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Description of the mature larva of *Austrolimnius nyctelioides* (Germain, 1892) and *A. elatus* Hinton, 1941 (Coleoptera: Elmidae)

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Abstract

Mature larvae of two species of the riffle beetle genus *Austrolimnius* Carter & Zeck, 1929 are described and illustrated for the first time: *Austrolimnius nyctelioides* (Germain, 1892) and *A. elatus* Hinton, 1941. A larval diagnosis for the genus is presented and both larvae are compared with each other and with the previously described larva of *A. mucubajiensis* Gómez & Bello, 2006. Comments on the species' habitats are included.

Key words: Elminae, riffle beetles, immature stages, taxonomy, Neotropical region, Patagonia

Introduction

Riffle beetles (Coleoptera: Elmidae) are common inhabitants of lotic ecosystems and have a worldwide distribution. Elmidae comprises two subfamilies: Larainae and Elminae. Larvae of both subfamilies and adult Elminae are considered strictly aquatic, while most adults of Larainae are riparian (Elliott 2008; Kodada et al. 2016). With 1498 species (Jäch et al. 2016), Elmidae is the fourth most speciose family of aquatic beetles (Jäch & Balke 2008). Regarding South American elmids, although in the last few years many contributions have been made, information is still far from complete (Monte & Mascagni 2012; Maier 2013). Knowledge of the immature stages of Elmidae is even scarcer; in South America larvae of only 22 genera are known (out of 41). In Argentina only nine elmid species have described larvae (Manzo 2013). However various papers have been published filling this gap (e.g. Manzo & Archangelsky 2001, 2008; Archangelsky & Manzo 2006, 2007; Archangelsky & Brand 2014). The inability to identify elmid larvae affects other areas of study such as ecology, biodiversity and systematics. Furthermore, since elmids are useful as bioindicators (García-Criado & Fernández-Aláez 1995; García-Criado *et al.* 1999, 2002; Miserendino & Archangelsky 2006; Dos Santos *et al.* 2011; Epele & Archangelsky 2012; Miserendino *et al.* 2012) studies to improve the identification of elmid larvae are urgently needed.

The genus *Austrolimnius* was described by Carter & Zeck (1929) and together with *Hydora* Broun, are the only genera that occur both in the Australian and Neotropical regions (Spangler & Brown 1981). *Austrolimnius* comprises 21 species in Central and South America (Manzo 2013), and only three species have been cited for Argentina: *A. eris* Hinton, 1971, *A. formosus* (Sharp, 1882) and *A. nyctelioides* (Germain, 1892) (Manzo 2007). The larvae of these species have not been described yet.

A. nyctelioides has the most restricted distribution of the three species and has been cited only for Chubut province, Argentina (Manzo 2007; Ottoboni Segura *et al.* 2013) and for Quillota province, Chile (Germain 1892; Ottoboni Segura *et al.* 2013). This species was originally described by Germain (1892) in the genus *Elmis* from Chile. Dèleve (1970) transferred the species to *Austrolimnius*. Manzo (2007) first cited *A. nyctelioides* for Argentina from Piedra Parada, Chubut province, and provided the first description of the male.

Austrolimnius elatus was described by Hinton (1941) with no details about the type locality except that it was in Chile. Hinton provided a detailed description of the male type but provided no illustrations. *A. elatus* was only known from the type locality.

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In this paper we describe the mature larva of *A. nyctelioides* and *A. elatus* for the first time.

Material and methods

Larvae and adults of *Austrolimnius nyctelioides* were collected in the Chubut River at Piedra Parada (Argentina, Chubut province, S 42º40'–70º05'W, 440 m) and in Nant y Fall creek, Road 17 to Corcovado (Argentina, Chubut province, 43°13'S–71°25'W, 665 m) in the fall and spring of 2004, 2007 and 2011. Adults and larvae of *A. elatus* were collected on the creeks La Cancha (S 42°46'–71°06'W), Rodeo (S 42°48'–71°07'W) and Esquel Viejo (S 42°51'–71°08'W) at the intersection with Road 40, in the summer of 2002.

Adults and larvae were collected either with a kick net or with a Surber sampler, and fixed *in situ* with 80% alcohol (specimens collected with the net) or with 4% formaldehyde (specimens collected with the Surber sampler). Specimens were cleared in lactic acid, dissected and mounted on slides for observation and description; the mounting media were Hoyer's and Polyvinyl-Lactoglycerol (PVLG). Observations (up to 1000 X) and illustrations were made using a Leica MZ6 dissecting microscope and DM500 and DMLB compound microscopes, all with a camera attached. Photographs were assembled using the freeware software CombineZP (Hadley 2010). In order to be consistent with the larval morphological nomenclature, we follow Kodada *et al.* (2016).

Adults were identified using the keys of Manzo (2007) and Hinton (1941); larvae were identified at genus level with the keys of Manzo & Archangelsky (2008, 2014).

Morphometric measurements were made on 5 specimens of each species.

Results

Diagnosis of mature larvae of *Austrolimnius* Carter & Zeck

Dorsal body surface with tubercles arranged in longitudinal rows. Anterior margin of head lacking tooth between base of antenna and clypeus. Second antennomere bearing a long sensorium, longer than the third antennomere and as long as or longer than second antennomere. Prothorax (Figs 6, 18) with six ventral sclerites (? divided basisternum, ? episterna and epimera), meso- and metathorax with five ventral sclerites (? basisternum, ? episterna and epimera); procoxal cavities open (Fig. 30). Pleural sclerites on abdominal segments I-VII. Apical emargination on segment IX extremely shallow. Abdominal segments with six dorsal, longitudinal rows of setiferous tubercles.

Austrolimnius nyctelioides, mature larva

Body (Figs 1, 2) elongate, slightly flattened dorsoventrally, widest at thorax, abdominal segments narrowing towards posterior end; body subtriangular in cross-section. Posterior margin of body segments with setiferous tubercles, dorsal ones 3.56 times as long as wide. Dorsal surface of thorax and abdomen with tubercles arranged in longitudinal rows (partially arranged in prothorax). Color yellowish to light brown. Length: 3.0–3.5 mm; maximum width: 0.6–0.7 mm.

Head capsule (Fig. 5) exposed to moderately covered by pronotum, anterior margin lacking tooth between base of antenna and clypeus. Surface with several ramose setae, mostly on disc of frons, and short slender setae on margin of parietale; various long ramose setae surrounding stemmata and two long blunt setae between frontal line and stemmata. Coronal line very short and broad, frontal lines long, extending to inner margin of antennal sockets. Frontoclypeal suture feeble; clypeal margin smooth, slightly convex. Gula subtrapezoidal, narrower than maxillo-labial complex; basal margin wider and concave, distal margin narrower and convex; gular sutures poorly defined. Eyes with five stemmata on each side of head behind base of antenna.

Labrum subrectangular, slightly wider in anterior third; anterior margin slightly convex, anterolateral margins rounded, each with a row of three strong dorsal setae, outer two ramose, inner one stout and blunt; dorsal surface with six strong ramose setae arranged in a transverse row on anterior third. Ventral surface with anterior row of ramose setae, rest of ventral surface covered by short pubescence oriented mediad and posteriorly.

FIGURES 1–4. Habitus of mature *Austrolimnius* larvae. 1) dorsal view of A. *nyctelioides*; 2) ventral view of *A. nyctelioides; 3*) dorsal view of *A. elatus*; 4) ventral view of *A. elatus*. Scale bars, Figs 1–4: 1 mm.

Antennae (Fig. 6) short, with three antennomeres, located on anterolateral corners of head capsule. Basal antennomere short, conical, wider than long, apically with a crown of ramose setae; second antennomere the longest, cylindrical, with a few short distal setae, bearing a long sensorium, subequal in length to second antennomere. Third antennomere the shortest, much shorter than sensorium of second antennomere, bearing a long apical seta.

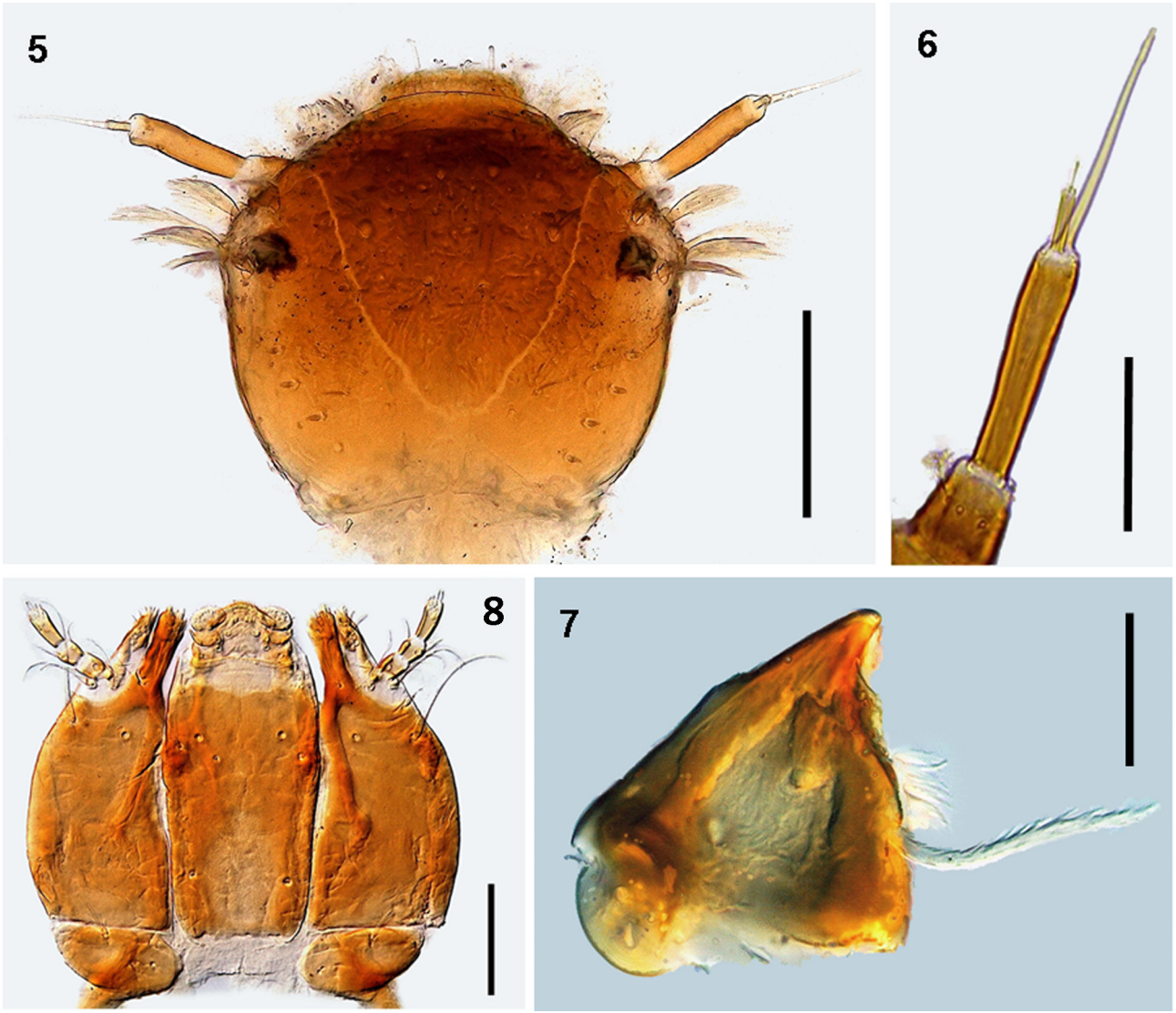
Mandibles (Fig. 7) symmetrical, subtriangular, as long as wide. Apex with three blunt teeth. Dorsal surface with inner margin straight and sharp. Ventral surface with inner margin slightly concave, bearing a comb of long stout submarginal setae. Inner margin with long plumose prostheca; outer margin lacking ramose setae close to midlength.

Maxillae (Fig. 8) with cardo short, irregularly suboval, transverse, 2.30–2.72 times wider than long, with stout ramose seta close to outer margin. Stipes the largest part, subrectangular, 1.60–1.74 times as long as wide, distal third with several setae distributed as follows: two long setae on outer margin (distal one ramose and shorter than basal one), inner margin with one short stout seta, distal margin with one ramose seta close to base of lacinia. Lacinia and galea well developed; lacinia subtriangular, fused to stipes, with inner margin bearing a group of stout setae; galea one-segmented, shorter than lacinia, elongate, with several apical setae. Palpus with four palpomeres, first palpomere the shortest, wider than long, second and third palpomeres slightly narrower, subequal in length, last palpomere slightly longer, narrower; first palpomere bearing one outer ramose seta, third palpomere with two setae, one on each anterolateral corner, last palpomere bearing several short apical setae and sensoria.

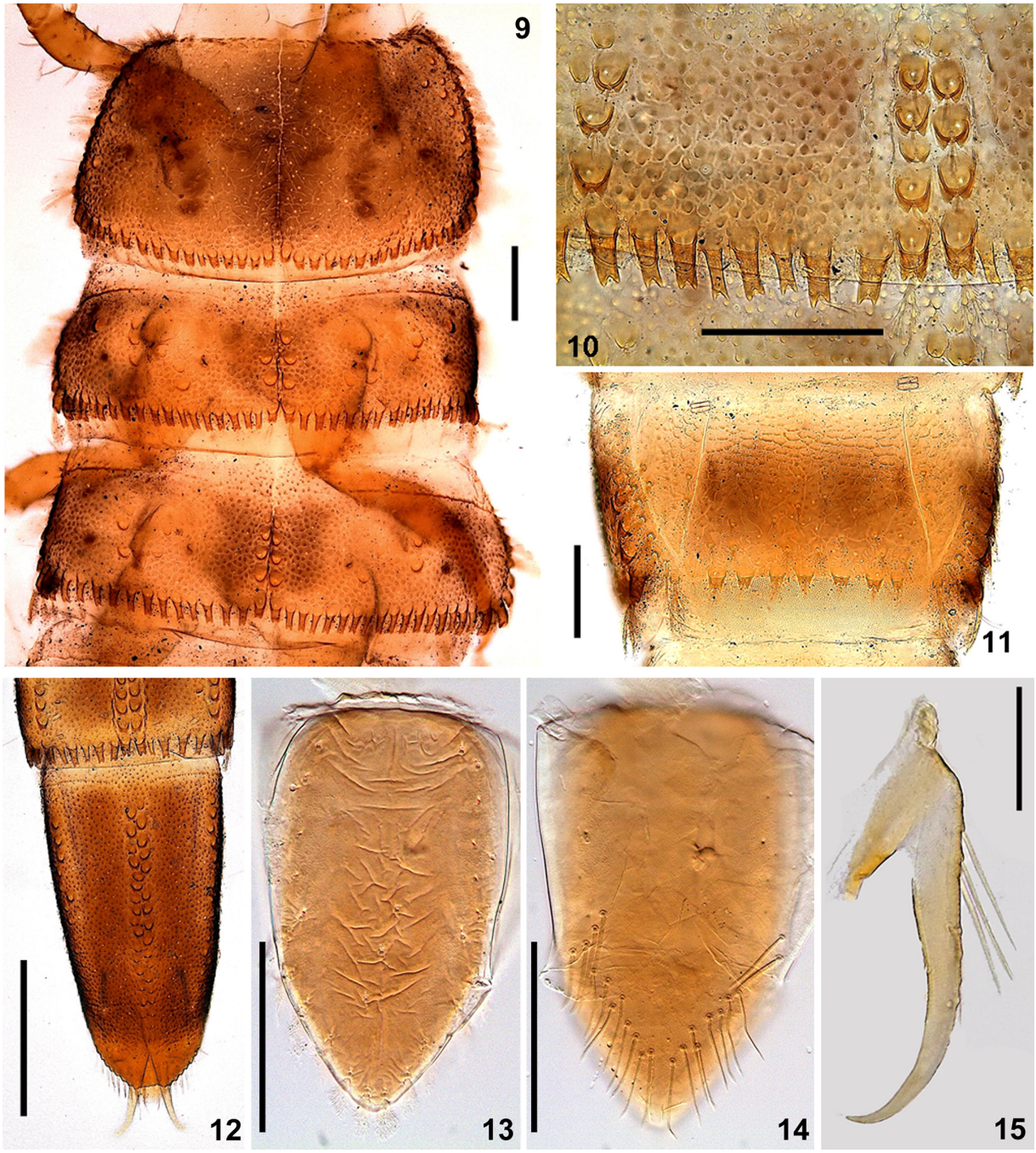
Labium (Fig. 8) large, subdivided into a longer postmentum and a shorter prementum, forming together with maxillae the maxillo-labial complex; postmentum subrectangular, 1.76–1.79 times as long as wide, ventral surface

with several short ramose setae at each side of midline, basal corners each with a large and stout ramose seta, distal corners each with a one long stout seta. Prementum short, poorly sclerotized, wider than long, distal margins densely setose; palps with two palpomeres, basal palpomere slightly shorter, distal palpomere with several distal setae and sensoria.

Thorax (Fig. 9) strongly sclerotized; tergal plates with sagittal lines and with large setiferous tubercles arranged as follows: pronotum with an irregularly arranged row of tubercles on lateral margins; meso- and metanotum with one row of tubercles on each side of sagittal line, a group of tubercles admedially, and a row sublaterally. Lateral margins of thoracic segments sagittate, with large ramose setae. Prothorax the largest segment, 1.5–1.81 times as wide as long; pronotum subtrapezoidal, anterior and posterior corners rounded; ventral side with six sclerites: one large and irregularly shaped transverse anterolateral pair (? divided basisternum), one small, triangular lateral pair (? episterna), one large posterolateral pair (? epimera); coxal cavities open. Meso- and metathorax shorter than prothorax, 3.09 times as wide as long; each segment ventrally with five sclerites: one large anterior subpentagonal sclerite (? basisternum), and two smaller subrectangular sclerites on each side (? episterna and epimera); coxal cavities open. Legs: five-segmented; coxa the largest segment, subtriangular; trochanter smaller, subtriangular; femur and tibia elongate, femur slightly longer and wider than tibia; claw stout, slightly shorter than tibia; surface with several short spines.

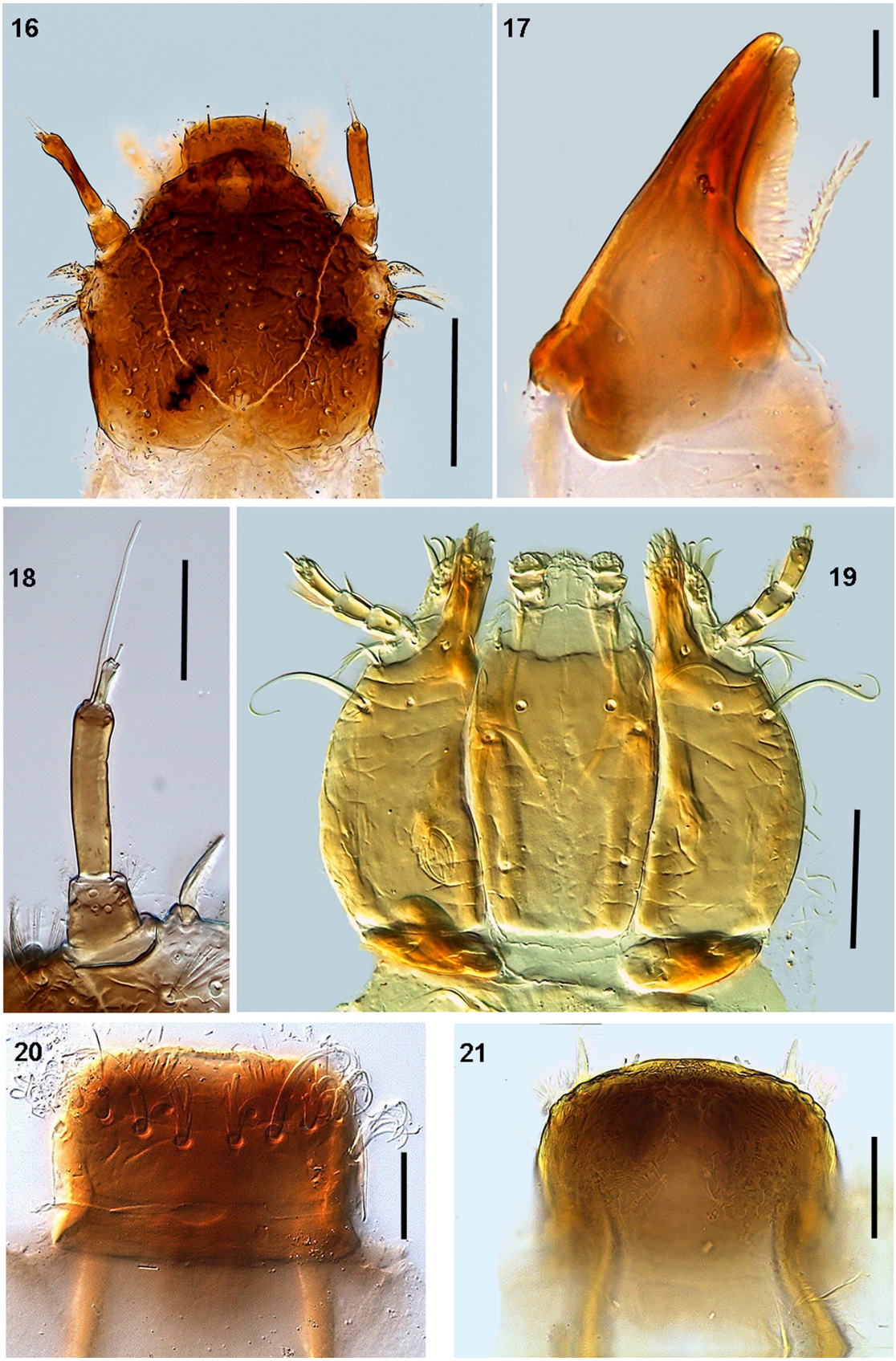


FIGURES 5–8. Mature larvae of *Austrolimnius nyctelioides*. 5) head capsule, dorsal view; 6) antenna, dorsal view; 7) right mandible, ventral view; 8) maxillo-labial complex, ventral view. Scale bars, Fig. 5: 0.1 mm; Figs 6, 8: 0.05 mm, Fig. 7: 0.025 mm.

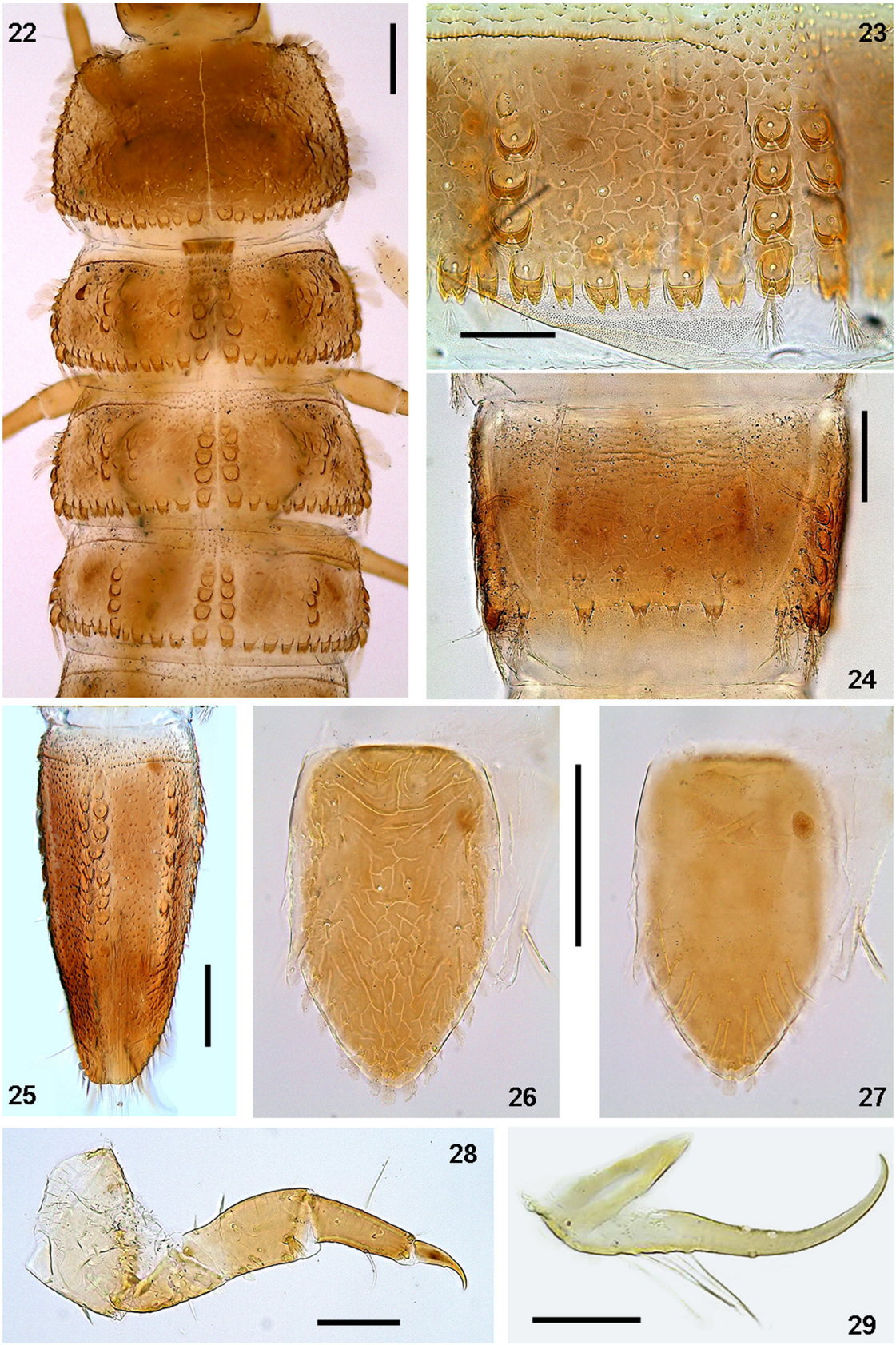


FIGURES 9–15. Mature larva of *Austrolimnius nyctelioides*. 9) thorax, dorsal view; 10) tergum of fifth abdominal segment, showing central and median rows of tubercles and setiferous tubercles on posterior margin; 11) sternum of abdominal segment VII; 12) abdominal segment IX; 13) operculum, external view; 14) operculum internal view; 15) hook of gill chamber. Scale bars: Figs 9–11, 13–14: 0.01 mm; Fig 12: 0.25 mm; Fig 15: 0.05 mm.

Abdomen (Figs 10–15) well sclerotized, composed of nine segments, tapering towards posterior end; segments I–VII with sagittal line poorly defined, subequal in length; segment IX the longest. Tergal plates with tubercles arranged in six longitudinal rows on segments I–IX, one central pair, one median pair and one lateral pair on each tergite; lateral tubercles less evident in the last segment. Pleural sclerites present on segments I–VII; sterna of segments I–VII subrectangular, wider than long. Segment VIII annular, ring-like. Segment IX elongate, 2.75–2.94 times as long as segment VIII, without dorsal keel, ventrally with several spines on distal half; sternal area with apical gill chamber, operculum subpentagonal, distal end smoothly pointed with several strong and ramose setae externally and marginal sharp setae internally, covering a pair of strong distal hooks with inner margin smooth and bearing several long setae on outer margin. Spiracles present on segments I–VIII.



FIGURES 16–21. Mature larvae of *Austrolimnius elatus*. 16) head capsule, dorsal view; 17) right mandible, ventral view; 18) antenna, dorsal view; 19) maxillo-labial complex, ventral view; 20) labrum, dorsal view; 21) labrum ventral view. Scale bars, Fig. 16: 0.1 mm; Fig. 17: 0.01 mm; Figs 18–19: 0.05 mm; Figs 20–21: 0.025 mm.



FIGURES 22–29. Mature larva of *Austrolimnius elatus*. 22) thorax and first abdominal segment, dorsal view; 23) tergum of abdominal segment V, showing central and median rows of tubercles and setiferous tubercles on posterior margin; 24) sternum of abdominal segment VII; 25) abdominal segment IX; 26) operculum, ventral view; 27) operculum dorsal view; 28) prothoracic leg; 29) hook of gill chamber. Scale bars: Figs 22, 24–28:0.1mm; Figs 23, 29: 0.05 mm.

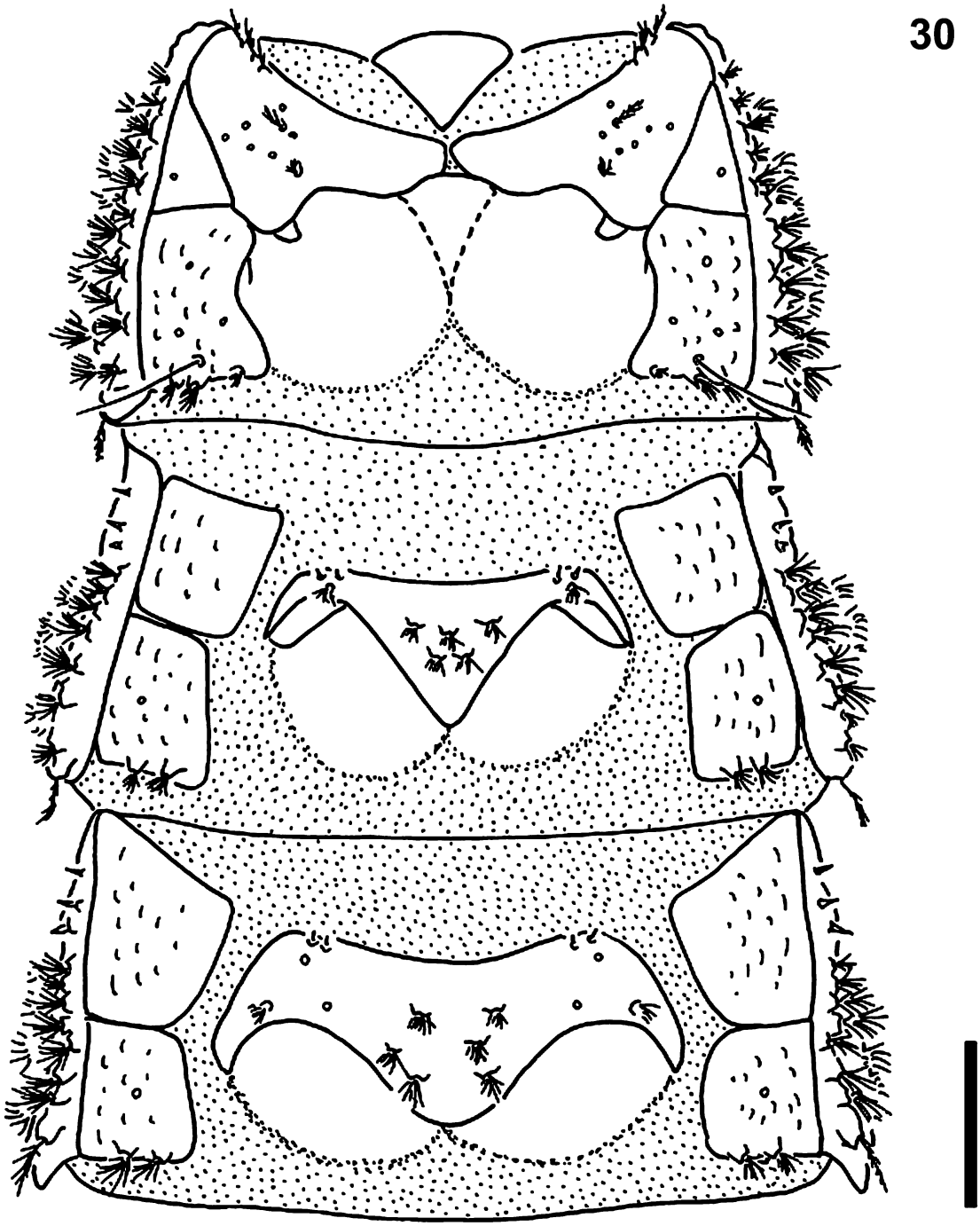


FIGURE 30. Thorax of mature larva of *Austrolimnius elatus*, ventral view. Scale bar: 0.1 mm.

Habitat. Nant y Fall stream is a 3rd order stream tributary of the Futaleufú river, and is located in a transitional mountain and piedmont area in the Northwest of Chubut province, Argentina. It belongs to the ecotone between the Subantarctic forest and the Patagonian steppe phytogeographical provinces. The substrate at the study site is composed of gravel, cobbles, and boulders, with sand and smaller fractions in pools and depositional areas. Aquatic vegetation is abundant and diverse, with *Isoetes savatieri* being the dominant species in riffles and *Myriophyllum quitense* and *Callitriche lechleri* in pools. Water flow ranged from 0.22 to 1.16 ms-1; water temperature ranged from 4 to 18 °C, with an annual average of 11.6 °C; and during the entire year the water

remains well oxygenated (9.95–13.88 mgl-1 O2) and above saturation (114.1–121.4 %) (Brand & Miserendino 2012, Brand *et al*. 2012).

The Chubut river flows from west to east and drains to the Atlantic Ocean. Major part of the river is located in the Patagonian steppe, where the lack of precipitation on the Patagonian Plateau produces a xerophytic vegetation. Water temperature ranged from 5 °C to 22.2 °C. Water flow ranged from 0.2 m s^{-1} to 2.1 m s^{-1} (Miserendino & Archangelsky, 2006). The specimens of *A. nyctelioides* were collected at Piedra Parada, which is near the middle-basin of the river. The substrate consisted mostly of boulders, cobbles, and pebbles (Miserendino & Archangelsky, 2006).

Austrolimnius elatus mature larva

Body (Figs 3, 4) with sides subparallel, semicircular in cross-section. Color pale yellowish. Posterior margins of body segments with setiferous tubercles, dorsal ones 1.20–1.47 times as long as wide. Length: 3.5 mm; maximum width: 0.4–0.5 mm.

Head (Figs 16–21): head capsule and head appendages similar to *A. nyctelioides*, except for: 1- mandibles (Fig. 20), narrower at base, maximum length / maximum width ratio of 1.66; 2- cardo proportionally shorter (Fig. 19), width / length ratio of 3.65; 3- stipes (Fig. 19) 1.60-1,78 times as long as wide; 4- postmentum slightly shorter (Fig. 19), 1.71–1.72 times as long as wide.

Thorax (Figs 22, 30): similar to *A. nyctelioides*, except for shorter and narrower prothorax, 1.64–1.86 times as wide as long.

Abdomen (Figs 23–29) similar to *A. nyctelioides*, except for segment IX longer, 2.76–3.07 times as long as segment VIII.

Habitat. Esquel Viejo, Rodeo and La Cancha creeks are shallow streams, in northwestern Chubut province, Argentina and are located in the ecotone between the Subantarctic forest and the Patagonian steppe phytogeographical provinces. This area is characterized by a gramineous steppe (dominant species *Festuca pallescens*) that gets into *Nothofagus spp* forest (León *et al*., 1998).

Comparative notes

As previously mentioned, the knowledge of immature stages in elmids is poor; this is particularly true for *Austrolimnius* since, until this contribution, the only described larva was that of *A. mucubajiensis*. Larvae of *A. mucubajiensis* differ from those of *A. elatus* and *A. nyctelioides* mostly in size, being longer (length: 4.48 mm) and wider (width: 0.77 mm). There are differences in color—the larva of *A. mucubajiensis* is reddish-yellow, while the larva of *A. nyctelioides* is yellowish-brown and that of *A. elatus* is pale-yellowish. There are also differences in the mandibles: according to the description of Gómez & Bello (2006) *A. mucubajiensis* has mandibles with bidentate apex, while *A. nyctelioides* and *A. elatus* have tridentate mandibles; nevertheless, this character must be verified for *A. mucubajiensis*. Finally, *A. nyctelioides* and *A. mucubajiensis* have in common a body that is subtriangular in cross section, while *A. elatus* has a body that is semicircular in cross section.

Between *A. nyctelioides* and *A. elatus*, the most prominent differences are morphometric: *A. nyctelioides* larvae are wider, with a mean body length-width ratio of 5.49 while *A. elatus* has a mean body length-width ratio of 7.50. Other distinguishing differences are related to the mouthparts: mandibles of *A. nyctelioides* are as long as wide while those of *A. elatus* are almost twice as long as wide; and, the cardo of *A. nyctelioides* is longer than that of *A. elatus*. Another difference is in the length of the setiferous tubercles on the posterior tergal margin, those of *A. nyctelioides* are longer than those of *A. elatus*.

With this contribution the number of described *Austrolimnius* larvae increases to three and the number of Argentinean elmid species with described larvae increases to eleven.

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