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First description of male worms of *Enterobius* (*Colobenterobius*) *serratus* (Nematoda: Oxyuridae), the pinworm parasite of proboscis monkeys

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Abstract

Males of *Enterobius* (*Colobenterobius*) *serratus* Hasegawa *et al.*, 2003 (Nematoda: Oxyuridae) are described for the first time based on six individuals collected from the feces of proboscis monkeys, *Nasalis larvatus*, in the Lower Kinabatangan Wildlife Sanctuary, Sabah, Malaysian Borneo. The males show identical cephalic morphology to females, being readily distinguishable from their congeners by the serrated inner margins of the lips. The bicolored esophageal corpus, long thin spicule and developed spicular pouch with paired muscular bands are also remarkable characteristics, presumably shared by other Asian members of the subgenus.

Key words: Pinworm, primates, Sabah, Borneo, Malaysia

Introduction

Enterobius (*Colobenterobius*) *serratus* Hasegawa *et al.*, 2003 was originally described based on female worms discharged in the feces after anthelmintic treatment of an infant proboscis monkey (*Nasalis larvatus*), a primate endemic to the island of Borneo (Hasegawa *et al.*, 2003). Since then, no additional worms have been collected, and hence male morphology remains unknown. During a survey of host-parasite community interactions, numerous fecal samples of wild primates living along the Kinabatangan floodplain in Sabah (Malaysian Borneo) were collected (Frias, 2019). Feces were filtered through nylon mesh and the filtrates were used for parasite egg counts (Frias, 2019), while pieces of mesh containing fecal residues were preserved separately for later examination to recover trapped minute worms (Hasegawa, 2009). This report presents the results of the mesh examination, along with the first description of male worms of *E.* (*C.*) *serratus*. Esophageal morphology and the spicular pouch were also compared among representative species of pinworms parasitic in primates to establish new criteria for subgeneric classification.

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Materials and Methods

Fecal sample collection was conducted in the Kinabatangan floodplain (Sabah, Malaysian Borneo) between September 2014 and November 2018. Collected feces were strained through a 330 μm Saran™ mesh (Asahi Kasei, Japan). Details of the fecal collection, filtration procedure, and results of coproscopic examination and egg counts are given in Frias (2019). A total of 341 mesh pieces used for fecal filtration were subjected to the present examination. Each mesh piece was washed with running tap water on a stainless strainer with an aperture size of 160 μm or 250 μm. The residues left on the strainer were transferred to a Petri dish and examined under a stereomicroscope to recover helminths (Hasegawa, 2009). The worms found were rinsed in 70% ethanol, cleared in glycerol-ethanol solution by evaporation of ethanol, mounted on a glass slide with 50% glycerol aqueous solution, and observed under an Olympus BX50 microscope equipped with a differential interference contrast apparatus and a drawing tube. *En face* cephalic morphology, lateral alae, caudal papillae arrangement and the spicular pouch were observed on hand-sectioned fragments.

Female specimens of the species listed in Table 1 were also processed in the same way as those described above and their esophageal morphology was examined for comparison.

The spicular pouch morphology was also observed in hand-sectioned males of *E.* (*C.*) *emodensis* Hasegawa *et al.*, 2018, E. (*Enterobius*) *vermicularis* (Linnaeus, 1758) and *Lemuricola* (*Protenterobius*) *nycticebi* Baylis, 1928, which were collected along with the females listed above.

TABLE 1. Female pinworms examined in this study.

Pinworm species	Host	Locality	Original report
Enterobius (*Colobenterobius*) *serratus* Hasegawa *et al.,* 2003	Proboscis monkey (*Nasalis larvatus*)	Sabah, Malaysian Borneo	Hasegawa *et al.* 2003
Enterobius (*Colobenterobius*) *emodensis* Hasegawa *et al.,* 2018	Himalayan langur (*Semnopithecus schistaceus*)	Uttarakhand, India	Hasegawa *et al.* 2018
Enterobius (*Colobenterobius*) *pygatrichus* Hasegawa *et al.,* 2002	Golden snub-nosed monkey (*Pygathrix roxellana*)	Kobe Zoo, Japan	Hasegawa *et al.* 2002
Enterobius (*Enterobius*) *macaci* Yen, 1973	Japanese macaque (*Macaca fuscata*)	Nara, Japan	Hasegawa *et al.* 2012
Enterobius (*Enterobius*) *anthropopitheci* Gedoelst, 1916	Chimpanzee (*Pan troglodytes*)	Kumamoto Sanctuary, Japan	Hasegawa and Udono 2007
Enterobius (*Enterobius*) *vermicularis* (Linnaeus, 1758)	Chimpanzee (*Pan troglodytes*)	Asahiyama Zoo, Japan	Nakano *et al.* 2005
Trypanoxyuris (*Buckleyenterobius*) *atelis* Cameron, 1929	Black-handed spider monkey (*Ateles geoffroyi*)	Asahiyama Zoo, Japan	Hasegawa *et al.* 2004
Trypanoxyuris (*Trypanoxyuris*) *microon* Linstow, 1907	Northern night monkey (*Aotus trivirgatus*)	Kobe Zoo, Japan	Hasegawa 2009
Lemuricola (*Protenterobius*) *nycticebi* Baylis, 1928	Philippine slow loris (*Nycticebus menagensis*)	Lower Kinabatangan Wildlife Sanctuary, Sabah, Malaysian Borneo	Frias *et al.* 2019
Lemuricola (*Madoxyuris*) *vauceli* Chabaud *et al.*, 1965	Common brown lemur (*Eulemur fulvus*)	Madagascar	Hasegawa 2009

Results

Pinworms were found in feces of three primate species (Table 2). The morphology of *L.* (*P.*) *nycticebi* has been reported elsewhere (Frias *et al.*, 2019). The female worm of *Pongobius* sp. recovered from orangutan feces was heavily decomposed, preventing detailed observation and species identification. The six male worms found in four samples from proboscis monkeys were well-preserved, except for the distal portion of the spicule that was lost in two individuals. Their morphology is described below.

TABLE 2. Pinworms recovered from the debris remaining in mesh pieces used for straining feces.

Primate species	Nº mesh samples examined	Nº mesh samples positive (%)	Pinworm species	Nº pinworms recovered
Cephalopacus bancanus	8	0	–	–
Nycticebus menagensis	16	12 (75.0)	*L.* (*P.*) *nycticebi*	204 (M129, F72, U3)*
Nasalis larvatus	245	4 (1.6)	*E.* (*C.*) *serratus*	6 (M6)
Trachypithecus cristatus	32	0	–	–
Macaca fascicularis	127	0	–	–
Macaca nemestrina	21	0	–	–
Hylobates sp.	25	0	–	–
Pongo pygmaeus	7	1 (14.3)	*Pongobius* sp.	1 (F1)

* M: male; F: female; U: sex undetermined.

Description

***Enterobius* (*Colobenterobius*) *serratus* Hasegawa, Matsuo et Onuma, 2003**
(ZooBank registration: urn:lsid:zoobank.org:pub:69E6C6D0-A122-4AB0-AE90-747E941EF26A)
(**Nematoda: Oxyuridae: Enterobiinae**)

Male (based on 6 males): Body minute, 1.96–2.91 ($\bar{x}$ =2.54) mm long and 163–178 (172) µm wide in midbody. Posterior end bent ventrally (Fig. 1). Cuticle transversely striated (Figs. 1, 6). Lateral alae single-crested, commencing at level of esophageal bulb and terminating ca. 200 µm anterior to posterior end (Figs. 1, 5). Cephalic expansion 150–200 (175) µm long by 100–128 (116) µm wide, with inner septa in posterior half. Cephalic end with three lips of almost equal size, forming round head with diameter of 63–70 (68) µm. Lips well set off from body, each with serrated inner margin (Figs. 2, 4). Dorsal lip with two cephalic papillae; subventral lips each with one cephalic papilla and amphidial pore; distance between amphidial pores 44–49 µm (n=2) (Fig. 3, 4). Slots present between lips and pharynx (Fig. 3). Pharynx with specific teeth composed of one large median and three pairs of side projections just beneath each lip (Figs. 2–4). Pharynx 23–35 (30) µm long; esophageal corpus 255–285 (271) µm long by 51–65 (58) µm wide with dark granules in posterior half; esophageal isthmus short, 5–10 (7) µm long by 21–25 (23) µm wide; esophageal bulb valved, 95–113 (102) µm long by 83–103 (90) µm wide (Fig. 1). Distance from cephalic apex to nerve ring 158–170 (165) µm and excretory pore 534–750 (662) µm. Testis extending to middle of body (Fig. 1). Spicule thin, slender, with ellipsoid light-refractile mass basally; distal portion pointed, slightly bent ventrally, 227–248 (241) µm long (n=4) (Fig. 6). Spicule portion inside body housed in spicular pouch with paired muscular bands (Figs. 6, 7). Caudal papillae comprised of four pairs: 1st pair large, pedunculated, projecting laterally at level of anus; 2nd and 3rd pairs slightly posterior to 1st pair, mostly flat, directing ventrally, surrounded by transparent cuticular thickenings; 4th pair pedunculated, smaller than 1st pair, directing posterolaterally (Figs. 6, 8). Phasmids arising from anterior base of 4th papillae, directing laterally (Figs. 6, 8).

Taxonomic summary

Host: *Nasalis larvatus* (Wurmb, 1787).

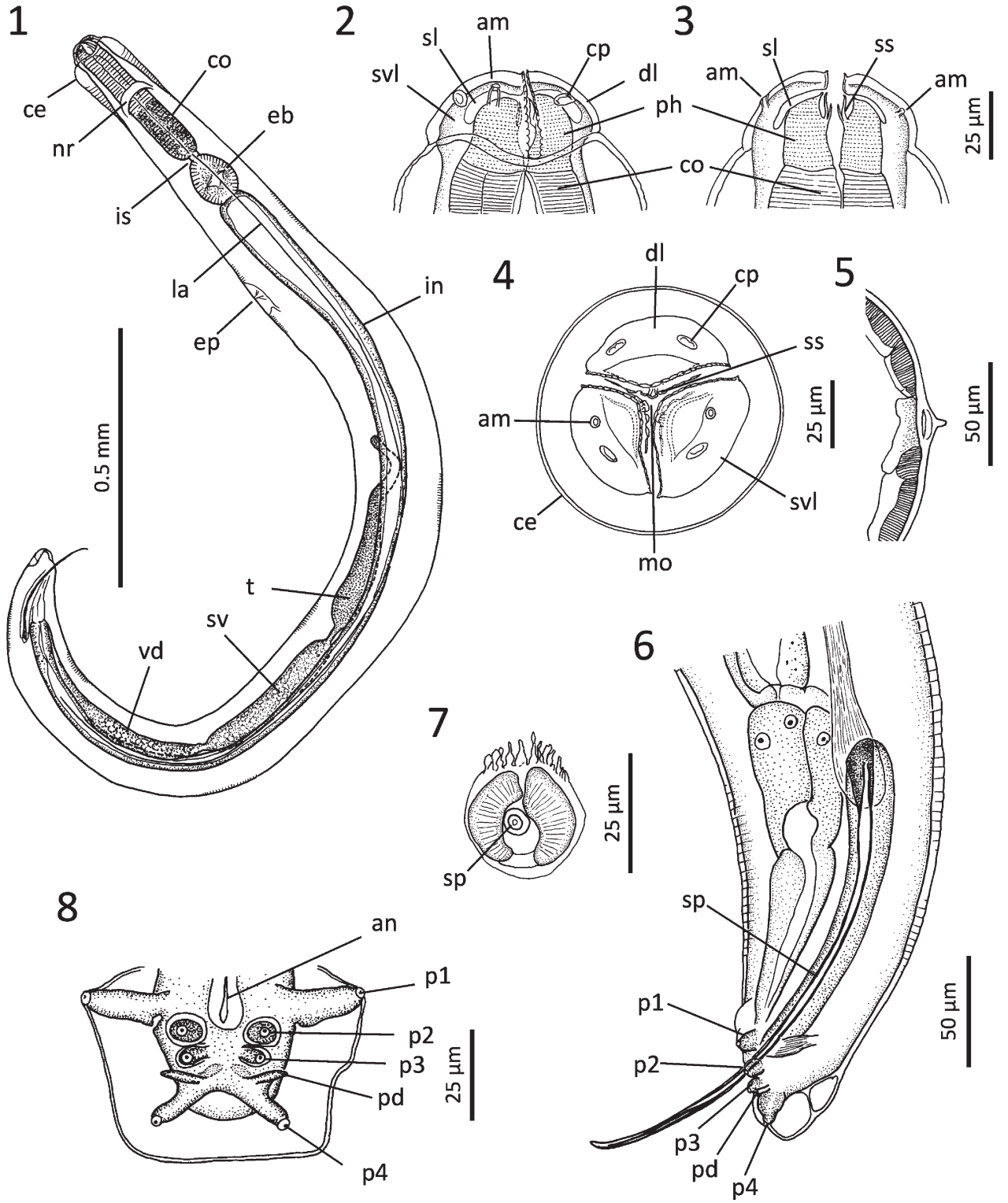
Site in host: Intestine (discharged in feces).

Localities and dates of collection: Lower Kinabatangan Wildlife Sanctuary, Sabah, Malaysia (5°24'59.2" N, 118°2'4.3" E, December 22, 2016; 5°24'46.6" N, 118°5'7.3" E, October 3, 2018; 5°25'42.1" N, 118°6'14.3" E, October 21, 2018; 5°25'42.1" N, 118°6'14.3" E, October 21, 2018).

Specimens deposited: PAR-00006 to PAR-00009 (6 adult males), BORNEENSIS, Natural History Collection, Universiti Malaysia Sabah (Kota Kinabalu, Malaysia).

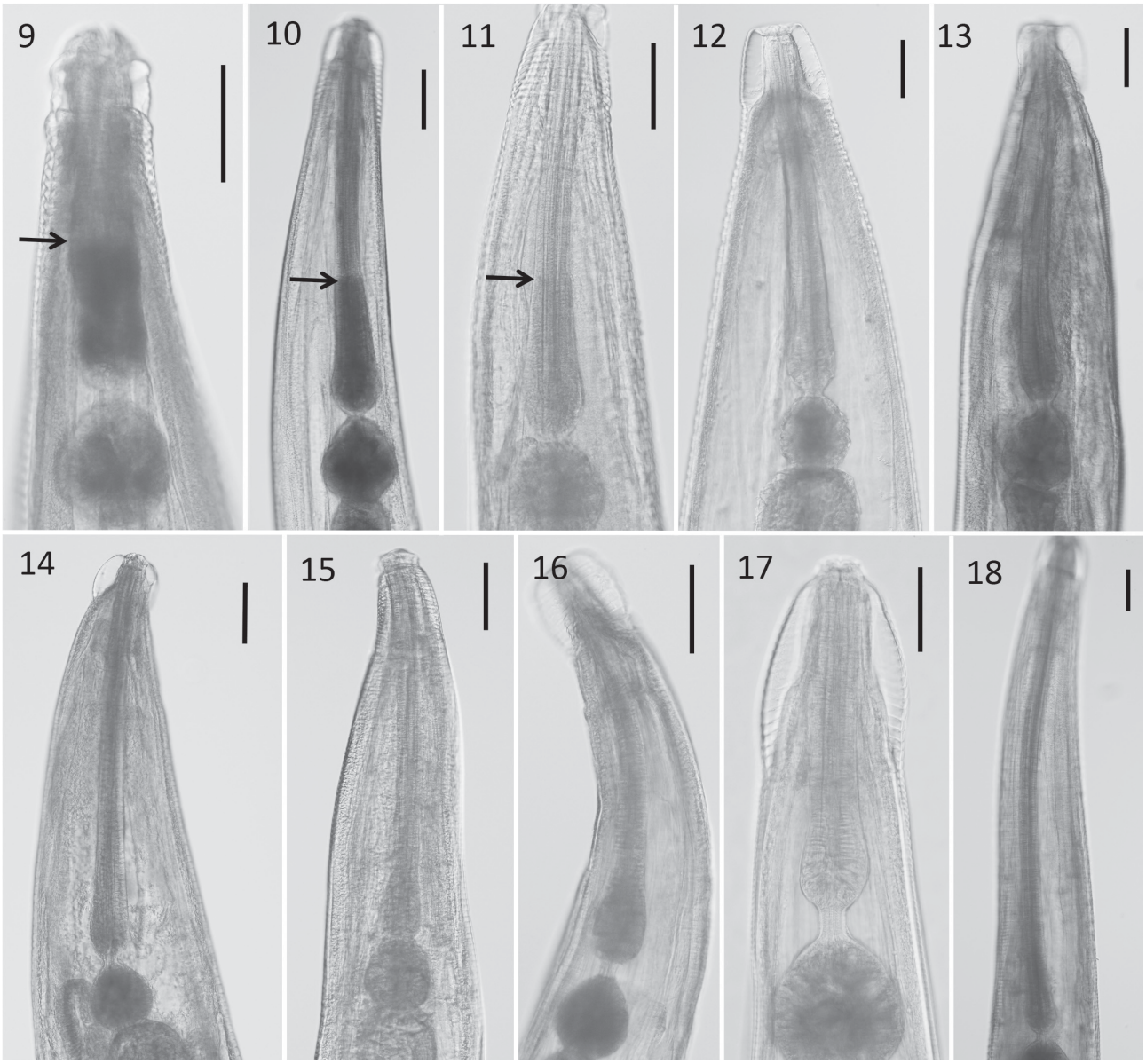
Comparison of esophageal morphology

The original description of female *E.* (*C.*) *serratus* did not report dark granules in the posterior half of the esophageal corpus. Upon re-examination of the female specimens, the presence of a bicolored esophageal corpus, similar to that observed in male worms, was confirmed (Fig. 9). This feature was also shared by *E.* (*C.*) *emodensis* (Fig. 10),



FIGURES 1–8. Male adult of *Enterobius* (*Colobenterobius*) *serratus* Hasegawa *et al.*, 2003, collected from the feces of *Nasalis larvatus* in Sabah, Malaysia. 1. Entire worm, left lateral view; 2–4. Cephalic extremity, left lateral view (2), optical frontal section (3) and apical view (4); 5. Lateral ala in cross section through midbody; 6. Caudal extremity, left lateral view; 7. Spicular pouch, cross section; 8. Caudal papillae arrangement, ventral view.

Abbreviations used: am. amphidial pore; an. anus; ce. cephalic expansion; co. corpus (of esophagus); cp. cephalic papilla; dl. dorsal lip; eb. esophageal bulb; ep. excretory pore; in. intestine; is. isthmus (of esophagus); la. lateral ala; mo. mouth; nr. nerve ring; pd. phasmidial duct; p1–p4. caudal papillae arrangement; ph. pharynx; sl. slot; sp. spicule; ss. specific structure (teeth) of pharynx; sv. seminal vesicle; svl. subventral lip; t. testis; vd. vas deferens.

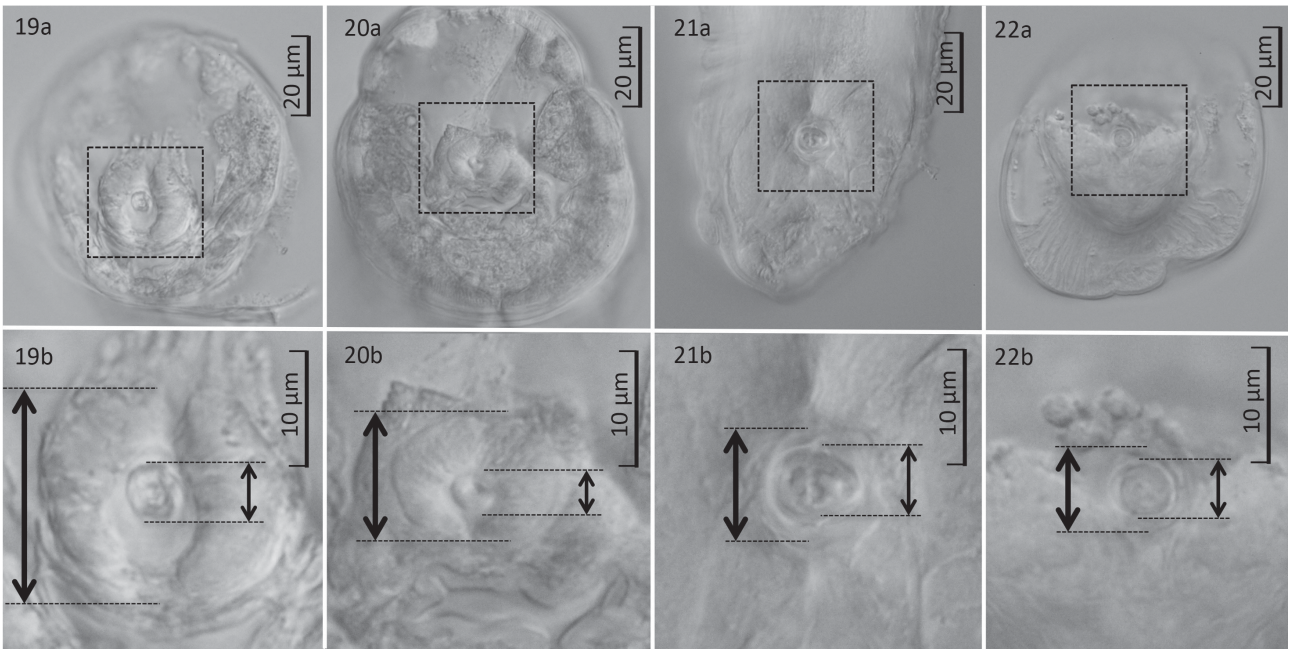


FIGURES 9–18. Esophageal region of female pinworms. 9. *Enterobius* (*Colobenterobius*) *serratus*; 10. *E.* (*C.*) *emodensis*; 11. *E.* (*C.*) *pygatrichus*; 12. *E.* (*Enterobius*) *macac*i; 13. *E.* (*E.*) *vermicularis*; 14. *E.* (*E.*) *anthropopitheci*; 15. *Trypanoxyuris* (*Buckleyenterobius*) *atelis*; 16. *T.* (*Trypanoxyuris*) *microon*; 17. *Lemuricola* (*Protenterobius*) *nycticebi*; 18. *L.* (*Madoxyuris*) *vauceli*. Arrow indicates junction between light and dark portions. Scale bar: 100 μm.

characterized by a clear junction between light and dark portions (Hasegawa *et al.*, 2018). Moreover, the female of *E.* (*C.*) *pygatrichus* Hasegawa *et al.*, 2002 was also found to possess a similar dark posterior portion of the esophagus, though less manifested (Fig. 11). Meanwhile, such a clear boundary was not observed in the esophageal corpuses of *E.* (*E.*) *macaci* Yen, 1973, *E.* (*E.*) *anthropopitheci* Gedoelst, 1916, *E.* (*E.*) *vermicularis*, *Trypanoxyuris* (*Buckleyenterobius*) *atelis*, *T.* (*Trypanoxyuris*) *microon* (Linstow, 1907), *L.* (*P.*) *nycticebi* or *L.* (*Madoxyuris*) *vauceli* Chabaud *et al.*, 1965 (Figs. 12–18).

Comparison of spicular pouch morphology

Among the four species observed, *E.* (*C.*) *serratus* showed the largest spicular pouch (Fig. 19), while that of *E.* (*C.*) *emodensis*, although somewhat smaller in size, was well developed and with paired muscular bands (Fig. 20). Meanwhile, those in *E.* (*E.*) *vermicularis* and *L.* (*P.*) *nycticebi* were thin-walled and lacked developed paired musculature (Figs. 21, 22).



FIGURES 19–22. Cross section near caudal extremity of male (a) and enlarged view of the boxed part (b) showing spicular pouch. 19. *Enterobius* (*Colobenterobius*) *serratus*; 20. *E.* (*C.*) *emodensis*; 21. *E.* (*Enterobius*) *vermicularis*; 22. *Lemuricola* (*Protenterobius*) *nycticebi.* Thick arrows indicate dorso-ventral height of spicular pouch; thin arrows indicate dorso-ventral height of spicule.

Discussion

The cephalic morphology of the males examined here, having serrated inner margins of the lips, coincides with that of female worms of *E.* (*C.*) *serratus* described from *N. larvatus* by Hasegawa *et al.* (2003). Hence, both male and female worms exhibit identical cephalic morphology, being readily distinguished from other known members of the subgenus by this feature. A long, narrow spicule over 200 µm in length seems to be the common feature of Asian species of the subgenus *Colobenterobius*: 350 µm in *E.* (*C.*) *zakiri* Siddiqi *et al.*, 1954; 200–210 µm in *E.* (*C.*) *presbytis* Yen, 1973; 290 µm in *E.* (*C.*) *longispiculum* Quentin *et al.*, 1979; 197–238 ($\bar{x}$ = 223) µm in *E.* (*C.*) *emodensis*, and 227–248 ($\bar{x}$ = 241) µm in *E.* (*C.*) *serratus* (Siddiqi *et al.*, 1954; Yen, 1973; Quentin *et al.*, 1979; Hugot, 1987b; Hasegawa *et al.*, 2018). Exceptionally, the spicule of *E.* (*C.*) *entellus* from a Hanuman langur (*Semnopithecus entellus*) reared in a zoological garden in Giza, Egypt, was only 110 µm long (Hugot, 1987b). In contrast, the African representatives of the subgenus possess shorter and/or stouter spicules except for *E.* (*C.*) *inglisi*, whose spicule was 300 µm long (Wahid, 1961; Hugot, 1987a).

The prominent spicular pouch is of special interest, as its paired muscular bands are presumed to act as protractors (cf. Gibbons, 2002). The development of a protractor may be related to the length of the spicule, as the eversion of a long spicule requires strong muscles. Such developed spicular pouch has also been suspected for the long-spicule species of the subgenus *Colobenterobius*, namely *E.* (*C.*) *longispiculum*, *E.* (*C.*) *zakiri*, *E.* (*C.*) *presbytis* and *E.* (*C.*) *emodensis*, but also in the short-spicule species *E.* (*C.*) *entellus* (see Fig. 1E of Quentin *et al.*, 1979, Figs. 2G, 4J, 6E of Hugot, 1987b, and Fig. 5 of Hasegawa *et al.*, 2018). A developed spicular pouch has not been observed in African members of *Colobenterobius* though its status in *E.* (*C.*) *inglisi*, the long-spicule species, remains unknown (Hugot, 1987a; Hasegawa *et al.*, 2008). The systematic significance of the spicular pouch should be further studied in various pinworms of primates.

Besides these features, the bicolored esophagus seems to be a remarkable characteristic, previously described in *E.* (*C.*) *emodensis* and regarded as a species-specific feature (Hasegawa *et al.*, 2018). However, the present observation demonstrates that the three members of *Colobenterobius* share this trait. Because such bicolored corpuses are not observed in the pinworms belonging to the subgenus *Enterobius* or in those of the genus *Trypanoxyuris* or *Lemuricola*, it is suspected to be specific of *Colobenterobius*. Although the esophagus of nematodes is often regarded as a pumping organ composed of muscular tissue, its walls also contain glandular cells (Chitwood and Chitwood, 1950;

Bird and Bird, 1991). In the future, it may be necessary to elucidate the nature, presumably secretory, of the colored granules. Moreover, attention should be paid to elucidate whether the granules are originally colored or secondarily stained with host bile (or foreign pigment) in the host feces after the death of the worm.

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