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Pinworms of the red howler monkey (*Alouatta seniculus*) in Colombia: Gathering the pieces of the pinworm-primate puzzle

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ABSTRACT

Pinworms of primates are believed to be highly host specific parasites, forming co-evolutionary associations with their hosts. In order to assess the strength and reach of such evolutionary links, we need to have a broad understanding of the pinworm diversity associated with primates. Here, we employed an integrative taxonomic approach to assess pinworm diversity in red howler monkeys in Colombia. Molecular and morphological evidence validate the presence of at least four different species of *Trypanoxyuris* occurring in red howler monkeys: *T. minutus*, a widely distributed species, and three new species, *T. seunimiii* n. sp., *T. kemuimae* n. sp. and *T. kotudoi* n. sp. The mitochondrial COI gene and the 28S ribosomal gene were used for phylogenetic assessments through Bayesian inference. The three new species were morphologically distinct and formed reciprocally monophyletic lineages. Further molecular lineage subdivision in *T. minutus* and *T. kotudoi* n. sp. without morphological correspondence, suggests the potential scenario for the existence of cryptic species. Phylogenetic relationships imply that the different species of *Trypanoxyuris* occurring in each howler monkey species were acquired through independent colonization events. On-going efforts to uncover pinworm diversity will allow us to test the degree of host specificity and the co-phylogenetic hypothesis, as well as to further unravel the primate-pinworm evolutionary history puzzle.

1. Introduction

Parasitic nematodes of the family Oxyuroidea, known as pinworms, are among the most common gastrointestinal parasites occurring in almost every primate genera (Hugot et al., 1996). They have a direct life cycle with no free-living stage; transmission occurs through the ingestion of eggs, with retro-infection and autoinfection being common forms of transmission (Felt and White, 2005; Vermund and Wilson, 2000). They display a series of reproductive strategies through their life cycle such as male progenesis, aggregation of egg output, and haplodiploidy where males are haploid and derive from unfertilized eggs, and females are diploid arising from fertilize eggs (Adamson, 1989).

Pinworms are highly host specific, with one genus parasitizing each major group of primates. i.e., *Enterobius* in Catarrhini, *Lemuricola* in Strepsirrhini, and *Trypanoxyuris* in Platyrrhini (Hugot, 1999). Currently, 21 species of *Trypanoxyuris* have been reported to infect Neotropical primates, with a particular species of pinworm for each genus

or even species of monkey (Solórzano-García and Pérez-Ponce de León, 2018). This host specificity and the fact that several aspects of the parasite life history have been moulded by their host traits (Sorci et al., 2003), suggests a tight evolutionary association between pinworms and their primate hosts (Brooks and Glen, 1982; Hugot, 1999).

In order to determine the strength of the evolutionary links between pinworms and Neotropical primates, and to be able to assess the level in which coevolution has driven co-speciation and diversification of these parasites and their hosts, it is fundamental to pursue a comprehensive estimation of the pinworm diversity occurring in these primates. Parasitological studies are common in Neotropical primates (Solórzano-García and Pérez-Ponce de León, 2018), and valuable efforts have been made to assess their pinworm diversity (Conga et al., 2016; Hugot, 1985, 1984; Hugot et al., 1994; Solórzano-García et al., 2016, 2015; among others). However, the species of pinworms parasitizing most Neotropical primate species remain unknown. Given their mostly arboreal life style, and their vulnerable conservation status, gathering

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parasitological material from free-ranging Neotropical primates is challenging. Moreover, performing taxonomic studies of parasites requires highly detailed morphological examinations, as well as the availability of adequate genetic data to accomplish an accurate species identification and description, without disregarding the difficulties imposed by the possible presence of cryptic species (Jorge et al., 2013; Pérez-Ponce de León and Nadler, 2010).

In this study, we applied non-invasive sampling methods to assess pinworm diversity of free-ranging red howler monkeys (*Alouatta seniculus*) from Colombia. Red howlers are one of the nine recognized species of howler monkeys (Cortes-Ortiz et al., 2015). They live in groups of 2–16 individuals within a variety of ecosystems including gallery forests, semideciduous forests and lowland rainforests, distributing from Colombia and Northwestern Venezuela through Brazilian Amazon, eastern Ecuador and Peru (Cortes-Ortiz et al., 2015; Defler, 2003). Three species of *Trypanoxyuris* have been reported to parasitize howler monkeys, i.e., *T. multilabiatus* in *A. palliata*, *T. pigrae* in *A. pigra* (Solórzano-García et al., 2016); and *T. minutus* in *A. belzebul*, *A. caraya*, *A. guariba*, *A. palliata*, *A. pigra*, and *A. seniculus* (Solórzano-García and Pérez-Ponce de León, 2018). The main objective of this study was to use morphological and molecular data to uncover pinworm diversity in *A. seniculus*. We describe three new species of *Trypanoxyuris* parasitizing *A. seniculus* from Colombia, and we assess their phylogenetic position through mitochondrial and nuclear ribosomal genes to further discuss the potential scenario for the existence of cryptic species. This study will contribute to a better understanding of the speciation processes and evolutionary relationships among members of *Trypanoxyuris* and will help to continue unfolding the evolutionary history of pinworm-primate associations.

2. Methods

2.1. Specimen collection

A total of 99 pinworm specimens (66 females and 33 males) were collected from eight free-ranging red howler monkey troops surveyed in forest fragments in San Juan del Carare, Santander Department, Colombia (Fig. 1). Pinworms were recovered from fresh howler monkey faeces in situ and fixed in 100% ethanol. Faecal samples were also collected in 15 ml tubes with 70% ethanol for further examination in the lab following the procedure suggested by Hasegawa (2009) for the recovery of minute pinworms, especially males. In order to establish a linkage between morphological features and DNA sequences of individuals, most specimens were cut in half with the anterior portion used for the morphological study (hologenophores sensu Pleijel et al., 2008), and the remainder used for DNA extraction. Full body photographs were taken using a Leica DM 1000 led microscope (Leica, Wetzlar, Germany) for all the specimens before performing the cuts.

2.2. Morphological analyses

Worms were cleared with alcohol-glycerol solution, and observed using an Olympus BX51 light microscope equipped with differential interference contrast (DIC). En face view observations were made following the technique proposed by Hasegawa et al. (2004). Specimens were also preserved and processed for scanning electron microscopy (SEM). Twenty two pinworms (6 of *T. seunimii* n. sp., 3 of *T. kemuimae* n. sp., 8 of *T. kotudoi* n. sp., and of *T. minutus*) were dehydrated through a graded series of ethanol and then critical point dried with carbon dioxide. The specimens were mounted on metal stubs with carbon adhesive, and then gold coated and examined in a 15 kV Hitachi Stereoscan Model SU1510 scanning electron microscope. Specimens were deposited in the Colección Nacional de Helmintos (CNHE), Instituto de Biología, Universidad Nacional Autónoma de México (UNAM).

2.3. DNA extraction and amplification

Individual pinworms were digested overnight at 56 °C in a solution containing 10 mM Tris-HCl (pH 7.6), 20 mM NaCl, 100 mM EDTA (pH 8.0), 1% Sarkosyl, and 0.1 mg/ml proteinase K. DNA was extracted from the supernatant using the DNAzol® reagent (Molecular Research Center, Cincinnati, OH) according to the manufacturer's instructions. A fragment of the mitochondrial cytochrome *c* oxidase subunit 1 gene (COI) and a region of the large subunit of the nuclear ribosomal gene (28S) were amplified by PCR using the primers and conditions specified in Table 1. PCR products were treated with Exo-SAP-IT (Thermo Scientific), according to the manufacturer's instructions, and sequenced at the sequencing facility of the Instituto de Biología, UNAM. All sequences obtained in this study were deposited in GenBank (Accession numbers in Supplementary material S1 and S2).

2.4. Phylogenetic analyses

In addition to the sequences obtained in this study, 42 sequences of *Trypanoxyuris* and seven sequences of *Enterobius* available from GenBank were also included in the analysis; *Oxyuris equi* was used as outgroup. Alignments of both genes were performed using MUSCLE (Edgar, 2004) through the EMBL-EBI web interface (Madeira et al., 2019). As an additional check on accuracy, COI sequences were translated into amino acids using MESQUITE v.3.2 (Maddison and Maddison, 2011) and the invertebrate mitochondrial genetic code.

Phylogenetic relationships were assessed using Bayesian inference (BI) for each single gene, and for the concatenated dataset. Since COI sequences obtained in this study were of different length than those retrieved from GenBank, missing data ("?") was allowed in the COI and concatenated data sets in order to expand the number of compared taxa. Most appropriate evolutionary models were inferred following the Akaike information criterion (AIC) in MrModeltest v. 2.3 (Nylander, 2004). The GTR + I + G substitution model was the best model for both genes. BI analyses were performed using MrBayes v.3.2.6 (Ronquist and Huelsenbeck, 2003) and the CIPRES Science Gateway (Miller et al., 2010). The analyses included two simultaneous runs of Markov chain Monte Carlo, each for four million generations, sampling trees every 4000 generations, a heating parameter value of 0.2, and a "burn-in" of 25%. A 50% majority-rule consensus tree was constructed from the post burn-in trees. BI outputs were imported to FigTree v. 1.4 (Rambaut, 2014) for graphical visualization and editing. Genetic divergence (uncorrected *p*-distance) between and within main genetic groups were calculated in Mega v.6 (Tamura et al., 2013) using the pairwise-deletion option; standard error of the distances was estimated by bootstrap resampling with 100 replicates.

3. Results

Four different species of *Trypanoxyuris* were found to occur in red howler monkeys in Colombia. Three of them represent undescribed species with unique morphologies and they have not been reported in any other howler or Neotropical primate species. One of these species, with 15 individuals (10 females, 5 males) out of the 99 recovered, morphologically resembles *T. minutus*. Molecular data distinguishes these four species from each other.

3.1. Morphological description

The four species of *Trypanoxyuris* found in *A. seniculus* share several morphological traits which are described below, followed by the description of the particular diagnostic features that characterise each of the new species. This was done in order to avoid redundancy in species descriptions. Measurements are shown in Tables 2 and 3, reported in micrometres (μm) unless otherwise noted, with the range followed by the mean (in parentheses).
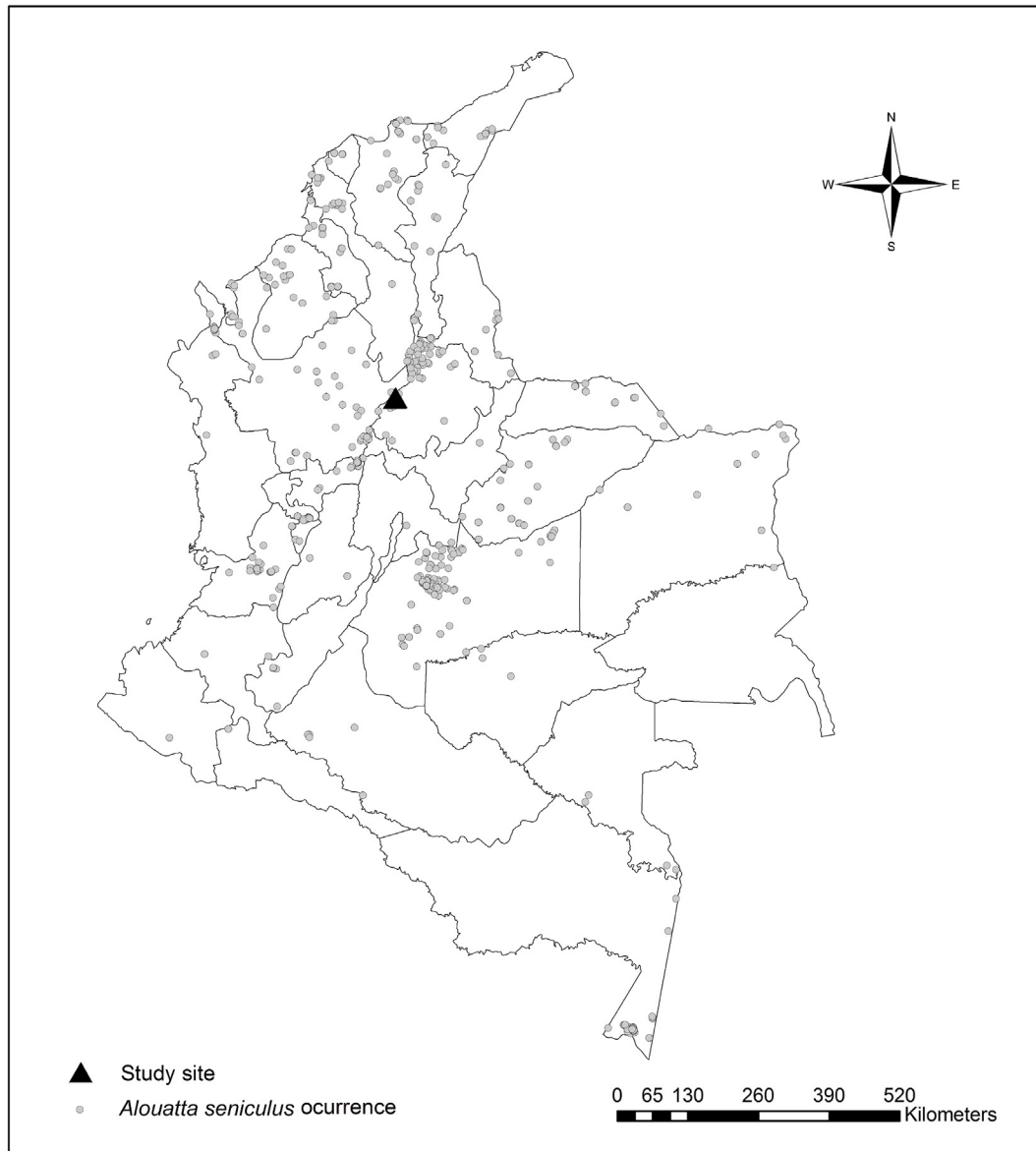
Fig. 1. Collecting site of *Trypanoxyuris* sp. from *Alouatta seniculus* (black triangle). Gray dots indicate sites of *A. seniculus* occurrence in Colombia.

Table 1
Primers and PCR conditions to amplify DNA sequences of *Trypanoxyuris* from red howler monkeys.

Gene	Primer	Sequence	PCR conditions	Reference
COI	COIintF	TGATTGGTGGTTTTGGTAA	(1 min at 94 ˚C, 1 min at 45 ˚C, 2 min at 72 ˚C) x 30	Casiraghi et al., 2001
	Pr-b	AGAAAGAACGTAATGAAAATGAGCAAC		Bessho et al., 1992
	TryCoxR*	AACCAACTAAAAACCTTAATMC		Solórzano-García et al. 2015
28s	391	AGCGGAGGAAAAGAAACTAA	(30 s at 94 ˚C, 30 s at 54 ˚C,1 min at 72 ˚C) x 33	Nadler and Hudspeth, 1998
	501	TCGGAAGGAACCAGCTACTA		Smythe and Nadler, 2006
	504*	CAGCTATCCTGAGGGAAAC		Smythe and Nadler, 2006
	503*	CCTTGGTCCGTGTTTCAAGACG		Smythe and Nadler, 2006

* Internal primers for sequencing only.

3.1.1. General description

White minute nematodes, females larger than males. Cuticle with transverse striations. Cephalic vesicle present. Cephalic tray quadrangular; buccal aperture triangular, delimited by three lips, one dorsal and two subventral. Labial structures surrounded by a circular furrow. Cephalic papillae readily visible, located in ventral and dorsal extremes of the cephalic tray with ventral papillae closest to the amphids. Two amphids, one on each side of the cephalic tray. Lateral alae present in both sexes. Oesophagus long with posterior spherical oesophageal bulb.

Females: Excretory pore located anterior to oesophageal bulb. Vulva located in the anterior 3rd of the body; muscular vagina longitudinally oriented, with distal vagina approximately perpendicular to longitudinal body axis. Tail long, conical. Eggs ellipsoidal, symmetric, with 3 longitudinal ridges forming a triangular contour in cross section.

Males: Lateral alae single crested. Excretory pore located posterior to oesophageal bulb. Four pairs of caudal papillae, all surrounded by

Table 2

Measurements of adult female *Trypanoxyuris* recovered from Colombian red howler monkeys (*Alouatta seniculus*) and its comparison with other closely related species of *Trypanoxyuris* from howler monkeys (*Alouatta* sp.). Measurements are presented in micrometres (μm) unless otherwise noted. Mean values in parentheses.

	T. seunimii n. sp.	*T. kemuimae* n. sp.	*T. kotudoi* n. sp.	*T. minutus*	*T. minutus*[a]	*T. pigrae*[a]
Females	n = 27	n = 10	n = 19	n = 10	n = 27	n = 22
Length (mm)	5.8–8.1 (6.9)	4.9 7-7 (6.3)	3.7–7.9 (5.9)	5.9–8.4 (7.2)	4.4–7.7 (5.6)	4.4–7.2 (5.8)
Width in midbody	207.1–553.1 (381.7)	246.9456.8 (352.9)	156–566.4 (332.1)	340.4–391.5 (362.5)	297.3–543.7 (431.7)	143.9–560.6 (405.7)
Nerve ring	193.6–346.7 (267.7)	238.4–286.3 (260.7)	164.4–300.9 (258.4)	252.6–306.9 (280.8)	156–243.2 (203.5)	178.7–262.7 (204.6)
Oesophagus length	1587.4–2066.5 (1744.6)	1642.5–1965.9 (1790.6)	1126.5–2038.2 (1663.7)	1729.2–2229.7 (2071.7)	1618–2051.5 (1836.2)	1212.5–1922.6 (1627.2)
Oesophagus corpus length	1479.7–1930.6 (1628.1)	1525 1829.6 (1683.4)	1017.4–1893.6 (1545.2)	1594–2085.5 (1937)	1483.8–1911.9 (1708.1)	1083.4–1784.6 (1497.5)
Oesophagus width at middle	31.8–51.4 (42.3)	38.3–52.6 (43.4)	30.3–48.1 (36.8)	42.1–56.7 (46.6)	37–54.5 (44.1)	31.5–49.3 (43.8)
Bulb length	107.7135.9 (121.2)	101.4149.3 (125.1)	97.9–144.6 (118.5)	123.5–145.7 (134.7)	113.7–148.2 (129)	109.4–166.5 (131.4)
Bulb width	97.5–136.3 (118.8)	106.9152.6 (122.9)	87.2–139 (116.9)	109.8–134.9 (122.4)	109.6–155.8 (137.2)	100–162.6 (133.8)
Excretory pore – anterior end	1789.6–2030.9 (1885)	1000.8–2114.3 (1587.7)	1312.8–2038.2 (1762.6)	1617.3–2399.4 (1941.7)	870.2–2210.1 (1292.4)	832.8–1910.9 (1317.2)
Vulva – anterior end	1433.5–3023.3 (2306.2)	1384.2–3016.4 (2117.1)	1196.4–2859.9 (2081.2)	1940.9–2977.4 (2567.6)	1300–2900 (1700)	1182.8–2168.5 (1685.1)
Tail length	1010.4–1639.9 (1415.1)	1232.2–1645.5 (1441.8)	1109.8–2085.2 (1529.4)	1415.9–1928.3 (1635.8)	1294–2444.8 (1517.7)	1268.2–1656.3 (1434.8)
Egg length	42.4 43.3 (42.9)	38.2–44.5 (41.9)	40.3–41.8 (41.1)	43.3	39.2–52.9 (47)	37.17–53.6 (45.7)
Egg width	21.7 22.4 (22.1)	21.4–23.2 (22.2)	21.1–22.2 (21.7)	22.3	20.8–29.9 (23.7)	16.4–27.3 (23.5)

[a] Solórzano-García et al., (2016).

ring shaped thickenings; first and fourth pairs pedunculated located at anterior and posterior extremes of the caudal bursa; second pair sessile, directed ventrally, flanking the cloacal aperture, surrounded by a large ring shaped thickening; third pair sessile, minute, directed postero-laterally. Spicule long, slightly wider in the middle. Short tail appendage.

3.1.2. Trypanoxyuris seunimii n. sp. (Fig. 2)

Description based on 37 specimens. Buccal aperture delimited by three notched and bilobulated lips, one dorsal and two subventral; lobes in the left ventral lip approximately symmetrical (Fig. 2).

Females (n = 27): Lips show discrete bilateral lateral indentations in the upper border, of the dorsal lip, while unilateral at the most ventral border of both ventral lips Lateral alae single crested (Fig. 2). Measurements are given in Table 2

Males (n = 10). Right ventral lip triangular-shaped; notches in ventral lips less conspicuous than in females and more subtle in dorsal lip; arch between lobes significantly smaller in the left ventral lip compared with the dorsal lip and discreet in the right ventral lip (Fig. 2). Measurements are given in Table 3.

3.1.2.1. Taxonomic summary. Type-host: *A. seniculus* (Linneus, 1766), red howler monkey.

Site of infection: Not determined (samples were obtained from faeces).

Type-locality: San Juan del Carare, Santander Department, Colombia (06° 43 N, 74° 09′W; 150 m.a.s.l.).

Type material: Holotype: CNHE: No 11212, paratypes CNHE: No. 11213.

No of individuals: 37/99 (37.4%) of the recovered specimens of *Trypanoxyuris* belong to this species.

Etymology: The specific epithet derives from the Tucano dialect for howler monkey "seunimi".

3.1.2.2. Remarks. Mixed infections with *T. seunimii* and other three species of *Trypanoxyuris* occurring in the same individual host were found in this study. *Trypanoxyuris seunimii* n. sp. was found as the most abundant pinworm parasitizing red howler monkeys and it is clearly distinguished by the presence of three bilobulated lips in females, instead of two bilobulated lips as in the other *Trypanoxyuris* species. Also, the single crested lateral alae in females differentiates this species from the rest. Males are difficult to distinguish; however, the triangular-

Table 3

Measurements of adult male *Trypanoxyuris* recovered from Colombian red howler monkeys (*Alouatta seniculus*) and its comparison with other closely related species of *Trypanoxyuris* from howler monkeys (*Alouatta* sp.). Measurements are presented in micrometres (μm) unless otherwise noted. Mean values in parentheses.

	T. seunimii n. sp.	*T. kemuimae* n. sp.	*T. kotudoi* n. sp.	*T. minutus*	*T. minutus*[a]	*T. pigrae*[b]
	n = 10	n = 7	n = 9	n = 7	n = 16[a]	n = 28[b]
Length (mm)	1.8–2.6 (2.4)	2.2–2.5 (2.3)	1.9–2.6 (2.4)	2.3–2.7 (2.5)	2.1–3 (2.6)	1.7–2.5 (2.2)
Width in midbody	91.2–140.9 (115)	94.5–137.7 (109.7)	90.7–131.1 (110)	101.5–145.9 (115)	117.3–173.3 (142.2)	74.7–130 (109.8)
Nerve ring	121.6–197.6 (162.9)	145.2–186.5 (170.5)	164.7–189.3 (177.5)	139.7–187.9 (164.6)	85.1–135.8 (108.3)	112.8–168.7 (145.5)-
Oesophagus length	578.9–750.1 (703)	676.9–755.2 (714.4)	567.1–787.8 (686.2)	679.1–776.1 (744.6)	659.6–834.3 (723.4)	485.3–750.2 (610.4)
Oesophagus corpus length	518.2–664.2 (623.2)	598.2–679.1 (634.7)	550.1–703. 5 (626.7)	603.6–690.2 (663)	555–735.4 (629.3)	420.3–662.3 (532)
Oesophagus width at middle	24.9–40.2 (349	27.4–48.6 (34.2)	27.7–41.7 (33.5)	27.8–34.7 (32)	17–34.3 (28.6)	14.9–35.1 (28.4)
Bulb length	60.7–86.3 (79.8)	74.8–90.6 (79.7)	69.4–87.4 (79.9)	75–93.1 (81.6)	70.7–93.6 (84.8)	58.6–88.3 (75.4)
Bulb width	61.4–89.7 (76.9)	79.8–106.2 (85.6)	73.7–89.1 (80.3)	64.5–91.1 (77.7)	66.3–93.6 (76.3)	48.1–83.5 (70.2)
Excretory pore	580.1–893.8 (753.9)	577.5–186.5 (170.5)	687.6–820.8 (749.6)	571.8–761.9 (692.8)	689.9–939.7 (814.3)	556.5–777.6 (667.5)
Spicule	35.6–50.9 (43.2)	29.2–56 (43.2)	32.6–52.8 (45.4)	42.1–50.5 (45.6)	27.3–79.9 (58.4)	27.4–57.5 (45.8)
Spike	10.7–15.2 (12.6)	10.4–12.6 (11.2)	–	11.7–15.2 (13.7)	11.6–15.6 (13.4)	6.7–18.5 (11.3)

[a] Solórzano-García et al., (2016).
[b] Solórzano-García et al., 2017.
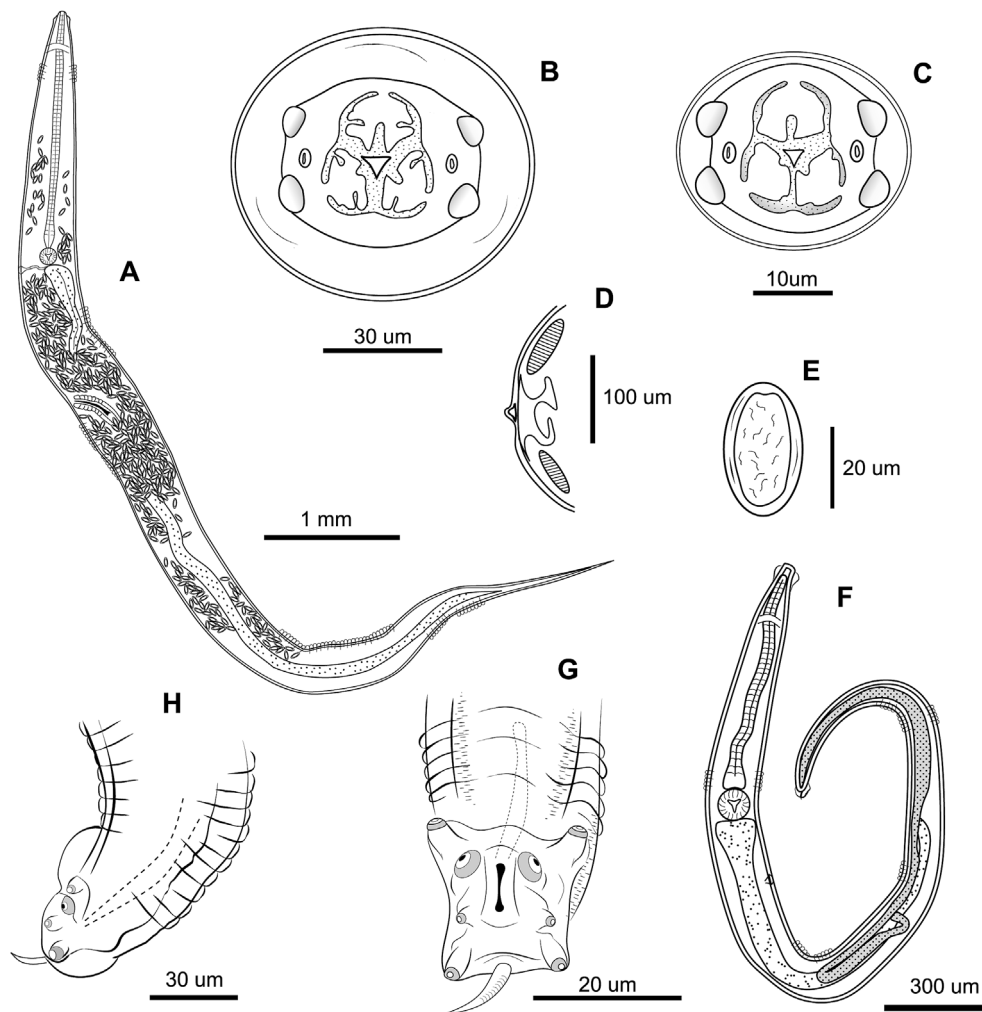
Fig. 2. *Trypanoxyuris seunimii* n. sp. (A) Female full body, lateral view. (B) Female cephalic end, apical view. (C) Male cephalic end, apical view, (D) Female cross section showing lateral ala; (E) Egg; (F) Male full body, lateral view; (G) Male posterior end, ventral view; (H) Male posterior end, lateral view.

shaped and slightly bilobulated right ventral lip, the symmetry between lobes in the left ventral lip, and the presence of notches in all three lips, differentiate *T. seunimii* n. sp. males from the those of the other species. The new species morphologically resembles *T. pigrae*; the main differences between the two species are the three bilobulated lips in *T. seunimii* n. sp. females; and the presence of sharp protuberances formed as a result of the notches in the lips of *T. pigrae* males which are not observable in males of the new species. Additionally, *T. pigrae* is only found in *A. pigra* and *T. seunimii* n. sp. is found in *A. seniculus*. Caudal structures in males of the new species are similar to several *Trypanoxyuris* found in howler monkeys, as well as egg size and shape, making these characters not useful for species diagnosis.

3.1.3. Trypanoxyuris kemuimae n. sp. *(Fig. 3)*

Description based on 17 specimens. Buccal aperture delimited by three lips, dorsal and left ventral lip are bilobulated; right ventral lip elongated; lobes in the left ventral lip asymmetrical with upper lobe larger than lower lobe (Fig. 3).

Females (n = 10): Lips show discrete lateral indentations in the upper border. Lateral alae double crested (Fig. 3). Measurements are given in Table 2.

Males (n = 7). Measurements are given in Table 3.

3.1.3.1. Taxonomic summary. Type-host: *A. seniculus* (Linneus, 1766), red howler monkey.

Site of infection: Not determined (samples were obtained from faeces).

Type-locality: San Juan del Carare, Santander Department, Colombia (06° 43 N, 74° 09′W; 150 m.a.s.l.).

Type material: Holotype: CNHE: No 11214, paratypes CNHE: No. 11215.

No of individuals: 17/99 (17.2%) of the recovered specimens of *Trypanoxyuris* belong to this species.

Etymology: The specific epithet derives from the Bora dialect for howler monkey "kemuime".

3.1.3.2. Remarks. This species is distinguished from *T. seunimii* n. sp. and *T. kotudoi* n. sp. by the absence of notches in the lips; however, it is morphologically similar to *T. minutus* (Fig. 4), which can be found in mixed infections in red howler monkeys. The main feature to discriminate between these species is the elongated shape of the right ventral lip in *T. kemuimae* n. sp., rather than a more circular shape of this lip showed by *T. minutus* (Fig. 4). Caudal structures among males are similar, as well as egg size and shape, making these characters not useful for discriminating among species.

3.1.4. Trypanoxyuris kotudoi n. sp. *(Fig. 5)*

Description based on 28 specimens. Buccal aperture delimited by three notched lips, dorsal and left ventral lip are bilobulated; right ventral lip with a salient square-shaped edge at the inner region; lobes in the left ventral lip approximately symmetrical.

Females (n = 19): Lips show discrete lateral indentations in the
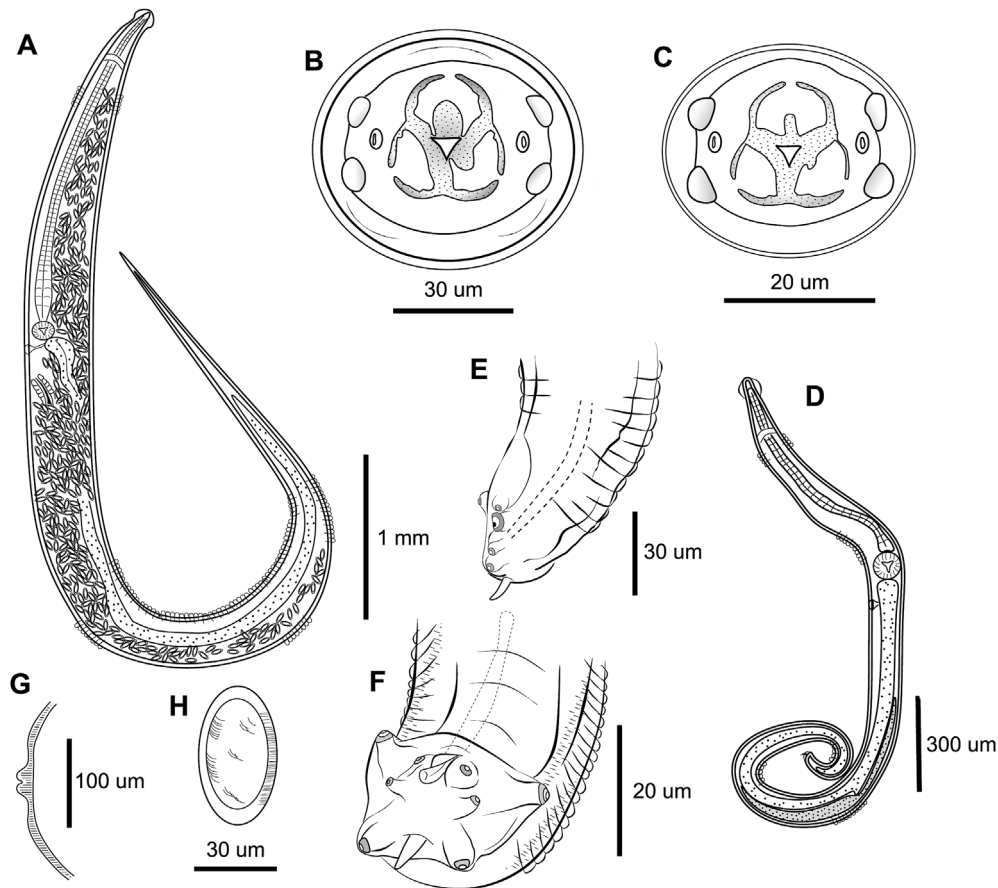
Fig. 3. *Trypanoxyuris keumimae* n. sp. (A) Female full body, lateral view. (B) Female cephalic end, apical view. (C) Male cephalic end, apical view, (D) Male full body, lateral view; (E) Male posterior end, lateral view, (F) Male posterior end, ventral view; (G) Female cross section showing lateral alae (H) Egg.

upper border, bilateral in the dorsal lip, while unilateral in the most ventral border in both ventral lips. Lateral alae double crested (Figs. 4 and 5). Measurements are given in Table 2.

Males (n = 9). Lips with discrete notches compared to females; indentations in the outer border are not observable; arch between lobes significantly smaller in the left ventral lip compared with the dorsal lip (Fig. 6). Tail appendage absent. Measurements are given in Table 3.

3.1.4.1. Taxonomic summary. Type-host: *A. seniculus (Linneus, 1766), red howler monkey.* *Site of infection*: Not determined (samples were obtained from faeces).

Type-locality: San Juan del Carare, Santander Department, Colombia (06° 43 N, 74° 09′W; 150 m.a.s.l.).

Type material: Holotype: CNHE: No 11216, paratypes CNHE: No. 11217.

No individuals: 28/99 (28.3%) of the recovered specimens of *Trypanoxyuris* belong to this species.

Etymology: The specific epithet derives from a combination of the Quechua word for red howler monkeys "kotu", and its common name in Colombia "mono cotudo".

3.1.4.2. Remarks. This species is morphologically similar to *T. seunimii* n. sp., and both species can be found in mixed infections in the same individual host. The presence of two bilobulated lips and double crested lateral alae in females of *T. kotudoi* n. sp. differentiates it from *T. seunimii* n. sp. Males of these two species are readily distinguished by the absence of a tail appendage in *T. kotudoi* n. sp.; additionally, the oval shape of the right ventral lip, rather than a triangular shape differentiate it from *T. seunimii* n. sp. (Fig. 6). *Trypanoxyuris kotudoi* n. sp. also resembles *T. pigrae,* but several traits can help to distinguish

between these two species: the square-shaped salient edge in the right ventral lip of *T. kotudoi* n. sp. rather than flat inner edge shown in *T. pigrae,* the symmetric versus asymmetric lobes of the left ventral lip in females of *T. kotudoi* n. sp. compared to *T. pigrae* (Fig. 4); the narrow arch between lobes in the dorsal lip of *T. kotudoi* n. sp. compared to a wider arch in *T. pigrae* (Fig. 4); and the double crested alae in females of *T. kotudoi* n. sp. while single crested in *T. pigrae.* Males of these two species distinguish from each other by the lack of tail appendage in the new species, and the sharp protuberances formed as a result of the notches in the lips, which are observable in *T. pigrae* (Fig. 6). Additionally, the host, with *T. pigrae* found in *A. pigra* and *T. kotudoi* n. sp. found in *A. seniculus.* Egg size and shape are highly similar among species, making these characters not useful for species level diagnosis.

3.2. Phylogenetic analyses

3.2.1. Concatenated data set

This data set consisted of sequences of the 28S rDNA plus the mitochondrial COI gene sequences. The final alignment included 31 sequences and 1994 base pairs (bp). Phylogenetic tree reconstructed by BI shows each of the *Trypanoxyuris* species found in red howler monkeys forming its own reciprocally monophyletic genetic lineage with high posterior probability support values (Fig. 7). *Trypanoxyuris seunimii* n. sp. and *T. kemuimae* n. sp. were recovered as sister species and grouped together with *T. pigrae,* while *T. minutus* and *T. kotudoi* n. sp. formed a separate genetic lineage. *Trypanoxyuris minutus* is further divided in two lineages, each corresponding to a different host species, with *T. minutus* obtained from *A. seniculus* in Colombia splitting from those obtained from the Mesoamerican howler monkeys (*A. palliata* and *A. pigra*) (Fig. 7). Likewise, *T. kotudoi* n. sp. subclade was also divided in two
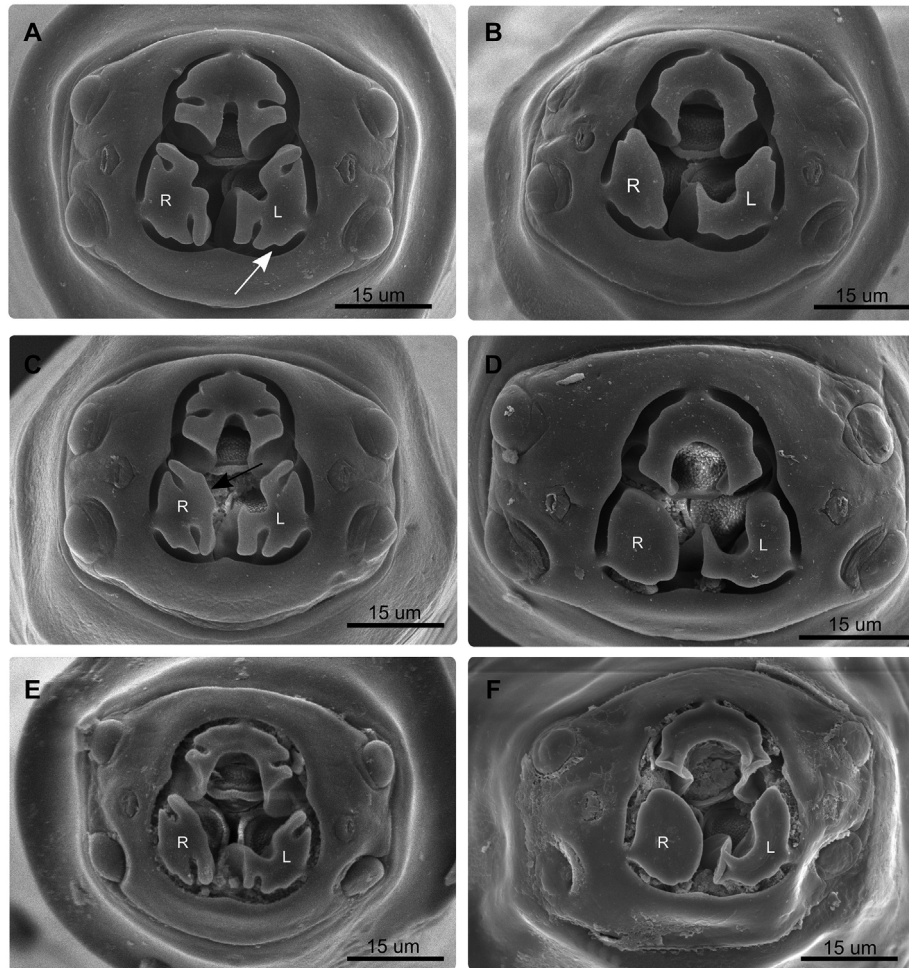
Fig. 4. SEM of buccal structures of females of *Trypanoxyuris* species found in howler monkeys (A) *Trypanoxyuris seunimii* n. sp. (B) *T. keumimae* n. sp. (C) *T. kotudoi* n. sp. (D) *T. minutus* from *Alouatta seniculus*. (E) *T. pigrae*. (F) *T. minutus* from Mesoamerican howler monkeys. R: right ventral lip; L: left ventral lip. White arrow pointing at the lateral indentations. Black arrow pointing at the square-shaped edge.

subgroups, each with high posterior probabilities. Individuals of both these subgroups could be found infecting the same individual host. The identity of these genetic lineages is maintained in the analysis conducted for each gene separately, with each species and lineage recovered consistently, and overall tree topology agrees with the tree topology for the concatenated data set; however, the phylogenetic relationships of some of these monophyletic groups vary in each case.

3.2.2. COI data set

This alignment included 49 sequences of 856 bp. Species of *Trypanoxyuris* in howler monkeys formed a monophyletic clade. Overall, the relationships among species of *Trypanoxyuris* of howler monkeys agreed with the tree topology for the concatenated data set (Fig. 7), and each species found in red howler monkeys formed its own genetic lineage with high nodal support values, except for *T. kotudoi* n. sp. where *T. kotudoi* Lineage 2 is sister taxa of *T. kotudoi* Lineage 1 plus *T. minutus* (Supplementary material S1).

3.2.3. Nuclear ribosomal DNA data set

The alignment included 34 sequences and 1138 bp. Reciprocal monophyly was also observed for each of the four species of *Trypanoxyuris* from red howler monkeys. Phylogenetic BI yielded *T. kemuimae* n. sp. and *T. pigrae* as sister species, forming a clade along with *T. seunimii* n. sp. This clade was recovered as sister of the *T. kotudoi* n. sp. clade. A separate group was then formed by *T. minutus*, which is further divided in two lineages according to host species. All

relationships shown in this phylogenetic analysis were supported by high posterior probabilities (Supplementary material S2).

3.3. Genetic divergence

Genetic divergence values estimated within and between lineages for both genes are presented in Table 4. Genetic *p*-distances within species of *Trypanoxyuris* from howler monkeys ranged from 0.2% to 1.7% in COI, while 28S isolates belonging to same species were identical. Genetic divergence among species ranged between 6% and 10.6% in COI, and from 0.4% to 4% in 28S. The COI genetic divergence between *T. minutus* from *A. seniculus* and *T. minutus* from Mesoamerican howler monkeys (5.9%), as well as the divergence between lineages of *T. kotudoi* n. sp. (5.3%), are relatively higher than the average within species divergence (Table 4); but they reached the lowest values of pairwise divergence with the other species. Moreover, 28S sequences within members of these lineages were not identical, with divergence values of 0.6% between lineages of *T. minutus*, and 0.2% between lineages of *T. kotudoi* n. sp. Despite the level of genetic divergence, no conspicuous morphological differences were detected when specimens of these genetically distinct groups were examined through light and scanning electron microscopy.

4. Discussion

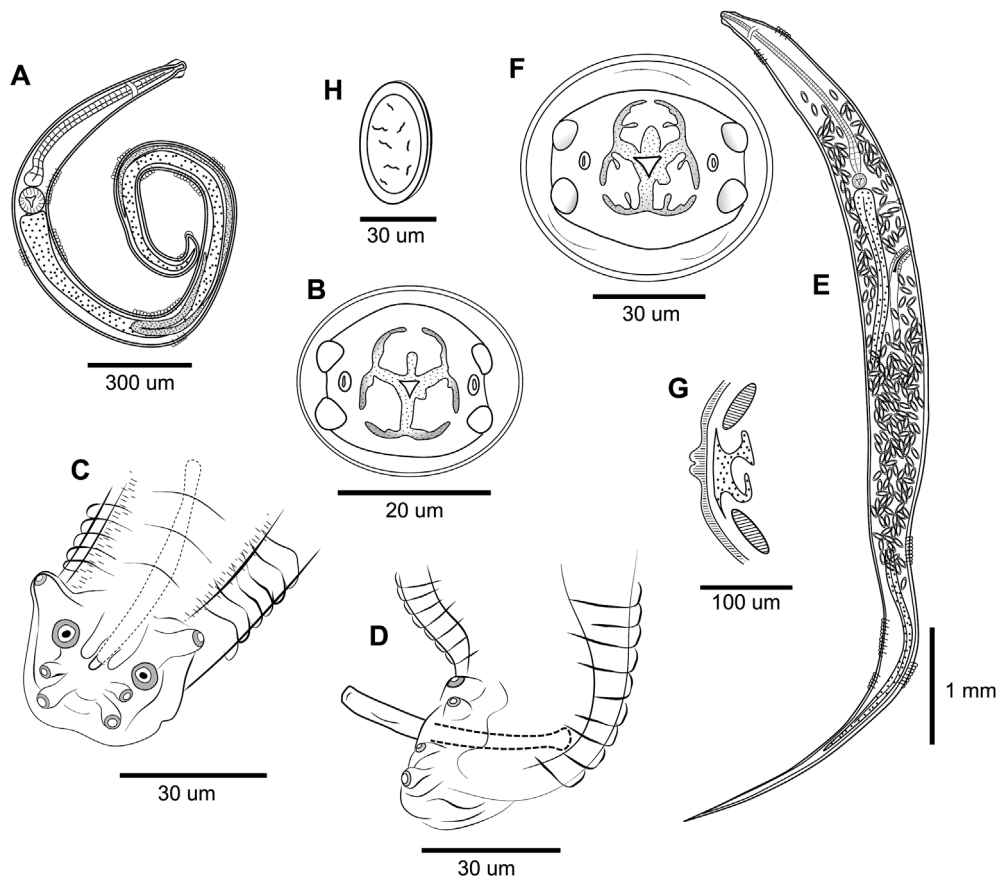Previous parasitological studies have reported the presence of

Fig. 5. *Trypanoxyuris kotudoi* n. sp. (A) Male full body, lateral view. (B) Male cephalic end, apical view. (C) Male posterior end, ventral view; (D) Male posterior end, lateral view, (E) Female full body, lateral view; (F) Female cephalic end, apical view; (G) Female cross section showing lateral alae; (H) Egg.

Oxyuridae eggs and *Trypanoxyuris* sp. eggs in faeces of red howler monkeys (*A. seniculus*) (De Thoisy et al., 2001; Rondón et al., 2017), with only one species level identification of *T. minutus* from red howlers in Brazil (Vicente et al., 1997). In this study, we showed that red howler monkeys from Colombia are parasitized by at least four different species of pinworms, *T. minutus, T. seunimii* n. sp., *T. kemuimae* n. sp. and *T. kotudoi* n. sp. The last three represent new species and were described using an integrative taxonomy approach through morphological and molecular evidence. The morphological diagnostic traits that differentiate these species reside mainly on the buccal structures, specifically the lips, and in the shape of the lateral alae in females. All four *Trypanoxyuris* species from *A. seniculus* have three lips; however, the shape of the lips, the presence of notches and the symmetry of the lobes constitute the main differences that enable us to distinguish among species. Hence, detailed enface view observations are needed in order to accurately designate species, and particularly the use of SEM microphotographs is a valuable tool to observe these structures in closer detail.

Furthermore, molecular phylogenetic analyses support the distinction of these species. Both the COI and 28S genes analyzed either separately or concatenated placed each of the species in reciprocally monophyletic lineages, most of them with high nodal support. Nonetheless, there are two cases in which molecular data suggest further species differentiation, although no morphological evidence was found to sustain the molecular differentiation. Phylogenetic assessments segregate individuals of *T. minutus* from *A. seniculus* from those found in the two species of Mesoamerican howler monkeys. Also, two linages of *T. kotudoi* n. sp. were recovered, although both were found in mixed infections within *A. seniculus* in Colombia. Overall morphological similarity between the examined specimens suggests the potential instance of the existence of cryptic species within both, *T. minutus*, and *T. kotudoi* n. sp. The evolutionary processes behind this pattern could be either a recent divergence or morphological stasis (Fišer et al., 2018; Struck et al., 2018; Struck and Cerca, 2019). In the case of the two lineages within *T. kotudoi* n. sp., a recent divergence might be invoked since both occur in concurrent infections in the red howler monkey; however, the two lineages of *T. minutus* are geographically separated and each correspond to a different host species and it seems that morphological stasis better explains the lack of morphological differentiation. The presence of cryptic species in parasite taxonomic studies is not unusual, due to the lack of easily detectable distinguishing characters (Pérez-Ponce de León and Nadler, 2010; Pérez-Ponce de León and Poulin, 2018); nevertheless, cryptic species can only be considered provisionally cryptic until additional studies reveal previously undetected diagnostic features (Nadler and Pérez-Ponce de León, 2011).

Even though the level of genetic divergence found between lineages of *T. minutus* and between lineages of *T. kotudoi* n. sp. is considerably high compared to the divergence observed within clades of the other *Trypanoxyuris* species (5.9% and 5.3%, respectively), it falls within the 6% maximum COI divergence previously reported within nematode species (Blouin, 1998), and within the 6.5% pairwise divergence in COI for *Enterobius vermicularis* (Nakano et al., 2006). Employing genetic distance thresholds can be useful for cryptic species prospecting (Criscione et al., 2005); however, it should not be taken as sole evidence for species delimitation. Values of intra and interspecific distances may overlap making it difficult to establish an appropriate divergence yardstick; furthermore, organisms with high evolutionary rates can show moderate distances that might only represent genetic variation among intraspecific genetic lineages, while relatively recently formed species can show minimal levels of genetic variation and morphological divergence (Nadler and Pérez-Ponce de León, 2011). Given
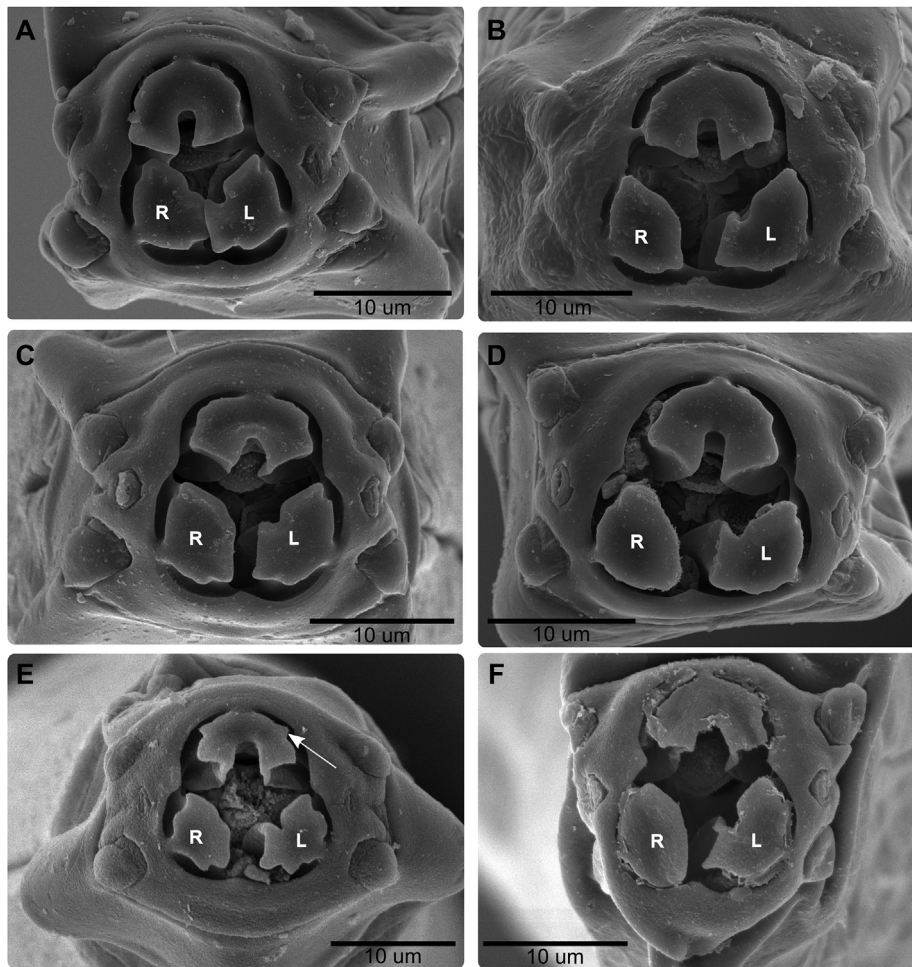
Fig. 6. SEM of buccal structures of males of *Trypanoxyuris* species found in howler monkeys (A) *Trypanoxyuris seunimii* n. sp. (B) *T. keumimae* n. sp. (C) *T. kotudoi* n. sp. (D) *T. minutus* from *Alouatta seniculus*. (E) *T. pigrae*. (F) *T. minutus* from Mesoamerican howler monkeys. R: right ventral lip; L: left ventral lip. White arrow pointing at the sharp protuberances formed as a result of the notches in the lips.

the moderate divergence, and the unnoticeable structural differences, we may consider the phylogenetic separation of these lineages as a result of an ongoing speciation process. Additional studies through detailed meristic analysis and high resolution microscopy remain essential to fully evaluate the cryptic nature of these lineages as has been done with other putative cryptic species of pinworms (Jorge et al., 2013). Also, examinations of *Trypanoxyuris* from different red howler monkey populations across their distribution range are needed to account for morphological and molecular variation within these pinworm species and lineages. For example, it is possible that the moderate differences observed within linages will become more evident as different host subspecies are sampled, as has happened with other *Trypanoxyuris* species (Solórzano-García et al., 2019); or that the species assemblages occurring in each population vary across the host range. Another possible approach might require the use of next generation sequencing (NGS) methods, gathering large molecular datasets (e.g., complete mitochondrial genomes) that could result in a more robust species delimitation (Ahmad et al., 2019; Duan et al., 2015; Gao et al., 2014; Jaleta et al., 2018).

The phylogenetic assessment conducted in this study showed that the species of *Trypanoxyuris* that parasitize each howler monkey species are not closely related. Two of the four pinworm species found in red howlers, *T. seunimii* n. sp. and *T. kemuimae* n. sp. are more closely related to *T. pigrae*, a parasite of black howlers (*A. pigra*) than to the other species of pinworms also occurring in red howlers (*A. seniculus*); while *T. minutus* from Mesoamerican howler monkeys is nested in a clade with

T. kotudoi n. sp., a parasite of red howlers, instead of *T. multilabiatus,* a pinworm described from *A. palliata* in southeastern Mexico (Fig. 7). These phylogenetic relationships suggest that howler monkeys acquired their pinworms in two or more independent colonization events. Interestingly, the species which are phylogenetically the closest are morphologically different but resemble distant species. For instance, *T. seunimii* n. sp.*, T. pigrae* and *T. kotudoi* n. sp. are characterized by having notched lips but are not sister species, while *T. minutus* and *T. kemuime* n. sp. lack such ornamentations. Nevertheless, since the main structural differences constitute buccal features, parasite feeding behavior and feeding environment could have driven pinworm speciation through resource competition and habitat partition (Solórzano-García et al., 2016). Thus, the howler's internal gut environment and feeding ecology could be key elements in the evolution and diversification of *Trypanoxyuris*.

The cumulative knowledge on primate-pinworm associations place *Trypanoxyuris* as host specific parasites, with a particular group of pinworms infecting each primate species (Hugot, 1985, 1984, 1999); the data gathered in this study appear to be concordant with this idea. The species of pinworms previously reported as exclusively from *A. palliata* and *A. pigra* (*T. multilabiatus* and *T. pigrae,* respectively) have not been found to parasitize Colombian red howler monkeys, which instead are parasitized by unique species of pinworms. However, the species described in the present study only correspond to a small area of red howler distribution, and a different pattern could emerge as different howler monkey populations are analyzed for pinworms. Thus, an
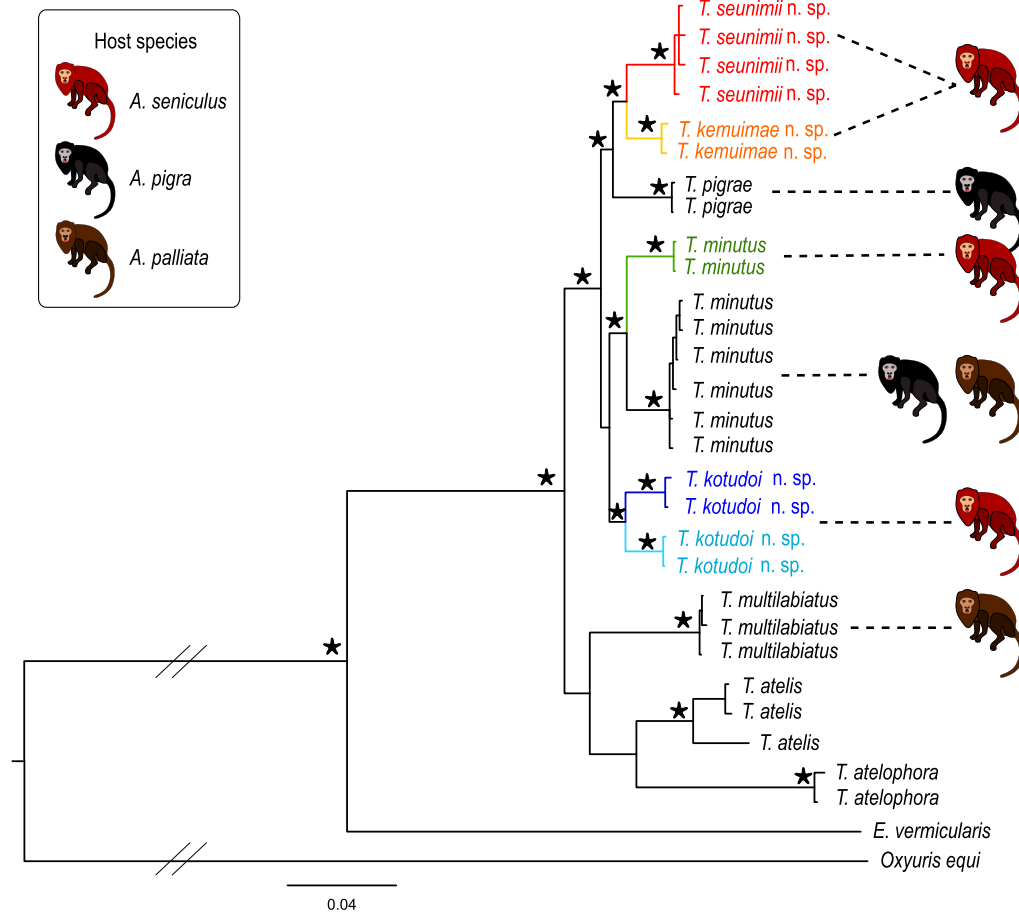
Fig. 7. Bayesian Phylogenetic tree of *Trypanoxyuris* species inferred with the concatenated data set (COI + 28S). Stars at the nodes represent posterior probability values higher than 0.95. Each colour indicates a lineage of *Trypanoxyuris* found in *Alouatta seniculus*. GenBank accessions numbers of the additional sequences are the same as those used in separated gen analysis (Supplementary material S1 and S2). Monkeys at the side represent host species. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

exhaustive sampling of red howler monkey populations across their range, and the examination of pinworms from neighboring populations of other howler species are required to properly assess such specificity patterns. Furthermore, *T. minutus* appears to be a more generalist parasite infecting howler monkeys along their entire distributional range. The results presented here suggest that *T. minutus* from Mesoamerican howlers and those from South American howlers could represent different lineages. A phylogenetic separation between trans- and cis-Andean organisms have been reported for howler monkeys (Cortés-Ortiz et al., 2003); the mechanisms underlying the diversification among species of *Alouatta* could also explain the divergence observed between *T. minutus* clades. Nevertheless, the suggestion of the

existence of more than one species in what has been considered *T. minutus* across the Americas remains valid (Solórzano-García et al., 2016).

5. Conclusion

Molecular and morphological evidence show that red howler monkeys are parasitized by at least four species of *Trypanoxyuris* which can occur in mixed infections, with the four species infecting the same individual host at the same time. Pinworms of howler monkeys appear to be host-specific with a unique set of species for each host species; however this notion needs further confirmation. Phylogenetic

Table 4
Mean genetic divergence between *Trypanoxyuris* from howler monkeys. Uncorrected p-distance expressed as percentage (± SE). 28S divergence above the diagonal; COI divergence below the diagonal. Bold numbers on diagonal indicate COI genetic divergence within species.

	T. multilabiatus	T. pigrae	T. minutus a	T. minutus b	*T. seunimii* n. sp.	*T. kemuimae* n. sp.	*T. kotudoi* n.sp. Clade 1	*T. kotudoi* n.sp. Clade 2
T. multilabiatus	**0.3 (0.1)**	3.9 (0.6)	3.8 (0.6)	4.0 (0.6)	3.8 (0.6)	4.0 (0.6)	3.8 (0.6)	4.0 (0.6)
T. pigrae	10.1 (0.9)	**0.3 (0.1)**	0.8 (0.3)	1.0 (0.3)	0.4 (0.2)	0.4 (0.2)	0.6 (0.3)	0.6 (0.2)
T. minutus[a]	9.3 (0.9)	7.9 (0.8)	**0.2 (0.2)**	0.6 (0.2)	0.8 (0.3)	1.0 (0.3)	0.8 (0.3)	0.8 (0.3)
T. minutus[b]	9.5 (0.9)	8.1 (0.9)	5.9 (0.8)	**0.6 (0.2)**	1.0 (0.3)	1.2 (0.3)	1.1 (0.3)	1.1 (0.3)
T seunimii n. sp.	10.5 (1.0)	8.3 (0.8)	8.6 (0.9)	7.1 (0.8)	**1.7 (0.3)**	0.4 (0.2)	0.6 (0.3)	0.6 (0.3)
T. kemuimae n. sp.	9.3 (1.0)	7.0 (0.7)	7.1 (0.9)	7.1 (0.9)	7.0 (0.8)	**0.6 (0.4)**	0.8 (0.3)	0.8 (0.3)
T. kotudoi n. sp.Clade 1	10.6 (0.9)	7.7 (0.8)	6.0 (0.8)	6.2 (0.8)	8.0 (0.9)	7.5 (0.8)	**0.6 (0.4)**	0.2 (0.1)
T. kotudoi n. sp.Clade 2	9.1 (0.9)	7.2 (0.7)	6.8 (0.8)	6.7 (0.8)	7.9 (0.9)	6.6 (0.7)	5.3 (0.7)	**0.2 (0.2)**

[a] *Trypanoxyuris minutus* from Mesoamerican howler monkeys (*Alouatta palliata*, and *A. pigra*).
[b] *T. minutus* from red howler monkeys (*A. seniculus*).

relationships among pinworms of howlers suggest a tight evolutionary association between pinworms and primates, and did not discard the co-evolutionary hypothesis. A complete understanding of the evolutionary patterns and dynamics between pinworms and primates could only be accomplished by a thorough sampling of the hosts to fully characterise pinworm biodiversity. The ongoing efforts to gather such information will allow us to disentangle phylogenetic relationships among *Trypanoxyuris,* to assess the level of host specificity, to test co-phylogenetic hypothesis, and to unveil co-evolutionary patterns.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2019.11.007.

References

Adamson, M.L., 1989. Evolutionary biology of the Oxyuridae (Nematoda): biofacies of a haplodiploid taxon. Adv. Parasitol. 28, 175–228.

Ahmad, A., Yang, X., Zhang, T., Wang, C., Zhou, C., Yan, X., Hassan, M., Ikram, M., Hu, M., 2019. Characterization of the complete mitochondrial genome of *Ostertagia trifurcata* of small ruminants and its phylogenetic associations for the trichostrongyloidea superfamily. Genes 10, 107. https://doi.org/10.3390/genes10020107.

Bessho, Y., Ohama, S., Osawa, S., 1992. Planarian mitochondria 1. Heterogeneity of cytochrome c oxidase subunit 1 gene sequences in the freshwater planarian, *Dugesia japonica*. Journal of Molecular Evolution 34, 324–330.

Blouin, M., 1998. Mitochondrial DNA diversity in nematodes. J. Helminthol. 72, 285–289.

Brooks, D.R., Glen, D.R., 1982. Pinworms and primates: a case study in coevolution. Proc. Helminthol. Soc. Wash. 49, 76–85.

Casiraghi, M., Anderson, T.TC, Bandi, C., Bazzocchi, C., Genchi, C., 2001. A phylogenictic analysis of filarial nematodes: comparison with the *Wolbachia* endosymbionts. Parasitology 122, 93–103.

Conga, D.F., Giese, E.G., Serra-Freire, N.M., Bowler, M., Mayor, P., 2016. Morphology of the oxyurid nematodes *Trypanoxyuris (T.) cacajao* n. sp. and *T. (T.) ucayalii* n. sp. from the red uakari monkey *Cacajao calvus ucayalii* in the Peruvian Amazon. J. Helminthol. 90, 483–493. https://doi.org/10.1017/S0022149X1500067X.

Cortés-Ortiz, L., Bermingham, E., Rico, C., Rodriguez-Luna, E., Sampaio, I., Ruiz-Garcia, M., 2003. Molecular systematics and biogeography of the Neotropical monkey genus. Alouatta. Mol. Phylogenet. Evol. 26, 64–81.

Cortes-Ortiz, L., Rylands, A.B., Mittermeier, R., 2015. The taxonomy of howler monkeys: integrating old and new knowledge from morphological and genetic studies. In: Kowalewski, M.M., Garber, P.A., Cortes-Ortiz, L., Urbani, B., Youlatos, D. (Eds.), Howler Monkeys, Developments in Primatology: Progress and Prospects. Springer, New York, pp. 55–80. https://doi.org/10.1007/978-1-4939-1957-4_3.

Criscione, C.D., Poulin, R., Blouin, M.S., 2005. Molecular ecology of parasites: elucidating ecological and microevolutionary processes. Mol. Ecol. 14, 2247–2257. https://doi.org/10.1111/j.1365-294X.2005.02587.x.

De Thoisy, B., Vogel, I., Reynes, J.M., Pouliquen, J.F., Carme, B., Kazanji, M., Vié, J.C., 2001. Health evaluation of translocated free-ranging primates in French Guiana. Am. J. Primatol. 54, 1–16. https://doi.org/10.1002/ajp.1008.

Defler, T., 2003. In: Primates de Colombia. Conservation International, Colombia.

Duan, H., Gao, J.F., Hou, M.R., Zhang, Y., Liu, Z.X., Gao, D.Z., Guo, D.H., Yue, D.M., Su, X., Fu, X., Wang, C.R., 2015. Complete Mitochondrial genome of an equine intestinal parasite, *Triodontophorus brevicauda* (Chromadorea: strongylidae): the first characterization within the genus. Parasitol. Int. 64, 429–434. https://doi.org/10.1016/j.parint.2015.06.006.

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32, 1792–1797.

Felt, S.A., White, C.E., 2005. Evaluation of a timed and repeated perianal tape test for the detection of pinworms (*Trypanoxyuris microon*) in owl monkeys (*Aotus nancymae*). J.

Med. Primatol. 209–214.

Fišer, C., Robinson, C.T., Malard, F., 2018. Cryptic species as a window into the paradigm shift of the species concept. Mol. Ecol. 27, 613–635. https://doi.org/10.1111/mec.14486.

Gao, J.F., Zhao, Q., Liu, G.H., Zhang, Yan, Zhang, Ying, Wang, W.T., Chang, Q.C., Wang, C.R., Zhu, X.Q., 2014. Comparative analyses of the complete mitochondrial genomes of the two ruminant hookworms *Bunostomum trigonocephalum* and *Bunostomum phlebotomum*. Gene 541, 92–100. https://doi.org/10.1016/j.gene.2014.03.017.

Hasegawa, H., 2009. Methods of collection and identification of minute nematodes from the feces of primates, with special application to coevolutionary study of pinworms. In: Huffman, M.A., Chapman, C.A. (Eds.), Primate Parasite Ecology. The Dynamics and Study of Host-Parasite Relationships. Cambridge University Press, United Kindom, pp. 19–46.

Hasegawa, H., Ikeda, Y., Dias-Aquino, J.J., Fukui, D., 2004. Redescription of two pinworms from the black-handed spider monkey, *Ateles geoffroyi*, with reestablishment of *Oxyuronema* and *Buckleyenterobius* (Nematoda: Oxyuroidea). Comp. Parasitol. 71, 166–174.

Hugot, J., 1985. Sur le genre *Trypanoxyuris* (Oxyuridae, Nematoda) III. Sons-genre *Trypanoxyuris* parasite de Primates Cebidae et Atelidae. Bull. Mus. natn. His.nat. Paris 4, 131–155.

Hugot, J., 1984. Sur le genre *Trypanoxyuris* (Oxyuridae, Nematoda) II . Sous-genre *Hapaloxyuris* parasite de Primates Callitrichidae. Bull. du Muséum Natl. d'Histoire Nat. 1007–1019.

Hugot, J.P., 1999. Primates and their pinworm parasites: the Cameron Hypothesis revisited. Syst. Biol. 48, 523–546.

Hugot, J.P., Gardner, S.L., Morand, S., 1996. The Enterobiinae subfam. nov. (Nematoda, Oxyurida) pinworm parasites of primates and rodents. Int. J. Parasitol. 26, 147–159. https://doi.org/10.1016/0020-7519(95)00108-5.

Hugot, J.P., Morand, S., Guerrero, R., 1994. *Trypanoxyuris croizati* n.sp. and *T. callicebi* Hugot & Vaucher, 1985 (Nematoda: Oxyuridae), two vicariant forms parasitic in *Callicebus* spp. (Primatia, Cebidae). Syst. Parasitol. 27, 35–43. https://doi.org/10.1007/BF02185666.

Jaleta, T.G., Rödelsperger, C., Abanda, B., Eisenbarth, A., Achukwi, M.D., Renz, A., Streit, A., 2018. Full mitochondrial and nuclear genome comparison confirms that Onchocerca sp. "Siisa" is Onchocerca ochengi. Parasitol. Res. 117, 1069–1077. https://doi.org/10.1007/s00436-018-5783-0.

Jorge, F., Perera, A., Carretero, M.A., James Harris, D., Roca, V., 2013. Cryptic species unveiled: the case of the nematode *Spauligodon atlanticus*. J. Zool. Syst. Evol. Res. 51, 187–202. https://doi.org/10.1111/jzs.12019.

Maddison, W., Maddison, D., 2011. Mesquite: a Modular System for Evolutionary Analysis.

Madeira, F., Park, Y., Lee, J., Buso, N., Tamer, G., Madhusoodanan, N., Basutkar, P., Tivey, A.R.N., Potter, S.C., Finn, D., Lopez, R., 2019. The EMBL-EBI search and sequence analysis tools APIs in 2019. Nucleic Acids Res. 47, w636–w641.

Miller, M., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop. New Orleans, pp. 1–8.

Nadler, S.A., Hudspeth, D.SS, 1998. Ribosomal DNA and phylogeny of the Ascaridoidea (Nemata: Secernentea): implications formorphological evolution and classification. Mol. Phylogenet. Evol. 10, 221–236.

Nadler, S.A., Pérez-Ponce de León, G., 2011. Integrating molecular and morphological approaches for characterizing parasite cryptic species: implications for parasitology. Parasitology 138, 1688–1709. https://doi.org/10.1017/S003118201000168X.

Nakano, T., Okamoto, M., Ikeda, Y., Hasegawa, H., 2006. Mitochondrial cytochrome c oxidase subunit 1 gene and nuclear rDNA regions of Enterobius vermicularis parasitic in captive chimpanzees with special reference to its relationship with pinworms in humans. Parasitol. Res. 100, 51–57. https://doi.org/10.1007/s00436-006-0238-4.

Nylander, J.A.A., 2004. MrModeltest v.2.

Pérez-Ponce de León, G., Nadler, S.A., 2010. What we don't recognize can hurt us: a plea for awareness about cryptic species. J. Parasitol. 96, 453–464. https://doi.org/10.1645/GE-2260.1.

Pérez-Ponce de León, G., Poulin, R., 2018. An updated look at the uneven distribution of cryptic diversity among parasitic helminths. J. Helminthol. 92, 197–202.

Pleijel, F., Jondelius, U., Norlinder, E., Nygren, A., Oxelman, B., Schander, C., Sundberg, P., Thollesson, M., 2008. Phylogenies without roots? A plea for the use of vouchers in molecular phylogenetic studies. Mol. Phylogenetics Evol. 48, 369–371. https://doi.org/10.1016/j.ympev.2008.03.024.

Rambaut, A., 2014. FigTree. Version 1.4.2.

Rondón, S., Ortiz, M., León, C., Galvis, N., Link, A., González, C., 2017. Seasonality, richness and prevalence of intestinal parasites of three neotropical primates (*Alouatta seniculus, Ateles hybridus* and *Cebus versicolor*) in a fragmented forest in Colombia. Int. J. Parasitol. Parasites Wildl. 6, 202–208. https://doi.org/10.1016/j.ijppaw.2017.07.006.

Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

Smythe, A.B., Nadler, S.A., 2006. Molecular phylogeny of Acrobeloides and Cephalobus (Nematoda: Cephalobidae) reveals paraphyletic taxa and recurrent evolution of simple labial morphology. Nematology 8, 819–836.

Solórzano-García, B., Güiris-Andrade, D.M., Pérez-Ponce de León, G., 2017. The Missing Fellow: First Description of the Trypanoxyuris pigrae Male (Nematoda: Oxyuridae), a Parasite of the Black Howler Monkey (Alouatta pigra) in Mexico. Journal of Parasitology 103 (4), 404–406.

Solórzano-García, B., Melin, A.D., Aureli, F., Pérez-Ponce de León, G., 2019. Unveiling patterns of genetic variation in parasite – host associations : an example with pinworms and Neotropical primates. Parasitology 146, 356–362.

Solórzano-García, B., Nadler, S.A., Pérez-Ponce de León, G., 2016. Pinworm diversity in

free-ranging howler monkeys (*Alouatta* spp.) in Mexico: morphological and molecular evidence for two new *Trypanoxyuris* species (Nematoda: Oxyuridae). Parasitol. Int. 65, 401–411. https://doi.org/10.1016/j.parint.2016.05.016.

Solórzano-García, B., Nadler, S.A., Pérez Ponce de León, G., 2015. *Trypanoxyuris atelis* and *T. atelophora* (Nematoda: Oxyuridae) in wild spider monkeys (*Ateles geoffroyi*) in tropical rain forest in Mexico: morphological and molecular evidence. Parasitol. Int. 64, 229–235. https://doi.org/10.1016/j.parint.2015.02.006.

Solórzano-García, B., Pérez-Ponce de León, G., 2018. Parasites of Neotropical primates: a review. Int. J. Primatol. 39, 155–182. https://doi.org/10.1007/s10764-018-0031-0.

Sorci, G., Skarstein, F., Morand, S., Hugot, J.P., 2003. Correlated evolution between host immunity and parasite life histories in primates and oxyurid parasites. Proc. R. Soc. Biol. Sci. 270, 2481–2484. https://doi.org/10.1098/rspb.2003.2536.

Struck, T.H., Cerca, J., 2019. In: Cryptic Species and Their Evolutionary Significance, https://doi.org/10.1002/9780470015902.a0028292. eLS 1–9.

Struck, T.H., Feder, J.L., Bendiksby, M., Birkeland, S., Cerca, J., Gusarov, V.I., Kistenich, S., Larsson, K.-H., Liow, L.H., Nowak, M.D., Stedje, B., Bachmann, L., Dimitrov, D., 2018. Finding evolutionary processes hidden in cryptic species. Trends Ecol. Evol. 33, 153–163.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729. https://doi.org/10.1093/molbev/mst197.

Vermund, S.H., Wilson, C.M., 2000. Pinworm (*Enterobius vermicularis*). Semin. Pediatr. Infect. Dis. 11, 252–256. https://doi.org/10.1053/spid.2000.9639.

Vicente, J.J., Rodrigues, H.O., Gomes, D.C., Pinto, R.M., 1997. Nematóides do Brasil. Parte V: Nematóides de mamíferos. Rev. Bras. Zoolo- gia 14, 1–452.