



Molecular phylogeny of the riffle beetle genus *Hexanchorus* revealed a presence of a new genus (Coleoptera: Elmidae)

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Abstract

The riffle beetle genus *Hexanchorus* Sharp is, with 25 known species, the most speciose genus of the subfamily Larinae in the Neotropics and the second largest globally. An analysis of its phylogeny, based on two mitochondrial (COI, 16S) and two nuclear (18S, 28S) markers, including *Hexanchorus*-like, but morphologically distinct specimens, supported presence of an unknown genus. The new genus, *Rumilara* **gen. nov.**, is described here with four new species (*R. obscura* **sp. nov.**, *R. paterna* **sp. nov.**, *R. riberai* **sp. nov.**, *R. suppressa* **sp. nov.**) and their larvae. The separate position of the new genus is, beside molecular differences, well substantiated by the morphology of adults (the absence of sexual dimorphism, structure of the pronotum) and larvae (pleurites never reaching to sixth abdominal ventrite). *H. sagittatus* **stat. nov.** is elevated to species rank based on molecular data, and *Rumilara leleupi* (Delève) **comb. nov.** is transferred from the genus *Hexanchorus* and redescribed, based on its morphology.

Key words

Ecuador, South America, Larinae, *Rumilara*, taxonomy, new genus, new species, molecular phylogeny, larva

1. Introduction

The family Elmidae is presently divided into two subfamilies: Larinae and Elminae. The Larinae consist mostly of active fliers with shorter life span, always associated with running water (Kodada et al. 2016). Globally, there are 29 known genera in the subfamily with well over 150 known species (Jäch et al. 2016; Barr and Shepard 2021). The most speciose and the widest spread genus in the Neotropics is *Hexanchorus* Sharp with 25 known

species. It is distributed from Mexico to southern Brazil and Argentina (Linský et al. 2019). The genus was erected on *H. gracilipes* collected in Mexico by Sharp (1882) who noticed that male abdomen has basal segments depressed in the middle, and the fifth ventrite emarginate on the apex. Female abdominal segments are convex, with the terminal one markedly less emarginate than in males. This sexual dimorphism is present in all described *Hex-*

anchorus species, except *H. leleupi* Delève from Ecuador (not specified in its original description). This species was described from Napo Province and was not reported since its collection (Delève 1968; Linský et al. 2019). It is also the only *Hexanchorus* species with elytral carinae (Maier 2013). The distribution of all known species was recently summarized (Linský et al. 2019).

Larva and pupa of *H. gracilipes* were described and illustrated for the first time in the study of Mexican rifle beetles by Hinton (1940). The distinctive, moderately compressed, elongate-ovate *Hexanchorus* larva superficially resembles that of *Stegoelmis* Hinton. Both larvae have lateral extensions bearing setae, and the same configuration of sclerites and pleurites on the ventral side but can be readily distinguished by the presence of two large tubercles on the eighth abdominal segment, a number of stemmata, and shape of the apex of the ninth abdominal segment (Manzo and Archangelsky 2008; Segura et al. 2011). This similarity confused Hinton (1940) who included two larvae of *Stegoelmis* in his key to the larvae of *Hexanchorus*. It was not until half a century later when the larva of *Stegoelmis* was described correctly by Spangler (1990). In the meantime, Bertrand (1972) redescribed *Hexanchorus* larva and Green (1972) described larva of *Hexanchorus caraibus* (Coquerel, 1851). Larvae of *H. gracilipes* and *H. caraibus* were redescribed and illustrated by Spangler and Santiago-Fragoso (1992).

The phylogeny of the subfamily Larainae, whether based on morphological or molecular characters, has not yet been properly analyzed. The only published molecular phylogeny of the family included only two Larainae genera resolved as not closely related to each other, indicating that the subfamily may be not monophyletic (Kobayashi et al. 2021). Molecular phylogeny of Byrrhoidea–Buprestoidea complex points to the same conclusion (Kundrata et al. 2017). At the genus level, with more information becoming available about *Hexanchorus*, relatively significant morphological variations appear, but the monophyly of the genus has not yet been seriously questioned. Hinton (1940) remarked that the genus *Potamophilops* Grouvelle could be congeneric with *Hexanchorus*, after he examined the type species *P. cinereus* Blanchard. Both genera were later separated by the presence of anal cell in the hind wing in Brown's (1981) key of the world genera of Larainae. Both genera share pronotum with distinct transverse impression in the anterior third, however, this character occurs also in *Hispaniolara* Brown and *Pseudodisersus* Brown not described in Hinton's time. The genus *Hexanchorus* can be distinguished from these three genera by the absence of anal cell and by its smaller size (Brown 1981).

The monophyly of *Hexanchorus* is supported by the following characters: smaller body size; pronotum with distinct transverse impression (Spangler and Santiago-Fragoso 1992); hind wing venation (Brown 1981); apex of fourth tarsal segment ventrally with a fine nearly erect seta; sexual dimorphism (Hinton 1940); membranous sac of penis with a laterally placed fibula (Linský et al. 2019, described as “oblong sclerotized structure”, hereafter referred to as fibula major; Fig. 10f); and larva with spiracles on apices of large posterolateral gibbositities

of the eighth abdominal segment (e.g., Manzo and Archangelsky 2008).

The use of molecular data, or more specifically DNA barcoding, has significantly improved discriminatory power in revealing true species diversity and phylogenetic relationships. This is increasingly being confirmed also in the study of the Elmidae taxonomy (e.g., Čiampor Jr et al. 2017, 2019; Hayashi et al. 2019; Linský et al. 2019, 2021). Unfortunately, we often encounter a lack of material suitable for DNA analysis as most known species are dry-pinned in museum collections, and the situation is similar in the genus *Hexanchorus*. Of the 25 known species, molecular data have been published for seven (Linský et al. 2019). The problem is not so much in extracting DNA from old specimens (see e.g., Mitchell 2015) as it is in accessing material from museums and obtaining permissions to use it for DNA analysis. In the current study, we attempt to bypass this limitation by using newly collected material. Although it comes from a relatively limited area of the genus range (Ecuador, Venezuela, Brazil), the samples comprise a great morphological variation and may contribute to the understanding of the phylogeny and taxonomy of the entire genus.

In this study, we (1) provide the first insight into the phylogeny of the speciose Larainae genus *Hexanchorus* using multilocus molecular data, (2) describe a new genus closely related to *Hexanchorus*, including *H. leleupi* and other morphologically distinct species from Ecuador, (3) describe larvae of four new species, (4) discuss relationships between the new genus and *Hexanchorus*.

2. Material and Methods

2.1. Morphological studies

The majority of the studied material was collected by net sampling in smaller streams flowing in primary or degraded forest of Ecuador. Larvae and adults were fixed in pure alcohol directly in the field. Material from previous studies (Laššová et al. 2014; Linský et al. 2019) was available for examination, and further material of *Hexanchorus* species was loaned from museums: *H. browni* Spangler & Santiago-Fragoso, 1992, *H. caraibus* (Coquerel, 1851), *H. crinitus* Spangler & Santiago-Fragoso, 1992, *H. dimorphus* Spangler & Staines, 2004, *H. emarginatus* Spangler & Santiago-Fragoso, 1992, *H. gracilipes* Sharp, 1882, *H. mcdiarmidi* Spangler & Staines, 2004, *H. shannoni* Spangler & Staines, 2004, *H. usitatus* Spangler & Santiago-Fragoso, 1992 from National Museum of Natural History, Washington, DC, USA (NMNH), *H. tarsalis* Hinton, 1937, *H. tibialis* Hinton, 1935 from the Museum of Natural History, London, UK (NHM), and *H. leleupi* Delève, 1968 from Royal Belgian Institute of Natural Sciences, Brussels, Belgium (RBINS). Specimens for the morphological study were cleaned and examined under a Leica M205C stereo microscope at magnifications up to 160×. Male and female genitalia were studied as temporary glycerine slides at magnifications up to 600×, using

a Leica DM1000 light microscope. Drawings were made with a drawing tube. Photographs of habitus were made using a Leica M205C with a Nikon D3s digital camera attached. Image stacks were combined using Zerene Stacker v1.04 and finalised in Adobe Photoshop CS5. The beginning and end of label texts are indicated by double quotation marks (“ ”); a double slash (/) separates the data on different labels; square brackets ([]) are used to indicate authors comments.

Morphological terms generally follow Kodada et al. (2016). The following measurements were taken: **CL** – combined body length (measured from anterior margin of pronotum to elytral apices); **EL** – elytral length; **EW** – maximum elytral width; **PL** – pronotal length; **PW** – maximum pronotal width.

2.2. DNA data and Phylogenetic analysis

For the DNA analyses, 44 specimens of *Hexanchorus*, 23 *Hexanchorus*-like specimens presumably belonging to the new genus and three specimens of related Larinae genera used as outgroups (Supplementary file 1: Table S1). DNA was extracted from the whole beetles using standard methods (see e.g., Čiampor and Ribera 2006). Primers used for PCR amplification are listed in Table 1. Amplification products were purified by alkaline phosphatase (FastAP) and exonuclease I and sequenced in both directions in Macrogen Europe Inc. (Amsterdam, Netherlands). Mitochondrial marker for COI was analysed for all 70 specimens. Three additional fragments, one mitochondrial (16S rDNA) and two nuclear (18S, 28S rDNA), were further analysed for 19 *Hexanchorus*, 8 *Hexanchorus*-like, and 3 outgroup specimens. Raw sequences were assembled and edited in Sequencher v.5.1. Genetic distances were calculated using K2P model and final matrices were done in MEGA v7 (Kumar et al. 2016).

A fast Maximum likelihood phylogenetic analysis (ML) was done for each marker separately in IQ-TREE v2.0 (Minh et al. 2020) on a web server (Trifinopoulos et al. 2016) with the optimal substitution model selected as a part of the analysis, and with 1000 ultrafast bootstrap replicates (Hoang et al. 2018) to assess branch support. Bayesian analyses were performed using BEAST v2.6.3 (Bouckaert et al. 2019) with the bModelTest module (Bouckaert and Drummond 2017) for the evaluation of the substitution model. We used the Relaxed Clock Log Normal model (Drummond and Suchard 2010) with standard mitochondrial rate (Clock.rate) for arthropod COI equal to 0.0115 substitutions/site/Myr (Brower 1994) and let estimate the rate for remaining partitions. The tree prior was set to the Yule model (Yule 1925). Four analyses were run for 100×10^6 generations, resampling every 1000 iterations and logging trace and tree every 1000 iterations. We checked for stationarity by using trace plots in Tracer v1.7.1 (Rambaut et al. 2018), ensuring that all values for effective sample size were >200 . The chains were combined with LogCombiner, discarding 25% of

Table 1. Primers used in PCR amplification.

Primer name	5' Sequence
BF3	CCH GAY ATR GCH TTY CCH CG
BR2	TCD GGR TGN CCR AAR AAY CA
LCO1490	GGT CAA CAA ATC ATA AAG ATA TTG G
HCO2198	TAA ACT TCA GGG TGA CCA AAA AAT CA
16S aR	CGC CTG TTT AWC AAA AAC AT
ND1 A	GGT CCC TTA CGA ATT TGA ATA TAT CCT
18S 5	GAC AAC CTG GTT GAT CCT GCC AGT
18S b5.0	TAA CCG CAA CAA CTT TAA T
28s Ka	ACA CGG ACC AAG GAG TCT AGC ATG
28S Kb	CGT CCT GCT GTC TTA AGT TAC

burn-in trees each. The maximum clade credibility tree with median node ages was calculated with TreeAnnotator v.2.6.3 (Bouckaert et al. 2019) and edited in FigTree 1.4 and Adobe Illustrator CS5.1.

2.3. Depository of digital data

The dataset DS-ELMRUMIL, with all information on sequences, was created in BOLD and was assigned with a DOI: <http://dx.doi.org/10.5883/DS-ELMRUMIL>.

2.4. Specimen Depositories

CCB – Coleoptera Collection Plant Science & Biodiversity Centre SAS, Bratislava, Slovakia (Fedor Čiampor Jr); **NHM** – Natural History Museum, London, UK (Christine E. Taylor); **NMNH** – National Museum of Natural History, Washington, DC, USA (Charyn J. Micheli); **PUCE** – Pontifical Catholic University of Ecuador, Quito, Ecuador (Giovanni Onore); **RBINS** – Royal Belgian Institute of Natural Sciences, Brussels, Belgium (Pol Limbourg).

3. Results

3.1. Phylogeny analysis

The material of *Hexanchorus* available for molecular analysis included only a third of the known species of the genus, yet it was the first and richest material to provide insight into the phylogeny of the genus using molecular data. In addition, the analyzed dataset contained samples of four *Hexanchorus*-like species that we suspected to represent an undescribed genus standing close to *Hexanchorus*. Our analyses confirmed this hypothesis: all *Hexanchorus* specimens formed a monophyletic clade sister to a clade formed by the aberrant species described below as *Rumilara* **gen. nov.**

All ML analyses, either with separate markers or concatenated data, clearly separated *Hexanchorus* – *Rumilara*

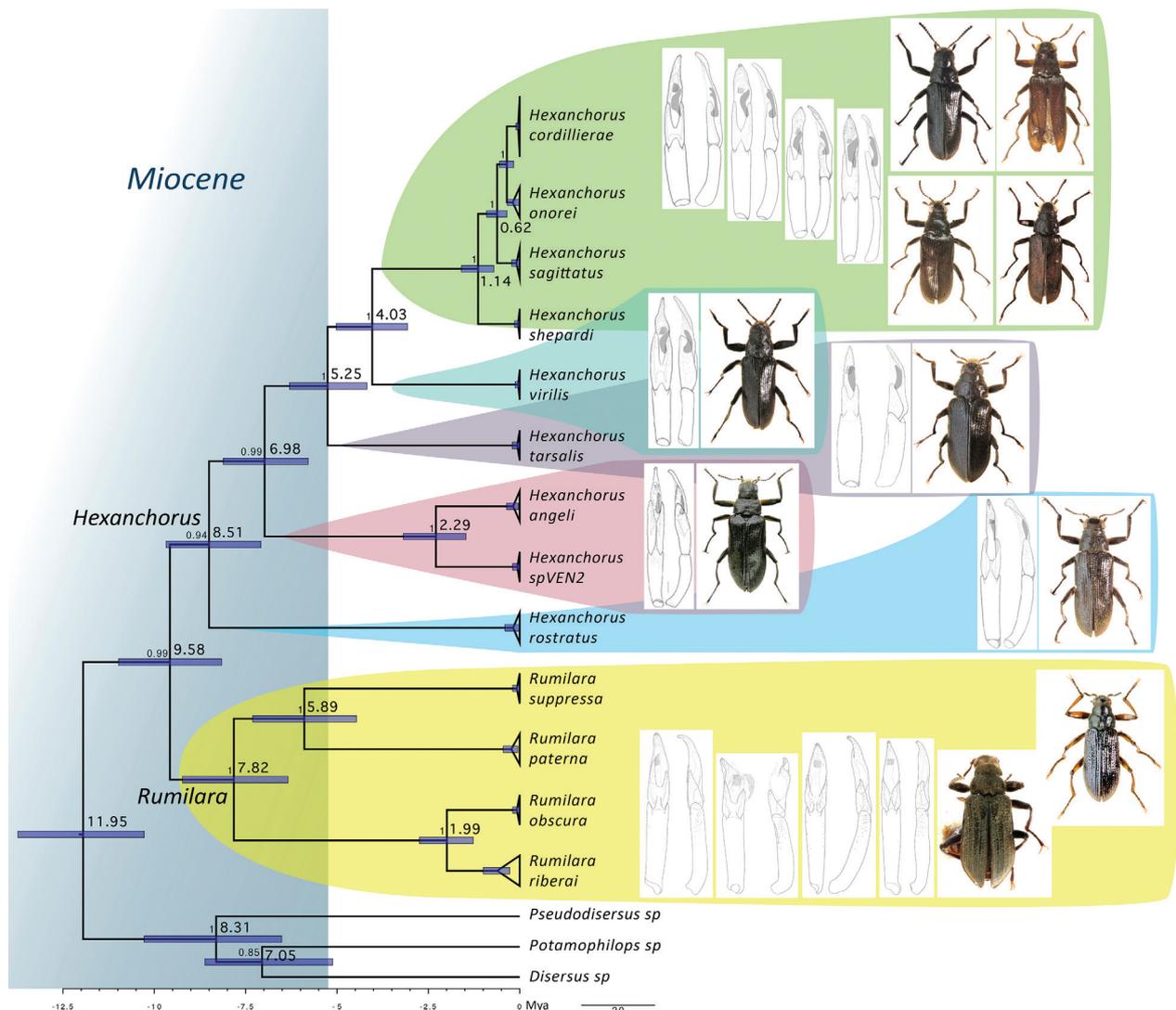


Figure 1. Time calibrated Bayesian phylogeny of *Hexanchorus* and *Rumilara* gen. nov. Genera *Disersus*, *Potamophilops*, and *Pseudodisersus* were used as an outgroup.

gen. nov. clade from the outgroup genera, the separation being well supported by bootstrap or posterior probability values. The separation of *Hexanchorus* and *Rumilara* gen. nov. was supported by 16S and concatenated data. 28S marker revealed the same results except for *H. rostratus* Linský, Čiamporová-Zaťovičová, Čiampor Jr that was grouped with *Rumilara* gen. nov. COI grouped all *Rumilara* gen. nov. into a highly supported clade nested within *Hexanchorus*. Partial, single-marker analyses revealed some incomplete lineage sorting, which suggests close relationships and common evolutionary history of both genera, concatenated data provided robust evidence for their separation.

Molecular data indicates presence of at least four distinct evolutionary lineages within *Hexanchorus* (Supplementary file 2: All markers), which are also supported by the presence of specific morphological characters: 1) a lineage represented by Venezuelan species characterized by the presence of a median process on the third female ventrite (see e.g., Maier and Short 2014); 2) a lineage including *H. cordillierae* (Guérin Méneville, 1843) characteristic by long serrate antennae (Figs 5g, k, l) and meso-

tibiae with a long posterior lateral pubescent area; 3) “*H. tarsalis* lineage” with shorter, more compact antennae (Fig. 5m) and mesotibiae with shorter posterior lateral pubescent area, never reaching behind the middle; and 4) “*H. rostratus* lineage” characterized by longer antennae (Fig. 5j) as in *H. cordillierae* group, but with shorter posterior pubescent area similar to that in *H. tarsalis* lineage. Based on morphological features characteristic for lineages recovered by molecular data, *H. bifurcatus* Maier & Short, 2014, *H. homaeotarsoides* Maier, 2013 and *H. inflatus* Maier, 2013 likely belong to the first lineage based on the presence of abdominal process in females. Species *H. crinitus* Spangler & Santiago-Fragoso, 1992 and *H. mcDiarmidi* Spangler & Staines, 2004 share structure of antennae and mesotibial pubescence with the second lineage. The rest of the known species fit the characteristics of third lineage (represented in molecular study only by *H. tarsalis*), but more detailed species examination or new molecular data are needed to sort them properly.

Hexanchorus onorei, *H. sagittatus* stat. nov. and *H. cordillierae* were uniquely distinguished by 16S. COI

merged *H. onorei* and *H. sagittatus* **stat. nov.** into a single cluster. According to the concatenated data, these three taxa are clearly although shallowly separated, with *H. onorei* and *H. cordillierae* representing sister taxa (Supplementary file 2: ML Trees).

The exclusive monophyly of *Rumilara* **gen. nov.** was revealed in ML analysis of the concatenated data, and 16S and 28S markers. Analysis based on 18S combined samples of *Rumilara* **gen. nov.** with those of *Hexanchorus*, likely due to its slow mutation rate too conservative for separation of Elmidae genera (also judging from the fact that it grouped the well-established *Disersus* and *Pseudodisersus* together). Within *Rumilara* **gen. nov.**, all analyses, except for 18S and 28S, proposed the presence of four species.

Bayesian analysis (Fig. 1) produced the same topology as maximum likelihood for concatenated data (Supplementary file 2: All markers). Molecular dating, using the beetle *cox1* rate (Brower 1994), indicated that the clade *Hexanchorus* + *Rumilara* **gen. nov.** separated from outgroups ~12 million years ago (Mya). Bayesian analysis recovered also the deep separation of lineages within *Hexanchorus* (e.g., *H. rostratus* ~8.5 Mya, species from Venezuela ~7 Mya). The long separation of these lineages supports the presence of old species groups within the genus. On the other hand, *Hexanchorus* also includes relatively young species, in this analysis represented by *H. cordillierae*, *H. onorei*, *H. sagittatus* **stat. nov.**, and *H. shepardi*, which appeared within the last 2 My.

The *Hexanchorus* and *Rumilara* **gen. nov.** separated ~9.5 Mya, which is comparable with the separation of other genera of Elmidae in South America (unpublished data of the authors including almost all Elmidae genera from the region). Within *Rumilara* a deep split was detected almost 8 Mya, separated groups each consisting of the two species. This pattern can be however caused by the low number of species available for the analysis and the molecular gap can be filled by adding new samples (e.g., *R. leleupi* **comb. nov.**) in the future.

3.2. Systematics and morphology

3.2.1. Genus *Hexanchorus* Sharp, 1882

Hexanchorus sagittatus Linský, Čiamporová-Zaťovičová & Čiampor Jr, 2019 **stat. nov.**

Hexanchorus onorei Linský, Čiamporová-Zaťovičová & Čiampor Jr, 2019: 100.

Diagnosis. This species can be distinguished from all known *Hexanchorus* species by combination of the following male characters: 1) smaller size (CL: 3.22–3.25 mm); 2) antennae long, serrate, with visible stalks; 3) anterior lateral pubescent area of mesotibia reaching to ca 3/5; 4) posterior lateral pubescent area of mesotibia almost reaching apex; 5) mesotibiae with distinct tubercle on inner apex; 6) metatibiae with small tubercle on inner apex; 7) elytral apex almost rounded, with inner margin arcuately produced; 8) fifth ventrite deeply and

very broadly emarginate; 9) aedeagus arrow-like in ventral view.

Remarks. As mentioned in its original description (Linský et al. 2019), this taxon clearly differs morphologically from other *Hexanchorus* species, but due to small genetic distance from *H. onorei* it was originally described as its subspecies. Phylogenetic analysis based on four markers (Fig. 1) recovered *H. sagittatus* **stat. nov.** as a sister to clade *H. cordillierae* + *H. onorei* and thus fully justifies its elevation to the species rank.

3.2.2. *Rumilara* **gen. nov.**

<https://zoobank.org/111B1F47-F9E2-4654-B112-17B9ACC6-D77A>

Type species. *Rumilara obscura* **sp. nov.**

Diagnosis. A new genus is recognized by the combination of the following characters: compact structure of antennae (Fig. 5a–e); mandibles with teeth fused (Fig. 4b); surface of pronotum microreticulate, with complete transverse impression before middle, outline of discal area elevated, partially or fully interrupted medially by a prescutellar indentation (Fig. 6), mesotibiae without pubescence on lateral sides, without tubercle on inner apex in males; fourth tarsomere with apicoventral, long, erect seta; abdomen convex, with apical segment rounded in both sexes (Fig. 8).

Description. Body elongate (Figs 2–3), 2.44–3.28 mm long, subparallel, widest near posterior fifth of elytra (EW), 0.83–1.37 mm wide, dorsum moderately convex. Dorsal surface covered, at least partially, with two types of setae: 1) short, prone, more or less densely pubescent, and 2) numerous moderately long, curved, semi-erect, hair-like. Ventral surface clothed with yellowish, densely set, short to moderately long, recumbent pubescence that is confined partly or fully to following areas: genae, ventral face of head, hypomera, epipleura, prosternum, mesoventrite, metaventrite, abdomen, coxae, trochanters, femora, tibiae and tarsi. **Head** narrower than pronotum, retractile, dorsally moderately convex, laterally rounded, ventrally nearly flat. Surface microreticulate, with fine sparse or very dense punctation partly or fully concealed by pubescence. Eyes well developed, hemispherical; with row of long, hair-like setae along dorsal margin (often peeled off in *R. riberae* **sp. nov.** and *R. suppressa* **sp. nov.** specimens). Labrum about 3 times wider than long; transverse; with row of long, yellowish, hair-like setae near anterior margin; anterolateral angles rounded; surface glabrous; anterior margin feebly emarginate medially. Clypeus shorter than labrum, transverse, surface microreticulate, fully or not at all concealed by pubescence, anterior margin with a row of moderately long, hair-like setae; frontoclypeal suture straight. Antennae (Fig. 5a–e) 11-segmented; clubbed, club ca. twice as long as combined length of scape and pedicel. Scape

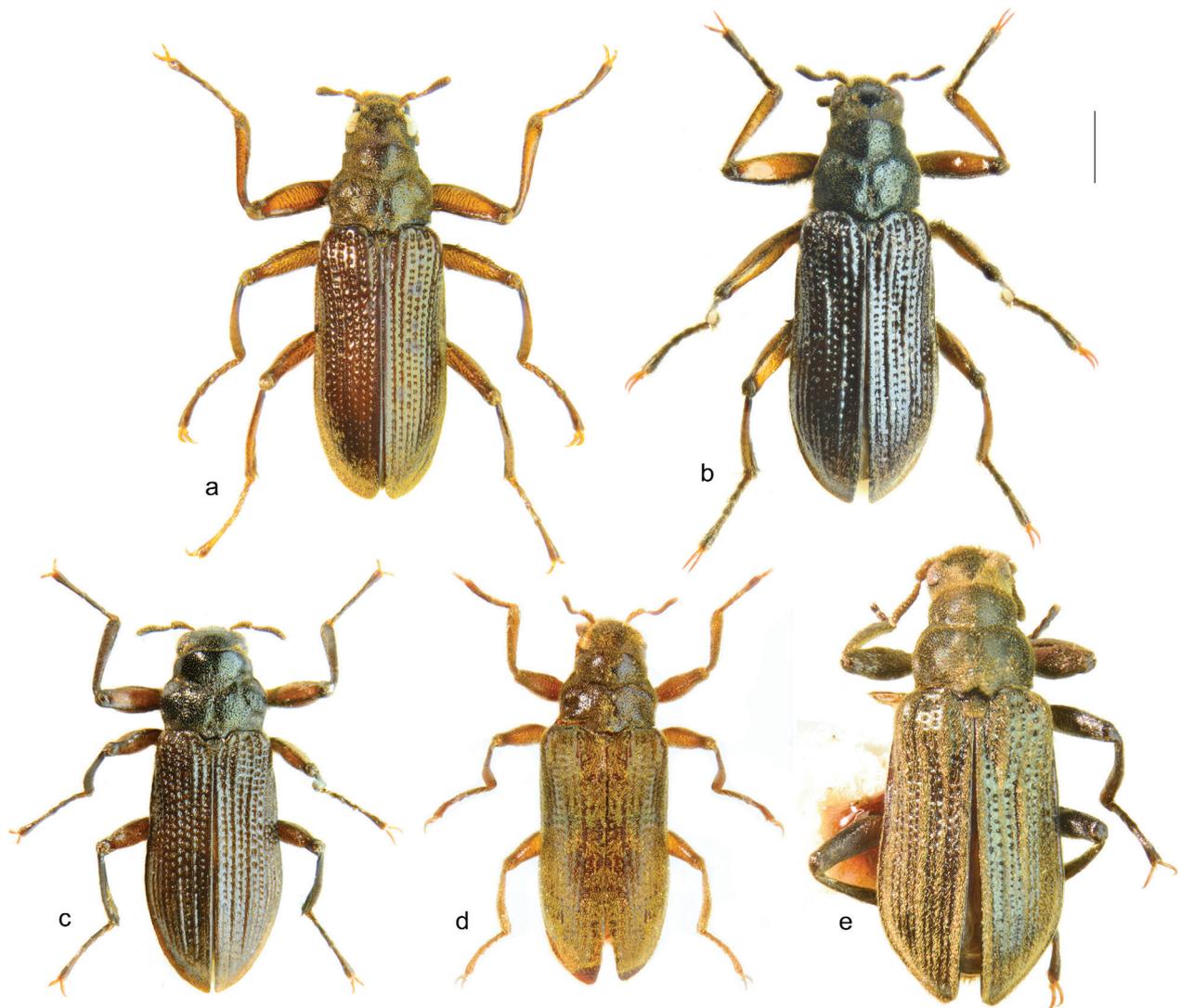


Figure 2. Dorsal habiti of *Rumilara* gen. nov. species: **a** *R. obscura* sp. nov. male holotype; **b** *R. suppressa* sp. nov. male holotype; **c** *R. riberai* sp. nov. male holotype; **d** *R. paterna* sp. nov. male holotype; **e** *R. leleupi* comb. nov. male holotype. Scale: 0.5mm.

curved, ca. 1.5 times as long as pedicel; pedicel subglobular. Segment 3 subtriangular, longer than wide; segment 4 about as long as wide; segments 5 to 10 wider than long, transverse; segment 11 about as long as wide, subglobular. All segments densely clothed with short, prone setae; scape and pedicel also with long, stout, hair-like setae; segments 5 to 11 with a few erect, hair-like setae. Mandible (Fig. 4b) short, moderately broad, nearly symmetrical with apical teeth fused; outer lateral margin strongly curved at base with a few long, curved, hair-like setae; ventral condyle bearing row of long, hair-like setae; molar part large, finely grooved. Prostheca hyaline, long, with numerous apical finger-like outgrowths. Maxilla (Fig. 4c) with cardo densely clothed with hair-like setae; stipes subtriangular, with moderately long, densely arranged, hair-like setae; palpifer nearly half as long as stipes, with moderately long, hair-like setae on external face, and with long, hair-like setae near anterior margin. Maxillary palpi 4-segmented; surface of segments 2 and 3 obscured by densely arranged, moderately long, hair-like setae; segment 1 very short, about as long as wide; segment 2 about twice as long as wide, widening apical-

ly; segment 3 slightly longer than the previous segment; terminal segment longest. Galea elongate, external outline convex, inner outline concave; apex rounded, with numerous slightly longer, finger-like setae; outer outline densely clothed with long, hair-like setae. Lacinia about twice as wide as galea, longer than wide, subrectangular; clothed with long, densely arranged, hair-like setae and apically with transverse rows of stout, bent, finger-like setae. Labium (Fig. 4d) with palpiger short, clothed with tuft of very long, densely set, hair-like setae. Labial palpi 3-segmented; segments 1 and 2 subequal in length, with tuft of conspicuous, long, hair-like setae; terminal segment largest, suboval, widest apically, apex truncate, with a field of short, finger-like setae. Ligula almost as long as palpi, widest anteriorly; anterolateral angles broadly rounded; anterior and anterolateral margin with densely arranged, moderately long, curved, hair-like setae; medial portion covered with short, stout, finger-like setae. Prementum short, transverse, widest posteriorly; mentum longer than prementum, densely clothed with long, semi-erect, hair-like setae. **Thorax.** Pronotum (Fig. 6) wider than long, subparallel, widest near mid-

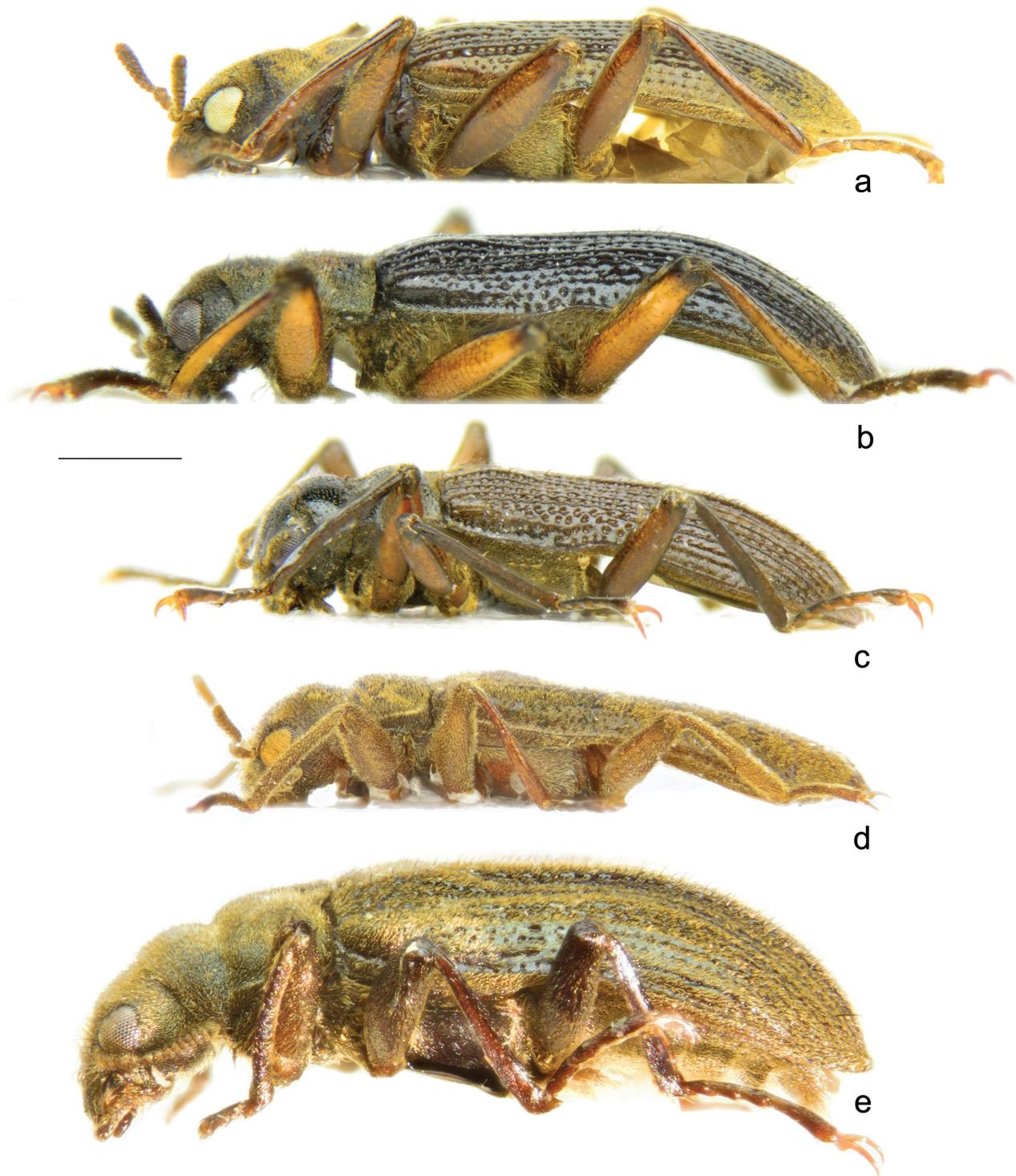


Figure 3. Lateral habits of *Rumilara* gen. nov. species. **a** *R. obscura* sp. nov. male holotype; **b** *R. suppressa* sp. nov. male holotype; **c** *R. riberai* sp. nov. male holotype; **d** *R. paterna* sp. nov. male holotype; **e** *R. leleupi* comb. nov. male holotype. Scale: 0.5mm.

dle; disc convex, posteriorly to transverse impression distinctly elevated, partially of fully interrupted medially by prescutellar indentation, surface microreticulate; with broad transverse impression in anterior third; sublateral carinae absent; with arcuate impression near posterolateral sides; anterior margin arcuate; anterolateral angles rounded; lateral margins almost straight in anterior third then broadly rounded; posterior margin trisinate, broadly arcuate on each side and narrowly in front of scutellum; posterior angles suborthogonal. Surface microreticulate

(feebly visible in *R. leleupi* comb. nov.). Hypomeron widest in anterior third, then distinctly narrowed, almost straight. Prosternum (Fig. 7) extremely short in front of procoxae; procoxae strongly transverse in lateral view; prosternal process wide; parallel-sided between procoxae, then straight or concavely tapering towards rounded apex. Mesoventrite short and wide, with deep narrow groove for reception of prosternal process. Metaventrite slightly wider than long, moderately smooth, disc convex with a narrow longitudinal impression on midline.

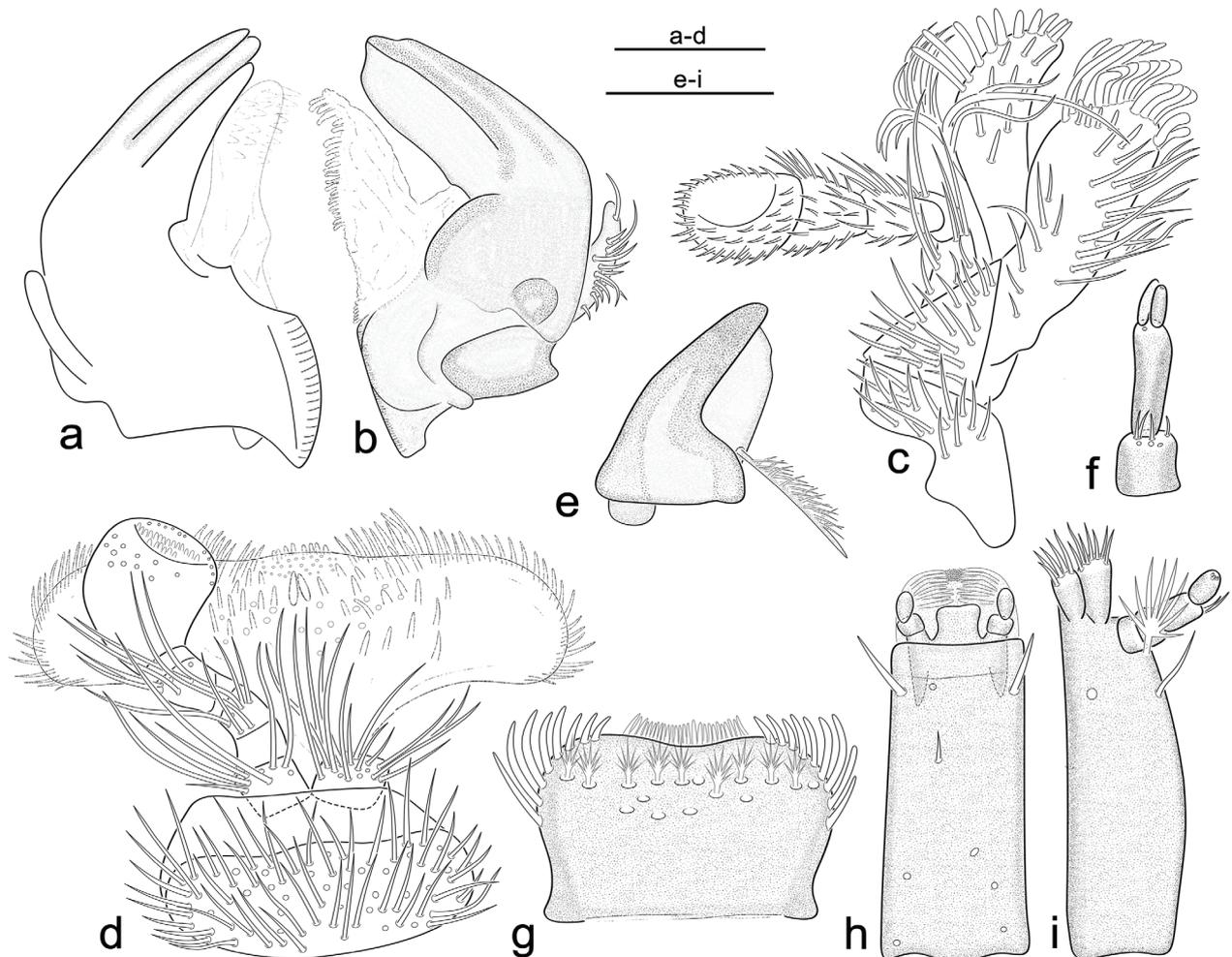


Figure 4. Mouthparts of *Hexanchorus cordillierae* (a) and *Rumilara obscura* sp. nov. (b–i); a adult mandible; b adult mandible; c adult maxilla; d adult labium (right palp omitted); e larval mandible; f larval antenna; g larval labrum; h larval labium; i larval maxilla. Scale: 0.1mm.

Elytra elongate, parallel or finely subparallel, widest in posterior 4/5, then evenly arched towards rounded apex. Each elytron with 10 rows of shallowly to deeply impressed punctures. Intervals nearly flat, slightly elevated or partly depressed. Scutellum wider than long, rounded. Epipleuron oblique until posterior margin of metaventre then inflexed horizontally, posteriorly tapering, not reaching elytral apex. Hind wing without radial cross vein; without anal cell; with first and second branches of anal vein 2 fused; anal vein 1 present only apically; cubito-anal cross vein incomplete or complete and joining cubitus to anal vein 1. Legs long, at least partially covered by pubescence; femora widest across middle; tibiae moderately bent, subequal in length with femora. Tarsi 5-segmented, fourth segment with an apicoventral, long, erect seta, fifth segment slightly shorter than remaining segments combined; claws strong, long, without basal or subbasal teeth. **Abdomen** (Fig. 8) with 5 ventrites; first ventrite with intercoxal process subtriangular, about twice as wide as long, feebly incised medially; ventrites 2–4 gradually shorter and slightly narrower than previous one; posterior margin of ventrite 5 broadly rounded. **Male.** Sternite VIII (Figs 11e, 12e, 13e, 14d, 15d) with long, rather wide medial apodeme; apically broad-

ly bisinuate, densely set with moderately long, curved, semi-erect, hair-like setae. Aedeagus of trilobate type; long and slender; penis long, tapering apically, corona present, with or without long, dorsal, rod-like sclerite, membranous sack with one smaller and one large, oblong ventral sclerites; parameres feebly to strongly asymmetrical, reaching from below half to 4/5 of penis; phallobase longer than penis. **Female.** Sternite VIII (Figs 11f, 12f, 13f) with long, narrow, medial apodeme; apically broadly bi- or quadrisinuate, densely set with moderately long, curved, semi-erect hairs. Ovipositor (Figs 11d, 12d, 13d) with coxites and styli short; coxites broad at base; struts long and slender.

Etymology. The name of the genus is inspired by the language of the indigenous Quechua people who live in the territory where the new genus originated. In the Quechua language (Gómez Rendón 2009), “*rumi*” means rock, referring to the nature of the type locality and other habitats of the species, i.e., steep rocks with water flowing in thin layer, or fast-flowing streams with bottoms composed largely of larger rocks. The adjective ‘*lara*’ refers to type-genus of the subfamily Larainae. Gender feminine.

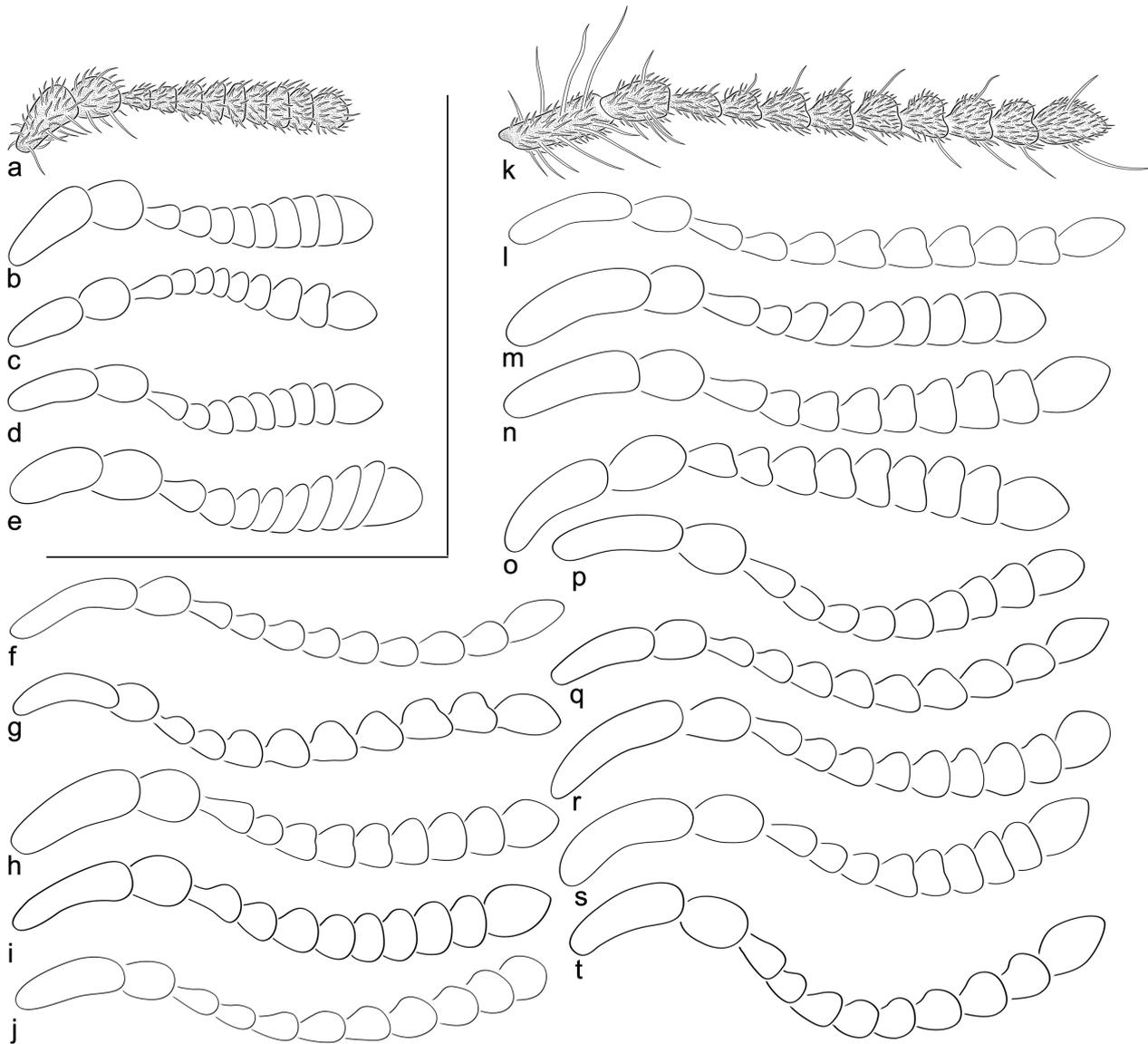


Figure 5. Antennae of *Rumilara* gen. nov. and *Hexanchorus*: **a** *R. obscura* sp. nov.; **b** *R. suppressa* sp. nov.; **c** *R. riberae* sp. nov.; **d** *R. paterna* sp. nov.; **e** *R. leleupi* comb. nov.; **f** *H. mdiarmidi*; **g** *H. virilis*; **h** *H. shannoni*; **i** *H. angeli*; **j** *H. rostratus*; **k** *H. cor-dillierae*; **l** *H. onorei*; **m** *H. tarsalis*; **n** *H. usitatus*; **o** *H. gracilipes*; **p** *H. caraibus*; **q** *H. crinitus*; **r** *H. dimorphus*; **s** *H. emarginatus*; **t** *H. browni*. (a, k based on dissected antennae; b–j, l–r based on photographs, not to scale, setae omitted).

Distribution. The genus is distributed in central Ecuador, so far known from four provinces (Cotopaxi Province, Napo Province, Morona-Santiago Province, Santo Domingo de los Tsáchilas Province).

Comparative notes. The new genus is the closest to *Hexanchorus*, both share a combination of following synapomorphies which separate them from remaining Larainae genera: smaller body size (length never reaches 5 mm); pronotum with distinct transverse impression in anterior half; hind wing venation without anal cell; apex of fourth tarsal segment ventrally with a fine nearly erect seta; and membranous sac of penis with laterally placed fibula. Adults of *Rumilara* gen. nov. differs from *Hexanchorus* mainly in: (i) microreticulate pronotum (Fig. 6) (less distinct in *R. leleupi* comb. nov.); outline of discal area elevated (nearly flat in *Hexanchorus*); discal area partially to fully interrupted by prescutellar indentation in middle

(medial impression only superficial in *Hexanchorus*); transverse impression shifted more to the middle (anterior third in *Hexanchorus*); (ii) mesotibiae without pubescence (e.g., Figs 3c–e) on lateral sides, without tubercle on inner apex (lateral pubescence is present in all *Hexanchorus* species, all males have tubercle on inner apex); (iii) abdomen (Fig 8) with fifth ventrite apically rounded, ventrites convex (in *Hexanchorus* fifth ventrite is apically emarginate in both sexes, male has ventrites impressed, female convex); (iv) male genitalia (Fig. 10f) with fibula major and minor (only major in *Hexanchorus*), some species with dorsal fibula (rod-like sclerite unknown in *Hexanchorus*); (v) antennae (Figs 5a–e) more compact, almost club-like (more loosely connected in *Hexanchorus*; Figs 5f–t), and (vi) mandibula (Fig. 4b) with apical teeth fused (as opposed to three apical teeth present in *Hexanchorus*; Fig. 4a).



Figure 6. Pronota of *Rumilara* gen. nov. species: **a** *R. obscura* sp. nov. male holotype; **b** *R. suppressa* sp. nov. male holotype; **c** *R. riberai* sp. nov. male holotype; **d** *R. paterna* sp. nov. male holotype; **e** *R. leleupi* comb. nov. male holotype. Scale: 0.2mm.

Remark. Three *Rumilara* gen. nov. species (*R. obscura* sp. nov., *R. riberai* sp. nov., *R. suppressa* sp. nov.) differ from *R. paterna* sp. nov. and *R. leleupi* comb. nov. by femoral pubescence, which is restricted to long, dark, densely set, hair-like setae along dorsal margin, and by

pro- and metatibial pubescence that is in a form of fringe at tibial apex.

Included species. *Rumilara leleupi* comb. nov., *R. obscura* sp. nov., *R. paterna* sp. nov., *R. riberai* sp. nov., *R. suppressa* sp. nov.

Key to the adults of species of the genus *Rumilara* gen. nov.

- 1 Pubescence on elytra arranged in stripes *R. leleupi* (Delève, 1968) comb. nov.
- Pubescence on elytra not arranged in stripes 2
- 2 Pro- and metatibiae fully pubescent..... *R. paterna* sp. nov.
- Pro- and metatibiae partly pubescent..... 3
- 3 Sides of pronotum parallel in basal 1/3, elytral intervals 2–4 partly depressed..... *R. suppressa* sp. nov.
- Sides of pronotum convex in basal 1/3, elytral intervals 2–4 not depressed 4
- 4 Head and clypeus fully clothed with pubescence *R. obscura* sp. nov.
- Head clothed with pubescence only behind eyes, clypeus bare..... *R. riberai* sp. nov.

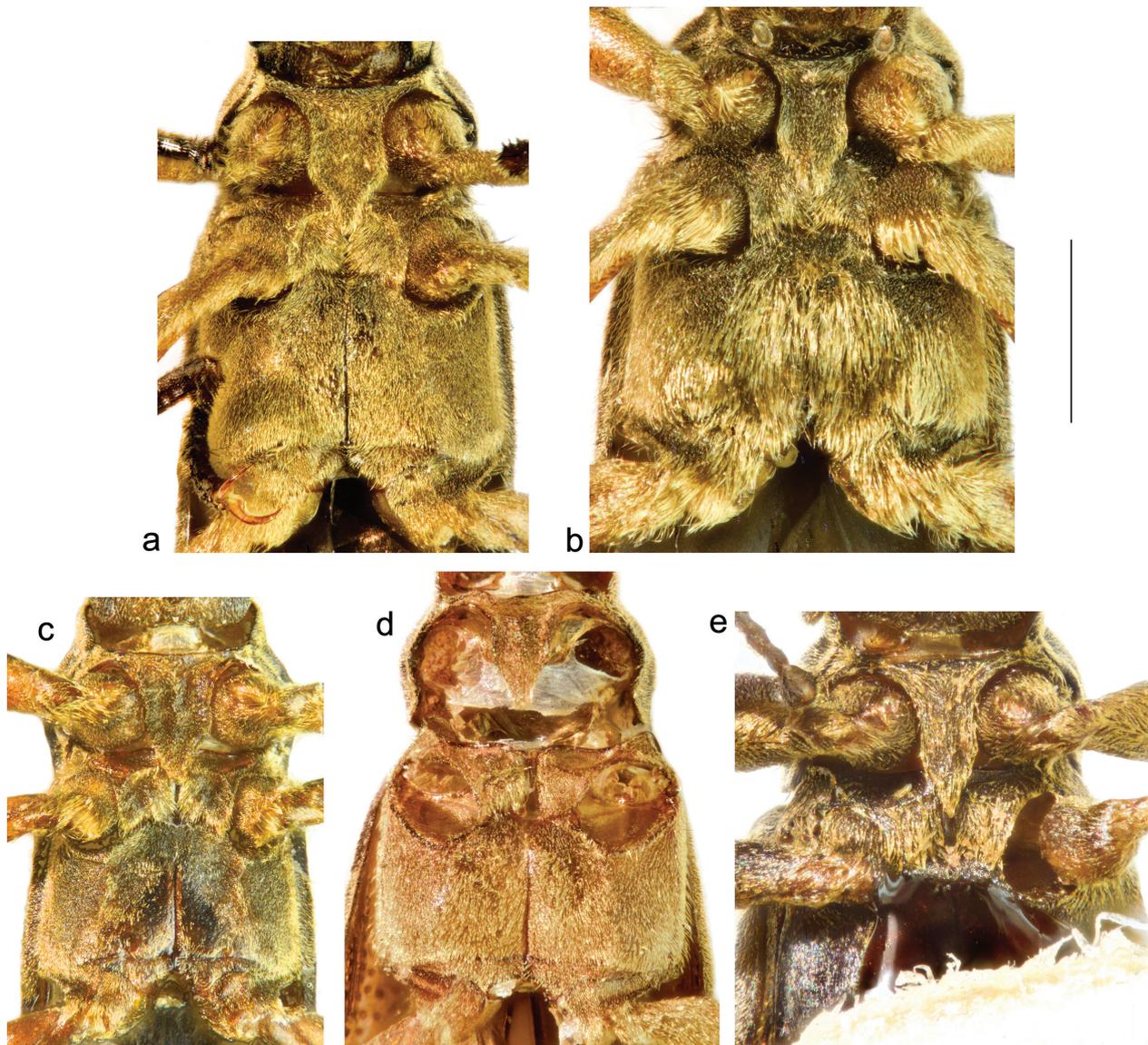


Figure 7. Ventral habits of *Rumilara* gen. nov. species: **a** *R. obscura* sp. nov. male paratype; **b** *R. suppressa* sp. nov. male paratype; **c** *R. riberai* sp. nov. female paratype; **d** *R. paterna* sp. nov. paratype; **e** *R. leleupi* comb. nov. male holotype. Scale: 0.5mm.

Larval morphology

Diagnosis. Body (Fig. 9) fusiform to subcylindrical, not flattened. Anterior margin between base of antenna and clypeus with a little tooth. Second antennomere with short sensorium as long as the third antennomere. Prothorax with eight ventral sclerites: one medial, cervical triangular sclerite; two large, broad anterior sclerites; two anterolateral sclerites; two posterolateral sclerites; and triangular sternellum. Procoxal cavities open, without posteromedial sclerite. Meso- and metathoracic pleura divided into two parts on each side. Abdominal ventrites 1–5 (1–4 in *R. suppressa* sp. nov.) bounded by tergo- and sternopleural sutures forming separate sclerites. Lateral margins of thoracic and abdominal segments (1–3 in *R. suppressa* sp. nov., 1–5 in *R. obscura* sp. nov., 1–6 in *R. riberai* sp. nov., 1–7 in *R. paterna* sp. nov.) explanate. Lateral margin of extensions serrated without setae. Thoracic tergites with two pairs of longitudinally arranged stick-like gibbosities, frontal pair of protergum in form of

massive gibbosities near anterior margin. Tergum of abdominal segments 1–7 each with a pair of longitudinally arranged gibbosities (indistinct in *R. suppressa* sp. nov.); segment 8 with pair of distinctly larger posterolaterally expanded gibbosities. Abdominal segment 9 elongate, subtriangular with rounded apex.

Description. Length (in profile) 2,92–4,88 mm, greatest width (at the base of metathorax) 0,95–1,28 mm. Body elongate, fusiform to subcylindrical, tapering posteriorly. Dorsum convex, venter slightly concave. Cuticle light brown to dark brown, ventral side, tergal gibbosities (except the pair on the abdominal segment 8), and lateral extensions yellowish. In *R. paterna* sp. nov. the surface around frontal pair of pronotal gibbosities yellowish. Surface densely granulate, with several smooth, dark spots scattered over thoracic terga. Basal margins of thoracic and abdominal segments with a row of seta-bearing granules. Spiracles present on mesothorax and abdominal segments 1–8. Mesothoracic spiracles present anterolaterally,

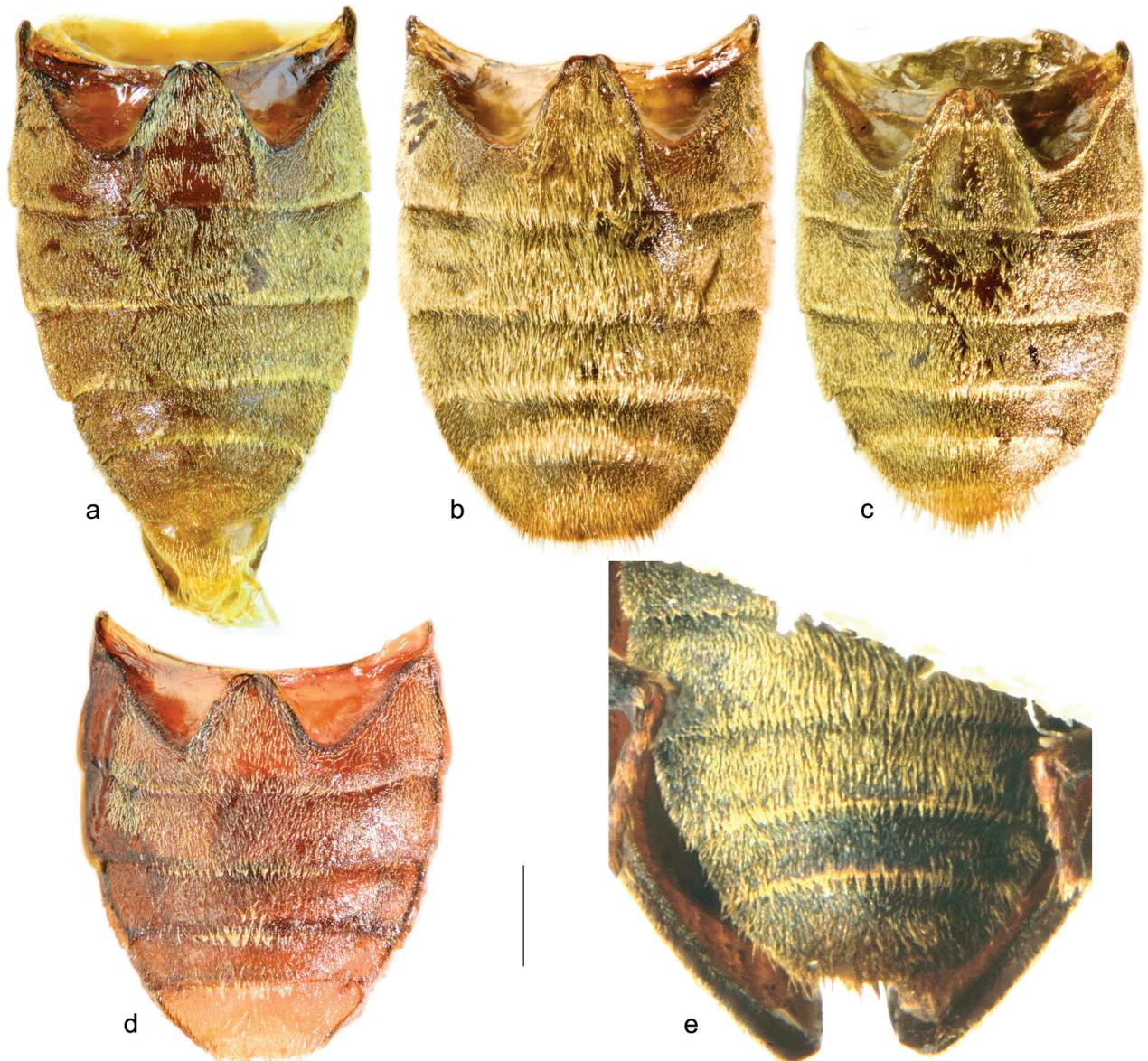


Figure 8. Abdomens of *Rumilara* gen. nov. species: **a** *R. obscura* sp. nov. male holotype; **b** *R. suppressa* sp. nov. male holotype; **c** *R. riberai* sp. nov. male holotype; **d** *R. paterna* sp. nov. male holotype; **e** *R. leleupi* comb. nov. male holotype. 0.2mm.

abdominal spiracles present laterally on posterior third of segments 1–7, and segment 8 with spiracles opening on apices of two large posterolateral gibbosities. — **Head** prognathous, partially retracted into prothorax, frons flattened. Head in ventral view obovate, widened in posterior portion, in lateral view subtriangular. Anterior margin with small tooth between base of antennae and clypeus; frontoclypeal suture well developed with numerous ramose setae. Frontal sutures beginning before posterior margin of antennal sockets and diverging forwards to its inner margin. Eyes composed of five stemmata. Antennae (Fig. 4f) 3-segmented; first segment widest, about as long as wide; second segment longest, about half as wide and twice as long as first segment, bearing a small sensorium, about as long as third segment. Labrum (Fig. 4g) with broadly rounded anterolateral angles, each side with numerous long setae; anteromedial margin emarginate, densely covered with short setae; with a row of ramose setae before anterior margin. Mandible (Fig. 4e) symmetrical without

distinct teeth; prostheca long and densely spinose. Maxilla (Fig. 4i) with palpus 4-segmented, with ramose seta at base; stipes long, transverse, without palpifer; galea and lacinia separate, each with apex densely spinose. Labium (Fig. 4h) with palpus 2-segmented; prementum with palpiger; postmentum undivided. Gula well developed. — **Thorax.** Protergum widest at base, convex, with two small prebasal gibbosities protruding medially and two massive gibbosities protruding fronto-medially. Lateral margins explanate, with angles rounded, pale coloured with dark brown serrate margin. Meso- and metatergum each with two pairs of longitudinally arranged small gibbosities, lateral margins as in protergum. Median suture well visible. Prothorax (Fig. 10a) with eight ventral sclerites: one medial, cervical triangular sclerite; two large, broad anterior sclerites; two anterolateral sclerites; two posterolateral sclerites; and triangular sternellum. Procoxal cavities open, without posteromedial sclerite. Meso- and metathoracic pleura divided into two parts on each side. — **Abdo-**



Figure 9. Larvae of *Rumilara* gen. nov. species: **a** *R. obscura* sp. nov., dorsal; **b** *R. suppressa* sp. nov., dorsal; **c** *R. riberai* sp. nov., dorsal; **d** *R. paterna* sp. nov., dorsal; **e** *R. obscura* sp. nov., ventral; **f** *R. suppressa* sp. nov., ventral. Scale: 1mm.

men with nine segments. Tergum of abdominal segments 1–7 each with a pair of longitudinally arranged gibbosities (indistinct in *R. suppressa* sp. nov.); segment 8 with a pair of distinctly larger posterolaterally expanded gibbosities. Abdominal segments 1–5 (1–4 in *R. suppressa* sp. nov.) bounded by tergo- and sternopleural sutures forming separate sclerites; segments 6–8 (5–8 in *R. suppressa* sp. nov.) forming complete sclerotized rings. Lateral margins of abdominal segments (1–3 in *R. suppressa* sp. nov., 1–5 in *R. obscura* sp. nov., 1–6 in *R. riberai* sp. nov., 1–7 in *R. paterna* sp. nov.) explanate, pale colored with dark brown serrate margin. Abdominal segment 9 elongate, subtriangular with rounded apex; dorsally with median keel; ventrally with apical gill chamber, subpentagonal operculum with rounded apex, and with a pair of strong distal hooks with serrate inner margin.

Comparative notes. The larvae of *Rumilara* gen. nov. are morphologically remarkably close to larvae of *Hexanchorus*. They can be distinguished from *Hexanchorus* by: 1) pleurites never reaching sixth abdominal ventrite; 2) lateral margin of thoracic and abdominal extensions never bearing setae; 3) lateral margin of thoracic and abdominal extensions distinctly paler than the rest of tergum.

Remark. Early instars have all tergal tubercles distinctly less developed than mature larvae, and lateral extension on their posteriormost segment is only vestigial. Larvae were assigned to conspecific adults based on matching COI DNA barcodes (Supplementary file 2,3 – COI tree all samples).

Key to the known larvae of species of the genus *Rumilara* gen. nov.

- 1 Pleurites on abdominal segments 1–5; lateral extensions on at least first five abdominal segments.....2
- Pleurites on abdominal segments 1–4 (Fig. 9f); lateral extensions on first three abdominal segments (Fig. 9b).....
..... *R. suppressa* sp. nov.
- 2 Surface around frontal pair of pronotal gibbosities of the same colour as the rest of pronotum; lateral extensions on abdominal segments not exceeding sixth abdominal segment.....3
- Surface around frontal pair of pronotal gibbosities paler than the rest of pronotum; lateral extensions on abdominal segments 1–7 (Fig. 9d).....
..... *R. paterna* sp. nov.
- 3 Lateral extensions on abdominal segments 1–5 (Fig. 9a)..... *R. obscura* sp. nov.
- Lateral extensions on abdominal segments 1–6 (Fig. 9c)..... *R. riberai* sp. nov.

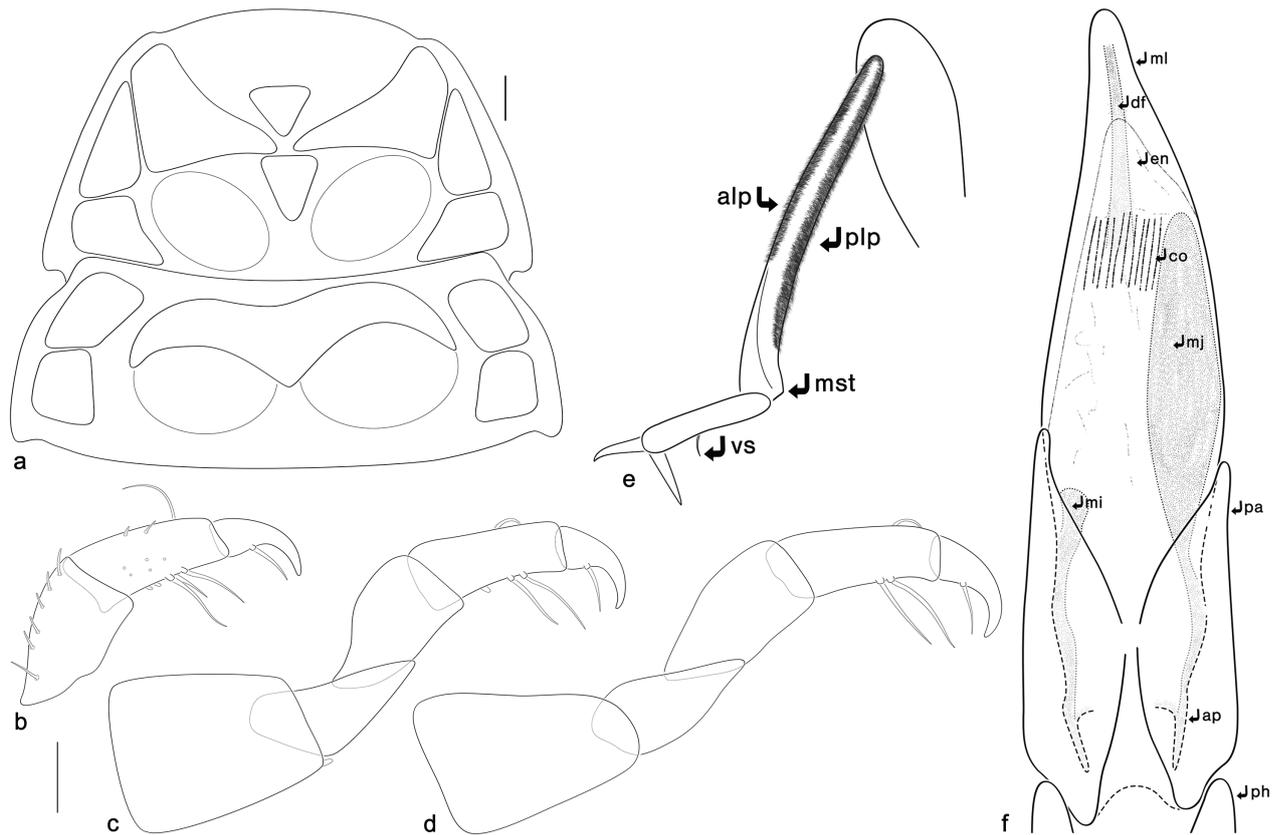


Figure 10. Larva of *Rumilara obscura* sp. nov. (a–d): **a** prosternum and mesosternum; **b** foreleg (without coxa); **c** midleg; **d** hindleg. Schematic illustrations: **e** midleg of *Hexanchorus cordillierae* (alp – anterior lateral pubescence; mst – mesotibial tubercle; plp – posterior lateral pubescence; vs – ventral seta); **f** aedeagus of *Rumilara riberai* sp. nov. (ap – apophyse; co – corona; df – dorsal fibula; en – endophallus; mi – fibula minor; mj – fibula major; ml – median lobe; pa – paramere; ph – phallobase). Scale: 0.1mm.

3.2.3. *Rumilara obscura* sp. nov.

<https://zoobank.org/8036845A-3CEA-4FE2-BFF9-A63C5E56-62A1>

Figs 2a, 3a, 4b–i, 5a, 6a, 7a, 8a, 9a, 9e, 10a–d, 11

Material examined. Type material: **Holotype** ♂ (PUCE) “Ecuador, Cotopaxi prov., Otonga env., Río Esmeralda, 00°25′10.4″ S, 78°59′46.9″ W, 1760m a.s.l., 8.8.2013, stream ca 3m wide, in primary forest, with gravel, stones, submerged wood, Čiampor Jr & Čiamporová-Zaťovičová lgt.”; **Paratypes** 4♂♂, 2♀♀ (PUCE, CCB), 2 ex with the same data as holotype; 4♂♂, 1♀, “Ecuador, Cotopaxi prov., Artos env., above confluence with Río Esmeralda, 00°25′09.6″ S, 78°59′40.2″ W, 1735m a.s.l., 8.8.2013, stream ca 2m wide, fast flowing from steep slope, with gravel, stones with moss, boulders, Čiampor Jr & Čiamporová-Zaťovičová lgt.” **Other material:** 4 larvae with the same data as holotype; 5 larvae “Ecuador, Cotopaxi prov., Artos env., above confluence with Río Esmeralda, 00°25′09.6″ S, 78°59′40.2″ W, 1735m a.s.l., 8.8.2013, stream ca 2m wide, fast flowing from steep slope, with gravel, stones with moss, boulders, Čiampor Jr & Čiamporová-Zaťovičová lgt.”.

Adult diagnosis. The species can be distinguished by a combination of following characters: 1) head and clypeus fully clothed by pubescence; 2) femora bare or with a row of dark, long hairs on dorsal margin; 3) elytra without dense pubescence; 4) aedeagus with rod-like sclerite; 5) parameres only feebly asymmetrical.

Adult diagnostic description. MALE. Colour: Body (Figs 2a, 3a) black; lateral sides of femora and tibiae brown to pale brown; tarsal claws pale brown with reddish tincture. **Pubescence:** Head and clypeus fully clothed with short, prone pubescence, most densely set behind eyes. Pronotum (Fig. 6a) partly covered by fine, short, moderately dense, prone pubescence. Elytra at lateral and apical edges with short, recumbent pubescence, and with numerous moderately long, curved, semi-erect, hair-like setae. Femora with long, dark, densely set, hair-like setae near dorsal margin; pro- and metatibiae apically with a fringe of long, dark, hair-like setae on inner margin; mesotibiae without pubescence. Metaventrte densely clothed with short, prone pubescence. **Surface:** Head, clypeus and pronotum sparsely microreticulate, partly concealed by pubescence. Elytra with rows of punctures deeply impressed; intervals nearly flat. Prosternal process (Fig. 7a) moderately wide, concave between procoxae, then subtriangular with rounded apex. **Aedeagus** (Figs 11a–c) with long, dorsal, rod-like sclerite on penis; parameres asymmetrical, left one slightly longer. — **FEMALE** externally similar to male, except slightly larger.

Measurements. ♂ – CL: 2.70–2.93 mm; PL: 0.67–0.70 mm; PW: 0.76–0.79 mm; EL: 2.02–2.24 mm; EW: 0.99–1.15 mm. ♀ – CL: 2.98–3.00 mm; PL: 0.68–0.72 mm; PW: 0.80–0.83 mm; EL: 2.25–2.31 mm; EW: 1.11–1.14 mm.

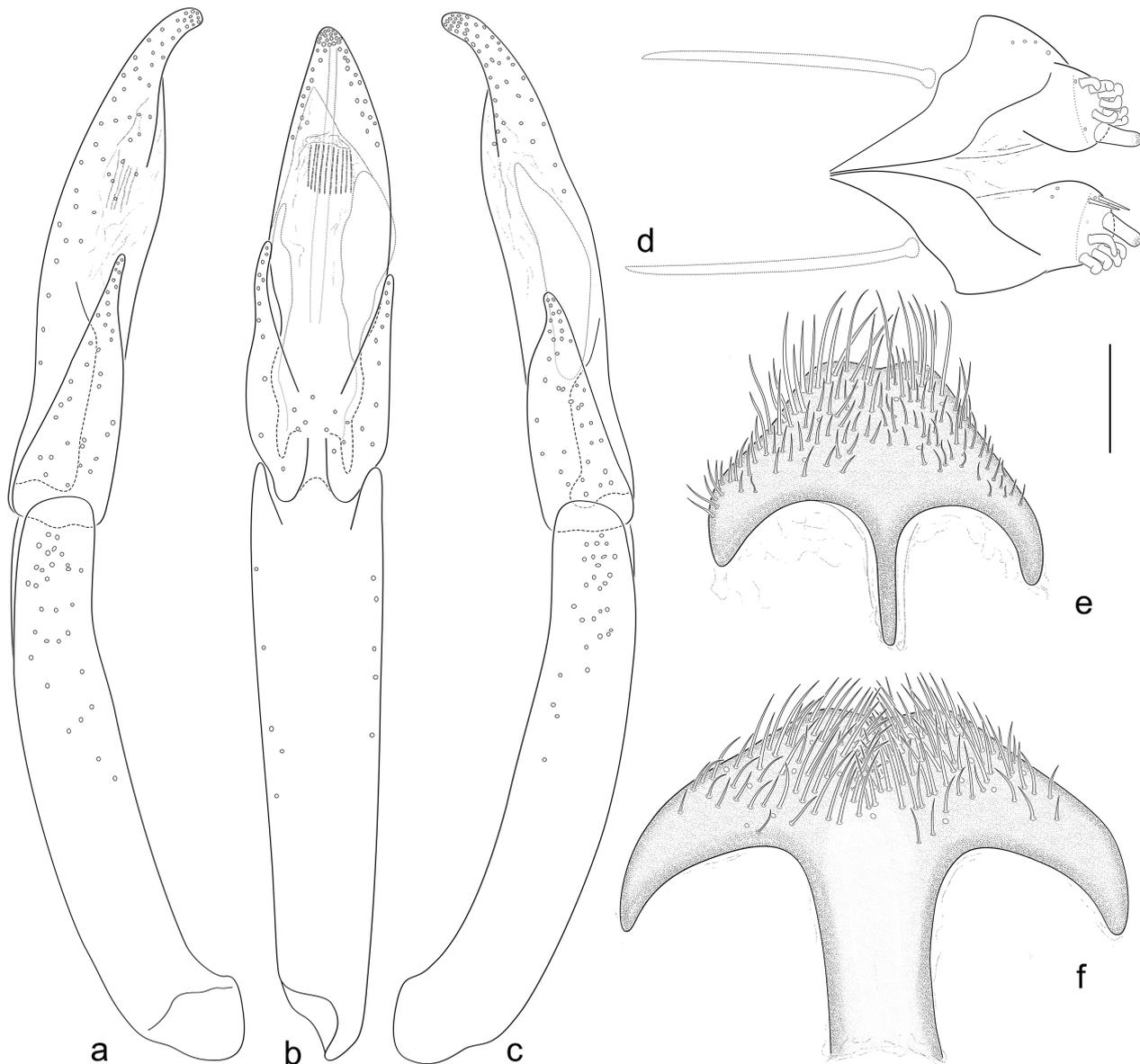


Figure 11. *Rumilara obscura* sp. nov.: **a** aedeagus, left lateral view; **b** aedeagus, ventral view; **c** aedeagus, right lateral view; **d** ovipositor; **e** male sternite VIII; **f** female sternite VIII. Scale: 0.1mm.

Variation. The greatest differences were observed in distribution of pubescence (mainly on legs, around eyes, partly on pronotum and elytra) due to its often removal.

Etymology. From Latin *obscurus* (dim, dark, obscure) due to its dark colour.

Larva (Fig. 9a, e) with length 3.86–4.04 mm, greatest width 1.04–1.16 mm. Pleurites on abdominal segments 1–5. Abdominal segments 1–5 explanate. Surface around the frontal pair of pronotal gibbosities of the same colour as the rest of pronotum. Tergum of abdominal segments 1–7 with a pair of distinct, longitudinally arranged small gibbosities.

Distribution. Known from two localities in Cotopaxi Province (Figs 16a–b) and one locality in Santo Domingo de los Tsáchilas Province (Fig. 16c).

3.2.4. *Rumilara suppressa* sp. nov.

<https://zoobank.org/B916C413-2EFD-4C32-A539-070228BB-D7C4>

Figs 2b, 3b, 5b, 6b, 7b, 8b, 9b, 9f, 12

Material examined. *Type material:* **Holotype** ♂ (PUCE) “Ecuador, Morona-Santiago prov., road Indanza – Gualaceo, second tributary of Río Tinajillas, 03°00′37.5″ S, 78°37′09.1″ W, 2783m a.s.l., 26.8.2013, smaller stream ca 1.5m wide, not fast flowing, with mossy waterfall, mainly mesolithal, Čiampor Jr & Čiamporová-Zaťovičová lgt.”; **Paratypes** 3♂♂, 2♀♀ (PUCE, CCB) with the same data as the holotype; 1♂, 1♀ “Ecuador, Napo prov., first stream in the Andes, 00°22′32.9″ S, 78°04′29.0″ W, 2723m a.s.l., 12.8.2013, fast flowing stream with a lot of smaller and larger rapids, Čiampor Jr & Čiamporová-Zaťovičová lgt.”; 1♀ “Ecuador, Morona-Santiago prov., road Indanza – Gualaceo, fourth tributary of Río Tinajillas, 03°00′41.9″ S, 78°36′32.9″ W, 2669m

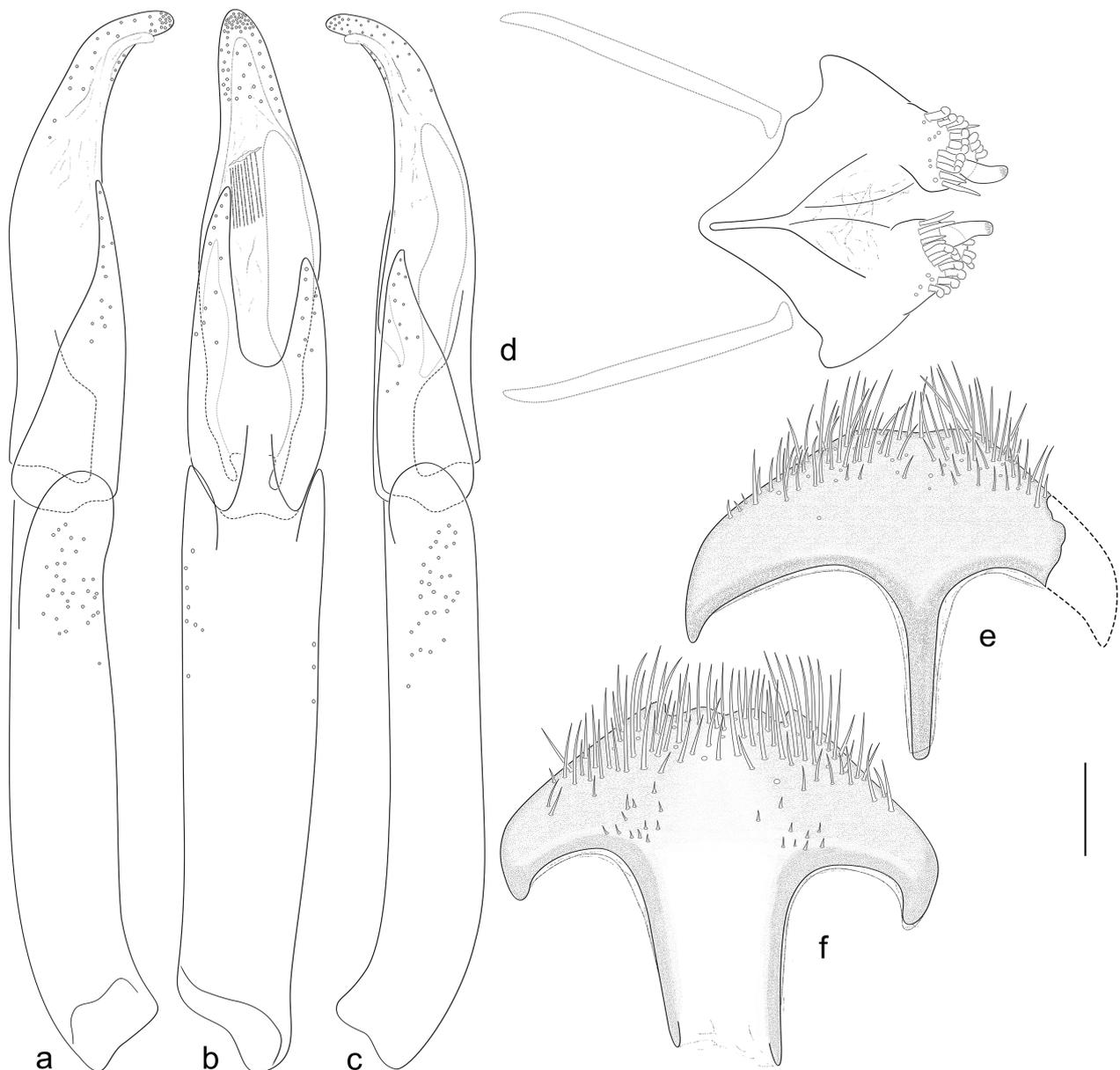


Figure 12. *Rumilara suppressa* sp. nov.: **a** aedeagus, left lateral view; **b** aedeagus, ventral view; **c** aedeagus, right lateral view; **d** ovipositor; **e** male sternite VIII; **f** female sternite VIII. Scale: 0.1mm.

a.s.l., 26.8.2013, tall, narrow waterfall, underneath a pool with pebbles, gravel, wood, detritus, roots, Čiampor Jr & Čiamporová-Zaťovičová lgt.” **Other material:** 20 larvae with the same data as the holotype; 10 larvae “Ecuador, Morona-Santiago prov., road Indanza – Gualaceo, third tributary of Río Tinajillas, 03°00'42.9" S, 78°36'48.3" W, 2739m a.s.l., 26.8.2013, montane stream ca 2m wide, mainly meso- and microlithal, occasional macrolithal, organic detritus, scarce mossy rocks, Čiampor Jr & Čiamporová-Zaťovičová lgt.”; 1 larva “Ecuador, Napo prov., first stream in the Andes, 00°22'32.9" S, 78°04'29.0" W, 2723m a.s.l., 12.8.2013, fast flowing stream with a lot of smaller and larger rapids, Čiampor Jr & Čiamporová-Zaťovičová lgt.”.

Adult diagnosis. The species can be distinguished by a combination of following characters: 1) head and clypeus fully clothed by pubescence; 2) femora bare or with a row of dark, long hairs on dorsal margin; 3) elytra without dense pubescence; 4) aedeagus without rod-like sclerite; 5) parameres strongly asymmetrical.

Adult diagnostic description. MALE. Colour: Body (Figs 2b, 3b) black; lateral sides of femora and tibiae brown to pale brown; tarsal claws pale brown with reddish tincture. **Pubescence:** Head, except middle of frons, and clypeus clothed with short, prone pubescence, most densely set behind eyes, and with numerous slightly longer, curved, semi-erect, hair-like setae. Pronotum (Fig. 6b) covered by short, moderately dense, prone pubescence, and with slightly longer, curved, semi-erect, hair-like setae. Elytra at lateral and apical edges with short, recumbent pubescence, and with numerous moderately long, curved, semi-erect, hair-like setae. Femora with long, dark, densely set, hair-like setae near dorsal margin; pro- and metatibiae apically with a fringe of long, dark, hair-like setae on inner margin; mesotibiae without pubescence. Metaventricle very densely clothed with moderately long, golden, prone pubescence. **Surface:** Head, clypeus and pronotum sparsely microreticulate, partly concealed by pubescence.

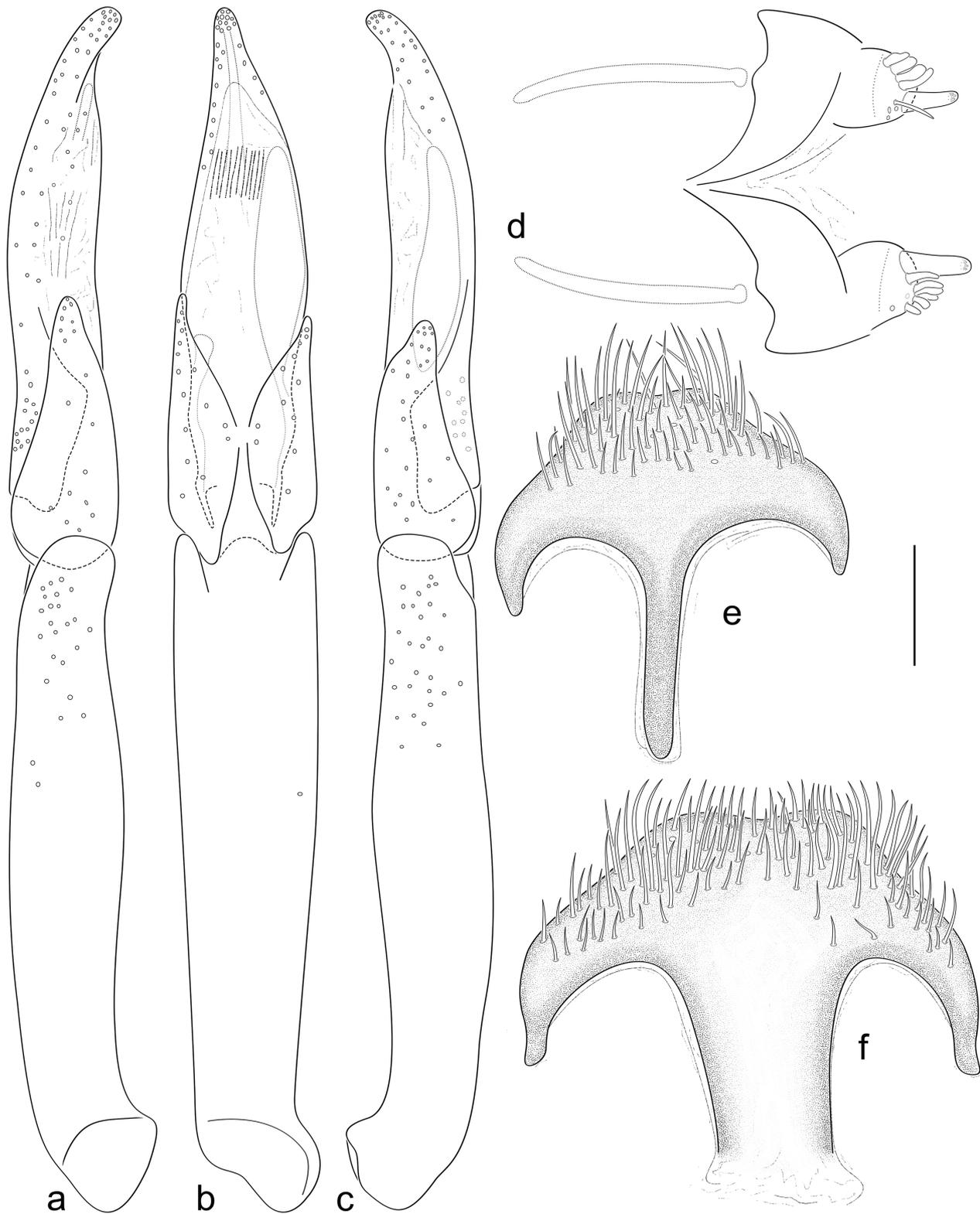


Figure 13. *Rumilara riberai* sp. nov.: **a** aedeagus, left lateral view; **b** aedeagus, ventral view; **c** aedeagus, right lateral view; **d** ovipositor; **e** male sternite VIII; **f** female sternite VIII. Scale: 0.1mm.

Elytra with rows of punctures deeply impressed; third interval slightly elevated in anterior 1/5; intervals 2–4 depressed from anterior 1/5 to 2/5 in lateral view. Prosternal process (Fig. 7b) subparallel between procoxae, then subtriangular with rounded apex. **Aedeagus** (Figs 12a–c) without long, dorsal, rod-like sclerite on penis; parameres

asymmetrical, left one distinctly longer. — **FEMALE** externally similar to male, except slightly larger.

Measurements. ♂ – CL: 2.87–3.10 mm; PL: 0.77–0.82 mm; PW: 0.82–0.91 mm; EL: 2.09–2.27 mm; EW: 1.12–1.22 mm. ♀ – CL: 2.86–3.18 mm; PL: 0.79–0.89

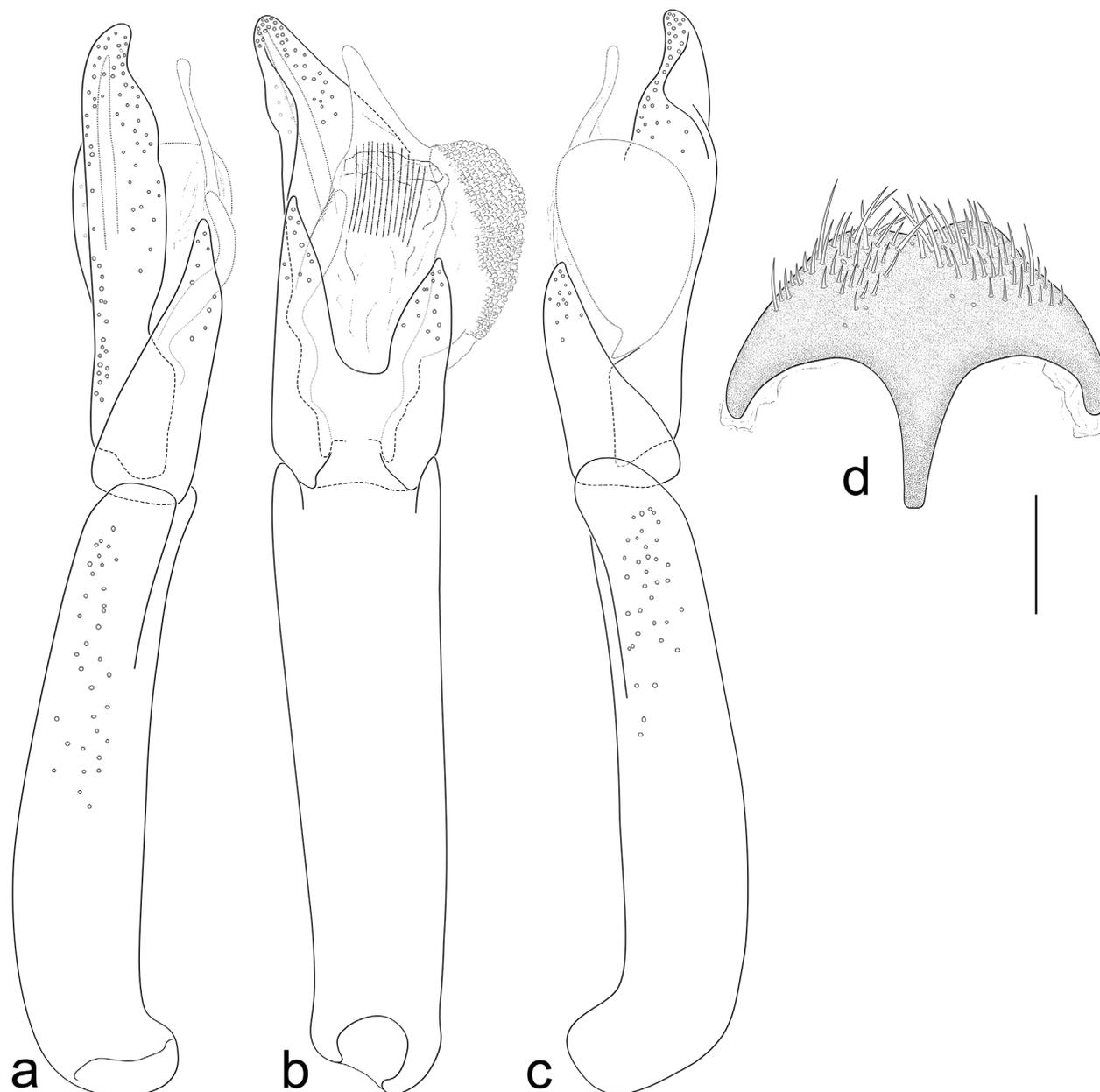


Figure 14. *Rumilara paterna* sp. nov.: **a** aedeagus, left lateral view; **b** aedeagus, ventral view; **c** aedeagus, right lateral view; **d** male sternite VIII. Scale: 0.1mm.

mm; PW: 0.89–1.01 mm; EL: 2.07–2.30 mm; EW: 1.19–1.33 mm.

Variation. The greatest differences were observed in distribution of pubescence (mainly on legs, around eyes, partly on pronotum and elytra) due to its often removal.

Etymology. From Latin *suppressus* (tucked in, contracted) due to its partly depressed elytral intervals 2–4.

Larva (Fig. 9b, e) with length 4.46–4.88 mm, greatest width 1.21–1.28 mm. Pleurites on abdominal segments 1–4. Abdominal segments 1–3 explanate. Surface around the frontal pair of pronotal gibbosities of the same colour as the rest of pronotum. Tergum of abdominal segments 1–7 without a pair of distinct, longitudinally arranged small gibbosities.

Distribution. Known from one locality in Napo Province (Fig. 16g) and three localities in Morona-Santiago Province (Figs 16h–i).

3.2.5. *Rumilara riberai* sp. nov.

<https://zoobank.org/7B43FDD9-535D-4D13-A19F-87573BF0-5C93>

Figs 2c, 3c, 5c, 6c, 7c, 8c, 9c, 10f, 13

Material examined. *Type material:* Holotype ♂ (PUCE) “Ecuador, Santo Domingo de los Tsáchilas prov., Otongachi env., tributary of Río Toachi, 00°19'59.7" S, 78°56'26.6" W, 917m a.s.l., 11.8.2013, stream ca 10m wide, with sand, gravel, boulders, Čiampor Jr & Čiamporová-Zaťovičová lgt.”; *Paratypes* 6♂♂, 1♀ (CCB), 3 ex with

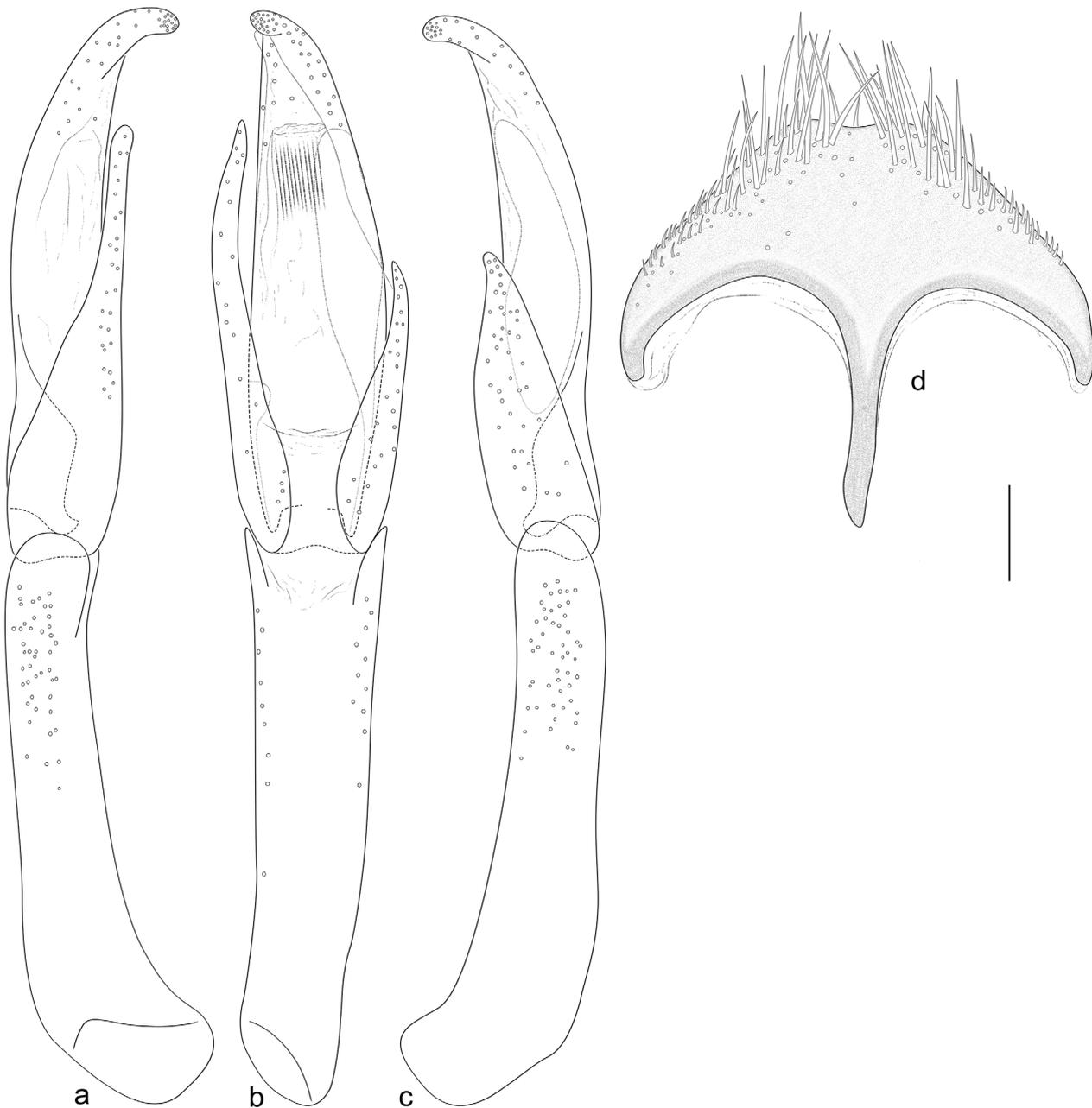


Figure 15. *Rumilara leleupi* comb. nov.: **a** aedeagus, left lateral view; **b** aedeagus, ventral view; **c** aedeagus, right lateral view; **d** male sternite VIII. Scale: 0.1mm.

the same data as the holotype; 4♀, 6 ex “Ecuador, Santo Domingo de los Tsáchilas prov., Otongachi env., next to the tributary of Río Toachi, 00°19'50.5" S, 78°56'41.6" W, 914m a.s.l., 11.8.2013, very shallow stream running along a rock covered by periphyton, Čiampor Jr & Čiamporová-Zaťovičová lgt.” **Other material:** 13 larvae with the same data as the holotype; 6 larvae “Ecuador, Santo Domingo de los Tsáchilas prov., Otongachi env., next to the tributary of Río Toachi, 00°19'50.5" S, 78°56'41.6" W, 914m a.s.l., 11.8.2013, very shallow stream running along a rock covered by periphyton, Čiampor Jr & Čiamporová-Zaťovičová lgt.”; 1 larva “Ecuador, Santo Domingo de los Tsáchilas prov., Otongachi env., tributary of Río Toachi, 00°19'34.0" S, 78°56'59.1" W, 852m a.s.l., 11.8.2013, wild river ca 20m wide, fast flowing, with large boulders, Čiampor Jr & Čiamporová-Zaťovičová lgt.”.

Adult diagnosis. The species can be distinguished by a combination of following characters: 1) head only par-

tially clothed by pubescence, clypeus bare; 2) femora bare or with a row of dark, long hairs on dorsal margin; 3) elytra without dense pubescence; 4) aedeagus with rod-like sclerite; 5) parameres only feebly asymmetrical.

Adult diagnostic description. MALE. Colour: Body (Figs 2c, 3c) black; lateral sides of femora and tibiae brown; tarsal claws pale brown with reddish tincture. **Pubescence:** Head behind eyes clothed with short, prone pubescence. Elytra very readily covered by short, recumbent pubescence, and with numerous moderately long, curved, semi-erect, hair-like setae. Femora with long, dark, densely set, hair-like setae near dorsal margin; pro- and metatibiae apically with a fringe of long, dark, hair-like setae on inner margin; mesotibiae without pubescence. Metaventricle readily clothed with short,

prone pubescence. **Surface:** Head, clypeus and pronotum densely punctate. Elytra with rows of punctures moderately deeply impressed; third interval slightly elevated in anterior 1/5; remaining intervals nearly flat. Prosternal process (Fig. 7c) moderately wide, concave between procoxae, then subtriangular with rounded apex. **Aedeagus** (Figs 13a–c) with long, dorsal, rod-like sclerite on penis; parameres asymmetrical, left one slightly longer. — FEMALE externally similar to male, except slightly larger.

Measurements. ♂ – CL: 2.16–2.53 mm; PL: 0.56–0.65 mm; PW: 0.71–0.79 mm; EL: 1.60–1.88 mm; EW: 0.83–0.97 mm. ♀ – CL: 2.68–2.69 mm; PL: 0.68–0.70 mm; PW: 0.82–0.85 mm; EL: 1.99–2.00 mm; EW: 1.04–1.06 mm.

Variation. The greatest differences were observed in distribution of pubescence (mainly on legs, around eyes, partly on pronotum and elytra) due to its often removal.

Etymology. Named after Ignacio Ribera Galán a great expert on systematics, phylogeny, evolution, biogeography, and conservation of water beetles, who untimely passed away.

Larva (Fig. 9c) with length 2.92–2.96 mm, greatest width 0.96–0.99 mm. Pleurites on abdominal segments 1–5. Abdominal segments 1–6 explanate. Surface around the frontal pair of pronotal gibbosities of the same colour as the rest of pronotum. Tergum of abdominal segments 1–7 with a pair of distinct, longitudinally arranged small gibbosities.

Distribution. Known from three localities in Santo Domingo de los Tsáchilas Province (Figs 16c–d).

Remarks. Several paratypes are markedly smaller than the rest (including holotype), however, they agree in all diagnostic characters.

3.2.6. *Rumilara paterna* sp. nov.

<https://zoobank.org/4BA8996F-8FA8-4A73-A7C0-3A20FAF7-CE92>

Figs 2d, 3d, 5d, 6d, 7d, 8d, 9d, 14

Material examined. Type material: **Holotype** ♂ (PUCE) „Ecuador, Morona-Santiago prov., Indanza env., 03°08'38.2" S, 78°32'10.5" W, 1299m a.s.l., 24.8.2013, stream ca 5m wide, with gravel, boulders, leaf debris, submerged wood, Čiampor Jr & Čiamporová-Zaťovičová lgt.“; **Paratype** 1 ex (CCB) „Ecuador, Morona-Santiago prov., Santa Rosa de Mamanguy env., Cascada la Encañada, 03°05'14.7" S, 78°24'36.0" W, 698m a.s.l., 25.8.2013, stream ca 2–3m wide under waterfall, with clay, gravel, stones, submerged wood, Čiampor Jr & Čiamporová-Zaťovičová lgt.“ **Other material:** 1 larva „Ecuador, Napo prov., road to Coca, 00°43'39.6" S, 77°45'56.1" W, 1129m a.s.l., 17.8.2013, small waterfall, Čiampor Jr & Čiamporová-Zaťovičová lgt.“; 2 larvae „Ecuador, Morona-Santiago prov., tributary of Río Crusado,

03°02'57.0" S, 78°30'03.2" W, 979m a.s.l., 24.8.2013, stream ca 2m wide, in forest, with gravel, stones, leaf debris, submerged wood with moss, Čiampor Jr & Čiamporová-Zaťovičová lgt.“; 1 larva “Ecuador, Morona-Santiago prov., Santa Rosa de Mamanguy env., Cascada la Encañada, 03°05'14.7" S, 78°24'36.0" W, 698m a.s.l., 25.8.2013, stream ca 2–3m wide under waterfall, with clay, gravel, stones, submerged wood, Čiampor Jr & Čiamporová-Zaťovičová lgt.”.

Adult diagnosis. The species can be distinguished by a combination of following characters: 1) head and clypeus fully clothed by pubescence; 2) femora fully pubescent; 3) elytra with dense pubescence, not arranged in stripes; 4) aedeagus with rod-like sclerite; 5) parameres moderately asymmetrical.

Adult diagnostic description. **MALE. Colour:** Body (Figs 2d, 3d) dark brown; coxae, trochanters, femora, tibiae, and tarsi brown; tarsal claws pale brown with reddish tincture. **Pubescence:** Head and clypeus fully clothed with short, prone pubescence, most densely set behind eyes, and with numerous slightly longer, curved, semi-erect, hair-like setae. Pronotum (Fig. 6d) covered by a short, moderately dense, prone pubescence, and with moderately long, curved, semi-erect, hair-like setae. Elytra with densely arranged, short, recumbent pubescence, and numerous moderately long, curved, semi-erect, hair-like setae. Femora, pro- and metatibiae densely covered with moderately long, recumbent, hair-like setae; mesotibiae with pubescence only at extreme base. Metaventricle densely clothed with short, prone pubescence. **Surface:** Head and clypeus sparsely microreticulate, concealed by pubescence. Pronotum densely punctate, partly concealed by pubescence. Elytra with rows of punctures shallowly impressed; intervals nearly flat. Prosternal process (Fig. 7d) subparallel between procoxae, then subtriangular with rounded apex. **Aedeagus** (Figs 14a–c) with long, dorsal, rod-like sclerite on penis; parameres asymmetrical, left one slightly longer. — FEMALE unknown.

Measurements. ♂ – CL: 2.67 mm; PL: 0.61 mm; PW: 0.75 mm; EL: 2.07 mm; EW: 0.83 mm.

Variation. No significant differences were observed between the two known specimens.

Etymology. From Latin *paternus* (of or connected with one's forefathers, ancestral) due to its similarity with the genus *Hexanchorus*.

Larva (Fig. 9d) with length 3.48–3.55 mm, greatest width 0.99–1.10 mm. Pleurites on abdominal segments 1–5. Abdominal segments 1–7 explanate. Surface around the frontal pair of pronotal gibbosities paler than the rest of pronotum. Tergum of abdominal segments 1–7 with a pair of distinct, longitudinally arranged small gibbosities.

Distribution. Known from one locality in Napo Province and three localities in Morona-Santiago Province (Figs 16e–f).



Figure 16. Sampling sites of *Rumilara* gen. nov. in Ecuador: **a** Cotopaxi prov., Otonga env., Río Esmeralda; **b** Cotopaxi prov., Artos env., stream above confluence with Río Esmeralda; **c** Santo Domingo de los Tsachilas prov., Otongachi env., tributary of Río Toachi; **d** Santo Domingo de los Tsachilas prov., thin water layer on rock, Otongachi env.; **e** Morona-Santiago prov., small stream near Indanza; **f** Morona-Santiago prov., Santa Rosa de Mamanguy env., Cascada la Encañada; **g** fast-flowing stream in Napo prov.; **h** Morona-Santiago prov., tributary of Río Tinajillas; **i** Morona-Santiago prov., tributary of Río Tinajillas; (localities of *R. obscura* – **a**, **b**; *R. riberai* – **c**, **d**; *R. paterna* – **e**, **f**; *R. suppressa* – **g**–**i**).

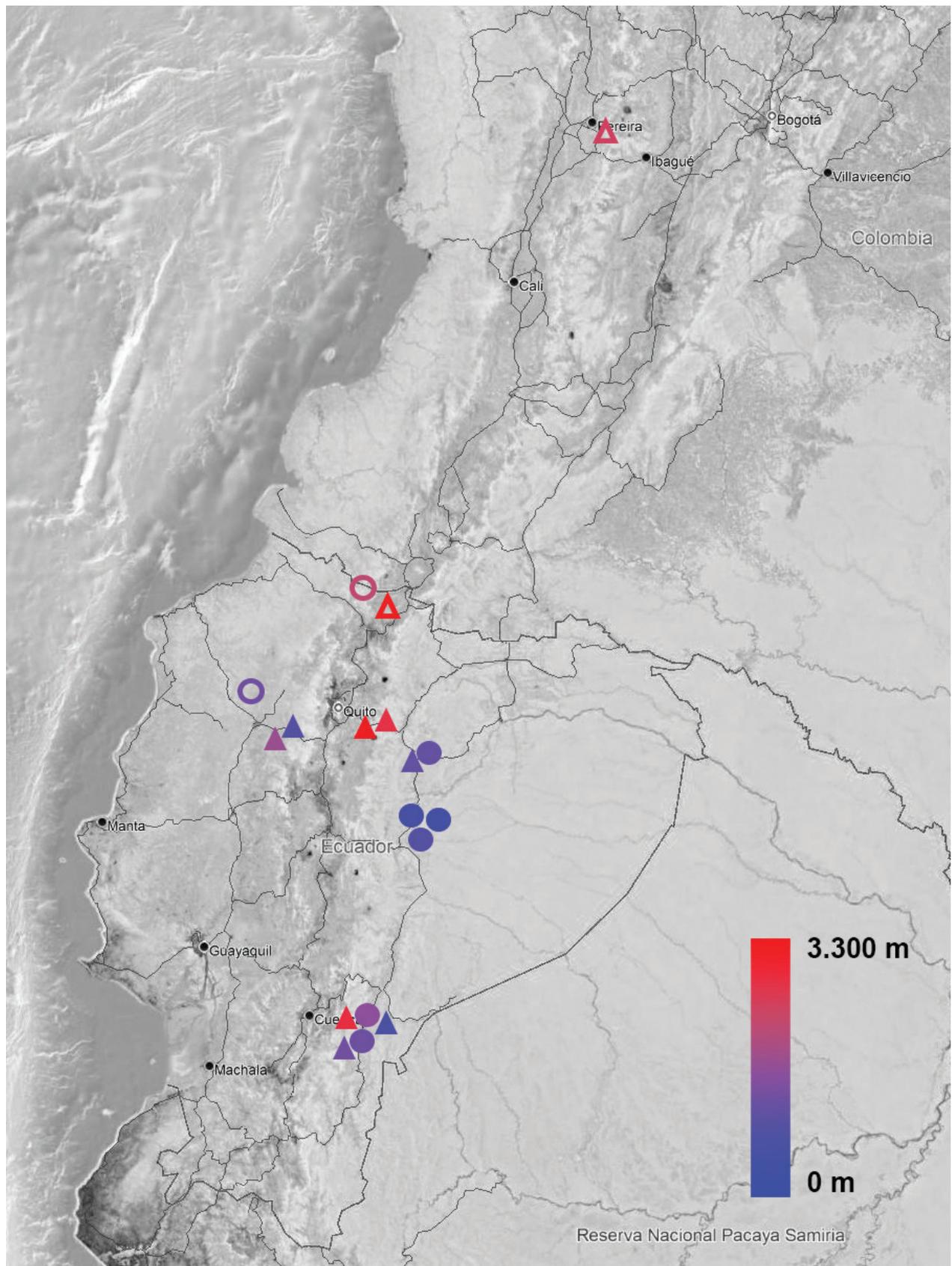


Figure 17. Altitudinal distribution of *Hexanchorus* and *Rumilara gen. nov.* in Ecuador (with one record from Colombia); circles – *Hexanchorus*, triangles – *Rumilara gen. nov.* Hollow symbols represent material not examined in this study (Modified from Google Earth).

Remarks. Only two specimens are known, one of them of unidentified sex, with abdomen lacking last two segments.

3.2.7. *Rumilara leleupi* (Delève, 1968) comb. nov.

Figs 2e, 3e, 5e, 6e, 7e, 8e, 15

Hexanchorus leleupi Delève, 1968: 214.

Type locality. Ecuador, [Napó Province], Papallacta, 3.300m a.s.l.

Material examined. Type material: Holotype ♂ (RBINS) “♂” // “Holotype” [red] // “Ecuador Papallacta III-1965. N. Leleup” // “Ruisseau torrentueux 3.300 m” // “R. I. Sc. N. B. I. G. 23.948” // “J. Delève det., 1966 *Hexanchorus leleupi* n.sp.” // “*Rumilara leleupi* (Delève, 1968)” [appended].

Condition. Generally, in good condition, except for following parts of legs that are missing: last segment of right protarsus, last two segments of left protarsus, left midleg (only coxa and trochanter present), and last two segments of left metatarsus.

Adult diagnosis. The species can be distinguished by a combination of following characters: 1) head and clypeus fully clothed by pubescence; 2) femora fully pubescent; 3) elytra with dense pubescence arranged in stripes; 4) aedeagus without rod-like sclerite; 5) parameres strongly asymmetrical.

Adult diagnostic redescription. MALE. **Colour:** Body (Figs 2e, 3e) black, tarsal claws pale brown with reddish tincture. **Pubescence:** Head and clypeus fully clothed with short, prone pubescence, most densely set behind eyes. Pronotum (Fig. 6e) covered by a short, moderately dense, prone pubescence. Elytra with short, recumbent pubescence, most densely arranged in stripes along intervals, and with numerous moderately long, curved, semi-erect, hair-like setae. Femora, pro- and metatibiae densely covered with moderately long, recumbent, hair-like setae; mesotibiae without pubescence. Metaventrite readily clothed with short, prone pubescence. **Surface:** Head, clypeus and pronotum sparsely microreticulate, concealed by pubescence. Elytra with rows of punctures deeply impressed; third interval slightly elevated in anterior 1/5; intervals 2–4 depressed from anterior 1/5 to 2/5 in lateral view. Prosternal process (Fig. 7e) subparallel between procoxae, then subtriangular with rounded apex. **Aedeagus** (Figs 15a–c) without long, dorsal, rod-like sclerite on penis; parameres asymmetrical, left one distinctly longer. — FEMALE unknown.

Measurements. ♂ – CL: 3.28 mm; PL: 0.92 mm; PW: 0.95 mm; EL: 2.36 mm; EW: 1.37 mm.

Larva unknown.

Distribution. So far known only from the type locality in Napó Province.

Remarks. Delève (1968) very likely interpreted distinctly shorter left paramere as broken and illustrated it with the same length as the right one. There are no signs of artificial ending of the left paramere, and even their shapes are dissimilar.

4. Discussion

This study has provided new insights into the taxonomy, diversity and phylogeny of riffle beetles, which play an important role in stream communities worldwide (Miserendino 2001; Masese et al. 2009; Luo et al. 2018; González-Córdoba et al. 2020). However, the study also showed how many gaps still exist in our knowledge of this specialized group of insects, so closely tied to globally threatened freshwater habitats.

The use of molecular data in Elmidae taxonomy has been proven to work well in the past (e.g., Čiampor Jr et al. 2019; Linský et al. 2021). The combination of DNA characters and morphological characterization contributed significantly to support the taxonomic claims presented here, again validating the feasibility of this approach and suggesting that it should become the standard for future studies of the diversity and evolution of these beetles.

4.1. Phylogeny of *Hexanchorus* and *Rumilara* gen. nov.

Phylogenetic analysis revealed a deep bifurcation between *Hexanchorus* and *Rumilara* gen. nov. that likely happened in the middle/late Miocene, in the similar time period as the separation of related genera *Pseudodisersus*, *Potamophilops*, and *Disersus* (Fig. 1). This period is associated with significant surface uplift of the Andes (Gregory-Wodzicki 2000), and it is likely that these processes played a key role in the diversification and speciation of the elmid fauna in the Andes and their vicinity as in other animal taxa (e.g., Fiedler and Strutzenberger 2013). Although Miocene evolutionary processes in South America have been studied relatively extensively, there is still much unknown about the evolution of freshwater faunas. Our results suggest that species closely tied to freshwater habitats may have evolved similarly to terrestrial taxa with lower dispersal ability (e.g., Wesselingh and Salo 2006; Santos et al. 2009).

The phylogeny reconstruction corroborates previous findings of Linský et al. (2019), that some Ecuadorian *Hexanchorus* species appeared very recently. Similar recent speciation events were found in Brazil (Linský et al., unpubl. data), indicating a possible young origin or Neotropical riffle beetle diversity. The most distant species within the genus is *H. rostratus*, which diverged ~8.5 Mya. The 28S sequences of both *H. rostratus* speci-

mens were identical with *Rumilara* **gen. nov.** species but differed from all *Hexanchorus* species. However, *H. rostratus* does not share morphological features typical for *Rumilara* **gen. nov.**, and other genetic markers and concatenated dataset assign the species in *Hexanchorus*. So, the similarity in the 28S rDNA agrees with the basal position of *H. rostratus* and the close relationship of *Hexanchorus* with *Rumilara* **gen. nov.** and could be attributed to incomplete lineage sorting.

Species groups within *Hexanchorus* have never been recognized except for the one consisting of the species from the Guiana Shield. This group includes *H. angeli*, *H. bifurcatus*, *H. homaetarsoides*, *H. inflatus* and one undescribed species from Venezuela (Maier and Short 2014). The group is characterized by distinct median process of the third female abdominal ventrite, and similar male genitalia (Maier 2013; Laššová et al. 2014; Maier and Short 2014). The group is only known from the table mountains from southern Venezuela and Suriname but possibly occurs also in Guyana and neighboring countries. Two species of this group were included in the analysis forming a distant clade, however, inclusion of more species is desired to properly evaluate their phylogenetic position.

The remaining six taxa of *Hexanchorus* were grouped together similarly to previous results (Linský et al. 2019), apart from *H. virilis* from Ecuador, which is now placed closer to the three Ecuadorian species than to *H. tarsalis* from Brazil. However, the results presented here have considerably higher support, and a closer relationship of *H. virilis* with other species, similar in the morphology of antennae (refer to 4.3.) and distribution, therefore seems more likely. The remaining Ecuadorian species (except *H. rostratus*) diversified very recently (within the last ~1.5 My). Contrary to the previous study, a more thorough molecular analysis proposed closer relation of *H. onorei onorei* to *H. cordillerae* than to *H. onorei sagittatus*. The latter taxon is thus elevated to species rank, as the morphological differences (body size, shape of ventrite 5, shape of aedeagus) support the separate status of the two taxa.

Other Larinae genera that are close to *Rumilara* **gen. nov.** and *Hexanchorus* morphologically and/or geneticaly include *Disersus*, *Hispaniolara*, *Potamophilops* and *Pseudodisersus*. Apart from *Hispaniolara*, they exhibit great variability in shape of elytral apices which often differ between species and sexes. Species of *Rumilara* **gen. nov.** have only simple, rounded elytral apices, but this state is also present in all related genera. *Hexanchorus* is the only genus with markedly depressed middle of the abdomen. It shares a tubercle on inner apex of metatibia in males with *Disersus* and *Potamophilops* (feature never mentioned in these genera before), its apically emarginate last abdominal segment is present in all mentioned genera except for *Pseudodisersus* and *Rumilara* **gen. nov.** (Spangler & Santiago-Fragoso 1987, 1992; Linský et al. 2019). *Rumilara* **gen. nov.** resembles *Pseudodisersus* in the lack of sexual dimorphism. The situation described above suggests that the use of morphological characters for assessing phylogenetic relationships can be misleading. Some characters

vary within genera, and reconstructed relationships may hence differ based on the taxon sampling used. Molecular characters help to solve this issue, avoiding the use of subjectively selected morphological characters. This supports the idea to prioritize DNA-based evolutionary relationships and confront them subsequently with morphology.

The *Rumilara* is undoubtedly close relative of *Hexanchorus* as both genera share many synapomorphies separating them from the rest of Larinae. However, molecular and morphological characters strongly support its reciprocal monophyly. *Hexanchorus* species display a striking sexual dimorphism (Hinton 1940), adults of *Rumilara* **gen. nov.** have microreticulate and differently shaped pronotum, convex abdomen with rounded apex, lack dense pubescence on mesotibiae, and tubercles on meso- and metatibiae. Furthermore, there are distinct differences in the structure of the male genitalia.

All but one *Rumilara* species (*R. paterna* **sp. nov.**) share a similar, almost glabrous dorsal side of body, and almost bare femora and tibiae. Although *R. paterna* **sp. nov.** superficially resembles *Hexanchorus* species in its pubescent body, its structure of antennae, pronotum, legs, abdomen, and male genitalia are typical for *Rumilara* **gen. nov.** From all described *Hexanchorus* species only *H. leleupi* does not have abdomen depressed and rounded distal margin of ventrite 5 in males. In fact, it is much more similar morphologically and ecologically with the new genus and especially with *R. suppressa* **sp. nov.** Based on this, *R. leleupi* **comb. nov.** is thus herein transferred to *Rumilara* **gen. nov.** despite the lack of molecular data.

The two distinct clades revealed by phylogenetic analysis within *Rumilara* **gen. nov.** are also supported by morphology. At least some species of both groups have aedeagus with rod-like dorsal fibula, a possible synapomorphy of the genus. The first clade can be characterized by a moderately wider prosternal process, and reduced femoral pubescence concentrated in a row of longer hairs along dorsal margin. The second clade shares narrower prosternal process but differs in almost all other studied characters. *R. suppressa* **sp. nov.** stands distinctly apart from the other species, differing in several morphological characters of both adults and larvae (see Figs 9, 12). Based on sharing several morphological features, its sister group relationship with *R. leleupi* **comb. nov.** can be expected. Furthermore, both species were collected in significantly higher altitudes (Fig. 17; *R. leleupi* **comb. nov.** 3300 m a.s.l., *R. suppressa* **sp. nov.** 2669–2783 m a.s.l.), which is in strong contrast to the habitats of *Hexanchorus* (0–1500 m a.s.l.; reported by Spangler and Santiago-Fragoso 1992) and the rest of *Rumilara* **gen. nov.** (698–1760 m a.s.l.).

4.2. Anatomy of legs

There are three taxonomically significant features that can be found on legs of *Hexanchorus* species (Fig. 10e): 1) presence and extension of pubescence on mesotibiae;

2) presence and size of tubercle on inner apex of meso- and metatibia; 3) species specific sexual dimorphism. None of these three traits are present in any of five recognized species of *Rumilara* **gen. nov.** and thus, further corroborate its separate position.

Pubescence on legs in *Hexanchorus* is relatively stable, with femora, protibiae, and metatibiae being fully and densely clothed with short hair-like setae, while pubescence of mesotibiae is very variable. Mesotibiae are in most cases bare in at least apical half, with most of the short hair-like setae densely clustered in two areas, firstly described by Spangler and Santiago-Fragoso (1992). The closer examination of available material revealed that the lateral and medial division is incorrect. The original division could be interpreted, through bilateral symmetry, that both lateral sides and both medial sides have the same distribution of pubescence. However, anterior and posterior lateral sides have, in most cases, asymmetrical extension of pubescence, and only sparse setae can be found medially. Our observation revealed many discrepancies between literature and extension of pubescence on mesotibiae. It is thus incorrect to only interpret lateral area as anterior lateral area and medial area as posterior lateral area. Spangler and Santiago-Fragoso (1992) proposed these characters only for males. In fact, the pubescence distribution is stable within species and is equal in both sexes.

In *Rumilara* **gen. nov.**, only *R. paterna* **sp. nov.** and *R. leleupi* **comb. nov.** have femora, pro- and metatibiae clothed as its sister genus, remaining three species have only apical tibial fringes and femora with dense, dark, long, hair-like setae along dorsal margin.

Presence of distinct carina on inner apex of meso- or metatibia was previously used as a diagnostic character for example for *H. tibialis* (Hinton 1935). Examination of majority of known species revealed that almost all males of *Hexanchorus* species have thorn-like carina on inner apex of mesotibia, and thin carina on inner apex of metatibia. The latter character is lacking in males of *H. angeli*, which could be characteristic for the species group from Guiana Shield.

Male sexual dimorphism was also observed on forelegs of three known *Hexanchorus* species. Distinct dilatation of femora was reported in *H. virilis*, *H. tarsalis*, and *H. caraibus* possess an enlarged last tarsal segment. Given their great differences in morphology, distribution, and genetic distances (latter two species not included in molecular analysis), it is likely that these characters have evolved independently within *Hexanchorus*.

4.3. Structure of antennae

Kodada et al. (2016) mention, that antennae of Larinae can be capitate, clavate, serrate or filiform, and this character is considered stable within genera. The degree of compactness of antennae (Figs 5f–t) varies between *Hexanchorus* species from relatively tight (e.g., *H. tarsalis*; Fig. 5m) to more relaxed (e.g., *H. usitatus*; Fig. 5n). Within species they are stable and not sex specific. What can

be clearly defined is a group of species having antennomeres with a visible basal stalk (Figs 5f, g, j, k, l, q; *H. mediarmedi*, *H. virilis*, *H. cordillerae*, *H. rostratus*, *H. onorei*, *H. crinitus*, *H. shepardii*, and *H. sagittatus* **stat. nov.**). Since *H. rostratus* is, according to the molecular analysis, the most basal *Hexanchorus* species, this could mean, that species with stalked antennomeres could be evolutionary closely related, however a more robust dataset is needed to confirm this hypothesis. *Rumilara* **gen. nov.** have advanced a bit further with compactness of antennae, by shortening of the segments and incorporating them into club-like structure (Figs 5a–e).

4.4. Larval morphology

The larvae of *Hexanchorus* and *Rumilara* **gen. nov.** are similar in having unique pair of large gibbosities on the eighth abdominal segment, but the character is less developed in early instars. Larvae of *Hexanchorus* can be misidentified in the genus *Stegoelmis* since both have similar shape, pleurites on abdominal segments 1–6, and lateral extensions bearing setae. On the other hand, *Rumilara* **gen. nov.** larvae (Fig. 9) do not have setae on lateral extensions, their pleurites never reach sixth abdominal segment, and lateral extensions are more prominent and distinctly paler. Larva of *R. suppressa* **sp. nov.** is more distinctly different from larvae of the remaining species, but adult morphology and the molecular data do not provide sufficient support that would justify its separate position.

4.5. Distribution

Spangler and Santiago-Fragoso (1992) stated that *Hexanchorus* occurs in altitudes from sea level to 1500 m a.s.l. From published records, only two species were found higher – *H. rostratus* (1522 m; Linský et al. 2019) and *H. flintorum* (1646 m; Maier 2013). However, given that most of the records are from Central America, the real altitudinal range of *Hexanchorus* could possibly extend above 2000 m a.s.l. Sites et al. (2003) collected *Hexanchorus* at two localities in northern Ecuador, one of them was 2195 m a.s.l., which corroborates previous claim (C. B. Barr personal communication). The studied material confirmed occurrence of *Rumilara* **gen. nov.** in four provinces of Ecuador. A syntopy with *Hexanchorus* was not recorded. In one case, they were collected only a few metres apart in a small stream and its tributary, which might suggest that their segregated occurrence is due to ecological rather than elevational preferences. It appears that *Rumilara* species partly occupy similar altitudinal range as *Hexanchorus* with two significant exceptions – *R. suppressa* **sp. nov.** and *R. leleupi* **comb. nov.** – which are known only from altitudes above 2600 m a.s.l. As they share also some morphological traits, they could form a species group within *Rumilara*. To support or reject this claim, more *R. leleupi* specimens are required, especially females (due to a different apex of eighth ventrite in *R. suppressa* **sp. nov.**) or larvae. Based on the available ma-

terial it seems that *Rumilara* **gen. nov.** could be a montane genus, with distribution limited along Andes (Fig. 17). Even though its species have similarly developed hind wings as much more widespread *Hexanchorus*, there are likely other factors limiting their distribution. Sites et al. (2003) collected few adult specimens that likely belong to *Rumilara* **gen. nov.** in Carchi (north Ecuador on border with Colombia) at 3252 m a.s.l. (C. B. Barr personal communication). Moreover, one larva of earlier instar, which resembles that of *R. suppressa* **sp. nov.**, was collected in the western central region of Colombia – in Risaralda Department at 2400 m a.s.l. (M. González-Córdoba personal communication). Based on these data it can be expected that the new genus exceeds Ecuador and occurs also in other Andean countries.

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Supplementary material 1

Table S1

Authors: Linský M, Čiamporová-Zaťovičová Z, Laššová K, Čiampor Jr F (2022)

Data type: .pdf

Explanation note: Table S1 is a list of all samples used in the phylogenetic analyses, all samples are provided with BIN and GenBank accession numbers, DNA markers available and geographic location.

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Link: <https://doi.org/asp.80.e84013.suppl1>

Supplementary material 2

ML trees

Authors: Linský M, Čiamporová-Zaťovičová Z, Laššová K, Čiampor Jr F (2022)

Data type: .pdf

Explanation note: ML trees includes all maximum likelihood trees produced for the dataset, including analysis all 70 samples available using *cox1* and analyses of the sample subset with single markers (*cox1*, 16S, 18S, 28S) and concatenated data.

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Link: <https://doi.org/asp.80.e84013.suppl2>

Supplementary material 3

Newick trees

Authors: Linský M, Čiamporová-Zaťovičová Z, Laššová K, Čiampor Jr F (2022)

Data type: .zip

Explanation note: Newick trees includes all original trees in newick format produced prior editing.

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Link: <https://doi.org/asp.80.e84013.suppl13>