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FAR FROM HOME? A NEW LOCALITY RECORD OF *FALCAUSTRA SANJUANENSIS* GONZÁLEZ, SANABRIA & QUIROGA, 2013 (NEMATODA: KATHLANIIDAE) IN A NEW HOST FROM THE BRAZILIAN AMAZON

¿LEJOS DE CASA? UN NUEVO REGISTRO DE LOCALIDAD DE *FALCAUSTRA SANJUANENSIS* GONZÁLEZ, SANABRIA & QUIROGA, 2013 (NEMATODA: KATHLANIIDAE) EN UN NUEVO HUÉSPED DE LA AMAZONÍA BRASILEÑA

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Titulillo: Far from home? A new locality record of *Falcaustra sanjuanensis*

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ABSTRACT

Studies on the helminth parasites of South American freshwater turtles are rare and punctual. The parasitic fauna of *Kinosternon scorpioides*' Linnaeus, 1766 nematodes from Brazil is restricted to only 4 species: *Serpinema magathi* Sprehn, 1932, *Serpinema monospiculatus* Freitas & Dobbin Jr., 1971, *Serpinema pelliculatus* Silva, Jesus & Melo, 2023 and *Spiroxys figueiredoi* Freitas & Dobbin Jr., 1962. The present work aimed to report the occurrence of the species *Falcaustra sanjuanensis* González, Sanabria & Quiroga, 2013 (Nematoda: Kathlaniidae), a parasite of an anuran from Argentina, in the large intestine of *K. scorpioides* from the Brazilian Amazon. The specimens of freshwater turtles were collected, anesthetized, and euthanized; the nematodes found were fixed in 90°GL ethanol and analyzed by light microscopy and scanning electron microscopy. The nematodes showed some morphological and morphometric variations when compared with the original description of the species. Here we present new locality and host records for *F. sanjuanensis* and a new parasite record for *K. scorpioides* from Brazil.

Keywords: Brazilian Amazon - *Falcaustra sanjuanensis* - Kathlaniidae - *Kinosternon scorpioides* – morphology - Turtle parasites

RESUMEN

Los estudios sobre los helmintos parásitos de las tortugas de agua dulce de América del Sur son escasos y puntuales. La fauna parasitaria de los nematodos de *Kinosternon scorpioides* Linnaeus, 1766 de Brasil está restringida a solo cuatro especies: *Serpinema magathi* Sprehn, 1932, *Serpinema monospiculatus* Freitas & Dobbin Jr., 1971, *Serpinema pelliculatus* Silva, Jesus & Melo, 2023 y *Spiroxys Figueiredoi* Freitas & Dobbin Jr., 1962. El objetivo del presente trabajo fue reportar la ocurrencia de la especie *Falcaustra sanjuanensis* González, Sanabria & Quiroga, 2013 (Nematoda: Kathlaniidae), parásito de un anuro de Argentina, en el intestino grueso de *K. scorpioides* de la Amazonía brasileña. Los ejemplares de tortugas de agua dulce fueron colectados, anestesiados y sacrificados; los nematodos encontrados se fijaron en etanol 90°GL y se analizaron mediante microscopía óptica y microscopía electrónica de barrido. Los nematodos mostraron algunas variaciones morfológicas y morfométricas al compararlos con la descripción original de la especie. Aquí presentamos nuevos registros de localidad y hospedante para *F. sanjuanensis* y un nuevo registro de parásito para *K. scorpioides* de Brasil.

Palabras clave: Amazonía Brasileña - *Falcaustra sanjuanensis* - Kathlaniidae - *Kinosternon scorpioides* – morfología - Parásitos de tortugas

INTRODUCTION

Vertebrate helminth parasites are a widely diverse but still poorly understood group. These organisms represent an important component of ecosystems and play vital ecological roles, impacting the structure and dynamics of food webs and contributing to

biodiversity and the functioning of ecological communities (Poulin & Morand, 2000; Hugot *et al*., 2001; Buck, 2019; Juaréz-Estrada *et al*., 2023; Lymbery & Smit, 2023).

Recently, many studies describing species of amphibian and reptile parasites have been developed; however, there is still a considerable gap in knowledge about the parasite diversity of turtles (Anjos, 2011). Studies on the helminth biodiversity of South American turtles are relatively recent and are generally represented by specific research in some countries such as Brazil, Uruguay, and Peru (Mascarenhas & Müller, 2021).

Kinosternon scorpioides Linnaeus, 1766 is a freshwater turtle belonging to the Family Kinosternidae Agassiz, 1857, widely distributed throughout the American continent, occurring from southern Mexico to northern Argentina (Turtle Taxonomic Work Group, 2017). The species is oviparous, has semi-aquatic and nocturnal habits, and prefers to inhabit clear water environments, but it can also occur in black waters (Ferrara *et al*., 2017).

In general, studies with *K. scorpioides* are directed to anatomical, food, ecological, and reproductive analyses, aiming at the monitoring, conservation, and management of this species, but parasitological data are still very scarce (Araújo *et al*., 2013; Sousa *et al.,* 2014; Chaves *et al*., 2020; Ferreira *et al*., 2020; Santos *et al.,* 2021).

Between the various groups of parasitic helminths, nematodes are the most found in turtles. Amidst them, the family Kathlaniidae Lane, 1914 is one of the most diversified, with the genus *Falcaustra* Lane, 1915 has about 100 parasitic species from the digestive tract of amphibians, fishes, and reptiles (Baker, 1986).

Currently, 12 valid species of the genus *Falcaustra* occur in the Neotropical region, namely: *F. belemensis* Baker & Bain, 1981; *F. caballeroi* (Caballero, 1935) Chabaud & Golvan, 1957; *F. condorcanquii* Ibanez & Córdova 1976; *F. costaricae*

Bursey, Goldberg & Miller, 2004; *F. guanacastensis* Bursey & Brooks, 2011; *F. guatamalana* (Caballero, 1953) Chabaud & Golvan, 1957; *F. intermedia* (Caballero, 1939) Freitas & Lent, 1941; *F. mascula* (Rudolphi, 1819) Freitas & Lent, 1941; *F. pumacahuai* Ibanez & Córdova 1976; *F. sanjuanensis* González, Sanabria & Quiroga, 2013; *F. tiahuanaquensis* Ibanez & Córdova 1976; and *F. tikasinghi* (Schoenecker, Schmidt & Everard, 1977) Baker & Bain, 1981 (Freitas & Lent, 1941; Chabaud & Golvan, 1957; Baker & Bain, 1981; Bursey *et al*., 2004; Bursey & Brooks, 2011; González *et al*., 2013). Of those, *F. guanacastensis*, *F. intermedia,* and *F. tikasinghi* are the only found in turtles, with no record of this genus parasitizing *Kinosternon* from Brazil (Mascarenhas & Müller, 2021).

Falcaustra sanjuanensis was described as parasitizing the large intestine of the anuran *Odontophrynus* cf. *barrioi* Cei, Ruiz & Beçak, 1982 from Argentina, which is the only record. In the present study, we found specimens of this nematode species in the large intestines of *K. scorpioides* from Brazil. Thus, we provide new morphological and morphometric data for *F. sanjuanensis*, a new location and host record.

MATERIAL AND METHODS

During an expedition to collect helminth parasites from amphibians and reptiles in Serra dos Carajás, Pará, Brazil (6°6'29"S 50°18'16"W), 4 specimens of *K. scorpioides* were collected (under SISBIO collection license: 53527-7), sent to the laboratory, anesthetized and euthanized. The nematodes collected from the large intestine were washed in 0.9% saline solution and fixed in 90°GL ethanol. For morphological and morphometric analysis, the nematodes were clarified in 50% Amann's lactophenol, mounted on temporary slides, and examined in an Olympus BX41 optical microscope (Olympus, Tokyo, Japan) with a drawing tube attached.

For scanning electron microscopy (SEM), 6 specimens of nematodes (3 males and 3 females) were postfixed in 1% OsO4, dehydrated in an ascending series of ethanol, critically dried with CO_2, coated with gold-palladium, mounted on metallic supports, and analyzed in a Vega3 microscope (TESCAN, Brno, Czech Republic) with acceleration voltage between 10-20 kV.

Measurements are presented in micrometers unless otherwise indicated, with the mean value followed by the minimum and maximum values in parentheses. The voucher specimens were deposited in the Collection Other Invertebrates of the Museu Paraense Emílio Goeldi (MPEG), Pará, Brazil.

Ethical aspects: All procedures contributing to this work comply with all applicable institutional, national, and international guidelines for animal care and use Animal Research Ethics Committee, Federal University of Pará, under license N8341260821CEUA/UFPa. The present study was approved by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), Brazil, and host specimens were collected under license number SISBIO: 53527-7.

RESULTS

Family Kathlaniidae Lane, 1914

Genus *Falcaustra* Lane, 1915

Falcaustra sanjuanensis González, Sanabria & Quiroga, 2013 (Figs. 1, 2)

General description: Large-sized nematodes; cylindrical body, robust. Cuticle with fine transverse striations. Truncated anterior end (Fig. 1A). Lateral alae absent. Triangular mouth opening, surrounded by 3 large lips; 2 papillae on each lip, 1 amphid on each subventral lip (Figs. 1B, 2A). Short, muscular pharynx. Divided esophagus, with elongated body, subspherical isthmus, spherical bulb, opening to the

intestine through a valve (Fig. 1A). Nerve ring located in the first third of the esophageal body. Deirids positioned in the posterior half of the esophageal body (Fig. 1A). Small excretory pore posterior to deirids (Figs. 1A, 2B). Short tail (Figs. 1E, G, 2D, F).

Males (based on 10 adult specimens): Body length 11.21 (9.93–12.41) mm. Width at esophagus-bowel junction 355 (300–394). Pharynx 78 (63–100) × 83 (63–100). Esophagus (except isthmus and bulb) 1.54 (1.32–1.66) mm × 101 (89–111). Isthmus 128 (103–152) × 114 (95–126). Bulb 210 (176–232) × 193 (168–216). Nerve ring, deirids and excretory pore located at 380 (345–426), 1.12 (0.97–1.26) mm and 1.27 (1.09–1.40) mm, respectively, from the anterior end. Well-developed pre-cloacal musculature; pre-cloacal pseudosucker present (Fig. 1E), length 387 (307–453). Distance from the beginning of the pseudosucker to the tail 2.86 (2.54–3.07) mm. Tail length 459 (395–495) (Fig. 1E). Eleven pairs of caudal papillae: 3 pre-cloacal, 2 ad-cloacal (1 anterior and 1 posterior to the cloaca), 6 post-cloacal (first ventral pair, second lateral pair located near the cloaca; third subventral pair near the middle of the tail; fourth dorsoventral pair, fifth lateral pair, sixth dorsoventral pair located near the posterior end); single median papilla immediately anterior to the cloacal opening (Figs. 1D, 2D, E). Gubernaculum length 208 (175–245) (Fig. 1F). Equal spicules, length 1.25 (1.18–1.32) mm (Fig. 1H).

Females (based on 11 adult specimens): Body length 12.58 (10.68–14.23) mm. Width at esophagus-intestine junction 366 (315–421). Pharynx 83 (63–116) × 87 (79–111). Esophagus (except isthmus and bulb) 1.72 (1.60–1.88) mm × 115 (95–153). Isthmus 148 (135–158) × 132 (116–153). Bulb 208 (179–242) × 204 (184–232). Nerve ring, deirids and excretory pore located 411 (384–453), 1.19 (1.09–1.33) mm and 1.37 (1.25–1.55) mm, respectively, from the anterior end. Vulva at the posterior half of the body, 4.70 (4.20–5.13) mm from the posterior end (Fig. 2C). Tail length 640 (595–695) (Figs. 1G, 2F). Vulva-anus distance 4.09 (3.57–4.59) mm. Oviparous. Uterus with

several eggs (Fig. 1C). Thick-shelled, non-embryonic eggs, length 79 (74–85), width 57 (46–61) (Fig. 1I).

Taxonomic summary

Host: *Kinosternon scorpioides* Linnaeus, 1766

Location: Serra dos Carajás (6°6'29"S 50°18'16"W), Pará, Brazil.

Voucher specimens: 10 males (MPEG 291) and 11 females (MPEG 292) deposited in the Collection Other Invertebrates of the Museu Paraense Emílio Goeldi.

Site of infection: Large intestine.

Prevalence: 4 infected hosts out of 4 analyzed (100%).

DISCUSSION

Falcaustra sanjuanensis was described as parasitizing the large intestine of *Odontophrynus* cf. *barrioi*, an anuran typical of arid and semiarid environments, in Pie de Palo, Quebrada de las Flores, Province of San Juan, Argentina. We found 34 specimens (16 males and 18 females) of this nematode in the large intestines of *K. scorpioides* from Brazil. The morphological and morphometric characteristics of these nematodes are similar to those reported in the original description. However, in our study, we also describe the deirids, which was not observed by Gonzalez *et al*. (2013).

According to Anderson *et al*. (2009), the presence of an oral opening with 3 well-developed lips, an esophagus with a short anterior pharynx, and a subspherical isthmus slightly anterior to the esophageal bulb are the main diagnostic characteristics of *Falcaustra*. The species of the genus are differentiated mainly by the distribution of caudal papillae, length of the spicules, and the presence or absence of a pseudosucker (Bursey & Brooks, 2011).

Among the 12 known species of the genus only *F. condorcanquii*, *F. intermedia*, *F. mascula*, *F. pumacahuai*, *F. sanjuanensis* and *F. tiahuanaquensis* have a pseudosucker. *Falcaustra sanjuanensis* have similar length of spicules when compared to *F. mascula*, but it is longer than in *F. pumacahuai* and *F. tiahuanaquensis*, and smaller than *F. condorcanquii* and *F. intermedia*. Additionally, *F. sanjuanensis* differs from all those mentioned species with pseudosucker by having a different male caudal papillae number and arrangement.

Among non-Neotropical *Falcaustra* species, *F. sanjuanensis* only resembles *F. andrias* (He, Liu & Ma, 1992) Liu, Zhang & Zhang, 2011 and *F. longispicula* Walton, 1927 by the pattern of caudal papillae. *Falcaustra andrias* from the Oriental realm has pseudosucker but differ by some metric data: smaller body length of males (9.93–12.41 mm in *F. sanjuanensis* vs. 5.47–7.78 mm) and females (10.68–14.23 mm in *F. sanjuanensis* vs. 7.60 –8.08 mm); smaller spicules length (1.18-1.32 mm in *F. sanjuanensis* vs. 0.48 to 0.57 mm); and length of the gubernaculum (175-245 in *F. sanjuanensis* vs. 100-109). Furthermore, these species have a different arrangement of post-cloacal papillae (first ventral pair, second lateral pair; third subventral pair; fourth dorsoventral pair, fifth lateral pair and sixth dorsoventral pair vs. 3 ventral pairs and 3 lateral pairs).

Falcaustra longispicula from the Nearctic realm resembles *F. sanjuanensis* from the present study by the length of spicules (1.18–1.32 mm vs. 1.20–1.21 mm). However, it has a larger length of spicules when compared to *F. sanjuanensis* described by González et al. (2013). Additionally, *F. longispicula* differ by the absence of pseudosucker, which is present in *F. sanjuanensis.*

SEM helped us to add new ultrastructural observations to *F. sanjuanensis* described by González *et al.* (2013). We observed details of the oral opening and the presence of deirids, positioned in the posterior half of the anterior end. The distribution

of the caudal papillae was also an important morphological characteristic observed by SEM to reinforce the similarity between the specimens found in Argentina and specimens from Brazil. Finally, we also obtained ultrastructural details of the excretory pore, vulva, anus, and tail.

Specimens of *F. sanjuanensis* from *K. scorpioides* show some intraspecific variations in metric data when compared to measurements presented by Gonzalez *et al.* (2013). In our specimens, males have shorter isthmus length (103–152 vs. 150–200), and longer spicules (1.18–1.32 mm vs. 0.45–0.67 mm) and tail (395–495 vs. 260–370); while in females the width of the pharynx (79–111 vs. 53–73) and the lengths of the tail (595–695 vs. 280–500) and eggs (74–85 vs. 63–72) are larger than in *F. sanjuanensis* described by González *et al*. (2013). Furthermore, the specimens described in our study have the largest spicule among the *Falcaustra* species with pseudosucker, and the second largest spicule among the neotropical species (Table 1).

González-Solís & Moravec (2004) suggest that intraspecific morphological and biometric variability among parasitic nematodes may be associated with the host's local ecological conditions. However, both the type locality and the new locality presented in this study are regions of mountain ranges, with similar climates, indicating that perhaps other factors are also related to these morphological and metric variations between specimens.

We consider plausible the hypothesis that *F. sanjuanensis* has switched from a chelonian (ancestral host) to an anuran (new host) since turtles originated first. For the host-switching, both hosts must coexist in an ecosystem, enabling the parasite to shift (Araujo *et al.,* 2015). *Kinosternon scorpioides* is widely distributed in South America, even in Argentina, the type locality of *F. sanjuanensis*, described as parasitizing an anuran of the genus *Odontophrynus* Reinhardt & Lütken, 1862. Both hosts have aquatic

habits and have relatively similar ecological niches and eating habits, which may increase the possibility of sharing the parasites among these animals (Aho, 1990).

When a parasite changes its host and colonizes a different niche, it enriches the local biodiversity and if this change is accompanied by strong isolation from the ancestral host, it favors genetic differentiation and possible speciations (Jaramillo & Rivera-Parra, 2018). However, it is possible that the host switch event of the nematode *F. sanjuanensis* was relatively recent and, therefore, possible genetic alterations have not yet manifested themselves phenotypically to the point where we consider that there was a morphological differentiation among different populations of this parasite species.

Another hypothesis is that the phenomenon of “ecological conservatism” has occurred, in which the exchange of hosts overcomes cospeciation; that is, the parasite manages to establish a host exchange but does not undergo changes. *Falcaustra sanjuanensis* managed to expand its distribution once it acquired a new host but remained morphologically indistinct from the parasites of the ancestral host. Thus, the interaction with a new vertebrate host taxon does not seem to have hampered the parasite's distribution, indicating that it is probably an organism with more generalist characteristics (Hoberg & Brooks, 2008).

To date, 8 species of nematodes have been reported in *K. scorpioides*, named: *Atractis impura* Caballero, 1944; *Klossinemella caballeroi* Brenes & Bravo-Hollis, 1960; *Serpinema kachugae* Baylis & Daubney, 1922; *Serpinema magathi* Sprehn, 1932; *Serpinema monospiculatus* Freitas & Dobbin Jr., 1962; *Serpinema pelliculatus* Silva, Jesus & Melo, 2023; *Spiroxys figueiredoi* Freitas & Dobbin Jr.,1962; and *Capillaria* sp. (Alho, 1965; Brenes & Bravo-Hollis, 1960; Freitas & Dobbin Jr., 1971; Hungría, 1978; Bursey & Brooks, 2011; Viana *et al.,* 2016; Pereira *et al*., 2018; Silva *et al*., 2023). Therefore, in this study, we present new morphologic data, location records, and a new host for the *F. sanjuanensis* species.

Author contributions: CRediT (Contributor Roles Taxonomy)

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Software: YW, LFSTC

Supervision: LMOS, RFJ, BN, LFSTC, JNS, FTVM

Validation: LMOS, RFJ, FTVM

Visualization: LMOS, RFJ, BN, FTVM

Writing – original draft: LMOS, RFJ, BN, FTVM

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Table 1. Morphometric data of *Falcaustra sanjuanensis* from the original description and the present study.

	Falcaustra sanjuanensis (present study)		*Falcaustra sanjuanensis* (González et al., 2013)	
Host	*Kinosternon scorpioides*		*Odontophrynus* cf. *barrioi*	
Locality	Brazil		Argentina	
Sex	Male	Female	Male	Female
Body length (mm)	9.93–12.41	10.68–14.23	11.17–13.45	10.10–15.50
Body width at esophagus-bowel junction	300–394	315–421	270–415	310–485
Pharynx length×width	63–100×63–100	63–116×79–111	86–110×50–72	85–110×53–73
Esophagus (except isthmus and bulb) length(mm)×width	1.32–1.66×89–111	1.60–1.88×95–153	1.55–1.94×50–98	1.67–1.98×60–100
Isthmus length×width	103–152×95–126	135–158×116–153	150–200×90–150	125–200×100–150
Bulb length×width	176–232×168–216	179–242×184–232	160–215×170–215	160–250×160–230
Nerve ring*	345–426	384–453	350–460	370–450
Deirids (mm)*	0.97–1.26	1.09–1.33	–	–
Excretory pore (mm)*	1.09–1.40	1.25–1.55	1.06–1.55	1.18–1.64
Tail length	395–495	595–695	260–370	280–500
Pseudosucker length	307–453	–	250–450	–
Gubernaculum length	175–245	–	175–230	–
Spicules length	1.18–1.32	–	450–675	–
Vulva (mm)*	–	4.20–5.13	–	3.0-5.4
Vulva-anus distance	–	3.57–4.59	–	–
Eggs length	–	74–85**	–	63–72
Eggs width	–	46–61**	–	50–58

*Distance from the anterior end.

** Measurmentss based on the average of 10 eggs from each specimen analyzed.

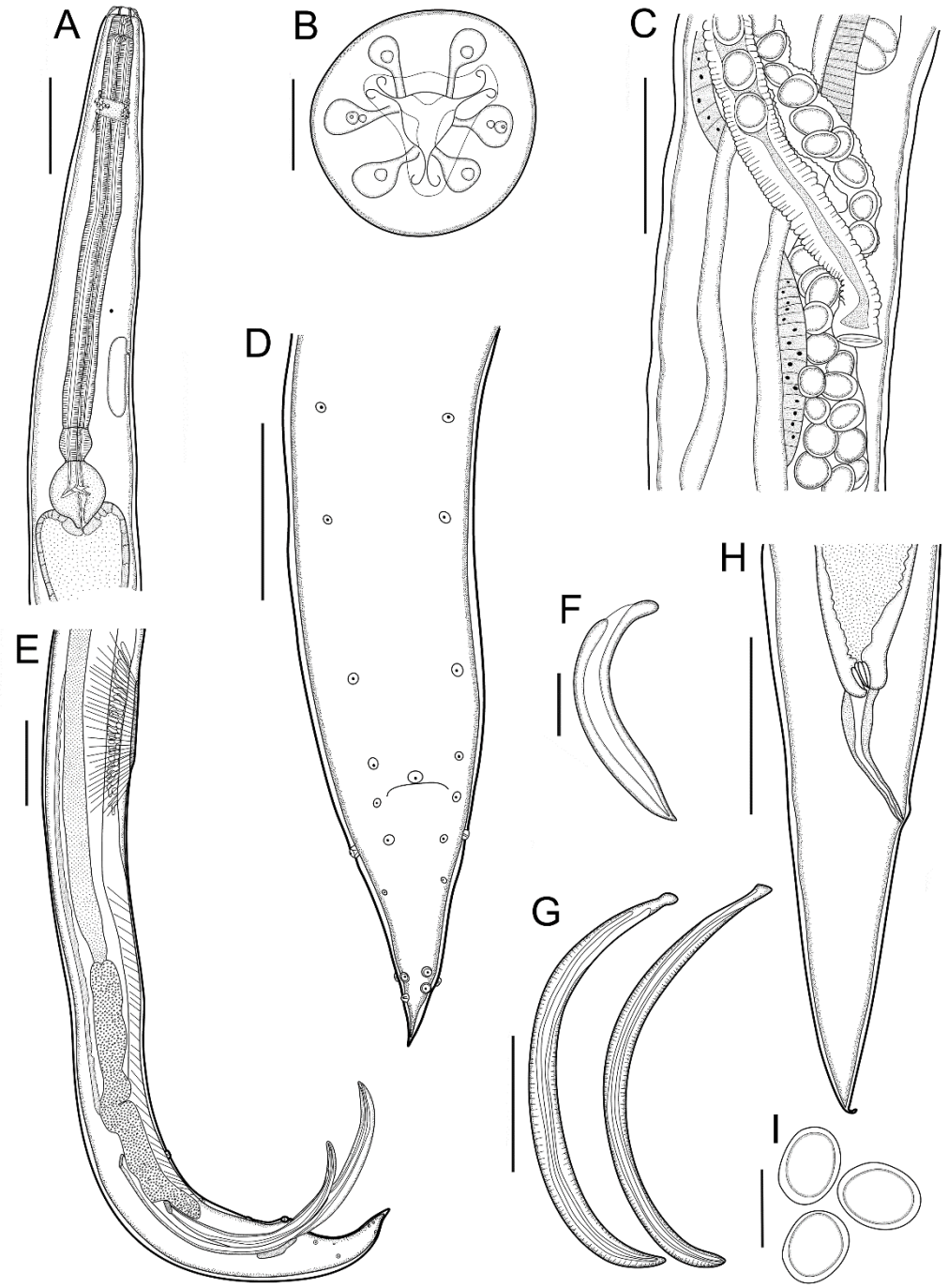

Figure 1. Light microscopy of *Falcaustra sanjuanensis* González, Sanabria & Quiroga, 2013 parasite of *K. scorpioides*. A, Anterior extremity of male, ventral view; B, Cephalic

extremity, apical view; C, Uterine region, side view; D, Posterior extremity of male, distribution of caudal papillae, ventral view; E, Posterior end of male, side view; F Gubernaculum, side view; G, Spicules, side view; H, Posterior extremity of female, side view; I, Eggs, front view. *Scale-bars*: A, C, D, E, G, H, 350 µm; B, F, 50 µm; I, 25 µm.

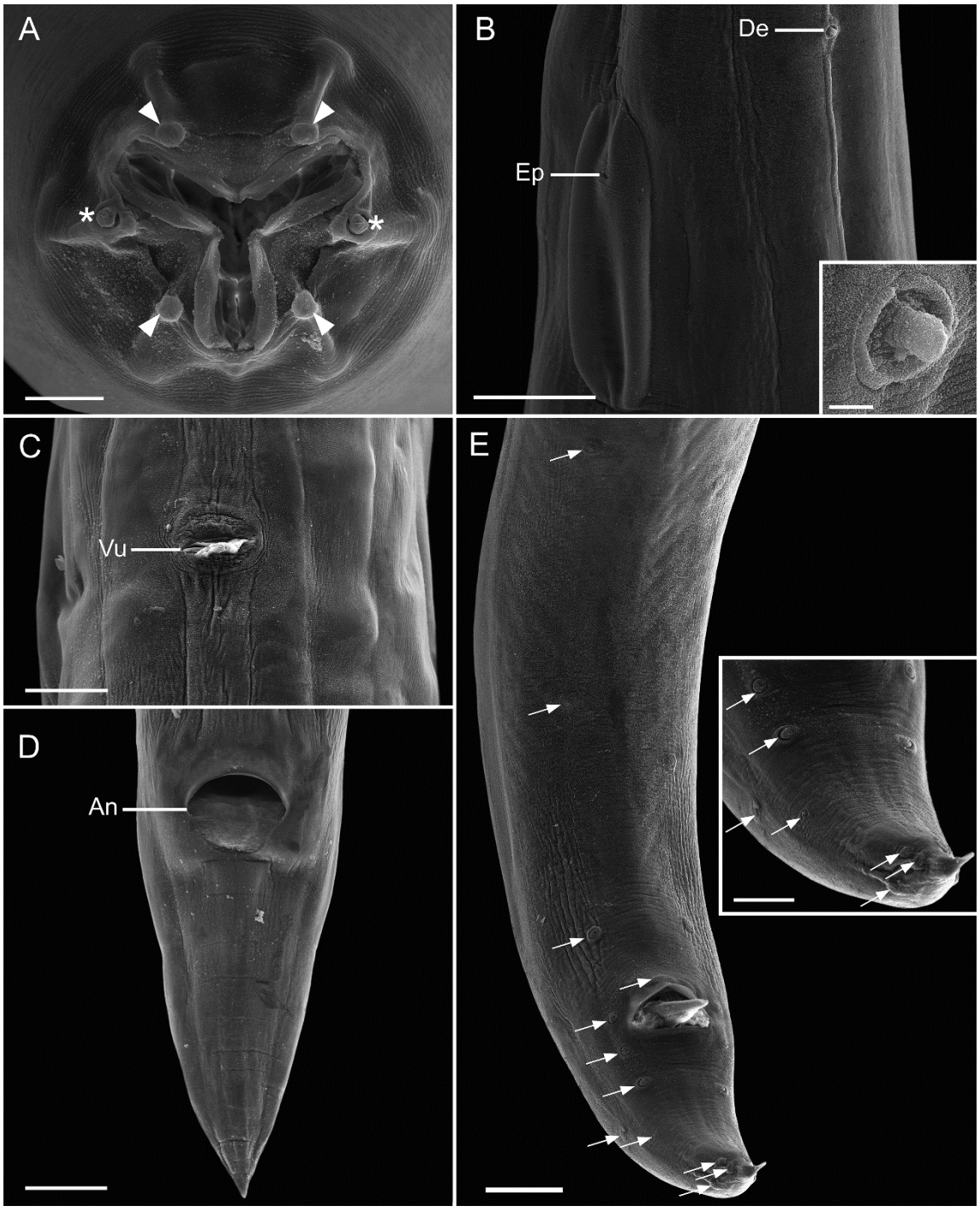

Figure 2. Scanning Electron Microscopy of *Falcaustra sanjuanensis* González, Sanabria & Quiroga, 2013 parasite of *K. scorpioides*. A, Cephalic end, apical view, showing cephalic papillae (arrow) and amphid (asterisk); B, Anterior extremity, ventrolateral view, excretory pore and deirid; C, Female, ventral view, vulva; D, Female, ventral view, anus and tail; E, Posterior region of the male, distribution of the caudal papillae (arrow). Inset:

detail of the deirid and papillae near the tip of the tail (arrow). Abbreviations: De, deirid; Ep, excretory pore; Vu, vulva; An, anus. *Scale-bars*: A, 20 µm; B, 100 µm; Inset, 5 µm; C, D, E, 100 µm; Inset, 50 µm.