



BIOLOGICAL SCIENCES

Negragrion *sagma* gen.n. and sp.n. from South America with a morphological phylogeny of the New World Ischnurinae (Odonata: Zygoptera: Coenagrionidae)

JAVIER MUZÓN & FEDERICO LOZANO

Abstract: A new coenagrionid genus, *Negragrion*, is described for *N. sagma* sp.n. found in Argentina and Brazil (Holotype and allotype, pair in tandem. Argentina: Corrientes: Santo Tomé, arroyo Ita Cuá sobre RP 94, 28°26'48.30"S 56°00'33.11"W, 24.ii.2003, Muzón & Pessacq coll., MLP). The new genus is characterized by the shape of male cerci (decumbent from base; saddle-shaped; in lateral view with an acute apophysis directed dorsally located at 0.3 from base). The presence of a vulvar spine on S8 of females places this genus within Ischnurinae. A cladistics analysis using morphological data was carried out to determine its phylogenetic position. *Negragrion* gen.n. is recovered within the clade 4 as the sister group of the clade (*Acanthallagma* *Acanthagrion* (*Oxyagrion*, *Fluminagrion*)).

Key words: Argentina, Brazil, Ischnurinae, *Negragrion*, phylogeny.

INTRODUCTION

Coenagrionidae is the most speciose family worldwide within the suborder Zygoptera, encompassing almost 1,300 species. In the Neotropical region it is represented by more than 650 species within 70 genera of which 60 are endemic or mainly distributed within the neotropics (Garrison et al. 2010, Dijkstra et al. 2013). This number is continuously increasing; for example, in the last decade 198 new species have been described.

The taxonomy of this family was recently redefined (Dijkstra et al. 2013, 2014) including within Coenagrionidae the former Pseudostigmatidae and the neotropical members of Protoneuridae. The cosmopolitan Ischnurinae, the largest clade into the core Coenagrionidae, includes all the genera with a

vulvar spine on S8, comprising over half of the described species.

In this contribution, a new genus and species of Ischnurinae from specimens collected in Argentina and Brazil are described and diagnosed. A cladistic analysis including all the New World Ischnurinae has been conducted to hypothesize about the position of the genus herein described within Ischnurinae.

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MATERIALS AND METHODS

Specimens were studied with the aid of a Leica MS5 stereomicroscope in the Laboratorio de Biodiversidad y Genética Ambiental of the Universidad Nacional de Avellaneda (BioGeA - UNDAV). Illustrations were made with the aid of a

digital camera coupled to the stereomicroscope and an open-source design program (Inkscape version 0.91. at <www.inkscape.org>) and are not to scale. Maps were created electronically using QGIS version 2.16.3.

Measurements are given in mm. Wing venation follows Riek & Kukalová-Peck (1984), modified by Bechly (1996); genital ligula terminology is that of Kennedy (1916). Color pattern terminology of thorax follows Westfall & May (2006), and color pattern of head and abdomen follows Lozano (2013).

In order to establish the phylogenetic position of the new genus a data matrix was constructed using as terminal taxa all the New World genera of Ischnurinae as established by Dijkstra et al. (2014). The genus *Coenagrion* was used as outgroup since it is a basal Coenagrionidae in the analysis performed by Dijkstra et al. (2014). The resulting data matrix is shown in Table I. A list of 32 morphological characters derived from the morphology of adults (head, wing venation, legs, thorax, genital ligula, female terminalia, male cerci and male paraprocts) was elaborated. No larval characters were used since little is known at the generic level for many New World Odonata. The information to assign character states was obtained from literature and direct observation of specimens. The resulting matrix is provided in Table I.

The data matrix was analyzed in the program TNT version 1.5 (Goloboff & Catalano 2016) applying implied weights as optimality criteria. To calculate the appropriate value for constant k a TNT script (propk.run) written by Salvador Arias was used (Spinelli et al. 2018). After running the script, a value of k=6 was obtained for the data set and it was selected for data analysis. To obtain the most parsimonious trees heuristic searches using TBR (Tree Bisection Reconnection) were performed using a Wagner tree as the starting tree, 1000 random

addition sequences keeping up 100 trees per replication. Support of clades was evaluated with frequency difference (GC, for “group present/contradicted”) (Goloboff et al. 2003) calculated with 1000 replicates by symmetric resampling of the matrix (not distorted by weights/costs).

Abbreviations used throughout the text are as follow: FW – forewing; HW – hindwing; pt – pterostigma; Ax – antenodals; Px – postnodals; S1–10 – abdominal segments 1 to 10.

Depositories. MLP – Departamento Científico Entomología, Museo de La Plata, Buenos Aires, Argentina; MCNU – Invertebrate Collection of the Univates Natural History Museum, Ríio Grande do Sul, Brazil; FAAL – Frederico A. A. Lencioni private collection, Jacareí, São Paulo, Brazil.

RESULTS

List of characters

Head. 0 Postocular spots: (0) present; (1) absent; 1 Location of most posterior point of head: (0) at eyes; (1) at postocular lobes.

Wings. 2 Wings: (0) hyaline; (1) colored; 3 Male HW: (0) without dense and dark venation; (1) with dense and dark venation; 4 CuA: (0) extending more than 6 cells; (1) extending less than 6 cells; 5 CuP: (0) reaching hind margin of wing; (1) reaching CuP&AA; 6 Vein descending from quadrangle: (0) forming an unbroken line to wing margin; (1) not forming an unbroken line to wing margin; 7 Petiolation: (0) short (proximal to CuP, by more than twice the length of CuP); (1) long (at CuP or proximal by distinctly less than twice the length of CuP).

Legs. 8 Metatibial spurs: (0) shorter than twice intervening spaces; (1) as long as, or longer than twice intervening spaces; 9 Pretarsal claw: (0) well developed; (1) small.

Thorax. 10 Posterior lobe of prothorax in males: (0) not projected medially in a bifurcated process; (1) projected medially in a bifurcated

Table I. Morphological data matrix used in the phylogenetic analysis of the New World Ischnurinae.

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
<i>Coenagrion</i>	0	1	0	0	0	1	1	0	0	0	0	[01]	0	1	1	[01]	0	1	1	1	0	0	0	0	1	1	0	[01]	0	1	[01]		
NEGRAGRION	0	0	0	0	0	[01]	1	0	0	0	0	0	0	1	1	1	0	1	1	0	0	1	0	0	0	1	1	0	1	0	0		
<i>Acanthagrion</i>	0	[01]	0	0	0	[01]	1	0	[01]	0	0	[01]	0	1	[01]	[01]	0	1	1	0	0	[01]	[01]	0	0	0	0	0	[01]	0	0		
<i>Acanthallagma</i>	0	1	1	0	0	1	1	1	0	0	0	[01]	0	1	1	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0		
<i>Andinagrion</i>	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	[01]	1	1	1	0	0	1	0	1	0	
<i>Anisagrion</i>	0	1	0	1	0	1	1	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	1	1	1	0	1	0	[01]	0	1	
<i>Apanisagrion</i>	[01]	1	0	1	0	1	1	0	0	0	0	0	0	1	0	[01]	0	1	0	0	0	0	0	0	1	1	1	1	0	0	1	0	
<i>Argentagrion</i>	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	1	1	0	0	1	0	1	1	1	0	0	1	[01]	1	0	
<i>Calvertagrion</i>	0	0	0	0	1	1	0	0	0	0	1	0	0	1	1	0	0	0	1	1	0	0	1	0	1	1	0	0	0	0	0	0	
<i>Cyanallagma</i>	0	1	0	0	0	1	1	0	0	0	0	0	0	1	1	[01]	1	1	1	0	[01]	1	[01]	1	[01]	1	0	1	[01]	0	1	0	
<i>Denticulobasis</i>	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	1	1	0	1	1	0	1	1	0	[01]	1	0	1	0	
<i>Dolonagrion</i>	0	1	0	0	[01]	1	1	0	0	1	1	0	0	1	1	1	0	1	1	1	0	0	2	0	0	1	0	0	0	0	0	0	0
<i>Enacantha</i>	0	0	0	0	0	1	1	0	0	0	0	0	0	1	1	0	0	1	1	0	0	1	1	1	1	1	0	1	0	0	0	0	0
<i>Enallagma</i>	[01]	[01]	0	0	0	[01]	1	0	0	0	0	[01]	0	1	[01]	[01]	0	1	1	1	0	1	0	1	1	1	0	[01]	0	[01]	0	[01]	
<i>Fluminagrion</i>	0	1	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	1	1	0	1	1	0	0	0	0	0	0	0	0	0	1	0
<i>Hesperagrion</i>	[01]	[01]	0	0	0	1	1	0	0	0	0	0	0	1	1	0	0	0	0	1	0	1	0	0	1	1	0	0	1	0	0	[01]	0
<i>Homeoura</i>	0	0	0	0	0	1	1	0	0	0	0	0	0	1	1	[01]	0	1	1	0	0	1	[01]	1	1	1	0	1	0	1	1	0	0
<i>Ischnura</i>	0	[01]	0	0	0	1	1	[01]	0	[01]	0	0	0	1	[01]	[01]	0	1	1	1	0	[01]	0	[01]	1	1	0	0	[01]	0	[01]	[01]	[01]
<i>Leptobasis</i>	[01]	0	0	0	1	0	1	0	0	1	0	0	0	[01]	[01]	0	0	0	1	1	0	[01]	2	[01]	1	1	0	1	0	0	0	[01]	0
<i>Leucobasis</i>	0	0	0	0	0	1	1	0	0	1	0	0	0	1	1	0	0	1	1	0	0	0	1	0	1	1	0	0	1	0	0	0	0
<i>Mesamphiagrion</i>	0	1	0	0	0	1	1	0	0	0	0	0	0	1	1	[01]	1	1	1	0	1	[01]	1	1	1	1	1	1	1	0	1	0	0
<i>Mesoleptobasis</i>	[01]	0	0	0	1	0	0	0	0	1	1	0	0	0	[01]	0	0	0	1	1	0	[01]	[12]	[01]	1	1	0	0	0	0	1	1	0
<i>Oreialtagma</i>	0	0	0	0	0	1	1	0	0	0	0	0	0	1	1	[01]	0	1	1	0	1	1	1	0	1	1	1	0	[01]	1	0	0	
<i>Oxyagrion</i>	[01]	0	0	0	[01]	0	1	0	0	0	0	0	0	[01]	[01]	0	[01]	1	1	0	1	[01]	[01]	0	0	0	0	0	0	1	0	0	
<i>Oxyallagma</i>	1	1	0	0	0	[01]	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	1	1	1	0	1	1	0	1	0	
<i>Protallagma</i>	0	1	0	0	1	1	1	0	0	0	0	0	0	[01]	0	0	0	0	1	1	1	1	0	0	1	1	0	1	0	0	0	0	
<i>Tigrigrion</i>	1	1	0	0	1	1	1	0	0	0	0	0	0	1	1	0	0	0	1	1	0	1	1	0	1	1	0	0	0	0	0	0	
<i>Tuberculobasis</i>	[01]	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	1	0	[01]	1	0	1	1	0	0	1	0	0	[01]	

process; 11 Mesepisternal fossae in females: (0) absent; (1) present; 12 Male mesanepisternum: (0) without horns or tubercles; (1) with horns or tubercles; 13 Middorsal dark stripe: (0) absent; (1) present; 14 Humeral dark stripe: (0) absent; (1) present; 15 Metapleural dark stripe: (0) absent; (1) present; 16 Mesanepisternal carina of females: (0) not defined; (1) well defined.

Genital ligula. 17 Lateral sclerotized flap-like projections distal to flexure directed anteriorly: (0) present; (1) absent; 18 Paired areas of sclerotized spinules at flexure: (0) present; (1) absent; 19 Inner fold proximal to flexure: (0) absent; (1) present; 20 Flexure of genital ligula: (0) short (distal segment 3 longer than flexure height); (1) C-shaped (distal segment as long as flexure height).

Female terminalia. 21 Female S8 (0) without modifications; (1) modified (either with vulvar spine or denticulate plate); 22 Ovipositor: (0) not surpassing S10; (1) ending between S10 and tip of cerci; (2) surpassing tip of cerci.

Male S10. 23 Postero-dorsal margin of S10: (0) without processes; (1) with processes.

Cerci. 24 Cerci: (0) decumbent from base; (1) horizontal; 25 Dorso-basal tubercle: (0) present; (1) absent; 26 Dorsal process: (0) absent; (1) present; 27 Ventro-basal process: (0) absent; (1) present; 28 Ventro-apical process: (0) absent; (1) present; 29 Length of cerci: (0) shorter than or equal to S10; (1) longer than S10.

Paraproct. 30 Paraproct: (0) entire; (1) not entire; 31 Length of paraproct: (0) shorter than or equal to cercus; (1) longer than cercus.

***Negragrion* gen. n.**

ZooBank Life Science Identifier (LSID) - urn:lsid:zoobank.org:act:951D469F-CBCA-4C8E-A8C8-58B99FDA2E96

Type species. *Negragrion sagma* sp. n. by monotypy.

ZooBank Life Science Identifier (LSID) - urn:lsid:zoobank.org:act:449B0373-E98E-412D-95FF-41F89D32D8C6

Etymology. From the Latin "*nigrum*" in honour of the first author's wife, Susana Claudia Diaz, which nickname is "*negra*", and "*agrion*" a neuter noun transliterated from the Greek "*agrios*" meaning wild, a noun used for many damselfly names.

Diagnosis. Head with frons rounded and postocular spots present. Synthorax light blue with well-defined black stripes. Flexure of genital ligula short (segment 3 longer than flexure height). Segment 3 of genital ligula slender, without lateral lobes; ental surface with a middle subrectangular lobe that reaches segment 2; distal margin with indentation; disto-lateral projections acutely pointed, not surpassing segment 2. Cerci decumbent from base, saddle-shaped; in lateral view with an acute apophysis directed dorsally located at 0.3 from base. Females without mesepisternal fossae, mesostigmal plates wide, with medial margin approximately equal to anterior margin; with a semicircular ridge on posterior margin; with vulvar spine.

***Negragrion sagma* sp. n.**

Etymology. From *sagma* (Greek) meaning saddle. The name refers to the male cercus shape.

Description of male holotype.

Head: Labrum pale brown with posteromedian spot and posterolateral margins black. Anteclypeus greenish light blue; postclypeus black. Antefrons light greenish blue with black T-shaped spot. Dorsum of head mostly light blue, spots as in Fig. 1a. Occipital bar brown. Antennifers anteriorly greenish light blue and posteriorly black. Antennae broken. Postocular spots light blue, slightly larger than ocellar triangle. Most posterior point of head located at compound eyes. Occipital area black.

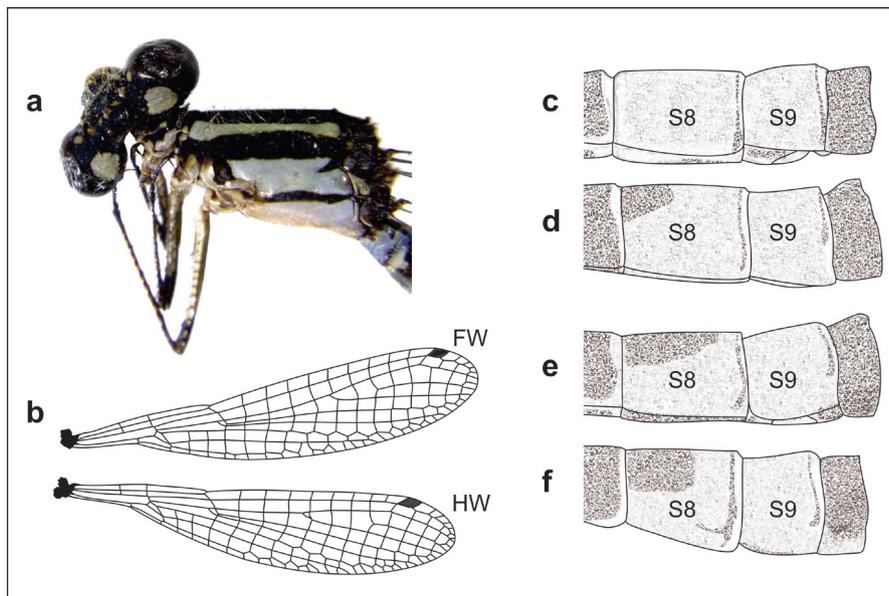


Figure 1. *Negragrion sagma* sp. n. a: male holotype head and thorax; b: male holotype wings; c: male holotype S8–10; d: male paratype from Querência, Córrego Sucuri; e: male paratype from Nova Xavantina; f: male paratype from Querência, Igarapé Neuri Mata.

Prothorax: Anterior lobe black with a large central light blue spot occupying almost all of dorsum. Middle lobe with trapezoidal geminate median light blue spots, and dorsolateral subrectangular light blue spots; propleuron light blue. Posterior lobe black with lateral streaks light blue and lateral margins rounded, without median projection.

Pterothorax (Fig. 1a): Predominant color light blue. Middorsal black stripe interrupted at middorsal carina by a dark brown line. Antealar sinus mostly black with lateral margins light blue. Antehumeral stripe light blue and entire, not reaching antealar crest. Humeral stripe black. Interpleural suture black. Stripe on metapleural suture complete. Mesinfraepisternum black, posteroventral angle light blue. Metinfraepisternum pale brown. Pectus pale brown with two black spots. Mesostigmal plates with inner half black and outer half light blue, without carinas. Interlaminar sinus triangular mostly black with center light blue; anterior margin of sinus slightly convex; lateral tips acute and projected anteriorly. Legs: Coxae and trochanters light blue. Femora extensor margin black; flexor margin light blue. Tibiae extensor

margin brown (paler towards tibia 3); flexor margin black. Tarsi dark brown. Leg spurs shorter than intervening spaces.

Wings (Fig. 1b): Hyaline, pt reddish brown; CuP reaching posterior margin of wing or CuP&AA'; arcus opposite Ax2. FW: Px 9; RP2 beginning between Px4 and Px5; IR1 beginning at Px7; 3–4 cells posterior to pt. HW: Px 8; RP2 beginning between Px3 and Px4; IR1 beginning at Px7; 4–5 cells posterior to pt.

Abdomen: S1: tergum light blue with anterior spot black, subrectangular, posterior margin separated from posterior margin; posterior stripe represented by a lateral J-shaped spot visible in lateral view; sternum pale light blue, with a diffuse dark spot on anterior margin. S2: tergum light blue; dorsal spot subrectangular, anterior margin reaching anterior margin of tergum; posterior margin separated from posterior stripe of S2; posterior stripe in contact with posterior margin of tergum; lateral margins in contact with ventrolateral margins of tergum in lateral view; laterals of tergum with elliptical pale brown spots; anterior lamina pale brown with external margins black; posterior hamulis pale brown. S3 to S7: terga with dorsal spots

subrectangular occupying most of the tergum; anterior margin of spots convex, separated from anterior margin of segments on S3 to S5; laterally reaching half the length of the terga, posterior fifth widened reaching ventral margin of terga; posterior stripes not visible; sterna pale brown with black midventral line, widening towards S7. S8 and S9 (Fig. 1c): terga light blue; posterior bands black, with a row of small spines posteriorly; sterna pale with a mid-ventral black stripe in S8, pale brown with genital valves paler in S9. S10: tergum subquadrangular; black with a small light blue spot visible on lateral view; sternum pale.

Genital ligula (Fig. 2a-b): Segment 1 with distal bristles. Segment 2 with basal bristles. Flexure short (segment 3 longer than flexure height). Segment 3: slender, without lateral lobes; ental surface with a middle subrectangular lobe that reaches segment 2; distal margin with indentation; disto-lateral projections acutely pointed, not surpassing segment 2.

Cerci (Fig. 2c-d): Black, approximately as long as S10; saddle-shaped. In lateral view decumbent from base, with acute apophysis directed dorsally located at 0.3 from base, tip of cerci rounded and pointing dorsally, therefore distal half forming a U-shaped concavity. Distal margin of cerci wide (visible in latero-dorsal

view), outer angle rounded, inner angle pointed and directed ventrally. Paraprocts (Fig. 2c): Black. Apophysis short (not reaching tip of cerci), tips acute pointing medially.

Measurements: Head: max. length 1.0; width between compound eyes along anterior margin 1.7. Legs: femur 1 length 1.5; femur 2 length 2.0; femur 3 length 2.7. Pterothorax: max. length along middorsal carina 2.7. Wings: FW length left 16.5, right 16.3; HW length left 15.2, right 15.1. Abdomen: max. length 23.4; S1 max. length 0.7; S2 max. length 1.9; S3 max. length 3.9; S4 max. length 4.1; S5 max. length 4.0; S6 max. length 3.7; S7 max. length 3.9; S8 max. length 1.3; S9 max. length 0.9; S10 max. length 0.5; S9 height 0.9; S10 height 0.9. Cerci: distance surpassing posterior margin of S10 in lateral view 0.4. Paraprocts: length in lateral view 0.2. Total length 29.5.

Description of female allotype.

Colour pattern: similar to that of male holotype. Thorax (Fig. 3a): Posterior lobe of prothorax not projected; mesostigmal plates wide (with medial margin approximately equal to anterior margin), posterior margin with a semicircular ridge; interlaminar sinus subrectangular, projected anteriorly; without mesepisternal fossae. Wings: CuP reaching posterior margin of wing; arculus opposite Ax2. FW: Px10; RP2 beginning at Px5; IR1 beginning

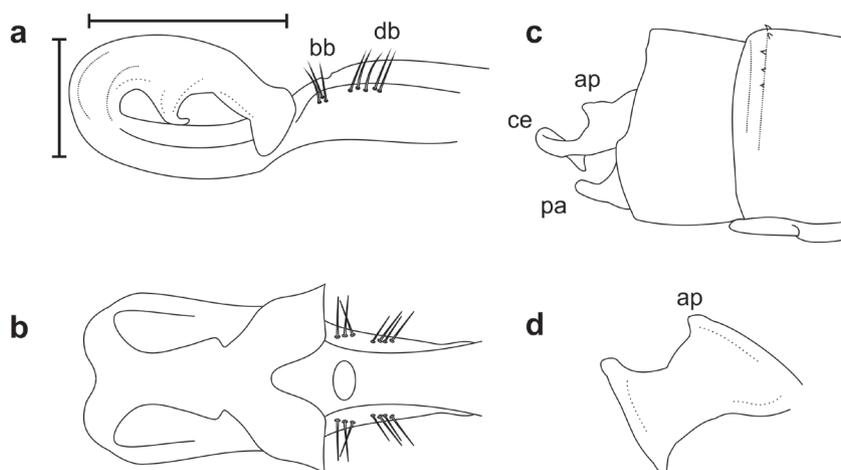


Figure 2. *Negragrion sagma* sp.n. a: holotype, genital ligula, lateral view; b: paratype from Rio Grande do Sul, genital ligula, ventral view; c: holotype, cerci, lateral view; d: holotype, cercus, inner view. — Abbreviations: ap – apophysis; bb – basal bristles of segment 2; ce – cercus; db – distal bristles of segment 1; pa – paraproct. — Symbols: Bars indicate relative length of flexure and segment 3.

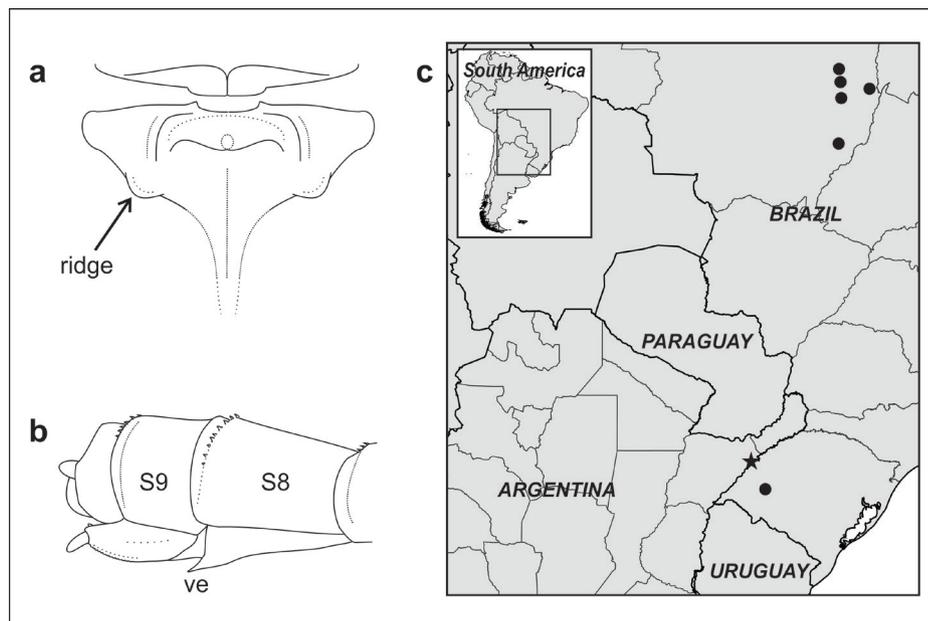


Figure 3. *Negragrion sagma* sp. n.: a: allotype, modifications of thorax, dorsal view; b: allotype, terminalia, lateral view; c: distribution map. — Abbreviations: ve – vulvar spine. — Symbols: star indicates type locality.

at Px8; 4–5 cells posterior to pt. HW: Px 8; RP2 beginning at Px4; IR1 beginning at Px6; 5–6 cells posterior to pt. pt pale brown. Terminalia (Fig. 3b): S8 tergum with dorsal black spot that extends posteriorly up to distal third and lateroventrally up to ventral third of tergum in lateral view; the rest is light blue; sternum pale brown with midventral line black; with well-developed vulvar spine. S9 tergum with a pair of L-shaped black spots that in lateral view occupy almost all of tergum; rest of tergum light blue. Anterior gonapophyses pale brown; posterior gonapophyses brown; valves surpassing S10 and extending to the tip of cerci; ventral margin slightly concave and serrated. S10 light blue. Cerci slightly shorter than length of S10; in lateral view subtriangular. Paraprocts short, subtriangular in lateral view.

Measurements: Head: max. length 1.2; width between compound eyes along anterior margin 1.6. Legs: femur 1 length 1.7; femur 2 length 2.2; femur 3 length 2.9. Pterothorax: max. length along middorsal carina 2.7; interlaminar sinus max. length 1.2; interlaminar sinus width between anterior angle of mesostigmal plates 0.5. Wings:

FW length left 17.1, right 17.2; HW length left 15.9, right 16.1. Abdomen: max. length 22.2; S1 max. length 0.6; S2 max. length 1.4; S3 max. length 3.7; S4 max. length 4.0; S5 max. length 3.9; S6 max. length 3.7; S7 max. length 2.8; S8 max. length 1.3; S9 max. length 0.8; S10 max. length 0.4. Cerci: distance surpassing posterior margin of S10 in lateral view 0.2. Paraprocts: length in lateral view 0.2. Total length 28.4.

Variation in paratypes.

Colour pattern. Aside from small differences in black markings of head and thorax, there is considerable variation in the black markings of S8. Some of the specimens do not have a dorsal black spot (as in the holotype) while in others there is a subrectangular black spot which can occupy from one 0.3 to 0.7 the length of the segment (Fig. 1d-f). Wings. FW: Px 8–10; RP2 beginning between Px4 and Px5 or at Px5; IR1 beginning at Px7 or at Px8; 3–4 cells posterior to pt. HW: Px 7–8; RP2 beginning between Px3 and Px4 or at Px4; IR1 beginning between Px6 and Px7 or at Px7; 4–5 cells posterior to pt.

Measurements. Wings: FW length 14.8–15.1; HW length 13.3–14.6. Abdomen: max. length 20.0–22.5.

Distribution and habitat notes. *Negragrion sagma* has been reported for Argentina (Corrientes) and Brazil (Mato Grosso and Rio Grande do Sul) (Fig. 3c). Specimens from Argentina were collected in a marshy area around a pine tree plantation which was crossed by a small creek (Muzón et al. 2008). Specimens from Rio Grande do Sul were obtained from the banks of a flooded area of a rice plantation. There is no information on the habitat of specimens collected in Mato Grosso.

Material examined. Type material: Holotype ♂ and allotype ♀ (pair in tandem). ARGENTINA: Corrientes: Santo Tomé, arroyo Ita Cuá sobre RP 94, 28°26'48.30"S 56°00'33.11"W, 24.ii.2003, Muzón & Pessacq (MLP). Paratypes (7 ♂♂ and 1 ♀). BRAZIL: 1 ♂ Rio Grande do Sul: Alegrete, Cerro do Tigre, 29°39'05.10"S 55°24'09.30"W, 02.iv.2015, Renner (MCNU). Mato Grosso: 1 ♂ Fazenda Manoel Taurino, 12°40'16.00"S 50°59'25.00"W, 25.v.2004, Batista (FAAL); 1 ♂ Nova Xavantina, 14°59'53.00"S 52°18'17.00"W, 06.v.2008, Batista (FAAL); 1 ♂ Querência, Córrego Sucuri, 11°49'50.00"S 52°17'02.00"W, 25.ix.2007, Juen & Cabette (FAAL); 2 ♂ 1 ♀ Querência, Córrego Transição, 13°03'35.00"S 52°12'03.00"W, 19.xii.2007, Juen & Cabette (FAAL); 1 ♂ Querência, Igarapé Neuri Mata, 12°22'28.00"S 52°13'23.00"W, 26.ix.2007, Juen & Cabette (FAAL).

Cladistic analysis

The cladistic analysis recovers one tree (Fit = 5.64; CI = 0.383; RI = 0.583) weakly supported (Fig. 4). The CI value is quite low indicating that there is a lot of homoplasy. A total of six synapomorphic characters were obtained: two derived from wing morphology: (character 3 and 6), two from the thorax (character 10 and 12), one from genital ligula (character 18), and one

from male cerci (character 25). The cladogram shows five distinct clades.

Clade 1 includes (*Leucobasis* (*Leptobasis* (*Dolonagrion* (*Mesoleptobasis*, *Calvertagrion*)))) it is supported by one homoplasious character, pretarsal claw small (character 9: 1). The clade (*Dolonagrion* (*Mesoleptobasis*, *Calvertagrion*)) is supported by the synapomorphy posterior lobe of prothorax projected medially in a bifurcated process (character 10: 1); and the clade (*Mesoleptobasis*, *Calvertagrion*) is supported by the vein descending from quadrangle forming an unbroken line to wing margin (character 6: 0).

Clade 2 (*Tuberculobasis*, *Denticulobasis*) is well supported (GC= 71) by one synapomorphy, male mesanepisternum with horns or tubercles (character 12: 1), and three homoplasious characters, (character 5: 0; character 13: 0, character 14: 0).

Clade 3 (*Tigriagrion*, *Oreiallagma*) is defined by two homoplasious characters (character 20: 1; character 28: 0).

Negragrion gen. nov. is recovered within the clade 4 as the sister group of the clade (*Acanthallagma* *Acanthagrion* (*Oxyagrion*, *Fluminagrion*)); there is no synapomorphy defining this clade since the male cerci decumbent from base [24 (0)] is shared with *Dolonagrion* and some species of *Cyanallagma*. The clade (*Acanthallagma* *Acanthagrion* (*Oxyagrion*, *Fluminagrion*)) is supported by one synapomorphy, dorsobasal tubercle present (character 25: 1), and one homoplasious character, ventro-apical process absent (character 28: 0).

Clade 5 is the largest; it is supported by two homoplasious characters postero-dorsal margin of S10 with processes (character 23: 1) and paraproct: not entire (character 30: 1). Within this two groups are recognized: ((*Andinagrion*, *Oxyallagma*) (*Mesamphiagrion*, *Cyanallagma*)) and (*Homeoura* (*Enacantha* ((*Protallagma*,

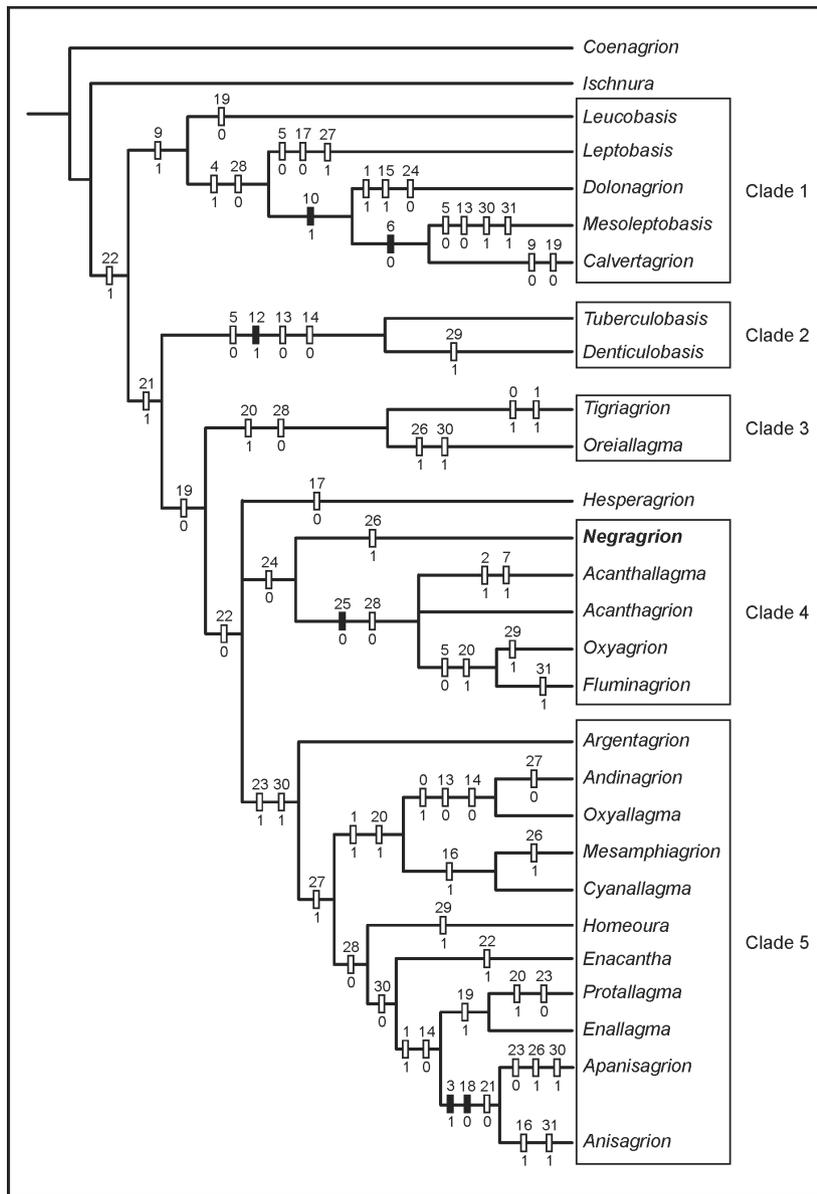


Figure 4.
Tree topology
obtained from
the phylogenetic
analysis.

Enallagma) (*Apanisagrion*, *Anisagrion*))). The clade (*Apanisagrion*, *Anisagrion*) is well supported (GC= 71) by two synapomorphies, male HW with dense and dark venation (character 3: 1), and presence of paired areas of sclerotized spinules at flexure (character 18: 0).

DISCUSSION

General morphology places *Negragrion* within Ischnurinae. Among the South American

Ischnurinae the combination of a rounded frons, presence of pale postocular spots, a trilobate prothoracic posterior lobe, striped pterothorax, and male cerci decumbent from base is common to *Acanthagrion* Selys, *Acanthallagma* Williamson & Williamson, some *Cyanallagma* Kennedy, *Dolonagrion* Garrison & von Ellenrieder, *Fluminagrion* Anjos Santos, Lozano & Costa, and some *Oxyagrion* Selys.

Males of *N. sagma* are readily recognized by the morphology of the male cerci: in lateral

view with an acute apophysis directed dorsally located at 0.3 from base (Fig. 2c), tip rounded and pointing dorsally; in dorso-medial view widened distally, outer angle rounded, inner angle pointed and directed ventrally. Genital ligula with flexure short (distal segment longer than flexure); segment 3 slender, without lateral lobes; ental surface with a middle sub-rectangular lobe that reaches segment 2; distal margin with indentation; disto-lateral projections acutely pointed not surpassing segment 2. Female mesostigmal plates wide (with medial margin approximately equal to anterior margin); with a semicircular ridge on posterior margin; without mesepisternal fossae; vulvar spine well developed.

Phylogenetic relations within Ischnurinae are still poorly supported due to lack of adequate generic characterization and highly homoplasious characters. A new extensive analysis, including molecular support is needed to firmly resolve the phylogenetic relationships of this subfamily.

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JAVIER MUZÓN

<https://orcid.org/0000-0002-3956-1986>

FEDERICO LOZANO

<https://orcid.org/0000-0002-8071-7567>

Universidad Nacional de Avellaneda, Laboratorio de Biodiversidad y Genética Ambiental/BioGeA, Mario Bravo 1460 esq. Isleta, 1870 Piñeyro, Buenos Aires, Argentina

Correspondence to: **Federico Lozano**

E-mail: flozano@undav.edu.ar

Author contributions

The author Javier Muzón is responsible for the collection of the holotype and allotype, the diagnosis of the new species, and the critical revision of the manuscript; the author Federico Lozano is responsible for the writing of the article, the cladistic analysis, and the making of figures. Both author contributed equally to the cladistics analysis provided herein.

