

New species and new stages of *Anacroneuria* (Plecoptera: Perlidae) from Southern Yungas (Argentina and Bolivia)

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Received 28 - VI - 2023 / Accepted 25 - IX - 2023 / Published 31 - XII - 2023

<https://doi.org/10.25085/rsea.820401>

Nuevas especies y nuevos estadios de *Anacroneuria* (Plecoptera: Perlidae) de las Yungas Australes (Argentina y Bolivia)

RESUMEN. Las Yungas Australes (sur de Bolivia y noroeste de Argentina) se caracterizan por su alta diversidad, paisaje empinado y baja accesibilidad. Las especies de *Anacroneuria* Klapálek (Plecoptera: Perlidae) en esta ecorregión han sido escasamente estudiadas. Nuestro objetivo es conocer su diversidad de especies (basada en caracteres de adultos) y explorar los caracteres larvales importantes para su reconocimiento. Hemos criado decenas de larvas de diferentes localidades y recolectado adultos con trampas de luz. Hemos identificado 13 especies (basadas en el edeago de los machos) y hemos asociado con éxito los estadios larvales y adultos de siete de ellas. En el presente artículo describimos siete nuevas especies: *Anacroneuria tafi* sp. nov., *A. flavinigra* sp. nov., *A. saican* sp. nov., *A. triplex* sp. nov., *A. calilegua* sp. nov., *A. lutea* sp. nov. y *A. tupacamaru* sp. nov. Para seis especies previamente descritas, presentamos nuevos datos y descripciones de estadios: *A. arrazayalensis* Orce, *A. handlirschi* Klapálek, *A. reedi* (Navás), *A. tucuman* Stark, *A. saltensis* Froehlich y *A. chimborazo* Stark. Se estudió la divergencia genética para algunas de estas especies utilizando la región del código de barras del gen mitocondrial COI. Se presenta una clave para los adultos machos de todas las especies de *Anacroneuria* del noroeste de Argentina.

PALABRAS CLAVE. Andes. Insectos acuáticos. Moscas de las piedras. Secuencias COI. Taxonomía.

ABSTRACT. Austral Yungas cloud forest (Southern Bolivia and NW Argentina) is known for its high diversity, steep landscape and low accessibility. *Anacroneuria* Klapálek (Plecoptera: Perlidae) species in this ecoregion have been seldom studied. We aim to know its species diversity (based on adult characters) and explore larval characters important for their recognition. We have reared dozens of larvae from different localities and collected adults with light traps. Reared adults and associated larval exuviae were studied, photographed and drawn. We have identified 13 species (based on male aedeagus) and successfully associated larval and adult stages for seven species. In the present paper we describe seven new species: *Anacroneuria tafi* sp. nov., *A. flavinigra* sp. nov., *A. saican* sp. nov., *A. triplex* sp. nov., *A. calilegua* sp. nov., *A. lutea* sp. nov., and *A. tupacamaru* sp. nov. For six species previously described we present new data and stage descriptions: *A. arrazayalensis* Orce, *A. handlirschi* Klapálek, *A. reedi* (Navás), *A. tucuman* Stark, *A. saltensis* Froehlich, and *A. chimborazo* Stark. Genetic divergence for some of these species was studied using the barcode region of the mitochondrial gene COI. A key to the male adults of all the species of *Anacroneuria* from Northwestern Argentina is presented.

KEYWORDS. Andes. Aquatic Insects. COI sequence. Stonefly. Taxonomy.

INTRODUCTION

The Perlidae genus *Anacroneuria* Klapálek, 1909 is dominant and diverse in Neotropical mountain rivers. It is distributed from the Southwestern United States to Central Argentina with more than 382 known species (Pessacq et al., 2019). In Argentina, Stark (2013) mentions 24 species that are distributed mainly in the biogeographical provinces of Yungas and Paranaense. The Austral Yungas cloud forest located in southern Bolivia and northwestern Argentina (Brown et al., 2001; Arana et al., 2021) is known for its high diversity, rugged landscape, and low accessibility. *Anacroneuria* in this ecoregion has rarely been studied and so far only 6 species have been cited: *Anacroneuria arrazayalensis* Orce, 2003, *A. handlirschi* Klapálek, 1922, *A. marshalli* Stark, 2007, *A. reedi* (Navás) 1919, *A. saltensis* Froehlich, 2002, and *A. tucuman* Stark, 2013.

The objective of this work is to study the diversity of species present in this ecoregion (based on adult characters), to explore important larval characters for their recognition, and to describe taxonomic novelties found (new species, new stages descriptions). Additionally, we present COI sequences for four of these species in order to evaluate genetic divergence between them.

MATERIAL AND METHODS

Specimen collection and preparation

Last instar larvae were reared in plastic cups with small windows covered by mesh (1 mm pore) to allow water flow. The cups were maintained in the river from few to several days floating on a perforated Styrofoam piece. Some larvae were reared in aquariums with small water pumps to maintain water current. After emergence, imagos were left in the cup for a few hours before fixing in ethanol. When possible, we used live specimens or recently fixed specimens, or photographs of live specimens to aid in the descriptions, as yellowish tints, for example, tend to fade with prolonged immersion in alcohol.

Male genitalia were removed from the abdomen after a 10-minute bath in 10% KOH (heated in a water bath), following the normal procedure for the group (e.g., Stark, 2013). The aedeagus is an eversible cylindro-conic structure with a long membranous base and an apical sclerotized portion (penial armature), in descriptions we call "aedeagus" to this apical structure. Female IX sternum was drawn or photographed without previous dissection. Larval parts (mouthparts, head capsule and antennae, legs and cerci) were dissected and mounted on microscope slides using Canada balsam. Photographs were taken with a Zeiss Axiocam ICc5 mounted on a Stemi 508 stereomicroscope. Drawings were made using an Olympus BX51 microscope with a camera lucida.

The species are treated in alphabetical order. Some characteristics are not drawn or described, because of

being the same in all the larvae studied: the small dark setae that cover the body and legs completely (e.g., Fig. 26c) and the rows of long setae at the margins of the legs (Fig. 26b). The small dark setae are easily removed and many areas may seem bare, but the small circular bases of these setae can be seen under magnification. Some areas do not present these setae and their bases, for example on a longitudinal median row of femora, these areas are marked in the figures with dotted lines.

All the material is housed in the entomological collection of Instituto de Biodiversidad Neotropical (IBN), Tucumán, Argentina, except some specimens deposited in the Museo de Entomología de la Universidad del Valle (MUSENUV), Cali, Colombia. Deposition of types belonging to species described by other authors are mentioned under the corresponding species section.

DNA sequencing and analyses

Total genomic DNA was extracted from ethanol-preserved specimens and purified using the salting-out protocol. In this protocol 300 μ L TNES lysis buffer (50 mM Tris, 400 mM NaCl, 20 mM EDTA, 0.5% SDS) and 16 μ L proteinase K (10 mg/mL final concentration) were added to each sample. Samples were vortexed for 30 seconds, and the tube spun down for 5 minutes at 14000 rpm. Tubes were incubated at 51°C overnight. Thereafter, 300 μ L NaCl (5 M) was added to each sample. The supernatant was transferred to fresh tubes. An equal volume of 99% ethanol was added to each sample, mixed well, and incubated at -4 °C for 1 h. Samples were then centrifuged for 5 min at 14000 rpm. The pellet was washed with 70% ethanol, dried, and finally resuspended in 20-50 μ L sterile dH₂O.

Fourteen sequences (Table 1fig11b) from the barcode region of the mitochondrial gene COI (Cytochrome c oxidase subunit I) were amplified based on standardized protocol of polymerase chain reaction (PCR), using the primers LCO-1490 and HCO-2198 (Folmer et al., 1994). Purification and bidirectional sequencing of amplicons was realized by Macrogen® (South Korea).

Using MEGA 11 (Tamura, et al. 2021), we manually edited the chromatograms to obtain consensus sequences and aligned them using ClustalW. A 535-base pair (bp) alignment of 14 sequences was obtained. For our samples, sequence distances were calculated using Kimura-2-parameter (K2P) (Table II). To assess species delimitation based on molecular data, we used: 1) Automatic Barcode Gap Discovery (ABGD) using the web interface (<http://www.wabi.snv.jussieu.fr/public/abgd/>) (Puillandre et al., 2012), considering recursive partition. ABGD is a tool designed to infer species hypotheses based on automatized identification of barcode gaps between inter- and intraspecific pairwise distances in partial sequence datasets. The method does not make assumptions about data structure or evolutionary history,

and only requires input data (a single locus alignment) to be sufficiently variable; and 2) Generalized Mixed Yule Coalescent (GMYC), model implementing single thresholds via web (<https://species.h-its.org/gmyc/>) on single ultrametric gene trees to infer GMYC species. The GMYC model aims to discern stochastic birth-death processes (effectively a pure-birth Yule model) between species from neutral coalescent processes within species. Phylogenetic relationships were inferred in a Bayesian framework in BEAST 1.10.4 (Suchard et al., 2018), with the clock model set to “Strict Clock”, Yule speciation model, and a 10% ‘burn-in’ as determined by examining ESS values and trace graphs in Tracer. Mega 11 was used to choose the best evolutionary models. The chosen model was HKY+I for all codons (Hasegawa et al., 1985).

To further support the Bayesian tree, we also inferred a phylogeny by using the Maximum Likelihood method. We used a sequence of *Anacroneuria subcostalis* downloaded from Genbank (access number: MW293829.1) to root the tree. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 67.01% sites).

RESULTS

Molecular comparisons

We obtained 14 cytochrome oxidase I barcodes with a full length of 535 base pairs (bp) (Table I). Two specimens of *A. flavinigra* sp. nov. shared the same haplotypes, as also do two specimens of *A. tucuman*, the remaining specimens presented unique haplotypes (Table II). Bayesian and ML yielded the same resulting tree, ML tree can be seen in Fig. 29. The overall average pairwise K2P distance was 11.8%. The average intraspecific pairwise K2P distance was 1.3% for *A. tucuman*, 2.1% for *A. flavinigra* and the distance between the two specimens of *A. tafi* sp. nov. was 0.2%. The average interspecies distance (K2P) between *A. tucuman* and *A. tafi* sp. nov. was 3.1%. Between *A. tucuman* and *A. flavinigra* sp. nov. was 10.7% and between *A. tafi* and *A. flavinigra* sp. nov. was 9.5%. Larger genetic differences were found for *A. lutea* sp. nov., for example between this species and the pair *A. tucuman* + *A. tafi* sp. nov. the genetic divergence was 22.5%, and between *A. lutea* sp. nov. and *A. flavinigra* sp. nov. was 22.9%. Finally, the number of entities obtained by both ABGD and GMYC were equal to five, but they differ in that ABGD split the specimens of *A. tucuman* into two lineages, while GMYC maintained *A. tucuman* as a single lineage and split the females AN028 and AN029 from the male here described as *A. lutea* sp.

Table I. Genbank access numbers for COI sequences obtained from *Anacroneuria* specimens that were sequenced.

Specimen	Genbank access number
AN008_A.flavinigra female	OQ920224
AN010_A.flavinigra female	OQ920230
AN004_A.flavinigra male holotype	OQ920231
AN003_A.flavinigra male	OQ920232
AN030_A.lutea male	OQ920233
AN020_A.tafi male	OQ920225
AN012_A.tafi male	OQ920229
AN015_A.tucuman male	OQ920221
AN848_A.tucuman male	OQ920226
AN849_A.tucuman female	OQ920227
AN011_A.tucuman male	OQ920228
AN022_A.tucuman female	OQ920234
AN028_sp female	OQ920222
AN029_sp female	OQ920223

nov. We preferred the last method, because it considered all the specimens morphologically attributed to *A. tucuman*, including a pair in copula as a single lineage.

Taxonomy

Anacroneuria arrazayalensis Orce (Figs. 1-2)

Anacroneuria arrazayalensis Orce, 2003: 227 (male adult); Stark, 2013: 98.

Diagnosis. The male of this species can be distinguished from other of the genus by: small size (male FW 8.2-10.0 mm), relatively dark body and wings, wing membrane brownish to ochraceous (rarely blackish, 1 male from Tucumán); hammer large and cylindrical (Fig. 1j-k); aedeagus stout, thinner towards the at apex, apex truncated more or less rectangular; with marked shoulders with ventrolateral microspines, and with lateral lobe distal to shoulder (Fig. 1a-i).

Type Material. Holotype male (IBN, FW 9.7 mm) from ARGENTINA, Salta, Finca Jakúlca, arroyo Arrazayal, -22.80889, -64.49278, 660 m, 13/XII/1999, Domínguez, Romero, Manzo cols.

Additional material. ARGENTINA. Tucumán: 2 male adults (IBN902CM, IBN893CM), Tapia, río Tapia, -26.609518, -65.265090, 931 m, 26.xi.2016, P. Rueda col.; 1 reared male adult (and larval exuvia slide IBN859CM) and 2 mature larvae, río Tapia, RN9, -26.609518, -65.265090, 12.ix.2021, Molineri col.; 1 male adult (slide IBN905CM, FW 8.5 mm); Río Nio, -26.44000, -64.99694, 984 m, 14.vi.2001, Molineri et al. cols. BOLIVIA. Tarija: 3 male adults (2 reared) with larval exuviae (IBN858CM), prov. O’Connor, río Salinas, ca. Entre Rios, -21.64500, -64.1522222, 1160 m, 5.x.2004, Molineri & Manzo cols.

Table II. Estimates of Evolutionary Divergence between Sequences.

		AN01	AN84	AN84	AN02	AN01	AN01	AN02	AN00	AN01	AN00	AN00	AN02	AN02
		-5	-8	-9	-2	-1	-2	0	-8	0	-4	-3	-8	-9
AN84	(8)	0.002												
AN84	(9)	0.021	0.019											
AN02	(2)	0.002	0.000	0.019										
AN01	-1	0.023	0.021	0.002	0.021									
AN01	-2	0.029	0.031	0.033	0.031	0.035								
AN02	0	0.027	0.029	0.031	0.029	0.033	0.002							
AN00	-8	0.099	0.101	0.104	0.101	0.101	0.086	0.088						
AN01	0	0.104	0.106	0.101	0.106	0.099	0.090	0.092	0.008					
AN00	-4	0.113	0.115	0.110	0.115	0.108	0.099	0.101	0.033	0.027				
AN00	-3	0.113	0.115	0.110	0.115	0.108	0.099	0.101	0.033	0.027	0.000			
AN02	-8	0.215	0.218	0.213	0.218	0.215	0.200	0.202	0.212	0.212	0.217	0.217		
AN02	-9	0.215	0.218	0.218	0.218	0.221	0.200	0.202	0.207	0.212	0.217	0.217	0.011	
AN03	0	0.220	0.223	0.239	0.223	0.242	0.212	0.215	0.222	0.227	0.233	0.233	0.059	0.055

The number of base substitutions per site between sequences are shown.

Adult habitus (Fig. 27c). General color dark, brownish yellow with wings slightly tinged with yellowish brown (except 1 male from Tucuman, with blackish wings). Head yellow shaded brownish medially. Antennae and mouthparts brownish. Pronotum mostly brown but with yellow broad median band; this band extends to mesonotum. Legs yellowish brown, subapical blackish mark on femora. Cerci yellowish with brownish joints.

Male adult. FW length 8.2-10.0 mm (n= 9). Hammer large and cylindrical (Fig. 1j-k). Aedeagus with subapical dorsal lobes, shoulders marked and with ventrolateral microspines (Fig. 1a-i); dorsal keel present and prominent, slightly serrated basally (Fig. 1d, i); with a pair of small ventral pouches.

Last instar larval exuvia. Measurements (mm). Male (n= 3): body length, 9.0-9.9; cerci length, 4.8 (broken); head length, 1.5-1.8; head width, 2.1-2.3; pronotum length, 1.2-1.4; pronotum width, 2.4. Cuticular pigmentation: head widely pigmented on central area between antennae (frons), on occipital margin and on labrum; paler areas include clypeus, and area between eyes and two larger oblong submedian marks anterior to epicranial suture. Coloration of fresh larva as in Fig. 26c. Head. Antennae: scape subquadrate (Fig. 2d), basal antennomeres with setae shorter than each segment, and with groups of 3 small acute microtrichiae arising at the same level than setae (Fig. 2e), and few single microtrichiae subapically at each segment. Labial palp with 3 blunt microtrichiae at apex as in Fig. 2a-c. Thorax. Margins of nota fringed with short and long spines, as in Fig. 2i-k. Legs with row of long setae on outer margins of femora and tibiae, more marked on tibiae; spines on dorsal surface of femora relatively long (Fig. 2g-h). Abdomen. Abdominal terga with very long and medium length spines along hind margin; sternum 8 with complete row of spines along hind margin. Cerci: from cercomere 6

to apex, two whorls of setae are present at each segment (Fig. 2f).

Distribution. Argentina (Salta, Tucumán **new provincial record**). Bolivia (**new country record**) (Fig. 30).

Discussion. *Anacroneuria arrazayalensis* was described from one male adult from Salta province. This male presents yellowish translucent wings, whilst most males newly reported here present darker wing membrane (yellowish brown), and even one male has almost blackish wings. Original description did not mention the microspines at ventrolateral margin of the aedeagal shoulders, and states that ventral pouches are absent. Nevertheless, after the re-study of the holotype and new material reported here, both structures are confirmed as present. The aedeagal apex with subdistal lobes, readily distinguishes this species from others in Yungas, as was noted by Orce (2003) (except from *A. saican* sp. nov. and *Anacroneuria triplex* sp. nov. described below). *Anacroneuria arrazayalensis* differs from *A. saican* sp. nov. by its large hammer (*A. saican* sp. nov. presents a small bubble-like hammer), and from *Anacroneuria triplex* sp. nov. by wing color (yellowish orange in the last species) and by having paired ventral pouches (an additional median pouch is present in *A. triplex* sp. nov.). Other species in Bolivian and Peruvian Andes present subdistal lobes (Stark & Sivec, 1998), from which the most similar to *A. arrazayalensis* is *A. x-nigrum* Klapálek, 1921. Nevertheless, this last species is readily distinguished by its pointed aedeagal apex (see Stark & Sivec, 1998, figures 165-168). *Anacroneuria amoro* Stark (2004) although having a similar aedeagus, differs by its pointed subdistal lateral lobes and the absence of ventral pouches. Finally, *A. caraja* Froehlich (2002) from Pará (Brazil) presents a similar aedeagus, but without dorsal keel.

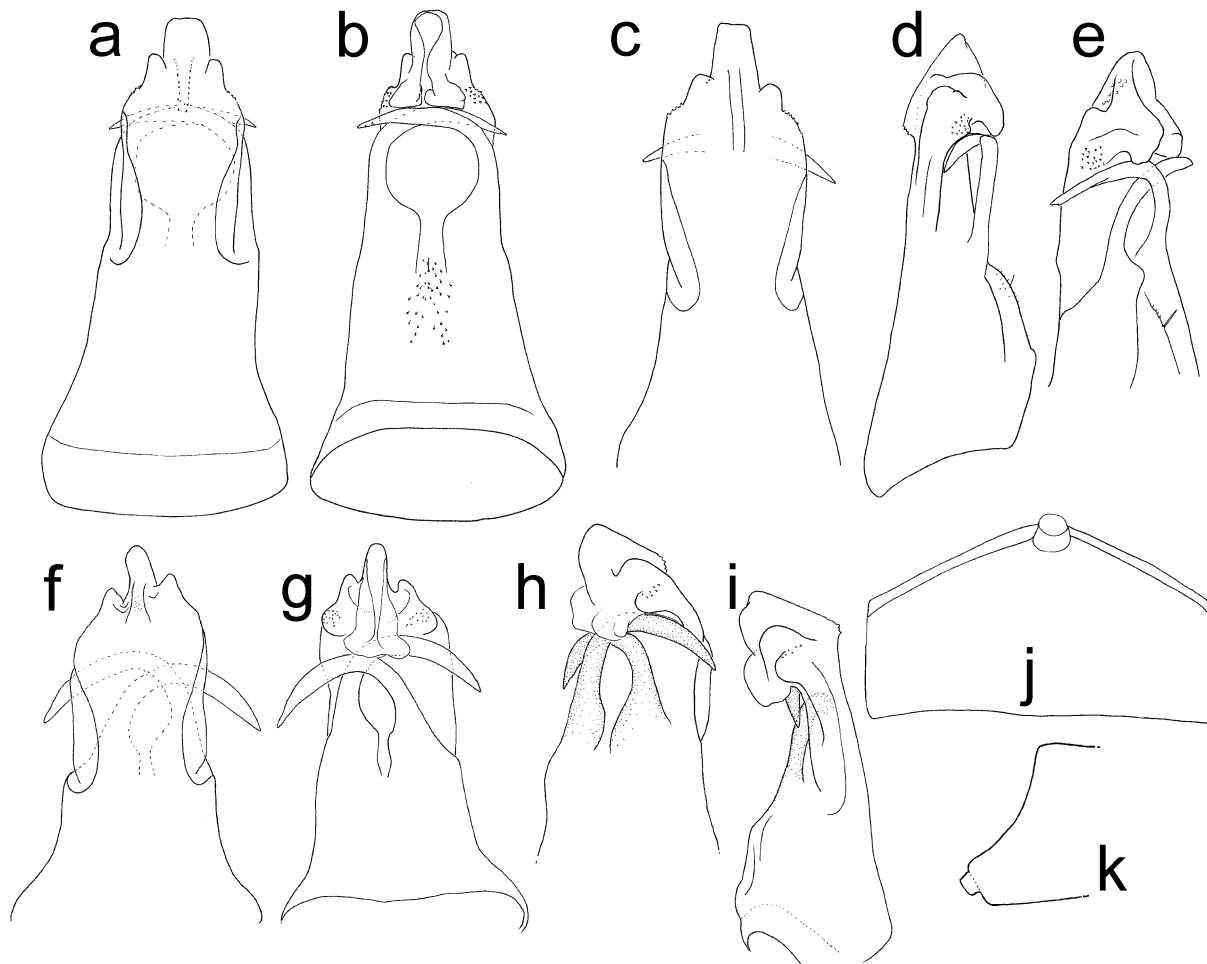


Fig. 1. *Anacroneuria arrayazalensis*. Aedeagus: a-b. dorsal and ventral view (Tapia, IBN902CM). c-e. dorsal, lateral and ventrolateral views (Tapia, IBN859CM). f-g. dorsal and ventral views (Burruyacu, IBN905CM). h-i. ventrolateral and ventral views (Bolivia, Tarija). Hammer: j. ventral view. h. lateral view.

Larva of *A. arrayazalensis* is similar to *Anacroneuria triplex* sp. nov., and *A. reedi* (both described below), in head color pattern, size and general aspect. It also shares with *A. reedi* the double whorl of setae distad to cercomere 7 (single whorl in other species).

Anacroneuria calilegua sp. nov. (Fig. 3)

urn:lsid:zoobank.org:act:123DB31D-B393-4E7A-A6D0-A5CC8566EA29

Diagnosis. *Anacroneuria calilegua* sp. nov., known from a single male, can be distinguished from other species in *Anacroneuria* by the following combination of characters: small sized (male FW 9.4 mm), light coloration with yellowish orange wings; hammer slender, cylindrical (Fig. 3d-f); aedeagus narrowing abruptly at the apex, with double and serrated dorsal keel (Fig. 3a, c); paired and medially partially fused ventral membranous lobes (Fig. 3b).

Type material. **Holotype** male adult (IBN897CM) from ARGENTINA, Jujuy, Parque Nacional Calilegua, arroyo Tres Cruces, -64.85089, -23.75230, 970 m, 28.xii.1997, light trap, Molineri col.

Adult habitus. Small species, coloration faded, but apparently a whitish to yellowish specimen; membrane of wings slightly tinged with orangish yellow, veins orangish. Head whitish yellow shaded orangish medially and on lappets. Antennae scape and pedicel yellowish, remainder of head and mouthparts whitish. Pronotum mostly orangish except wide whitish median band; mesonotum whitish yellow except laterally and posteriorly orangish. Legs whitish yellow, subapical blackish mark on femora. Cerci yellowish white.

Male adult. FW 9.4 mm. Hammer slender, cylindrical (Fig. 3d-f). Aedeagus narrowing distally in dorsal view, without dorsal lobes or shoulders (Fig. 3a-c); in dorsal view apex of aedeagus straight to slightly concave (Fig. 3a); dorsal keel double, short and serrated (Fig. 3c); with two membranous ventral pouches, partially fused in the middle (Fig. 3b); forceps slender, somewhat widened subdistally (Fig. 3b).

Etymology. From the type locality Calilegua, probably from an Aimara voice referring to the pronounced cliffs present in the area.

Distribution. Argentina (Jujuy) (Fig. 30).

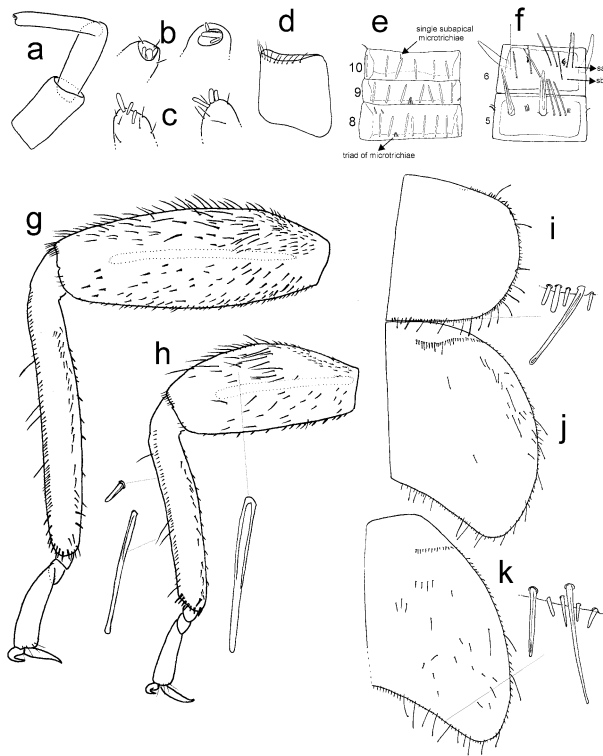


Fig. 2. *Anacroneuria arrayazalensis*, last larval exuvia (reared male from Tarija). a. labial palp. b-c. detail of apex of labial palp. d. scape, ventral. e. antennomeres 8-10. f. cercomeres 5-6. g. hind leg, dorsal. h. fore leg with details, dorsal. i. pronotum with detail. j. mesonotum. k. metanotum with detail.

aymara); but differs in the double serrated keel present in *Anacroneuria calilegua* **sp. nov.** Other similar species include *Anacroneuria marshalli* Stark, 2007 from Salta (Argentina) except that this last species presents wider forceps, and a smooth single keel, not serrated dorsally. Additionally, the apex of aedeagus narrows more abruptly in *Anacroneuria calilegua* **sp. nov.**

Anacroneuria chimborazo Stark, 2001 (Fig. 4)

Diagnosis. Male adults of *Anacroneuria chimborazo* Stark can be distinguished from other species in the genus by: FW length 10.0-16.0 mm; wing membrane hyaline to yellowish, body and legs pale yellowish white; hammer well developed, cylindrical; aedeagus stout, broad, with apex broadly truncated.

Material studied. ARGENTINA. Jujuy: 1 male (IBN) Tiraxi, río Tiraxi, -23.99972, -65.33333, 13.xii.2001, light trap, Molineri & Romero cols.

Distribution. Ecuador and Argentina (Jujuy) (**new country record**) (Fig. 30).

Discussion. A male (FW length 10.0 mm) was collected along with several other males of *A. tucuman* and *A. saltensis* using a light trap. The aedeagus of this male (Fig. 4a-c), together with other features (color pattern of head and pronotum) coincide with the description and figures of *A. chimborazo* Stark, 2001. Nevertheless, size and color of wings are different. The types of *A. chimborazo* present FW length of 16 mm, transparent wing membrane, and brown R and Sc veins (Stark, 2001). The male reported here is much smaller (FW 10 mm) and the wings are slightly tinged yellowish with paler veins.

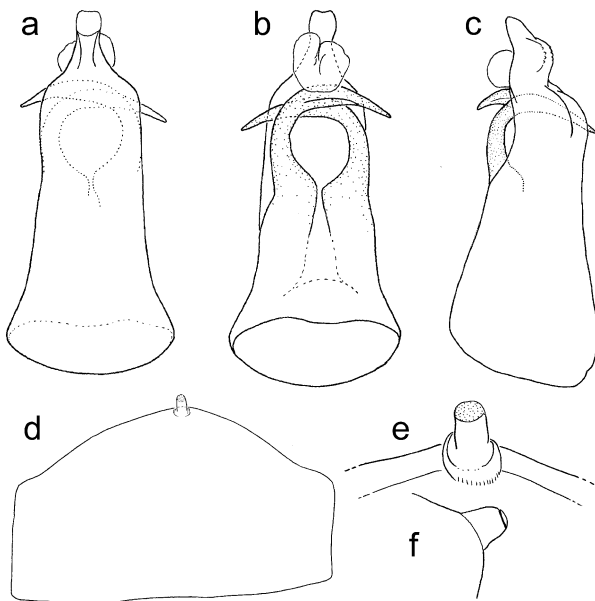


Fig. 3. *Anacroneuria calilegua*, male. Aedeagus: a. dorsal view. b. ventral view. c. lateral view. Hammer: d. ventral view. e. same, detail. f. lateral view.

Discussion. *Anacroneuria calilegua* **sp. nov.** is similar to *A. aymara* Stark & Sivec, 1998, both share general coloration, slender hammer, apically narrow penes, and presence of ventral membranous lobes (smaller in *A.*

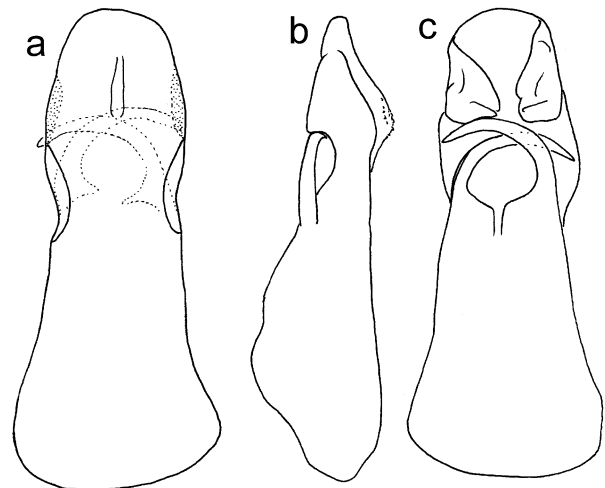


Fig. 4. *Anacroneuria chimborazo*, male aedeagus. a. dorsal. b. lateral. c. ventral.

Anacroneuria flavinigra **sp. nov.** (Figs. 5, 27a)

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Diagnosis. Male adults of *Anacroneuria flavinigra* sp. nov. can be distinguished from other species in the genus by: FW length 8.9-9.9 mm; dark coloration, wing membrane blackish to grayish brown, body and legs dark yellow (Fig. 27a); hammer well developed, cylindrical (Fig. 5i-j); aedeagus with apex broadly truncated, with large slightly serrated dorsal keel, ventral pouches subtriangular and flat (Fig. 5a-h). Females paler, wing membrane slightly tinged grayish yellow, subgenital plate bi-lobed, with V-shaped notch (Fig. 5k).

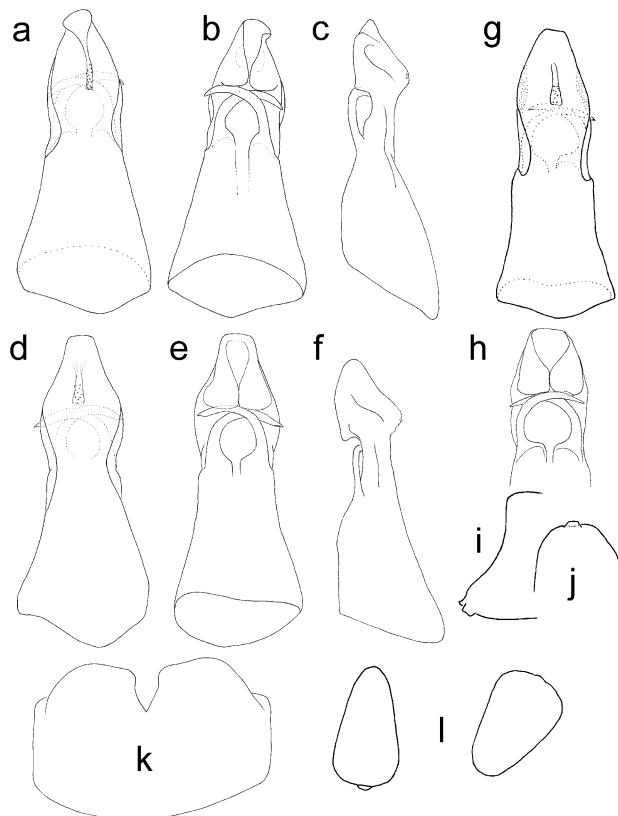


Fig. 5. *Anacroneuria flavinigra*. Aedeagus in dorsal, ventral and lateral view: a-c. male from Horco Molle (IBN478CM). d-f. male from Raco (IBN901CM). g-h. male from Burruyacu (IBN891CM). Hammer: i. lateral view. j. ventral view. Female (AN010): k. subgenital plate. l. eggs.

Type material. **Holotype** male adult (IBN901CM, COI AN004) from ARGENTINA, Tucumán, Raco, arroyo Yerbabuenita, -26.665958, -65.413941, 990 m, 24.xii.2021, Molineri col. **Paratypes:** 2 male adults from same locality, light trap, 31.xii.2021; Molineri col.; 1 male and 3 females (slide IBN933CM, COI AN008, AN010) same locality except 2.i.2022; 1 male adult (slide IBN874CM) from Tucumán, Horco Molle, Arroyo Anta Yacu, -26.77203, -65.32748, light trap 5am, 19.xi.2019, Molineri col.; 1 male adult (IBN891CM, COI AN003) from Tucumán, Altos de Medina, arroyo del Tigre, -26.42583, -65.05556, 1400 m, 29.xii.2019, Molineri & Rodríguez cols.

Additional material. ARGENTINA. Jujuy: 4 males, Tiraxi, río Tiraxi, -23.99972, -65.33333, 13.xii.2001, light trap, Molineri & Romero col.

Adult habitus (Fig. 27a). General color of male dark bright yellow with blackish wings, female paler, whitish yellow with translucent wings. Head yellow shaded brownish on frons and lappets. Antennae and mouthparts brownish. Pronotum mostly brown but with yellow broad median band; this band extends to mesonotum. Male legs yellow, subapical blackish mark on femora. Female legs yellowish white with small subapical blackish mark on femora, and small brownish subbasal mark on tibiae. Male wings blackish, veins blackish brown. Female wing translucent, FW slightly tinged with grayish yellow and with brownish veins (except C, Sc and basal $\frac{2}{3}$ of R, whitish). Female HW with yellowish veins. Cerci brownish.

Male adult. FW length 8.9-9.9 mm (n= 6). Hammer strong, cylindrical (Fig. 5i-j). Aedeagal apex rounded and truncated, without shoulders (Fig. 5a, d, g); dorsal keel strongly projected and slightly serrated (Fig. 5c, f). Pouches flat and subtriangular (Fig. 5b, e, h).

Female adult. FW length 13.4-13.7 mm (n= 3). Subgenital plate bi-lobed, inner lobes broadly rounded, with deep median V-shaped notch, lobes roundly projected medially on apex (Fig. 5k).

Eggs. Length 340-370 μ m; maximum width 210-230 μ m (length/max. width ratio 1.6-1.7). Brown, surface smooth at 100X, drop-shaped (Fig. 5l).

Etymology. From the Latin words *flavum* (yellow) and *nigrum* (black), because of the strong contrasting colors of the live specimens.

Distribution. Argentina (Tucumán and Jujuy) (Fig. 30).

Discussion. Males of *Anacroneuria flavinigra* sp. nov. are easily distinguished from other *Anacroneuria* from NW Argentina and Southern Bolivia by their general smaller size and strong yellow coloration of body and blackish wings. Only *Anacroneuria lutea* sp. nov. (described below) presents these characteristics in the studied region, but *Anacroneuria flavinigra* sp. nov. presents a rounded and truncated aedeagal apex (Fig. 5a-h), ventral pouches triangular and flat (Fig. 5b, e, h), and does not show microspines in aedeagal shoulders (in *Anacroneuria lutea* sp. nov. the aedeagal apex is elongated and not subrectangular, ventral pouches are elongated and not flattened, and shoulders present microspines). Additionally, the genetic distance between both species is very high (23.1%, Fig. 29). *Anacroneuria flavinigra* sp. nov. females are much paler than males, they are rather similar to *A. tucuman* and *A. tafi*, in the bi-lobed nature of the subgenital plate. Nevertheless, *Anacroneuria flavinigra* sp. nov. females present V-shaped notch in subgenital plate, with the distal portion of the lobes somewhat projected medially (see arrow in Fig. 5k). *Anacroneuria tucuman* presents a U-shaped notch, *A. tafi* presents a V-shaped notch but without distal projections of the lobes.

Anacroneuria flavinigra **sp. nov.** presents an aedeagus similar to *A. quechua* Stark & Sivec (1998) from Peru, but in this last species the ventral pouches are smaller and do not touch medially (Figs. 140-142 in Stark & Sivec, 1998). *Anacroneuria brunneilata* Jewett, 1959 also presents a similar aedeagus except that the apex is more truncated (figures 19-21 in Stark & Sivec, 1998). *Anacroneuria flavinigra* **sp. nov.** males further differ from both species by their blackish wing membrane.

Anacroneuria handlirschi Klapálek (Fig. 6)

Anacroneuria handlirschi Klapálek, 1922: 94; Zwick, 1973 (synonym of *A. angusticollis*); Stark & Sivec, 1998: 28 (species revalidated); Stark, 2013 (Salta, Argentina).

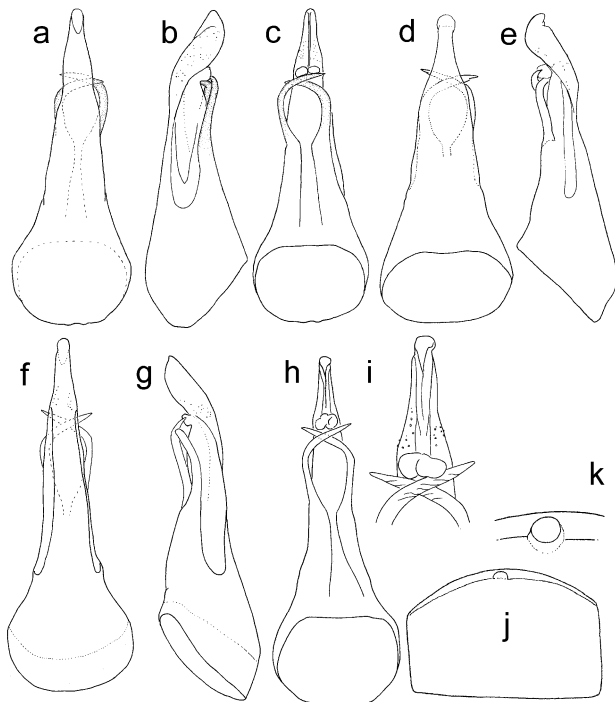


Fig. 6. *Anacroneuria handlirschi*. Male (Tucuman, río Tapia): a. aedeagus, dorsal. b. same, lateral. c. same, ventral. j. hammer, ventral. k. hammer, detail. Male (Tarija, río Orosa): d. aedeagus, dorsal. e. same, lateral. Male (Tarija, río Salinas): f., aedeagus, dorsal. g. same, lateral. h. same, ventral. i. detail of aedeagal apex, ventral. j. male subgenital plate. k. detail of hammer.

Diagnosis. *Anacroneuria handlirschi* Klapálek is known from male and female adults and can be distinguished from other species in *Anacroneuria* by the following combination of characters: relatively large sized (male FW 12.2-14.0 mm), light coloration with hyaline wings, veins yellowish except C whitish; head whitish except shaded yellowish brown anteriorly to ocelli and antennal bases; pronotum light brownish with a wide central whitish band; hammer well developed, not longer than basal width (Fig. 6j-k); aedeagus long and slender; without keel, small ventral membranous lobes (Fig. 6a-i).

Material examined. ARGENTINA. Tucumán: 2 male adults, río Tapia, -26.609518, -65.265090, 931 m, 26.ii.2016, Rueda col. Salta: 1 male, Arroyo Arrayazal,

Finca Jakulica, -22.80889, -64.49278, 660 m, 7.xii.1984, Dominguez col. BOLIVIA. Tarija: 8 males and 25 females, río Orosa, ca. La Mamora, -22.20256, -64.62683, 1100 m, 4.x.2004, light trap, Molineri & Manzo col.; 1 female río Conchas, -22.29569, -64.38853, 828 m, 2.iii.2006, Rueda & Nieto cols.; 1 male río Orosa, La Mamora, -22.16833, -64.66806, 1219 m, 28.ii.2006, Rueda & Nieto cols.; and 1 female río Salinas, ca. de Salinas, -64.24161, -21.78691, 6.iii.2006, Domínguez & Nieto cols.

Distribution. Argentina (Salta, Tucumán **new provincial record**). Bolivia (Fig. 30).

Discussion. This species was previously reported from Bolivia, Peru (Stark & Sivec, 1998), and Salta in Argentina (Stark, 2013). In Austral Yungas, it is rare in Argentina but is the most frequent species in Southern Bolivia. Easy to recognize for the slender penes of a unique shape (Fig. 6a-h), it is a large and light-colored species, with relatively small hammer.

Anacroneuria lutea **sp. nov.** (Figs. 7, 27b)

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Diagnosis. Male adults of *Anacroneuria lutea* **sp. nov.** can be distinguished from other species in the genus by: FW length 9.2-10.0 mm, dark coloration, wing membrane blackish, body and legs dark yellow (Fig. 27b); hammer well developed, cylindrical (Fig. 7i-k); aedeagus narrowing abruptly from shoulders toward apex, shoulders with microspines; keel pronounced and short, serrated; ventral pouches suboval, touching in the midline (Fig. 7a-h).

Type material. **Holotype** male (IBN926CM) from ARGENTINA, Tucumán, Tafí del Valle, Apeadero Muñoz, -26.98722, -63.66361, 1630 m, 26.ii.2022, Molineri & Fontanarrosa cols. **Paratypes:** 1 male (MUSENUV, COI AN030) same data as holotype except: 17.ii.2016, Hankel & Rueda cols.; and 1 male (IBN) from Tucumán, Horco Molle, arroyo Piedras, -26.761746, -65.32634, 740 m, 21.xi.2017, Molineri col.

Adult habitus (Fig. 27b). General color yellow with blackish wings. Head yellow shaded brownish on frons and lappets. Antennae and mouthparts yellowish brown. Pronotum mostly brown but with yellow broad median band. Legs yellow, subapical blackish mark on femora. Wings blackish, veins blackish brown. Cerci brown.

Male adult. FW length 9.2-10.0 mm (n= 2). Hammer large, cylindrical (Fig. 7i-k). Aedeagal apex narrowing abruptly from shoulders towards apex (Fig. 7a-b, e-f), shoulders with lateral microspines (Fig. 7h); dorsal keel projected, short and serrated (with two strong teeth. Fig. 7c, g, h). Ventral membranous lobes suboval (Fig. 7b, d, f).

Etymology. The specific epithet is derived from the Latin word *luteum*, meaning yellow.

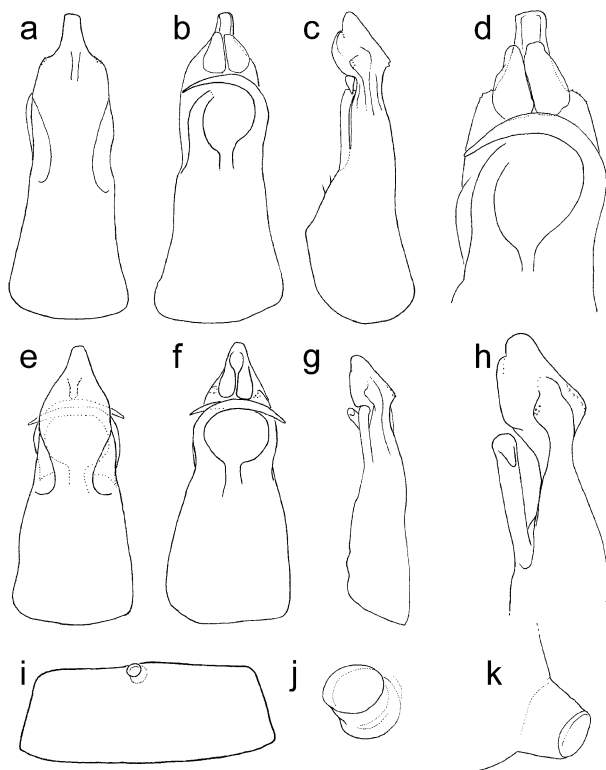


Fig. 7. *Anacroneuria lutea*. Aedeagus (IBN926CM, Tafí del Valle): a. dorsal view. b. ventral view. c. lateral view. d. ventral detail. Male (Horco Molle): e. dorsal. f. ventral. g. lateral. h. lateral detail. i. hammer, ventral. j. same, detail. k. same, lateral.

Distribution. Argentina (Tucumán) (Fig. 30).

Discussion. *Anacroneuria lutea* sp. nov. is similar to *A. flavinigra* sp. nov. because of its general aspect (yellow body, blackish wings) and small body size. Nevertheless, the structure of the aedeagus is different in both species (see discussion under *A. flavinigra*). The aedeagus of *A. lutea* sp. nov. resembles that of *A. cotacachi* Stark, 2001, but this last species presents a less pronounced dorsal keel, more pronounced ventral pouches that are visible dorsally and more separated in the midline than in *A. lutea* sp. nov. (not visible dorsally, and touching in median line).

In our molecular analysis (Fig. 29), two female specimens (1 female adult, AN029, FW 12.0 mm, from Jujuy, Calilegua, tributario de arroyo Sauzalito, -23.64361, -64.58194, 500 m, 14.xi.14, E. Dominguez & C. Molineri col.; and 1 female adult, AN028, FW 13.2 mm, from Tucumán, Raco, arroyo Yerbabuenita, -26.665958, -65.413941, 990 m, 6.xi.2014, Molineri col.) resulted nearly related to the male paratype of *A. lutea* from Apeadero Muñoz. Nevertheless, relatively large molecular differences and one of the lineage models used (GMYC) suggest that they may be from a different species. We prefer not to describe or name these females until the corresponding male could be known.

Anacroneuria reedi (Navás) (Figs. 8-12)

Neoperla reedi Navás, 1919:4 (female adult, Tucumán); Froehlich, 2010: 169 (catalogue).

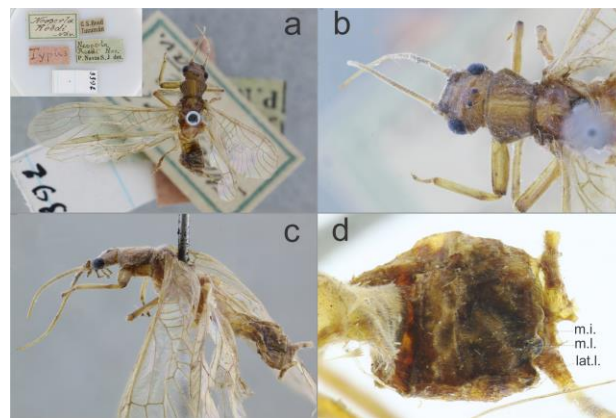


Fig. 8. *Anacroneuria reedi*, female adult holotype. a. general dorsal view and detail of labels. b. detail of head and thorax, dorsal. c. general lateral view. d. subgenital lobe. References= m.i.: median indentation, m.l.: median lobe, lat.l.: lateral lobe.

Anacroneuria reedi, Illies, 1964:491 (probably an *Anacroneuria*); Cabo & Wais, 1988:10 (records from Catamarca, Argentina).

Diagnosis. Male adults of *Anacroneuria reedi* can be distinguished from other species in the genus by: FW length 12.8 mm, light coloration, wing membrane hyaline, body and legs whitish yellow; hammer well developed, cylindrical (Fig. 9f); aedeagus narrowing abruptly on apex (Fig. 9a), shoulders with lateroventral microspines (Fig. 9e); keel low, with a V-shaped fold around (Fig. 9b-c); ventral membranous lobes present and small (Fig. 9d-e). Female adults: FW length 12.0-13.3 mm, with 4-lobed subgenital plate, lobes rounded and separated by shallow emarginations (Figs. 8d, 9g). Larval stage with (Fig. 10f) or cercomeres with relatively thick microtrichiae, shorter than

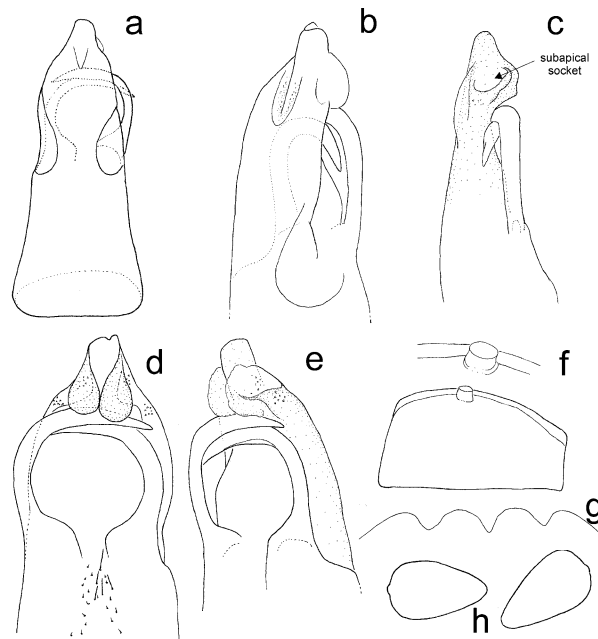


Fig. 9. *Anacroneuria reedi*. Male (IBN847CM): a. aedeagus, dorsal. b. detail of aedeagus, laterodorsal. c. same, lateral. d. same, ventral. e. same, ventrolateral. f. male subgenital plate and detail of hammer, ventral. Female (IBN857CM): g. ventral outline of apex of subgenital plate. h. eggs.

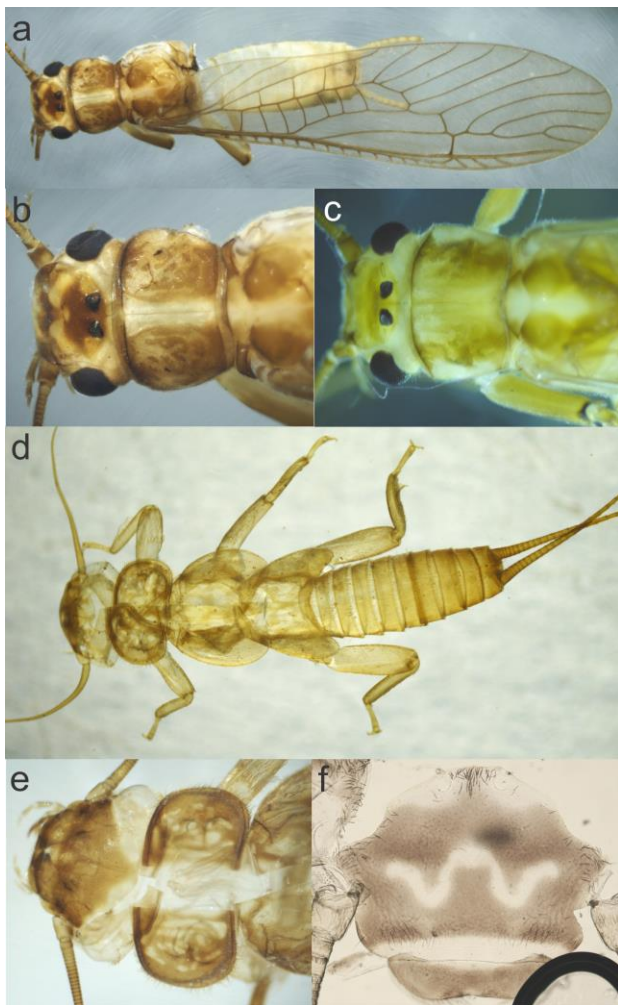


Fig. 10. *Anacroneuria reedi*. a. female adult (AN030), dorsal habitus. b. same, detail of head and pronotum. c. teneral male, head and pronotum (IBN847CM, Raco). d. last instar larval exuvia female (IBN855CM, río Medina), dorsal habitus. e. larval exuvia detail of head (reared female from Bolivia). f. detail of head in larval exuvia (male IBN847CM, Tucuman, Raco).

each segment (Fig. 11e); cercomerites with spines and microtrichiae as in Fig. 11b, c, h.

Type material. Holotype (photographs) female adult (pinned) with the following labels, 1) "Neoperla Reedi" (white paper, hand written), 2) "Typus" (red paper, hand written), 3) "C.S.Reed Tucumán", 4) "Neoperla Reedi Nav. P. Navás S.J. det." (green paper), and 5) "3392" (white paper). Deposited in Museo de La Plata (La Plata, Argentina).

Additional material. ARGENTINA. Tucumán: 1 reared male adult (IBN892CM, FW 12.8 mm) with larval exuvia (IBN847CM) Raco, arroyo Yerbabuenita, -26.665958, -65.413941, 990 m, 2.xii.2014, Molineri col.; 1 reared female adult (FW 13.2 mm) with larval exuvia (IBN855CM) from río Medina, no more data; 9 female adults (2 vials) El Siambón, río Grande, -26.77222, -65.46667, 900 m, 25.x.2003, light trap, Molineri col.; 1 female adult (FW 13.3 mm) Potrero de las Tablas, río Membrillos, -26.85639, -65.43139, 17.ix.2004, Molineri & Giordano cols.; 1 reared

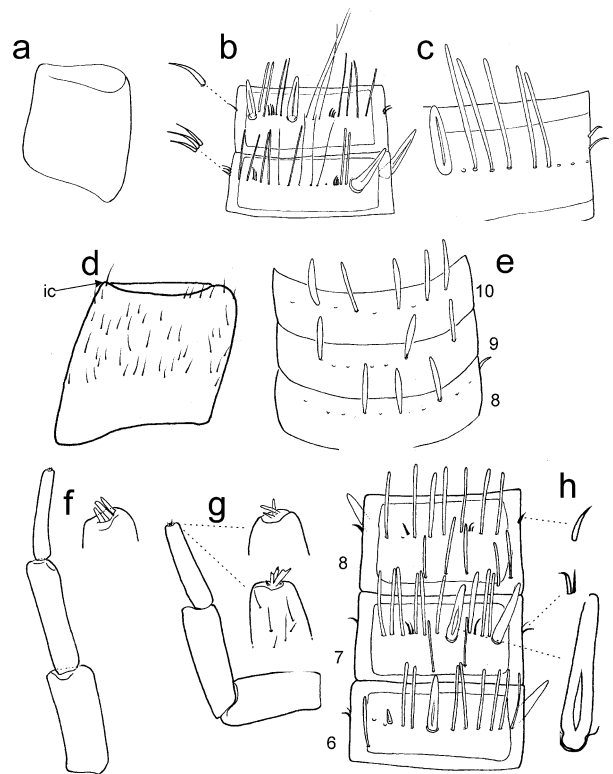


Fig. 11. *Anacroneuria reedi*. Larva (IBN847CM): a. scape outline, dorsal. b. cercomerites 7-8 with detail of double and triple microtrichiae. c. same, detail of spines and setae. f. labial palp with detail. Larva (IBN855CM): d. scape, dorsal (ic= inner corner). e. antennomeres 8-10, dorsal. g. labial palp and details of apex. h. cercomerites 6-8 with details of microtrichiae and spine.

female adult (IBN934CM, FW 13.0 mm) with larval exuvia (IBN855CM) Simoca, río Balderrama, -27.179597, -65.359622, 400 m, 18.ix.2015, Molineri col. BOLIVIA. Tarija: 1 reared female adult (FW 12.8 mm) with larval exuvia (IBN934CM) Prov. O'Connor, -21.64514, -64.15228, río Salinas, ca. de Entre Ríos, 1160 m, 5.x.2004, lighth trap, Molineri & Manzo cols.

Adult habitus. General coloration pale yellow brown. Head pale yellowish brown, darker on lappets. Antennae and mouthparts yellowish white. Pronotum pale brown. Legs pale brown except femora whitish with a narrow subdistal dark brown band. Wings pale, membrane light yellowish; veins yellowish brown.

Male adult. FW length 12.8 mm (n= 1). Hammer large, cylindrical (Fig. 9f). Aedeagal apex narrowing from shoulders towards apex, shoulders with ventral microspines (Fig. 9e); with a subapical socket at each side (Fig. 9c); keel low, with a V-shaped fold around it (Fig. 9b-c). Ventral membranous lobes small and subtriangular, rounded basally (Fig. 9d-e).

Female adult. FW length, 12.0-13.3 mm (n= 6) (holotype 12.8 mm). As in male except abdomen dark brown when full of eggs. Subgenital plate four-lobed; shallow median and submedian U-shaped notches (Figs. 8d, 9g).

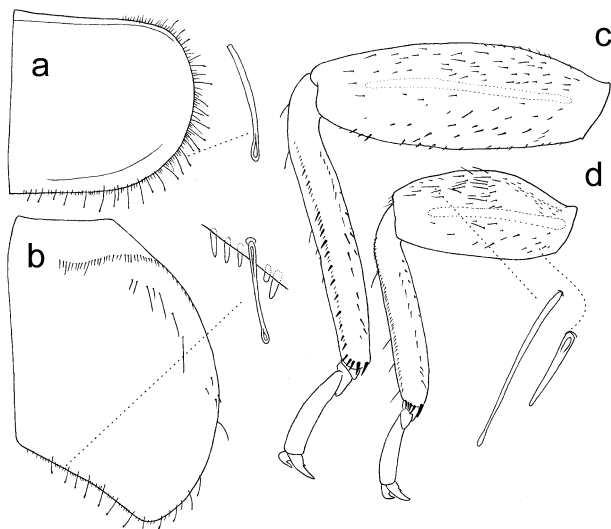


Fig. 12. *Anacroneuria reedi*, larval exuvia (IBN855CM). a. pronoto with detail of seta. b. mesonoto, with detail of posterior margin. c. hind leg. d. foreleg, with detail of setae.

Eggs. Length: 330-360 μ , maximum width, 190-220 μ . Dark brown, drop-shaped (Fig. 9h), surface smooth at light microscope.

Last instar larval exuvia (Fig. 10d-f). Measurements (mm, n= 3). Body length (from anterior margin of pronotum to apex of abdomen), 11.0-12.5; cerci length, >8.0; head width 2.2-2.8; head length, 1.8-2.0; pronotum length, 1.5-1.6; pronotum width, 2.8-3.0. Cuticular pigmentation: head with orangish central part (from antennae toward ecdysial suture); paler on a pair of anteromedian dots, a pair of submedian marks, and submedian larger marks on epicranial suture (Fig. 10e) or with a complete pale W-mark (Fig. 10f). Antennae: scape rounded on apical corners (Fig. 11a, d), antennomeres with a subdistal whorl of short relatively thick setae (Fig. 11e). Labial palpi distally with 3 to 4 microsetae (Fig. 11f-g). Thorax with small scattered pale areas, with many slender spines along margins of nota (Fig. 12a-b), dorsum with few spines. Legs with very thin and medium-length setae along outer margins of femora and tibia, spines as in Fig. 12c-d. Abdomen uniformly colored orangish with long spines along posterior margins of all terga, on sterna forming complete transverse rows only on segments 8-10, in sterna 1-7 the spines are only on lateral parts of posterior margins. Cerci with one whorl of microspines on cercomerites 1-6 (Fig. 11b-c); from 7th cercomerite toward apex, two whorls are present in each segment (Fig. 11h).

Distribution. Argentina (Tucumán, Jujuy **new provincial record**). Bolivia (**new country record**) (Fig. 30).

Discussion. Navás (1919) described *Neoperla reedi* from a single female from Tucuman (Argentina), without further locality data. The type specimen deposited in MLP is in good condition, this female presents a FW length of 12.8 mm and a pale coloration (Fig. 8a-c). Locality data in the original labels (Fig. 8a) only indicate "Tucumán". The

size and general coloration of the specimen, and most importantly the female subgenital plate allowed us to identify newly collected material as conspecific with this pinned female. *Anacroneuria reedi* (Navás) is similar to the most frequently found species in NW Argentina, *A. tucuman* Stark (2013), but the subgenital plate of both are very different (4-lobed in *A. reedi*, 2-lobed in *A. tucuman*). In the studied area, only *A. handlirschi* shows a four-lobed subgenital plate, as *A. reedi*. Nevertheless, the aforementioned species is much larger (FW 15-20 mm, Stark & Sivec, 1998) and the shape of the lobes are different.

Cabo & Wais (1988) cited adults (2 males and 1 female) identified by Dr. Claudio Froehlich as *A. reedi* from Catamarca (Singuil and Las Chacritas, January 1987, Goloboff et al. cols.). At that moment the male of *A. reedi* was not known, nevertheless none of these authors seem to have described it. This material could not be located by us.

Anacroneuria saican sp. nov. (Fig. 13)

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Diagnosis. Male adults of *Anacroneuria saican* sp. nov. can be separated from other species in the genus by medium size (FW length 9.5-10.7 mm), light coloration, hammer small and bubble-like (Fig. 13d-g); aedeagus with shoulders and subapical lobes (Fig. 13a), pronounced but short and smooth dorsal keel (Fig. 13c); ventral pouches spherical and small (Fig. 13b), short and robust forceps (Fig. 13b).

Type material. **Holotype** male (slide IBN906CM, FW 10.5 mm), and **paratype** male (slide IBN907CM, FW 10.6 mm) from BOLIVIA, Dpto. Tarija, Prov. O'Connor, río Saican, ca. Entre Ríos, 1000 m, -21.77458, -64.0900, 6.x.2004, light trap, Molineri & Manzo cols. Other **paratypes** 5 males and 1 female from Tarija, río Conchas, -22.29569, -64.38853, 828 m, 2 iii.2006, Rueda & Nieto cols.

Adult habitus. General color whitish yellow, wings whitish translucent with light brownish veins. Head whitish yellow shaded brownish medially. Antennae and mouthparts whitish yellow. Pronotum mostly light brown but with pale broad median band and brownish mediolongitudinal line; mesonotum whitish yellow except anterolateral margins light brown. Legs yellowish white, subapical blackish mark on femora. Cerci yellowish white.

Male adult. FW length 9.5-10.7 mm (n= 6). Hammer small and bubble like (Fig. 13d-g). Aedeagus with subapical dorsal lobes and marked shoulders (Fig. 13a); dorsal keel pronounced and short, without crenulations (Fig. 13c); with a pair of small spherical ventral pouches (Fig. 13b); forceps stout (Fig. 13b).

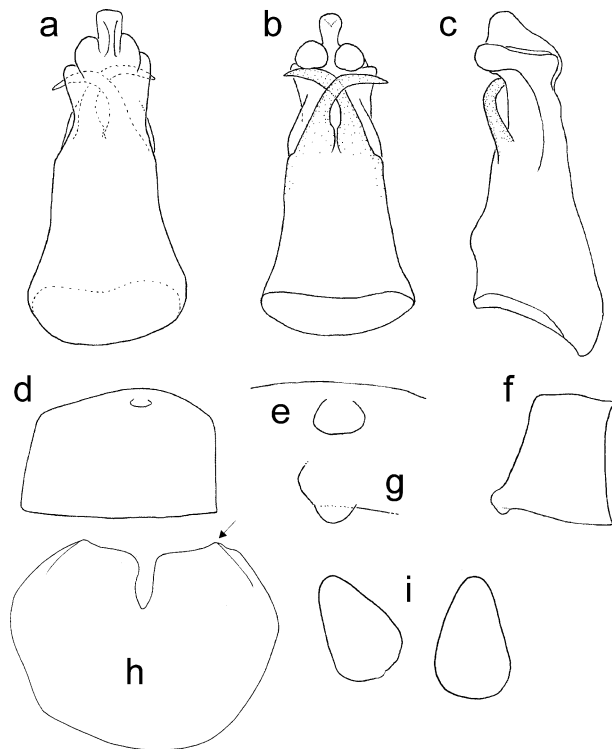


Fig. 13. *Anacroneuria saican*. a. aedeagus, dorsal. b. same, ventral. c. same, lateral. d. male subgenital plate. e. detail of hammer, ventral. f-g. hammer, lateral view. h. female subgenital plate. i. eggs.

Female adult. FW length 11.2 (n= 1). Subgenital plate bilobed but with small sublateral projections (arrow in Fig. 13h), with deep median V-shaped notch, surrounded by a glabrous area.

Eggs. Length 320-330 μm ; maximum width 200-210 μm (length/max. width ratio 1.5-1.6). Light brown, surface smooth at 100X, drop-shaped (Fig. 13i).

Etymology. From the type locality, *Saicán* river, name in apposition.

Distribution. Bolivia (Fig. 30).

Discussion. *Anacroneuria saican* sp. nov. is similar to *A. arrazayalensis* and *Anacroneuria triplex* sp. nov., this last species collected in the same light trap. *Anacroneuria saican* sp. nov. can be separated from both species by its small bubble-like hammer, light coloration, including wings, and aedeagal features such as the absence of microspines on shoulders (present in *A. arrazayalensis*) and absence of central ventral pouch (present in *Anacroneuria triplex* sp. nov.). *Anacroneuria pakitza* Stark and Sivec, from Peru, has similar aedeagus but presents slender forceps and less marked shoulders, and its hammer is large and conical (figure 128 in Stark & Sivec, 1998). *Anacroneuria cushueme* Stark & Gill (in Stark et al. 2012) also has a similar aedeagus but with shoulders more marked and truncated apex. *Anacroneuria clarki* Stark & Baumann (2011) from Bolivia and *Anacroneuria cayapa* Stark (2001) from Ecuador, with similar aedeagus, can be differentiated by the greater separation of lateral

apical lobes from the median one (the three lobes are partially fused in *A. saican* sp. nov.). *Anacroneuria zunigae* Stark (2001) from Colombia and Ecuador also presents a similar aedeagus, but this species presents slender forceps and more projected aedeagal apex (see figure 129 in Stark, 2001). The female collected in the same light trap than the males, here tentatively associated because of general coloration and size, is similar to the *A. flavinigra* sp. nov., in the shape of subgenital lobe, which is bi-lobed and with a deep V-shaped notch in both. Nevertheless *A. saican* sp. nov. presents a small sublateral projection on hind margin (Fig. 13h) and slightly smaller eggs.

Anacroneuria saltensis Froehlich (Figs. 14-17)

Anacroneuria saltensis Froehlich, 2002: 98 (male, female).

Diagnosis. *Anacroneuria saltensis* can be distinguished from other species in the genus by FW length in male 9.6-15.5 mm; pale coloration, wing membrane pale ochraceous (Fig. 14); hammer well developed, cylindrical (Fig. 15e-g); aedeagus with large dorsally projected keel, in lateral view the dorsal margin of the keel is concave (arrow in Fig. 15b).



Fig. 14. *Anacroneuria saltensis*, male holotype. a. dorsal view. b. lateral. c. frontal view of head. d. labels.

Type material (photographs). **Holotype**, 1 male, Argentina, Salta, Peña Baya, Cañada La Gotera, 16-17.x.1973, Flint (NMNH) (Approx. -25.10886, -65.60280, ca. Chicoana). **Paratypes**: Same data as holotype, 1 male (NMNH); La Viña, 5 Females, 25.xi.1983, Peña (NMNH) (Approx. -25.45196, -65.56484).

Additional material (IBN). ARGENTINA. Jujuy: 1 reared male adult with larval exuvia (slide IBN837CM) Yala, río Yala, -24.12063, -65.43821, 12.xii.2001, Romero & Molineri cols. (this male presents a teratological hammer, formed by two small humps); 3 male adults (IBN927CM and IBN910CM) same data; 1 male (FW 9.5) Tiraxi, río Tiraxi, -23.99972, -65.33333, 13.xii.2001, light trap, Molineri & Romero. Salta: 2 males (IBN886CM) Los Toldos, río Huaico Grande, -22.281105, -64.721189, 1650 m, 26.x.1999, light trap, Molineri col.

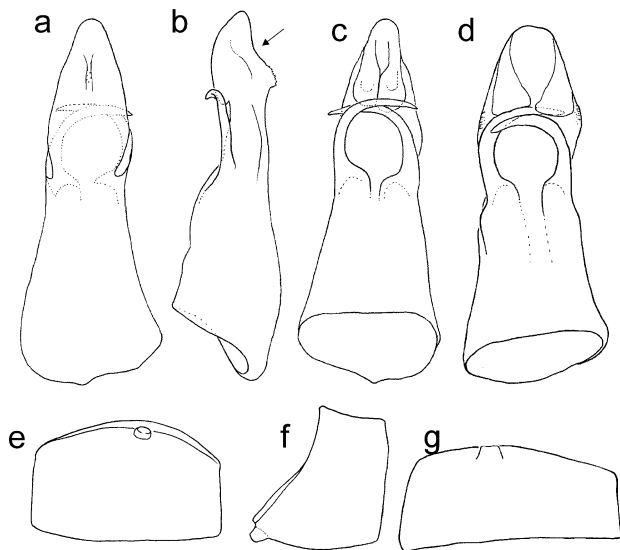


Fig. 15. *Anacroneuria saltensis*. Male (IBN837CM): a. aedeagus, dorsal. b. same, lateral (arrow indicates concave margin). c. same, ventral. Male (IBN910CM): d. aedeagus, ventral. e. hammer, ventral. f. same, lateral. Male (IBN927CM): g. hammer, ventral.

Adults. Described in Froehlich (2002). Photographs of the holotype can be seen in Fig. 14.

Last instar larval exuvia (male). Measurements (mm, n= 1): body length, 11.5; cerci length, >7.0; head length, 2.0; head width, 2.5; pronotum length, 1.5; pronotum width, 2.6. Cuticular pigmentation: head medially darker, no W-shaped mark visible. Scape subrectangular (Fig. 16a). Small dark setae around antennal segments slightly

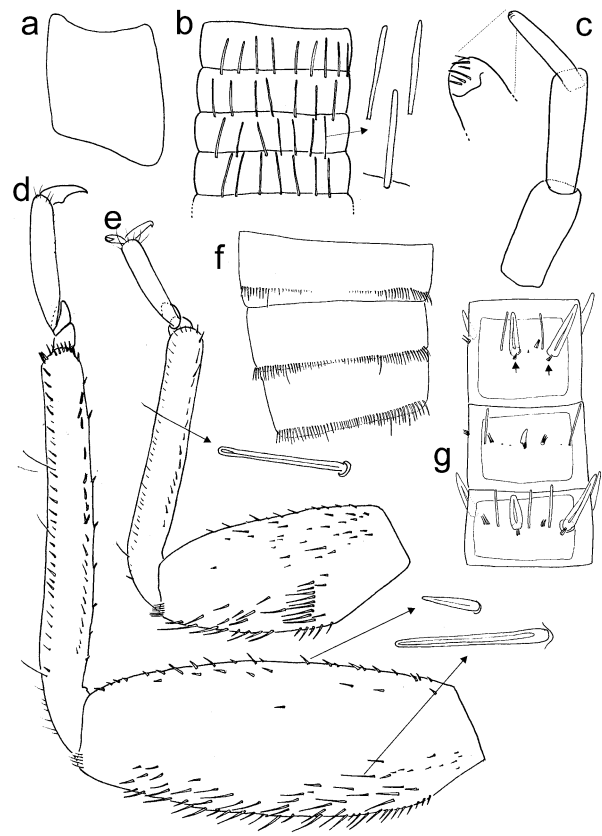


Fig. 16. *Anacroneuria saltensis*, male larval exuvia (IBN837CM). a. scape dorsal. b. antennomeres 5-8 with detail of setae. c. labial palp and detail of apex. d. hind leg. e. fore leg and detail of setae. f. abdominal sterna 6-9. g. cercomerites 6-9.

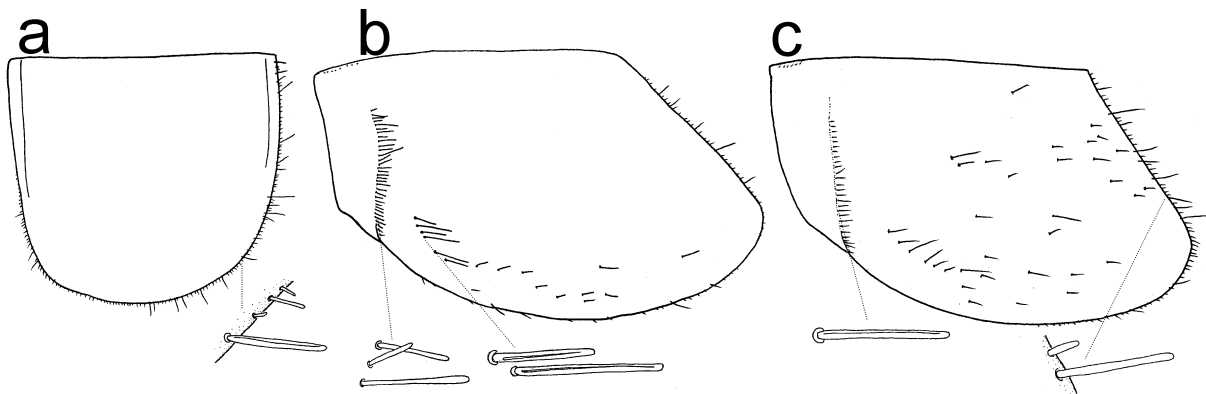


Fig. 17. *Anacroneuria saltensis*, male larval exuvia (IBN837CM). a. pronotum with details. b. mesonotum with details. c. metanotum with details.

shorter than segment (5th to seventh segments) (Fig. 16b). Mouthparts. Labial palp with 5-6 setae of subequal size at apex (Fig. 16c). Thorax with large pale areas, dorsum and margins with small slender spines (Fig. 17). Legs profusely fringed with long setae along outer margins of femora and tibia, tarsus with shorter setae (Fig. 16d-e). Abdomen uniformly colored orangish with spines along posterior margins of all terga, on sterna forming complete transverse rows only on segments 8-10, in sterna 1-7 the spines are only on lateral parts of posterior margins (Fig.

16f). Cerci with one row of bi- and trifid microtrichiae, stiff short setae and stout spines as in Fig. 16g.

Distribution. Argentina (Salta, and Jujuy **new provincial record**) (Fig. 30).

Discussion. Froehlich (2002) described this species from 2 males (one of them is the holotype) from Salta (Cañada La Gotera, October 1973) and 3 females from a different locality in the same province (La Viña, November 1983). *Anacroneuria saltensis* is a relatively rare species

that was never reported again until the records here provided for Salta and the neighboring province of Jujuy. The females described by Froehlich present a four-lobed subgenital plate (figure 91 in Froehlich, 2002), as we report here for *A. reedi* (Navás). Nevertheless, female subgenital plate of *A. saltensis* presents a much deeper median notch. The true identity of *A. saltensis* females remain doubtful, until a direct association with males could be attained. The male of *A. saltensis* is similar to *A. tucuman*, but can be distinguished by the presence of a well-developed hammer (mostly reduced and bubble-like in *A. tucuman*) and by the concave dorsal margin of the aedeagal keel (when looked laterally) (margin straight in *A. tucuman*). *Anacroneuria cusí* Stark, 2004 also presents a similarly concave aedeagal keel, but its forceps are stouter and the keel is not serrated. The larva of *A. saltensis*, described here for the first time, is not easily distinguished from other of the genus, by its large size is similar to *A. tucuman*, but setae and spines on cerci are shorter in *A. saltensis*; the apex of the labial palp presents 6 microtrichia in *A. saltensis* (as in *A. tafi*), but 3 in *A. tucuman*.

Anacroneuria tafi sp. nov. (Figs. 18-19, 26b, 28b)

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Diagnosis. *Anacroneuria tafi* sp. nov. can be distinguished from other species in the genus by the following combination of characters: relatively large body size (male FW length 10.8-14.7 mm, female FW length 15.3-17.3 mm); blackish brown coloration on thorax, wing membrane and legs contrasting with pale yellow head and abdomen (Fig. 28b); low hammer (Fig. 18g-h); aedeagus with slightly serrated keel, ventral pouches in lateral view basally projected (arrow in Fig. 18f), with ventral paired spines near base (arrow in Fig. 18e); apex of aedeagus rounded and without marked shoulders (Fig. 18a, d). Female with V-shaped notch on subgenital plate (Fig. 18j-k). Larvae without small acute projection on distal inner corner of antennal pedicel (better visualized in exuviae.), but sometimes with a small lappet on outer corner (Fig. 19d-e).

Type material. Holotype male (IBN) from ARGENTINA, Tucumán, Tafí del Valle, arroyo Apeadero Muñoz, -26.98722, -63.66361, 1630 m, 26.ii.2015, Molineri col. **Paratypes:** 3 male adults (IBN890CM, COI AN012) same data; 3 female adults, same data; 12 male adults (in 8 vials, COI AN020) and a reared female with larval exuvia (IBN924CM y IBN925CM), same data except 17.ii.2016, Rueda et al. cols.; 1 male and 2 female adults (IBN, not dissected) same, 8.xii.2015, Molineri col.; 1 male adult (MUSENUV) and 1 female adult same locality, 22.xii.2015, Molineri col.; 2 male adults (IBN887CM, IBN889CM), same locality, 21.iv.2016, Molineri; 1 male adult (slide IBN888CM) same data except 22.ii.2017; 3 males and 1

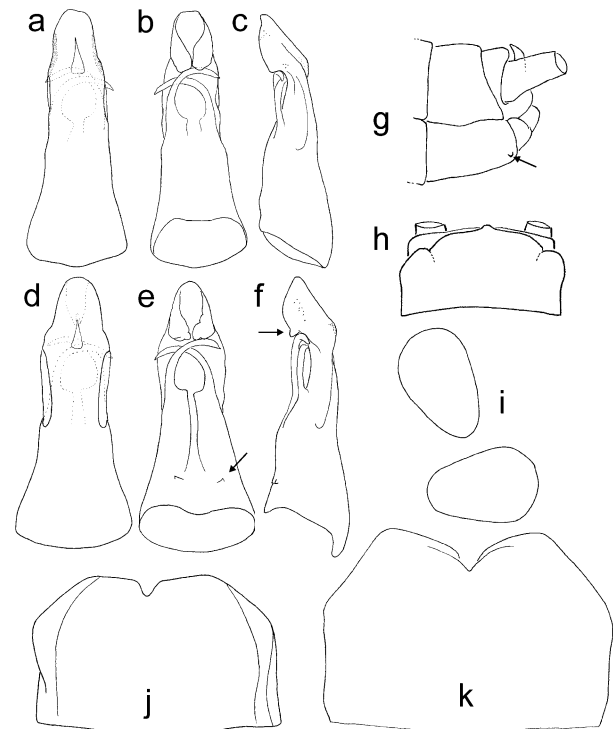


Fig. 18. *Anacroneuria tafi*. Aedeagus: a, d. dorsal. b, e. ventral (arrow indicates basal spine). c, f. lateral (arrow indicates pouch projection). Male terminalia: g. lateral. h. ventral (arrow indicates hammer). Eggs: i. outline under light microscope. j-k. female subgenital plates.

female adult (IBN918CM), same locality except 26.ii.2022, Molineri & Fontanarrosa cols.; 2 male and 1 female adults, same locality except: 09.iii.16, Hankel et al. cols; 1 male and 1 female adults (IBN) same locality except date: 4.v.2016, Molineri et al. cols.; 2 female adults same data except 1.xii.2016, Hankel et al. cols.

Additional material. ARGENTINA. Tucumán: 1 female adult Tafí del Valle, Cerro Muñoz en Santa Cruz, 2400 m, -26.90000, -65.77833m, 6.i.2001, Molineri col.; 4 larvae from arroyo Azucenas, -27.01506, -65.67250,1605m, 26.ix.2009, Molineri & Emmerich cols; 1 larva, PN Los Alisos, arroyo Hondo, -27.22667, -65.91583,1530 m, 22.ix.2006, Molineri col.; 4 larvae PN Los Alisos, río Pavas, -27.21083, -65.92750,1655 m, 24.ix.2006, Molineri col.; 10 larvae PN Los Alisos, río Pavas, 1500 m, 24.ix.2006, Molineri col; 7 larvae PN Los Alisos, tributario de arroyo Grande, -27.21722, -65.92972, 1625 m, 23.ix.2006, Molineri col.; 8 larvae Chasquivil, río Chasquivil, -26.64517, -65.63239, 2270 m, i.2004, Molineri & Fernández col.; 4 larvae Chasquivil, río Chasquivil, -26.63617, -65.62672, 2160 m, 9-10.xi.2002, Molineri col.

Adult habitus (Fig. 28b). General color brown yellow. Head pale yellow dorsally, except anterior margin of frons and lappets brown; with a whitish M-shaped diffuse mark near anterior margin. Antennae and mouthparts brown. Pronotum mostly brown but with paler median and paramedian longitudinal lines; these marks present slightly

elevated margins (rugose) (Fig. 28b). Legs brown. Wings light brown, veins brown.

Male adult. FW length 10.8-14.7 mm (n= 25). Hammer low, not sclerotized, rounded (Fig. 18g-h). Aedeagal apex rounded and simple, with just a slight constriction towards shoulders (Fig. 18a, d); dorsal keel nose-shaped (Fig. 18a, d), not strongly projected in lateral view, with small serration visible at 100X (Fig. 18c, f). Apical ventral lobes paired (Fig. 18b, e) and slightly projected at its proximal margin (better seen at lateral view, Fig. 18c, f). A pair of small submedian ventral spines present at base of aedeagus (Fig. 18e). Hooks slender from base (Fig. 18b, e).

Female adult. FW length 15.3-17.3 mm (n= 2). Subgenital plate bilobed, with deep median V-shaped notch (Fig. 18j-k).

Eggs. Length 380-390 μm ; maximum width 240 μm ; ratio length/maximum width = 1.6. Blackish brown, surface smooth at 100X, drop-shaped (Fig. 18i).

Last instar larval exuvia. Measurements (mm). Female (n= 1): body length, 18.5; cerci length, broken and lost; head length, 2.7; head width, 4.0; pronotum length, 2.3; pronotum width, 4.0. Cuticular pigmentation: head orangish except on central W-shaped mark on frons. Scape with inner distal corner rounded and outer corner with a small lappet (Fig. 19c-e). Small dark setae around antennal segments, these setae are subequal to slightly longer than the respective segment (Fig. 19f-g). Mouth parts. Labial palp with 5-6 setae of subequal size at apex (Fig. 19a-b). Thorax orangish brown, with pale markings on nota; spines as in Fig. 19k-l. Legs profusely fringed with long setae along outer margins of femora and tibiae, tarsus with shorter setae (Fig. 19h-i), dorsal surface of femora with many spines (Fig. 19h-i). Abdomen yellowish brown. Cercomeres with many thin setae, thicker ones and spines (Fig. 19j).

Larval coloration (Fig. 26b): the pale marks on head and thorax visible in the exuvia are not so marked in the fresh larvae, the W-mark in head is hardly visible, only a pair of sublateral pale marks on occipute are visible.

Etymology. "Tafi", name in apposition, from the type locality. "Tafi" derives from the Aimara word "Thaui", meaning "place where cold air blows or it is very cold."

Distribution. Argentina (Tucumán) (Fig. 30).

Discussion. Several *Anacroneuria* species in Yungas, such as *A. saltensis* Froehlich and *A. tucuman* Stark have similar aedeagal shape but none have the combination of large body size (*A. tucuman* is also large), dark coloration on thorax, wings and legs (*A. tucuman* and *A. saltensis* are pale species), low hammer (*A. tucuman* presents low hammer also), slightly serrated keel (*A. tucuman* and *A. saltensis* may present serrated keel, but variable), ventral pouches basally projected (only one specimen of *A. tucuman* presented this character too), and ventral spines

on aedeagus (sometimes present in *A. tucuman*). The female, besides coloration, also can be distinguished from *A. tucuman* (with U-shaped notch) by the V-shaped notch on the subgenital plate. The coloration of *A. tafi* sp. nov. is dark in comparison with *A. tucuman*, almost without pale marks on head and thoracic nota. Larvae are morphologically very similar to *A. tucuman*, except for the presence of a small lappet on the outer distal corner of antennal pedicel (absent in *A. tucuman*, better visualized in exuviae). The apex of the labial palp in *A. tafi* sp. nov. larvae show 5-6 small setae (as in *A. saltensis* but opposed to *A. tucuman* with 3 larger ones). Finally, the antennal segments present whorls of longer setae in *A. tafi* sp. nov. (those in *A. tucuman* are shorter than each segment). These setae should be compared in the same segments since they vary in length at different positions along the flagellum. Genetic divergence between *A. tafi* sp. nov. and *A. tucuman* is low (3.1%, Fig. 29), showing a recent divergence between both groups. This difference together with coloration, V-shaped notch in female, nose-shaped keel in aedeagus and larval characters discussed above are considered by us sufficient to support the description of this new species. Furthermore, in most localities where *A. tafi* sp. nov. is present, we have also found specimens of *A. tucuman*, suggesting that specimens from both species do not breed.

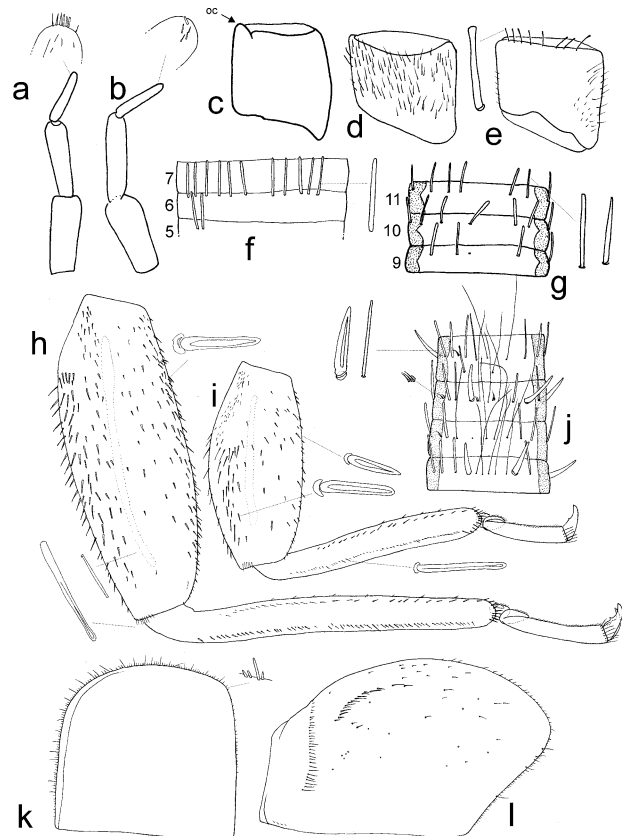


Fig. 19. *Anacroneuria tafi*. Larva (IBN925CM): a-b. labial palpi with detail of apex. c. scape, dorsal (oc=outer corner). f. antennomeres 5-7, with detail of seta. k. pronotum with detail. l. mesonotum. h. hind leg. i. fore leg with details. Specimen IBN935CM: d. scape, dorsal. e. scape, ventral with detail. g. antennomeres 9-11 with detail. j. cercomeres 9-12 with details.

Anacroneuria triplex sp. nov. (Figs. 20-21).

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Diagnosis. *Anacroneuria triplex* sp. nov., known from male and female adults and larva, can be distinguished from other species in *Anacroneuria* by the following combination of characters: medium sized (male FW length 9.8-10.7 mm, female FW length 14.5 mm), light coloration with yellowish orange wings; hammer well developed, cylindrical (Fig. 20e-h); aedeagus with shoulders and subapical lobes (Fig. 20a); dorsal keel pronounced and serrated basally (Fig. 20c); with a dorsal semicircular fold basad to keel (Fig. 20d); three ventral pouches present, one median and two sublateral (Fig. 20b); female subgenital plate four-lobed (Fig. 20i).

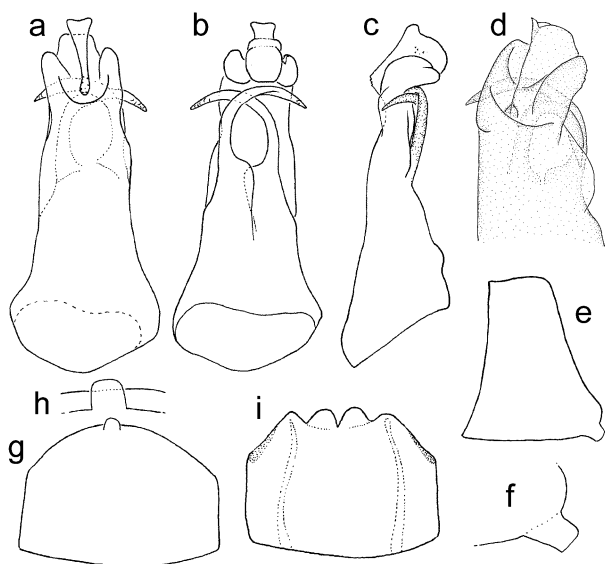


Fig. 20. *Anacroneuria triplex*. Aedeagus (Tarija, IBN895CM): a. dorsal view. b. ventral view. c. lateral view. d. dorsolateral detail. Hammer: e-f. lateral view. h-g. ventral view. i. female subgenital plate.

Type material. **Holotype** reared male adult and larval exuvia (IBN826CM) from ARGENTINA, Jujuy, arroyo Las Lanzas, -24.45472, -65.29667, 1250 m, 3.iii.2000, Molineri et al. col. **Paratypes:** 1 male (IBN895CM) and 1 female (not associated, slide IBN896CM) from BOLIVIA, Dpto Tarija, Prov. O'Connor, río Saican ca. Entre Ríos, 1000 m, -21.77458333, -64.09000, 6.x.2004, ligh trap, Molineri & Manzo cols.

Adult habitus. General color yellowish orange, wings orangish translucent with yellowish veins. Head whitish yellow shaded orangish medially. Antennae basally yellowish, remainder and mouthparts whitish. Pronotum mostly orangish except laterally whitish, with brownish middle longitudinal line; mesonotum whitish yellow except anterolateral margins orangish. Legs whitish yellow, subapical blackish mark on femora. Cerci yellowish white.

Male adult. FW length 9.8-10.7 mm (n= 2). Hammer large, cylindrical (Fig. 20e-h). Aedeagus with subapical

dorsal lobes and marked shoulders (Fig. 20a); in dorsal view apex of aedeagus subapically constricted, distal margin slightly concave; in lateral view the apex bends ventrally; dorsal keel pronounced and short, basally serrated (Fig. 20c); with three ventral pouches and slender forceps (Fig. 20b).

Female adult. FW length 14.5 mm. Subgenital plate four-lobed, all lobes subequal in length; median notch V-shaped, sublateral notches slightly broader (Fig. 20i).

Last instar larval exuvia. Measurements (mm). Male (n= 1): body length, 9.4; cerci length >4.0 (broken); head length, 1.6; head width, 2.3; pronotum length, 1.2; pronotum width, 2.2. Cuticular pigmentation: head almost completely pigmented except for a pair of small submedian dots on epicranial suture, anterior margin of clypeus and a very slightly marked and diffuse pale W-shaped mark is visible under certain illuminations in the center of frons. Antennae with whorls of relatively long and thin setae (Fig. 21e). Apex of labial palp with 2-4 rounded microtrichiae (Fig. 21a-b). Thorax. Nota fringed with short and medium length spines as in Fig. 21i-k. Legs with row of medium length thin setae on outer margin of tibiae, only few setae on outer margin of femora; long and acute spines on femora as in Fig. 21g-h. Abdominal sternum 8 with distal row of setae almost complete, interrupted in the middle by a short distance. Cerci. Basal cercomeres with a submedian whorl of long and thin setae, medium length spines and single to double hook-like microtrichiae (Fig. 21f).

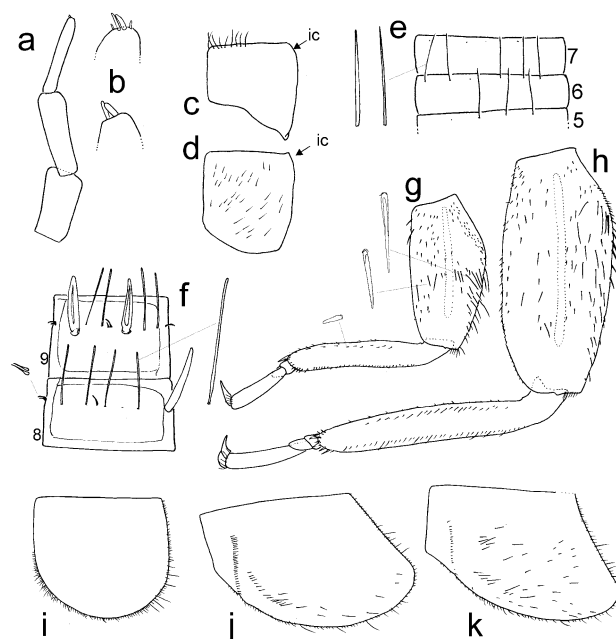


Fig. 21. *Anacroneuria triplex*, last larval exuvia. a. labial palp. b. details of apex of labial palp. c. scape ventral. d. same, dorsal. e. antennomeres 5-7 with details. f. cercomeres 8-9, with details. g. fore leg with details, dorsal. h. hind leg. i. pronotum. j. mesonotum. k. metanotum.

Etymology. From the Latin word *triplex*, because of the triple distal ventral pouches present on aedeagus.

Distribution. Argentina (Jujuy) and Bolivia (Fig. 30).

Discussion. *Anacroneuria triplex* sp. nov. is similar to *A. saican* sp. nov. in aedeagal structure but can be readily separated by the serrated keel, the additional median ventral pouch, the slender forceps, and the larger hammer. The female of *A. saltensis* (Fig. 91 in Froehlich, 2002) and *A. reedi* (Fig. 9g above) are the only other known in Austral Yungas with four-lobed subgenital plate. The median lobes of *Anacroneuria triplex* sp. nov. are semicircular and the median notch is shallower and V-shaped, contrasting with more acute median lobes and deeper U-shaped median notch in *A. saltensis*. Lobes in female subgenital plate of *A. reedi* are all of similar length and shape, but in *A. triplex* sp. nov., the medial lobes are slightly longer than the lateral ones. Both sexes are associated here by this general coloration; additionally, both were captured in the same light trap, together with other males from *A. saican* sp. nov. The association of adults of both sexes nevertheless should be treated as tentative. Larva is similar to other small species in the region, with head widely pigmented dorsally. *Anacroneuria triplex* sp. nov. can be separated from other similar larva because the setae on antennal segments are relatively slender, the head is more widely pigmented than in other species (pale areas are reduced to anterior band on clypeus and sublateral dot on epicranial suture); and on cerci the small hook-like microtrichiae are single to double (single to triple in *A. arrayazalensis*).

Anacroneuria tucuman Stark (Figs. 22-24, 26a, 28a)

Anacroneuria tucuman Stark, 2013: 97 (male adult, Tucumán); Pessacq & de Paul, 2015: 271 (larva, Jujuy).

Diagnosis. *Anacroneuria tucuman* can be distinguished from other species in the genus by the following combination of characters: relatively large body size (male FW length 10.0-15.0 mm, female FW length 13.0-18.7 mm); pale yellow brown coloration (Fig. 28a); low bubble-like hammer (Fig. 22j-k); aedeagus with slightly serrated keel (Fig. 22c, f, i), ventral pouches in ventral view notched basally (arrow in Fig. 22e), generally without ventral paired spines, but variable; apex of aedeagus rounded and with slightly marked shoulders (Fig. 22a, d, g). Female with U-shaped notch on subgenital plate (Fig. 22l-m). Larvae with inner distal corner of scape rounded (Fig. 23e) to very slightly and acutely projected (Fig. 23d).

Type material (not studied). Primary types: *Anacroneuria tucuman* Stark (2013) (male adult holotype and paratype) from Tucumán, Horco Molle, 700 m, 15.i.1980, Stange col., deposited in FSCA (Approx. -26.77203, -65.32748). Attempts to study photographs of this material were unsuccessful.

Additional material (IBN). ARGENTINA. Tucumán: 1 reared female adult and larval exuvia (slide IBN841CM, IBN879CM), arroyo Azucenas, -27.01083, -65.65722, 1405 m, 16 x.2007, Rodriguez col.; 1 female adult (COI

AN022), same data except 26.ii.2022, Molineri & Fontanarrosa cols; 1 reared male adult and larval exuvia (slide IBN840CM, IBN880CM) same data; 1 reared male adult with larval exuvia (slide IBN838CM, IBN915CM) Siambón, río Grande, -26.77222, -65.46667, 900 m, 5 xii.2014, Molineri col.; 1 reared male adult and larval exuvia (slide IBN842CM, IBN867CM), same data except: 28 x.2003, Giordano col.; 1 reared female and larval exuvia, same data; 2 female same data except: 25.x.2003; 4 males same data except: 9.xii.2007, Giordano & Rodriguez col.; 1 reared male adult with larval exuvia (slide IBN827CM, IBN866CM), Siambón, -26.73917, -65.44750, 4.viii.2004; 2 reared female adults and larval exuvia, and 2 male adults (in 2 vials) same data; 1 male adult (FW 12.5 mm, penes drawn), same data except 25.x.2003; 1 male adult same data except date 9.xii.2007; 8 male and 1 female adults (IBN875CM), Siambón, arroyo Melocoton, -26.75358, -65.45392, 1040 m, 3.iii.2009, light trap, Molineri, Rueda & Tejerina cols.; 4 male adults (IBN865CM, FW12 mm; IBN919CM; IBN920CM, COI AN015) and 2 females (IBN922CM, IBN923CM) from arroyo Apeadero Muñoz, -26.98722, -63.66361, 1630 m, 17 ii.2016, C. Molineri col; 1 reared male adult and larval exuvia (slide IBN830CM, IBN862CM), río Los Sosa, -27.13222, -65.55722, 402 m, 4.v.2005 (emerged 16.v.2005), Domínguez et al. cols.; 1 reared female adult with larval exuvia from río Los Sosa, 18.vi.2006, Giordano col; 1 reared male adult (COI AN848) and 1 reared female adult (COI AN849) with larval exuviae (IBN848CM, IBN869CM, IBN849CM, IBN868CM), Horco Molle, arroyo Anta Yacu, -26.77203, -65.32748, 30. ix.2019, Molineri col.; 1 male adult (slide IBN873CM) Horco Molle, arroyo Anta Yacu, -26.77203, -65.32748, light trap 5am, 19.xi.2019, C Molineri col.; 1 reared female same data except date 19.v.2022; 1 reared female adult same data except date 15.v.2022; 1 male adult (FW12.8 mm, IBN912CM), Horco Molle, arroyo Piedras, -26.76175, -65.32634, 740 m, 28 ix.2017; 1 reared male with larval exuvia (IBN839CM), same locality except date 23.ix.2017; 2 reared female (FW 17-18 mm) and exuviae same locality except 28.ix.2017; 1 reared female adult and larval exuvia, same locality except, emerged in lab on 19.x.2017; 1 reared female and larval exuvia (IBN851CM) Trancas, San Pedro de Colalao, arroyo Ceibalito, -26.3111, -65.53611, 1377 m, 29.x.1999, Romero, Manzo & Nieto cols; 1 reared male adult with larval exuvia (slide IBN831CM, IBN863CM), Tafí Viejo, La Toma, 27. ii.2005; 1 reared male adult with larval exuvia (slide IBN828CM) same data except 29.iv. 2006 (emerged 1 May 2006), Giordano col.; 1 reared male adult with larval exuvia (slide IBN829CM, IBN864CM) Burruyacu, arroyo Artaza, 8.vi.2004, C. Molineri col.; 1 male adult, Chicligasta, río Jaya, -22.30639, -65.82889, 760 m, 9-10.ii.2000, light trap, Molineri et al. col.; 1 male adult (IBN917CM), Pueblo Viejo, arroyo Yacuchina, -27.14083, -65.76806, 10.ii.2000, light trap, Molineri et al col.; 1 reared male and exuvia (slides IBN843CM and IBN911CM) from río Medina, -26.54083, -65.02917, 860 m, 14.vi.2001, Molineri et al.

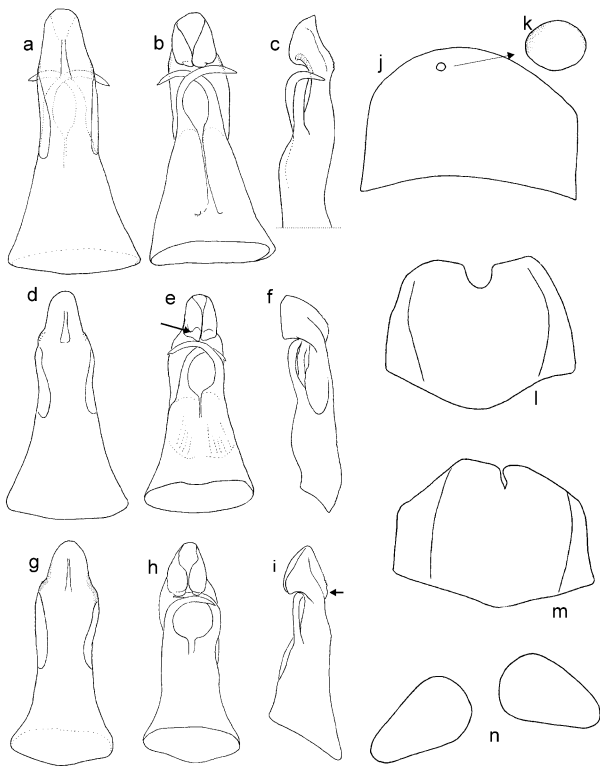


Fig. 22. *Anacroneuria tucuman*. Aedeagus variations in dorsal, ventral and lateral view, all males from Tucumán: a-c. Horco Molle (IBN839CM). d-f. Siambón (IBN838CM). g-i. Burruyacu (IBN911CM). j. male subgenital plate and hammer, ventral. k. bubble-like hammer enlarged. l. female subgenital plate, ventral, normal shape. m. same but with median emargination closed. n. outline of eggs.

col.; 1 reared female and larval exuvia (FW 14.5mm), (IBN856CM) río Nio, no more data (FW 16.3mm); 1 reared female and larval exuvia (IBN850CM) arroyo Artaza, -26.60222, -65.04389, 750 m, 14.vi.2001, Molineri et al. cols.; 1 reared female (FW17mm) and larval exuvia same data; 1 reared female and larval exuvia (IBN852CM) Raco, arroyo Yerbabuenita, -26.665958, -65.413941, 990m, 24.ix.2000, Molineri col.; 1 male same except date: 3.iii.2001; 1 male (IBN904CM) same locality except date 2.i.2022; 1 male (IBN913CM, COI AN011) and 1 female same locality except date: 30.i.2022, Molineri col.; 1 reared female (FW15.5) and larval exuvia (IBN854CM) Burruyacu, near río Nio, 8.v.2000, Orce col. Jujuy: 1 reared female with larval exuvia (IBN853CM) río Zora, -23.75111, -64.69083, 410 m, 2.vi.2000, Molineri et al. cols.; 1 reared male adult with larval exuvia (slide IBN824CM, 870), Bananal, río Piedras, -23.513056, -64.55250, 2.vi.2000, Molineri col.; 1 reared male adult with larval exuvia (slide IBN825CM, 872, FW 12.5mm), Yala, río Yala, -24.120631, -65.438208, 12. xii.001, Romero & Molineri cols.; 14 male and 1 female Tiraxi, río Tiraxi, -23.99972, -65.33333, 13.xii.2001, ligh trap, Molineri & Romero col.; 1 male adult (IBN882CM, FW 13.5 mm) Calilegua, río Jordan, -23.65694, -64.92889, 1510 m, 2 iv.2004, light trap, Nieto & Mesa cols.; 8 male (IBN881CM, IBN909CM) and 6 female adults (IBN885, FW 18.5 mm; IBN883CM, FW 14.3mm; IBN884CM, FW16.5 mm),

Calilegua, río Jordan, -23.65694, -64.92889, 1510 m, 2 iv.2004, light trap, Nieto & Mesa cols.; 1 male Calilegua, arroyo Aguas Negras, -23.76083, -64.85083, 700 m, 1.iv.2004, Nieto & Mesa cols. Salta: 1 male (IBN877CM), Santa Victoria, Lipeo, arroyo Los Naranjos, -22.42972, -64.73889, 1109 m, 13.xi.2004, Molineri & Romero cols. Catamarca: 2 reared male adult and larval exuvia (2 vials, slide IBN846CM), Andalgalá, río Potrereros, -27.496629, -66.310491, 25. iii.2006, Molineri col.; 1 reared male adult (IBN861CM) and 1 female adult (IBN860CM), same data; 1 reared male adult with exuvia, and 2 female adults, same data except 28.iii.2006. BOLIVIA. La Paz: 1 male adult (IBN914CM) Reserva Carrasco, -15.719167, -67.518056, 940 m, 29.xi.2000, Molineri et al. cols. Tarija: 4 females río Conchas, -22.29569, -64.388528, 828 m, 2 iii.2006, Rueda & Nieto cols.; 1 male río Orosa, ca. La Mamora, -22.202556, -64.62683, 1100 m, 4 x.2004, ligh trap pm, Molineri & Manzo cols.

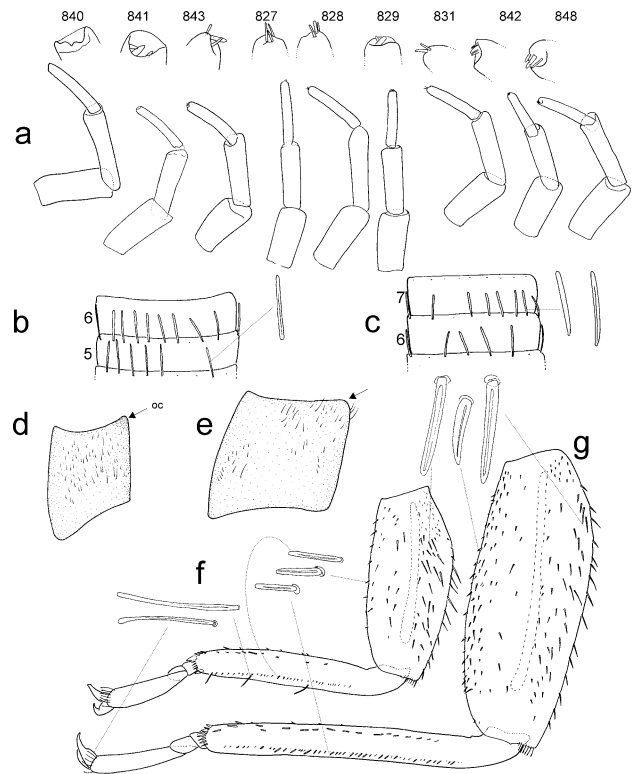


Fig. 23. *Anacroneuria tucuman*. Larva. a. labial palps with detail of apical groove, ventral (from left to right, specimens: IBN840CM, IBN841CM, IBN843CM, IBN827CM, IBN828CM, IBN829CM, IBN831CM, IBN842 CM, IBN848CM). b. antennomeres 5-6 with detail of microtrichia (IBN848CM). c. antennomeres 6-7 with detail of microtrichia (IBN841CM). d. scape, dorsal (IBN848CM). e. same (IBN841CM). f. fore and hind legs, with details of spines (IBN824CM). References= oc: outer corner

Adult habitus (Fig. 28a). General coloration pale yellow brown. Head variable in coloration including orangish and brownish tints, most frequently pale yellowish brown, darker on lappets. Antennae and mouthparts yellowish to light brown. Pronotum pale brown but with pale lateral margins, pale median longitudinal band and with irregular rugose

pale markings sublaterally. Legs pale brown except femora whitish with a narrow subdistal dark brown band. Wings pale, membrane light yellowish to whitish brown; veins pale brown to yellowish except for C and R veins, whitish.

Male adult. FW length 10.0-15.0 mm. Hammer absent to slightly marked, low and bubble-like (Fig. 22j-k). Aedeagus with dorsal keel, slightly serrated in lateral view, sometimes with a basal short semicircular portion not serrated and a longer distal part slightly serrated (Fig. 22c, f, i); apex of aedeagus rounded; shoulders variable, frequently well-marked, with tiny spines ventro-laterally (Fig. 22a, d, g); ventral pouches paired, prominent, elongated and triangular, with a small subbasal fold ventrally (Fig. 22b, e, h).

Female adult. FW length 13.0-18.7 mm. As in male except abdomen blackish when full of eggs. Subgenital plate bilobed; deep median U-shaped notch (when fully expanded, Fig. 22l) but may seem V-shaped or even be completely closed depending on fixation and hardness (Fig. 22m).

Eggs. Length: 330-350 μ m, maximum width, 200-215 μ m. Ratio Length/maximum width= 1.6-1.7. Dark brown, oval, surface smooth at light microscope.

Last instar larval exuvia. Measurements (mm). Male (n= 18): body length, 10.0-12.8; cerci length, 6.5-8.5; head length, 1.6-2.2; head width, 2.1-3.0; pronotum length, 1.3-1.7; pronotum width, 2.3-3.0. Female (n= 6): body length, 14.0-18.0; cerci length, 9.5-11.0; head length, 2.1-2.7; head width, 2.6-3.7; pronotum length, 1.8-2.1; pronotum width, 3.0-4.0. Cuticular pigmentation: head pigmented (yellowish to brownish) except on pair of anteromedian dots, and central W-shaped mark on frons (variations: the lateral sides of this mark may be separated or fused together), also with a pair of submedian pale marks on epicranial suture (Fig. 26a). In younger larvae pale markings on head may be missing. Scape with inner distal corner rounded (Fig. 23e) to very slightly and acutely projected (Fig. 23d), outer corner rounded. Small dark setae around antennal segments, shorter than segment (5th to seventh segments) (Fig. 23b-c). Mouth parts. Labial palp with three distinct setae at apex, frequently one of them is wider than the others (Fig. 23a). Thorax yellow to brown, with pale markings on nota; spines as in Fig. 24a-f. Legs profusely fringed with long setae along outer margins of femora and tibiae, tarsus with shorter setae (Fig. 23f-g), with many spines on dorsum of femora (Fig. 23f-g). Abdomen yellow to brown. Cercomeres with setae, stout spines, and hooked microtrichiae as in Fig 24g-h.

Distribution. Argentina (Tucumán, Jujuy; Catamarca and Salta **new provincial records**). Bolivia (**new country record**) (Fig. 30).

Discussion. *Anacroneuria tucuman* is the most frequent species in NW Argentina, occupying different altitudinal levels, and showing wide size ranges. Pessaqc & de Paul (2015) described the larvae of this species. See characters useful to distinguish larvae in the discussion of *A. tafi* sp. nov. Males are similar to *A. tafi* sp. nov., but can be distinguished by the pale coloration (yellow with black markings in *A. tafi* sp. nov.), the aedeagus with slightly marked shoulders (shoulders absent in *A. tafi* sp. nov.). Females are here described for the first time, the U shaped notch on the subgenital plate is unique in the region.

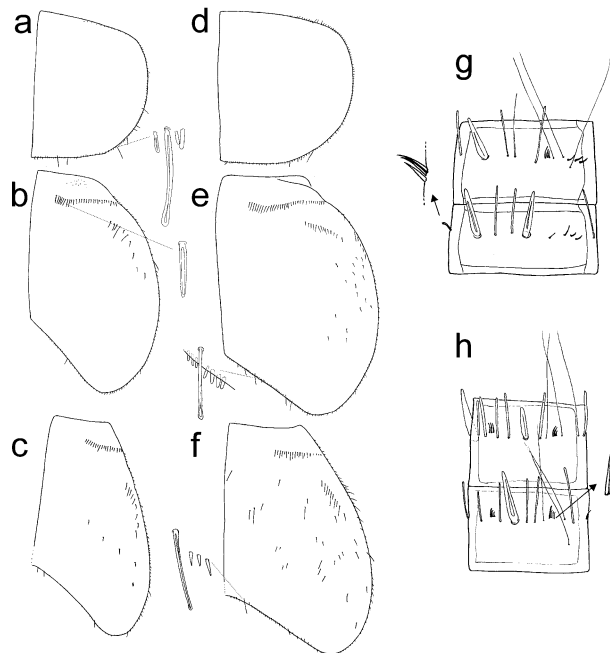


Fig. 24. *Anacroneuria tucuman*, larva. Specimen IBN848CM: a. pronotum outline with detail of setae. b. mesonotum with detail. c. metanotum. Specimen IBN839CM: d., pronotum outline. b. mesonotum with detail. c. metanotum with detail. g. cercomeres 7-8 with detail of microtrichia (IBN840CM). h. same (IBN848CM).

Anacroneuria tupacamaru sp. nov. (Fig. 25)

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Diagnosis. Male adults of *Anacroneuria tupacamaru* sp. nov. can be distinguished from other species in the genus by: FW length 10.5-11.5 mm, medium coloration, wing membrane orangish yellow, body and legs light yellowish brown; hammer relatively long and slender, cylindrical (Fig. 25g-i); aedeagus gradually narrowing distally, apex truncated, shoulders absent (Fig. 25a, d) and with lateroventral microspines (Fig. 25b, e); keel large and pronounced (Fig. 25b, e); ventral membranous lobes large, largely projected ventrally (Fig. 25c, f).

Type material. **Holotype** male (IBN) and 6 male **paratypes** (IBN) from BOLIVIA, Tarija, río Conchas, -22.295694, -64.388528, 828 m, 2 iii.2006, Rueda & Nieto col.

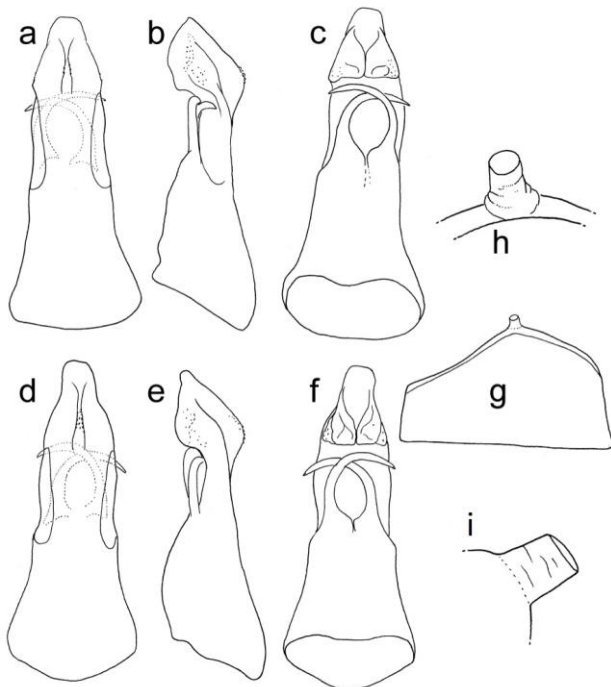


Fig 25. *Anacroneuria tupacamaru*. Male 1: a. aedeagus, dorsal. b. same, lateral. c. same, ventral. Male 2: d. aedeagus, dorsal. e. same, lateral. f. same, ventral. g. hammer and subgenital plate, ventral. h. detail of hammer, ventral. i. same, lateral.

Adult habitus. Medium sized species with medium light coloration. Wing membrane orangish yellow. Head light yellowish brown, lappets and antennae brownish, palps yellowish. Pronotum yellowish brown, paler medially, with thin dark line medially and on margins. Legs light yellowish brown, paler towards base of femora with small subapical black mark on femora.

Male adult. FW length 10.5-11.5 mm. Hammer (Fig. 25g-i) large, slender, surrounded by stiff setae. Aedeagus (Fig. 25a-f) with slender forceps, pronounced and serrated keel; ventral membranous lobes large and subtriangular.

Etymology. The specific epithet is derived from the great Incas Tupac Amaru I and II. From the *quechua* voice *tupac amaru* meaning 'glowing snake'.

Discussion. *Anacroneuria tupacamaru* **sp. nov.** presents an aedeagus similar in shape to other species in Austral Yungas: *A. tucuman*, *A. tafi*, *A. saltensis*, but its forceps are somewhat slender and ventral membranous lobes are more projected than in those species. The large and slender hammer of *Anacroneuria tupacamaru* **sp. nov.** is useful to distinguish from these and other species in the region, except *A. calilegua*, but this last species presents a unique aedeagus with double keel.

DISCUSION

The number of *Anacroneuria* species in Yungas is surely higher than reflected in the present paper. A number of specimens from at least 5 different morphotypes were

studied but could not be assigned to species, and the material is incomplete or badly preserved to be formally described. There are still unexplored areas with little or no knowledge about this genus, for example in southern Bolivia and in Argentina, the northern extreme, and Cuyo and Central region. The species diversity in Bolivia and Argentina has been revised by Stark and co-authors (Stark & Sivec, 1998; Stark, 2004, 2013; Stark & Baumann, 2011).

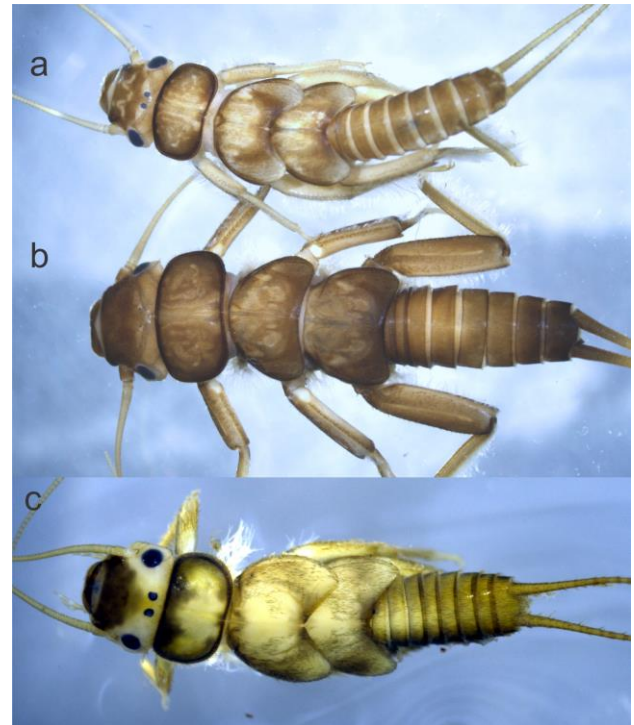


Fig. 26. *Anacroneuria*, larvae of the three most common morphotypes in NW Argentina. a. *A. tucuman*. b. *A. tafi*. c. *A. arrayazalensis*.

The larvae we have studied are almost indistinguishable in their morphology, but coloration of fresh material is useful to distinguish some species, mainly maculation pattern of the head. The larvae of less than 10% of the described species are known, and most frequent diagnostic characters for larvae are from coloration of body and legs (Stark, 1995; Zúñiga et al., 2007; Ribeiro & Gorayeb, 2014). There are few useful morphological characters to separate species at this stage, those most commonly used are setation of legs, shape and number of the setae in antenna and cercomeres, apex of labial palp, and setae on abdominal segments.

Some studies have found low levels of genetic differentiation in some species of *Anacroneuria*, but high differentiation in others (Gamboa & Arrivillaga, 2019), similar to our results. This gives support for the delimitation of species, we preferred the GMYC model, among other reasons, because it joins under the same lineage the male AN848 and female AN849, which were observed and photographed in copulation. We therefore deduce that the ABGD model that separates the

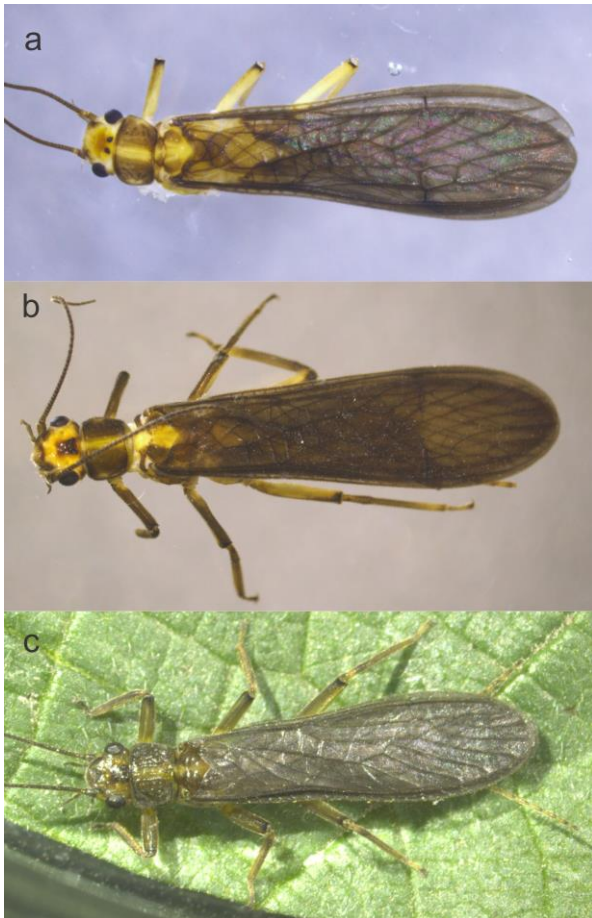


Fig. 27. *Anacroneuria*, male habitus. a. *A. flavinigra* (Raco). b. *A. lutea* (Apeadero Muñoz). c. *A. arrayazalensis* (Tapia).

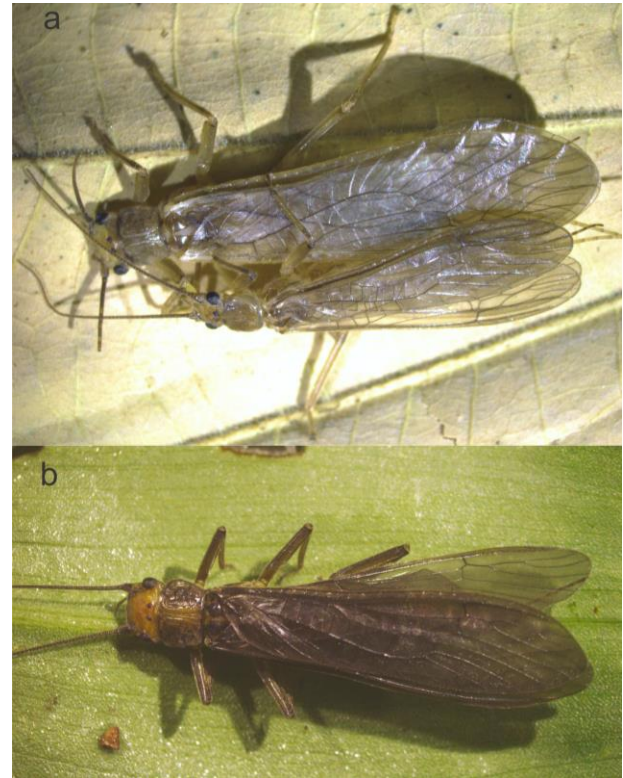


Fig 28. *Anacroneuria*, adults. a. *A. tucuman*, male and female (Horco Molle). b. *A. tafi* (Apeadero Muñoz).

specimens mentioned into two different lineages is not suitable for our data. For the same reason, we have not described the females AN28 and AN029 as conspecific with the male AN030 (*Anacroneuria lutea*).

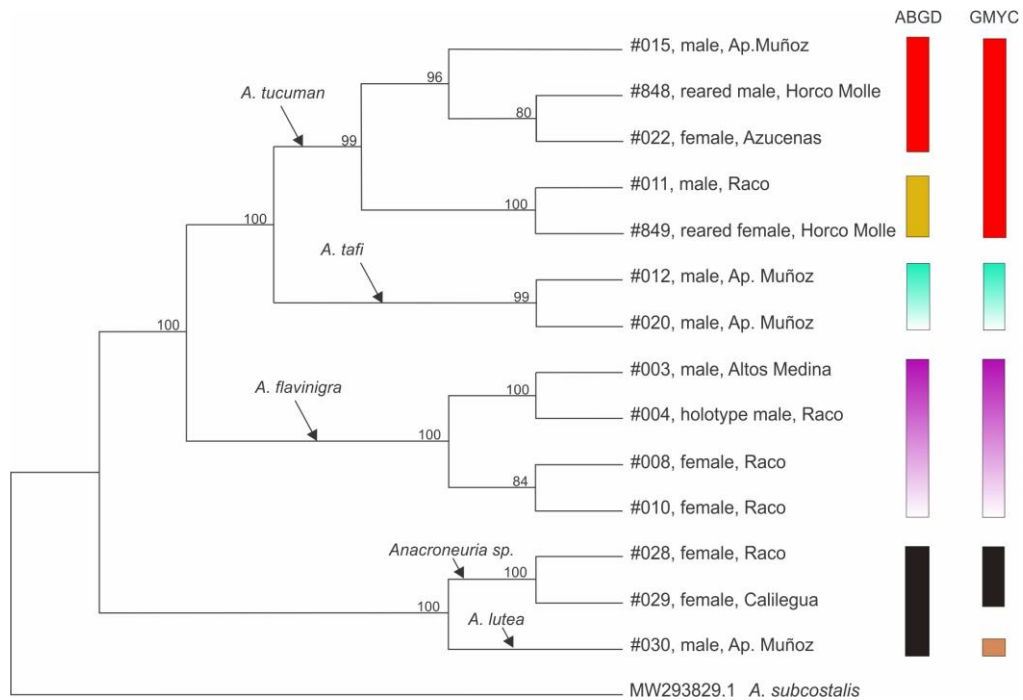


Fig. 29. Tree with the highest log likelihood. The percentage of trees in which the associated taxa clustered together is shown above the branches. Lineage delimitation by two different models can be seen at right (ABGD=Automatic Barcode Gap Discovery, and GMYC=Generalized Mixed Yule Coalescent).

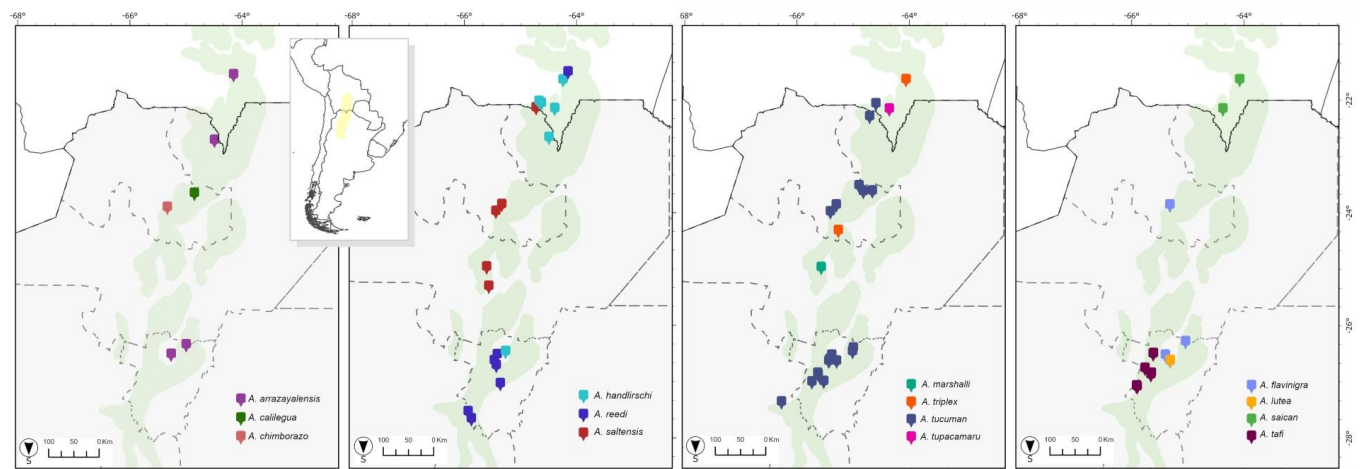


Fig. 30. Distribution maps of *Anacroneuria* species present in North Western Argentina and South Bolivia.

Key to male adults of *Anacroneuria* species present in North Western Argentina

- 1 - Wings membrane blackish translucent (Fig. 27a-b).....2
- 1' - Wings membrane variable but never blackish (Figs. 27c, 28a-b).....3
- 2(1) - Aedeagal apex rounded and broadly truncated (Fig. 5a, d, g); ventral membranous lobes triangular and relatively flat(Fig. 5b, e, h); aedeagal shoulders without microspines (Fig. 5a-h).....*A. flavinigra* sp. nov.
- 2' - Aedeagal apex elongated and subrectangular (Fig. 7a, e); ventral membranous lobes elongated, not flattened (Fig. 7b, d,f); aedeagal shoulders with microspines (Fig. 7h).....*A. lutea* sp. nov.
- 3(2') - Ventral membranous lobes projecting anteriorly (as in figure 4 in Stark, 2007).....*A. marshalli* Stark
- 3' - Ventral membranous lobes only ventrally projected (e.g., Figs. 1, 3, 4).....4
- 4(3') - Hammer poorly developed, bubble-like not prominent (Fig. 18g-h).....5
- 4' - Hammer well developed, cylindrical and prominent (Figs. 3d-f, 20e-h).....7
- 5(4) - Aedeagus with subapical lobes (Fig. 13a).....*A. saican* sp. nov.
- 5' - Aedeagus without subapical lobes (Fig. 18a, d, 22a, d, g).....6
- 6(5) - Dark coloration on thorax, wings and legs (Fig. 28b); aedeagus without shoulders (Fig. 18a-d).....*A. tafi* sp. nov.
- 6' - Paler coloration (Fig. 28a); aedeagus with slightly marked shoulders (Figs 22a, d, g).....*A. tucuman* Stark
- 7(4') - Hammer conical (Fig. 3d-f); dorsal keel at subapex of aedeagus double (Fig. 3c).....*A. calilegua* sp. nov.
- 7' - Hammer cylindrical (e.g. Fig. 20e-h); dorsal keel simple (e.g. Fig. 20a, c).....8
- 8(7') - Ventral pouches separated in the middle by an additional large median pouch (Fig. 20b).....*A. triplex* sp. nov.
- 8' - Additional median pouch absent (e.g., Figs. 6, 9).....9
- 9(8') - Aedeagus long and slender (Fig. 6a-h).....*A. handlirschi* Klapálek
- 9' - Aedeagus stouter (e.g., Figs. 1, 4).....10
- 10(9) - Aedeagus dorsally with shoulders and subapical lateral lobes (Fig. 1a, c, f).....*A. arrazayalensis* Orce
- 10' - Aedeagus may show marked shoulders but not subapical lateral lobes (e.g., Figs. 4, 9).....11
- 11(10') - Aedeagus short and wide, apex widely truncated (Fig. 4a-c).....*A. chimborazo* Stark
- 11' - Aedeagus slender, apex variable, rounded or narrow and truncated (Figs. 9, 15, 25).....12
- 12(11') - Aedeagus narrowing abruptly on apex, low smooth dorsal keel, and small ventral pouches (Fig. 9a-d).....*A. reedi* (Navás)
- 12' - Aedeagus not narrowing abruptly, dorsal keel high and serrated, and with large ventral pouches (Fig. 15a-d, 25a-c)....13
- 13(12') - In lateral view the distal superior margin of aedeagus is straight (Figs. 25e, b).....*A. tupacamaru* sp. nov.
- 13' - In lateral view the distal superior margin of aedeagus is concave (Fig. 15b).....*A. saltensis* Froehlich

ACKNOWLEDGEMENTS

We thank the following colleagues that helped us during these years: Carolina Nieto, Pablo Pessacq, Mariana Orce, José Giordano, José Rodríguez, Paola Rueda, Eduardo Domínguez, Veronica Manzo. We are grateful to Pablo Pessacq and Lucas Almeida for manuscript revisions. We also have received support from the following institutions: IBN, FML, CONICET (PIP845 to CM, PIP652 to C. Nieto), ANPCyT (PICT2021-058 to C. Nieto).

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