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A new dracunculus species (Nematoda: Dracunculoidea) in neotropical otters (*Lontra longicaudis*) from Argentina: morphological and molecular characterization

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ABSTRACT

A new species of *Dracunculus* is described in wild neotropical otters, *Lontra longicaudis*, occurring in Corrientes, Argentina, based on morphological and molecular characteristics. Worms were located in the subcutaneous tissue from two of five investigated otters. *Dracunculus jaguape* n. sp. is differentiated from the 14 species of *Dracunculus* described from mammals and reptiles by the prominent dorsal and ventral papillae on the head; deirids posterior to nerve ring; male with long needlelike spicules and presence of gubernaculum; and long first stage larva. Phylogenetic analysis using the 18S rRNA positioned *Dracunculus jaguape* n. sp. in an anterior position to the rest of *Dracunculus* sequences available and COI positioned it in a separated clade sister to *Dracunculus lutrae* sequences. This is the first report on the presence of this nematode in *Lontra longicaudis* in Argentina.

1. Introduction

The genus *Dracunculus* Reichard, 1759 commonly parasitizes tissues and serous cavities of a wide variety of mammals and reptiles (Cleveland et al., 2018). Dracunculosis caused by the parasitic worm *Dracunculus medinensis* Linnaeus, 1758, the human "Guinea-worm" is the most studied species and has been diagnosed in Africa and Asia (Cleveland et al., 2018). Although the disease is not fatal, it causes significant morbidity, and infected people can be unwell and in significant pain for weeks and even months (WHO, 2022). Female worms are located in the subcutaneous tissues of the definitive vertebrate host, forming an ulcer on the skin of a limb. Females release first-stage larvae into the water. The definitive host becomes infected by drinking water containing the infected copepod with the third-stage larvae. Male and female larvae mature and mate 60–90 days after infection. The male worm dies shortly after mating, and the female matures over the subsequent 10–14 months, slowly migrates to the surface of the body and emerge through the skin. When affected body parts are submerged in water, the female worm releases larvae, which are ingested by copepods, thus completing the life cycle. Frogs could be paratenic hosts, and fish may serve as either paratenic or transport hosts in *Dracunculus* spp. cycles (Anderson, 2000; Cleveland et al., 2018; Box et al., 2021). In South America, there are a few reports that mention the presence of *D. medinensis* in humans from Brazil (Costa 1956; Watts, 2000) and Argentina (Riveros et al., 1981; Bono Battistoni et al., 2011); however, none of these reports contained sufficient morphological descriptions or have been molecularly confirmed.

There are at least three species of *Dracunculus* infecting wild mammals in North and South America: *Dracunculus insignis* Leidy, 1858, *Dracunculus lutrae* Crichton and Beverley-Burton (1973) and *Dracunculus fuelleborni* Travassos (1934); Muller (1971); Crichton and Beverley-Burton (1973); Cleveland et al., (2018). *Dracunculus insignis* was found in raccoons (*Procyon lotor*) from Canada (Gibson and McKiel, 1972; Crichton and Beverly-Burton, 1974; Elsasser et al., 2009) and USA (Chitwood, 1950; Tumlison and Surf, 2018) as well as in mink (*Mustela vison*), fisher (*Martes pennanti*), North American river otter (*Lontra canadensis*) and Virginia opossum (*Didelphis virginiana*) (Cleveland et al., 2018). Likewise, *D. lutrae* was reported in North American river otter

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from Canada (Crichton and Beverly-Burton, 1973, 1974; Elsasser et al., 2009), meanwhile, *D. fuelleborni* was found in the big-eared opossum (*Didelphis aurita*) from Brazil (Travassos, 1934). Recently, Paiva et al. (2021) reported a possible new species of *Dracunculus* in a dog from Mato Grosso do Sul, Brazil, based mainly on molecular sequences; and in the same geographical area Fagundes-Moreira et al. (2023) reported *Dracunculus* sp. in a jaguar (*Panthera onca*). There are also species of *Dracunculus* that infect reptiles, such as *Dracunculus ophidensis* Brackett (1938) reported in snakes from USA (Brackett, 1938) and Mexico (Pérez-Ponce de León et al., 2001; Jiménez-Ruiz et al., 2002; Moravec, 2006), *Dracunculus brasiliensis* Moravec and Santos (2009) in snakes from Brazil (Moravec and Santos, 2009; Quirino et al., 2018) and *Dracunculus globocephalus* Mackin (1927) in snapping turtle from USA (Moravec and Little, 2004). There are reports associated with *Dracunculus* in Argentina parasitizing domestic dogs from Formosa and Santa Fe provinces (Hoyos et al., 1995; Bono Battistoni et al., 2011, 2020), plus a record in a cougar (*Puma concolor*) from Formosa (Rosster et al., 1981).

The neotropical otter (*Lontra longicaudis*) is part of the Mustelidae family and is distributed from northwestern Mexico to Uruguay and throughout the northern part of Argentina (Rheingantz et al., 2017). These versatile otters are found in a very wide variety of environments, always associated with the presence of water (Vezzosi et al., 2014). Otters are opportunistic predators, the most common prey items are fish and crustaceans, while molluscs, amphibians, reptiles, birds, and mammals are consumed in less quantity (Gori et al., 2003; Vezzosi et al., 2014; Rheingantz et al., 2017). The species may occur in areas with variable degrees of human activity and habitat degradation (Rheingantz et al., 2017).

Available information on parasites from *L. longicaudis* is scarce in South America. In Brazil, Vieira et al. (2008) mentioned *Dioctophyme renale* Goeze 1782, *Dirofilaria* spp. Freitas and Lent 1949, *Dracunculus* spp. (Nematoda) and *Hexaglandula mutabilis* Rudolphi 1819 (Acanthocephala). While Uchôa et al. (2004) in a coprological study, found oocysts of *Eimeria* spp., and eggs from *Hymenolepis* spp., *Strongyloides* spp., Ancylostomatidae and *Toxocara* spp.

Herein, we describe a new species of *Dracunculus* based on morphological and molecular data, parasitizing the neotropical otter *Lontra longicaudis* in Corrientes province, Argentina, recovered from road-killed animals.

2. Materials and methods

2.1. Samples

We performed the necropsy of five opportunistically collected road-killed neotropical otters (*L. longicaudis*) in the northwest of Corrientes during 2021–2022 (Figs. 1–2). We recovered a complete nematode and fragments from otters #3 and #1 respectively and measured the total length of each worm. A piece of the middle part of the male specimen was cut for DNA extraction and the rest of the specimens were washed in physiological saline solution and fixed in 10% formalin. For morphological identification, worms were cleared in lactophenol, observed under a light microscope (LEICA DM500), and photographed with a Leica camera (ICC50W). Measurements were taken with the Leica Application Suite software (2016) and given in millimeters, unless otherwise stated. The helminths were deposited at the Helminthological Collection of the "Museo de La Plata", La Plata, Buenos Aires, Argentina (MLP-He). The following specimens deposited in the Helminthological Collection from Instituto Oswaldo Cruz (CHIOC) were consulted: *Dracunculus medinensis* CHIOC 8285, *Dracunculus* sp. CHIOC 8124 and CHIOC 12236. Host specimens were deposited at the "Colección

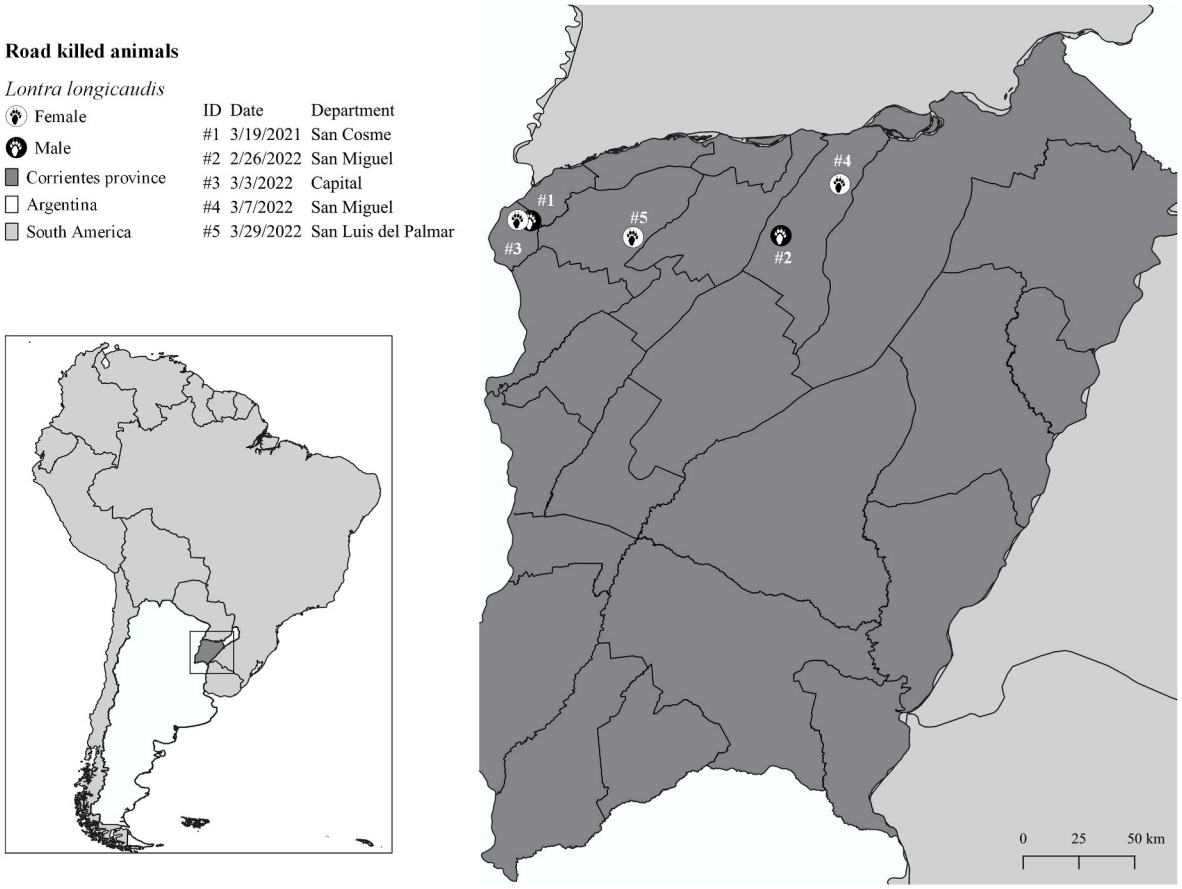

Fig. 1. Location of Corrientes province, Argentina, in South America (inset). Map of Corrientes showing the road-killed animals.

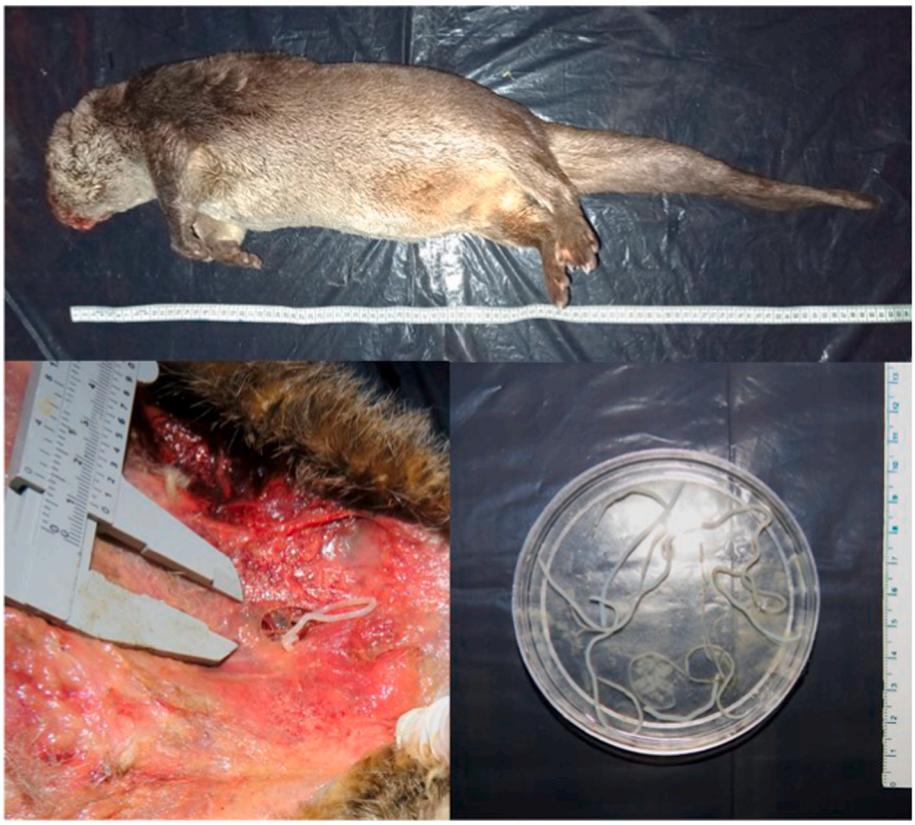

Fig. 2. Neotropical otters (*Lontra longicaudis*) dead and *Dracunculus* parasites in subcutaneous tissues.

Mamíferos" CECOAL, Corrientes, Argentina.

2.2. Molecular and phylogenetic analyses

A piece of one adult male specimen was processed using the BeadBug™ homogenizer (Benchmark Scientific) and DNA was extracted using the Accuprep® Genomic DNA Extraction Kit (Bioneer) according to the manufacturer's instructions. Genomic DNA concentration and quality were assessed using the SPECTROstar Nano and the MARS Data Analysis Software (BMG Labtech, Germany). Partial sequences of the 18S rRNA and the cytochrome oxidase I (*COI*) genes were amplified using primers and conditions previously reported (Floyd et al., 2005; Diekmann et al., 2020) (Table 1). Samples were subjected to agarose gel electrophoresis; PCR products were column purified and sequenced directly using amplifying primers. Phylogenetic trees were constructed by using MEGA 7.0 (https://www.megasoftware.net), and best-fitting substitution models were determined with the Akaike Information Criterion, using the maximum-likelihood model test.

3. Results

3.1. Morphological identification

We recovered from otter #1 fragments of females (two anterior extremities of a gravid and subgravid specimens) in subcutaneous tissues near the neck and thoracic cavity, and from otter #3 one male specimen in subcutaneous tissues near hind limbs.

3.1.1. *Dracunculus jaguape n sp*

General description: Body whitish, filiform, without any anterior constriction; anterior end rounded. Cuticle smooth. Peribuccal ring indistinct. Dorsal and ventral papillae projected anteriorly (Fig. 3 b, d, e). Male with needlelike spicules, gubernaculum present. Oesophagus consisting of a short, narrow anterior muscular portion, and a very long glandular portion (Table 2); the glandular oesophagus consists of a prominent swelling, anterior to nerve ring (Fig. 3 a, c). Deirids posterior to nerve ring. Excretory pore not observed.

3.1.2. *Male holotype (n = 1)*

Oral opening surrounded by a cuticularized plate (Fig. 4 a). Salient dorsal and ventral papillae close to the oral opening; external circle of papillae constituted by four notorious papillae. Amphids conspicuous.

Table 1
Primers and cycling conditions.

Gene	Primer	Sequence	Cycling conditions	Fragment size	Reference
18S rRNA	Nem_18S_F Nem_18S_R	CGCGAATRGCTCATTACAACAGC GGGCGGTATCTGATCGCC	94 °C for 5 min; 44 cycles of 94 °C for 15 s, 55 °C for 30 s and 72 °C for 45 s; final extension at 72 °C for 5 min.	933 bp	Floyd et al. (2005)
COI	COI_Nema_Fw COI_Nema_Rv	GAAAGTTCTAATCATAARGATATTGG ACCTCAGGATGACCAAAAAAYCAA	94 °C for 5 min; 44 cycles of 94 °C for 15 s, 55 °C for 30 s and 72 °C for 45 s; final extension at 72 °C for 5 min.	704 bp	Diekmann et al. (2020)

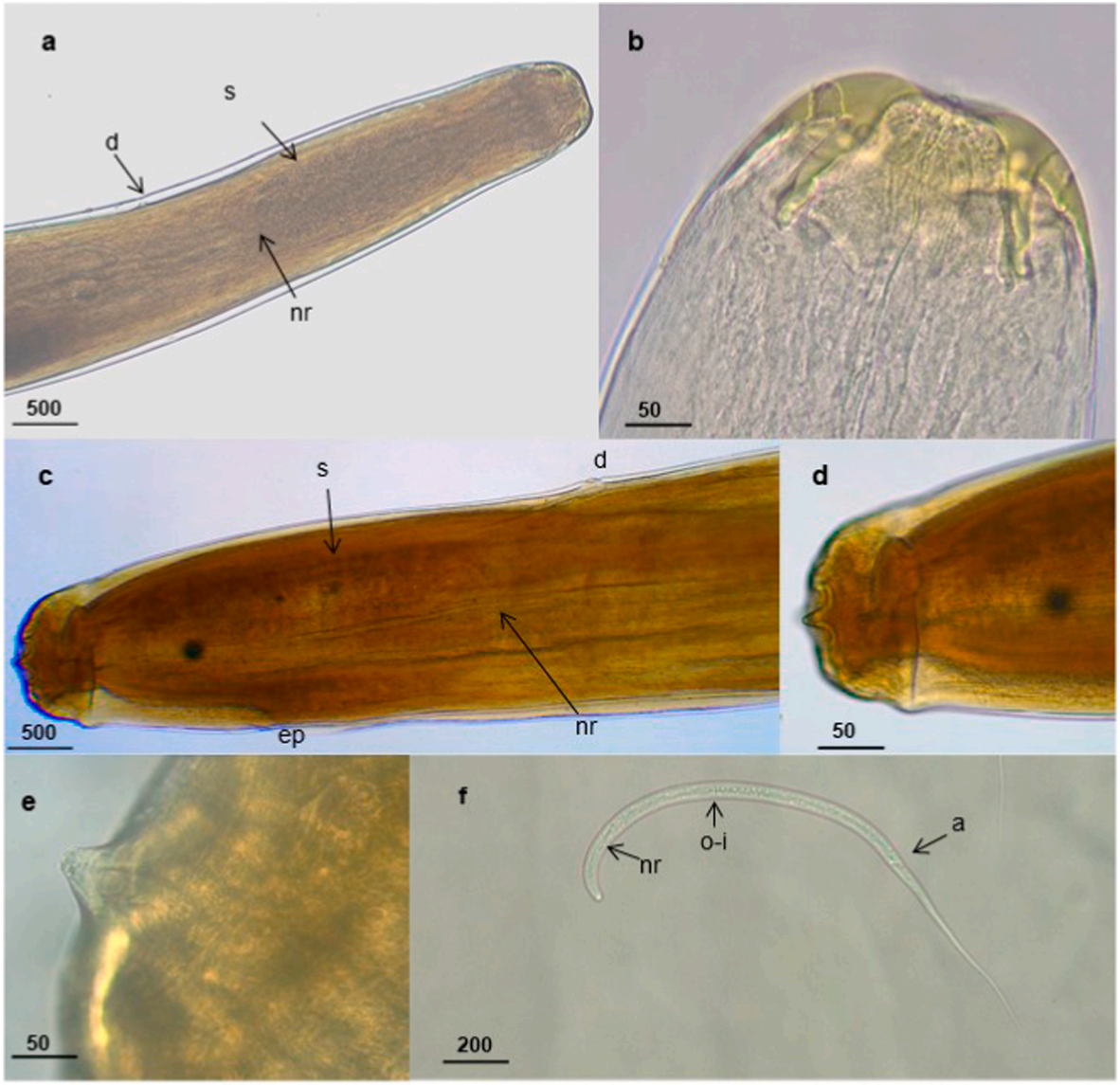

Fig. 3. *Dracunculus jaguape* n. sp. (a) Anterior extremity of a male showing the oesophagus swelling, deirid and nerve ring. (b) Cephalic extremity, lateral view showing the cephalic papillae. (c) Anterior extremity of a gravid female. (d) Cephalic extremity, lateral view. (e) Detail of dorsal papillae, (f) Larvae with long and tapered tail, removed from the uterus. a: anus, d: deirid, ep: excretory pore, nr: nerv ring, s: glandular oesophagus swelling, o-i: oesophagus-intestine junction, p: papillae.

Oesophagus divided in muscular and glandular portions. Muscular oesophagus 0.256 long. Glandular oesophagus 9.87 long; swelling developed, 0.448 long by 0.152 wide (Table 2). Distance from the anterior extremity to the nerve ring and deirids 0.748 and 0.896 respectively. Excretory pore not observed. Tail conical, with sharply pointed tip, 0.422 long. Spicules similar in morphology, needlelike, 0.549 and 0.526 long (Fig. 4 b, e). Gubernaculum 0.133 long, rounded at distal extremity, with two lateral infoldings partially enclosing the spicules, infoldings well cuticularized, wider at proximal end than at distal end. Caudal papillae arrangement as follows: two pairs of preanal papillae; four pairs of subventral papillae immediately posterior to anus, one pair of ventrolateral papillae immediately behind the preceding papillae; one pair of lateral papillae and two pairs of subventral papillae near caudal extremity; a pair of lateral papillae close to the tip tail, and phasmids (Fig. 4 b, c).

3.1.3. *Female allotype (anterior extremity)*

Anterior extremity of 50 long, by 1.21 wide. Head papillae arranged in two circles: the internal circle formed by two pairs of large dorsal and ventral papillae, respectively, 60 μm high projecting anteriorly (Fig. 3 d, e), and a pair of small lateral simple papillae close to the amphids; the external circle formed by four submedian papillae; amphids small. Muscular oesophagus 0.45 long. Glandular oesophagus extending all along the fragment of the worms; length of glandular swelling 1.70 by 0.67. Distance from the anterior extremity to the nerve ring and the deirids 2.21 and 2.33, respectively. Vulva not observed. Uterus extending through major part of body, being filled with larvae. *Paratype* (anterior extremity): Anterior extremity 70 long, by 1.13 wide. Muscular oesophagus 0.36 long. Glandular oesophagus extending all along the fragment of the worms; length of glandular swelling 1.61 by 0.89. Distance from the anterior extremity to the nerve ring and the deirids 1.92 and 1.95, respectively. Vulva not observed.

3.1.4. *First-stage larva (n = 10)*

Anterior end of larvae rounded, with distinct dorsal larval tooth, posterior end slender, with a pair of prominent phasmids open just behind the anus. Body length 706 (668–771) μm, body width 21 (19–25) μm; nerve ring 78 (57–110) μm; oesophagus 187.55 (141–244) μm long;

Table 2
Measurements of males and females of all known species of *Dracunculus*. (in millimeters unless otherwise indicated).

Species	Sex	Muscular oesophagus L	Glandular oesophagus swelling L	Nerve ring	Deirids	Right spicule	Left spicule	Gubernaculum	Tail	Reference
Mammalia										
D. jaguape n. sp.	M‡	0.256	0.448	0.748	0.896	0.549	0.526	0.133	0.422	Present study
	F§	0.36–0.45	1.61–1.70	1.91–2.21	1.95–2.33	–	–	–	NK	
	F	0.3*	NK	NG	NG	–	–	–	1.2	
D. insignis†	M	0.15–0.21	NK	0.39–0.54	0.50–0.71	0.46–0.55	0.43–0.52	0.10–0.12	0.22–0.34	Crichton and Beberley-Burton (1973)
	F	0.24–0.35	NK	0.83–1.09	0,96–1.31	–	–	–	0.70–1.0	
D. lutrae	M	0.25–0.29	0.43‡	0.74–0.87	0.89–1.13	0.59–0.72	0.51–0.68	0.16–0.18	0.42–0.52	Crichton and Beberley-Burton (1973)
	F	0,30–0.46	0.50§	0.80–0.99	0.93–1.25	–	–	–	0.70–1.20	
D. medinensis	M	0.215*	0.30*	0.51*	0.60	0.49–0.73	0.49–0.73	0.2	0.25	Moorthy (1937)
	F	NK	NK	NK	NK	–	–	–	0.25–0.90	
Squamata										
D. alii	M	0.17–0.28	0.17*	0.34–0.58	NK	0.23–0.30	0.20–0.29	0.05–0.07	0.15–0.24	Deshmukh (1969)
	F	NK	NK	NK	NK	NK	NK	NK	NK	
D. brasiliensis	M	NK	NK	NK	NK	NK	NK	NK	NK	Moravec and Santos (2009)
	F	0.367	0.517	0.925	NK	–	–	–	0.92	
D. coluberensis	M	0.21	0.29*	0.48	NK	0.7	0.8	Small	0.18	Desportes (1938)
	F	NK	NK	NK	NK	NK	NK	NK	NK	
D. dahomensis	M	NK	NK	NK	NK	0.40	0.425	NK	0.24*	Moorthy (1937)
	F	NK	NK	NK	NK	–	–	–	NK	
D. doi	M	0.33	0.30	0.70	0.71	0.46	0.46	0.13	0.24	Chabaud (1960)
	F	NK	NK	NK	NK	NK	NK	NK	NK	
D. houdemeri	M	NK	NK	NK	NK	NK	NK	NK	NK	Hsü (1933)
	F	0.38	0.50	0.93	NK	–	–	–	NK	
D. oesophageus	M	0.20	0.30	Anterior to	0.65	0.283	0.297	0.65	0.15*	Desportes (1938)
	F	0.19–0.30	0.32–0.42	deirids	0.91	–	–	–	0.30–0.325	
D. ophidensis	M	0.19	0.28	0.465*	Posterior to nerve	0.554	0.523	0.9	0.17	Bracket, 1038
	F	0.17*	0.60*	0.47*	ring	–	–	–	NK	
D. mulbus	M	0.18–0.22	0.24–0.28	0.76–0.80	NK	0.40–0.48	0.40–0.48	0.08–0.11	0.13–0.18	Jones and Mulder (2007)
	F	0.30–0.34	0.45–0.63	0.76–0.96	1–1.2	–	–	–	0.20–0.27	
Testudines										
D. globocephalus	M	0.22–0.28	0.39–0.43	0.73–0.76	0.84–0.89	0.96–1.06	0.18–0.21	absent	0.36–0.38	Moravec and Little (2004)
	F	0.40–0.54	1.04–1.15	1.55–2.16	1.97–2.24	–	–	–	0.76–0.87	

M = male; F = female; * measurements taken from drawings; NK = not known; † measurements from mink; ‡ measurement from holotype; § measurement from allotype.

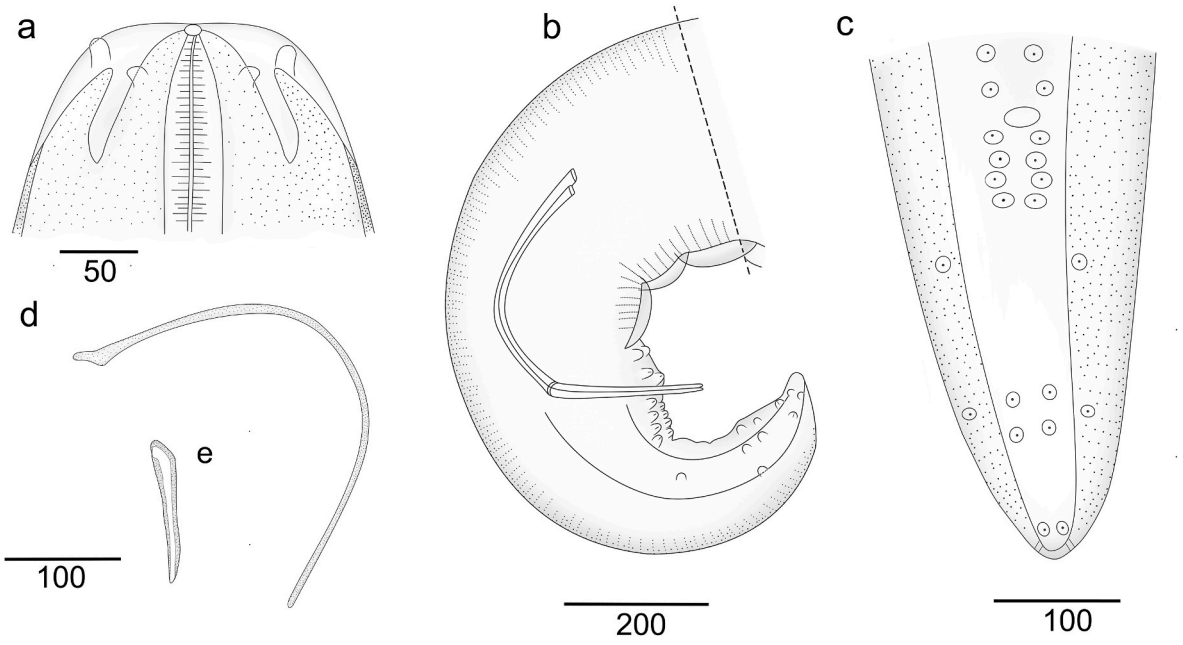

Fig. 4. *Dracunculus jaguape* n. sp. (a) Cephalic extremity of a male, lateral view. (b) Male tail, lateral view showing the spicules, and papillae. (c) Schematic male tail in ventral view. (d) Detailed of the spicule. (e) Detail of the gubernaculum.

anus to the tip tail 438 (406–494) μm; tail takes up about last third of the body being 268 (250–286) μm long (see Table 3 for measurements; Fig. 3 f).

3.1.5. Type host

Lontra longicaudis (Mustellidae), male (specimen harboring holotype) field number CECOAL – AC 208 deposited at Colección Mamíferos CECOAL, Corrientes, Argentina. Other specimens: *Lontra longicaudis*, female, field number CECOAL – AC 232 specimen harboring allotype.

3.1.6. Type locality

San Cosme department (−27.4715 S; −58.6715 W). *Other locality*: Santa Ana (−27.4519 S; −58.7279 W), Corrientes, Argentina.

3.1.7. Type data and depository

Male holotype deposited in the Helminthological Collection of the "Museo de La Plata", La Plata, Argentina (MLP- He 7985), female allotype (MLP- He 7986) and female paratype (MLP-He 7987).

3.1.8. Etymology

The specific name of this species refers to the local name of the host in Guarani language "jagua pe", meaning snub beast or flat beast.

3.2. Differential diagnosis

Dracunculus jaguape n. sp. is different from the four species of *Dracunculus* mentioned in mammals. The new species differs from *D. insignis* and *D. lutrae* by the longer muscular oesophagus, the posterior position of the nerve ring, by the longer tail in male, and by a different arrangement of the caudal papillae (Chitwood, 1950; Crichton and Beverley-Burton, 1973). Moreover, *D. jaguape* n. sp. possesses spicules shorter than *D. lutrae, D. fuelleborni and D. medinensis*, also *D. fuelleborni*

Table 3
Measurements of first stage larva from all known species of *Dracunculus* (in micrometers unless otherwise indicated).

Species	Body length (μm)	Body width (μm)	Anus (μm)	Reference
Mammalia				
D. jaguape n. sp.	668–771	19–25	406–494	Present study
D. fulleborni	300–429	60	96	Travassos (1934)
D. insignis†	673–749	20–23	371–446	Crichton and Beberley-Burton (1973)
D. lutrae	608–722	16–28	330–420	Crichton and Beberley-Burton (1973)
D. medinensis	550–760	15–30	127	Moorthy (1937)
Dracunculus sp. Pantanal-Br	488–614	17–20	110–151	Paiva et al. (2021)
Dracunculus sp.	507.2–582.6	19.1–22.6	NK	Fagundes-Moreira et al. (2023)
Squamata				
D. alii	NK	NK	NK	Deshmukh (1969)
D. brasiliensis	394	14	150	Moravec and Santos (2009)
D. coluberensis	NK	NK	NK	Desportes (1938)
D. dahomensis	400–425	12–15	100	Neumann (1895)
D. doi	NK	NK	NK	Chabaud (1960)
D. houdemeri	330–363	15–18	NK	Hsü (1933)
D. oesophageus	475	18	NK	Desportes (1938)
D. ophidensis	430–450	NK	NK	Bracket, 1038
D. mulbus	340–400	12–16	100	Jones and Mulder (2007)
Testudines				
D. globocephalus	666–721	21	258–288	Moravec and Little (2004)

NK = not known; † measurements from mink.

and *D. medinensis* differ by having longer tail in males (Travassos, 1934; Moorthy, 1937; Crichton and Beverley-Burton, 1973).

Furthermore, our species is distinguished from the 10 species of *Dracunculus* occurring in Squamata and Testudines reptiles. From those species with known documented male morphological characters, *D. jaguape* n. sp. differs from *Dracunculus alii* Deshmukh (1969), *Dracunculus oesophageus* Polonio, 1859, *Dracunculus doi* Chabaud (1960), *Dracunculus mulbus* Jones and Mulder (2007), *Dracunculus dahomensis* Neumann (1895), *Dracunculus coluberensis* Deshmukh (1969), *D. ophidensis*, *D. globocephalus* in having a longer tail (0.422 mm in our species versus less than 0.380 mm, Table 2), and with the six first species named above in having longer spicules (0.52 and 0.54 mm in our species versus less than 0.48 mm in the remaining) (Brackett, 1938; Desportes, 1938; Chabaud, 1960; Deshmukh, 1969; Muller, 1971; Moravec and Little, 2004; Jones and Mulder, 2007). Our male differs from *D. globocephalus* because left and right spicules are similar in size and possesses gubernaculum instead of different size and lacking off gubernaculum (Moravec and Little, 2004). The remaining two species described from reptiles are based on females and they are also different from the new species. *Dracunculus jaguape* n. sp. differs from *Dracunculus houdemeri* Hsü (1933) and from *D. brasiliensis* by the length of the glandular oesphagus swelling and the position of the nerve ring (1.61–1.70 versus 0.50 and 0.517 respectively; 1.91–2.21 versus 0.93 and 0.925 respectively) (Hsü, 1933; Moravec and Santos, 2009).

Considering the first stage larva, *D. jaguape* n. sp. possesses a longer larva than *D. oesophageus*, *D. houdemeri*, *D. mulbus*, *D. ophidensis*, *D. brasiliensis* and *D. fuelleborni* (mean of 706 μm versus less than 475 μm, Table 2). Furthermore, our larvae are slightly longer than those from *D. insignis*, *D. lutrae* and *D. medinensis* (mean of 706 μm versus a mean of 698 –from mink–, 665 μm and 600 μm, respectively, Table 3) (Moorthy and Sweet, 1938; Crichton and Beverley-Burton, 1973). Finally, our first stage larva has a longer body and longer tail than *Dracunculus* sp. Pantanal-Br (Table 3), found in a dog and a yaguar from Brazil (Paiva et al., 2021; Fagundes-Moreira et al., 2023).

3.3. Molecular identification and phylogenetic analyses

The 18S rRNA sequence obtained (901 bp) was 99.78 % identical to the corresponding sequences of *D. medinensis* and *Dracunculus* sp. PDB 20–070 reported to parasitize humans (MT530443, MW685454), and 99.56 % identical to the corresponding sequences of *D. lutrae* (*Lutra lutra*, JF934737), *Dracunculus* sp. V3104 (*Lontra canadensis*, DQ503457) and *D. insignis* (*Procyon lotor*, AY947719). Regarding the *COI* sequence (615 bp), it was 91.06–91.54% identical to *D. medinensis* (*Canis lupus familiaris*, KF77021; *Homo sapiens*, MT983884; HQ216219) and 91.54% to *Dracunculus* sp. Pantanal−Br (*Canis lupus familiaris*, MW018870).

Phylogenetic analysis using 18S rRNA sequences positioned *Dracunculus jaguape* n. sp. in a separated clade, anterior to the rest of *Dracunculus* sp. sequences (Fig. 5 a). However, the low number of 18S rRNA sequences available for these taxa precluded a more accurate phylogenetic positioning. The phylogenetic analysis using *COI* sequences also positioned *Dracunculus jaguape* n. sp. in a separated clade sister to the clade composed by *D. lutrae* sequences, both clades in an anterior position to the rest of *Dracunculus* sequences available (Fig. 5 b). Partial sequences of the 18S rRNA and *COI* genes of *Dracunculus jaguape* n. sp. obtained in this study were deposited in GenBank (accession numbers OR575632 and OR575050).

4. Discussion and conclusions

Based on morphological and molecular characters we identified our specimens as a new species of *Dracunculus*. Morphological characters of *Dracunculus jaguape* n. sp. are distinguished by prominent dorsal and ventral papillae on the head; deirids posterior to nerve ring; male with long needlelike spicules and gubernaculum; and long first stage larva. Furthermore, when comparing the ratio between the length of swelling and the length of muscular oesophagus, all the species from Squamata display a ratio between 1 and 1.7 in females and 1 to 1.5 in males – meaning that the muscular oesophagus is shorter than the length of the swelling. *Dracunculus globocephalus*, a parasite from turtles in United States, exhibits a ratio of 2.5 in females and 1.75 in males. Similarly, *D. jaguape* n. sp. displays a ratio ranging from 3.7 to 4.4 in females and 1.75 in males. The ratio represents a useful character that contributes to differentiate species.

In Argentina, unfortunately, the specimens of *Dracunculus* sp. reported from puma and dogs were never described, nor stored in any collection (Riveros et al., 1981; Rosster et al., 1981; Hoyos et al., 1995; Bono Battistoni et al., 2011, 2020), making it impossible to review its morphology. However, we had the opportunity to obtain information on specimens from Brazil deposited as *Dracunculus* sp. at the Helminthological Collection from Instituto Oswaldo Cruz, Brazil. Vial CHIOC 8124 contains specimens parasitizing *L. longicaudis* from Rio de Janeiro, Brazil (Noronha et al., 2002; Vieira et al., 2008). The vial only contains pieces of females broken and very damaged without anterior or posterior ends, not even with larval stages (M. Knoff and D. Almeida pers. com.). These specimens were collected by L. Travassos in 1925 but never described. In his autopsy book, Travassos noted that male had two subequal spicules. Later, Teixeira de Freitas in 1941 re-examined the vial and wrote in the

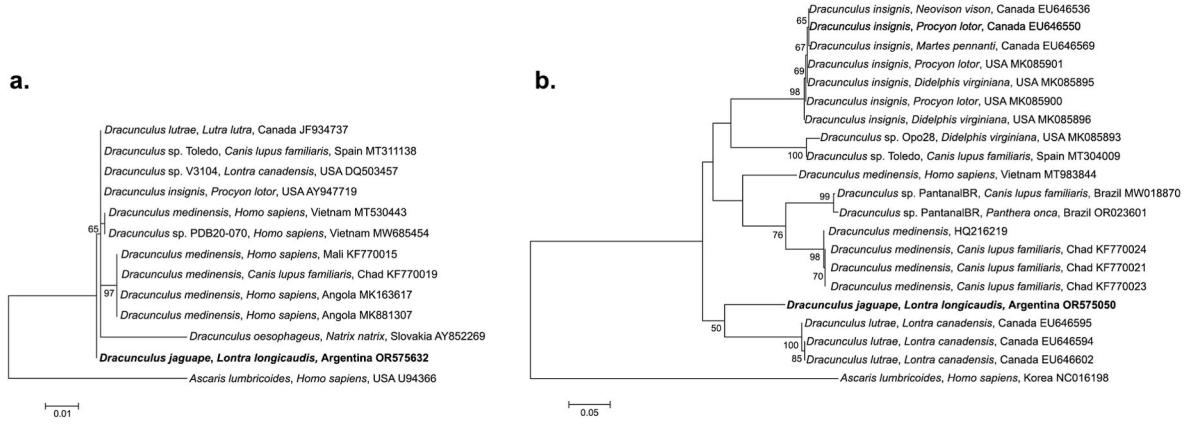

Fig. 5. Maximum-likelihood trees constructed from (a) 18S rRNA and (b) *COI* sequences of *Dracunculus jaguape* n. sp. from *Lontra longicaudis* in Argentina compared with available sequences. Best-fitting substitution models using the Maximum-Likelihood model test were determined with the Akaike Information Criterion. Kimura 2-parameter was selected as the best model for 18S rRNA and Tamura-Nei with a discrete Gamma distribution was selected as the best model for COI. Numbers represent bootstrap support generated from 1000 replications. GenBank accession numbers are shown. Boldface indicates the strain identified in this study. Scale bars indicate nucleotide substitutions/site.

same book, that there were no males, only a young female with a vulva at midbody, closer to rear end of body than front (probably third body); short ovijector, amphidelphic uterus, and anterior uterus more developed than posterior. Unfortunately, the information provided in the autopsy book does not allow us to identify them and it is not possible to infer whether those specimens are similar to our specimens, especially considering that both findings were in the same host species.

Another vial examined was CHIOC 8285 from a human. As stated Knoff et al. (2017), the specimens were collected by Oswaldo Cruz and determined by Travassos. However, to date there were no whole specimens and only remains of fibers were detected. Vial CHIOC 12236 was reported to contain specimens collected by Travassos in 1925 from the body cavity of a caninana snake (genus *Chironius*), unfortunately, there were no *Dracunculus* specimens in the vial. Actually, the vial contains specimens of *Kalicephalus costatus costatus* published by Vicente et al. (1993). However, in the autopsy book Travassos wrote: "In the general cavity *Dracunculus* (2♂, 1♀ broken), acanthocephalan larvae. In the small intestine *Kalicephalus*. In the oesophagus two *Opistogonimus*" (Original text in Portuguese).

Although species of *Dracunculus* spp. are geographically widespread, literature reports of infection in South America are scarce and morphological descriptions or molecular data are insufficient. Human and dog cases of *Dracunculus* in South America do not count with any morphological or genetic information (Paiva et al., 2021), with the aggravating circumstance that they are not deposited in reference collections (Bono Battistoni et al., 2011; Watts, 2000). Moreover, specimens of *D. medinensis* from the Old World are not well enough morphologically characterized (Moorthy, 1937; Muller, 1971; Bimi et al., 2005), making the comparison even more difficult. In Brazil, the unique species morphologically well described is *D. fuelleborni* with males and females (Travassos 1934), *D. brasiliensis* based only in females, while the two recent molecular reports from *Dracunculus* sp. Pantanal-Br in a dog and a jaguar from Mato Grosso do Sul present a detailed description of the first stage larva (Paiva et al., 2021; Fagundes-Moreira et al., 2023).

The present work also provides molecular evidence of the separation of *Dracunculus jaguape* n. sp. from South American *Lontra* from all other *Dracunculus* species currently found in various host species, including humans. *COI* analysis revealed that *Dracunculus jaguape* n. sp. from *L. longicaudis* in Argentina and *D. lutrae* from *L. canadensis* share the same clade, distinct from the rest of the *Dracunculus* species, including those found in other Mustelidae. The analysis of 18S rRNA demonstrated that *Dracunculus jaguape* n. sp. belongs to a separate clade from the rest of the *Dracunculus* species. In this analysis, *Dracunculus* sp. from *L. canadensis* was in a different clade; however, the limited number of 18S rRNA sequences available prevents a more in-depth analysis. These results suggest that the *Dracunculus* of *Lontra* of South American origin represents a distinct species, phylogenetically related to *D. lutrae*, which is known to parasitize another member of the *Lontra* genus, *L. canadensis*, in North America.

As otters feed mainly on fish, but also on crustaceans and molluscs (Gori et al., 2003), then it is possible that these preys are involved in the transmission. Further studies are warranted to clarify the life history, biology, and possible pathological effects of *Dracunculus jaguape* n. sp. on their host. Finally, this is the first time a *Dracunculus* species is described for Argentina using morphological and molecular data.

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 "Dirección de Recursos Naturales" of Corrientes (Guía Tránsito 00022702 and 00022703).

Authors' contributions

M.B.N. and J.N. identified the parasites, wrote the main manuscript text, M.B.N., A.B.V., F.S.G. carried out the necropsies and the field work, L.D.M. performed the molecular analysis, M.B.N., J.N. and L.D.M. prepared figures and M.M.K. supervised, reviewed and edited. All authors reviewed and contributed to the final manuscript.

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Conflicts of interest

The authors declare that they have no conflict of interest.

Declaration of competing interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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