



New species of *Raphidascaris* (*Sprentascaris*) (Nematoda: Anisakidae) in *Rineloricaria steinbachi* (Actinopterygii: Loricariidae) from Northwest Argentina

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Abstract

During a parasitological survey, we reported specimens of a new species of *Raphidascaris* (*Sprentascaris*) in *Rineloricaria steinbachi* from La Caldera River, province of Salta, Northwest Argentina. The new species, *R. (S.) saltaensis* **sp. nov.**, is characterized by possessing 16 pairs of preanal papillae, lips with lateral protrusions and membranous elevations, and mature eggs rough-shelled with striations. This is the sixth species of the genus described in the Neotropical Region, and the second from Argentina. *Rineloricaria steinbachi* is reported as host of this nematode genus for the first time. A key to the species of *Raphidascaris* (*Sprentascaris*) in the Neotropical realm is provided. Also, zoogeographical and host information of the species, belonging to the subgenus *Sprentascaris*, is summarized.

Keys words: *Raphidascaris* (*Sprentascaris*) *saltaensis* **sp. nov.**, Siluriformes, River Bermejo basin, Salta

Introduction

Raphidascaris Railliet & Henry, 1915 includes parasite species of marine and freshwater fishes (Moravec 1998). Currently, five valid species—all belonging to the subgenus *Sprentascaris* (Petter & Cassone, 1984)—have been reported occurring in Neotropical freshwater fishes: *Raphidascaris* (*Sprentascaris*) *hypostomi* (Petter & Cassone, 1984), *R. (S.) mahnerti* (Petter & Cassone, 1984); *R. (S.) pimelodi* (Petter & Cassone, 1984); *R. (S.) lanfrediae* Carvalho de Melo, Nascimento dos Santos, Guerreiro Giese, Nunes dos Santos & Portes Santos, 2011; and *R. (S.) marano* Ramallo, 2009. The first three species were recorded for the first time in siluriform fishes (Loricariidae and Pimelodidae) in Paraguay (Petter & Cassone 1984; Moravec *et al.* 1990). *Raphidascaris* (*S.*) *lanfrediae* parasitizes *Satanoperca jurupari* Heckel, (Cichlidae) in the Guama River, Brasil, (Melo *et al.* 2011), while the last species (*R. (S.) marano*) was isolated from *Hypostomus cordovae* Günther, from Marapa River, Tucumán, Argentina (Ramallo 2009). With the exception of *R. (S.) lanfrediae*, whose host belongs to the Order Perciformes, the remaining known species were registered frequently in Siluriformes, and also in Characiformes (Moravec 1998; Eiras *et al.* 2010).

Siluriformes is one of the most widely distributed groups of Actinopterygii in all continents. In Argentina, the order consists of 12 families, one of them is Loricariidae. This family includes *Rineloricaria steinbachi*, (= *Ixinandria steinbachi* Regan, recorded in Northern Argentina and Southern Bolivia (Monasterio de Gonzo 2003, Rodríguez *et al.* 2008). Covain *et al.* (2016) synonymized *Ixinandria* with *Rineloricaria* based on molecular analysis. Consequently, the geographical distribution of *R. steinbachi* was extended to include the river basins of the Bermejo River; Juramento River; Dorado del Valle River and Pilcomayo River.

During parasitological surveys performed on freshwaters fishes in the province of Salta, we found specimens of a new species of *Raphidascaris* (*Sprentascaris*) in specimens of *R. steinbachi* from the province of Salta herein described. A taxonomic identification key for the known species of the *Raphidascaris* (*Sprentascaris*) is provided, modifying the key proposed by Moravec (1998).

Materials and methods

During June 2014 and October 2015, 67 specimens of *Rineloricaria steinbachi* (standard length 54.96 ± 20.23 (24.85–148.81) mm; weight 5.28 ± 9.42 (0.20–74) g) were collected from La Caldera River (24°35'40.07"S 65°22'11"W), province of Salta (Argentina). Fish samples were fixed in 10% formalin for 24 hrs and then preserved in 70% ethanol for later helminthological examination. Isolated nematodes from the intestine were preserved in 70% ethanol. Nematodes were cleared with lactophenol, examined with a light microscope (LM). Drawings were made using a camera lucida. Measurements are given in millimetres (mm) unless otherwise indicated and are presented as mean \pm SD followed by minimum and maximum values in parentheses. Quantitative indicators of infections were estimated based on definitions by Bush *et al.* (1997). Some specimens were dehydrated throughout an ethanol series, acetone and ether, coated with gold and examined in a Zeiss Supra 55VP SEM (scanning electronic microscopy).

Nematodes were deposited in the Colección Helmintológica Fundación Miguel Lillo (CH-FML), San Miguel de Tucumán, Argentina. Fish samples were stored in the Colección Ictiológica, Instituto de Bio y Geociencias del NOA (IBIGEO-I), Salta, Argentina.

Order Ascaridida Skrjabin & Schulz, 1940

Family Anisakidae Railliet & Henry, 1912

Genus *Raphidascaris* Railliet & Henry, 1915

Subgenus *Sprentascaris* Petter & Cassone, 1984

Raphidascaris (*Sprentascaris*) *saltaensis* sp. nov.

(Figs 1–2)

Type material. Holotype: female CH-FML # 07663; paratypes: eight males and five females, of which nine specimens were used for SEM, CH-FML # 07665.

Type host. *Rineloricaria steinbachi* Regan, (Siluriformes: Loricariidae) (IBIGEO-I # 355–422).

Type locality. La Caldera River (24°35'40.07"S; 65°22'11"W), La Caldera Department, province of Salta, Northwest Argentina.

Etymology. The new species is named for its geographical location in the province of Salta.

Site of infection. Intestine.

Infection parameters. Prevalence: 11.94% (8/67); media intensity: 2.63 per fish.

Measurements. Table 1.

General description. Medium sized nematodes. Cuticle bearing transverse striations. Mouth aperture triangular, two ventrolateral lips and one dorsal lip. Lips well-developed and oval-shaped; dorsal lip slightly smaller than the two ventrolateral ones. Dorsal lip bearing two double papillae, ventrolateral lips with one double papilla, one single papilla and one amphid. Lips with lateral membranous margins forming finger-shaped protrusions at each side. Membranous extensions at base of lips. Interlabia absent (Figs. 1A–C; 2A–C). Excretory pore slightly posterior to nerve ring level. Lateral alae present in both sexes, beginning in lateral region below lips and reaching almost to tail tip. Muscular oesophagus short with expanded posterior half. Caudal alae absent in both sexes. Ventriculus shorter than wide, ventricular appendix longer than wide. Both sexes with conical tail, its distal tip slender and sharply pointed.

Adult males (eight specimens): Spicules equal, simple with pointed distal tips. Twenty pairs of caudal papillae. Preanal papillae: Sixteen subventral pairs; counting from cloaca opening, the first until the seventh pair are closer to each other than the remaining pairs. Adanal papillae: only one subventral pair. Postanal papillae: three small subventral pairs. Lateral alae present, without the partition in the caudal region. Caudal alae absent. Conical tail (Fig. 1G).

Adult female (six specimens): Vulva preequatorial, posterior to oesophagus end level (Fig. 1E). Eggs oval-shaped, with thick and rough shell provided with membranous striations, mature eggs larvated (Figs. 1D). Conical tail (Figs. 1F; 2D).

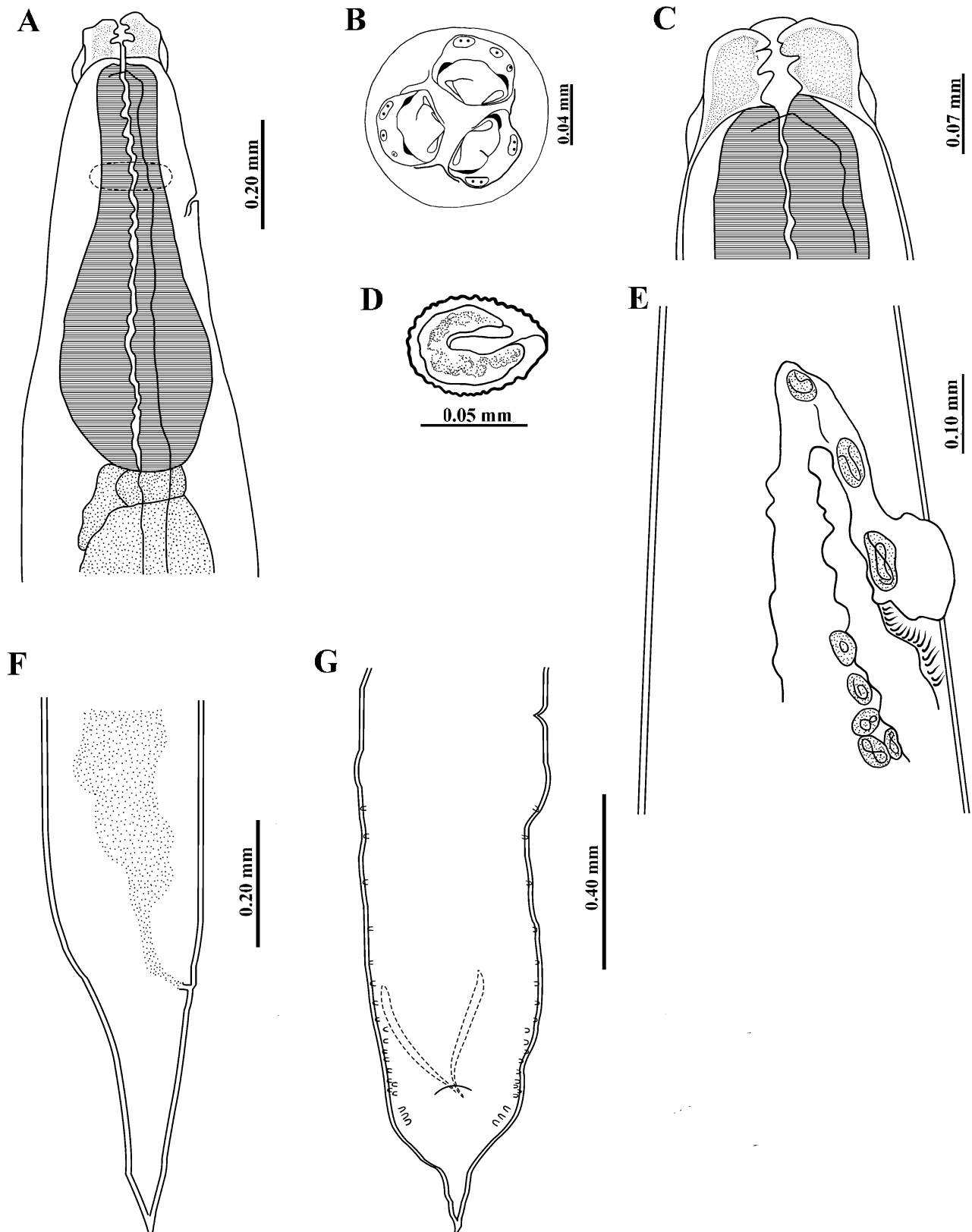


FIGURE 1. *Raphidascaris (Sprentascaris) saltaensis* sp. nov. (A) Female, anterior end, lateral view. (B) Male, apical view. (C) Female, head, lateral view. (D) Mature and larvated egg. (E) Female, vulva, lateral view. (F) Female, tail, lateral view. (G) Male, posterior end with spicules and papillae, ventral view.

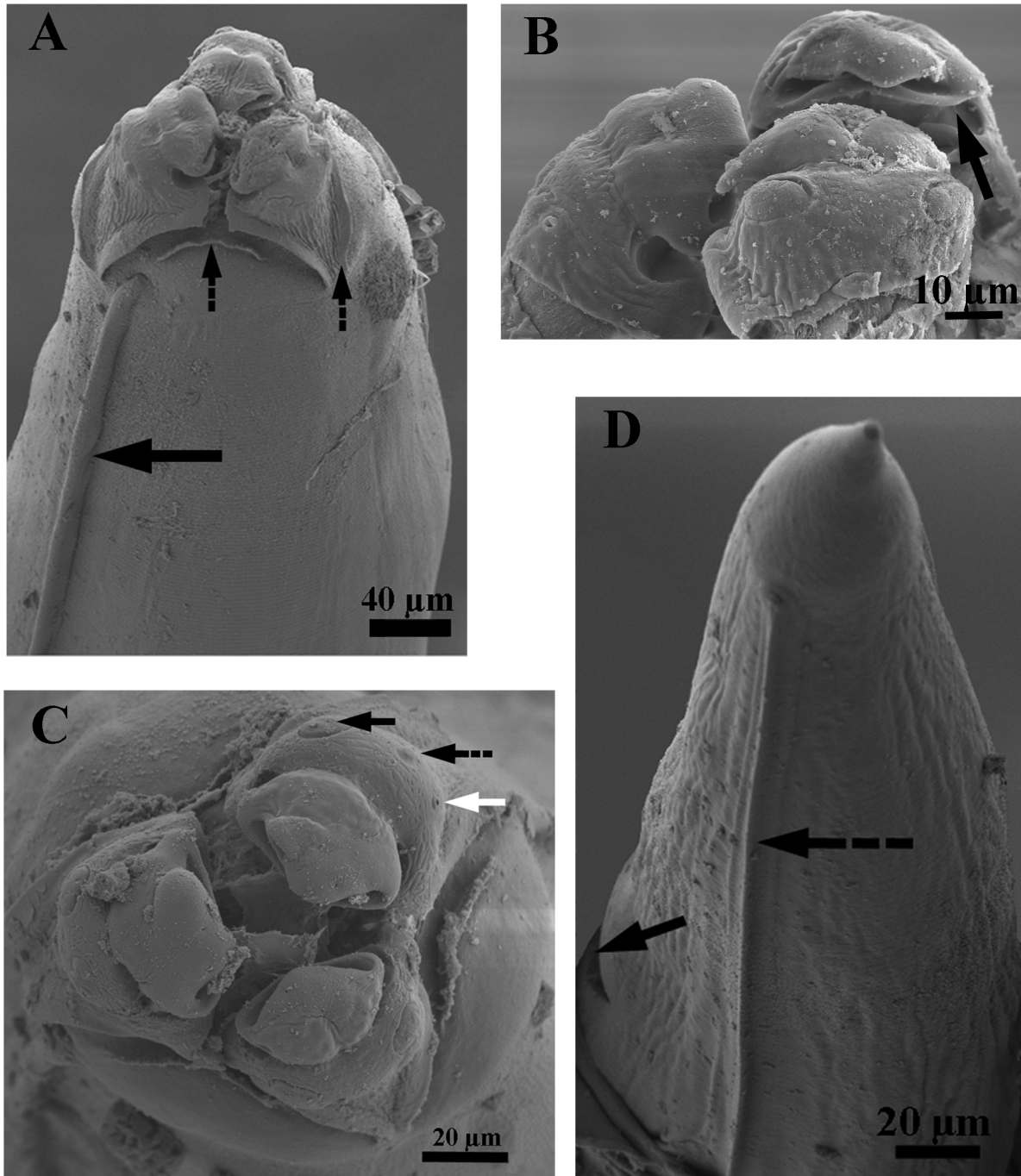


FIGURE 2. *Raphidasca (Sprentasca) saltaensis* sp. nov. (SEM micrographs) (A) Male, cephalic end. Lateral alae (black arrow), membranous elevations below lips (discontinuous black arrow). (B) Anterior end of female, dorsal view. Lips with lateral membranous margins forming finger-shaped protrusions at each side (black arrow). (C) Cephalic end of male, apical view. Subdorsal double papillae (black arrow), single papillae (white arrow), amphids (discontinuous black arrow). (D) Female, posterior end, sublateral view. Anus (black arrow), lateral alae (discontinuous black arrow).

Remarks. *Raphidasca (Sprentasca) saltaensis* sp. nov. is distinguished from their congeners, by possessing 16 pairs of preanal papillae, membranous elevations below the lips, mature eggs with striated shell and the size of spicules (0.24 mm). For more detailed description of morphometric differences between the new species and other species of *Raphidasca (Sprentasca)* see Table 2.

Raphidasca (S.) saltaensis sp. nov. differs from *R. (S.) mahnerti* and *R. (S.) lanfrediae* by the absence of caudal alae in the males; but shares this feature with *R. (S.) pimelodi*, *R. (S.) hypostomi* and *R. (S.) marano*. The new species presents lateral alae, which begins in the base of ventrolateral lips and extends almost to tail tip; no

partition of this structure is observed, hence caudal alae are not present. *Raphidascaris* (*S.*) *saltaensis* **sp. nov.** differs from *R. (S.) pimelodi* and *R. (S.) hypostomi* by the presence of lateral alae, and by the number of postanal papillae pairs; *R. (S.) saltaensis* possess three pairs, while *R. (S.) pimelodi* and *R. (S.) hypostomi* present five pairs and lack lateral alae (Moravec 1998).

TABLE 1. Measurements of *Raphidascaris (Sprentascaris) saltaensis* **sp. nov.** Given in mm, mean \pm SD (range). (* measurements with a unique value).

Character	Holotype	Paratypes	
	♀ 1	♀ 5	♂ 8
Total body length	6.86	6.59 \pm 1.37 (5.62–7.56)	5.65 \pm 0.32 (5.12–5.92)
Body width	0.57	0.58 \pm 0.06 (0.54–0.63)	0.46 \pm 0.15 (0.28–0.67)
Lateral alae width	0.01	0.02*	0.02 \pm 0.02 (0.01–0.04)
Lips length	0.06	0.05 \pm 0.02 (0.04–0.06)	0.06 \pm 0.00 (0.06–0.07)
Oesophagus total length	0.75	0.92 \pm 0.25 (0.71–1.27)	0.79 \pm 0.09 (0.63–0.88)
Oesophagus total width	0.30	0.36 \pm 0.06 (0.30–0.43)	0.22 \pm 0.06 (0.14–0.34)
Nerve ring—anterior end	0.29	0.37 \pm 0.08 (0.32–0.43)	0.34 \pm 0.03 (0.29–0.37)
Excretory pore—anterior end	0.36	0.45 \pm 0.02 (0.44–0.47)	0.40 \pm 0.05 (0.32–0.47)
Ventriculus length	0.05	0.07*	0.06 \pm 0.01 (0.06–0.07)
Ventriculus width	0.13	0.13*	0.19 \pm 0.06 (0.15–0.24)
Ventricular appendix length	0.16	0.10*	0.14 \pm 0.06 (0.10–0.19)
Ventricular appendix width	0.06	0.04*	0.05 \pm 0.00 (0.04–0.05)
Ventricular appendix/ oesophagus length ratio	1: 0.21	1:0.13*	1:0.21 (1:0.12–0.3)
Vulva—anterior end	1.20	1.74*	-
Spicules length	-	-	0.24 \pm 0.02 (0.22–0.27)
Tail length	0.37	0.30 \pm 0.13 (0.15–0.39)	0.25 \pm 0.02 (0.22–0.27)
Eggs length	0.07	0.06 \pm 0.02 (0.04–0.09)	-
Eggs width	0.04	0.03 \pm 0.01 (0.03–0.04)	-

Raphidascaris (S.) saltaensis **sp. nov.** resembles *R. (S.) marano*, both species have lateral alae and three pairs of postanal papillae; but can readily be distinguished by the number of preanal papillae (16 vs. 22), the shape and size of lips (smaller and different lips with protrusions vs. longer, symmetrical, simple and equal lips), shape of eggs (rough-shelled vs. thin-shelled) and the spicule length (0.24 vs. 0.27) (Moravec 1998; Ramallo 2009).

Discussion. Five genera of Anisakidae have been reported from the intestine of Neotropical freshwater fishes: *Goezia* (Zeder, 1800), *Hysterothylacium* (Ward and Magath, 1917), *Terranova* (Leiper and Atkinson, 1914), *Raphidascaroides* (Yamaguti, 1941) and *Raphidascaris* (Railliet and Henry, 1915). *Goezia*, *Hysterothylacium* and *Raphidascaris* show a broad geographical distribution including Mexico, Guyana, Venezuela, Brazil, Paraguay, Peru and Argentina; in contrast *Raphidascaroides* and *Terranova* were only recorded in Brazil and Venezuela (Moravec 1998). *Raphidascaris* has two subgenera: *Raphidascaris* (not in the Neotropical region), and *Sprentascaris* whose members are restricted to South America and were only reported in freshwater fishes (Ramallo 2009). Species of *Raphidascaris (Sprentascaris)* are characterized by possessing a cuticular ring without spines, the presence of small postlabial or interlabial cuticular elevations, absence of true interlabia; the excretory pore slightly posterior to nerve ring level, a muscular oesophagus with ventriculus and ventricular appendix; and no intestinal caecum (Moravec 1998).

All species of *Raphidascaris (Sprentascaris)*, except *R. (S.) lanfrediae*, have Siluriformes fishes as hosts. *Raphidascaris (S.) hypostomi* was isolated from specimens of the subfamilies: Hypostominae and Ancistrinae (all Loricariidae) (Moravec 1998). Eiras *et al.* (2010) found nematodes identified as *R. (S.) hypostomi* and *R. (S.) mahnerti* in *Metynnis lippincottianus* Cope (Characiformes, Serrasalminidae) from Brazil. *Raphidascaris (S.) mahnerti* was also reported in Loricariidae fishes; moreover Moravec *et al.* (1993) recorded the third larval stage of

TABLE 2. Morphometrics of *Raphidascaris (Sprentascaris)* species. Given in mm, minimum value–maximum value.

Character	<i>R. (S.) hypostomi</i>		<i>R. (S.) mahneri</i>		<i>R. (S.) pimelodi</i>		<i>R. (S.) marano</i>		<i>R. (S.) langfrediae</i>		<i>R. (S.) saltaensis sp. nov.</i>	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Total body length	11.22–14.27	6.06–7.90	6.91–9.43	5.28–8.12	13.6–19.0	8.90	13.2–14.8	4.50–10.1	2.11–6.52	3.92–6.45	5.62–7.56	5.12–5.92
Body width	0.80–1.14	0.34–0.83	0.19–0.31	0.15–0.22	0.40–0.60	0.20	0.74–1.34	0.44–0.94	0.10–0.29	0.15–0.25	0.54–0.63	0.28–0.67
Lips length	0.10–0.11	0.05–0.10	0.04–0.05	0.03–0.05			0.17–0.20	0.11–0.14	0.02–0.04	0.02–0.030	0.04–0.06	0.06–0.07
oesophagus total length	1.22–1.39	0.80–1.33	0.88–1.16	0.69–1.02	1.40–1.60	0.96	0.94–1.27	0.50–1.00	0.29–0.71	0.40–0.70	0.71–1.27	0.63–0.88
oesophagus total width	0.35–0.41	0.17–0.35	0.11–0.15	0.07–0.11			0.31–0.43	0.20–0.40	0.05–0.14	0.07–0.10	0.30–0.43	0.14–0.34
Nerve ring— anterior end	0.41–0.50	0.27–0.44	0.25–0.40	0.25–0.32	0.38–0.50	0.30	0.22–0.37	0.22–0.37	0.14–0.29	0.23–0.30	0.29–0.43	0.29–0.37
Excretory pore— anterior end	0.46–0.49	0.35–0.46	0.28–0.41	0.27–0.34	0.41–0.55	0.37	0.35–0.44	0.35–0.44	0.16–0.44	0.21–0.33	0.36–0.47	0.32–0.47
Ventriculus length	0.12–0.14	0.08–0.14	0.05–0.07	0.03–0.07	0.10–0.15	0.07	0.11–0.15	0.11–0.12	0.02–0.04	0.03–0.05	0.05–0.07	0.06–0.07
Ventriculus width	0.30–0.41	0.23–0.33	0.10–0.12	0.07–0.11	0.12–0.15	0.07	0.15–0.27	0.10–0.19	0.05–0.08	0.08–0.10	0.13	0.15–0.24
Ventricular appendix length	0.19–0.20	0.15–0.20	0.06–0.12	0.07–0.14	0.21–0.24	0.15	0.24–0.28	0.22–0.34	0.12–0.31	0.16–0.21	0.10–0.16	0.10–0.19
Ventricular appendix width	0.08–0.10	0.06–0.10	0.02–0.07	0.03–0.04			0.10–0.17	0.10–0.16	0.03–0.05	0.03–0.06	0.04–0.06	0.04–0.05
Vulva— anterior end	1.86–2.31		1.82–3.94		3.30–4.80		1.70–2.54		0.40–1.06		1.20–1.74	
Spicules length		0.27–0.29		0.12–0.15		0.14		0.25–0.30		0.13–0.16		0.22–0.27
Tail length	0.44–0.53	0.20–0.26	0.20–0.31	0.08–0.14	0.30–0.35		0.53–0.58	0.17–0.23	0.12–0.34	0.09–0.12	0.15–0.39	0.22–0.27
Egg length	0.05–0.07		0.08–0.09				0.05–0.07		0.01–0.02		0.04–0.09	
Egg width	0.05–0.06		0.05–0.08				0.04–0.05		0.01–0.01		0.03–0.04	

Raphidascaris (*Sprentascaris*) in the intestine of the cichlid *Geophagus brasiliensis* Quoy & Gaimard, (Perciformes, Cichlidae). The larva is probably conspecific with *R. (S.) mahnerti* and *G. brasiliensis* could be a paratenic host (Moravec 1998). This is not the only time *Raphidascaris* (*Sprentascaris*) was detected in cichlids; Melo *et al.* (2011) reported the adults of *R. (S.) lanfrediae* in *Satanoperca jurupari*. Possibly, this species share the host spectrum with *R. (S.) mahnerti*. Both species might be closely related but they can be differentiated by morphological features and their geographical distribution (Melo *et al.* 2011). *Raphidascaris (S.) pimelodi* is distinguished from other known species of the subgenus, because it was only detected in a pimelodid species.

Although *R. (S.) marano* and *Raphidascaris (S.) saltaensis* **sp. nov.** have both been recorded in Northwest of Argentina, they inhabit different fluvial systems; *R. (S.) marano* in Marapa River (basin of Sali River, provinces of Tucumán and Catamarca), and *R. (S.) saltaensis* **sp. nov.** in La Caldera River (basin of the Bermejo River, province of Salta). Ramallo (2009) proposed that *R. (S.) marano* would be an endemic species in Argentina, following the endemic character of its host, *H. cordovae* (Loricariidae). However, *H. cordovae* is synonymous of *H. paranensis*, thus the geographical distribution was extended to include Paraguay (Paraguay River basin, the main tributary of Paraná River) (Ferraris 2007). The new parasite species was found from *Rineloricaria steinbachi* (Loricariidae), whose geographical distribution is limited to southern Bolivia and northwestern Argentina, including the basins of the Bermejo (Rivers Bermejo, San Andrés, La Caldera, Mojotoro and Vaqueros and streams Gallinato and Pucheta), Juramento (Rivers Salado, Piedras, Arias Arenales, Rosario, Aguas Negras and Calchaquí), Pilcomayo River, and also the Dorado River basin (La Sala stream and Popayan River).

In this paper, we propose the erection of the sixth species of *Raphidascaris (Sprentascaris)* from the Neotropical region and the second from Argentina. *Raphidascaris (S.) saltaensis* **sp. nov.** differs from all congeners in the number of preanal papillae, the shape and size of lips, the egg's shell and the spicules length. The new identification key presented in the article incorporates *R. (S.) lanfrediae*, *R. (S.) marano* and *R. (S.) saltaensis* **sp. nov.**

Key to species of the subgenus *Sprentascaris* parasitic in Neotropical freshwater fishes

- 1 Males with caudal alae and six pairs of postanal papillae. 2
- Males without caudal alae and less than six pairs of postanal papillae. 3
- 2 Ventricular appendix/oesophageal length ratio in males and females is 1:0.16. Caudal alae well-developed. Vulva situated at 1.82–3.94 mm from anterior extremity, approximately at 1/3 of body length *R. (S.) mahnerti*
- Ventricular appendix/oesophageal length ratio in males is 1:0.34 and in females 1:0.36. Caudal alae rudimentary. Vulva at short distance below oesophagus, at 0.40–1.06 mm from anterior extremity *R. (S.) lanfrediae*
- 3 Lateral alae well developed (broad). In males, three pairs of postanal papillae. 4
- Lateral alae absent or rudimentary. In males, five pairs of postanal papillae. 5
- 4 Symmetrical and equal lips. In males, 22 pairs of preanal papillae. In females, mature eggs embryonated, thin-shelled.
- *R. (S.) marano*
- Dorsal lips smaller than ventrolateral ones. In males, 16 pairs of preanal papillae. In females, mature eggs embryonated with rough shell and membranous striations. *R. (S.) saltaensis* **sp. nov.**
- 5 Narrow oesophagus. Rounded tail in both sexes. Posterior end of males with ventral cuticular ornamentation. Length of spicules 0.14 mm. Parasites of Pimelodidae fishes. *R. (S.) pimelodi*
- Oesophagus markedly broad. Pointed tail tip in both sexes. Males with smooth posterior ventral surface. Length of spicules between 0.15–0.25 mm *R. (S.) hypostomi*

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