

Two remarkable pinworms (Nematoda: Enterobiinae) parasitizing orangutan (*Pongo abelii*) in the Sumatra (Indonesia) including *Lemuricola (Protenterobius) pongoi* n.sp.

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Summary

Two species of pinworms (Enterobiinae) were collected from fresh faeces of semi-wild orangutans *Pongo abelii* Lesson living in northern Sumatra (Indonesia). The female of *Enterobius (Enterobius) buckleyi* Sandosham, 1950 is redescribed. *Lemuricola (Protenterobius) pongoi* n. sp. is described on the basis of females (no males are available) and distinguished from *L. (P.) nycticebi* (Baylis, 1928) by cephalic and mouth morphology (head and teeth superstructures), body (9.85 – 15.46 mm) and tail (2.34 – 2.95 mm) length, smaller eggs (48 – 56 x 22 – 28 µm), longer vulva distance from anterior extremity (2.05 – 3.09 mm) and other features. Characteristic is the total body length/oesophagus length ratio (1 : 15.3 – 22.0). Both nematode species were studied using scanning electron microscopy for the first time.

Key words: Orangutan, Oxyuridae, Sumatra, *Lemuricola (Protenterobius) pongoi* n.sp., *Enterobius (Enterobius) buckleyi*

Introduction

For critically endangered great apes of the genus *Pongo* Lacépède (Hominidae: Ponginae) data on occurrence of their parasites are patchy and mainly limited to captive animals. The first field-based study of parasites in wild orangutan populations were presented in the unpublished PhD thesis by Rijksen (1978) and Foitová (2002). There is only one study published on the parasites of wild, captive and newly reintroduced orangutans in Indonesia (Kalimantan and Sumatra) by Collet *et al.* (1986), and Mul *et al.* (2007).

In hosts of the order Primates, pinworm subfamily Enterobiinae Hugot, Gardner et Morand, 1996 is very interesting for the systematic and cospeciation point of view (Hugot *et al.* 1996, Hugot 1999). Four species have been reported from orangutans: *Enterobius buckleyi* Sandosham, 1950, *E.*

foecundus (Linstow, 1879), *E. simiae* MacCallum, 1921 and *Pongobius hugoti* Baruš, Foitová, Koubková, Hodová, Šimková et Nurcahyo, 2007 (see Baruš *et al.*, 2007). In the present study, descriptions of two species of pinworms (based on females) parasitizing the native Sumatran orangutan *Pongo abelii* Lesson are provided, including scanning electron microscopy data.

Materials and methods

The locality of investigation, the village Bukit Lawang (former home to a rehabilitation centre for orangutans) is situated on the southwestern border of the Gunung Leuser National Park (northern Sumatra, Indonesia) (03°32.983' N, 098°06.908' E) at an altitude of 323 m above sea level. Temperatures range between 21 °C and 28 °C and relative humidity between 80 % and 100 %. Annual rainfall is 2000 – 3200 mm. The majority of orangutans in this area live in a semi-wild population. From the fresh faeces of three adult female orangutans, 12 worms were collected in 2002 and immediately fixed in 70 % ethanol. These nematodes were cleared in glycerine (mixture 1:1 with 70 % ethanol) and examined under a light microscope equipped with differential interference contrast (DIC), digital image analysis system (analySIS FIVE DOCU) and a drawing attachment was used for morphometric analysis.

Parasite specimens used for scanning electron microscopy (SEM) were washed several times in 0.6 % saline, fixed with hot 4 % formaldehyde solution and stored at room temperature. Prior to examination, the parasites were subsequently dehydrated through ethanol, dried in a CPD 030 critical point drying apparatus (Bal-tec) using liquid CO₂, mounted on aluminium stubs with double sided adhesive disc, coated with gold in a SCD 040 sputter coating unit (Balzers) and examined in a VEGA (Tescan) scanning electron microscope operating at 20 kV.

Results

Oxyuridae Cobbold, 1864

1. *Enterobius (Enterobius) buckleyi* Sandosham, 1950 (Figs. 1, 2)

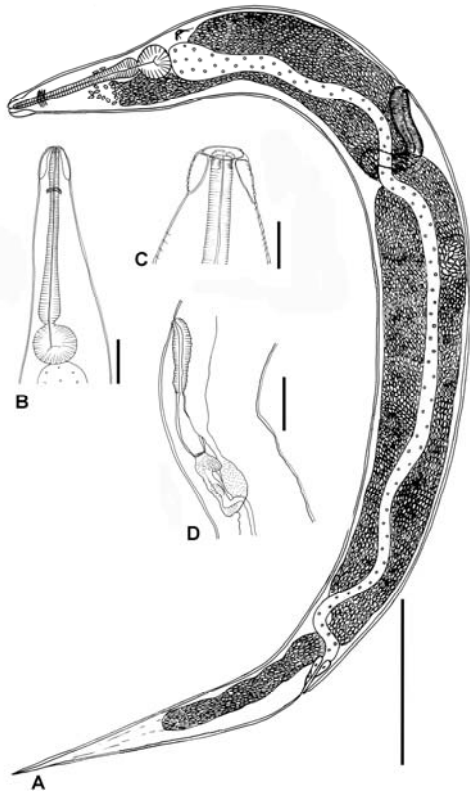


Fig. 1. *Enterobius (E.) buckleyi*, female. A - total view; B - anterior extremity; C - cephalic region; D - vulva region, lateral view. Scale bars: A = 1000 µm; B, D = 200 µm; C = 100 µm.

Description

Female (based on 6 ovigerous specimens; measurements of one juvenile nonovigerous specimen in parentheses): Medium sized, whitish nematodes. Cuticle with clear transverse striations, cephalic vesicle present and well developed, with transverse striations. Lateral alae in cervical part consisting of single crest, and posterior part with double crests. Mouth triangular, surrounded by three obtuse lips; in mouth cavity, dental apparatus present, corresponding to respective lips, and consist of three wide and obtuse teeth opposed to lips. Dorsal lip is wider, with two hemispherical papillae; two subventral lips each with two cephalic papillae, one dome-shaped, one incorporating amphid. Oesophagus with short pharyngeal part, corpus slightly enlarged and continuing in very short isthmus, bulb rounded with valvular apparatus. Excretory pore situated at bulbal or slightly postbulbal level. Vulva pre-equatorial, muscular vagina and uterine tube divided into two parts by cellular wall forming diaphragm; no spermatheca observed. Length of body: ovigerous specimens 7.35 – 9.35 mm (range), juvenile specimen (measurements in parentheses) 5.35 mm, maximum width 572 – 806 (386) µm; width at bulb 393 – 691 (381), at vulva 565 – 791

(361), at anus 266 – 536 (183); cephalic vesicle length 129 – 193 (118), cephalic vesicle width 150 – 190 (128). Total oesophagus length 1026 – 1169 (1089); pharynx length 62 – 93 (65); width 40 – 52 (34); corpus length 737 – 901 (890), corpus width 70 – 124 (87); isthmus length 14 – 41 (17), width 31 – 47 (39); bulb length 169 – 217 (169), width 164 – 235 (143). Nerve ring 230 – 320 (260); excretory pore 858 – 1374 (1046); vulva 1668 – 2394 (1710) from anterior extremity. Oviduct directed posteriorly, muscular vagina length 314 – 526 (433); vagina width 97 – 141 (50). Amphidelphic. Distance vulva to anus 3764 – 5261 (1363); tail long, conical, with pointed apex, 182 – 2344 (1280). Eggs numerous, ellipsoidal, with flattened side, 50 – 55 long, and 23 – 27 wide, having three longitudinal thickenings.

Ratios: Total body length/total oesophagus length 6.83 – 8.95, (4.12); total body length/tail length 3.69 – 4.91, (4.18); total body length/vulva distance from anterior extremity 3.40 – 4.40, (3.13); vulva - anus distance/tail length 1.72 – 2.76, (1.07).

Host: *Pongo abelii* Lesson, 1827 (Primates: Hominidae).

Locality: Northern Sumatra, Bukit Lawang (03°32.983' N, 098°06.908' E)

Site of infection: discharged in faeces

Material: Voucher specimens (n = 6) from 3 orangutans in the helminthological collection of the Research Centre for Biology LIPI, Zoology Division, Puslit Biology LIPI, Cibinong, Indonesia (Cat. No. MZBNa 412).

Remarks

Sandosham (1950) noted that material for description of *E. buckleyi* consisted of three males and 8 females obtained from the appendix of Bornean orangutan *Pongo pygmaeus* (Linnaeus), which died in the laboratory of Professor Buckley in London. Borneo was designated in the original description as the type locality (= *terra typica*). Our finding is the second report of this pinworm species, and the first from native territory and new host (Sumatra, *P. abelii*).

The morphology of the worms (females) in our material is generally the same as that described by Sandosham (1950). In the head morphology the species is clearly consubgeneric with members of the subgenus *Enterobius (Enterobius)* Leach, 1853 as is evidenced by its SEM study, and from a comparison with the type species of the genus (*E. vermicularis* Linnaeus, 1758), studied by Tenora *et al.* (1981).

Identical features of subgeneric weight were found in form of lips, numbers and distribution of cephalic papillae on lips, presence of three teeth with rounded margins in the mouth cavity, and the form of female genital characteristics of the genus *Enterobius* (see Hugot *et al.*, 1996; Fig. 4A, pp. 154 – 155). Small difference was found in the excretion pore location. According to Sandosham (1950) "is at the level of the posterior bulb of the oesophagus". In our material (n = 7) is situated at the bulb level (n = 3), or slightly postbulbal, at distance 86-280 postbulbal (mean 174) (n = 4). We consider these character as intraspecific variability. The measured features in our material reflected

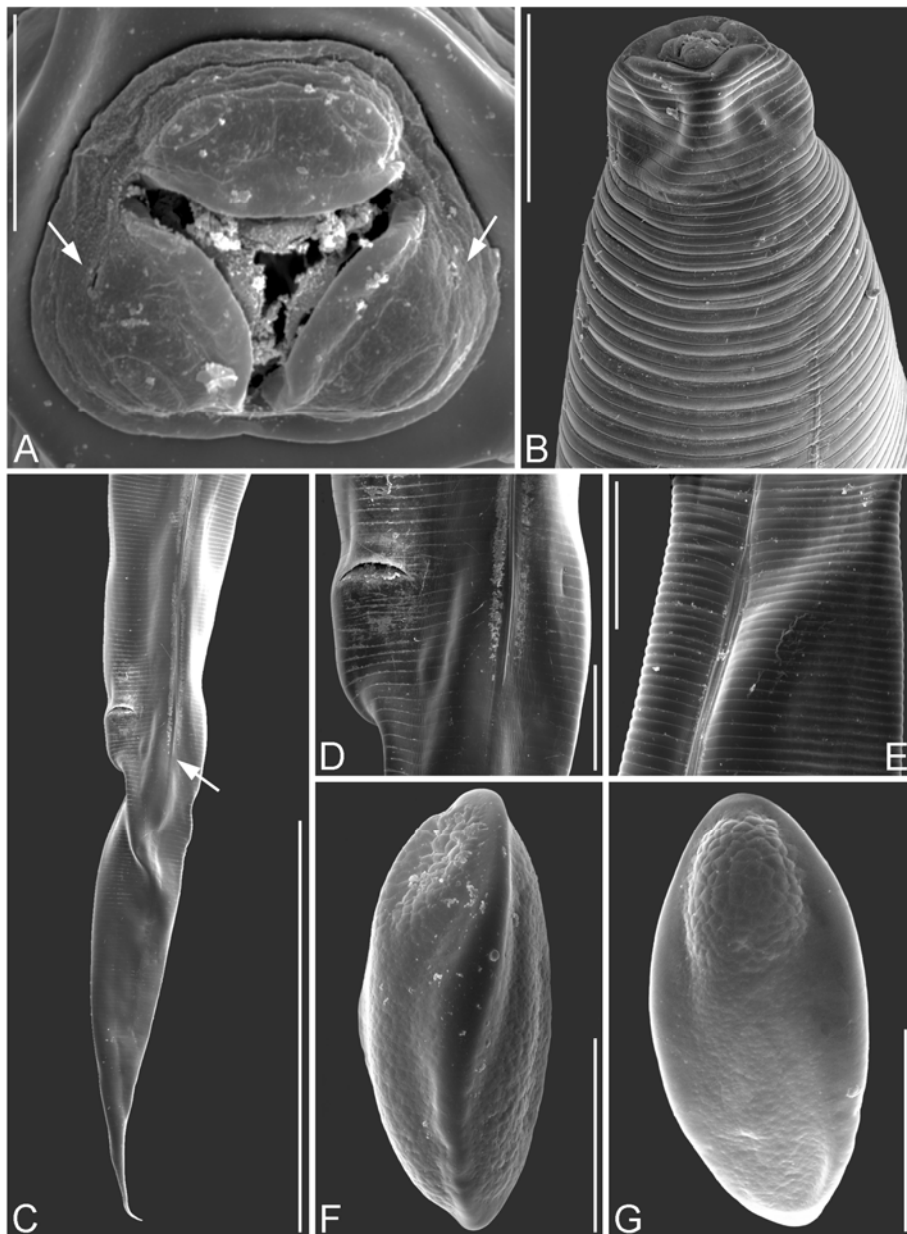


Fig. 2. *Enterobius buckleyi* female; scanning electron micrographs. A - cephalic end, papillae, teeth (apical view, arrows – amphids); B - forebody, dorsolateral view; C - tail end, lateral view, arrow - end of lateral ala; D - detail of anus, ventrolateral view; E - detail of lateral ala, transition from single to double crest; F - egg, total view; G - egg, egg pole with granular structure, total view;
Scale bars: A, F, G = 20 μ m; B, D = 100 μ m; E = 200 μ m; C = 1 mm.

a higher variability in relation to the pinworm specimens' body length. Females studied by Sandosham (1950) attain body length 6.96 – 7.25 mm and their body is shorter at 386 – 2.096 mm than ovigerous specimens in our material. Nonovigerous female (length 5.353 mm) is shorter of 1.600 mm in comparison with minimum length in original description. Our specimen has maximum body width of 16 μ m higher than reported by Sandosham (1950) (370 – 420 μ m). This difference doesn't have any taxonomic significance and it's probably results of compression during sample preparation; in females without eggs in uterus is very common. Body length affect oesophagus length less distinctly, which in comparison with original description (0.9

– 0.99 mm length) in our studied specimens is longer of 126 – 179 μ m; transparently affect tail length, which is longer of 474 – 864 μ m (in Sadosham description-1.350 – 1.480 mm). From ratios value we remark the difference in values $R = \text{prevulval body length/postbulbal body length}$. According to Sandosham (1950) the range is 1.7 – 1.78 (females 6.96 – 7.25 of the body length). Adult females in our material cover ratio range 2.39 – 3.40; in the juvenile specimen (body length 5.353) is ratio 1.55 only. Ratio value rises on body grow. Small oesophagus length difference is considered as result of intraspecies variability. Our measurements of eggs present the variability range (50 – 55 x 23 – 27); Sandosham (1950) states only one measure

(56 x 26). Differences described in the egg structure (three longitudinal thickenings present) and presence of a sub-polar lid noted in our material result from a more detailed SEM study of our material.

Pinworm parasites of the great apes and man (Hominidae: genera *Gorilla* Geoffroy, *Pan* Oken, *Homo* Linnaeus and *Pongo* Lacépède manifested under cladistic analysis of the Enterobiinae by Hugot (1999) support the Cameron's hypothesis in its assertion of "association by descent" (Cameron 1929). In the apes and humans group, the *Enterobius* contain four host-specialised species: *E. lerouxi* Sandosham, 1950 in *Gorilla gorilla* (Savage et Wyman); *E. anthropopitheci* (Gedoelst, 1916) in *Pan paniscus* Schwartz and *P. troglodytes* Blumenbach; *E. buckleyi* Sandosham, 1950 in *P. pygmaeus* and *P. abelii*; and *E. vermicularis* in *Homo sapiens* Linnaeus. Nakano *et al.* (2006) documented that the synonymy of *E. gregorii* Hugot, 1983 with *E. vermicularis* was supported by the molecular evidence. All these pinworm species are well described or recently redescribed (Skrjabin *et al.* 1960; Hugot and Tourte-Schaeffer 1985, Hugot 1993; Hasegawa *et al.* 2005) and are clearly different.

Two other species of *Enterobius* have been described from the orangutan (*P. pygmaeus*) kept in captivity (ZOO); namely *E. foecundus* (Linstow, 1879) and *E. simiae* (MacCallum, 1921). Cameron (1929) did not provide any different or additional data on these two species in his review of *Enterobius* in Primates. He expressed the opinion that *E. simiae* is possibly identical with *E. foecundus*. The description by McCallum (1921) is very inadequate compared to current standards, containing only data on the body length (6.00 mm) and width (0.30), statement that "the vagina is at about the beginning of the posterior third of the worm" and "both sexes are much alike in appearance". His illustrations of the anterior and posterior ends of the female without any accompanying scale add little information of taxonomic value to the verbal description.

In the original description of *E. foecundus* (syn. *Oxyuris foecunda*) by Linstow (1879), the mouth shows two small rounded lips, and no lateral alae. Body length of the female is 6.5 mm and width 0.66 mm. The oesophagus in the female is 1/6.4 (= 1.015 mm) of the total body length and tail 1/6 (= 1.083 mm) of the body length; eggs measures 56 x 26 µm and have a smooth shell. The females of *E. buckleyi* in our material differ in all above-mentioned features and mainly by a longer tail. In comparison with Sandosham (1950) description, males differ in the spicule length (0.1225 vs. 0.052 mm). For this reason, *E. foecundus* is not considered conspecific with *E. buckleyi*, but we support the opinion of Inglis (1961) "that *E. simiae* was rightly treated by Sandosham (1950) as *nomen inquirendum*, it is better to treat it and probably *E. foecundus* also, as *nomina dubia*".

2. *Lemuricola (Protenterobius) pongoi* n.sp. (Figs. 3, 4)

Description

Female (holotype; and 5 paratypes): Small worm, whitish

coloured, tapering to both extremities; body (holotype) 15.46; paratypes (range and mean) 9.85 – 12.11, 11.57 mm length and 880; 700 – 801, 755 mm maximum width. Width at bulb 594; 404 – 505, 474, at vulva 826; 693 – 808, 752, at anus 400; 308 – 430, 379. Cuticle with clear transverse striations on cephalic vesicle and body. Lateral alae developed, originating slightly posterior to vesicular end, in cervical part consisting of single crest, and posterior part with two parallel crests, terminating slightly posterior to anus. Mouth triangular, rounded by three small lips (wider than higher) and leading into shallow bucal cavity. Dorsal lip with two hemispherical papillae, two subventral lips bears a hemispherical papilla near ventral rim, and other papilla near lateral rim of lips, with amphid conspicuous opening. Mouth cavity armed with three tooth structures arising from each sector of oesophagus. Wide, leaf-like and flat tooth to the inner wall of each lip adjoining and slightly overlaps top margin of the lips. Inner tooth width is 1/3 of width of corresponding tooth. Inner tooth element protrude from the bottom of prostom funnel to the mouth cavity (having common base with external tooth). Inner teeth are short, triangular and flat with top oriented medially. Tip is armed by 5 – 6 very small teeth like spurs. Cephalic vesicle 125; 110 – 146, 127 length, and 204; 159 – 191, 179 maximum width. Total oesophagus length 703; 625 – 709, 676, pharynx length 71; 35 – 89, 69 and width 56; 44 – 58, 51; corpus length 422; 255 – 398, 339 and width 69; 58 – 108, 74. Short isthmus present, 75; 34 – 51,

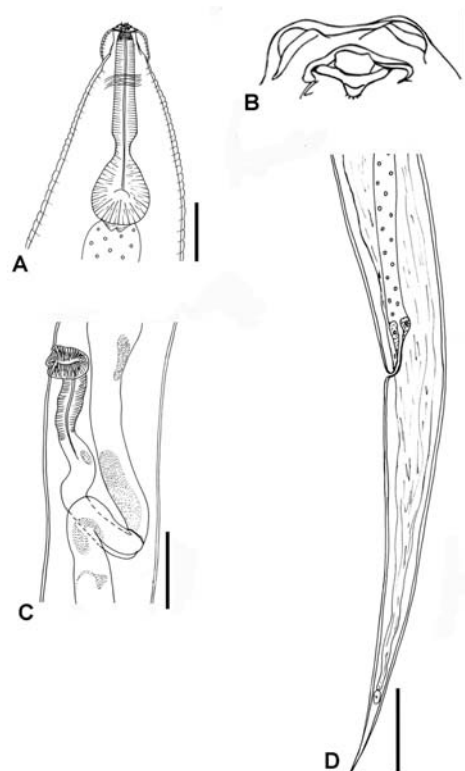


Fig. 3. *Lemuricola (P.) pongoi* n.sp., female. A - anterior extremity; B - detail of mouth opening - dorsal lip with two hemispherical papillae, the tip of inner tooth with very small tooth like spur; C - vulva region - lateral view; D - tail end - ventrolateral view; Scale bars: A = 100 µm; B = 10 µm; C, D = 500 µm.



Fig. 4. *Lemuricola (P.) pongoi* n.sp., female; scanning electron micrographs. A - cephalic end (apical view), cephalic papillae and teeth with superstructures; B - lateral ala, forepart of body; C - tail end of female, lateral view, arrow - end of lateral ala; D - detail of anus, ventrolateral view; E - egg, total view, arrow - subpolar plug with granular structure; Scale bars: A, E = 20 μ m; B = 100 μ m; D = 200 μ m; C = 1 mm.

47 length, and 39; 32 - 71, 43 width, containing valve in piriform bulb, 246; 222 - 264, 247 length, and 205; 156 - 243, 196 width. Nerve ring 243; 156 - 226, 196, excretory pore 1126; 653 - 1037, 869, vulva 3093; 2046 - 2936; 2488, from anterior extremity. In females longer than 10 mm (n = 5) the vulva closed by dark brown cement. Ovijector directed posteriorly; vagina muscular, 881; 543 - 956, 722 length, and 123; 81 - 139, 117 in maximum width. Vagina uterina divided into two portions by thick cellular wall (diaphragm). Distance between vulva and diaphragm longer than that between diaphragm and uterine opening. Amphidelphic. Distance of vulva to anus 9413; 5092 - 6729, 6338. Tail conical with pointed apex, 2951; 2339 - 2786, 2610 long. Eggs (n = 25) ellipsoidal, with flattened side, 48 - 56; 53.4 long and 22 - 28, 24.8 wide; having three longitudinal thickenings, the lid is subterminal, has a coarsely granulated texture, an irregular elliptic form.

Ratios: Total body length/total oesophagus length 21.98;

15.3 - 19.0, 17.1; total body length/tail length 5.24; 3.6 - 5.2; 4.4; total body length/vulva-head distance 4.99; 3.6 - 5.0; 4.7; vulva-anus distance/tail length 3.2; 1.8 - 2.7; 2.44.

Type host: *Pongo abelii* Lesson, 1827 (Primates: Homini-
dae).

Type locality: Northern Sumatra, Bukit Lawang
(03°32.983' N, 098°06.908' E)

Site of infection: discharged in faeces

Number of specimens studied: measured, 6 adult females
and 1 juvenile female; SEM studied by 1 female.

Type material: Holotype and four paratypes deposited in
the helminthological collection of the Research Centre for
Biology LIPI, Zoology Division, Puslit Biology LIPI, Ci-
binong, Indonesia (Cat. No. MZBNa 411).

Discussion

Although the male was not found, the present species is
assigned to the genus *Lemuricola* subgenus *Protenterobius*

Inglis, 1961 (Enterobiinae) which has a mouth opening surrounded by three non-prominent lips (wider than higher), prominent cephalic papillae on each lip, and three distinct tooth-like structures projecting into the buccal cavity. The oesophagus in the subgenus has distinct isthmus, lateral alae with double crest and vulva is closed by brown cement in the gravid females. These characters coincide well with subgenus *Protenterobius* by Inglis (1961) and Inglis & Dunn (1963).

The type species of this subgenus is *L. (Protenterobius) nycticebi* (Baylis, 1928), parasitizing *Nycticebus coucang* (Boddaert) in Malaya and Borneo (Baylis 1928; Inglis 1961; Inglis & Dunn 1963; Hugot 1999). Only in this species closed vulva by brown cement is recorded by Inglis & Dunn (1963). We are not agreeing with Inglis and Dunn (1963) that *Protenterobius* is synonymous with *Lemuricola* Chabaud et Petter, 1959. We prefer and agree with conclusions by Hugot *et al.* (1996, p. 147) that "subfamily Enterobiinae includes the following genera or (subgenera): *Enterobius* Leach, 1853; *Colobenterobius* Quentin, Berterton et Krishnasamy, 1979; *Rodentoxyuris* Quentin et Tenora, 1974; *Xeroxyuris* Hugot, 1995; *Lemuricola* Chabaud et Petter, 1959; *Protenterobius* Inglis, 1961; *Madoxyuris* Chabaud, Brygoo et Petter, 1965; *Trypanoxyuris* Vevers, 1923; *Hapaloxuyuris* Inglis et Gosgrove, 1965; and *Paraoxyuronema* Artigas, 1936". Having the well visible isthmus is *Protenterobius* clearly different from *Pongobius* (Baruš *et al.* 2007), which has a special form of oesophagus in the Enterobiinae (isthmus absent and piriform bulb present).

Females of *L. (P.) nycticebi* differ (Baylis 1928; Inglis & Dunn 1963) from the *L. (P.) pongoi* by shorter body (4.5-6.0 mm), and tail (1.0 - 1.3) length, vulva distance from anterior extremity 1.50-1.75, and bigger eggs (69-87 x 29-37). Morphology of the head and mouth is different by a simple structure of teeth in *L. (P.) nycticebi*, and by presence of teeth superstructures in *L. (P.) pongoi*. Ratio of body length/total oesophagus length (8.49 - 9.29) is different, too.

It is believed that pinworms and their host primates have coevolved (Cameron 1929; Sandosham, 1950; Chabaud *et al.* 1961; Hugot *et al.* 1996; Sorci *et al.* 1997; Hasegawa *et al.* 2005). *L. (P.) nycticebi* is specialist parasitizing genus *Nycticebus* Geoffroy (Lorisidae), his distribution includes: S. Philippines; Assam (India) to Vietnam and Malayan Peninsula; W Indonesia; Yunnan perhaps Kwangsi (China) (Wilson & Reeder, 2005). Possible divergent co-speciation of ancestral *Protenterobius* pinworm takes place by host-switching during the course of evolution of Lorisidae and Ponginae (Hominidae). The family Hominidae, consisting of the great apes and man, contains four genera of hosts that are parasitised by following pinworm species: *Enterobius lerouxi*, *E. vermicularis*, *E. anthropopitheci*, *E. buckleyi* and *Pongobius hugoti* (see Gedoelst 1916; Sandosham 1950; Hugot 1993, 1999; Hugot *et al.*, 1996; Hasegawa *et al.*, 2005; Nakano *et al.*, 2006; Baruš *et al.*, 2007). *Lemuricola (Protenterobius) pongoi* differs from all above mentioned species in the generic characters defined

by Hugot *et al.* (1996).

The following nominal pinworm taxa were found in the orangutans (*Pongo*) also: *Enterobius foecundus* and *E. simiae*. The differences of these two species discussed in this paper in relation to *E. buckleyi* are valid also for *L. (P.) pongoi*. As described Linstow (1879) and McCallum (1921) both species are smaller in body length (6.0-6.5 mm); *E. foecundus* has longer oesophagus (1173µm) and shorter tail (1083) than *L. (P.) pongoi*. *E. simiae* differs in form of oesophagus, having a much shorter isthmus as majority of *Enterobius* species. We prefer treated both latter pinworms as *species inquirendae*. The newly described taxon *L. (P.) pongoi* is morphologically and metrically well characterised, and specialised to the host genus *Pongo* in its native distribution in Sumatra and probably Borneo also (Indonesia).

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