partitioning criterion of discrete morphological characters (see Rosa et al. 2019). For the partition scheme, the homoplasy scores were calculated as implemented in TNT, with default concavity parameter ($k=3$). The characters were distributed into 11 partitions based on their homoplasy values (non-informative characters were assigned to their own partition) (see Supplementary file 4). The analyses were conducted in MrBayes 3.2.7 (Ronquist et al. 2012). The Mk model (Lewis 2001) with coding correction *lset coding=variable* (which corrects for lack of non-variable characters) was used, with equal rate variation across characters. We ran analyses for two independent runs (four chains each) of 5.000.000 generations each and sample frequency of 1.000. Burn-in was set to the first 25% of all samples and convergence was checked in Tracer 1.7.2 (Rambaut et al. 2013). In some of our preliminary and final analyses, *Paracymus* branches independently from Chaetarthriinae whereas *Tormus* appears deeply nested as a sister group of *Guyanobius*. Therefore, in order to avoid artificial topologies due to the inclusion of a taxon with convergent morphology, *Tormus* was excluded from these analyses.

3. Results

3.1. Third instar larva of *Guyanobius adocetus* Spangler, 1986

Diagnosis. The following combination of characters distinguishes *Guyanobius* larvae from any other known hydrophilid larvae.



Figures 1–2. *Guyanobius adocetus*, third instar larva. (1) head capsule, dorsal view; (2) detail of clypeolabrum, dorsal view.

Larval morphology. Head capsule subquadrate (Fig. 1); frontal lines inversely bell-shaped, converging towards occipital foramen but not coming together, coronal line absent; clypeolabrum symmetrical, nasale bearing five sharp teeth (Fig. 2), lateral ones slightly shorter; lateral lobes of epistome symmetrical, not projected farther than nasale, bearing a sharp spine projecting mesally; posterior tentorial grooves close to midline, subapical. Cervical sclerites present. Antenna short, first and second antennomeres subequal in length, basal one slightly wider; third antennomere short and narrow; first and second antennomeres bearing sharp cuticular spines on dorsal surface. Mandibles symmetrical, with three inner teeth, basal one smaller. Maxilla with large stipes, longer than palpus, dorsal face with sharp cuticular spines, apically with a stout spine on inner margin; first palpomere slightly longer, wider than long, incompletely sclerotized and bearing small cuticular spines on dorsal and inner faces, remaining palpomeres subequal in length. Labium stout, submentum wide, mentum subtrapezoidal, much wider than prementum, with strong cuticular projections on dorsal face; prementum wider than long with cuticular spines on anterior corners; palpi with few small cuticular spines, basal palpomere the shortest; ligula much longer than palpi, strongly sclerotized. Prothoracic plate large, covering most of pronotum, with sagittal line, sternal sclerite subrectangular, with sagittal line; meso- and metathorax

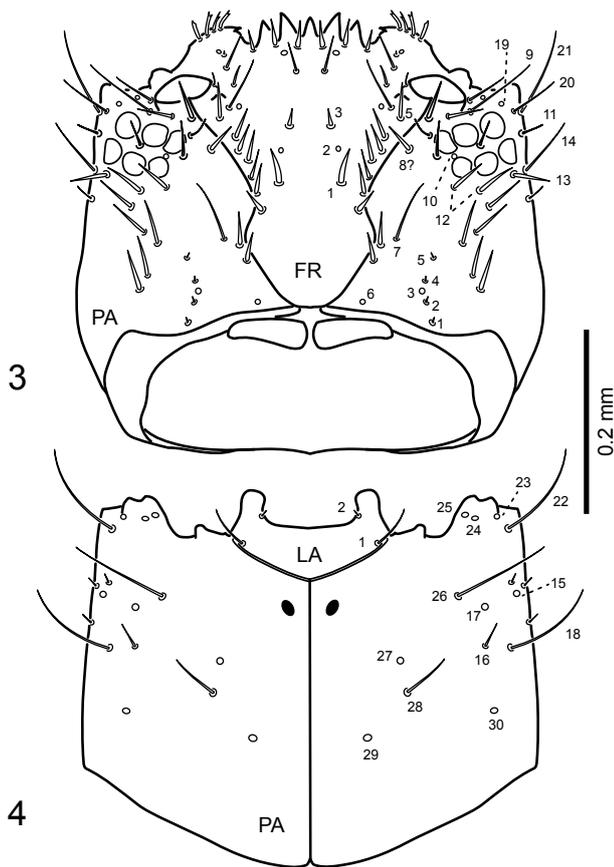
Table 1. Measurements (in mm) and ratios for different structures of third instar larva of *G. adocetus*. Abbreviations: see Material and Methods section.

Measure	<i>G. adocetus</i> L3	Measure	<i>G. adocetus</i> L3
TL	5.02	SL/MPL	1.00
MW	0.89	MP1L	0.04
HL	0.32	MP2L	0.03
HW	0.51	MP3L	0.03
HL/HW	0.63	MP4L	0.04
AL	0.20	ML	0.28
A1	0.08	LPL	0.04
A2	0.08	LP1L	0.01
A3	0.04	LP2L	0.03
SeL	0.04	LP2L/LP1L	3.00
SeL/A3	1.00	LigL	0.07
A1/A2	1.00	LigL/LPL	1.75
A1/(A2+A3)	0.67	MtW	0.11
HL/AL	1.60	PrmtW	0.06
HW/AL	2.55	PrmtL	0.04
SL	0.14	PrmtW/PrmtL	1.50
MPL	0.14	PrmtW/MtW	0.55

with pleural areas strongly lobed, with one pair of narrow subrectangular tergites, those of metathorax narrower. Legs short, five-segmented. Abdominal segments poorly sclerotized, with one pair of small oval sclerites dorsally and pleural areas strongly lobed; segment eight covered by a large dorsal plate; pleura and lateral margins of abdominal tergites bearing stout asperities.

Chaetotaxy. Frons with six secondary setae on each side along inner margin of frontal lines; gFR1 with eight setae, six stout, dorsal setae and two smaller setae ventrally, below two dorsal innermost setae; gFR2 with four stout setae, bifid apically; FR1 short and stout; pores FR15 not closely aggregated. Parietale with setae PA13 and PA14 closely aggregated; seta PA16 short; PA26–28 forming a triangle. Antenna with AN9 absent; SE1 as long as A3. Mandible with MN1 rather long, on basal fifth; minute seta MN5 closer to pore MN4 than to apex; MN2–4 forming a triangle. Maxilla with seta MX7 slender; setae MX8–11 stout and bifid apically; two secondary setae on ventral side near seta MX5; seta MX24 very long. Labium with 18 secondary setae on mentum along outer and anterolateral corners; seta LA5 rather long; seta LA10 at base of ligula; pore LA11 sub-basal. Morphometric measures are detailed in Table 1.

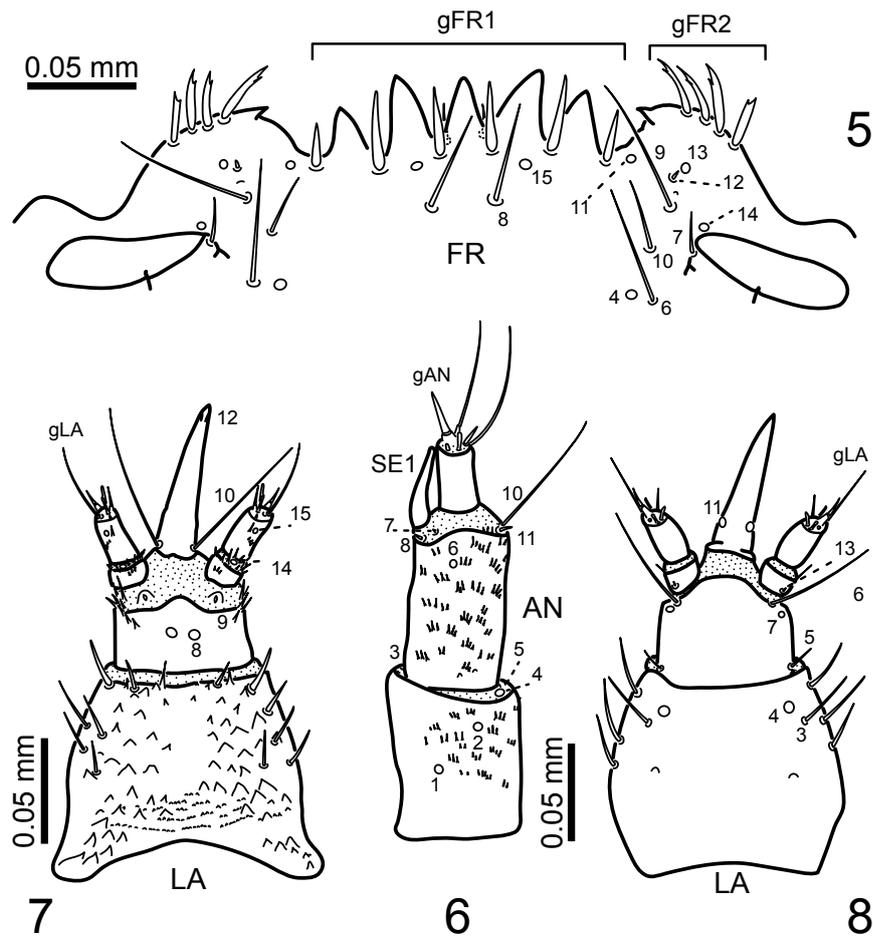
Description of chaetotaxy. Head capsule (Figs. 3–5). Frontale with 30 primary sensilla and 12 secondary setae: two stout setae at midlength close to frontal lines (FR1); two pores (FR2) and two short setae (FR3) closer to midline on distal half; two pairs of setae (FR5 stout and short, FR6 slender and rather long) and one pore (FR4) close to base of antennal socket; short seta (FR7) on inner margin of antennal socket; distal area of frontale in front of antennal socket with three setae (FR9 short, FR10 long,



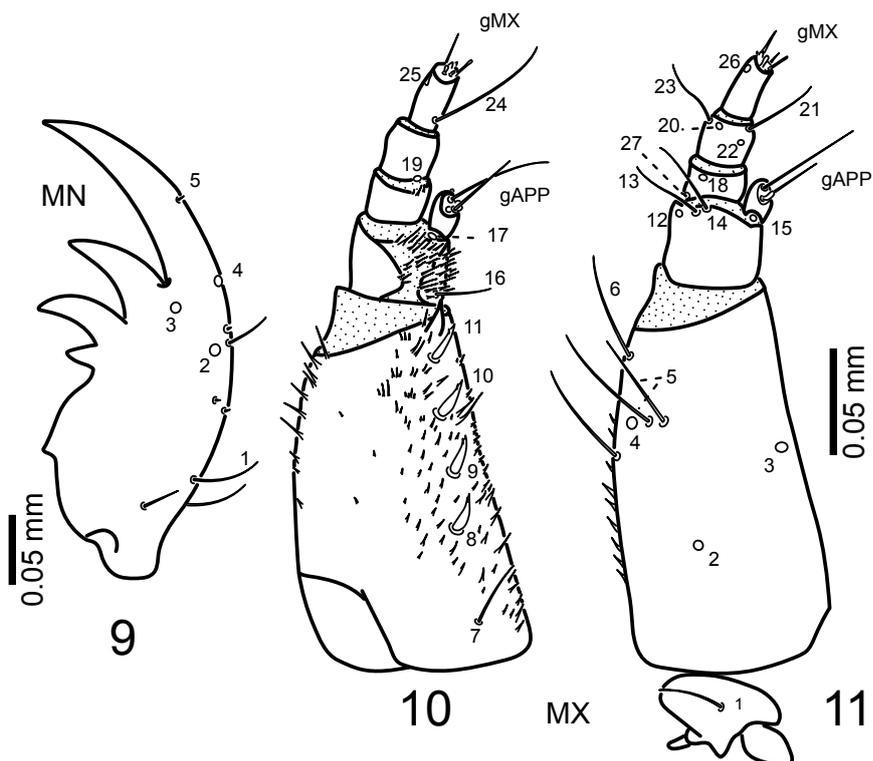
Figures 3–4. *Guyanobius adocetus*, third instar larva, head capsule. (3) dorsal view; (4) ventral view. Scale bar: 0.2 mm.

FR12 minute) and two pores (FR11 and FR13); central area behind nasale with one pair of rather long setae (FR8) and one pair of pores (FR15); nasale dorsally with six stout setae intercalated between teeth and two short setae ventrally, below dorsal two innermost setae (gFR1); each epistomal lobe with four stout and apically bifid curved setae (gFR2). Each parietale with 30 primary sensilla and 17–19 secondary sensoria on dorsal and lateral surfaces (16–18 setae and on pore). Dorsal surface with a basal longitudinal row of four minute setae (PA1, PA2, PA4, PA5) and one pore (PA3); one sub-basal pore (PA6) close to but not touching frontal lines; four setae arranged in an oblique row behind stemmata (PA7 rather long and slender, close to frontal line, PA12 and PA13 short and stout, PA14 rather long and slender on outer face); one stout and short seta (PA8) close to frontal line on distal third of parietale; one pore (PA10) between two innermost stemmata; three long setae (PA9, PA20, PA21) and one pore (PA19) distal to stemmata; one short seta (PA11) between PA14 and PA20; secondary setae arranged as follows: 3–4 along frontal lines, 6 behind stemmata, one between middle stemmata, two distal to stemmata and one on outer face; one secondary pore between seta PA9 and pore PA19. Ventral surface with three pores (PA23, PA24, PA25) and one long seta (PA22) on anterolateral corner, behind mandibular acetabulum; three setae (PA26 and PA18 long, PA16 short) and three pores (PA15, PA17, PA30) along outer margin; one rather short seta (PA28) and two pores (PA27, PA29) forming a triangle closer to

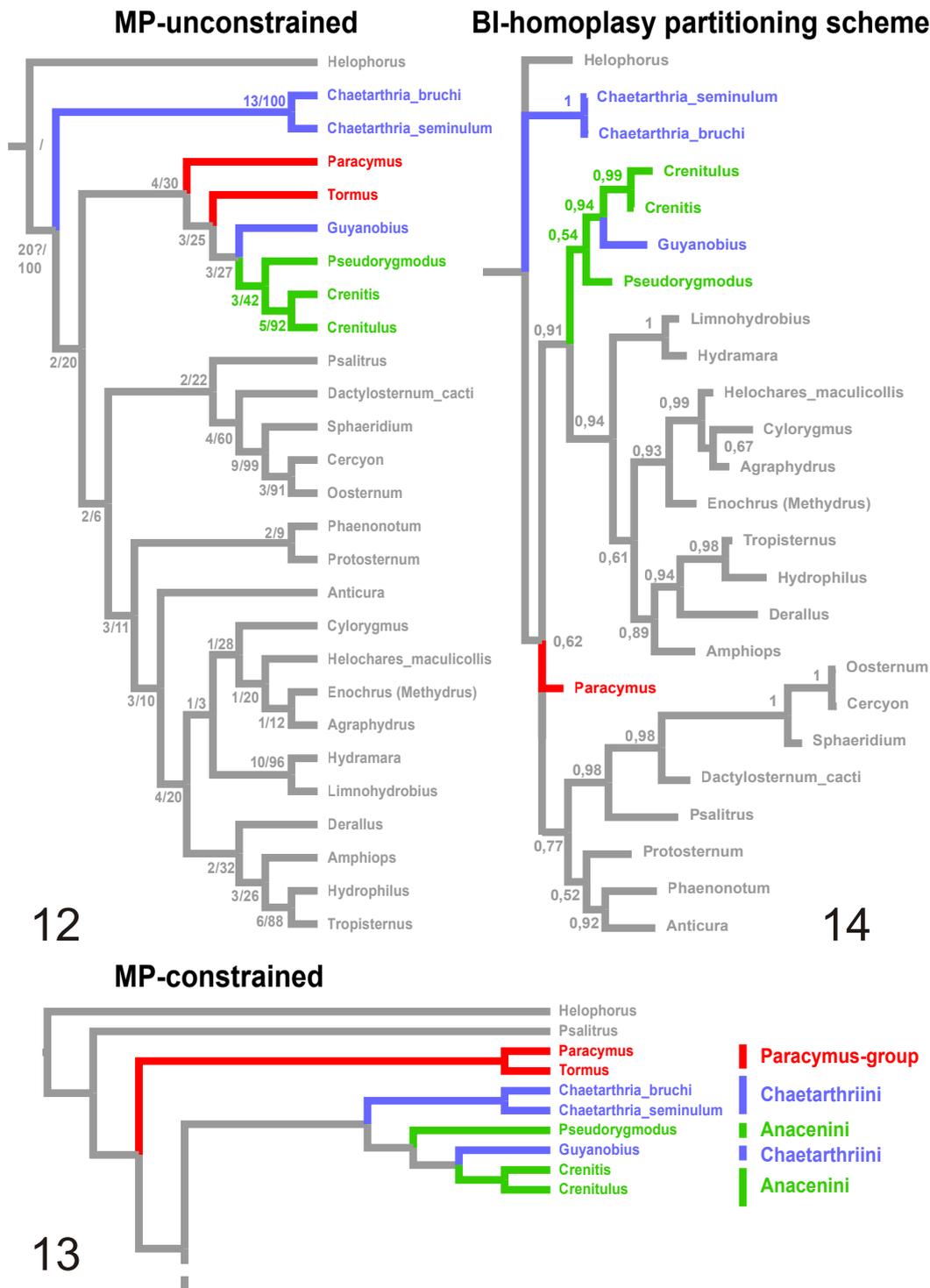
midline; three minute secondary setae on outer face close to pore PA15. **Antenna** (Fig. 6). A1 with five pores, three dorsal (AN1 at midlength closer to outer margin, AN2 subapical closer to inner margin, AN4 distally on membrane of inner margin) and two ventral on distal margin (AN3 on outer margin, AN5 on inner margin). A2 with one dorsal pore (AN6) on distal fourth, two minute apical setae on outer margin (AN7, AN8) close to base of SE1, and two apical setae on inner margin (AN10 long, AN11 very short); AN9 absent. A3 bearing a group of at least four short setae, one pore and two long setae (gAN); SE1 as long as A3. **Mandible** (Fig. 9). Bearing five primary sensilla and six or seven secondary setae; three dorsal pores on retinacular area arranged in a triangle (MN2 and MN4 on outer margin, MN3 at base of distal retinaculum); one rather long seta (MN1) on outer margin at basal fifth of mandible and one minute seta (MN5) on outer margin at distal quarter; pore MN6 not found; two secondary short sub-basal setae on outer margin and four or five more secondary setae near pore MN2, two or three minute behind MN2, one minute and one rather short between pores MN2 and MN4. **Maxilla** (Figs. 10–11). Cardio with a rather long seta (MX1); stipes with an inner row of five setae (MX7–11), MX7 slender, remaining ones stout and bifid apically; ventrally with three pores (MX2 at basal third, MX3 at midlength on inner margin, MX4 at distal third on outer margin) and four long setae on outer margin (MX6 subapical, MX5 close to MX4 and two secondary setae). MP1 dorsally with one basal hair-like seta (MX16) and one pore at base of appendage (MX17); ventrally with two long subapical setae (MX13, MX14) and two pores (MX12 on outer margin and MX15 at base of appendage); inner appendage with two long setae and two short sensoria (gAPP). MP2 with two pores, one ventral and apical (MX18) and one dorsal on membrane connecting with MP3 (MX19); minute seta MX27 basal on outer margin. MP3 ventrally with two rather long setae (MX21 on inner margin, MX23 on outer margin) and two pores (MX20, MX22). MP4 with one basal long seta (MX24) on inner margin and two sub-apical pores on outer face (MX25 digitiform and dorsal, MX29 ventral); a group of at least seven short sensoria constitute gMX. **Labium** (Figs. 4, 7–8). Submentum with two pairs of setae, one long (LA1), the other very short, on anterior margin (LA2). Mentum ventrally with two rather long setae (LA3) and two pores (LA4) close to anterolateral angle; distal and outer margins with nine pairs of stout secondary setae. Prementum ventrally with two pairs of setae (LA6 long and subapical, LA5 short, basal) and one pair of pores on distal margin (LA7); dorsally with one pair of pores on disc (LA8) and one pair of minute seta-like sensilla (LA9) on membrane connecting with labial palpi. Ligula with three pairs of sensilla, one pair of very long basal setae (LA10) and two pairs of pores (LA11 sub-basal and ventral, LA12 subapical and dorsal). LP1 with one minute seta (LA13, ventral) and one distal pore (LA14, dorsal) on membrane connecting with LP2; LP2 dorsally with one subapical pore on outer face (LA15); distally with a group of at least six or seven sensoria (gLA).



Figures 5–8. *Guyanobius adoce-tus*, third instar larva. (5) detail of clipeolabrum, dorsal view; (6) left antenna, dorsal view; (7) labium, dorsal view; (8) labium, ventral view. Scale bars: 0.05 mm.



Figures 9–11. *Guyanobius adoce-tus*, third instar larva. (9) right mandible, dorsal view; (10) left maxilla, dorsal view; (11) left maxilla, ventral view. Scale bars: 0.05 mm.



Figures 12–14. Unconstrained-MP analyses, most parsimonious tree (549 steps) with Bremer and Bootstrap values of support (12); Constrained-MP analyses, forcing Chaetarthriinae monophyly (13); unconstrained-BI analysis with homoplasy as partitioning scheme and mapped posterior probabilities (pp).

3.2. Cladistic analysis

The unconstrained MP analysis produced one most parsimonious tree (549 steps); the tree with support values is shown in Fig. 12. Chaetarthriinae appears as non-monophyletic, with *Chaetarthria* branching early on. The remaining genera of Chaetarthriinae cluster in one clade but including *Paracymus* + *Tormus* (Hydrophilinae: Lacobiini). The tribes Chaetarthriini and Anacaeniini were

not recovered as monophyletic. *Guyanobius* appears with low support as the sister group of the Anacaeniini (*Pseudorygmodus* (*Crenitis* *Crenitulus*)), and forms a clade with *Paracymus* and *Tormus*, which diverge earlier into two successive branches.

Figure 15 shows a detailed diagram of the Chaetarthriinae clade with the synapomorphies mapped on it. The complete most parsimonious tree with all synapomorphies is shown in Supplementary file 5.

To test if the resultant topology of the unconstrained analysis was not affected by the inclusion of *Paracymus* + *Tormus*, we performed a constrained MP forcing Chaetarthriinae monophyly. This analysis generated two most parsimonious trees (554 steps) with no differences in the divergence patterns for the subfamily; a detail of the Chaetarthriinae clade is shown in Figure 13. In this case, *Guyanobius* appears nested within Anacaenini, forming a cluster with *Crenitis* + *Crenitulus*.

The unconstrained BI with homoplasy as a partitioning scheme does not recover neither the subfamily nor the tribes as monophyletic (Fig. 14). *Chaetarthria* appears as a basal taxon, sister to all taxa included, and *Guyanobius* is revealed to be nested within Anacaenini. *Guyanobius* clustered with *Crenitis* + *Crenitulus* with a rather high support (pp=0.94) and *Pseudorygmodus* appears as sister group of all of them.

4. Discussion

4.1. Comparative notes between Chaetarthriinae

Larval knowledge of Chaetarthriini is rather limited, of the six recognized genera (four if *Apurebium* and *Venezuelobium* are considered variants of *Chaetarthria*) only *Chaetarthria* and *Guyanobius* have known larvae. Within *Guyanobius*, only the larva of *G. adocetus* has been described; therefore, within the tribe it can only be compared with larvae of *Chaetarthria*. Archangelsky (2002) provided a table comparing *Chaetarthria* and *Guyanobius* larvae but only a few morphological traits were mentioned. We provide an updated table (Table 2) that contains several morphological and chaetotaxic features that allow us to distinguish between *Chaetarthria* and *Guyanobius* larvae. Additionally, because our findings show that *Guyanobius* may be more closely related to (or potentially a member of) the Anacaenini, we include a comprehensive comparison with known Anacaenini larvae (*Pseudorygmodus*, *Crenitis*, *Crenitulus*).

4.2. Cladistic analysis

Chaetarthriinae was raised to subfamily level quite recently (Short and Fikáček 2013), grouping most genera previously included in the tribes Chaetarthriini and Anacaenini by Hansen (1991, 1999), and a few genera originally described in other subfamilies (i.e., *Pseudorygmodus*, *Horelophus*, *Phelea*) (Fikáček and Vondráček 2014; Fikáček and Watts 2015). Out of the 14 genera (13 if *Apurebium* and *Venezuelobium* are considered synonyms of *Chaetarthria* (Short 2009)) that comprise the subfamily only the larvae of five genera (38.5 % of the genera: *Chaetarthria*, *Guyanobius*, *Crenitis*, *Crenitulus* and *Pseudorygmodus*) could be included in this analysis since the remaining are unknown.

Neither the monophyly of Chaetarthriinae nor that of its tribes is supported by larval characters. Our results are in contrast to those analyses based on molecular data (Short and Fikáček 2013, Clarkson et al. 2019) in which Chaetarthriinae is recovered as monophyletic. The monophyly of Chaetarthriini and Anacaenini needs to be clarified. In the phylogeny of Short and Fikáček (2013) Chaetarthriini was resolved as a monophyletic grouping in the maximum parsimony analysis, but as a paraphyletic grade of two successively branching clades (*Chaetarthria*-group, *Guyanobius*-group) subordinate to the Anacaenini in the Bayesian analysis. On the other hand, the preliminary results of a more recent molecular study of Chaetarthriinae, do not support the monophyly of both tribes, being *Guyanobius* more closely related to a clade formed by *Anacaena*, *Notohydrus* and *Pseudorygmodus* (Clarkson et al. 2019). All analyses performed here support the clustering of *Guyanobius* with the tribe Anacaenini rather than with the tribe Chaetarthriini. Unconstrained MP analysis placed *Guyanobius* as sister of the Anacaenini with rather low statistical support. However, the topology is probably affected by the presence of *Tormus* and *Paracymus*. When these taxa are forced out of Chaetarthriinae in the constrained MP analysis (Fig. 13), the internal topology changes, and *Guyanobius* is revealed as sister of *Crenitis* + *Crenitulus*, deeply nested within Anacaenini. The same internal pattern appears with strong support in the unconstrained Bayesian analysis when *Tormus* is excluded (Fig. 14), indicating that (((*Pseudorygmodus*(*Guyanobius*(*Crenitis* *Crenitulus*))) is the most likely internal topology. Despite the fact that the results of these analyses do not agree with those obtained with the molecular data (Short and Fikáček 2013, Clarkson et al. 2019) some interesting characters can be discussed over the most parsimonious tree of the unconstrained MP analysis (Fig. 15).

The unusual morphology of *Chaetarthria* places it in a basal position, diverging early from the other taxa. This was expected since these larvae are unique within Hydrophilidae and display a high degree of modifications to the labroclypeus, stemmata, labium and legs, all of which are most likely related to a riparian lifestyle. For instance, these larvae present three unique apomorphies (Fig. 15): character 33(1), prementum incompletely sclerotized, this character is unique for *Chaetarthria* within Hydrophilidae, with the exception of the sphaeridiine genus *Protosternum* Sharp, in which second instar larvae have an incompletely sclerotized mentum, first instar larvae are unknown, and third instar larvae have a completely sclerotized mentum (Fikáček et al., 2018); character 39(1), a globular ligula, not found in other hydrophiloid genera; character 43(1), legs reduced, three-segmented, the only other genus with three-segmented larvae is *Georissus*, belonging to the hydrophiloid family Georissidae, a convergence. Other homoplastic characters worth mentioning are: character 3(1) stemmata closely aggregated forming one or two groups, shared with some Megasternini and Omicrini, although in these taxa the stemmata are clustered in two groups whereas in *Chaetarthria* the stemmata are aggregated forming only one; character 13(1) teeth of nasale dissimilar, this feature was coded for other hy-

Table 2. Comparative table of morphological and chaetotaxic characters among known Chaetarthriinae larvae (third instars). *Anacaena* is not included since its chaetotaxy has not been described.

Character	Chaetarthriini		Anacaenini		
	<i>Chaetarthria</i>	<i>Guyanobius</i>	<i>Crenitis</i>	<i>Crenitulus</i>	<i>Pseudorygmodus</i>
Nasale, symmetry	symmetrical	symmetrical	asymmetrical	asymmetrical	asymmetrical
Nasale, teeth	3 teeth, middle one much longer	5 teeth	5 teeth	5 teeth	5 teeth, lateral ones grouped
Epistomal lobes with inner spines	present	present	absent	absent	absent
Frontal lines	subparallel, widely separated basally	lyriform, converging towards base of head capsule			
Stemmata	closely aggregated, difficult to count	6 distinctly separated	6 distinctly separated	6 distinctly separated	6 distinctly separated
Antennal sculpture	A1 and A2 smooth	A1 and A2 with sharp cuticular spines	A1 and A2 with sharp cuticular spines	A1 and A2 with sharp cuticular spines	A1 and A2 smooth
Antenna	A1 not wider than A2, A3 as long as wide	A1 distinctly wider than A2, A3 longer than wide	A1 distinctly wider than A2, A3 longer than wide	A1 distinctly wider than A2, A3 longer than wide	A1 slightly wider than A2, A3 longer than wide
Mandible	with 2 retinacula	with 3 retinacula	with 3 retinacula	with 3 retinacula	with 2 retinacula
Stipes	without spine	with a stout apical spine on inner margin	without spine	without spine	without spine
Maxillary palpomere 1	smooth	with sharp dorsal and mesal cuticular spines			
Maxillary palpomere 1	incompletely sclerotized	incompletely sclerotized	completely sclerotized	incompletely sclerotized	incompletely sclerotized
Mentum	as wide as prementum	much wider than prementum	much wider than prementum	much wider than prementum	much wider than prementum
Prementum	incompletely sclerotized dorsally	completely sclerotized	completely sclerotized	completely sclerotized	completely sclerotized
Ligula	round, as long as labial palpi	elongate, subtriangular, longer than labial palpi	elongate, subtriangular, longer than labial palpi	elongate, subtriangular, longer than labial palpi	elongate, subtriangular, longer than labial palpi
Pronotal plate	without lateral projections	without lateral projections	with lateral projections	with lateral projections	with lateral projections
Legs	reduced, 3-segmented	normal, 5-segmented	normal, 5-segmented	normal, 5-segmented	normal, 5-segmented
Thoracic and abdominal pleura	slightly lobed	strongly lobed	strongly lobed	slightly lobed	strongly lobed
Abdominal tergum VIII	divided	entire	entire	entire	entire
Posterior margin of tergum VIII	not trifurcated	not trifurcated	trifurcated	trifurcated	trifurcated
Head capsule, latero-ventrally with many secondary setae	absent	absent	present	present	absent
gFR1	with 6 setae	with 8 setae	with 6 setae	with 6 setae	with 8 setae
gFR2	3–4 simple setae	4 bifid apically setae	4 simple setae	4 simple setae	3 simple setae
Secondary setae along frontal lines	at most 5 setae	at least 8 stout setae	3–4 setae	1 seta	no secondary setae
Setae FR4-6	arranged in a triangle	arranged in a straight line	arranged in a straight line	arranged in a triangle	arranged in a triangle
Seta FR12 position	posterior to FR13	posterior to FR13	anterior to FR13	anterior to FR13	anterior to FR13
Pore PA6 position	distant from frontal lines	distant from frontal lines	close to frontal lines	close to frontal lines	close to frontal lines
Setae MX8-11	apex simple	bifid apically	apex simple	apex simple	apex simple
Seta MX21 position	closer to inner edge of palpomere	closer to inner edge of palpomere	closer to inner edge of palpomere	closer to outer edge of palpomere	closer to outer edge of palpomere
Seta LA3	very long, slender	short, stout	short, slender	long, slender	?
Pore LA8 position	distal	at midlength	distal	distal	distal
Seta LA10 position	on intersegmental membrane	at base of ligula	on intersegmental membrane	on intersegmental membrane	?
Pore LA11 position on ligula	at midlength	basal	at midlength	at midlength	?

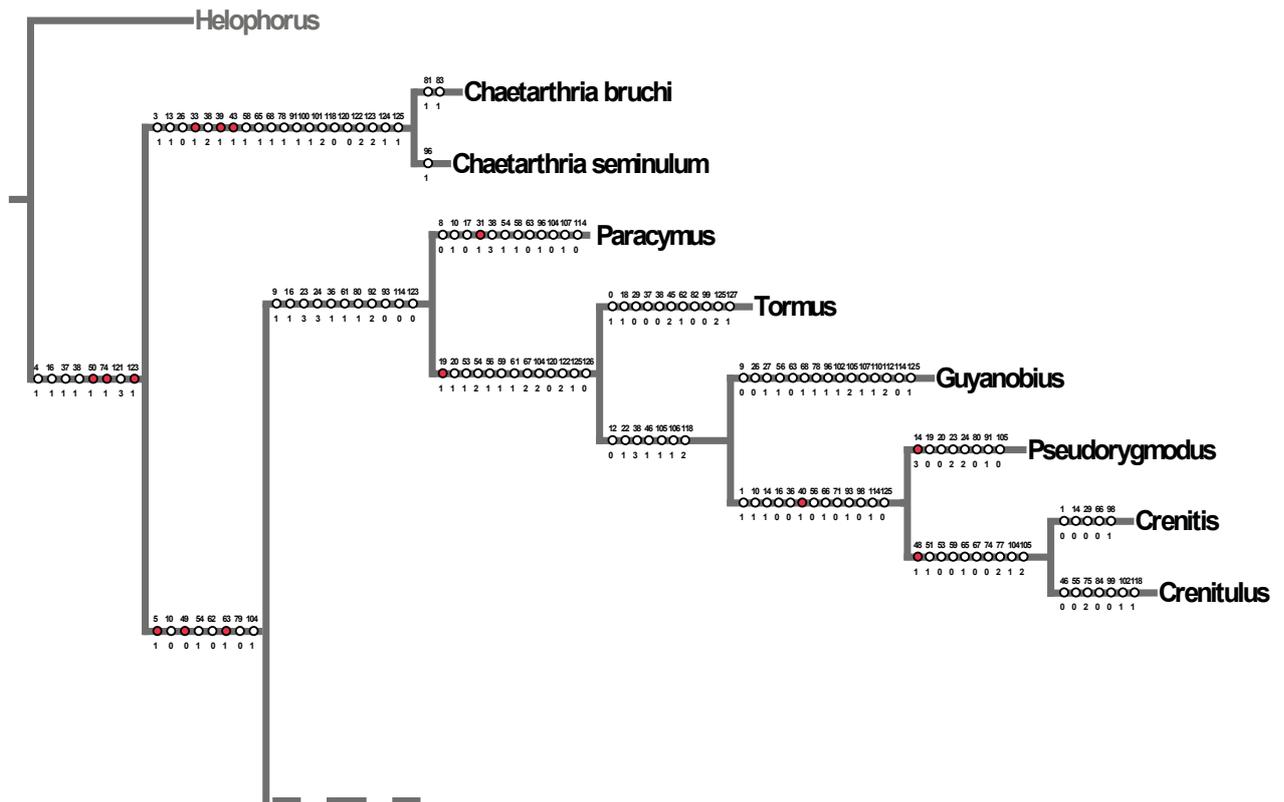


Figure 15. Detail of the tree that includes Chaetarthriinae with synapomorphies mapped. Characters in red indicate unique transformations; characters in white indicate homoplasious transformations.

drophilids but the configuration of the teeth of *Chaetarthria* is unique with the middle tooth being much larger than the others; character 58(1) FR7 rather long, present also in *Crenitis* + *Crenitulus*, *Cylorygmus* and *Acidocerinae*; character 68(1) seta FR12 posterior to pore FR13, shared with *Guyanobius* and only a few unrelated genera belonging to other subfamilies (*Enochrus*, *Agraphydrus* and *Cylorygmus*), a convergence; character 101(2) seta MN1 posterior to retinacular area, this character is unique within Chaetarthriinae, and is shared with only a few Sphaeridiinae genera (i.e., *Phaenonotum*, *Cercyon*). These features are strong enough to break the connection between *Chaetarthria* and the remaining genera of Chaetarthriinae; larvae with highly modified morphologies resulting from adaptations to live in new habitats generate topological problems in phylogenetic reconstructions (Archangelsky et al. 2021; Rodriguez et al. 2021).

The convergent larval morphology of *Paracymus* and *Tormus* with Chaetarthriinae (excluding *Chaetarthria*) also causes many problems in reconstructing the phylogeny of the group. Until quite recently, *Paracymus* was considered part of Anacaenini based on adult morphology (Hansen 1991, 1999). Subsequent molecular studies placed it close to the *Laccobius*-group in the subfamily Hydrophilinae (Short and Fikáček 2013). However, the position of the *Paracymus*-group (which also includes *Tormus*) of Laccobiini has always been problematic (see in figure 2 of Short and Fikáček 2013, the *Paracymus*-group is sister to the clade formed by Hydrophilini and Hydrobiusini). Furthermore, in an unpublished recent study based on larval characters (Rodriguez 2021), *Para-*

cymus and *Tormus* nest in a clade together with *Chaetarthria* and *Guyanobius*.

Paracymus larvae share several character states in common with Chaetarthriinae larvae. However, all of these characters are highly homoplastic and no unique synapomorphy can be mentioned. All of these features are shared with several genera of the family Hydrophilidae such as character 9(1) asymmetric nasale; characters 23(3) and 24(3) right and left mandibles with 3 retinacular teeth; character 61(1) pore FR2 inserted equidistant from FR1 and FR3; character 80(1) short sensilla PA20, character 92(2) pore MX3 in line with MX4 on the stipes; among others. In the case of *Tormus*, in addition to several homoplastic synapomorphies, there is a unique feature that supports the grouping with Chaetarthriinae (except *Chaetarthria*): character 19(1) surface of A1 with groups of fine cuticular spicules. Although not present in this work, this character state was also reported for *Enigmahydrus* and *Saphydrus* (Hydrophilidae: Cylominae) (see Seidel et al. 2020) and is likely to be a convergent character associated with semi-aquatic environments. *Tormus* larvae were described by Fikáček et al. (2013) and in many aspects are close to those of *Paracymus*. In the present study, *Tormus* is the most problematic taxon as it groups with *Guyanobius* in all the analyses, modifying the internal relationships of the clade, and does not nest with *Paracymus*. This could be related to the fact that while *Paracymus* larvae are aquatic *Tormus* larvae are not (Fikáček et al. 2013). In fact, *Tormus* and *Guyanobius* inhabit much more similar environments such as leaf packs lodged against rocks, logs in small streams near dense

rainforest (*Guyanobius*) and moss within the forest (*Tormus*) (Archangelsky 1997, Fikáček et al. 2013) although *Tormus* does not inhabit extremely water-soaked environments as *Guyanobius*. Therefore, *Tormus* shows several morphological characters associated with adaptations to a terrestrial life style (i.e. reduction of ligula and dense pubescence on the antennae, maxillae and labium). However, the relationship between *Tormus* and *Paracymus* is strongly supported by molecular studies and also by adult characters (Short and Fikáček, 2013; Toussaint and Short, 2018).

In spite of having two alternative positions within the Chaetarthriinae, all analyses agree that *Guyanobius* appears to be more closely related to Anacaenini than to Chaetarthriini. This clade is supported by nine homoplastic synapomorphies in the unconstrained MP analysis (Fig. 15). Some features worth mentioning are: character 12(0), nasale with five teeth, this is shared with only a few other genera (*Amphiops*, *Hydrarama*, *Limnohydrobius* and *Cylorygmus*), which belong to other subfamilies, a probable convergence; character 38(1) ligula longer than labial palpi, very long ligulas are unique for Chaetarthriinae, (only shared with *Paracymus*), but in *Chaetarthria* the ligula is slightly shorter than the labial palpi, related to a change in the shape of the ligula, which is round in *Chaetarthria* instead of elongated as in the other genera; character 46(1) abdominal segments with lateral conspicuous lobes, this feature is also found in *Derallus* but in this case the lobes are longer with several cuticular projections and is a convergence; character 106(1) setae LA1 short, only shared with few other unrelated genera (i.e., *Sphaeridium*, *Oosternum* and *Protosternum*); 118(2), ratio A1/A3 in third instar larvae, A1 much longer than A3, with a reversal in *Crenitulus* that has a slightly shorter A1.

The clade *Pseudorygmodes* + *Crenitis* + *Crenitulus* (Fig. 12) is defined by a unique synapomorphy and more than 10 homoplastic characters. The presence of pronotal lateral projections is a distinctive feature of this group, which is not shared with any other hydrophilid. *Crenitis* and *Crenitulus* are strongly supported by a unique synapomorphy, the posterior margin of abdominal tergite VIII trifurcate (character 48(1)), and 10 other homoplastic characters.

4.3. Concluding remarks

This paper provides a first insight into the relationships among the Chaetarthriinae based on larval morphological characters. The results of our work are affected by the low taxon sampling and the effect of evolutionary convergence. However, some interesting conclusions can be mentioned:

- (1) Our study does not support the monophyly of Chaetarthriinae and neither of its two tribes. Larval characters place *Guyanobius* closer to Anacaenini than to Chaetarthriini, making further analysis necessary to test their tribal position.
- (2) Larval characters are informative. Nonetheless, they are affected by derived or convergent morphologies re-

lated to different life strategies that generate topological problems.

- (3) Different sources of characters are necessary (larval, adult, molecular) to generate robust phylogenetic hypotheses.
- (4) Homoplasy partitioning seems to be an efficient strategy to analyze morphological data sets. The analysis implementing the homoplasy partitioning scheme proved to be more useful for solving some artifacts generated by convergent morphologies than the other alternative.

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Supplementary material 1

List of taxa studied for the analyses

Authors: Archangelsky M, Rodriguez G, Torres PLM (2022)

Data type: .docx

Explanation note: List of taxa (genera and species) examined for the study and source of information.

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Link: <https://doi.org/10.3897/asp.80.e76826.suppl1>

Supplementary material 2

List of characters and character states used in this study

Authors: Archangelsky M, Rodriguez G, Torres PLM (2022)

Data type: .docx

Explanation note: Characters and states used in the phylogenetic analyses.

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Link: <https://doi.org/10.3897/asp.80.e76826.suppl2>

Supplementary material 3

Data matrix (27×128) analyzed for this study

Authors: Archangelsky M, Rodriguez G, Torres PLM (2022)

Data type: .nex

Explanation note: Chaetarthriinae TNT data matrix

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Link: <https://doi.org/10.3897/asp.80.e76826.suppl3>

Supplementary material 4

Chaetarthriinae partitioning scheme and data matrix for the Bayesian inference analysis

Authors: Archangelsky M, Rodriguez G, Torres PLM (2022)

Data type: .docx

Explanation note: Partitioning scheme and data matrix for the Bayesian inference analysis.

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Link: <https://doi.org/10.3897/asp.80.e76826.suppl4>

Supplementary material 5

Chaetarthriinae most parsimonious tree with all synapomorphies mapped

Authors: Archangelsky M, Rodriguez G, Torres PLM (2022)

Data type: .pdf

Explanation note: Most parsimonious tree with all synapomorphies mapped. Characters in black indicate unique transformations; characters in white indicate homoplasious transformations.

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Link: <https://doi.org/10.3897/asp.80.e76826.suppl5>