

First genetic characterisation and phylogenetic position of *Aplectana hylambatis* (Nematoda: Cosmocercidae), infecting *Pithecopus azureus* (Anura: Hylidae) from the Brazilian Pantanal
P.S. dos Santos, I.C.O. Silva, V.L. Ferreira, L.E.R. Tavares, F. Paiva, and F.B. Pereira

Table S1. Genetic sequences of nematodes of the family Cosmocercidae and of the outgroup, used for phylogenetic reconstructions, associated with the genetic markers and GenBank accession numbers.

Taxon	18S rDNA	28S rDNA
<i>Aplectana chamaeleonis</i>	OK045518	OK045533
<i>Aplectana dayaoshanensis</i>	OK045516	OK045530
<i>Aplectana hylambatis</i>	PQ037030	PQ037031
<i>Aplectana xishuangbannaensis</i>	MW329991	MW329038
<i>Cosmocerca ornata</i>	MW326676	MW326675
<i>Cosmocerca simile</i>	MN839758	MN839755
<i>Cosmocercoides dukae</i>	FJ516753	-
<i>Cosmocercoides pulcher</i>	MH178322	LC018444
<i>Cosmocercoides qingtianensis</i>	MH032769	MW325956
<i>Cosmocercoides tonkinensis</i>	AB908160	AB908160
<i>Cosmocercoides wuyiensis</i>	MK110872	-
<i>Dichelyne grandistomis</i> *	KX752094	KX752093
<i>Nemhelix bakeri</i>	DQ118537	-

*Used as an outgroup. This species appears as *Cucullanus grandistomis* in GenBank.

Table S2. Comparative morphometry of *Aplectana hylambatis* from previous taxonomic studies carried out in South America (mainly Argentina), and from the original description. Measurements are in micrometres, unless otherwise indicated.

Reference	Present study	Baylis (1927) ^a	Aguiar <i>et al.</i> (2015)	Gutiérrez (1945)	Baker (1980)	González and Hamann (2010)	González <i>et al.</i> (2013)
Host	<i>Pithecopus azureus</i>	<i>Leptopelis aubryi</i>	<i>Dermatonotus muelleri</i>	<i>Rhinella arenarum</i>	<i>Rhinella achalensis</i>	<i>Physalaemus santafecinus</i>	<i>Rhinella arenarum</i>
Locality	Brazil	French Guinea, West Africa	Brazil	Argentina	Argentina	Argentina	Argentina
Male	n = 10	n = 3	n = ?	n = ?	n = 5	n = 11	n = 11
Total L (mm)	1.6–2.4	4.0–4.2	2.2–4.0	4.0–4.8	4.2–5.0	1.9–2.7	3.0–3.8
Maximum W	42–86	260–300	137–282	181–265	NM	120–205	210–300
Pharynx L	32–47	75–85	NM	45–52	44–56	28–37	30–54
Pharynx W	20–33	NM	NM	23–30	NM	17–22	23–34
Oesophagus L	363–513	700	503–610	640–755	465–506	306–390	450–540
Corpus L	239–383	NM	NM	NM	NM	NM	NM
Isthmus L	30–51	NM	NM	NM	NM	NM	NM
Bulb L	77–90	140–150	91–131	92–102	100–119	71–80	75–102
Bulb W	72–94	100–120	106–145	92–109	NM	67–82	84–101
Excretory pore*	284–372	530–650	382–496	238–334	472–559	306–420	475–600
Nerve ring*	167–229	300–330	NM	238–288	259–278	281–311	200–300
Spicule L	201–276	240–280	228–340	238–349	319–350	255–298	240–348
Spicule ratio (%)**	11.5–12.6	6.0–6.7	8.5–10.1	6.0–7.3	7.0–7.6	11.0–13.4	8.0–9.2
Gubernaculum L	60–97	90–100	53–85	NM	109–116	67–89	48–78
Gubernaculum ratio (%)**	3.8–4.0	2.3–2.4	2.1–2.4	NM	2.3	3.3–3.5	1.6–2.1
Tail L	132–177	200	160–279	NM	244–303	115–160	213–258
Female	n = 10	n = 4	n = ?	n = ?	n = 5	n = 10	n = 10
Total L (mm)	1.3–2.4	5.3–6.0	2.4–3.9	4.4–6.2	4.8–6.3	2.5–3.1	3.7–5.4
Maximum W	102–201	300–370	188–299	232–423	NM	183–324	325–460
Pharynx L	21–50	75–85	NM	43–60	47–63	28–41	36–60
Pharynx W	20–39	NM	NM	29–36	NM	21–28	26–36
Oesophagus L	232–518	760–850	612–661	764–863	553–616	306–402	485–565
Corpus L	160–365	NM	NM	NM	NM	NM	NM
Isthmus L	14–54	NM	NM	NM	NM	NM	NM
Bulb L	58–117	180–190	115–132	115–119	122–137	85–103	84–132
Bulb W	56–120	140–150	136–169	122–135	NM	90–108	92–132
Excretory pore*	167–342	530–650	415–509	238–334	481–616	342–620	470–610
Nerve ring*	90–205	300–330	NM	241–334	281–311	204–270	265–325
Vulva (mm)**	0.8–1.7	1.8	1.6–2.5	NM	3.2–4.2	1.6–2.1	2.2–3.5
Vulva ratio (%)	61.5–70.8	66.7	64.1–66.7	NM	66.7	64.0–67.8	59.4–64.8
Tail	121–197	280–310	182–219	174–274	209–272	177–218	235–350

*Distance from anterior end. ^aThis is the original description of *A. hylambatis* (originally nominated as *Oxysomatium hylambatis*).

**Ratio to total body length.

Abbreviations: L = length; W = width; NM = not measured

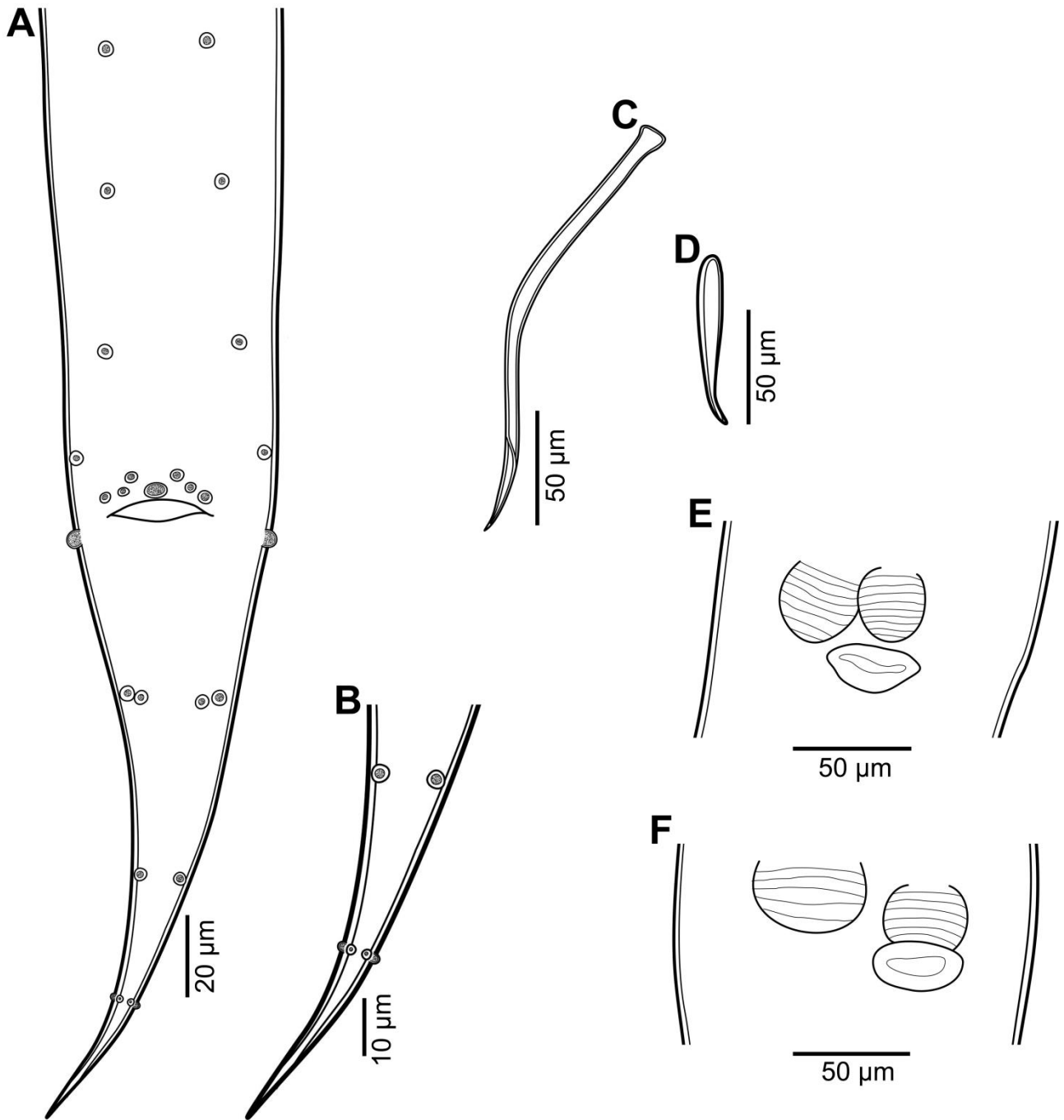
Table S2 continued

Reference	González <i>et al.</i> (2019)	González <i>et al.</i> (2019)	González <i>et al.</i> (2019)	González <i>et al.</i> (2019)	González <i>et al.</i> (2019)	Castillo <i>et al.</i> (2023)
Host	<i>D. muelleri</i>	<i>Rhinella major</i> ^b	<i>Rhinella arenarum</i> ^b	<i>Leptodactylus bufonius</i> ^b	<i>Physalaemus santafecinus</i>	<i>Rhinella spinulosa</i>
Locality	Argentina	Argentina	Argentina	Argentina	Argentina	Argentina
Male	n = 20	n = 80	n = 40	n = 60	n = 20	n = 2
Total L (mm)	3.0–3.8	2.1–3.3	2.9–3.8	2.1–3.1	2.0–2.8	3.7–3.9
Maximum W	210–300	120–240	200–300	90–200	120–250	340–355
Pharynx L	NM	NM	NM	NM	NM	NM
Pharynx W	NM	NM	NM	NM	NM	NM
Oesophagus L	521–621	342–453	470–582	322–517	341–418	700–784
Corpus L	NM	NM	NM	NM	NM	NM
Isthmus L	NM	NM	NM	NM	NM	NM
Bulb L	84–132	69–92	75–97	69–120	71–80	117–120
Bulb W	92–132	67–92	78–101	74–114	67–82	117–120
Excretory pore*	342–414	342–570	438–600	300–485	306–420	550–552
Nerve ring*	162–216	156–270	200–350	138–275	174–222	178–275
Spicule L	184–242	207–327	230–348	150–246	255–298	400–402
Spicule ratio (%)**	6.1–6.4	9.9–9.9	7.9–9.2	7.1–7.9	10.6–12.8	10.3–10.8
Gubernaculum L	58–81	69–104	48–104	45–78	67–89	NM
Gubernaculum ratio (%)**	1.2–2.1	3.2–3.3	1.7–2.7	2.5–2.1	3.2–3.4	NM
Tail L	NM	NM	NM	NM	NM	NM
Female	n = 20	n = 80	n = 40	n = 60	n = 20	n = 2
Total L (mm)	3.7–5.4	2.6–4.1	3.4–5.4	2.1–3.6	2.5–3.1	4.9–5.0
Maximum W	325–460	140–290	150–460	110–285	183–324	567–570
Pharynx L	NM	NM	NM	NM	NM	NM
Pharynx W	NM	NM	NM	NM	NM	NM
Oesophagus L	521–621	376–496	503–621	391–594	336–435	637–660
Corpus L	NM	NM	NM	NM	NM	NM
Isthmus L	NM	NM	NM	NM	NM	NM
Bulb L	84–132	81–115	84–132	90–140	85–103	137–140
Bulb W	92–132	81–113	92–132	83–145	90–108	117–120
Excretory pore*	378–468	378–540	470–610	348–550	342–620	544–550
Nerve ring*	180–240	174–258	240–325	150–315	204–270	225–230
Vulva (mm)**	2.0–2.3	1.8–2.8	2.2–3.5	1.5–2.4	1.6–2.1	3.4–3.5
Vulva ratio (%)	42.6–54.1	68.3–69.2	64.7–64.8	66.7–71.4	64.0–67.7	69.4–70.0
Tail	NM	NM	NM	NM	NM	NM

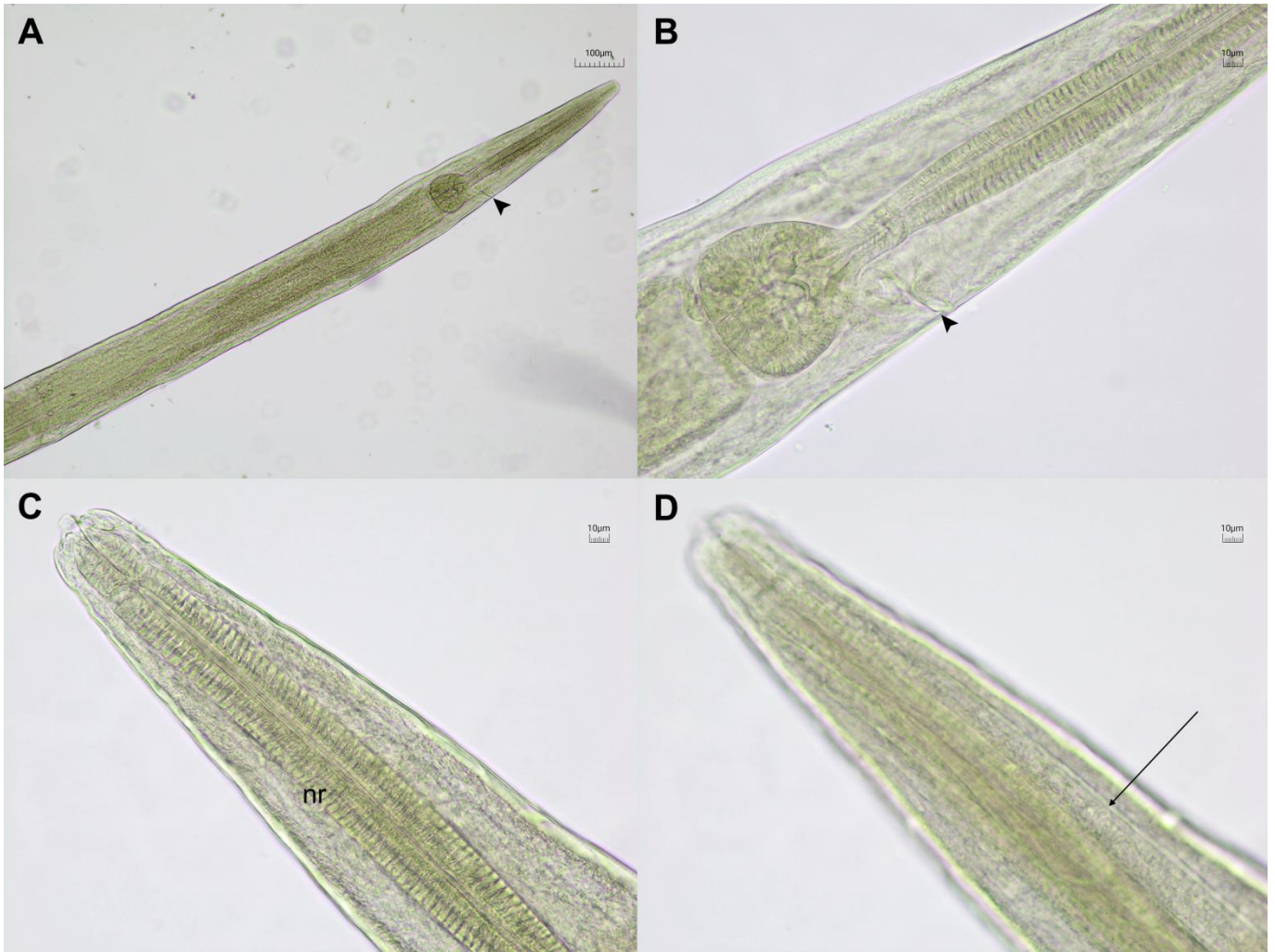
*Distance from anterior end. ^bThese hosts were collected from different localities in Argentina, the measurements are presented as minimum and maximum values considering all of them.

**Ratio to total body length.

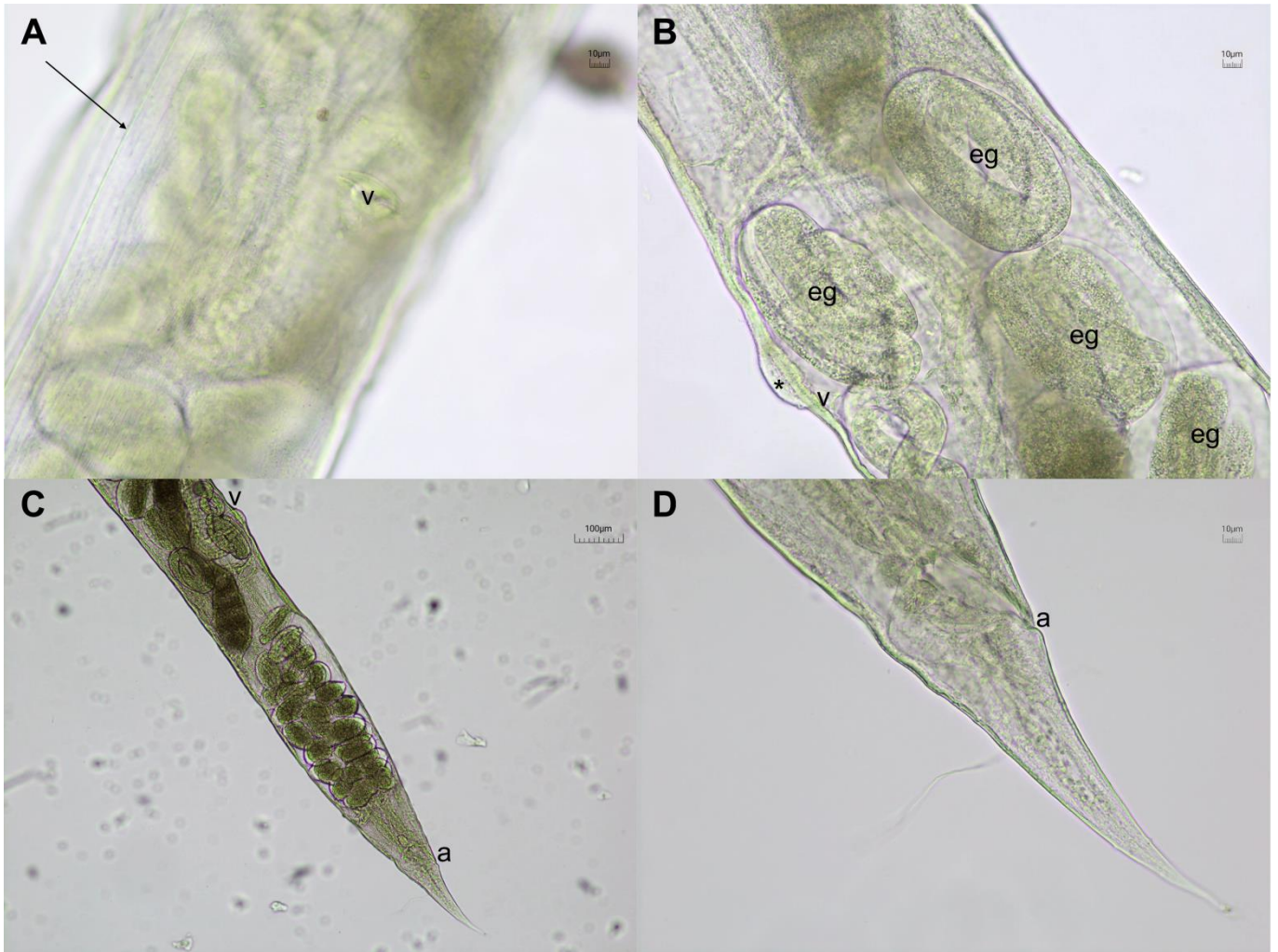
Abbreviations: L = length; W = width; NM = not measured



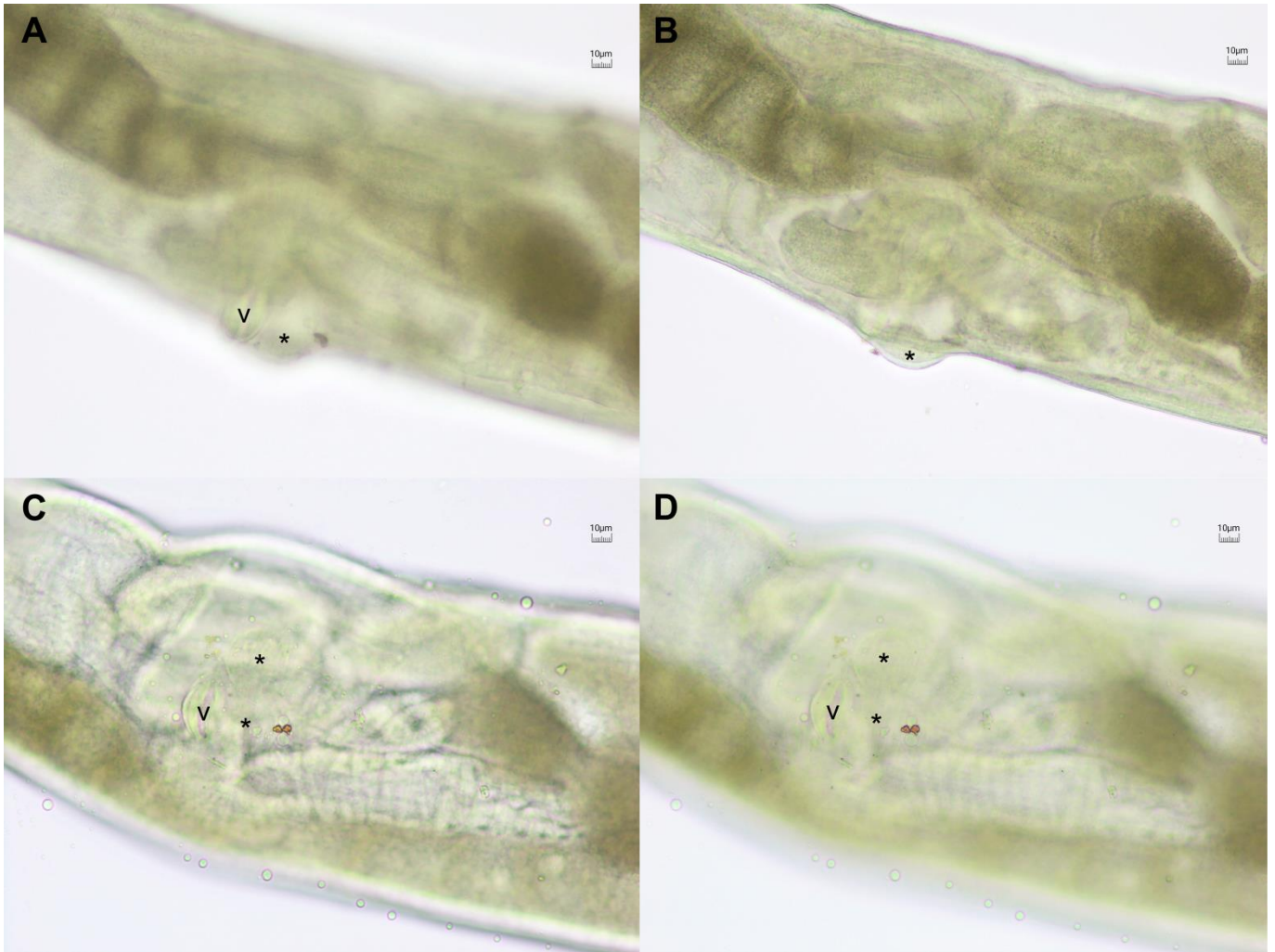
Supplementary Figure S1. *Aplectana hylambatis* parasite of *Pithecopus azureus* from Pantanal wetlands, Brazil. Line drawings: A, B, posterior and tail end of male showing number and arrangement of caudal papillae, ventral views; C, spicule showing the point of articulation at the distal part, lateral view; D, gubernaculum, lateral view; E, F, region of vulva from two different females showing the number and arrangement of mamelon-like structures, ventral views.



Supplementary Figure S2. *Aplectana hylambatis* parasite of *Pithecopus azureus* from Pantanal wetlands, Brazil. Light micrographs: A, anterior end, lateral view (arrowhead indicates excretory pore); B, posterior end of oesophagus, lateral view (arrowhead indicates excretory pore); C, D, anterior end, sublateral views (arrow indicated lateral ala). Abbreviation: nr = nerve ring.

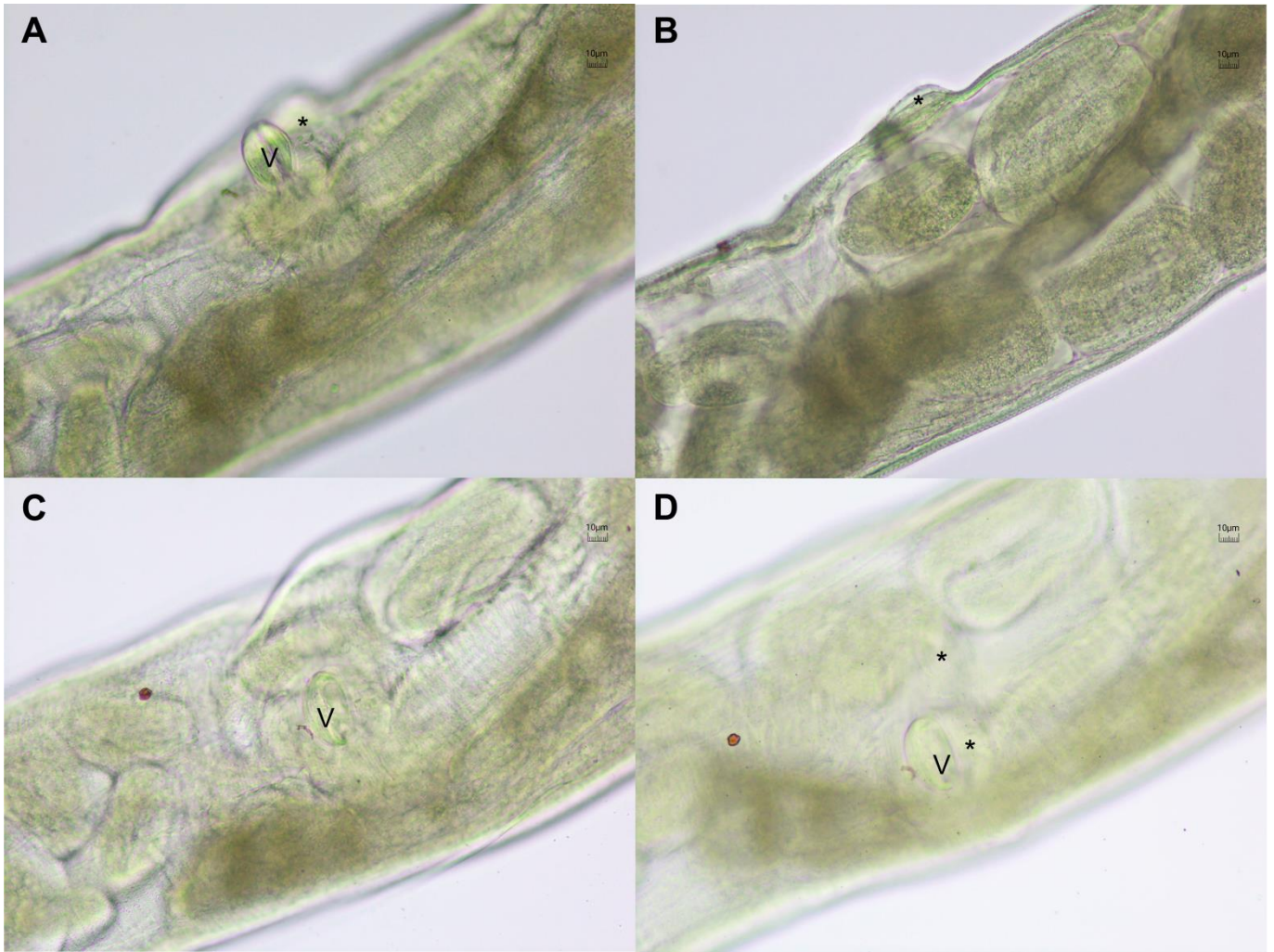


Supplementary Figure S3. *Aplectana hylambatis* female parasite of *Pithecopus azureus* from Pantanal wetlands, Brazil. Light micrographs: A, B, vulvar region subventral and lateral views, respectively (arrow indicates lateral ala; asterisk indicated mamelon-like structure); C, posterior end, lateral view; D, tail, lateral view. Abbreviations: a = anus; eg = egg containing larva; v = vulva.



Supplementary Figure S4. *Aplectana hylambatis* female parasite of *Pithecopus azureus* from Pantanal wetlands, Brazil. Light micrographs: A, B, vulvar region, subventral view (asterisks indicate mamelon-like structures); C, D, vulvar region, ventral view (asterisks indicate mamelon-like structures).

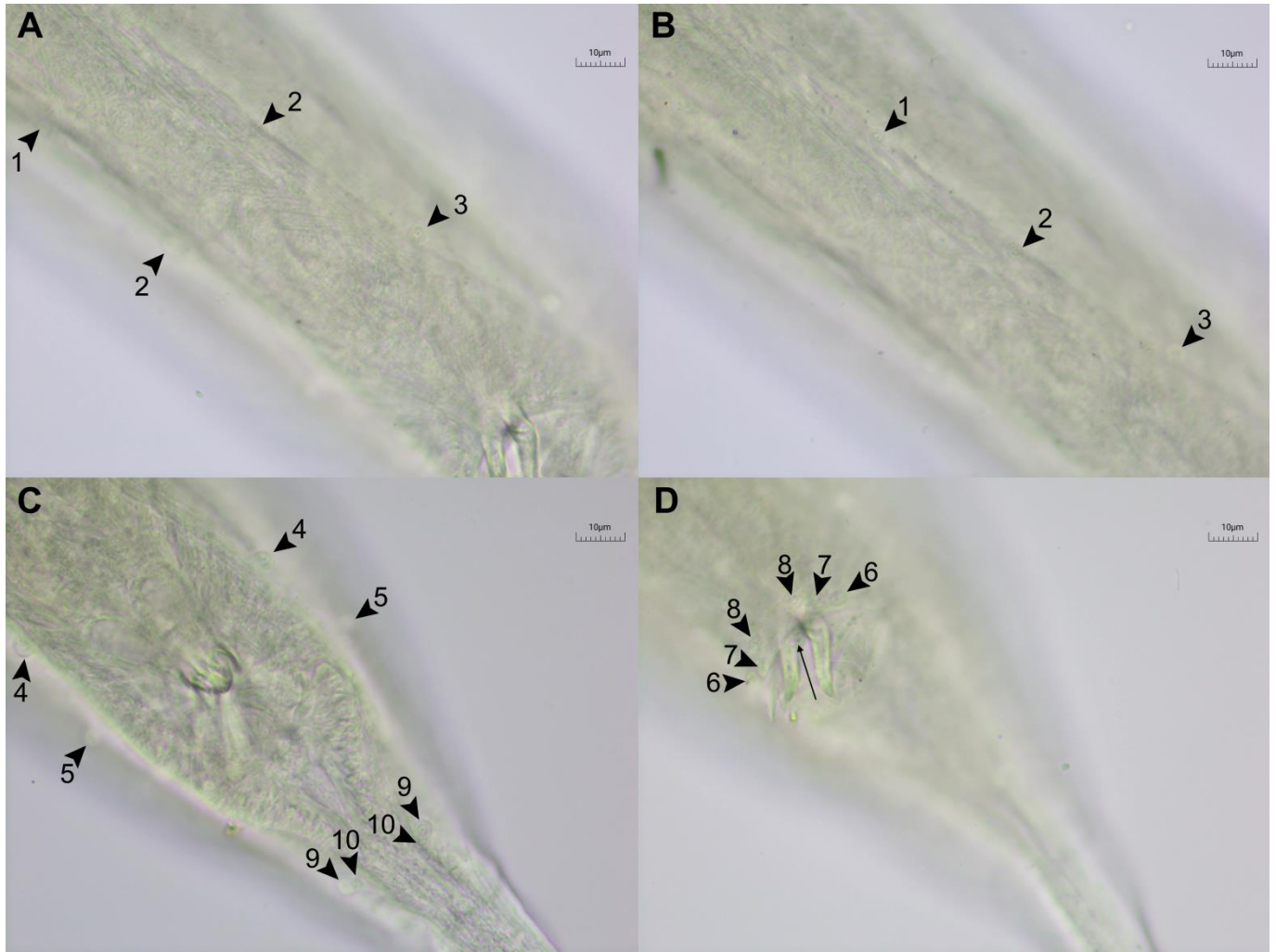
Abbreviations: v = vulva.



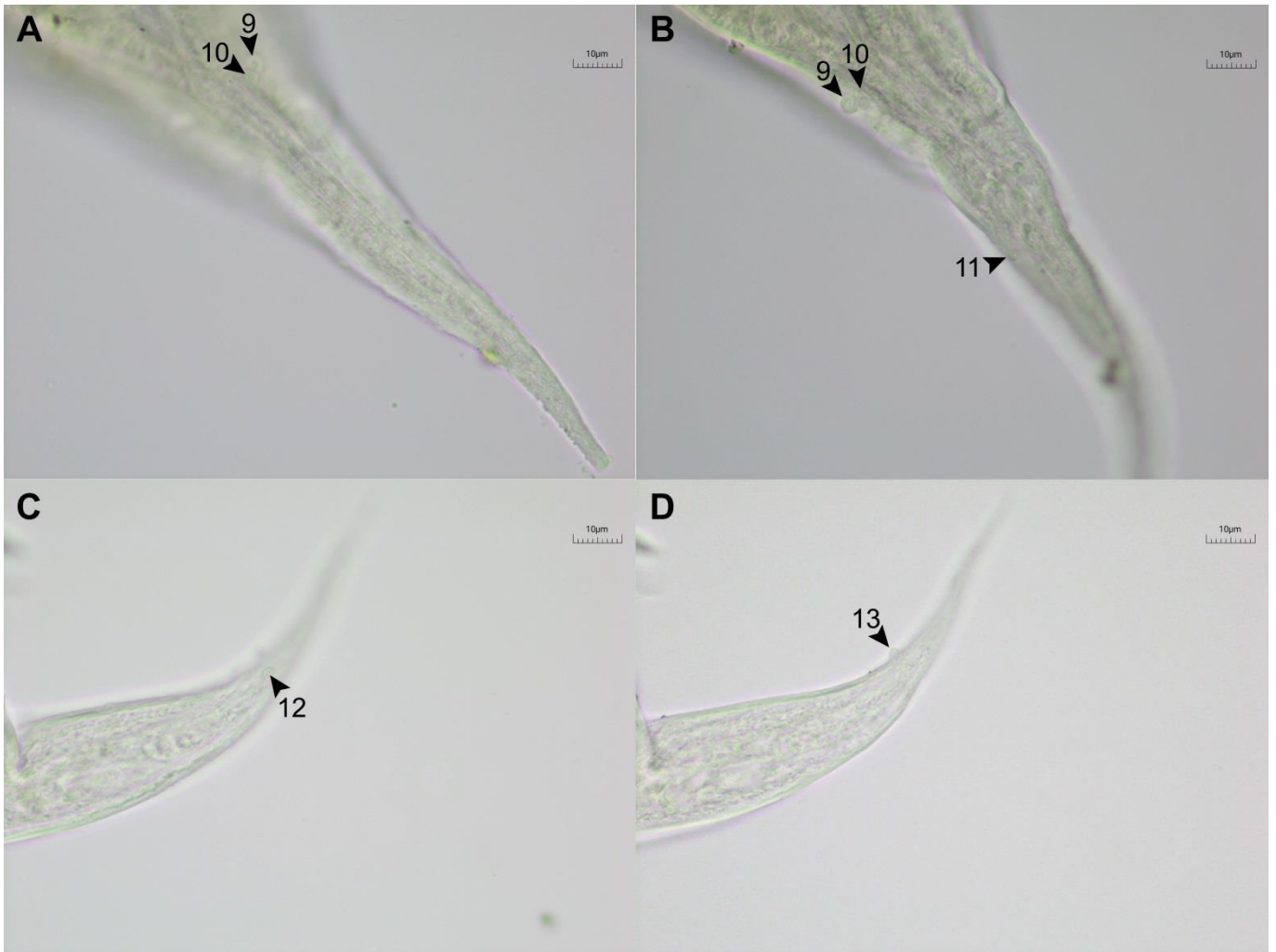
Supplementary Figure S5. *Aplectana hylambatis* female (different from that showed in Figure S3) parasite of *Pithecopus azureus* from Pantanal wetlands, Brazil. Light micrographs: A, B, vulvar region, subventral view (asterisk indicate mamelon-like structure); C, D, vulvar region, ventral view (asterisks indicate mamelon-like structures). Abbreviations: v = vulva.



Supplementary Figure S6. *Aplectana hylambatis* male parasite of *Pithecopus azureus* from Pantanal wetlands, Brazil. Light micrographs: A, whole specimen, lateral view; B, Anterior end, lateral view; C, D, posterior end, lateral and ventral views (arrowheads indicate caudal papillae; arrow indicates articulation point at distal part of the spicule). Abbreviations: e = spicule; g = gubernaculum.



Supplementary Figure S7. *Aplectana hylambatis* male parasite of *Pithecopus azureus* from Pantanal wetlands, Brazil. Light micrographs: A, B, posterior end showing precloacal papillae, subventral views (arrowheads indicate caudal papillae); C, D, cloacal region, subventral views (arrowheads indicate caudal papillae; arrow indicates the large unpaired precloacal papilla). Caudal papillae are numbered according to pairs from the first to the tenth.



Supplementary Figure S8. *Aplectana hylambatis* male parasite of *Pithecopus azureus* from Pantanal wetlands, Brazil. Light micrographs: A, B, tail end, ventral views (arrowheads indicate caudal papillae); C, D, tails end, lateral views (arrowheads indicate caudal papillae). Caudal papillae are numbered according to pairs from the ninth to the thirteenth.



Supplementary Figure S9. *Aplectana hylambatis* male parasite of *Pithecopus azureus* from Pantanal wetlands, Brazil. Scanning electron micrographs: A, posterior end, ventral view (arrowheads indicate articulated distal part of the spicules); B, tail, lateral view (arrowhead indicates articulated distal part of the spicule); C tail, ventral view (arrowheads indicate articulated distal part of the spicules). Note that lateral ala is visible in the micrographs A and C.

Supplementary Material S1. [Genetic alignment, output of bModelTest, output of BEAST2 after running the MCMC in 2 runs and raw phylogenetic trees from the present study.](#)

References cited in Table S2

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