Running head: Syphacia (Syphacia) semiadii n.sp from Halmahera Island, Indonesia

SYPHACIA (SYPHACIA) SEMIADII N.SP (NEMATODA: OXYURIDAE) FROM HALMAHERAMYS BOKIMEKOT FABRE ET AL., 2013 (RODENTIA: MURIDAE) ON HALMAHERA ISLAND, INDONESIA AND A KEY TO THE SPECIES PRESENT IN SULAWESI AND THE AUSTRALIAN BIOREGION.

KARTIKA DEWI^{1,3)}, MITSUHIKO ASAKAWA²⁾ AND YULI. S. FITRIANA¹⁾

¹⁾ Zoology Division (Museum Zoologicum Bogoriense), RC Biology-LIPI, Jl. Raya Jakarta-Bogor, Km. 46. Cibinong, West Java, 16911.
 ²⁾ Department of Pathobiology (Wild Animal Medical Centre), School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, 069-8501, Japan
 ³⁾Corresponding author. E-mail: kartika_mzb@yahoo.co.id

Correspondence to : Kartika Dewi, Zoology Division (Museum Zoologicum Bogoriense), RC Biology-LIPI, Jl. Raya Jakarta-Bogor, Km. 46. Cibinong, West Java, 16911. Phone: +62-21-8765056; Fax: +62-21-8765068; E-mail: kartika_mzb@yahoo.co.id

Abstract

Syphacia (Syphacia) semiadii n.sp. is described from Halmaheramys bokimekot, an endemic murine of Halmahera Island, Maluku, Indonesia. Among the New Guinea/Australian congeners, S. (S.) semiadii n. sp. most closely resembles S. (S.) mamelonitenuis and S. (S.) longaecauda by having a circular cephalic plate without dorsoventral constriction laterally and by lacking lateral alae in both sexes. However, this species differs from S.(S.) mamelonitenuis, which has a shorter tail in both sexes and larger eggs, and from S. (S.) longaecauda, which has a longer tail in both sexes. To aid identification, we also provide a dichotomous key to the species of Syphacia in Sulawesi and the Australian bioregion.

Introduction

Halmahera is the largest island of the Maluku Islands (also known as the Moluccas or

Moluccan Islands). Tectonically, they are located on the Halmahera Plate within the Molucca

Sea Collision Zone. Geographically, they are located east of Sulawesi (Celebes), west of New

Guinea, and north of Timor (Anon., 2010). Biogeographically, Halmahera is a unique transition

area between Sulawesi and New Guinea, and some endemic species of *Rattus* and *Melomys* occur there.

A new species of *Nippostrongylus* and several species of *Odilia*, which showed affinity to New-Guinea/Australian representatives, have been recorded from the endemic rat, *Rattus* cf. *morotaiensis*, of Halmahera (Hasegawa & Syafruddin, 1995a,b; Hasegawa, 1996). In the biodiversity project in Weda Bay, Halmahera, carried out in 2010, a nematode species of the genus *Syphacia* was obtained from the caecum of *Halmaheramys bokimekot* Fabre *et al.*, 2013.

To date, *Halmaheramys* is the only known endemic genus of Halmahera with *H. bokimekot* Fabre *et al.*, 2013 as the described species. Because oxyurids often have coevolutionary relationships with their hosts (Hugot, 1988), this parasite may have importance in understanding the biogeography of the Moluccas. We describe and discuss the morphology of this nematode from a coevolutionary and biogeographical perspective.

Material and Methods

The rats were captured using small cage traps, 28 x 12 x 12 cm and then killed using ether alcohol. The viscera were removed in the field, fixed in 4% formalin and then examined for helminths in the laboratory. Nematodes recovered were stored in70% ethanol. Later, the worms were examined under a compound Olympus BH-2 series microscope with a drawing tube and a JEOL JSM5310LV scanning electron microscope (SEM) at an accelerating voltage of 20 kV. For light microscopy, the specimens were cleared in glycerine. Measurements were made with an ocular micrometer. For SEM studies, the specimens were post-fixed in glutaraldehyde, dehydrated through an ethanol series and freeze dried. The dried specimens were then coated with gold at 5–8mA for 5 min. Measurements (range, followed by mean in parentheses) are given in micrometres unless otherwise stated. The type specimens and voucher host specimens are deposited in Museum Zoologicum Bogoriense (MZB), Bogor, Indonesia.

RESULTS

Syphacia (Syphacia) semiadii n. sp. (Figs: Plate 1: A-K; Plate 2: A-B)

Diagnosis

Small worm with transverse cuticular striations. Cuticle forming vesicular widening at head which extends to nerve ring. Mouth leading directly into small pharynx. Oesophagus with pharynx, corpus and posterior bulb. Cervical and lateral alae absent in both sexes. Deirids not seen. Cephalic plate round; mouth surrounded by 3 lips with 'teeth'-like structure on apical margin, 1 dorsal and 2 subventral. Four large cephalic papillae; 2 placed at dorsal lip and 1 at each subventral lip, amphidial pores situated between cephalic papillae with porous patches laterally. Excretory pore posterior to oesophago-intestinal junction.

Description

Male (holotype and 9 paratypes): Length 0.88-1.06 (0.93) mm, maximum width 79-95 (89). Total oesophagus including pharynx, corpus and bulb168-221 (203) long: pharynx 13-16 (14) long, corpus 125-153 (144) long and 20-26 (23) wide, bulb 46-53 (49) long by 42-47 (45) wide. Nerve ring 87-100(93), and excretory pore 341-452 (385) from cephalic end, respectively. Three hemispherical mamelons with transverse striations at ventral posterior body, anterior mamelon 54-62 (57) long, middle mamelon 54-65 (58) long and posterior mamelon 50-56 (53) long. Distance from anterior end to anterior, middle and posterior edges mamelons 366-488 (390), 488-615 (511), and 604-708 (643), respectively. Posterior extremity bent ventrally. Spicule thin, needle-shaped anterior proximal portion broad compared to the posterior distal portion which is pointed, 61-78 (70) long, i.e. 5.7-6.5 (6.1)% of total body length (TBL); gubernaculum stout, hook-shaped, 30-36 (33) long; accessory piece of gubernaculum relatively thin, unornamented. Caudal papillae in 3 pairs, 2 pairs adanal close together and 1 posterior pair protruding posterolaterally. Tail whip-like, 117-134 (126) long, i.e. 11.0-14.6 (13.5)% of TBL. *Female (10 paratypes)*: Length 1.93-2.37 (2.22) mm, maximum width 169–249 (208). Distance between amphidial pores 20. Lateral alae absent. Total oesophagus including pharynx, corpus and bulb 271–288 (280) long: pharynx 21-25 (23) long, corpus 171–206 (189) long and 32–40 (35) wide, bulb 66-71 (68) long by 74-79 (77) wide. Nerve ring 114–148 (137), excretory pore 415-485 (452), from cephalic end. Vulva lip salient, 527– 647 (590), i.e. 24.7-29.2 (26.7) % of TBL, from cephalic end; vagina and ovejector directed posteriorly. Cephalic vesicle 253-294 (273) long. Distance between excretory pore and vulva 90–145 (139), i.e. 4.0–8.2 (6.26) % of TBL. Eggs numerous with a flattened side, operculated, embryonated in uteri, 68–70 (69) x 23– 29 (27). Uterus extending anteriorly to the oesophageal bulb and ending posteriorly near anus. Tail long, tapering to a slender point, 440–556 (480) long, i.e. 19.0-25.0 (21.7) % of TBL.

Taxonomic summary

Type Host : *Halmaheramys bokimekot* Fabre *et al.*, 2013 (Mammalia: Muridae) Site: Caecum

Type locality : Tofu Blewen, East Halmahera, Indonesia (00°48'11.8" N; 128°01'27.6"S) The type specimen was collected 15 km NW of Sagea village, (central 29 Halmahera, Halmahera Island, North Moluccas, Indonesia), at 723 m elevation. Coordinates: 00°36'42.60"N, 128°2'49.00"E. Date of collection : 26 January 2010

Etymology: The new species is named after Prof. G. Semiadi (MZB) for his kind help in providing the host specimens.

Collector: G. Semiadi, Y. S. Fitriana and N. Supriatna (MZB)

Type specimens: MZB Na 483 (holotype); MZB Na 484 (paratype)

Symbiotypes: MZB 33249, MZB 33251, MZB 33255.

Remarks

The following features of this species, round cephalic plate, less developed lips, absence of cervical alae and absence of well-developed deirids, place it in the subgenus *Syphacia* (see Hugot, 1988). *Syphacia semiadii* n. sp. clearly differs from *S. (S.) muris*, a cosmopolitan pinworm of species of *Rattus*, by having a round cephalic plate (Quentin, 1971). It also easily distinguished from *S. sulawesiensis* Hasegawa & Tarore, 1996 and *S. rifaii* Dewi & Hasegawa, 2010, *S. taeromyos* Dewi & Hasegawa, 2012, *S. paruromyos* Dewi & Hasegawa, 2012 from endemic rats in Sulawesi, by lacking vesicular lateral alae in both sexes (Hasegawa & Tarore, 1996; Dewi & Hasegawa, 2010; 2012). Among the New Guinea/Australian congeners, *S. semiadii* n. sp. most closely resembles *S. mamelonitenuis* Smales 2010 and *S. (S.) namelonitenuis*, which has a shorter tail in both sexes. However, this species differs from *S.(S.) mamelonitenuis*, which has a shorter tail in both sexes and larger eggs, and from *S. (S.) longaecauda*, which has a longer tail in both sexes (Smales, 2001; 2010).

Discussion

Halmahera and other Moluccan islands were not connected by a land bridge to the surrounding land masses during the Pleistocene (Voris, 2000). Hence, dispersal of non-volant mammals in Moluccan islands might have occurred only accidentally, probably by drifting. The endemic murines of the Moluccas have been predicted to be allied with those on New Guinea and small surrounding islands (Musser, 1981). However, based on phylogenetic studies, *Halmaheramys* ancestors probably colonized Halmahera from the west (probably from Sulawesi) during the Pliocene (Fabre *et al.*, 2013). Based on molecular reconstruction including most murine genera of Indo-Pacific group within *Rattus* Division, a new group has been defined by Fabre *et al.* (2013) which places *Halmaheramys* in one group with *Bullimus, Bunomys, Paruromys, Halmaheramys, Sundamys*, and *Taeromys*. It differs from other endemic murids of Halmahera *R. morotainensis* and undescribed species of *Melomys*, which probably colonized the North Moluccas in the Pleistocene from the east (Sahul).

It is hence surprising that *Syphacia* (*S*.) *semiadii* is morphologically similar to *S*. (*S*.) *mamelonitenuis* and *S*. (*S*.) *longaecauda*, which have been recorded from Papua New Guinea. Presumably, the ancestor of *S*. (*S*.) *semiadii* originated in New Guinea and dispersed to Halmahera with its original host murine endemic to New Guinea. Subsequently, host switching may have happened, and thereafter the pinworm coevolved with *Halmaheramys* on this island. It is to be expected that more species of *Syphacia* exist in endemic rats of the Moluccas. In order to prove their phylogenetic relationship, DNA sequence analysis will be necessary.

A key to the *Syphacia* (*Syphacia*) species present in the Australian bioregion was given by Weaver and Smales (2010). After that key was published, seven new species of *Syphacia* (*Syphacia*) from that region were described (Dewi & Hasegawa, 2010; 2012; Smales, 2010; 2011). So herein, we provide an updated key for identification.

Key to species of *Syphacia* in Sulawesi and the Australian bioregion (revised after Weaver & Smales, 2010)

- Cephalic plate absent......Syphacia sp. 1 of Weaver & Smales (2008) (Host: Pseudomys; Locality: Australia)
- Cephalic plate present......2
- 2. Cephalic plate square......3
- Cephalic plate oval or round......4
- 3. Eggs without longitudinal ridge......Syphacia muris (Rattus; cosmopolitan)
- Eggs with longitudinal ridge......Syphacia australasiensis (Rattus; Papua New Guinea and Australia)
- 4. Cephalic plate uniformly round, without dorsoventral constriction......5
- Cephalic plate extending laterally with dorsoventral constriction.......13
- 5. Lateral alae present......6
 - Lateral alae absent.....11
- 6. Lateral alae large.....7
- Lateral alae small.....10
- 7. Lateral alae present in both sexes8
- Lateral alae present only in male9
- 8. Having protunded lips...... Syphacia taeromyos (Taeromys; Sulawesi)
- Lips not protunded......Syphacia sulawesiensis (Rattus; Sulawesi)

- Tail male long with whip like appendages...... Syphacia paruromyos (Paruromys; Sulawesi)
- 10. Male with two mamelons...... Syphacia darwini (Melomys; Australia
- Male with three mamelons, first without thick muscular body wall..... *Syphacia lorentzymyos (Lorentzymys*; Papua New Guinea)
- 11. Female tail length >600, male tail length >350..... Syphacia longaecauda (Melomys;Australia and Papua New Guinea)
- Female tail length <500, male tail length <150.....12
- 12. Male tail thin, >100 long; female tail length >400...... Syphacia semiadii (Halmaheramys; Halmahera Island, the Moluccas, Indonesia)
- Male tail thick, <100 long, female tail length <200...... Syphacia mamelonitenuis (Lorentzymys: Papua New Guinea)
- 13. Alae (either lateral and cervical) absent...... 14
- Alae present.....16
- 14. Male spicule length >75; female tail length >550...... Syphacia boodjamullensis (Zyzomys; Australia)
- Male spicule length <70; female tail length <500......15
- 15. Male with two pairs of postanal papillae; female with excretory pore close-set to oesophageal bulb........... *Syphacia brevicauda (Pseudomys*; Australia)

- Male with one pair of postanal papillae; female with excretory pore posterior to oesophageal bulb.......... *Syphacia pseudomyos (Pseudomys*; Australia)
- 16. Cervical and lateral alae present...... Syphacia coccymyos (Coccymys; Papua New Guinea)
- Only lateral or cervical alae present......17
- 17. Lateral alae present; cervical alae absent...... Syphacia helidonensis (Pseudomys; Australia)
- Cervical alae present; lateral alae absent......18
- Cervical alae wide; male tail more than 100; spicule length <60; egg length
 <100 Syphacia abertoni (Zyzomys; Australia)

Acknowledgment

We would like to express our sincere thanks to Prof. H. Hasegawa, Oita University, Japan, for revision and suggestions on the manuscript. We also wish to thank Prof. G. Semiadi and Nanang Supriatna, Zoology Division, RC Biology-Indonesian Institute of Sciences who collected the rodent host specimens, to Prof. (Em.) L. R. Smales (South Australian Museum) and Dr. H.J. Weaver (The Australian National University) for providing relevant literature. Thanks are also owed to Yuni Apriyanti for her assistance in the laboratory. This research was a part of a fauna diversity study in the Weda Bay sponsored by Weda Bay Nickel. The SEM observation was funded by the Indonesian government through DIPA Research Centre for Biology-LIPI 2010.

Supported in part by the RONPAKU Project from the Ministry of Education, Science and Culture of Japan. The first author had been donated as the project researcher (ID N0. LIPI-11317) from 2013-2015.

References

- Anonymous. (2010) Moluccas Island. <u>http://indonesiaexplore.com/mollucas-island/</u>. Cited 1 June 2010.
- Dewi, K & H. Hasegawa. (2010) *Syphacia (Syphacia) rifaii* n. sp. (Nematoda: Oxyuridae) collected from *Bunomys* spp. (Rodentia: Muridae) in Central Sulawesi, Indonesia. *Journal of Parasitology* **96**(1):125-128.
- Dewi, K. & H. Hasegawa. (2012) Two new species of Syphacia (Nematoda: Oxyuridae) in endemic murid rodents from Sulawesi, Indonesia. *Journal of Helminthology*. 1-9. doi:10.1017/S0022149X12000703
- Fabre, P-H., Pagès, M. G.G. Musser, Y.S. Fitriana, J. Fjeldså, A. Jennings, K. A. Jønsson, J. Kennedy, J. Michaux, G. Semiadi, N. Supriatna & K. M. Helgen. (2013) A new genus of rodent from Wallacea (Rodentia: Muridae: Murinae: Rattini) and its implication for biogeography and Indo-Pacific Rattini systematic. *Zoological Journal of the Linnaean Society* 169:408-447.
- Hasegawa, H. (1996) Notes on the morphology of three nematode species of the subfamily Nippostrongylinae (Heligmosomoidea: Heligmonellidae) collected from an endemic rat of Halmahera Island, Indonesia. *Biological Magazine Okinawa* **34**: 13-21.

& Syafruddin. (1995a) Nematode fauna of the two sympatric rats, *Rattus rattus* and *R. exulans*, in Kao district, Halmahera Island, Indonesia. *Journal of the Helminthological Society of Washington* **62:** 27-31.

& ______. (1995b) *Nippostrongylus marhaeniae* sp. n. and other nematodes collected from *Rattus* cf. *morotainensis* in North Halmahera, Molucca Islands, Indonesia. *Journal of the Helminthological Society of Washington* **62**: 111-116.

<u>Karane</u> & Tarore, D. (1996) *Syphacia (Syphacia) sulawesiensis* n. sp. and *S. (S.) muris* (Yamaguti, 1933) (Nematoda Oxyuridae) collected from *Rattus xanthurus* (Gray 1867) (Rodentia: Muridae) in North Sulawesi, Indonesia. *Tropical Zoology* **9**: 165–175.

- Hugot, J. P. (1988) Les nématodes Syphaciinae, parasites de rongeurs et de Lagomorphes. *Memoires du Museum National d'Histoire Naturelle*, Série A. *Zoologie* **141**: 1–148.
- Musser, G. G. (1981) The giant rat of Flores and its relatives east of Borneo and Bali. *Bulletin of the American Museum of Natural History* **169**:62-176.
- Quentin, J. C. (1971) Morphologie comparée des structures céphaliques et génitales des Oxyures du genre *Syphacia*. *Annales de Parasitologie Humaine et Comparée* **46**: 15–60.
- Smales, L. R. (2001) Syphacia longaecauda n. sp. (Nematoda: Oxyuridae) Syphacinea from Melomys spp. (Muridae: Hydromyinae) from Papua New Guinea and Irian Jaya, Indonesia. Parasite 8: 39–43.

_____. (2004) Syphacia (Syphacia) australasiensis sp. nov. (Nematoda: Oxyuridae) from *Rattus leucopus* (muridae) from Papua New Guinea and Australia. *Transactions of Royal Society of South Australia* **128**:47–51.

(2010) The Gastrointestinal helminths of *Lorentzimys nouhuysi* (Rodentia: muridae) with Descriptions of Two New Genera and Three New Species (Nematoda) From Papua New Guinea. *Journal of Parasitology* **96** (3): 602-613.

(2011) Gastrointestinal nematodes of *Coccymys ruemmleri* (Rodentia, Muridae) with the description *Montistrongylus giluwensis* sp. nov. (Heligmonellidae) and *Syphacia coccymyos* sp. nov. (Oxyuridae) from Papua New Guiena. *Acta Parasitologia* **56** (4): 418-426.

- Voris, H. K. (2000) Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. *Journal of Biogeography* **27:** 1153–1167.
- Weaver, H. J., & L. R. Smales. (2006) Syphacia (Syphacia) abertoni n.sp. (Nematoda: Oxyuridae) from Zyzomys argurus (Thomas) (Rodentia: Muridae) from Northern Australia. Transactions of Royal Society of South Australia 131: 206–210.

<u>&</u>_____. (2008) New species of *Syphacia* (*Syphacia*) Seurat (Nematoda: Oxyuridae) from *Pseudomys* species (Rodentia: Muridae) from central Australia. *Zootaxa* **1775**: 39–50.

<u>&</u>_____. (2010) Three new species of *Syphacia (Syphacia)* (Oxyurida: Oxyuridae) from Queensland, Australia, and a key to the species present in the Australian Bioregion. *Comparative Parasitology* **17**(1): 9-19.

LEGEND

Plate 1. *Syphacia semiadii* n.sp. collected from *Halmaheramys bokimekot* on Halmahera Island, Indonesia. A. Cephalic end of female (apical view), B. Cephalic end of female (right lateral view), C. Female (paratype) (left lateral view), D. Anterior portion of female (right lateral view). E. midbody in cross section of female, F. midbody in cross section of male G. Egg, H. Male (holotype) (left lateral view), I. Posterior end of male (ventral view), J. Spicule and gubernaculums (right lateral view), K. Posterior end of male (right lateral view). Scale bars : A: 10μm; B, E, I, K: 50 μm; C, D: 200 μm; F, J: 25 μm, G: 20 μm; H: 100 μm.

Plate 2. Scanning electron microscopy of *Syphacia semiadii* n.sp. collected from *Halmaheramys bokimekot* on Halmahera Island, Indonesia. A. Cephalic end of female (apical view), B. Anterior portion of female (lateral view). Scale bars : A: 10µm; B: 20µm.



