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A new species of *Microtetrameres* (Nematoda, Tetrameridae) parasitizing *Buteogallus urubitinga* (Aves, Accipitridae) from northeastern Argentina

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ABSTRACT

A new tetramerid nematode, *Microtetrameres urubitinga* n. sp., is described from specimens recovered from the proventriculus of the great black-hawk, *Buteogallus urubitinga* (Aves: Accipitridae), from Formosa Province, Argentina. The males of the new species are characterized by having spicules unequal (length ratio of spicules 1:3.8–5.9) and dissimilar in shape (right spicule with a simple tip, left spicule with a symmetrical bifurcated tip), caudal papillae arranged asymmetrically (two pairs precloacal and two pairs postcloacal) and cloacal lips highly protruded forming a tube. The gravid females are permanently coiled clockwise or counterclockwise in a spiral and having a tail tapering gradually to a sharp point, with a cuticular fold. This is the first nominal species of *Microtetrameres* (Travassos, 1915) described parasitizing birds from Argentina. The relationship between the diet of *B. urubitinga* and the low prevalence of *M. urubitinga* n. sp. is discussed.

Key words: Accipitridae, Argentina, *Buteogallus urubitinga*, *Microtetrameres urubitinga* n. sp., Tetrameridae.

INTRODUCTION

The great black-hawk, *Buteogallus urubitinga* (Gmelin) (Accipitridae), is a diurnal bird of prey found in the open savanna and swamp edges of the Neotropical Region, from Mexico through Central America to Bolivia, Uruguay and central Argentina (Thiollay 1994). The helminth fauna of the great black-hawk is scarcely known, so far seven species of helminths have been recorded parasitizing this species, *Thelazia (Thelaziella) aquilina* Baylis, 1934 (Nematoda-Thelaziidae), *Contracaecum caballeroi* Bravo-Hollis, 1939 (Nematoda-Anisakidae), *Neodiplostomum microcotyle* Dubois, 1937 (Digenea-Diplostomidae), and *Oligacanthorhynchus iheringi* Travassos, 1916 (Acanthocephala-Oligacanthorhynchidae) from Brazil, *Choanotaenia trapezoides* (Fuhrmann, 1906) (Cestoda- Dilepididae) from British Guiana and Brazil, *Parastrigea macrobursa* Drago and Lunaschi, 2011 and *Strigea proteolitica* Drago, Lunaschi and Draghi, 2014 (Digenea, Strigeidae) from Argentina (Travassos 1917, Vevers 1923, Dubois 1937, Pinto et al. 1994, Drago and Lunaschi 2011, Drago et al. 2014, Justo et al. 2017).

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Among nematodes, several members of the family Tetrameridae Travassos, 1914 are frequently found parasitizing birds. Chabaud (1975) recognized three subfamilies: Crassicaudinae Yorke and Maplestone, 1926; Geopetitinae Chabaud, 1951 and Tetramerinae Raillet, 1915. The subfamily Crassicaudinae contains two genera, *Crassicauda* Leiper and Atkinson, 1914 found in the urogenital system and the cranial pterygoid sinuses of cetaceans, and *Placentonema* Guvanov, 1951 reported in the placenta, uterus, mammary glands and subdermis of sperm whales (Hermosilla et al. 2015, Keenan-Bateman et al. 2016). The subfamily Geopetitinae contains only one genus, *Geopetitia* Chabaud, 1951, found in capsules attached to the serosa of the oesophagus, proventriculus and gizzard from birds (Anderson 2000). The subfamily Tetramerinae contains three genera, *Microhadjelia* Jogis, 1965, *Tetrameres* Creplin, 1846 and *Microtetrameres* (Travassos, 1915).

The members of *Microhadjelia* are found in the digestive tract of passeriform birds. They are filiform worms, the females are totally coiled, while the males only have the posterior end coiled (Quentin and Wertheim 1975). The members of *Tetrameres* and *Microtetrameres* are parasites of the proventriculus of birds. The females are typically embedded in the gastric glands, with the tails directed towards the lumen of the proventriculus. The filiform males are generally found on the mucosa, in the lumen of the proventriculus, or in the crypts (associated or not with females). The evidence suggests that males move about freely, to reach and fertilize the stationary females (Anderson 2000). The taxonomic status of genus *Tetrameres* is closely related to that of the genus *Microtetrameres*. These nematodes originally were grouped by Diesing (1835) under the name *Tropidurus*, including species from Brazilian birds. Creplin (1846) renamed the genus as *Tetrameres*, because the name *Tropidurus* was preoccupied. Travassos (1915) separated the genus into two subgenera, *Tetrameres* and *Microtetrameres*. Cram (1927) raises to generic category both subgenera based on female body form; globular or spindle-shaped in *Tetrameres* and with its longitudinal axis spirally coiled in *Microtetrameres*.

The genus *Microtetrameres* currently includes over 50 nominal species parasitic in birds worldwide (Mawson 1977, Clark et al. 1979, Martínez Gómez et al. 1982, Mahdy and El-Ghaysh 1998).

The aim of this paper is to describe a new species of *Microtetrameres* obtained from the proventriculus of the great black-hawks, *B. urubitinga*, from northeastern Argentina.

The ZooBank Life Science Identifier (LSID) of this publication is: urn:lsid:zoobank.org:pub:0F813D24-C6EF-4D08-B26A-191924F8D143.

MATERIALS AND METHODS

Six specimens of *B. urubitinga* were collected between 2004 and 2012 at La Marcela farm (26°17'35"S, 59°08'38"W), Pirané, Formosa Province, Argentina, with authorization of Ministerio de la Producción y Ambiente, Dirección de Fauna y Parques of Formosa Province. The birds were captured with a shotgun and dissected in the field, the viscera preserved in 10% formalin and transported to the laboratory for examination. Nematodes were removed from the proventriculus and preserved in 70% alcohol. For examination, the males were cleared by immersion in glycerine-alcohol and the females in Amman's lactophenol. Measurements are given in micrometres (μm) unless otherwise stated, as the range followed by mean in parentheses. Drawings were made with the aid of a drawing tube. The helminths were deposited in the Helminthological Collection of the Museo de La Plata (MLP–He) and the hosts in the Ornithological Collection of the Museo de La Plata (MLP–Or), La Plata, Argentina. Additionally, one

specimen of *Microtetrameres* sp. stored in MLP–He was examined (MLP–He 6734).

RESULTS

MORPHOLOGICAL DESCRIPTION

Tetrameridae Travassos, 1914
Microtetrameres (Travassos, 1915)
***Microtetrameres urubitinga* n. sp.** (Figs. 1–5, Tables I, II)
ZooBank Life Science Identifier (LSID) - urn:lsid:zoobank.org:act:40436225-3CC7-4CC1-A4C2-E613527FBB9C.

Diagnosis

Male (based on 4 entire worms and 3 damaged specimens): Whitish worms. Body elongated, tapering towards ends. Cuticle with fine transverse striations. Total length 2020–2540 (2354). Maximum width 60–71 (65). Buccal capsule cylindrical, moderately sclerotized, 19–24 (21) in depth and 5–7 (6) of inner diameter. Oesophagus 660–780 (712) long, muscular portion 250–280 (268), about 32–42% (38%) of oesophagus length and glandular portion 380–530 (445) long. Nerve ring at 124–180 (146) from the anterior end. Excretory pore not seen. Spicules unequal and dissimilar in shape. Right spicule slightly curved, 130–260 (189) long, with a simple tip. Left spicule thin, 770–1040 (953), 32–50% (41%) of body length, with a symmetrical bifurcated tip. Length ratio of spicules 1:3.8–5.9 (1:5.2). Cloacal lips highly protruded forming a tube. Two pairs precloacal papillae and two pairs postcloacal, all arranged asymmetrically. Tail ventrally curved forming a loop, 140–160 (150) long. (Figures 1, 2, Table I).

Female (based on 8 specimens): Red. Gravid specimens permanently coiled clockwise or counterclockwise in a spiral. Cuticle with fine transverse striations. Body without longitudinal furrows, 1285–2430 (1678) long by 743–1029 (885) wide in coiled position. Buccal capsule rounded, moderately sclerotized, 17–21 (19) in depth and 10–11 (10) of inner diameter. Oesophagus 770–1020 (885) long, muscular portion 160–210 (190), about 20–25% (22%) of oesophagus length and glandular portion 590–800 (673). Nerve ring at 119–220 (148) from the anterior end. Excretory pore not observed. Tail tapering gradually to a sharp point, with a cuticular fold, 161–240 (203) long. Vulva at 161–240 (203) from posterior end. Eggs elongate, with a thin eggshell, 1-1.5, near parallel sides, and without polar filaments. Young females with non-embryonated eggs. Fully gravid females with eggs containing fully developed larvae, 48–52 (49) long by 24 wide. (Figures 3-5, Table II).

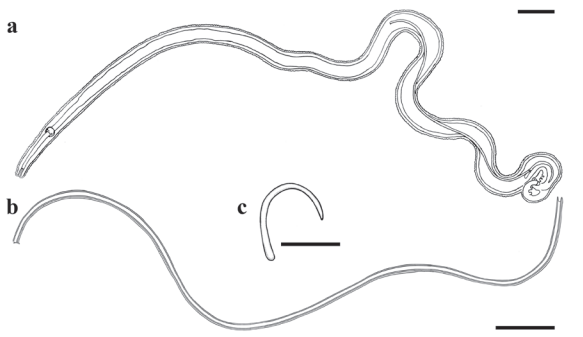

Figure 1 - Male of *Microtetrameres urubitinga* n. sp. **(a)** Entire worm. **(b)** Left spicule. **(c)** Right spicule. Scale bars: 100 μm.

TAXONOMIC SUMMARY

Type host: *Buteogallus urubitinga* (Gmelin) (Accipitridae), (great black-hawk).

Site of infection: Proventriculus. Females within the glands, males free in the lumen.

Type locality and collection date: La Marcela farm (26°17'35"S, 59°08'38"W), Pirané, Formosa Province, Argentina. 19 June 2012.

Prevalence and mean intensity: 1 of 6 birds examined (16.7%), females 80, males 22.

Specimens deposited: Holotype (male) MLP–He 7447; allotype (female) MLP–He 7448;

TABLE I
Comparative data for males of *Microtetrameres urubitinga* n. sp. and South American *Microtetrameres* spp.

	M. urubitinga n. sp.	*M. cruzi*	*M. minima*	*M. pusilla*
Source	Present study	Travassos 1914	Travassos 1914	Travassos 1915, 1919
Country	Argentina	Brazil	Brazil	Brazil
Hosts	*B. urubitinga*	*N. swainsoni*, *M. flavifrons*	*T. c. brunneus*	*T. rufiventris*, *T. flavipes*
Body length	2020–2540	1170–1400	1400	3500–4000
Body width	60–71	85	-----	100–120
Buccal capsule depth	19–24	12	-----	17
Buccal capsule inner diameter	5–7	4–5	-----	7
Oesophagus	660–780	383	-----	900***
Muscular portion	250–280	93	-----	300
Glandular portion	380–530	290	-----	600**
Glandular/muscular portion	1.5–1.7	3.1*	-----	2***
Oesophagus/body length (%)	33	27–33*	-----	23–26***
Left spicule	770–1040	651–787	990	1320
Right spicule	130–260	82	100	85
Length ratio of spicules	3.8–5.9	7.9–9.6*	9.9*	15.5*
Body/left spicule length	2–3.2	1.5–2.2*	1.4*	2.7–3*
Tail	140–160	132	-----	170

* Calculated from original descriptions. **Estimated from plate XXV, figure 3 of Travassos (1919) given that Travassos (1915) described an oesophagus of 0.042 mm long, but evidently refers to width. *** Calculated from plate XXV figure 3 of Travassos (1919) and original description.

paratypes MLP–He 7449; voucher specimens: MLP–He 7450.

Etymology: The new species is named after the specific name of the host.

REMARKS

In South America, only three species of *Microtetrameres* are known parasitizing Piciform and Passeriform birds from Brazil: *Microtetrameres cruzi* (Travassos, 1914) in *Notharchus swainsoni* (Gray) (as *Bucco swainsoni*) and *Melanerpes flavifrons* (Vieillot); *Microtetrameres minima* (Travassos, 1914) in *Tachyphonus cristatus brunneus* (Spix) and *Passer domesticus* (Linnaeus); and *Microtetrameres pusilla* (Travassos, 1915) in *Turdus rufiventris* Vieillot and *Turdus flavipes* (Vieillot) (as *Platycichla flavipes*) (Travassos 1914, 1915, Brasil and Amato 1992). Also, there are four reports of *Microtetrameres* sp. parasitizing Passeriform birds, *Cyanocorax chrysops* (Vieillot), *Coryphospingus cucullatus* (Müller) and *Sturnus vulgaris* Linnaeus from Argentina (Boero and Led 1968, Valente et al. 2014), and *P. domesticus* from Peru (Sarmiento et al. 1999).

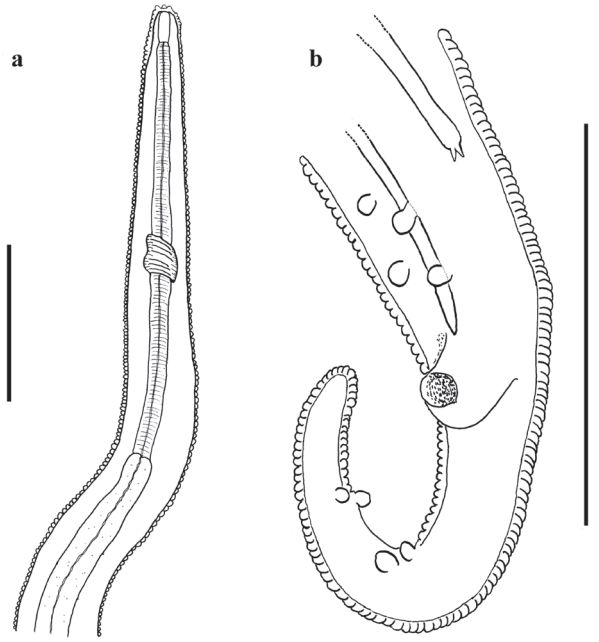

Figure 2 - Male of *Microtetrameres urubitinga* n. sp. **(a)** Detail of anterior end. **(b)** Detail of posterior end. Scale bars: 100 μm.

Microtetrameres cruzi can be differentiated from *M. urubitinga* n. sp. by having shorter males, shorter right spicule, higher spicule length ratio, different distribution of caudal papillae (1 pair precloacal and 3 pairs postcloacal arranged symmetrically) and females with shorter tail without cuticular fold (Tables I, II).

Microtetrameres minima can be distinguished from the new species by having shorter males, shorter right spicule, and higher ratio between spicules. Females differ from those of *M. urubitinga* n. sp. by being smaller and by having shorter tail and smaller eggs (Tables I, II).

Microtetrameres pusilla can be differentiated from *M. urubitinga* n. sp. by having longer males with longer left spicule, shorter right spicule, higher spicular ratio and different distribution of caudal papillae (two pairs precloacal, 1 pair adcloacal and 2 pairs postcloacal), and wider females with longitudinal furrows (Tables I, II).

The specimens of *Microtetrameres* sp. reported by Boero and Led (1968) in *C. chrysops* and *C. cucullatus* were not described; only a female and

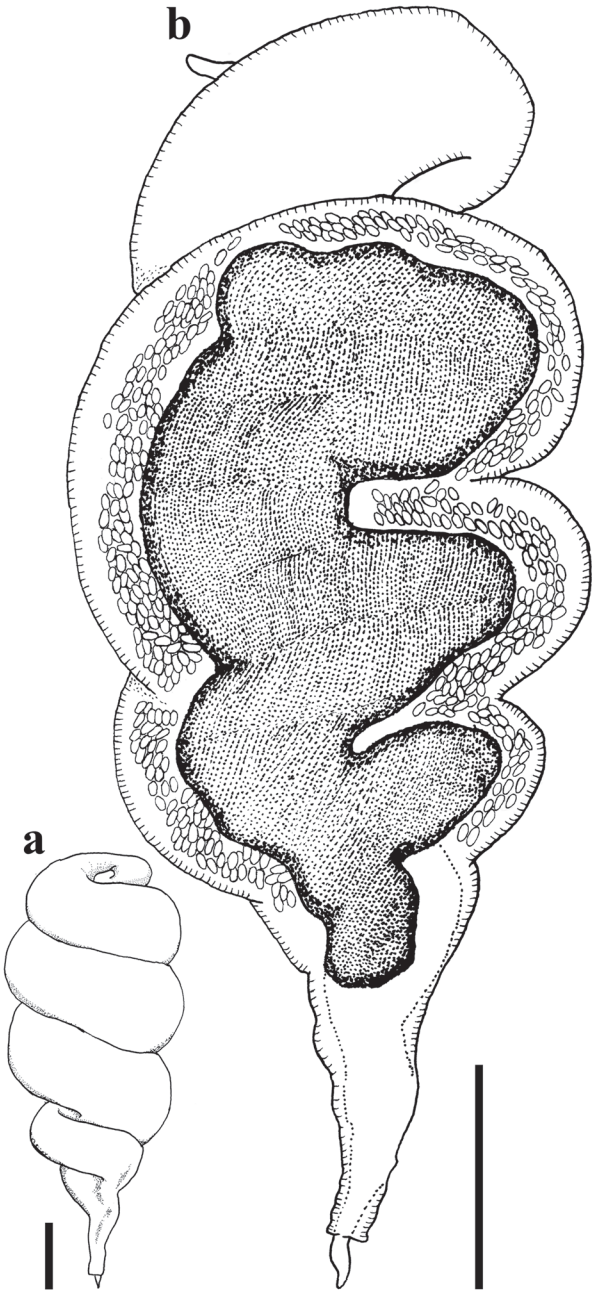

Figure 3 - Female of *Microtetrameres urubitinga* n. sp. **(a)** Entire worm showing spiral coiling. **(b)** Gravid female. Scale bars: 300 μm.

eggs were drawn without scale. Unfortunately, these specimens cannot be reviewed because no specimens were deposited at the appropriate time. The specimens of *Microtetrameres* sp. from *S. vulgaris* reported by Valente et al. (2014) were not described or drawn. The material deposited

TABLE II
Comparative data for females of *Microtetrameres urubitinga* n. sp. and South American *Microtetrameres* spp.

	M. urubitinga n. sp.	*M. cruzi*	*M. minima*	*M. pusilla*
Source	Present study	Travassos 1914	Travassos 1914	Travassos 1915, 1919
Country	Argentina	Brazil	Brazil	Brazil
Hosts	*B. urubitinga*	*N. swainsoni*, *M. flavifrons*	*T. c. brunneus*	*T. rufiventris*, *T. flavipes*
Body length	1285–2430	2000	780	2000
Body width	743–1029	1500	640	1500
Buccal capsule depth	17–21	16–20	12	10–19
Buccal capsule inner diameter	10–11	8	-----	9–12
Oesophagus	770–1020	780	563	
Muscular portion	160–210	160	73	210–273
Glandular portion	590–800	620	490	530–974
Glandular/muscular portion	3–3.9	3.9*	6.7*	2.5–3.6*
Distance nerve ring-anterior end	119–220	-----	-----	-----
Distance vulva- posterior end	161–240	300	-----	-----
Tail	161–240	74–100	68	140
Eggs	48–52 x 24	50–60 x 24–28	45 x 24	42–49 x 28–35

* Calculated from original descriptions.

by these authors (MLP-He 6734) consists of a single immature female; therefore it is not possible to compare with our specimens. The specimens of *Microtetrameres* sp. from *P. domesticus* listed by Sarmiento et al. (1999) were not described or drawn.

In accipitrid birds, several species of *Microtetrameres* have been reported: *Microtetrameres aquila* Schell, 1953 in *Aquila chrysaetos* (Linnaeus) and *Microtetrameres accipiter* Schell, 1953 in *Accipiter gentilis* (Linnaeus) from USA; *Microtetrameres paraccipiter* Mawson, 1977 in *Accipiter fasciatus* (Vigors and Horsfield), *Microtetrameres cerci* Mawson, 1977 in *Circus assimilis* Jardine and Selby from Australia, *Microtetrameres cloacitectus* Oschmarin, 1956 in *Buteo buteo* (Linnaeus) from Eurasia, *Microtetrameres creplini* (Vavilova, 1926) in *Accipiter nisus* (Linnaeus) from Russia, *Microtetrameres inermis* (Linstow, 1879) parasitizing *A. gentilis* and *A. nisus* from Europe, also reported in other groups of birds from Asia and Africa (Cram 1927, Schell 1953, Yamaguti 1961,

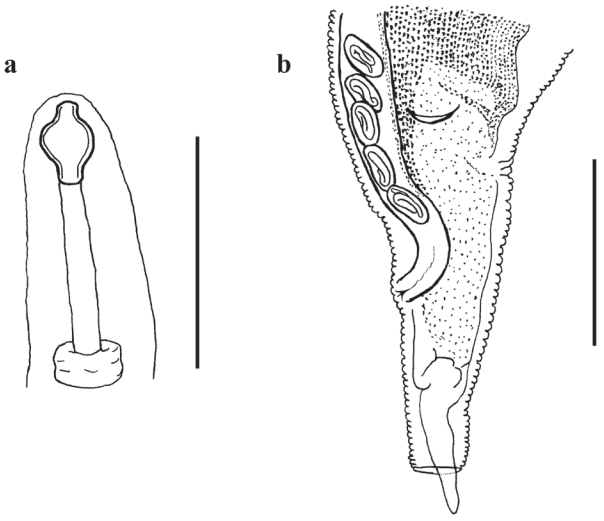

Figure 4 - Female of *Microtetrameres urubitinga* n. sp. **(a)** Detail of anterior end, scale bar: 50 µm. **(b)** Detail of posterior end, scale bar: 200 µm.

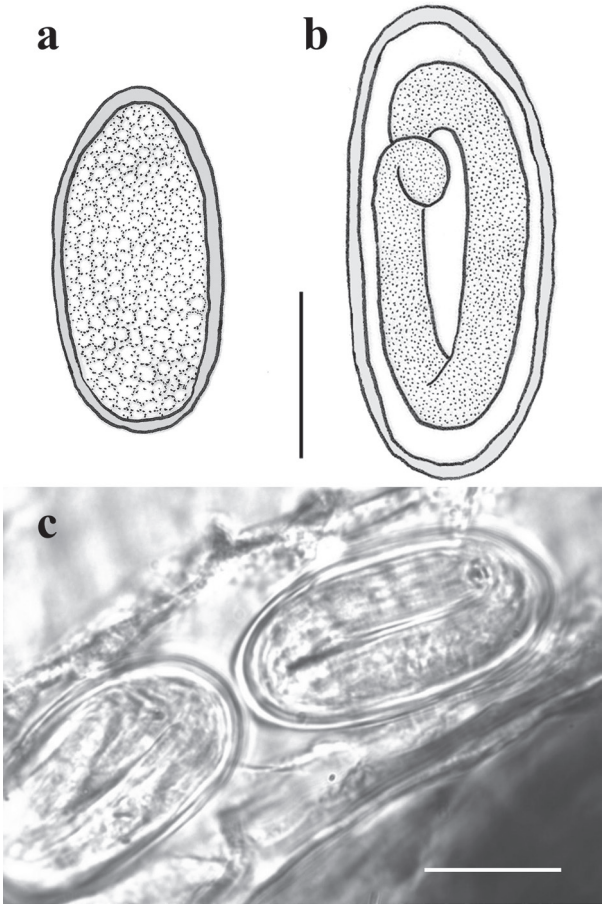

Figure 5 - Eggs of *Microtetrameres urubitinga* n. sp. **(a)** Egg non-embryonated. **(b)** Egg embryonated containing first-stage larva. **(c)** Microphotograph of embryonated eggs in the uterus. Scale bars: 20 µm.

Barus 1966, Mawson 1977, Zhang et al. 2012, Komorová et al. 2017).

Microtetrameres aquila mainly differs from the new species by having larger males (3600–4300 x 90–100 vs. 2020–2540 x 60–71) and longer left spicule (1480–1800 vs. 770–1040) (Schell 1953).

Males of *M. accipiter* differ from those of *M. urubitinga* n. sp. by being larger (3600–4400 x 79–04 vs. 2020–2540 x 60–71), having longer left spicule (2030–2400 vs. 770–1040), shorter right spicule (100–105 vs. 130–260), and higher spicular ratio (19–24 vs. 4–6). The females mainly differ in having a cuticular ruffled longitudinal flange and smaller eggs (43 x 25 vs. 48–52 x 24) (Schell 1953).

Microtetrameres paraccipiter differs from the new species by having longer males (3400–3700 vs. 2020–2540), higher spicular ratio (18–20 vs. 4–6), longer left spicule (2000–2400 vs. 770–1040), shorter right spicule (110–120 vs. 130–260) and smaller eggs (44–46 vs. 48–52) (Mawson 1977).

Males of *M. cerci* can be mainly distinguished from those of *M. urubitinga* n. sp. by having an undivided tip of left spicule and a higher spicular ratio (10.4–16 vs. 4–6). The females are unknown (Mawson 1977).

Males of *M. cloacitectus* described by Barus (1966) mainly differ from those of *M. urubitinga* n. sp. by the number of caudal papillae (five pairs vs. four pairs), the morphology of left spicule (tip leaf-shaped vs. symmetrically bifurcated) and the presence of a tongue-shaped prominence close to cloaca. Also, females have shorter and wider eggs (42–46 x 26–29 vs. 48–52 x 24).

Males of *M. creplini* can be mainly distinguished from those of *M. urubitinga* n. sp. by having a higher spicular ratio (19 vs. 4–6) and by the number and distribution of caudal papillae (two symmetrical pairs and one unpaired precloacal papillae and two pairs of postcloacal papillae, the first symmetrically, and the second asymmetric arranged). The females are unknown.

Microtetrameres inermis differs from the new species by having males with longer left spicule (1187 vs. 770–1040), which tip is undivided, shorter right spicule (75 vs. 130–260), and by the number and distribution of caudal papillae (two pairs and one unpaired precloacal papillae, and two pairs postcloacal slightly asymmetrical) (Cram 1927).

Based on all these morphological and morphometrical differences, a new species *Microtetrameres urubitinga* n. sp. is proposed.

This is the first nominal species of *Microtetrameres* described from Argentina.

DISCUSSION

The known life cycle of *Microtetrameres* spp. involve grasshoppers and cockroaches, as experimental intermediate hosts and birds as natural definitive hosts (Anderson 2000).

Cram (1934) described the experimental life cycle of *Microtetrameres helix* Cram, 1927 from North America. This author gave embryonated eggs of this species, to earthworms, isopods, grasshoppers [*Melanoplus bivittatus* (Say) and *Melanoplus femurrubrum* (De Geer)] and a cockroach [*Blattella germanica* (Linnaeus)]. She obtained several infective third-stage larvae in the grasshoppers and only a single larva in the cockroach; while infections in annelids and isopods were negative.

Ellis (1969) studied the life cycle of *Microtetrameres centuri* Barus, 1966 in USA. He obtained infective third-stage larvae in grasshopper nymphs (*Melanoplus* spp.) feed with embryonated eggs obtained from natural hosts, *Sturnella neglecta* Audubon and *Sturnella magna* (Linnaeus).

Bethel (1973) described the experimental life cycle of *Microtetrameres corax* Schell, 1953 in USA. He obtained infective third-stage larvae in grasshoppers (*Melanoplus* spp.) fed with embryonated eggs found in the natural host, *Pica hudsonia* (Sabine). However, the examination of grasshopper from natural environments revealed no infections with this nematode.

Quentin et al. (1986) studied the life cycle of *M. inermis* from Togo, Africa. They obtained infective third-stage larvae in orthopterans [*Metaxymecus patagiatus* (Karsch) and *Locusta migratoria* Linnaeus] fed with embryonated eggs found in the natural host, *Ploceus aurantius* (Vieillot).

The diet of the great black-hawks consists mainly of vertebrates (reptiles, amphibians, birds and mammals), and in lesser extent large insects (Carvalho Filho et al. 2006). The low prevalence of *M. urubitinga* (16.7%) in *B. urubitinga* would indicate the occasional ingestion of insects, which act as intermediate hosts.

Pathogenicity of *Microtetrameres* spp. is mainly related to mechanical effects caused by the location of the females in the proventricular glands. The poor physical appearance and loss of weight in the experimentally infected birds may have been due to their inability to compete for food and/or digest it (Bethel 1973)

The pathological effects of a few species of *Microtetrameres* are known, among them *M. centuri* and *Microtetrameres nestoris* Black and Rutherford, 1979 (Ellis 1970, Bethel 1973, Clark et al. 1979). These authors detected glandular atrophy in the proventriculus but no marked inflammatory response, despite finding females with red blood cells in the intestine.

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