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First report of *Potorolepis* Spassky, 1994 (Eucestoda: Hymenolepididae) from China, with description of a new species in bats (Chiroptera: Rhinolophidae)

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Abstract: *Potorolepis gulyaevi* sp. n. (Cestoda: Hymenolepididae) is described from the Chinese horseshoe bat, *Rhinolophus sinicus* Andersen (Chiroptera: Rhinolophidae), from southern China. The new species differs from known species of the genus by the shape, number and size of rostellar hooks, the relative position and length of the cirrus-sac and the morphology of gravid uterus. This is the first report of a member of the genus from non-marsupial mammals and the first record of a *Potorolepis* Spassky, 1994 from eastern Asia. The generic diagnosis of *Potorolepis* is amended.

Keywords: Cestoda, Hymenolepididae, *Potorolepis gulyaevi*, new species, morphology, bats, *Rhinolophus*, China

Currently, majority of the hymenolepidid cestodes with armed rostellum parasitic in bats are included in the genus *Vampirolepis* Spassky, 1954 (Vaucher 1992, Sawada 1997). In addition to *Vampirolepis*, representatives of two other genera of hymenolepidids with an armed rostellum have been reported from bats, namely *Staphylocystis* Villot, 1877 and *Rodentolepis* Spassky, 1954 (Sawada 1997).

During a survey of the helminth fauna of Chiroptera in southern China (Yunnan Province), we found a previously unrecognized hymenolepidid species with an armed scolex in Chinese horseshoe bat, *Rhinolophus sinicus* Andersen. Based on morphological characteristics, this cestode differed from *Vampirolepis*, *Staphylocystis* and *Rodentolepis*. We attribute the specimen to the genus *Potorolepis* Spassky, 1994 (see Spassky 1994, Vaucher and Beveridge 1997) based on the characteristic hook shape, testes arranged in triangle, a fan-shaped ovary and a bilobed developing uterus.

At present, *Potorolepis* includes ten species which are regarded as specific parasites of dasyurid, peramelid and potoroid marsupials. Six species were described from Australia and one from Tasmania, and all had been originally placed in the genus *Hymenolepis* Weinland, 1858 (see Nybelin 1917, Beveridge and Barker 1975, Vaucher et al. 1984) and subsequently transferred to *Potorolepis* by Spassky (1994). Later, Vaucher and Beveridge (1997) described two additional species in marsupials from Aru Islands and New Guinea, including *P. aruensis* Vaucher et Beveridge, 1997 and *P. woolleyae* Vaucher et Beveridge, 1997; they also transferred *P. peroryctis* (Jones et Anderson, 1990) (syn.: *Vampirolepis peroryctis* Jones et Anderson, 1990) from the same locality to this genus. Additionally, Vaucher and Beveridge (1997) modified the generic diagnosis of *Potorolepis* with regard to the arrangement of the testes (either linear or triangular) and cirrus armature (armed or unarmed).

The new species discovered in Chinese bats is largely consistent with *Potorolepis*, but differs substantially from congeners in the shape, number and size of rostellar hooks, the relative position and length of the cirrus-sac, and morphology of gravid uterus. Comparative studies of *Potorolepis*, including this newly recognised species, revealed new details of morphology, which could be used as additional differential characters for the genus. Herein, we provide the description of a new species of *Potorolepis* and amend the generic diagnosis of the genus.

MATERIALS AND METHODS

A single cestode specimen was obtained from the small intestine of one of 18 Chinese horseshoe bats, *Rhinolophus sinicus*, collected in China (Yunnan Province). During June and July 2006 we examined 141 specimens of bats belonging to the genera *Tadarida* Rafinesque (1 specimen), *Rhinolophus* Lacépède (71), *Miniopterus* Bonaparte (6), *Aselliscus* Tate (3) and *Myotis* Kaup (52).

Bats were dissected within one hour after their death. The cestode specimen was isolated, washed and relaxed in water, and then fixed in 70% ethanol. It was stained in Ehrlich's haematoxylin, differentiated in a 3% aqueous solution of ferric-ammonium sulphate 12-hydrate, dehydrated in an ethanol series, cleared in clove oil and mounted in Canada balsam. The scolex

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and terminal proglottides were mounted separately in Berlese's medium to facilitate the examination of the rostellar hooks and eggs. The holotype was deposited in the helminthological collection of the Natural History Museum, Geneva, Switzerland (MHNG-PLAT). Measurements are given in micrometres unless otherwise stated, with mean and number of measurements in parentheses.

The following type materials and vouchers of previously described species were studied (all from MHNG-PLAT): type series of *Potorolepis potoroi* (Vaucher, Beveridge et Spratt, 1984) (holotype 14669, paratype 14670), *P. bettongiae* (Vaucher, Beveridge et Spratt, 1984) (holotype 14722, paratype 14723, vouchers 14724 (four slides)), *Rodentolepis cercarteti* (Vaucher, Beveridge et Spratt, 1984) (holotype 14401, paratypes 14402), *P. peramelidarum* (Nybelin, 1917) (vouchers 13870, 13871, 13478, 13479), *P. antechini* (Vaucher, Beveridge et Spratt, 1984) (holotype 13480), *P. aklei* (Beveridge et Barker, 1975) (vouchers 13873, 13471), *P. bradleyi* (vouchers 13483, 13874, 14672), *P. isoodontis* (Vaucher, Beveridge et Spratt, 1984 (paratype 14725).

RESULTS

Potorolepis gulyaevi sp. n. Figs. 1, 2

Description (based on one specimen). Strobila of fully-developed gravid individual 112 mm long, maximum width 2.3 mm in posterior part of strobila. Strobila consisting of about 1000 craspedote proglottides. Segments wider than long. Strobilation gradual, with internal segmentation appearing prior to external segmentation.

Scolex rounded, 520 in diameter, weakly differentiated from neck (Fig. 1A). Suckers unarmed, cup-shaped, 170–180 × 160–170 (175 × 165, n = 4). Rhynchus short, armed with 15 rostellar hooks arranged in single row. Rostellar hooks (Fig. 1B) of diorchoid type, relatively large, 47–50 (48.3, n = 11) long, with curved blade, 16–18 (16.8, n = 11) long, longer than guard, 8.5–9.5 (9.1, n = 11) long; guard situated almost perpendicular to blade; handle relatively long, 19–21 (19.8, n = 11). Rostellum sac-like, 140 × 170, situated in rostellar pouch, 210 × 200, slightly reaching beyond level of posterior margins of suckers. Neck 450 wide.

Two pairs of osmoregulatory canals, without transverse anastomoses. Dorsal osmoregulatory canals narrow, 4–5 (4.6, n = 5) wide, situated predominantly in same sagittal plane as ventral canals. Ventral osmoregulatory canals 24–30 (27, n = 5) wide. Position of dorsal osmoregulatory canals not always constant; their loops may be situated lateral to ventral canals. Genital pores unilateral, dextral. Genital ducts pass dorsal to osmoregulatory canals (Fig. 1C, D). Development of proglottides gradual, protandrous. Strobilar part containing juvenile proglottides lacks external segmentation; proglottides become externally distinct at level of premature proglottides.

Male mature proglottides 110–130 × 800–830, with well-developed velum (Fig. 1C). Length/width ratio 1 : 7. Testes relatively large, usually three in number, almost equal in size, 117–160 × 74–101 (136 × 85, n = 18), oval or pear-shaped, situated in triangle or, rarely, in row; poral testis separated from two antiporal testes by female gonads.

Hermaphroditic mature proglottides 102–130 × 1 018–1 100 (116 × 1 054, n = 10), with well-developed velum (Fig. 1D). Testes 119–160 × 74–101 (135 × 85, n = 18), commonly situated in triangle or, rarely, in row. Cirrus-sac relatively short, 168–217 × 31–38 (189 × 34, n = 10), antiporal part of cirrus-sac reaching ventral osmoregulatory canal, rarely overlapping or slightly crossing it (Fig. 1D, E). Genital atrium simple, 8–17 × 6–9, infundibular, deep, opens laterally about middle of lateral proglottis margin. No evaginated cirrus present in material studied. Invaginated cirrus 25–27 × 6–7 (26, n = 5), armed with small (less than 1 long) spines (Fig. 2A). Internal seminal vesicle ovoid, 90–134 × 25–31 (109, n = 8), more than half of cirrus-sac length (Fig. 1E). External seminal vesicle transversely elongate, 74–125 × 34–44 (95 × 43, n = 8), clearly outlined from vas deferens, distinctly smaller than seminal receptacle.

Ovary 437–503 (457, n = 15) wide, median, fan-shaped, irregularly lobed, ventral to male genital organs, occupying substantial part of median field, overlapping testes (Fig. 1D). Vitellarium 137–153 × 47–76 (142 × 63, n = 15), postovarian, median, scarcely lobed. Vagina tubular, clearly distinct from seminal receptacle, ventral to cirrus-sac. Copulatory part of vagina 24–25 × 4–6 (24.4 × 4.8, n = 5), tubular, clearly distinct from seminal receptacle; ventral to cirrus-sac (Figs. 1E, 2A). Seminal receptacle transversely elongate, 416–489 × 72–110 (452 × 84, n = 13), reaching median line of proglottis.

Uterus first appears as perforated two-lobed sac, positioned dorsal to other organs and does not extend beyond osmoregulatory canals (Fig. 2B). With development of proglottides, uterus forms numerous diverticula on dorsal and ventral side (Fig. 2C, D). Testes remain in postmature proglottides; cirrus-sac and vagina persist in gravid proglottides. Gravid proglottides transversely elongate, 297–356 × 1 900–2 100 (334 × 1 971, n = 10). Fully developed uterus occupies entire median field, does not extend beyond osmoregulatory canals, saccate, with one row or rarely two rows of ventral and dorsal diverticula; gravid uterus not perforated (Fig. 2D). Uterus contains numerous (up to 500) small oligolecithal eggs. Eggs 55–63 × 55–61 (59 × 58, n = 17), subspherical (Fig. 2E), with relatively thick outer coat (up to 4 in thickness); egg surface smooth; oncosphere 37–41 × 27–38 (34 × 31, n = 14). Embryophore subspherical, thin. Embryonic hooks 13–15 (14, n = 14) long (Fig. 2F).

Type host: Chinese horseshoe bat (*Rhinolophus sinicus* Andersen) (Chiroptera, Rhinolophidae).

Type locality: Longxu Cave, near Fagudian village, Kunyang Town, Yunnan Province, China; *ca.* 24°30'N; 102°20'E.

Type material: Holotype: MHNG-PLAT 82283, collected on 17 June 2006.

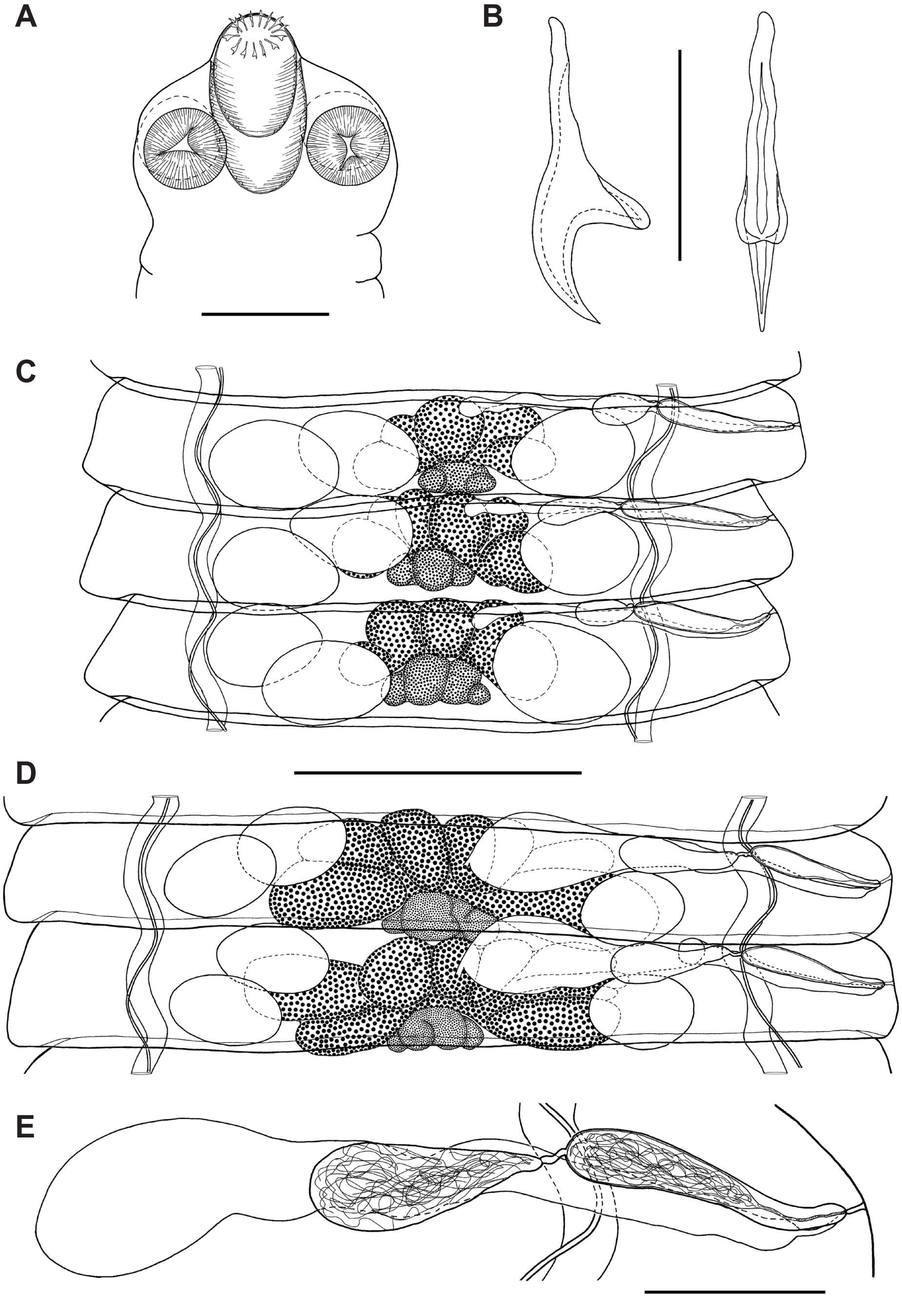

Fig. 1. *Potorolepis gulyaevi* sp. n. from *Rhinolophus sinicus*, China; holotype (MHNG-PLAT 82283). **A** – dorsoventral view of scolex; **B** – rostellar hooks in profile and view from posterior surface showing enlarged hook guard; **C** – male mature proglottides, from dorsal side; **D** – hermaphroditic mature proglottides, from dorsal side; **E** – genital ducts, from dorsal side. Scale bars: A = 250 µm; B = 30 µm; C, D = 300 µm; E = 100 µm.

Etymology: This species is named in honour of late Dr. Vladimir Gulyaev in recognition of his important contributions to studies on hymenolepidid cestodes.

Remarks. The specimen of *Potorolepis gulyaevi* sp. n. has the primary characters of *Potorolepis*, i.e. large diorchoid hooks, testes situated in triangle, fan-shaped ovary and bilobed developing uterus (see Spassky 1994, Vaucher and Beveridge 1997). Among ten recognized species of *Potorolepis*, only *P. aklei* and *P. aruensis* have rostellar hooks similar in shape and number to those seen of *P. gulyaevi* (see Table 1). Rostellar hooks in the new species, however, are substantially smaller (2×) than those of *P. aklei* and *P. aruensis*. Additionally, the ovary is wider and embryonic hooks in the oncosphere are significantly

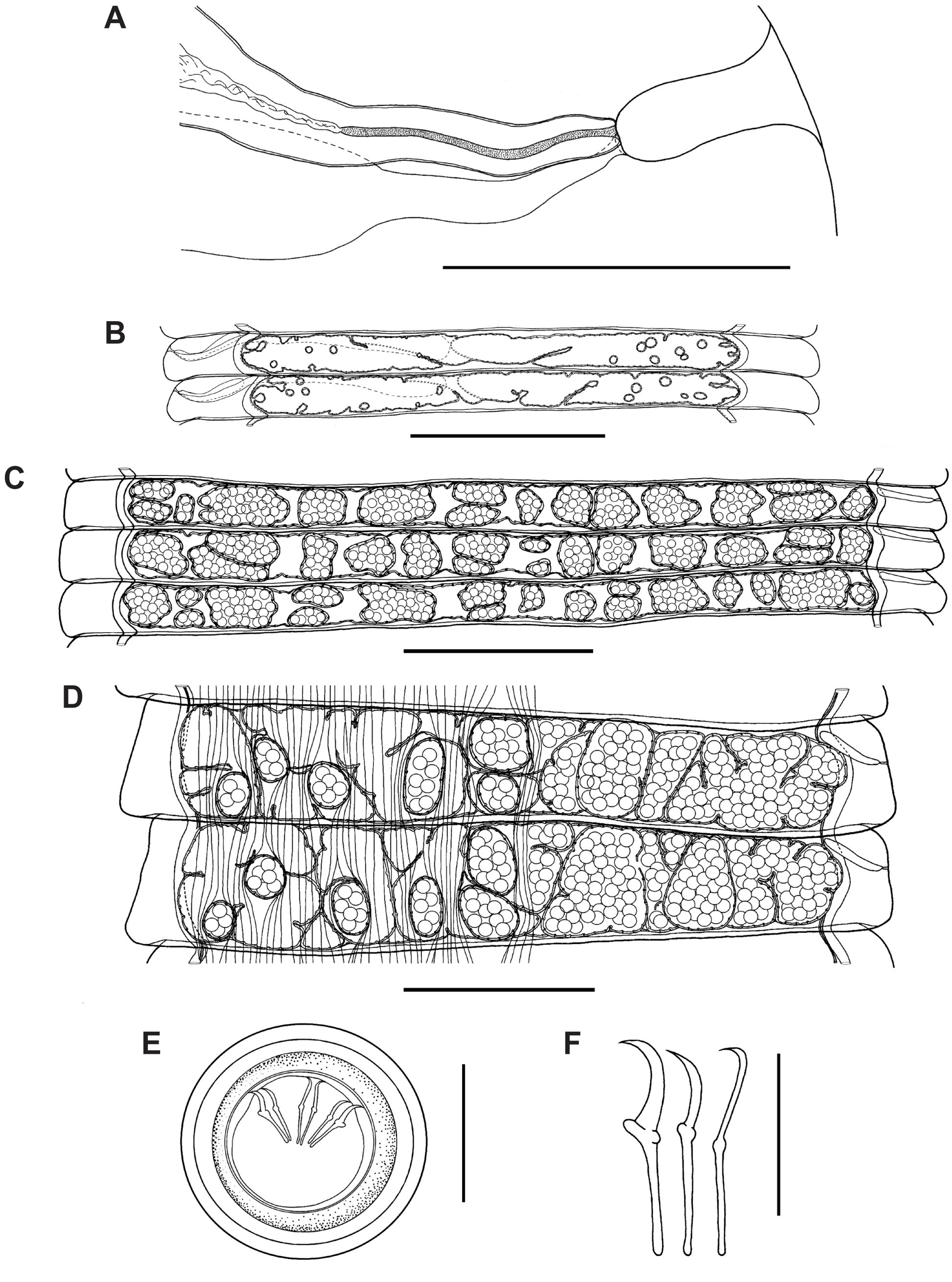

Fig. 2. *Potorolepis gulyaevi* sp. n. from *Rhinolophus sinicus*, China; holotype (MHNG-PLAT 82283). **A** – cirrus and vagina; **B** – postmature proglottides from dorsal side, showing uterus development; **C** – pregravid proglottides from dorsal side, showing appearance of uterine diverticula; **D** – gravid proglottides from dorsal side, showing saccate uterus with dorsal uterine diverticula (muscle fibres of the inner longitudinal musculature are illustrated only on the left side of the proglottis); **E** – egg; **F** – embryonic hooks. Scale bars: A = 30 µm; B–D = 500 µm; E = 30 µm; F = 10 µm.

smaller in *P. gulyaevi* compared to those of *P. aklei*. The cirrus-sac of *P. gulyaevi* is shorter than that of *P. aruensis*. *Potorolepis gulyaevi* is also distinguished from *P. aruensis* by its testes being arranged in a triangle; in the latter species the testes are situated in one row.

Currently, the genus *Vampirolepis* includes a number of poorly described species mainly from bats from south-eastern Asia (see Sawada 1997). We assume that *Vampirolepis* represents an artificial generic complex since some species placed in this genus do not correspond to its diagnosis (Spassky 1954, Vaucher 1992, Czaplinski and Vaucher 1994). To specify generic allocation of such species, it is necessary to re-examine their type materials because most of the original descriptions are inadequate.

Table 1. Morphometric data distinguishing species of *Potorolepis* (measurements in micrometres unless otherwise stated).

Characters	*P. peramelidarum*	*P. aklei*	*P. bradleyi*	*P. potoroi*	*P. antechini*	*P. bettongiae*	*P. isoodontis*	*P. peroryctis*	*P. aruensis*	*P. woolleyae*	***P. gulyaevi* sp. n.**
Strobila: length (mm)	60–130	80–122	80–135	30	87–100	160	60	12–15	60	35	**112**
Strobila: width (mm)	1.5–2	1.1–1.6	0.9–1.7	1.4	0.9–1.2	1.2	0.8	2.5	0.93–1.63	0.76–0.9	**2.3**
Scolex: width	700–840	370–510	390–680	409–665	–	483–851	590–900	486–761	520—650	440–550	**520**
Rostellum: size	–	130–200 × 100–170	150–220 × 90–170	180–206 × 117–188	–	156–213 × 172–261	166–245 × 130–278	240 × 360	180–260 × 170–230	130–200 × 190–280	**140 × 170**
Rostellar hooks: no.	44–46	11–17	10–15	29–33	22–23	24–27	33–39	40	16–18	15–19	**15**
Rostellar hook: length	100	83–100	103–128	98–103	56–59	79–91	71–82	124–192	128–147	163–182	**47–50**
Sucker: size	–	110–180	170–210	237–360 × 123–327	–	212–466 × 183–376	212–368 × 186–270	212–466 × 183–376	130–160 × 150–180	130–210 × 100–190	**170–180 × 160–170**
Cirrus-sac: size	140	130–210 × 20–40	140–250 × 15–30	228–293 × 55–80	115 × 12	150–277 × 36–78	81–168 × 29–71	264–308 × 44–88	270–420 × 40–50	140–170 × 36–42	**168–217 × 31–38**
Testes: size	130–180	100–180	80–110	–	–	–	–	80–200 × 172–280	110–170 × 80–110	80–130 × 40–50	**119–160 × 74–101**
Ovary: width	200–240	70–140	90–170	–	–	–	–	200–388	110–190	140	**437–503**
Uterus: shape	horseshoe-shape	saccate	bilobed sac	saccate, with diverticula*	bilobed sac	bilobed sac	–	bilobed sac	saccate, with diverticula	saccate, with diverticula	**saccate, with diverticula**
Egg: size	78 × 81	40–70	50–90	–	–	–	–	67–72	–	32–45	**55–61 × 56–63**
Egg: shape	spherical	spherical	spherical	–	–	spherical*	–	–	–	spherical	**spherical**
Oncosphere: size	30 × 26	–	–	–	–	–	–	34–38	–	–	**27–38 × 37–41**
Embryonic hook: size	14.8	25–35	–	–	–	–	–	–	–	–	**13–15**
References	Nybelin (1917)	Beveridge and Barker (1975)	Beveridge and Barker (1975)	Vaucher et al. (1984)	Vaucher et al. (1984)	Vaucher et al. (1984)	Vaucher et al. (1984)	Jones and Anderson (1990)	Vaucher and Beveridge (1997)	Vaucher and Beveridge (1997)	**present study**

* Characters observed in the present study.

However, revision of *Vampirolepis* is beyond the scope of the present study. Herein, we differentiate *P. gulyaevi* from some *Vampirolepis* (*sensu lato*) species because we believe that the latter genus may include cestodes that should belong to *Potorolepis*.

There are several species of *Vampirolepis* from bats, which may be similar to *P. gulyaevi* based on the armature of rhynchus. These are *V. guarany* (Rego, 1961) in *Molossus molossus* (Pallas) (syn. *Molossus crassicaudatus* Geoffroy) and *Molossus* sp. from South America; *V. isensis* Sawada, 1966 in *Rhinolophus cornutus* Temminck, *R. ferrumequinum* (Schreber) and *R. monoceros* Andersen from Taiwan and Japan; *V. kobayashii* Sawada, Harada et Kobayashi, 1984 in *Rhinolophus creaghi* Thomas from Malaysia; and *V. acollaris* Sawada et Harada, 1985 in *Rhinolophus coelophyllus* Peters from Thailand. The size of rostellar hooks of *P. gulyaevi* is close to those in *V. guarany* (50 µm according to Rego 1962 and 46–52 µm according to Vaucher 1986). However, *V. guarany* should be excluded from potential congeners of the new species since it has characters of *Vampirolepis* (fraternoid rostellar hooks and testes situated in one row and not separated by female gonads).

Vampirolepis isensis, *V. acollaris* and *V. kobayashii* are similar to *P. gulyaevi* based on the shape of the rostellar hooks. *Potorolepis gulyaevi* is distinguished from these three species by a smaller number of rostellar hooks (22–25 in *V. isensis*, 25 in *V. acollaris* and 20 in *V. kobayashii*) and greater hook length (32 in *V. isensis*, 28–32 in *V. acollaris* and 38 in *V. kobayashii*). *Potorolepis gulyaevi* can be distinguished from *V. isensis* and *V. kobayashii* by the antiporal end of the cirrus-sac that reaches the ventral osmoregulatory canal but rarely overlaps it; in the two latter species the cirrus-sac overlaps the osmoregulatory canal. Furthermore, the testes of *P. gulyaevi* are arranged in a triangle in contrast to those in *V. isensis*, which are situated in a row.

Our comparative studies of the type species, *P. potoroi* and other congeners, and inclusion of *P. gulyaevi* have required amendation of the original generic diagnosis of *Potorolepis* proposed by Spassky (1994) and then modified by Vaucher and Beveridge (1997). Previously unrecognized but distinctive generic-level characters for *Potorolepis* now include attributes of the gravid uterus and structure of the eggs. For example, the gravid uterus may have diverticula in *P. potoroi* (our observations), *P. peramelidarum* (our observations), *P. aruensis*, *P. woolleyae* and *P. gulyaevi*. The eggs are spherical and with a thick outer coat in *P. peramelidarum*, *P. bradleyi*, *P. woolleyae*, *P. bettongiae* (our observations) and *P. gulyaevi*.

Amended diagnosis *Potorolepis* Spassky, 1994

Diagnosis (after Spassky 1994, with modifications): Hymenolepididae of medium size. Development of proglottides gradual. Proglottides numerous, transversely elongate, craspedote. Rhynchus armed with one row of large diorchoid hooks. Suckers unarmed, muscular, commonly not prominent. Dorsal and ventral osmoregulatory canals located on same sagittal plane. Ventral canals without transverse anastomoses. Genital pores unilateral, dextral, genital ducts pass dorsal to osmoregulatory canals. Three testes situated in triangle or in one row; poral testis separated from two antiporal testes by female gonads. Cirrus-sac does not reach median line of proglottis. Cirrus armed or rarely smooth. External and internal seminal vesicles present. Ovary median, usually fan-shaped, lobed, sometimes slightly lobed. Vitellarium postovarian, median, slightly lobed. Uterus initially bilobed sac, fully developed uterus usually with lobes and diverticula not extending beyond osmoregulatory canals. Eggs numerous, spherical or subspherical, with thick outer coat. Embryophore subspherical. Parasites of marsupials and bats in Australasia and Indo-Malaya. Type species: *Potorolepis potoroi* (Vaucher, Beveridge et Spratt, 1984).

DISCUSSION

Potorolepis gulyaevi sp. n. represents the first record of this genus in chiropterans and considerably extends distribution area of the genus outside the Australasian region. Previously known geographic range of *Potorolepis* generally corresponded to the distributions of their definitive hosts in Australia, Tasmania, Aru Islands and New Guinea (Beveridge and Barker 1975, Vaucher et al. 1984, Jones and Anderson 1990, Vaucher and Beveridge 1997). Phylogenetic relationships of these tapeworms with other hymenolepidids are not yet established, and thus currently cannot provide further insights into the evolutionary history of this cestode group. The demonstrated host and geographic range of *Potorolepis* suggests an association with Marsupialia that may pre-date the breakup of Gondwanaland; such a distribution for the Hymenolepididae was proposed by Beveridge and Jones (2002).

There are no described species of *Potorolepis* species in marsupials from South America (Schmidt 1986, Gardner and Campbell 1992, Gardner et al. 2003). However, incomplete host sampling and confused taxonomy of these cestodes may obscure the larger biogeographic history for *Potorolepis*. In this regard, Beveridge and Spratt (2003) made the assumption that some specimens in South American marsupials of the genus *Dromisiops* Thomas were apparently attributable to *Potorolepis*. This is of interest given the Trans-Antarctic distributions for species of the anoplocephalid genus *Linstowia* Zschokke, 1899, which demonstrates ecological continuity and a link between the South American and Australian faunas in the late Cretaceous (Beveridge 1983, Gardner and Campbell 1992).

Potorolepis gulyaevi represents the first member of the genus occurring in non-marsupial hosts and outside the Australasian ecozone. We assume that the occurrence of *Potorolepis* in bats (*Rhinolophus* Lacépède) represents an event of host colonization and is an example of the "hostal radiation" of parasites (Hoberg and Brooks 2008). Alternatively, the apparently rare occurrence of this new hymenolepidid in chiropterans may suggest that it has not yet been found in its typical definitive host. Further survey and inventory of cestodes in chiropterans and other small insectivorous mammals is required to completely resolve the relationships of *P. gulyaevi*. Ecological similarity and geographic sympatry for some chiropterans, marsupials and other small mammals suggest the possibility of mutual exchanges of helminths, which may influence the structure of faunas over the evolutionary time.

For example, some hymenolepidids of Australian marsupials are morphologically similar to those of the genus *Vampirolepis* in bats (see Sawada 1997) as exemplified by *Rodentolepis cercarteti* (Vaucher, Beveridge et Spratt, 1984). The occurrence of species attributed to *Hymenolepis*, *Rodentolepis* and *Staphylocystis* in various Chiroptera supports the assumption of the important role of hostal radiation in the evolution of hymenolepidids (see Sawada 1997, Makarikova et al. 2010).

A strong volant capacity and dispersal potential of bats allow them overcome significant geographical barriers (including aquatic) that are almost impenetrable for other terrestrial mammals (Breed et al. 2010). In turn, wide distribution of chiropteran hosts could become an important determinant of geographic distributions of their parasitic worms including cestodes. Rhinolophids inhabit temperate and tropical regions from Europe and Africa, to southeast Asia and Japan, to the Philippines, New Guinea and Australia. Phylogenetic analyses suggest that the horseshoe bats arose in Asia or in southeastern Asia and subsequently expanded into Europe and Africa (Maree and Grant 1997, Stoffberga et al. 2010). The oldest fossils of *Rhinolophus*, however, were recorded from late Oligocene and early Miocene in South Australia (Hall 1989, Sazali et al. 2011). In any case, these data demonstrate that events of *Rhinolophus* dispersal between Australia and Asia have occurred historically, which provided a basis for the concurrent expansion of their cestode fauna.

In summary, results of the present study do not support the transfer of *V. isensis*, *V. kobayashii* and *V. acollaris* into *Potorolepis*. Although these three species are similar to *P. gulyaevi* in having diorchoid hooks and also occur in bats of the genus *Rhinolophus* from East and Southeast Asia, their generic allocation requires additional study.

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