

Short notes

Presence of *Kalicephalus subulatus* Molin, 1861 (Nematoda, Diaphanocephalidae) in Wagler's snake, *Xenodon merremi* from Argentina

Cynthia E. González, Eduardo F. Schaefer, Marta I. Duré

Centro de Ecología Aplicada del Litoral (CECOAL), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Ruta Provincial Número 5, km 2,5; W 3400 AMD; Corrientes, Argentina

Corresponding Author: Cynthia González; e-mail: cynthyaelizabethg@hotmail.com

ABSTRACT. This is the first record of the nematode parasite *Kalicephalus subulatus* (Strongylida, Diaphanocephalidae) in Wagler's snakes, *Xenodon merremi* (Squamata, Colubridae), collected in Corrientes, Argentina. The species was observed using light and scanning electron microscope (SEM) and compared to previous studies from Tropical America. As for the metric characters, the specimens collected in Argentina are smaller than all of those found so far in other hosts. However, despite the differences in the measurements, some characters such as the dorsal lobe type III and the amphidelphic uterus allow its specific identification. Previously, this genus was found in *X. merremi* from Argentina but the absence of males precluded their specific identification.

Key words: nematode parasites, Strongylida, *Xenodon merremi*, Corrientes

Introduction

Studies about helminths in Argentinean snakes refer mainly to trematodes collected in different host species from the northeast region. Lunaschi and Drago [1,2] reported 23 species of digenean for 33 species of Argentinean snakes. Although studies on nematodes are scarce for snakes, there are some reports for this country, as in the case for nematodes of the genus *Camallanus* Railliet et Henry, 1915 found in *Hydrodynastes gigas* (Duméril, Bibron and Duméril, 1854) and *Aplectana travassosi* (Gomes and Motta, 1967) Baker, 1980, *Physaloptera liophis* Vicente and Santos, 1974 and *Kalicephalus* sp. collected from *Xenodon merremi* (Wagler, 1824) both collected in the Province of Chaco; and *Kalicephalus costatus* (Rudolphi, 1819) collected from *Erythrolamprus semiaureus* (Cope, 1862) (= *Liophis miliaris semiaureus*) in the Province of Entre Ríos [3–5].

The genus *Kalicephalus* Molin, 1861, is a parasite of the esophagus, intestine and stomach in snakes and lizards [6]. Schad [7], revised this genus, described 5 new species and synonymized many species. The

results were 23 species arranged into 6 groups according to the following morphological characters: buccal capsule, uterus, spicules and bursa. At present, the genus comprises 33 species distributed around the world [8].

Xenodon merremi is a colubrid (Dipsadinae) snake whose distribution spans Guyana, Surinam, French Guiana, Brazil, Venezuela, Paraguay, North of Argentina, Bolivia, and Uruguay [9]. Specifically, in Argentina, this species is distributed in the provinces of Buenos Aires, Catamarca, Chaco, Córdoba, Corrientes, Entre Ríos, Formosa, Jujuy, La Rioja, Misiones, Salta, San Juan, San Luis, Santa Fe, Santiago del Estero, and Tucumán [10]. These are snakes with particularly variable color patterns, robust body that can reach 130 cm in length, females larger than males, aggressive, and with the most frequent mimetic color pattern with poisonous species of the genus *Bothrops* Wagler, 1824 [11–13]. They have annual oviparous reproduction and the clutch is recorded between October and December in Argentina [14]. It has terrestrial habits [15,16] and feeds mainly on several species of toads and frogs; however, it mainly ingests toads of the genus

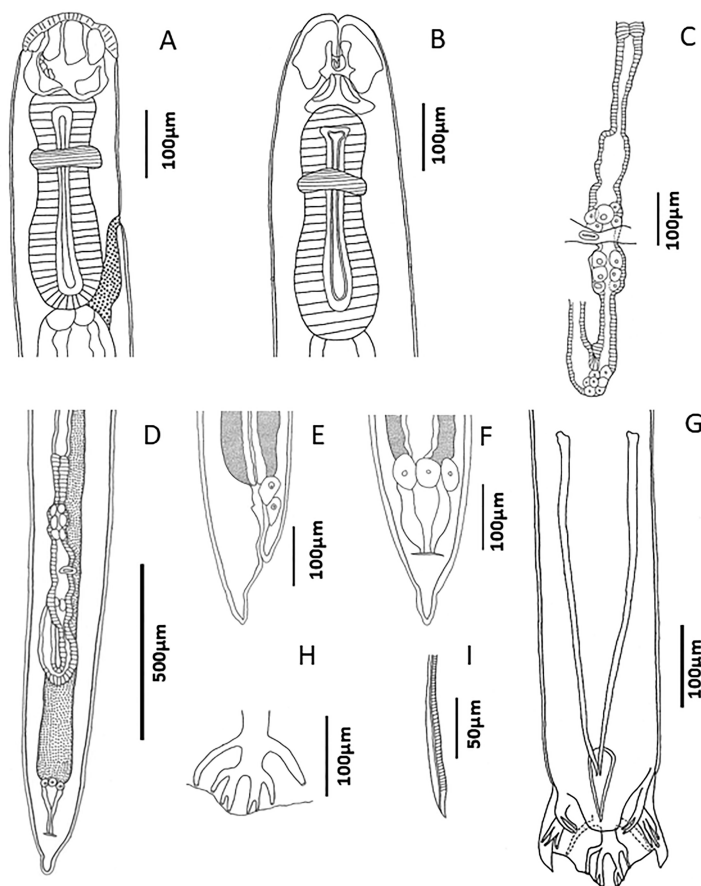


Fig. 1. *Kalicephalus subulatus* Molin, 1861 from *Xenodon merremi* from Corrientes, Argentina. A. Anterior end of female, lateral view. B. Anterior end of female, dorsal view. C. Reproductive system of female, ventral view. D. Posterior end of female, ventral view, showing vulva and anus. E. Posterior end of female, lateral view. F. Posterior end of female, ventral view. G. Posterior end of male, ventral view, showing the spicules and gubernaculum. H. Detail of dorsal ray, ventral view; I. Spicule, detail of distal end.

Rhinella [11,13,15,17]. It is also interesting to mention, some bibliographical references in which the presence of arthropod remains (ants, coleopteran, and spiders) was mentioned [18], although they would be part of the stomach content of the anurans ingested by the snake, and not items in the diet of *Xenodon merremi* [19].

The aim of the present study is to provide morphological details of *K. subulatus* Molin, 1861 on the basis of specimens recovered from a colubrid snake *X. merremi* captured in the Province of Corrientes, Argentina.

Materials and Methods

Five snakes belonging to species *Xenodon merremi* were collected in Corrientes, Corrientes province (27°29'33,89"S; 58°45'33,63"W), between May, 2015 and June, 2016. Snakes were transported alive to the laboratory and then euthanized by intraperitoneal administration of anesthesia (carticaine L-adrenaline). At necropsy, hosts were sexed and the esophagus, stomach, gut, lungs, liver, urinary bladder, kidneys, body cavity, and musculature skin examined for parasites. Taxonomy of the host is in accordance with Uetz et al. [9]. Nematodes were fixed in 10% hot formaldehyde solution and preserved in 70% ethanol and cleared with lactophenol for examination under light microscope.

Illustrations were made using a Leica microscope DM 2500 with the aid of a lucid camera. For examination with scanning electron microscope (SEM), the nematodes were postfixed in 1% OsO₄, dehydrated through an ethanol and an acetone series and then subjected to critical point drying. The specimens were coated with gold and examined with a Jeol 5800LV scanning electron microscope. All measurements are given in micrometers unless otherwise indicated, as the mean ±SD with the range in parentheses. Nematodes were deposited in the Helminthological Collection of Centro de Ecología Aplicada del Litoral (CECOAL), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Corrientes City, Corrientes, Argentina; accession number CECOAL 16061001 (2 females, 2 males). Snakes were deposited in the Colección Herpetológica of the Universidad Nacional del Nordeste; accession number UNNEC 13116.

Results and Discussion

Eighteen specimens of *K. subulatus* (12 females and 6 males) were found in the intestine of one examined specimens (1/5: prevalence 20%). Measurements of the specimens in the present study and those provided by previous authors are shown in Table 1. Morphological details, observed with light microscopy and SEM, are shown in Figures 1 and 2.

General: Both sexes with similar size. Body cuticle finely transversely striated, with no inflations. Slightly rounded head (Fig. 1A,B). Buccal capsule consists of two lateral valves with the following chitinous pieces: two large lateral plates, anterior ventral plate and small anterior

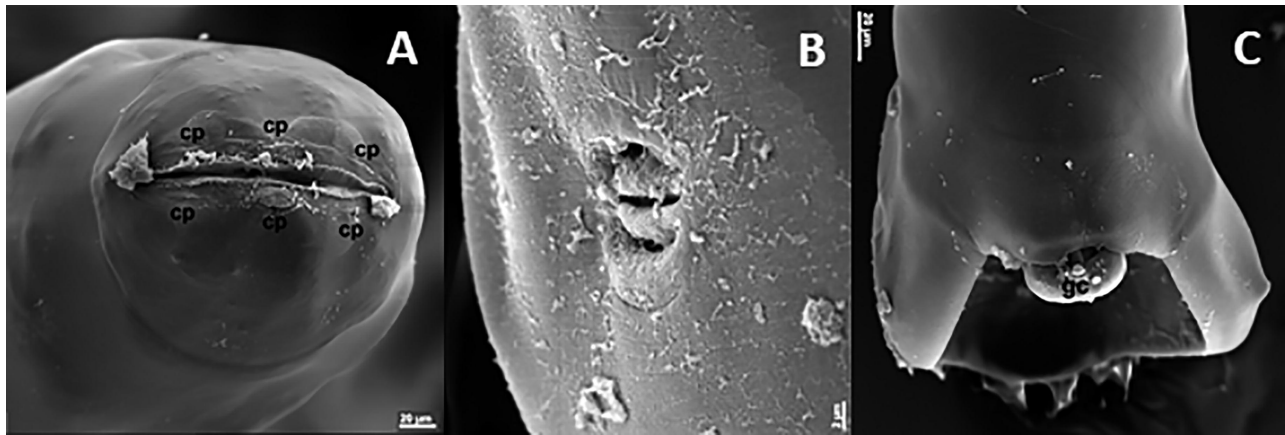


Fig. 2. *Kalicephalus subulatus* Molin, 1861 from *Xenodon merremi* from Corrientes, Argentina by SEM. A. Cephalic end of female, apical view; B. Detail of vulva, ventral view; C. Posterior end of male, ventral view. Abbreviations: cp: cephalic papilla; gc: genital cone.

dorsal plate small, largely triangular posterior dorsal plate and posterior ventral plate, smoothly rounded posteriorly, not forming posterior extension. Anterior ridges relative wide; the entire ridge, in lateral view, is seen as an arc. Buccal capsule with prominent dorsal gutter. Six cephalic papillae, three in each lateral valve, one dorsal, one medial and one ventral (Fig. 2A). Esophagus club-shaped, muscular, with prominent egg-shaped bulb (Fig. 1A,B). Excretory pore situated slightly posterior to nerve ring (Fig. 1A). Nerve ring surrounding esophagus between its anterior and middle thirds. Deirids minute, situated slightly posterior to the nerve ring.

Male: Bursa typical of the genus (Figs. 1G–2C). Ventral rays directed forward, thinner than lateral rays, applied throughout most of their length, separate only at tips; these tips evenly spaced, they do not reach the margin of bursa. Lateral ray with short common stem. Externolateral set off at base, blunter, more thumb-like; short antero-lateral ray, directed forward, rounded tip; medial and postero-lateral rays directed backwards. Type III dorsal ray of Schad [7] (Fig. 1G,H). Rounded genital cone, papillae are not evidenced (Fig. 2C). Spicules and gubernaculum well sclerotized; equal long thin alate spicules, they are gradually sharpened and end in spatulate tip (Fig. 1I); gubernaculum appears as a long narrow wedge (Fig. 1G), telamon is not evidenced.

Female: Vulva near beginning of posterior third of body, lips only slightly raised, often in shallow depression (Fig. 1D,2B). Amphidelphic; short and stout ovoiders (Fig. 1C). Anterior ovarian coils crowded to anterior uterus. Females analyzed in this study did not have eggs.

Schad [7] expressed that *K. subulatus* was

restricted to the *Boa constrictor* Linnaeus, 1758; however, Fernandes and Artigas [20] found this species of nematode in serpentes of the genus *Epicrates* Wagler, 1830 and *Corallus* Daudin, 1803, two genera that also belong to the Boidae family. Subsequent works such as Gómez and Sánchez [21] and González-Solís et al. [22] carried out in Peru and Mexico, respectively by reported the finding of *K. subulatus* also in boids, in both cases of *Boa constrictor*. The present study refers to the finding of *K. subulatus* in a non-boid snake species, *Xenodon merremi* from the province of Corrientes, Argentina. Previously, the genus *Kalicephalus* was found in *X. merremi* but in this case the specimens were not identified at species level [5].

The measurements of morphological characters of females and males of *K. subulatus* studied here are smaller than those found in the specimens studied in other hosts and localities. This may be due to the fact that the specimens studied in this work are immature (no eggs were found in any of the females examined). Intraspecific variation in adult nematode parasites has been attributed to different factors, such as size, age, physical condition, metabolic rate, and food supply of the host at the time of the entrance and growth of the parasite, in addition to climate or locality [23–25].

Of the South American species of the genus *Kalicephalus*, *K. subulatus* is distinguished from others because the females of *K. appendiculatus* Molin, 1861 and *K. costatus* are prodelphic; while the females of *K. subulatus*, *K. inermis* Molin, 1861 and *K. rectiphilus* Harwood, 1932 are amphidelphic. These last two species are different because *K. inermis* has the dorsal ray type V, and *K. rectiphilus* has dorsal ray type II.

Table 1. Comparative morphometrics of females and males of *Kalicephalus subulatus* from different hosts and localities

Host	Schad [7]		Fernandes and Artigas [20]			Gómez and Sánchez [21]		Present study
	<i>Boa constrictor</i>	<i>Boa constrictor</i>	<i>B. constrictor</i>	<i>Epicratus cenchria cenchria</i>	<i>Corallus caninus</i>	<i>Boa constrictor</i>	<i>Xenodon merremi</i>	
Locality	*	Manaus, Amazonas State, Brazil	Pará State, Brazil	Vale do Guaropé, Acre State, Brazil	Marabá, Pará State, Brazil	Nayta, Iquitos Province, and Lluylucuecha, Moyabamba Province, Perú	Corrientes, Corrientes Province, Argentina	
Females (n)	25	4	2	3	2	?	11	
Total length	6.93-10.47mm	3.22-8.44mm	5.08-5.49mm	6.80-7.67mm	8.16-8.48 mm	8.18mm (7.0-9.25)	4.57±0.55mm (3.74-5.52)	
Width	340-500	360-480	410	430	390-450	440 (400-520)	204.6±23.8 (166.3-232.7)	
Anterior end in lateral view	230-300	300-520	340-360	280-320	280	300 (280-320)	160.9±14.8 (140-190)	
Buccal capsule depth	170-220	200-320	220-250	180-210	210-240	200 (180-220)	142.3±14.6 (115-160)	
Deirids L	450-590						361.0±33.5 (320-410)	
Esophagus length	380-490	350-510	600-610	440-460	470-480	460 (430-480)	347.4±22.3 (315-385)	
Width of esophagus at anterior end							98.9±6.28 (90-108)	
Width of esophagus at midlength							83.2±7.2 (70-92)	
Maximum width of bulb	210-280	250-300	310-320	270-290	240-280		127.0±8.92 (110-138)	
Nerve ring L	250-380						259.5±17.1 (230-285)	
Excretory pore L	400-530					360 (350-380)	351.3±31.7 (305-400)	
Vulva †		2.54-3.01mm	1.82-2.04	2.33-2.97mm	2.82-2.90mm	2.78mm (2.70-2.88)	0.72±0.079 mm (0.61-0.83)	
Tail length	240-390	260-300	220-260	270-470	300	300 (260-320)	100.7±10.9 (85-120)	
Egg length		76	-	78-86	68	70 (60-70)	-	
Egg width		40	-	47-48	41-42	50 (40-50)	-	

Table 1. continuation

Males (n)	25	2	2	2	2	1	?	4
Total length	6.41-8.31 mm	6.04-6.18 mm	4.89-5.88 mm	6.0-6.86 mm	5.27 mm	7.18 mm (6.25-8.25)	3.65±0.38 mm (3.4-4.1)	
Width	220-390	310-340	300-340	300-320	270	370 (310-430)	168.0±8.23 (160-179.5)	
Anterior end in lateral view	200-270	270-280	240-260	250-260	250	260 (270-280)	135.7±4.3 (132-142)	
Buccal capsule depth	150-190	200	110-180	190-210	170	170 (140-190)	121.2±6.3 (115-130)	
Deirids* \bar{L}	410-520	-	-	-	-	-	295.0±7.07 (290-300)	
Esophagus length	340-420	400-450	420-430	420-440	390	420 (360-450)	317.5±11.9 (305-330)	
Width of esophagus at anterior end	-	-	-	-	-	-	88.2±8.65 (80-98)	
Width of esophagus at midlength	-	-	-	-	-	-	73.7±4.7 (70-80)	
Maximum width of bulb	180-230	230	190-230	230-250	240	-	112.2±4.1 (109-118)	
Nerve ring \bar{L}	250-310	-	-	-	-	300 (280-330)	220±21.6 (200-250)	
Excretory pore \bar{L}	390-510	-	-	-	-	-	308.7±37.9 (280-360)	
Spicules length	510-650	570-580	590	-	540	580 (530-630)	458.7±25.3 (430-490)	
Gubernaculum length	-	-	-	-	-	-	122.3±11.6 (112-135)	

*Schad [7] author examined specimens deposited in 15 different collections from different localities.

 \bar{L} from anterior end; † from posterior end.

Table 2. Helminth parasites found in *Xenodon merremi* from Argentina

Helminth species	Locality	References
Nematoda		
<i>Aplectana travassosi</i> (Gomes and Mota, 1967) Baker, 1980	Colonia Las Mercedes, Chaco province	Lamas et al. [5]
<i>Physaloptera liophis</i> Vicente and Santos, 1974		
<i>Kalicephalus</i> sp.		
<i>Kalicephalus subulatus</i> Molin, 1861	Corrientes, Corrientes province, Argentina	Present study
Digenea		
<i>Catadiscus Freitaslenti</i> Ruiz, 1943	Córdoba, Santa Fe, and Chaco provinces	Lunaschi and Drago [1]
<i>Catadiscus longicoecalis</i> Caubisens Poumarau, 1965	Entre Rios and Chaco provinces	
<i>Opisthgonimus fonsceai</i> Ruiz and Leão, 1942	Misiones province	
<i>Opisthgonimus interrogativus</i> (Nicoll, 1914) Pereira, 1928	Misiones province	
<i>Opisthgonimus lecithonotus</i> Lühe, 1900	Córdoba, Entre Rios, Santa Fe, Chaco, and Corrientes provinces	
<i>Opisthgonimus megabothrium</i> Pereira, 1928	Misiones province	
<i>Opisthgonimus misionesensis</i> Lunaschi and Drago, 2001	Misiones province	
<i>Styphlodora condita</i> Faria, 1911	Chaco, Córdoba, Entre Rios, Salta, and Corrientes provinces	
<i>Travtrema stenocotyle</i> (Cohn, 1902) Goodman, 1951	Córdoba, Misiones, Entre Rios, Corrientes, Santa Fe, and Chaco provinces	

The life cycle of this species is not defined but Jacobson [26] suggests that they have a direct cycle and that the infecting form is acquired by water or food. However, Sánchez et al. [27] suggest a possible active penetration of larvae through the skin.

So far, the helminthfauna of *X. merremi* from Argentina comprises twelve species; nine digenean and three nematodes (see Table 2). With this work we add the species *K. subulatus* to the helminths that parasitizing *X. merremi*. Also, we add a new locality for this genus of nematode.

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