A pinworm's tale: The evolutionary history of *Lemuricola (Protenterobius) nycticebi*

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ABSTRACT

Lemuricola (Protenterobius) nycticebi is the only pinworm species known to infect strepsirrhine primates outside Africa, and the only pinworm species yet described in slow lorises. Here, we provided a detailed morphological comparison of female and male worms, and a first description of fourth-stage larvae collected from free-living slow lorises (*Nycticebus menagensis*) in Sabah, Malaysian Borneo. Using mitochondrial and nuclear markers, we also reconstructed the species' phylogenetic relationship with other pinworms infecting primates. Both morphological and molecular results indicated a distinct association between *L. (P.) nycticebi* and its host. However, while taxonomy identified this species as a member of the *Lemuricola* clade and grouped pinworms infecting lemurs and slow lorises together, phylogenetic reconstruction split them, placing *L. (P.) nycticebi* within the *Enterobius* clade. Our results suggest that *L. (P.) nycticebi* may represent a different taxon altogether, and that it is more closely related to pinworm species infecting Old World primates outside Madagascar. *Pongobius pongoi* (Foitová et al., 2008) n. comb. is also proposed.

1. Introduction

Pinworms are exceptional among nematodes in that they have conquered both vertebrate and invertebrate realms, having undergone repeated radiations in several hosts (Adamson, 1990). At the same time, their life cycle has remained fairly conservative, characterized by direct transmission and no free-living stages in the external environment. Pinworms' limited dispersal abilities imply that contact with infected conspecifics and reinfection are the most common transmission routes (Cook, 1994; Felt and White, 2005; González-Hernández et al., 2014), virtually "trapping" them in their host lineages over long timescales. This close association between pinworms and their hosts has been extensively studied in primates, where their cophylogenetic structure has led researchers to infer strong patterns of cospeciation, with occasional

cross-clade host switching (Cameron, 1929; Sandosham, 1950; Inglis, 1961; Brooks and Glen, 1982; Hugot, 1999; Ashford, 2000, but see Brooks et al., 2015; Nylin et al., 2018).

Oxyurids infecting primates have been classified through standard morphological characters and morphometric variables under the subfamily Enterobiinae, and subdivided into three monophyletic genera, closely underlining the primate classification: *Enterobius* comprising the parasites of the catarrhines, *Trypanoxyuris* comprising the parasites of the platirrhines, and *Lemuricola* comprising the parasites of strepsirrhines (Hugot, 1999). Only recently was the genus *Pongobius* established based on the description of pinworms parasitizing Sumatran orangutans (Baruš et al., 2007). The genus *Lemuricola* is further divided into three subgenera (Table 1), based on the cephalic and caudal papillae and characteristics of the lips, esophagus and tip of the tail in

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Table 1
Members of the genus *Lemuricola*.

Genus	Subgenus	Species	Type host
Lemuricola Chabaud and Petter, (1959)	*Lemuricola* Chabaud et al. (1965)	*L. (L.) contagiosus* Chabaud and Petter (1959)	*Cheirogaleus major*
		L. (L.) microcebi Hugot et al. (1995)	*Microcebus murinus*
		L. (L.) sp. of Hugot et al. (1995)	*Galago senegalensis*
	Biguetius Chabaud et al. (1961)	*L. (B.) trichuroides* Chabaud et al. (1961)	*Propithecus verreauxi*
	Madoxyuris Chabaud et al. (1965)	*L. (M.) lemuris* Baer (1935)	*Eulemur macaco*
		L. (M.) vauceli Chabaud et al. (1965)	*E. fulvus*
		L. (M.) baltazardi Chabaud et al. (1965)	*E. fulvus*
		L. (M.) bauchoti Chabaud et al. (1965)	*Hapalemur simus*
		L. (M.) daubentoniae Petter et al. (1972)	*Daubentonia madagascariensis*
	Protenterobius Inglis (1961)	*L. (P.) nycticebi* Baylis (1928) [syn. *P. malayensis* Inglis and Dunn (1963)]	*Nycticebus menagensis*
		L. (P.) pongoi Foitová et al. (2008)[a]	*Pongo abelii*

[a] New combination with *Pongobius* is proposed herein.

males (Chabaud and Petter, 1959; Inglis, 1961; Chabaud et al. 1965). The only species known to occur in a strepsirrhine host outside Africa is *Lemuricola (Protenterobius) nycticebi*, which was described from a free-living Philippine slow loris (*Nycticebus menagensis*) in Sarawak, Malaysian Borneo (Baylis, 1928), and later redescribed from a Sunda slow loris (*N. coucang*) in Peninsular Malaysia (Inglis and Dunn, 1963).

Here, and almost one hundred years after the original publication, we provide a detailed morphological comparison of *L. (P.) nycticebi* infecting free-living slow lorises in Sabah (Malaysian Borneo), including a new description of fourth-stage larvae. We then assess its phylogenetic relationship among members of the subfamily Enterobiinae.

2. Methods

2.1. Study subject and parasite collection

Slow lorises (*Nycticebus* spp.) are nocturnal arboreal primates distributed throughout Southeast Asia, from northeastern India and southern China to the Thai-Malay Peninsula and extending further south to the islands of Sumatra, Java and Borneo (Nekaris et al., 2008; Munds et al., 2013). Based on morphological similarities among different lorisiform primates coupled with their nocturnal lifestyle, lorises are regarded as cryptic primates that are difficult to detect moving throughout the forest (Nekaris and Bearder, 2007). From the eight currently recognized species (Groves, 1998; Ravosa, 1998; Chen et al., 2006; Munds et al., 2013; Pozzi et al., 2014, 2015), only the Philippine slow loris (*N. menagensis*) has been recorded inhabiting the Lower Kinabatangan Wildlife Sanctuary in Sabah, Malaysian Borneo (Munds et al., 2013).

As part of a radio-tracking study of nocturnal primates in the area, animals were continually captured and feces were opportunistically collected. Female pinworms were discharged in the feces and subsequently collected and stored in ethanol and EcoFix® (Meridian Bioscience, Inc., USA), a fixative that preserves the morphological characteristics of the specimen. For examination of helminth eggs, feces were strained through a 330 μm SARAN™ mesh (Asahi Kasei, Japan) and the remaining fecal debris was processed by the 'gauze-washing'

method (Hasegawa, 2009) to recover minute worms such as male pinworms.

2.2. Morphological observation

The retrieved worms were cleared in glycerol-ethanol solution by evaporating the ethanol from preserved specimens. They were mounted on glass slides with 50% glycerol aqueous solution and observed under an Olympus BX50 microscope equipped with a differential interference contrast apparatus. Free-hand sections were made using a disposable scalpel blade for observation of en-face view of cephalic end and cross slices of the body. Figures were made with the aid of a drawing tube (Olympus U-DA).

2.3. Phylogenetic analyses

Genomic DNA was extracted from two individual female pinworms from two different slow lorises using a QIAamp DNA micro kit (Qiagen, Japan) according to the manufacturer's instructions. A fragment of the mitochondrial cytochrome c oxidase subunit 1 gene (*cox1*), the D1 and D2 domains of the 28S ribosomal DNA gene (28S rDNA), and partial 18S ribosomal DNA gene (18S rDNA) were amplified by PCR using the primers shown in Table 2.

Each PCR reaction (15 μl) was prepared using a master mix that consisted of 10 mM buffer, 2.5 mM dNTPs, 5 μM of each primer, TaKaRa *Taq* HS polymerase (0.5 units), and the DNA template. PCR conditions for the *cox1* region consisted of an initial denaturation at 94 °C for 2 min, followed by 20 cycles at 94 °C for 60 s, 55 °C for 60 s, 68 °C for 60 s, and a final extension at 68 °C for 7 min, following Hasegawa et al. (2012); for 28S rDNA gene, an initial denaturation at 94 °C for 1 min, followed by 35 cycles at 94 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, and a final extension for 7 min at 72 °C, following Okamoto et al. (2009), and for 18S rDNA gene, an initial denaturation at 94 °C for 5 min, followed by 35 cycles at 94 °C for 30 s, 54 °C for 30 s, 72 °C for 60 s, and a final extension for 10 min at 72 °C, following Floyd et al. (2005). Following PCR amplification, nonspecific products were removed from the amplicons using the Agencourt AMPure system (Agencourt Bioscience Corp., Beverly, MA), and aliquots were sequenced in a ABI-PRISM 3130

Table 2
Primers used in this study.

Gene	Primer name	Sequence (5′-3′)	Reference
Cox1	StrCoxAfrF	GTGGTTTTGGTAATTGAATGGTT	Hasegawa et al. (2010)
	pr-b	AGAAAGAACGTAATGAAAATGAGCAAC	Nakano et al. (2006)
18S rDNA	Nem18SF	CGCGAATRGCTCATTACAACAGC	Floyd et al. (2005)
	Nem18SR	GGGCGGTATCTGATCGCC	
28S rDNA	C1	ACCCGCTGAATTTAAGCAT	de Bellocq et al. (2001)
	D2	TCCGTGTTTCAAGACGG	

Genetic Analyzer (Applied Biosystems, CA, USA). Sequences obtained in this study were deposited in the DNA Database of Japan (DDBJ), under accession numbers LC416074-LC416079.

Cox1 and 18S rDNA sequences were aligned using CLUSTALW (Thompson et al., 1994), but multiple sequence alignment for 28S rDNA sequences was conducted in MAFFT to account for the secondary structure of non-coding RNA when constructing the alignment (Katoh and Toh, 2008; Okamoto et al., 2009). Maximum likelihood (ML) and neighbor-joining (NJ) trees were inferred with bootstrap values calculated using 1000 replicates. To provide phylogenetic context to the analysis, we included sequences of *cox1*, 28S rDNA and 18S rDNA from other members of the Enterobiinae, and also included five sequences of the 28S rDNA gene of *L. vauceli* and *L. bauchoti* from feces of ring tailed lemurs (*L. catta*) collected in Madagascar, and *E. vermicularis*, *T. atelis* and *T. microon* from feces of a captive chimpanzee (*Pan tronglodytes*), black spider monkey (*Ateles ater*) and night monkey (*Aotus azarae*), respectively (Accession numbers LC416069-LC416073). *Syphacia frederici* (Oxyuridae: Syphaciinae), a parasite of rodents, was used as an outgroup.

3. Results

3.1. Morphological identification

The morphology of adult worms was identical to previous descriptions of *Lemuricola (Protenterobius) nycticebi* by Baylis (1928), Inglis and Dunn (1963) and Hugot et al. (1995), and therefore, only measurements are provided for comparative purposes with those reported by previous studies (Table 3). Because the worm reported as fourth-stage male larva by Inglis and Dunn (1963) was actually in the adult stage, by having fully developed caudal papillae and spicule, fourth-stage larvae are described for the first time as follows.

Fourth-stage larva: Body is tapered to both extremities (Figs. 1–3, 8). The cephalic vesicle is absent. Four cephalic papillae, amphidial pores and one pair of minute papillae between amphidial pores are observed; the mouth is triangular and encircled by three lips (Fig. 5). The esophagus is as it occurs in the adult stage (Figs. 1 and 4). Lateral alae are single-crested in both sexes, commencing slightly posterior to the nerve ring and terminating preanally in males and postanally in females (Figs. 1–4, 6, 8). In females, the genital primordium is formed at the primordial vulva (Fig. 7). *Males* (n = 2): length 1.14–1.42 mm, width 107–125 μm, cephalic diameter 18–24 μm, pharynx 9–10 μm long, esophageal corpus 111–185 μm long by 24–31 μm wide; esophageal isthmus 28 μm long by 13 μm wide (n = 1); esophageal bulb 50–75 μm long by 43–60 μm wide; nerve ring 78–80 μm and excretory pore 310–330 μm from cephalic apex; tail abruptly narrowed posterior to anus, 93–95 μm long (Fig. 2 and 3). In molting larva, the inside adult possesses caudal papillae and a spicule of 80 μm in length and lacks a manubrium basally (Fig. 3). *Females* (n = 3): length 1.47–1.73 mm, width 98–105 μm, cephalic diameter 25–28 μm, pharynx 6–8 μm long, esophageal corpus 108–111 μm long by 24–26 μm wide; esophageal isthmus 19–20 μm long by 12–13 μm wide; esophageal bulb 56–58 μm long by 41–54 μm wide; nerve ring 83–85 μm, excretory pore 291–383 μm and primordial vulva 0.44–0.57 mm (n = 2) from cephalic apex; tail is gradually narrowed and 317–353 μm in length (Fig. 8).

3.1.1. Taxonomic summary
Host: Philippine slow loris, *Nycticebus menagensis* (Lydekker, 1893).
Site in host: alimentary canal (discharged in feces).
Locality: Lower Kinabatangan Wildlife Sanctuary (Lot 6), Sabah, Malaysia (5°25′8.0″ N, 118°2′18.4″ E).
Specimens deposited: ITBC PAR-00003 (6 females and 9 males) and PAR-00004 (1 female, and 2 male fourth-stage larvae), Borneensis, Universiti Malaysia Sabah (Kota Kinabalu, Malaysia).

3.2. Phylogenetic analyses

Partial *cox1* mtDNA (845 bp), 18S rDNA (761 bp) and 28S rDNA (748 bp) of *L. (P.) nycticebi* were successfully amplified and sequenced. Phylogenetic analyses for each gene consisted of an alignment of 33 sequences trimmed to 636 bp for *cox1*, 30 sequences trimmed to 740 bp for 18S rDNA, and eight sequences aligned to 828 bp for 28S rDNA, to ensure comparison among homologous regions of the genes. Phylogenetic trees reconstructed by ML and NJ methods yielded similar

Table 3
Morphometric comparison of *Lemuricola* (*P.*) *nycticebi* collected from a Philippine slow loris (*N. menagensis*), in micrometers unless stated otherwise. a) Recorded as *Lemuricola* (*P.*) *malayensis*; b) Range followed by mean in parenthesis; c) Probably an error by Inglis and Dunn (1963); d) Combined length of pharynx, corpus, isthmus and bulb; e) Ratio to worm length; f) Distance from cephalic end.

Sex (Nº measured)	Male (12)	Male	Male (4)[a]	Female (10)	Female	Female (3)[a]
Host	*N. menagensis*	*N. menagensis*	*N. coucang*	*N. menagensis*	*N. menagensis*	*N. coucang*
Locality	Sabah, Malaysia	Sarawak, Malaysia	Malay Peninsula	Sabah, Malaysia	Sarawak, Malaysia	Malay Peninsula
Source	Present study	Baylis (1928)	Inglis and Dunn (1963)	Present study	Baylis (1928)	Inglis and Dunn (1963)
Length, mm	2.11–2.91 (2.66)[b]	2.2–2.4	1.82–2.92 (2.35)	4.53–7.58 (6.29)	4.5–6.0	4.1–5.4 (4.9)
Width	195–255 (222)	220–250	93–98 (95)[c]	310–460 (384)	400	320–340 (330)
Cephalic diameter	35–40 (38)		28–38 (33)	45–53 (49)		48–63 (57)
Cephalic vesicle diameter	70–88 (81)			95–150 (121)		
Pharynx length	13–14 (13)			14–18 (15)		
Esophageal corpus length	240–285 (268)			328–385 (359)		
Esophageal corpus width	50–60 (57)			70–83 (76)		
Esophageal isthmus length	25–33 (28)			23–35 (27)		
Esophageal isthmus width	20–30 (24)			28–35 (32)		
Esophageal bulb length	107–130 (122)	120–140		148–168 (157)	160–170	
Esophageal bulb width	88–110 (101)	100–120		125–145 (138)	140–150	
Total esophagus length, mm[d]	0.39–0.45 (0.43)	0.4–0.45	0.41–0.49 (0.45)	0.52–0.59 (0.56)	0.6–0.65	0.53–0.60 (0.56)
Total esophageal length (%)[e]	14.6–18.3 (16.3)		16.8–20.7 (19.6)	7.6–11.4 (9.1)		10.0–12.9 (11.4)
Nerve ring[f]	105–148 (123)	120	121–199 (145)	135–193 (156)	170	126–156 (136)
Excretory pore, mm[f]	0.54–0.76 (0.66)	0.7	0.66–0.74 (0.70)	0.73–1.24 (1.00)	0.95–1.05	0.84–1.08 (0.96)
Spicule length	84–108 (97)	ca. 100	84–96 (88)			
Vulva, mm[f]				1.34–2.18 (1.74)	1.5–1.75	1.30–1.68 (1.51)
Vulva (%)[e]				23.9–29.9 (27.8)		29.4–31.7 (30.7)
Tail length, mm				1.04–1.51 (1.33)	1.0–1.3	1.00–1.16 (1.10)
Tail length (%)[e]				19.4–23.8 (21.3)		20.9–24.4 (22.4)
Egg length				70–80 (75.3)	87.5	69–75
Egg width				35–39 (36.7)	37.3	29–32
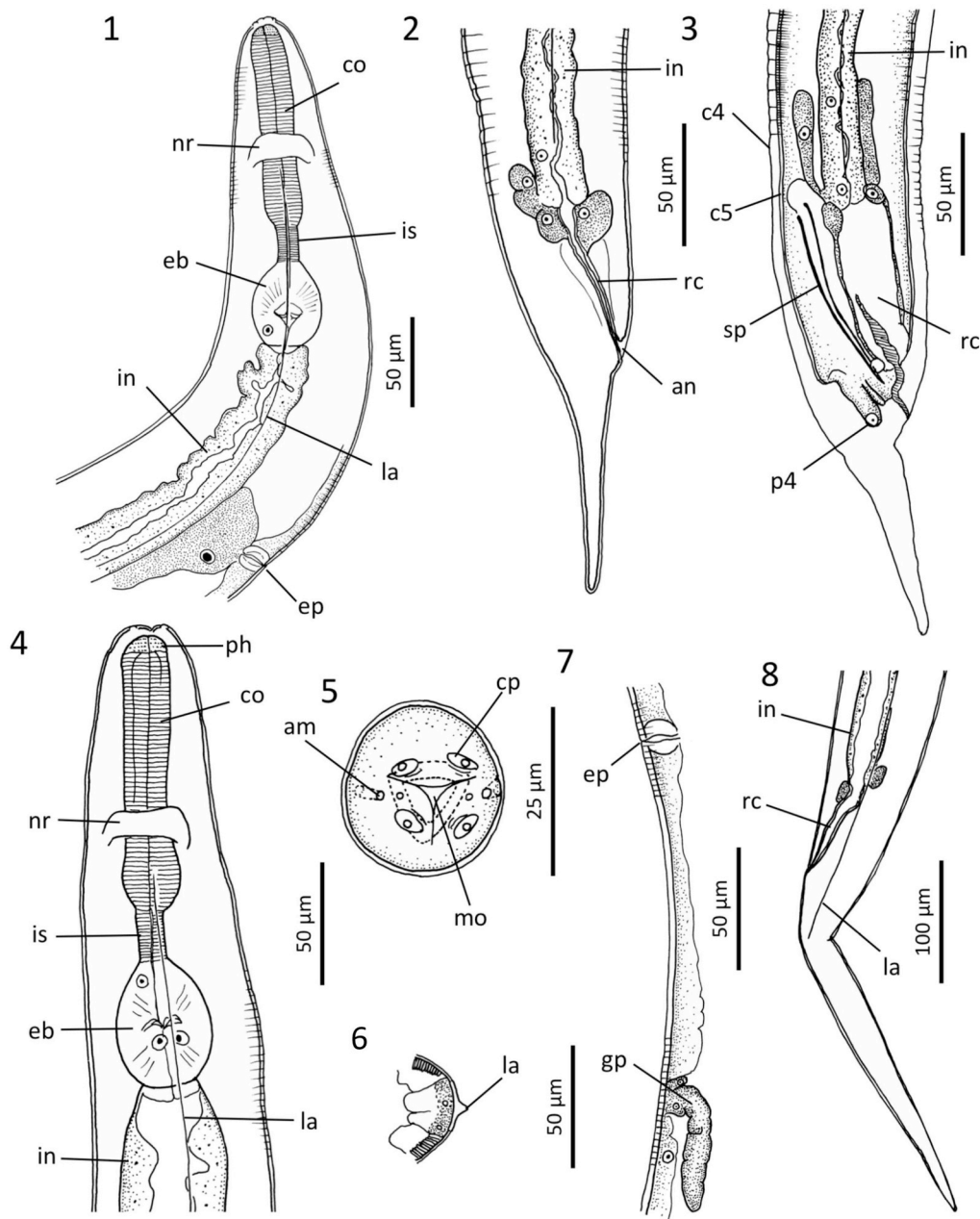
Figs. 1–8. Figs. 1–3: Fourth-stage males. 1. Anterior body, lateral view; 2. Posterior body, lateral view; 3. Posterior body of molting stage, lateral view. Figs. 4–8: Fourth-stage females. 4. Anterior body, lateral view; 5. Cephalic end, apical view; 6. Lateral ala in cross section through midbody; 7. Excretory pore and primordial genital organ at presumptive vulval region, lateral view; 8. Tail, lateral view.

Abbreviations: am. amphidial pore; an. anus; c4. cuticle of 4th stage; c5. cuticle of 5th (adult) stage; co. esophageal corpus; cp. cephalic papilla; eb. esophageal bulb; ep. excretory pore; in. intestine; gp. genital primordium; is. esophageal isthmus; la. lateral ala; mo. mouth; nr. nerve ring; p4: 4th caudal papilla; ph: pharynx; rc. rectum; sp. spicule.

topologies, therefore only ML trees are shown (Figs. 9-11). The analysis of both mitochondrial and nuclear genes resulted in trees with similar branching patterns, where *Trypanoxyuris* and *Enterobius* lineages split first. *Cox1* phylogeny further divided *Enterobius* infecting great apes and monkeys, and although not strongly supported, placed *L. (P.) nycticebi* as a different taxon (Fig. 9). The phylogenies for 18S and 28S rDNA gene sequences also confirmed this scenario (Figs. 10 and 11). For the former, there were two *Lemuricola* sequences available other than this study; *Lemuricola* sp., recovered from *Eulemur* sp. in Madagascar, forming a separate cluster than that of *L. (P.) nycticebi*, and *L. pongoi*, infecting Sumatran orangutans, included within the *Enterobius* clade with *E. buckleyi* and *P. hugoti*, which are also orangutan pinworms. Overall, sequences of slow loris pinworms did not form a basal group, branching out from the *Enterobius* lineage instead. This was also the case when other *Lemuricola* species from Madagascar lemurs were included in the analysis (Fig. 11), suggesting that the genus is not monophyletic if *L. (P.) nycticebi* is included.

4. Discussion

The evolution of Oxyurida has been largely driven by the hosts they inhabit and, unlike other nematode lineages, without further diversification in terms of microhabitat or life cycle. The limited dispersal capacity of pinworms further predicts a strong congruence between host and parasite phylogenies, as they have fewer opportunities to encounter new hosts. In the case of primate pinworms, such congruence originates from both parasite- and host-specific attributes that would seem to provide a buffer against host switching and successful exploitation of novel hosts. For instance, even though humans and chimpanzees are closely related, infection with the human pinworm *E. vermicularis* is usually of mild pathogenicity in humans but often results in fatalities in chimpanzees (Murata et al., 2002; Yaguchi et al., 2014). The contrasting infection outcomes in these two closely related species suggests differences in host specificity.

Reports on parasites of free-living slow lorises remain scarce due to the elusive nature of their hosts. However, reports of oxyurids in slow
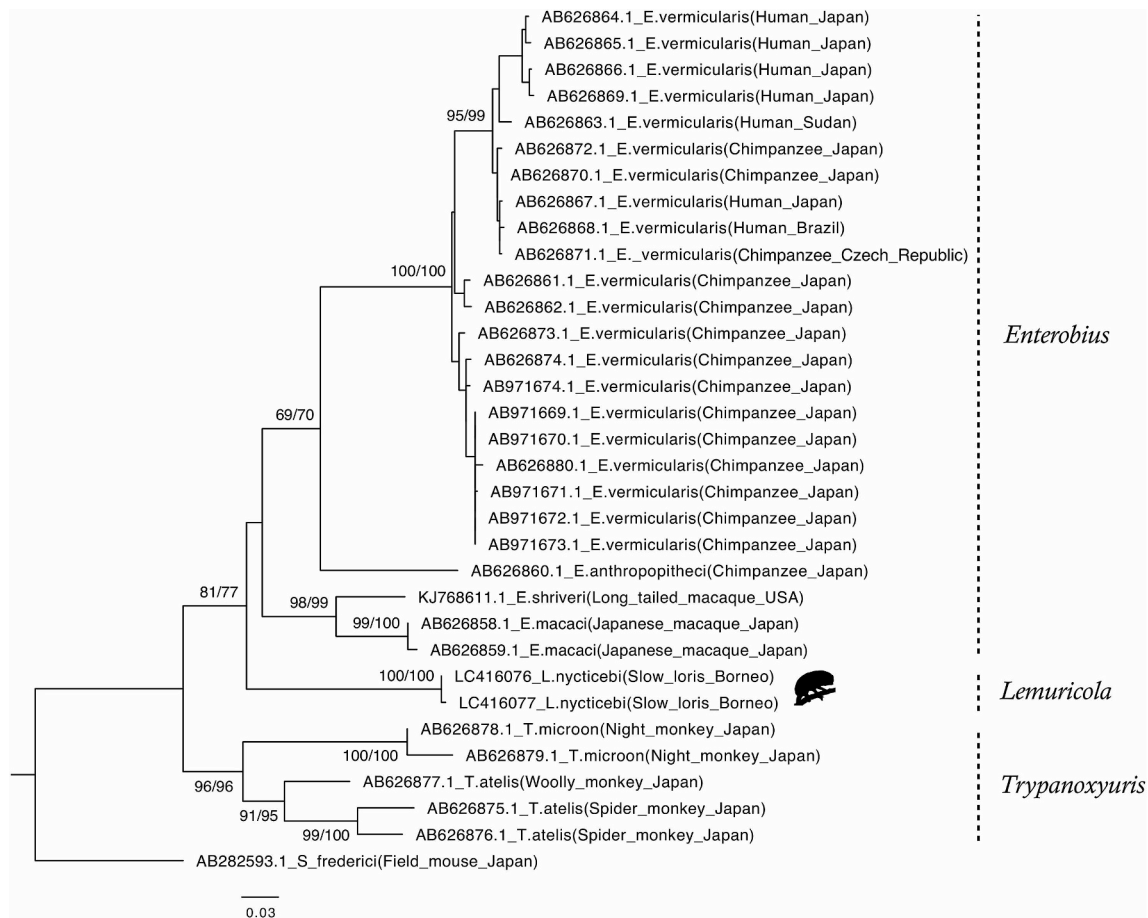
Fig. 9. Phylogenetic relationships among primate pinworms inferred from *cox1* gene sequences. Numbers at the nodes represent ML/NJ bootstrap values, respectively.

lorises are not rare and exist for both captive (Sutherland-Smith and Stalis, 2001) and wild individuals (Baylis, 1928; Inglis and Dunn, 1963; Rode-Margono et al., 2015). Unlike studies on pinworms infecting other mammals, sampling pinworms from primates is usually constrained by various factors; *e.g.* pinworms are uncommonly shed in feces, which can be collected non-invasively, and there are ethical considerations when capture is necessary. By collecting several specimens from different free-living slow lorises in Sabah, we were able to complete previous descriptions of *L. (P.) nycticebi*, including a morphological description of fourth-stage larvae and the genetic characterization of worms. Classifying biodiversity according to the evolutionary history of different organisms has been a task pursued from the days of the early naturalists, and remains highly relevant and more urgent than ever (Deans et al., 2012) as we keep losing species at unprecedented rates (Dirzo and Raven, 2003; Ceballos et al., 2017). The close association with their primate hosts makes pinworms vulnerable to extinction, particularly when host populations are in decline and density-dependent transmission is compromised (Stork and Lyal, 1993; Dunn et al., 2009; Koh et al., 2004).

Taxonomy, based on the morphological characteristics of organisms, has long been the traditional approach towards classification but now molecular tools are being increasingly used, sometimes leading to discrepancies. Numerous studies of various organisms have documented substantial incongruences between molecular phylogenies and morphological classifications, stimulating controversy over which method should be preferred (Seberg et al., 2003; Tautz et al., 2003; Dunn, 2003; Hebert and Gregory, 2005). In this study, morphological and molecular results agree in that they both indicate a clear association between *L. (P.) nycticebi* and its host. However, differences

between the two approaches place *L. (P.) nycticebi* under different phylogenetic scenarios: while taxonomy clusters slow loris and lemur pinworms together, phylogenetic reconstruction of both mitochondrial and nuclear markers places *L. (P.) nycticebi* as a different taxon, distinct from other members of the genus *Lemuricola* and nested within the *Enterobius* clade. Furthermore, the inclusion of *L. pongoi* within the *Enterobius* clade indicates that this species may belong to the *Enterobius* lineage instead of the *Lemuricola* lineage, suggesting greater diversity among orangutan pinworms than previously recognized. It is worth noting that *L. pongoi* possesses a nearly hexagonal cephalic plateau with very large cephalic papillae at four corners in both sexes, an oblong esophageal bulb connecting to the esophageal corpus without strong constriction, and a long tail appendage in the male (Foitová et al., 2008, 2010). These morphological features suggest close affinity of *L. pongoi* with *Pongobius*, which has been known only from orangutans (see Baruš et al., 2007; Kuze et al., 2010). In phylogenetic analyses, *L. pongoi* also shows a close relationship with *Pongobius* based on sequences of *cox1* and 18S rDNA (Foitová et al., 2014; this study), and therefore, we would like to propose here a new transfer for this species as *Pongobius pongoi* (Foitová et al., 2008) n. comb. If the position of *Pongobius* within the lineage of *Enterobius* reflects actual phylogeny, it should be suppressed to subgeneric rank.

Molecular data support the hypothesis that there are three monophyletic clusters within the primate pinworms, however, the *Lemuricola* clade does not seem to be monophyletic. In this regard, not only lorises slow lorises but also their pinworms diverged from their African counterparts ~40 mya (Perelman et al., 2011; Pozzi et al., 2014), later colonizing Asia. The morphological similarities between pinworms from lemurs and slow lorises may not be the product of a shared
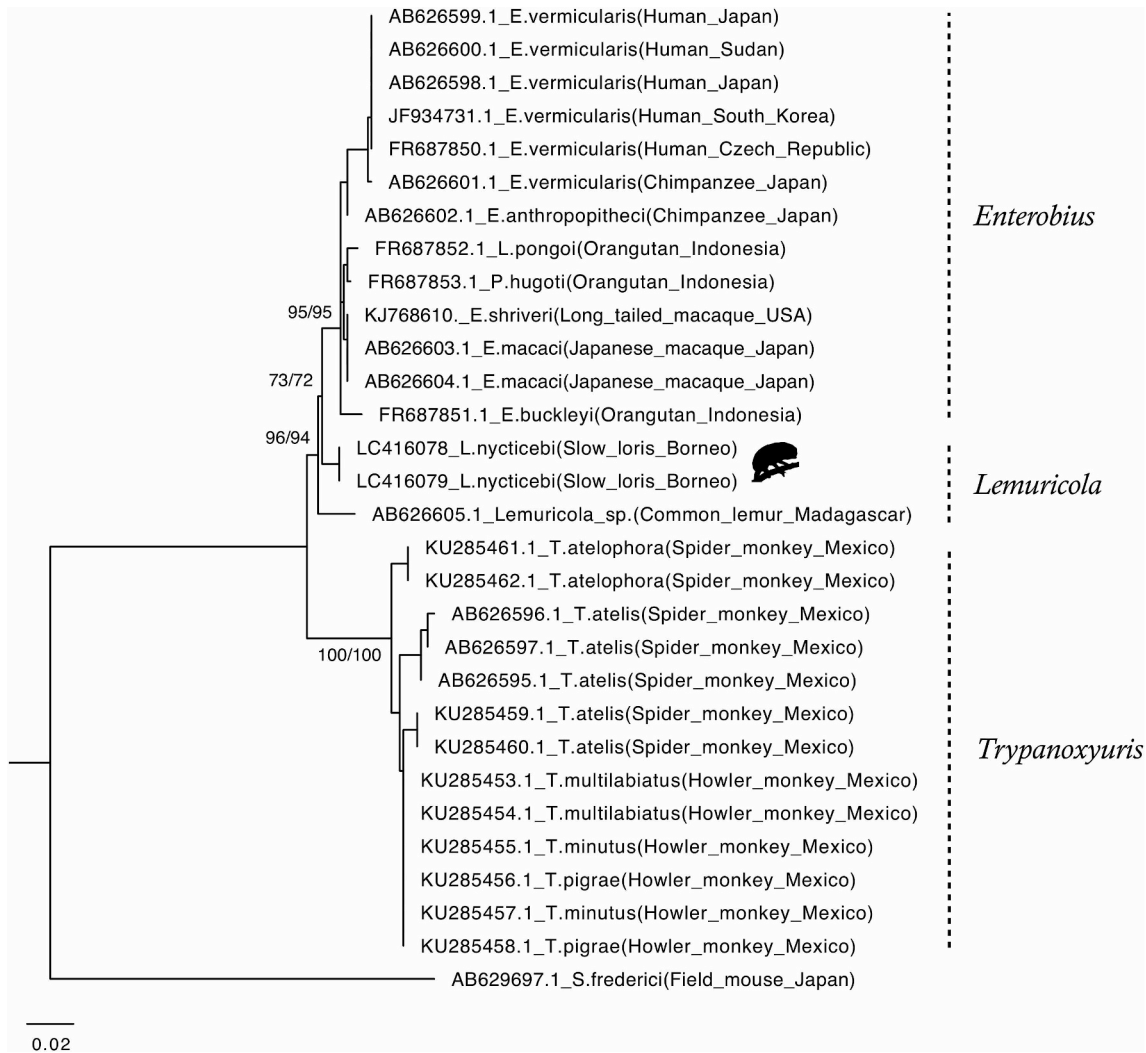
Fig. 10. Phylogenetic relationship among primate pinworms inferred from 18S rDNA gene sequences. Numbers at the nodes represent ML/NJ bootstrap values, respectively.

phylogenetic history but instead the result of convergence, *i.e.* independent adaptations to similar environments, in this case their strepsirrhine hosts. If convergence was the actual case, *Protenterobius*

should be transferred to *Enterobius* or elevated to generic rank. The subdivisions within the *Enterobius* clade are likely to become clearer with a stronger sampling and sequencing effort. Accurate
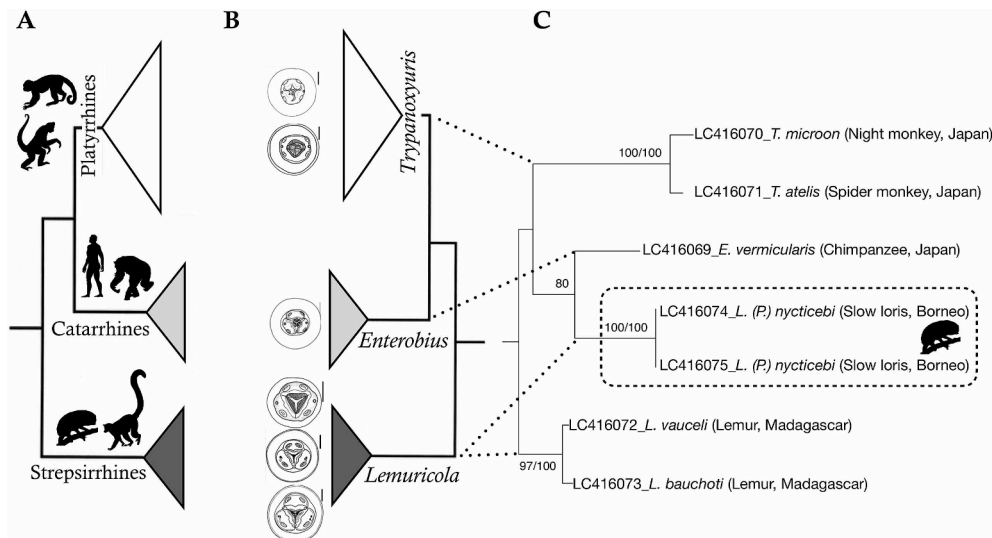
Fig. 11. Comparison between (A) primate phylogeny, (B) pinworm phylogeny derived from cladistics, and (C) 28S rDNA gene sequences (outgroup not shown). Female pinworm cephalic ends shown in B correspond to (top to bottom): *Trypanoxyuris (Trypanoxyuris) microon*(Linstow 1907),*Trypanoxyuris (Buckleyenterobius) atelis* (Cameron 1929), *Enterobius (Enterobius) vermicularis*(Linnaeus 1758)*,Lemuricola (Protenterobius) nycticebi*(Baylis 1928)*,Lemuricola (Madoxyuris) bauchoti*(Chabaud et al. 1965)*,Lemuricola (Madoxyuris) vauceli* (Chabaud et al. 1965). Scale: 20 μm. Line drawings reprinted with permission of Cambridge University Press from Hasegawa (2009), Methods of collection and identification of minute nematodes from the feces of primates, with special application to coevolutionary study of pinworms. In: Huffman Chapman (eds.) Primate Parasite Ecology. Cambridge University Press, pp. 29–46.

classifications, involving morphological and phylogenetic descriptions, are the basis for comparative biology, biodiversity studies and conservation efforts, and the identification of phylogenetic associations is part of the exploration and understanding of biological diversity. As they enable the reconstruction of the evolutionary history of organisms, molecular characterization and morphological description should be conducted in concert wherever possible.

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