Two new species of *Trichuris* (Nematoda: Trichuridae) collected from endemic murines of Indonesia

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Abstract

Two new species of the genus *Trichuris* (Nematoda: Trichuridae) parasitic in the old endemic murids of Indonesia are described: *T. musseri* sp. nov. from *Echiothrix centrosa* (Murinae: Rattini) in Sulawesi and *T. mallomyos* sp. nov. from *Mallomys rothschildi* (Murinae: Hydromyini) in Papua Indonesia. Both species are characterized by having a gradually tapered and sharply pointed distal end of the spicule, being readily distinguished from most of the congeners known from murid rodents. *Trichuris musseri* is readily distinguished from *T. mallomyos* by having a much smaller body and large number of nuclei per subdivision of stichosome. The resemblance in spicule morphology between the two new species is of special interest because both hosts belong to different tribes and have different habitats and habits. It remains to be elucidated whether the resemblance is merely homoplasy or actually reflects close phylogenetic relationship of the parasites.

Key words: *Trichuris musseri* sp. nov., *Trichuris mallomyos* sp. nov., Echiothrix, Mallomys, old endemic murids, Sulawesi, Papua, Indonesia, zoogeography

Introduction

Nematodes of the genus *Trichuris* (Trichuridae) are parasitic in the large intestine of various mammals including murine rodents (Anderson & Bain, 1982). Indonesia is known as an area with very high diversity of murines with more than 170 species, especially many endemic species in Sulawesi and Papua Indonesia (=West New Guinea) (Suyanto et al., 1998; Musser & Carleton, 2005; Fabre et al., 2013). These endemic species are classified into old endemics and new endemics. The old endemic murines have many archaic features and exhibit unique combinations of specializations, and have no close living relatives in continental Asia and islands on Sunda Shelf (see Musser, 1981, 1987). It has been considered that ancestors of the old endemics arrived in the Miocene and early Pliocene, whereas the new endemics colonized later (see Rowe et al., 2016 and literature cited therein). Because a new whipworm, *T. germani* Smales, 2013, was recently described from *Pogonomys* spp., old endemic murines, in Papua New Guinea (=East New Guinea) (Smales, 2013), it is expected that more *Trichuris* species may be present in the endemic murines in these areas. On examination of nematode material collected from old endemic rats of Indonesia, two new species of *Trichuris* were recovered. Their morphology is described herein with a zoogeographical discussion.

Materials and methods

Two individuals of *Mallomys rothschildi* were purchased at a local market in Wamena, Papua, and their alimentary canals were fixed in 10% formalin (Hasegawa & Syafruddin, 1994). Individuals of *Echiothrix centrosa* were captured in Malakosa, Kuala Navusu, Sulawesi, by Dr. Guy G. Musser, and their carcasses were fixed in 10% formalin and preserved in 95% ethanol at the American Museum of Natural History (AMNH), New York, (Musser
The contents of the cecum and large intestine were removed and washed with running tap water on a fine sieve. The residues left on the sieve were transferred to a petri dish and examined under a stereomicroscope. Nematodes found were stored in 70% ethanol, cleared in glycerol-ethanol solution by evaporation of ethanol, and mounted on glass slides with a 50% glycerol solution. Measurements, given in micrometers unless otherwise stated, are averages followed by the ranges in parentheses. Type specimens were deposited in the National Museum of Natural History (USNM), Washington, D.C., U.S.A., and Museum Zoologicum Bogoriense (MZB), Bogor, Indonesia.

Description

Trichuris musseri sp. nov.

(Figs. 1–12)

General. Small sized as a Trichuris in murids. Cuticle with fine transversal striation. Anterior part of body narrow, thread-like, tapering to cephalic end; gradually increasing to widest portion in posterior body and again slightly tapered to posterior end (Figs. 1, 2, 9). Minute cephalic stylet present. Stichosome with 1 row of stichocytes, and 1 pair of conspicuous cells at esophago-intestinal junction level (Figs. 2–5, 10). Nuclei of stichosome numerous, almost 1 nucleus per 1 to 2 subdivisions, in middle to posterior portions (Figs. 3–5). Bacillary band commencing just after cephalic end, well developed in middle portion of anterior body (Figs. 2–5), becoming narrower and invisible anterior to esophago-intestinal junction. Round cuticular inflations of various size bordering bacillary band present in anterior portion of body (Fig. 2).

Male (9 specimens): Length 8.79 (7.89–9.24) mm. Anterior portion of body 5.32 (4.93–5.65) mm long, corresponding to 61 (58–63) % of body length. Width at middle of anterior body 57 (44–69), esophago-intestinal junction 129 (99–162) and thick portion of posterior body 182 (162–210). Round cuticular inflations present in area from 192–258 to 618–860 from anterior end. Stichosome with ca. 160 nuclei. Testis recurved posterior to esophago-intestinal junction, directed posteriorly forming convolutions, ending near anterior end of proximal cloacal tube (Fig. 1). Proximal cloacal tube stout, 0.66 (0.45–0.91) mm long, united laterally to distal cloacal tube of 0.53 (0.35–0.71) mm long (Fig. 1). Spicular pouch 0.18 (0.09–0.28) mm long (Fig. 1). Spicule slender, 1.11 (0.90–1.28) mm long, corresponding to 12.7 (10.0–14.1) % of body length, sharply pointed distally (Figs. 6-7). Distal portion of spicule with pit-like structures on one side (Fig. 7). Spicular sheath densely spinose (Figs. 6-7). Distal end of fully extended spicular sheath devoid of spines, with faint reticulate markings (Fig. 8). Cloaca subterminal with 1 pair of simple papillae (Fig. 6).

Female (10 specimens): Length 10.9 (9.3–12.4) mm. Anterior body 6.20 (5.55–7.22) mm long, corresponding to 57.0 (54.6–59.6) % of body length. Width at middle of anterior body 58 (51–67), esophago-intestinal junction 135 (121–158) and thick portion of posterior body 202 (184–218). Round cuticular inflations present in area from 162–300 to 684–1039 from anterior end. Stichosome with ca. 160–170 nuclei. Vulva 40 (10–71) posterior to esophago-intestinal junction, with anterior lip of varying degree of development and minute posterior lip (Figs. 9, 10). Vagina muscular, winding posteriorly, 0.67 (0.56–0.78) mm long (Figs. 11, 12). Ovary extending to preanal level (Figs. 9, 11). Anus subterminal (Fig. 11). Eggs lemon-shaped, thick shelled, brownish, with polar plugs, 73.6 ± SD 1.7 (71–77) by 33.0 ± SD 1.4 (30–35) (n=25), with exception of one egg sized 91 by 38 (Fig. 12).

Taxonomic summary

Type host: Echiothrix centrosa Miller & Hollister, 1921 (large-bodied shrew rat) (Rattini: Murinae: Muridae). Site in host: Cecum.

Type locality: Kuala Navusu (00˚58’S, 120˚27’E; 38–155 m elevation), Malakosa, Central Sulawesi, Indonesia.

Date of collection: October 1975.

Type specimens: USNM 1422103 (hototype male and allotype female), USNM 1422104 (5 male and 6 female paratypes), MZB Na 720 (3 male and 3 female paratypes).


Etymology. The species epithet is dedicated to Dr. G. G. Musser, an outstanding mammalogist, who has made invaluable contributions on the systematics of murid rodents of Indonesia.
FIGURES 1–8. Male of *Trichuris musseri* sp. nov. collected from *Echiothrix centrosa* in Sulawesi, Indonesia. 1. Holotype. 2. Anterior end of holotype. 3. Middle portion of stichosome showing bacillary band at left side. 4. Enlarged view at posterior one-third of stichosome, showing surface of bacillary band partially; two-way arrow indicating width of bacillary band at this level. 5. Esophago-intestinal junction. 6. Caudal end. 7. Distal end of spicule; arrows showing pit-like structures. 8. Distal end of spicular sheath fully extended.

Remarks. By having a stichosome, tubular intestine, vulva positioned near esophago-intestinal junction, monodelphic reproductive system and eggs with polar plugs but without membranous envelope or polar filaments, the present species belongs to the family Trichuridae in the superfamily Trichinelloidea (Chabaud, 1974; Anderson & Bain, 1982). By possessing much thicker posterior portion of body, a bacillary band at esophageal portion, stichocytes similar in form and arranged in a single row and unembryonated eggs in the uterus, it belongs to the subfamily Trichurinae which includes only the genus *Trichuris* Roederer, 1761 (Anderson & Bain, 1982). By having a small body, the present species is readily distinguished from all congeners known from murids because all have average body length over 10 mm in males and 15 mm in females (cf. Skrjabin *et al*., 1957; Quentin, 1966; Bernard, 1969; Tenora, 1969; Johnson, 1973; Sadighian *et al*., 1974; Feliu *et al*., 2000; Robles, 2011; Ribas *et al*., 2013; Smales, 2013; Robles *et al*., 2014). The present species is characteristic by having a gradually-tapered and sharply pointed distal end of the spicule, being readily distinguished from most congeners parasitic in murids, which have dull or round distal end or suddenly narrowed near distal end of the spicule (cf. Quentin, 1966; Robles, 2011; Robles *et al*., 2014; Smales, 2013; Feliu *et al*., 2000; Ribas *et al*., 2013). Among the congeners parasitic in
murids, only *T. petrowi* in *Arvicola terrestris* of Tatarstan, Russia, and *T. spalacis* in the mole rat, *Spalacis microphthalmus*, of Ukraine, have been known to have gradually tapered and pointed spicule (Petrov & Potekhina, 1953; Skrjabin *et al.*, 1957). However, the former species has a cephalic expansion in the male and much longer distance (>1 mm) between the anus and posterior end of body in female, and the latter species has a smaller ratio (<58%) of anterior body to worm length in males and smaller eggs (62–65 by 29 µm), differing from the present species (Petrov & Potekhina, 1953; Skrjabin *et al.*, 1957).


*Trichuris mallomyos* sp. nov.
(Figs. 13–22)

**General.** Usual size as a *Trichuris* in murids. Cuticle with fine transversal striation. Anterior part of body narrow, thread-like, tapering to cephalic end; posterior part of body stout (Figs. 13, 19). Minute cephalic stylet present. Stichosome with 1 row of stichocytes, and 1 pair of conspicuous cells at esophago-intestinal junction level (Figs. 13, 19). Nucleus of stichosome present per 4 to 5 subdivisions, in middle portion (Fig. 14). Bacillary band commencing just after cephalic end, well developed in middle portion of anterior body (Figs. 14, 15), becoming narrower and invisible anterior to esophago-intestinal junction. Round cuticular inflations of various sizes bordering bacillary band present in anterior portion of body.

**Male (10 specimens):** Length 28.8 (24.6–32.4) mm. Anterior portion of body 18.7 (15.3–21.0) mm long, corresponding to 65 (62–68) % of body length. Width at middle of anterior body 101 (95–111), esophago-intestinal junction 227 (202–259) and thick portion of posterior body 359 (331–397). Round cuticular inflations present in area from 258–370 to 1287–1990 from anterior end. Stichosome with ca. 180–200 nuclei. Testis recurved posterior to esophago-intestinal junction, directed posteriorly forming convolutions, ending near anterior end of proximal cloacal tube (Fig. 13). Proximal cloacal tube stout, 2.53 (2.25–2.77) mm long, united laterally to distal cloacal tube of 0.92 (0.75–1.37) mm long (Fig. 13). Spicular pouch 0.28 (0.09–0.73) mm long (Fig. 13). Spicule length 2.89 (2.75–3.13) mm long, corresponding to 10.1 (8.5–11.9) % of body length, sharply pointed distally (Figs. 16–18).
Distal portion of spicule with pit-like structures on one side (Fig. 17). Spicular sheath densely spinose; distal end of fully extended spicular sheath devoid of spines, with reticulate markings (Fig. 18). Cloaca subterminal with 1 pair of simple papillae (Fig. 16).

**Female (10 specimens):** Length 35.1 (28.1–41.4) mm. Anterior body 22.0 (17.2–27.1) mm long, corresponding to 62.6 (59.6–66.9) % of body length. Width at middle of anterior body 100 (86–109), esophago-intestinal junction 243 (202–274) and thick portion of posterior body 420 (372–486). Round cuticular inflations present in area from 198–455 to 1505–2109 from anterior end. Stichosome with ca. 190–210 nuclei. Vulva slightly elevated, 81 (0–137) posterior to esophago-intestinal junction (Fig. 20). Vagina muscular, winding posteriorly, 1.37 (1.01–1.65) mm long (Fig. 21). Ovary extending to preanal level (Figs. 19, 21). Anus subterminal (Fig. 20). Eggs lemon-shaped, thick shelled, brownish, with polar plugs, 90.1 ± SD 2.9 (83–95) by 39.2 ± SD 1.2 (36–41) (n=50) (Fig. 22).

**FIGURES 13–18.** Male of *Trichuris mallomyos* sp. nov. collected from *Mallomys rothschildi* in Papua, Indonesia. 13. Holotype. 14. Middle portion of stichosome showing bacillary band at left side. 15. Enlarged view at middle showing surface of bacillary band partially; two-way arrow indicating width of bacillary band at this level. 16. Caudal end. 17. Distal end of spicule; arrows showing pit-like structures. 18. Distal end of spicular sheath fully extended.

**Taxonomic summary.**
Type host: *Mallomys rothschildi* Thomas, 1898 (Rothschild’s woolly rat) (Hydromini: Murinae: Muridae).
Site in host: Cecum.
Type locality: Highland forest near Wamena (4˚11’S, 138˚58’E; 1500 m elevation), Papua Indonesia, Indonesia.
Date of collection: 4 August 1993.
Type specimens: USNM 1422105 (hototype male and allotype female), USNM 1422106 (6 male and 6 female paratypes), MZB Na 721 (3 male and 3 female paratypes).
Symbiotypes: AMNH M-267742, M-267743.


**Etymology.** Species epithet is derived from the generic name of the type host.

**Remarks.** This species is also assigned to *Trichuris* for the same reasons as for the preceding species. By having a gradually tapered and sharply pointed spicule, it resembles *T. musseri*, *T. petrowi* and *T. spalacis* among those parasitic in murids (Petrov & Potekhina, 1953; Skrjabin et al., 1957). It is easily distinguished from *T. musseri* in that the body is much larger and the number of nuclei per divisions of stichosome is fewer. It also differs from the latter two species by the following features: *T. petrowi* has a cephalic expansion and a larger ratio of anterior body to worm length (>70 %) in male, and much longer distance (>1 mm) between the anus and posterior end of body in female; *T. spalacis* has a smaller ratio of anterior body to worm length (<60 %) in both sexes and much smaller eggs (62–65 by 29 µm) (Petrov & Potekhina, 1953; Skrjabin et al., 1957). Morphology of the distal end of the spicule has not been described or figured in some *Trichuris* species in murids, namely, *T. neotomae* Chandler, 1945 and *T. peromysci* Chandler, 1946. Although their males have body length comparable to the present males (22–23 mm in *T. neotomae* and 14.7–31.8 mm in *T. peromysci*), they possess a much shorter spicule (1.15–1.23 mm and 0.86–1.4 mm long, respectively), being readily distinguished from *T. mallomyos* sp. nov. (Chandler, 1945, 1946). *Trichuris germani*, an endemic congener parasitic in the endemic murids of Papua New Guinea, differs from the present species by having smaller eggs (42.5–63 by 25.5–27.7 µm) in addition to the rounded distal end of the spicule (Smales, 2013).
Discussion

Trichuris spp. are widely distributed among mammals regardless of their dietary habits and habitat preference. Echinothrix centrosa is a ground dweller, feeding mainly on earthworms (Musser & Durden, 2014) while M. rothschildi is of arboreal nature principally, consuming a mainly abrasive vegetable diet (Flannery et al., 1989). Trichuris eggs are unembryonated when passed in the host feces and take several weeks to one month in a humid environment to become infective, but require no intermediate host (Anderson, 2000). The hosts acquire the infection by eating diets or drinking water contaminated with embryonated eggs. This life history pattern may facilitate host-shifts. Actually, occurrence of such host-shift events in Trichuris evolution has been suggested by molecular phylogenetic studies (cf. Callejón et al., 2015; Doležalová et al., 2015).

The present two new species resemble each other by having a sharply pointed spicule with lined pit-like structures on one side near distal end. The pit-like structures are surmised to be of sensory nature as are those observed at the dull distal end of the spicule of T. muris (see Wright, 1978). The morphological resemblance in the spicule morphology between the present two new species suggests their close phylogenetic relationship. Although Echinothrix and Mallomys are old-endemic murine genera of the area, they belong to different tribes, Rattini and Hydromini, and were considered to have diverged in the Tortonian era of the Miocene about 12.5 million years ago (Fabre et al., 2013; Rowe et al., 2016). It remains to be elucidated whether the morphological resemblance between the two new species of Trichuris described herein is only homoplasy or actually reflects a close phylogenetic relationship of the parasites.

Identification of Trichuris species is generally difficult because their cephalic end is very minute for observation by light microscopy and the caudal region of the male lacks multiple caudal papillae or bursal rays that provide distinguishing characteristics in other groups of parasitic nematodes. Presence or absence of a cephalic stylet and protuberance at the vulval portion are often used as key characters. The taxonomic significance of these features remains unsure, however, because the former was often difficult to be observed and the latter may vary depending on the maturation and physiological condition of worms (cf. Skrjabin et al., 1957). The number of stichocytes has been also regarded as a key feature. However, the number of stichocytes is difficult to count because a stichocyte is usually subdivided, these subdivisions often being indistinguishable from the actual divisions between stichocytes. Instead, the number of nuclei of stichosomes is rather easy to be counted. The number of nuclei does not coincide with the number of stichocytes because plural nuclei are often present in a stichocyte. The number of subdivisions per nucleus differed between the present two species. Taxonomical significance of the nuclei number should be further investigated in other congeners.

Published records of Trichuris in murids of Indonesia are very limited even in commensal rats. Wiroreno (1978) examined many rats including 66 Rattus tanezumi (recorded as Rattus rattus) and 10 Rattus exulans, but did not find species of Trichuris. Hasegawa & Syafruddin (1995) examined 29 R. tanezumi (recorded as R. rattus) and 20 R. exulans on Halmahera Island, but could not collect species of Trichuris. In the checklist of murine helminths of Indonesia by Dewi & Purwaningsih (2013), only one record of T. muris from Bumonis chrysoconus, an endemic rat in Sulawesi, was cited. Meanwhile, T. muris has been recorded from commensal murines, i.e., Rattus norvegicus, R. rattus and Mus musculus in Australia (cf. Mackerras, 1958), as well as old endemic rats, Hydromys chrysogaster, Melomys cervinipes and Uromys caudimaculatus (Smales & Cribb, 1997; Smales, 2005; Smales & Spratt, 2008) in Australia and a new endemic rat, Rattus leucopus, of New Guinea (Smales, 1997; Smales & Cribb, 1997; Smales & Spratt, 2004). The presence of T. muris in the Australian endemic rats has been surmised to be a secondary adaptation which occurred after introduction by commensal murines (Smales, 2012). However, it is also possible that so-called T. muris in these endemic rats represent cryptic species. DNA sequence analysis of Trichuris species parasitic in murids in the Indonesia-Australian region is essential to understand their phylogeny and biogeographical relationship with their hosts.

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