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ORIGINAL PAPER

A new species of *Stilestrongylus* (Nematoda, Heligmonellidae) from the Atlantic Forest of Misiones, Argentina, parasitic in *Euryoryzomys russatus* (Cricetidae, Sigmodontinae)

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Abstract
A new species of Heligmonellidae (Trichostrongylina, Heligmosomoidea), *Stilestrongylus kaaguyporai* n. sp. is described from the small intestine of *Euryoryzomys russatus* (Rodentia, Cricetidae, Sigmodontinae) from the Argentine Atlantic Forest, in the Misiones province. The new species was found at Campo Anexo Manuel Belgrano, Reserva de Vida Silvestre Urugua-í and Parque Provincial Urugua-í, with a prevalence of 73% in 15 hosts examined. *Stilestrongylus* includes 24 Neotropical species, all parasitic in rodents, mostly Sigmodontinae. *Stilestrongylus kaaguyporai* n. sp. can be differentiated from its congeners by the following characters: caudal bursa dissymmetrical with right lobe larger and pattern of type1–4 in both lobes, rays 6 not forming a lateral trident with rays 4 and 5, rays 8 with dissymmetrical pathway, genital cone hypertrophied with a conspicuous hood-like projection and females with a marked dorso-ventral torsion of the posterior end. This report is the second record of a *Stilestrongylus* species in *E. russatus,* increasing to nine the number of parasitic species known from this host.

Keywords Helminths · *Stilestrongylus kaaguyporai* n. sp. · Nippostrongylinae · *Euryoryzomys russatus* · Sigmodontinae · Atlantic forest

Introduction

Recently, Panisse et al. (2017) described a parasitic assemblage of seven helminth species in *Euryoryzomys russatus* (Wagner, 1848) (Cricetidae, Sigmodontinae) in the Atlantic Forest of Misiones, Argentina. Those records increased to nine the number of species known for this host, for which only two species of helminths from Brazil were known prior to that study (Pinto et al. 1982; Gomes et al. 2003; Costa et al. 2014). *Euryoryzomys russatus* is distributed along the eastern Brazilian coastal region from Bahia to Rio Grande do Sul and from there to the western highlands of São Paulo state, reaching Paraguay and Argentina (Misiones) (Percequillo 2015). From Brazilian hosts, the species *Guerrerostrongylus zetta* (Travassos, 1937) and *Hassalstrongylus luquei* Costa, Maldonado, Bóia, Lucio, and Simões, 2014 (Trichostrongylina, Nippostrongylinae) were recorded. Whereas in the Misiones assemblage, the following species were recorded: *Trichuris* sp. (Enoplida, Trichuridae); *Syphacia evaginata* Hugot and Quentin, 1985 (Oxyurida, Syphaciinae); *Tapironema coronatum* Durette-Desset, Chabaud and Sutton, 1997 (Trichostrongylina, Obeliscoidinae); *Guerrerostrongylus ulysi* Digiani, Notarnicola and Navone, 2012; *Stilestrongylus lanfrediae* Souza, Digiani, Simões, Luque, Rodrigues-Silva and Maldonado, 2009 (Trichostrongylina, Nippostrongylinae); *Stilestrongylus* n. sp. (2) and a Nippostrongylinae n. sp. Of these, the species identified as *Stilestrongylus* n. sp. (2) is described herein.

Materials and methods

Rodents ($n = 15$) were collected between September 2011 and August 2013 in the localities of Campo Anexo

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Manuel Belgrano of the Instituto Nacional de Tecnología Agropecuaria- INTA San Antonio (CAMB) (26° 03′ S, 53° 46′ W), Reserva de Vida Silvestre Urugua-í (RVSU) (25° 58′ S, 54° 07′ W) and Parque Provincial Urugua-í (PPU) (25° 58′ S, 54° 06′ W), Misiones, Argentina. The trapping, using traps of live capture was carried out over a period of 23 days during the fall and winter with a sampling effort of 4086 trap nights (Jones et al. 1996).

Parasites were fixed in 10% formaldehyde and preserved in 70% alcohol. The synlophe was studied following Durette-Desset (1985), and the nomenclature referring to the axis of orientation and other characters of the synlophe follows Durette-Desset and Digiani (2005) and Durette-Desset et al. (2017). The use of terms for the caudal bursa follows Durette-Desset and Digiani (2012). The nomenclature above the family group for the Strongylida (Nematoda) follows Durette-Desset and Chabaud (1993). Ridges are considered as dorsal or ventral with respect to the axis of orientation and not to the lateral hypodermal cords.

Measurements are given in micrometres, except where stated otherwise. Type specimens of parasites are deposited in the Helminthological Collection of the Museo de La Plata, La Plata, Argentina (MLP-He). The type host is deposited in the Mammal Collection of the Centro Nacional Patagónico (CNP), Puerto Madryn, Chubut and is mentioned in this manuscript under the field number (CG 553).

The research was conducted in compliance with Argentine laws (see Compliance with ethical standards).

Results

Parasitized rodents were found in all three localities sampled (CAMB, RVSU and PPU). The specimens studied were found in the small intestine of 11 of 15 ($P = 73\%$) specimens of *E. russatus* harbouring 3–215 (mean = 67.3) worms.

***Stilestrongylus kaaguyporai* n. sp.**

Figures 1–15

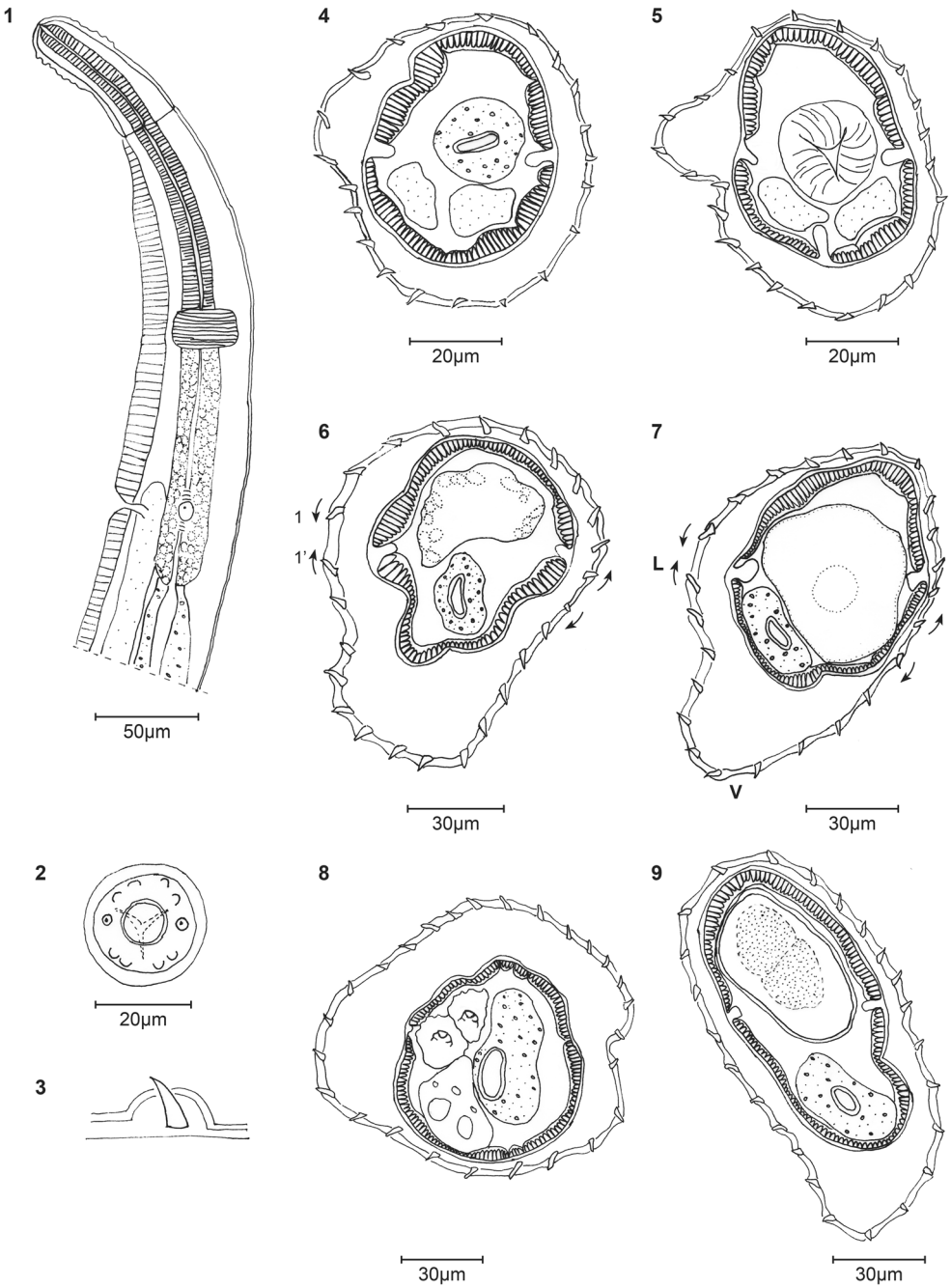
Description

General: Small nematodes, strongly coiled sinistrally along ventral side in 3–5 spirals in males, more loosely and irregularly coiled in females. Excretory pore 68–90% of oesophageal length in males, 63–90% in females. Deirids are situated at level of excretory pore (Fig. 1).

Head: Cephalic vesicle elongate. In apical view, rounded buccal opening surrounded by very thin ring. Two amphids, four externo-labial papillae and four submedian cephalic papillae observed (Fig. 2). Synlophe: (studied in one male and one female paratypes). In both sexes, cuticle bearing longitudinal, uninterrupted ridges appearing mainly on left side posterior to cephalic vesicle, up to oesophago-intestinal junction, disappearing just anterior to caudal bursa in male and anterior to vulva in female. Ridges with cuticular struts. Struts usually flanked by cuticular folds (Fig. 3). Number of ridges: at level of oesophago-intestinal junction, 21 in male and 24 in female (Figs. 4 and 5), at mid-body, 25 in male and 24 in female (Figs. 6 and 7). At about 530 anterior to bursa, 26 in male, and in female, at mid-uterus level, 24 ridges (Figs. 8 and 9). At oesophago-intestinal junction and at mid-body, ridges slightly unequal in size, with right-ventral ridges smaller. Within distal third of body, ridges subequal in both sexes. Most ridges with marked orientation. In male, at oesophago-intestinal junction, and in female at level of uterus, right-ventral ridges oriented more perpendicularly to body surface. Double axis of orientation of ridges; right axis inclined at about 65° and left axis at 83° to sagittal axis in both sexes.

Holotype male: 3.72 mm long, 104 wide at mid-body, cephalic vesicle 70 long and 28 wide, nerve ring, excretory pore and deirids situated 200, 270 and 255 from apex, respectively. Oesophagus 335 long (Fig. 1). Caudal bursa dissymmetrical though not markedly, with right lobe larger and pattern of type 1–4 in both lobes (Fig. 10a). Prebursal papillae not observed. Rays on both lobes with similar development. Rays 3 straight, similar in length to rays 2, rays 4 and 5 divergent at extremities. In right lobe, ray 3 arising more distally than ray 6 from common trunk of rays 3 to 6, ray 6 similar in length to ray 3; in left lobe, rays 3 and 6 arising at about same level, ray 6 markedly shorter (Fig. 10a). Dorsal lobe medium sized. Rays 8 with different pathways, both arising from proximal third of dorsal ray: right ray 8 arising slightly proximally, close to lateral trunk of rays 2–6 and parallel to it; left ray 8 arising somewhat distally and separated at its origin from lateral trunk of rays 2–6 (Fig. 10b). Both rays 8 contact ray 6 at the origin of the latter then directed posteriorly (Fig. 10a). Dorsal ray divided at distal third into two branches, these latter dividing apically into rays 9 (external) and 10 (internal). Genital cone 46 long by 30 wide at base, hypertrophied, with hood-like projection on dorsal lip, bearing sessile papillae 7 laterally (Fig. 11). Papilla 0 not observed. Gubernaculum 49 long and 26 wide at base in ventral view (Fig. 11). Spicules subequal, alate, 860 long, representing 23.4% of body length. Tips of spicules with marked distal bend and joined but not fused. Separated tips reveal left spicule fitting into right one (Fig. 12).

Measurements (range and average) of 11 male paratypes: 3.58–3.97 (3.81) mm long, 99–127 (114) wide at mid-body. Cephalic vesicle 60–78 (69) long, 25–30 (28) wide. Nerve ring, excretory pore and deirids situated 177–205 (190),

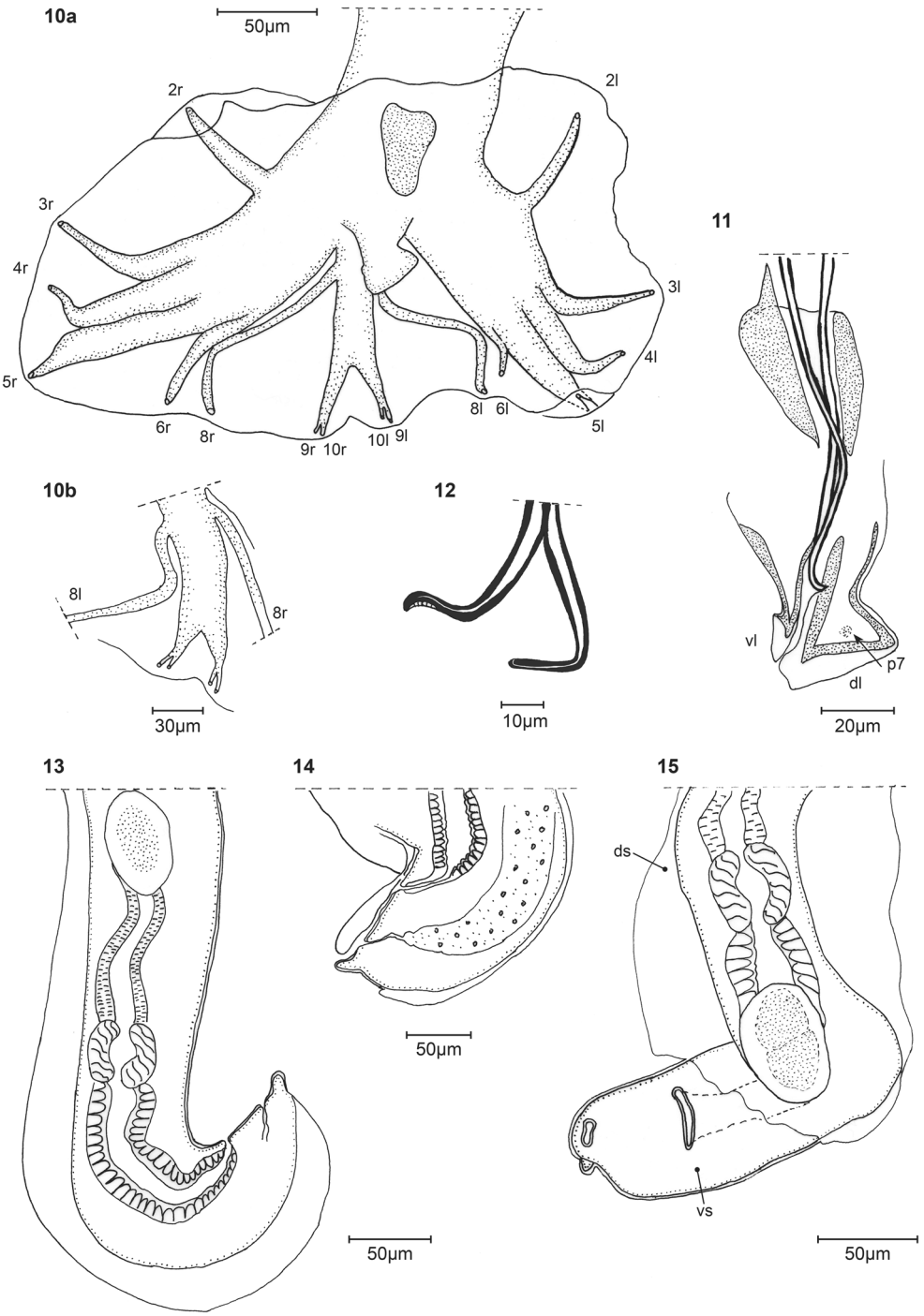


Figs. 1-9 *Stilestrongylus kaaguyporai* n. sp. **1** Male, anterior extremity, left lateral view. **2** Female, head, apical view. **3** Single cuticular ridge in cross section showing strut flanked by cuticular folds. **4–9** Synlophe in sections at different body levels, cuticular folds flanking struts omitted for bettter observation and counting of the ridges. 4–5 at oesophago-intestinal junction, 4, male, 5, female; 6–7 at mid-body, 6 male, 7 female; 8–9 within posterior third of body length, 8 male at level of spicules, 9 female at level of mid-uterus. Arrows indicate ridges pointing in opposite directions, delimitating axes of orientation. Symbols: *1*, *1'*: first dorsal and first ventral ridge, respectively; *L*, left, *V*, ventral. Figs. 2 and 4–9 oriented as Fig. 7

240–296 (277) and 247–296 (278) from apex, respectively. Oesophagus 320–355 (332) long. Genital cone 41–50 (45) long by 30–35 (32) wide at base. Gubernaculum 40–54 (45) long and 28–31 (30) wide at base in ventral view. Spicules 790–920 (839) long, 20.2–24.7% of body length.

Allotype female: 4.99 mm long, 104 wide at mid-body. Cephalic vesicle 75 long, 30 wide. Nerve ring, excretory pore and deirids situated 204, 313 and 310 from apex, respectively. Oesophagus 360 long. Reproductive tract monodelphic. Vulva situated 42 from caudal extremity. Vagina vera 27 long, vestibule 108, sphincter 48 long and 50 wide, infundibulum 123 long (Fig. 13). Infundibulum folded, proximal end not clearly observed. Uterus 880 long, 18% of body length, containing 19 eggs, 62.8 long and 42.8 wide. Tail broadly conical, ending in a mucron, 24 long (Fig. 14). Distal part of body bent and twisted at level of vestibule, involving lateral shift of 90° leftwards plus dorso-ventral torsion of the distal part. When whole posterior part of body is observed in dorsal view, post-vestibular portion is seen ventrally (Fig. 15).

Measurements (range and average) of 9 female paratypes: 4.65–5.62 (5.06) mm long, 93–130 (109) wide at mid-body. Cephalic vesicle 60–74 (70) long, 26–38 (31) wide. Nerve ring, excretory pore and deirids situated 150–200 (177), 226–301 (268) and 220–300 (265) from apex, respectively.



Figs. 10-15 *Stilestrongylus kaaguyporai* n. sp. **10**–**12** Male. 10 Bursa. **a** Entire bursa, ventral view, spicules omitted. **b** Detail of dorsal lobe and rays 8, dorsal view. 11 Detail of gubernaculum and genital cone, gubernaculum in ventral view, genital cone displaced in left lateral view. 12 Tips of everted spicules. **13**–**15** Female, posterior end. 13 Showing parts of ovejector, right lateral view, torsion not represented. 14 Distal extremity, behind torsion, showing detail of vulva, anus and tail, left lateral view. 15 Posterior extremity showing shift leftwards and dorso-ventral torsion of the distal part, dorsal view. Symbols: *2l*–*10l*, left rays 2 to 10; *2r*–*10r*, right rays 2 to 10; *dl*, dorsal lip; *vl*, ventral lip; *p7*, left papilla 7; *ds*, dorsal surface; *vs*, ventral surface

Oesophagus 334–365 (347) long. Vulva situated 50–141 (77) from caudal extremity. Vagina vera 22–60 (34) long, vestibule 108–130 (120), sphincter 34–48 (42) long, 40–50 (43) wide, infundibulum 105–176 (133). Uterus 801–1137 (975) long, 16–22% (19%) of body length. Number of eggs 16–21 (18), segmented, 65–80 (64.8) long, 35–50 (41.5) wide. Tail 25–61 (40) long. Distal end retracted to different degrees or unretracted (Figs. 14, and 15).

Taxonomic summary

Types: male holotype CHMLP-He 7444; female allotype CHMLP-He 7445; paratypes: 11 males, 9 females CHMLP-He 7446.

Type-host: *Euryoryzomys russatus* (Wagner, 1848) (Rodentia, Sigmodontinae) field number (CG553)

Site: Small intestine

Type-locality: Parque Provincial Urugua-í (PPU) (25° 58′ S, 54° 06′ W), Misiones, Argentina.

Etymology: from Ka-águy póra, according to the Guaraní ethnic group, the protector of the forests. In honour of the Guaraní people, first walkers of these forests.

Other material deposited: CHMLP-He 7341, 5 males, 5 females from *E. russatus* (CG561).

Diagnosis

This species belongs to the genus *Stilestrongylus* Freitas, Lent and Almeida, 1937 (Heligmonellidae: Nippostrongylinae) by having a synlophe with 24–25 small ridges of equivalent size, and a dissymmetrical caudal bursa with a hypertrophied genital cone. *Stilestrongylus* includes 24 neotropical members of which 23 species are parasitic in the Sigmodontinae and 1 in the Echimyidae. Among these species, five share a bursal pattern of type 1–4 on both lobes. These species are *S. barusi* Durette-Desset, 1970 in *Sigmodontomys alfari* (Allen, 1897) from Colombia; *S. dessetae* Yoyotte Vado, 1972 in *Melanomys caliginosus* (Tomes, 1860) from Colombia, *S. eta* (Travassos, 1937) in *Akodon montensis* Thomas, 1913, *A. cursor* Winge, 1887 and *Oligoryzomys nigripes* Olfers, 1818 from Brazil (Gomes et al. 2003, Simões et al. 2011), *S. peromysci* Falcón and Sanabria, 1999 in *Peromyscus difficilis* Allen, 1891 from Mexico and *S. renaudae* Durette-Desset, 1970 in *Rhipidomys latimanus* Tomes, 1860 from Colombia.

Stilestrongylus barusi is readily differentiated from these specimens by having a strongly dissymmetrical bursa with the left lobe very poorly developed, implying that the left lobe is smaller but also the left rays are markedly smaller than the right ones, in contrast with our specimens, in which rays on both lobes have a similar degree of development. *Stilestrongylus dessetae* and *S. renaudae* differ from these specimens by possessing subsymmetrical bursae and having, in both lateral lobes, rays 3, 4 and 5 parallel and apposed throughout most of their length, diverging distally at about the same level and forming a distinct lateral trident. This same pattern is observed in the left lobe of *S. peromysci*, in which the bursa has not been illustrated fully opened, and only the left lobe shows a more or less clear pattern of the rays 2 to 6. Concerning *S. eta*, there are four different illustrations of the bursa for this species provided by Travassos (1937) (three figures) and by Simões et al. (2011) (one figure), which account for some differences in the bursal symmetry and the disposition of the right lateral rays. However, all of them differ markedly from our specimens by having the left rays 4, 5 and 6 arising at the same level (vs. rays 6 markedly proximal in our specimens), and both rays 8 with a similar pathway, parallel to the lateral trunk of rays 2–6 (vs. left ray 8 bent posteriorly). In addition, the spicule length is half that of our specimens.

Furthermore, two characters mentioned in the Description seem to be peculiar to our specimens: the conspicuous dorsal hood-like projection on the male genital cone, and the marked bend and torsion of the posterior end of the female. We consider that the whole of these differences allows us to designate a new species, for which we propose the name *Stilestrongylus kaaguyporai* n. sp.

Since another species of *Stilestrongylus*, *S. lanfrediae*, can be found in the same host and area, it may be worthwhile to remark that *S. lanfrediae* is differentiated from the new species by a bursal pattern of type 2–2-1 in both lobes, a marked dissymmetry of the dorsal lobe involving both the rays 8 and the branches of the dorsal ray, and spicules thick and markedly striated.

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Compliance with ethical standards

The research has been conducted according to Argentine laws. Sample collection was carried out during fieldwork under official permits granted by the Ministerio de Ecología, RNR y Turismo, Provincia de Misiones (autorización #23, Guía Tránsito 000685 and 000699). The study was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The specimens obtained with methods for live capture were studied and humanely sacrificed following the procedures and protocols approved by national laws (LN 14.346 and references in the provincial permits) and by the Comité de Ética of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) (Resolución 1047, sección 2, anexo II).

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