Scanning electron microscope observations of *Gongylonema neoplasticum* and *Heterakis spumosa*, nematode parasites of an endemic murine rodent from Sulawesi

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Abstract. Sulawesi has unique mammalian fauna with high level of endemism. One of the endemic fauna in Sulawesi is *Bunomys chrysocomus* (Rodentia: Muridae). This study aimed to observed the nematode species parasitizing *B. chrysocomus* by using scanning electron microscope. Based on the observation nematodes obtained from gastric mucosa was *Gongylonema neoplasticum* Fibiger and Ditlevsen, 1914, while from the caecum was *Heterakis spumosa* Schneider, 1866. Both nematodes are cosmopolite nematodes which have wide host range and worldwide distribution. The detail morphology and taxonomic status of both nematodes from *B. chrysocomus* of Sulawesi will be presented here.

1. Introduction

Sulawesi, formerly known as Celebes, is the largest and oldest island within Wallacea. This island harbor many unique mammalian fauna with a very high level of endemism [1]. The murine rodents represent about 30% of the total mammalian species and approximately 52% of all the endemic species in this island [2]. Consequently, helminth parasites of murine rodents are also rich in this region.

Some researches stated that 60% of human diseases to be of zoonotic origin [3]. Nematode parasites have ability for host shifting with potential effect for human and wildlife health. The interesting one is nematode parasites of murin rodents because some reports have been recorded for the human infection by these nematodes. For example, *Cyclodontostomum purvisi*, a nematode parasitic in the cecum of murines, has been recorded from human body [4]. A case of human infection with *Rictularia* was found in New York [5]. Moreover, human cases of angiostrongyloidiasis were reported from North Sumatra [6] and Java [7].

Specimens of *Gongylonema neoplasticum* (Fibiger and Ditlevsen, 1914) and *Heterakis spumosa* Schneider, 1866 were collected from gastric mucosa and caecum of *Bunomys chrysocomus*, an endemic murine of Sulawesi, respectively. The presence of both species as the parasite in *B. chrysocomus* in Sulawesi already reported by Purwaningsih and Dewi [8] and Dewi [9]. Furthermore, in this study, both nematode species were observed by using scanning electron microscopy (SEM). To date, there was no study about the SEM observation of *G. neoplasticum* and *H. spumosa* based on Indonesian specimens. This study aimed to know the detail morphology by SEM and to know the
taxonomic status of both species. This study is the first description of *G. neoplasticum* and *H. spumosa* from Indonesian specimens.

2. Materials and Methods

*Heterakis spumosa* and *G. neoplasticum* from Pakuli, Gumbara, Donggala, Central Sulawesi deposited in Museum Zoologicum Bogor were observed by SEM. SEM observation was done under a scanning electron microscope, type JSM 5310LV for detailed examination. Measurements were made by software ImageJ v. 1.51m9 software (NIH, USA). In order to measure the character, we also observed the nematode by using a light microscope Nikon SE100 with an ocular micrometer. Measurements (minimum, maximum, followed by mean in parentheses) were given in micrometers unless otherwise stated. For light microscopic study, nematodes were cleared in glycerine until sufficiently transparent for examination. For SEM study, specimens were fixed in glutaraldehyde, dehydrated through a series of ethanol and freeze-dried. Dried specimens were then coated with gold using standard techniques.

3. Results and Discussion

3.1. Results

**Gongylonematidae** (Hall, 1916)

**Gongylonema** Molin, 1857

*Gongylonema neoplasticum* (Fibiger & Ditlevsen, 1914)

Syn. *G. orientale* Yokogawa, 1925, *G. problematicum* Schulz, 1924

Site of infection: gastric mucosa

**General.**—Small, long filariform nematodes with many cuticular fold in the anterior region (Figs. 1A, B). Mouth opening, small, triangular with 6 small ear-shaped lips, three lips on each side, one big interlabial on each dorsal and ventral side. Large lateral amphids each with transverse slit-like opening with thick wall. Four pairs of cephalic papilla arranged in two lateral rows; four big outer papilla and four smaller inner papilla (Fig. 1C). Lateral alae present. Pharinx short, cylinder. Oesophagus long, divided into two parts; small anterior part and long muscular oesophagus part.

**Male** *(n=10)*—Total length 1.3 (1.1–1.6) cm long, 165 (157–170) wide. Mouth 14.3 by 8.2 *(n=1)*. Diameter amphid 2.6 *(n=1)*. Distance between amphids 13 *(n=1)*. Buccal capsule 47.6 (40–58) by 14.6 (14–15). Excretory pore 181 (170–190), cervical papilla and nerve ring at the same level, 187 (180–190) from anterior end. Muscular oesophagus 256 (220–310) long, 49.4 (49–50) wide. Glandular oesophagus 1.58 (1.30–2.17) mm, width 66 (60–70). Caudal alae subsymmetric, right alae wider than left, and supported by 10 pairs papillae; four pairs precloacal papillae and six pairs post cloacal papillae (Fig. 1D). Spicules dissimilar and unequal, right spicula 88 (86–90) long, and the left 688 (670–710) long.

**Female** *(n=10)*—Total length 4.8 (3.2–7.5) cm, width 212 (200–220). Head width 44.6 *(n=1)*. Buccal capsule 48 (45–60). Cervical papilla as same lavel as nerve ring, 300 (250–410) from anterior end. Muscular oesophagus 340 (300–400), width 47 (40–50), glandular oesophagus 3.12 (2.9–3.25) mm, width 89 (81–93). Cuticle striation in the mid body apart 6.7 (6.3–7.1). Vulva at posterior part of body, 420 (390–490) from posterior end (Fig. 1H). Vagina long, uterus direct opposed. Eggs oval, thick shell, contain coil larva in vagina, embryonated eggs in utery 56.6 x 41.0. Tail conical 150 (145–180) (Fig. 1G).

3.1.1. Remarks. Species of *Gongylonema* are characterized by the presence of numerous cuticular bosses in the anterior part, a gubernaculum, and markedly unequal spicules [10,11]. The detail observation of *G. neoplasticum* by SEM had been done by da Costa Cordeiro et al. [10] from *R. rattus* and *R. tanezumi* from urban areas of the eastern Brazilian Amazon. The morphology and the
Figure 1. Gongylonema neoplasticum. A. Anterior portion, B. Anterior posterior with cuticular bosses (lateral view), C. End face, D. Posterior end of male with pre and post caudal papillae (lateral view), E. Posterior tip of male with post cloacal papillae, F. Cloaca and tip of spicule, G. Posterior end of female (lateral view), H. Vulva
Bars: A, G: 50 µm, B: 100 µm, C, F: 10 µm, D: 200 µm, E: 20 µm, H: 30 µm.
Abb: cb= cutical bosses, l= lip, il= interlabial lip, icp= inner cervical papilla, ocp= outer cervical papilla, am= amphid, clo= cloaca, spi= spicule.
measurements of \textit{G. neoplasticum} in this study agreed well with that description, except in the number of cloacal papillae of the males. Cloacal papillae of the males in this study have constant number i.e., 10 pairs consist of four pairs precloacal papillae and six pairs post cloacal papillae, while \textit{G. neoplasticum} of the eastern Brazilian Amazon has nine pairs of cloacal papillae (four pre- and five post-cloacal) [10].

The variation of the number of cloacal papillae in \textit{G. neoplasticum} has been recorded by the previous researches. \textit{Gongylonema orientale} by Yokogawa (1925) (syn: \textit{G. neoplasticum}) has eight pairs of caudal papillae [10]. Eira et al. [11] described \textit{G. neoplasticum} from \textit{Oryctolagus cuniculus} (Lagomorpha, Leporidae) with number of cervical papillae by 8–9 pairs of lateral papillae arranged asymmetrically; 5–6 precloacal and 3 postcloacal.

Heterakidae Ralliet and Henry, 1914
\textit{Heterakis} Dujardin, 1845
\textit{Heterakis spumosa} Schneider, 1866
Syn. \textit{Ganguleterakis gangula} Lane, 1914

\textit{General.} - Anterior end bluntly rounded with terminal mouth (Fig. 2D). Three dome-shaped lips present. Two cephalic papillae at dorsal lip and one cephalic papilla at each sub ventral lips (Fig. 2B). Cervical alae well developed as long as body (Fig. 2A). Amphids at sub ventral lips, same in side with cephalic papilla. Oesophagus divided into three part; short pharynx, cylinder oesophagus and a strong posterior bulb with valvular apparatus. Cuticle transversal striation smooth.

\textit{Male} (n=10) – Total length 4.7 (4.1–5.8) mm. Distance of amphids 31 (n=1). Nerve ring and excretory pore 193 (180–210) and 222 (195–230) from anterior end, respectively. Total length of oesophagus 589 (568–710) consist of pharynx 44 (39–46) long and bulb with diameter 136 (129–140) x 152 (141–150). Cuticle striations in the middle part of body 4.9 (4.6–5.2) apart (Fig. 2A). Posterior end broad. Caudal alae well developed, symmetrical, and supported by ten pairs papillae; two pairs pedunculate caudal papilla at lateral pre-cloacal sucker; three pairs of lateral pedunculate papillae (one pair precloacal, one pair adcloacal, and one pair postcloacal), two pairs sessile papillae close to cloaca, three pairs lateral papillae at the tip of tail (Figs. 2E, F, H). Precloacal sucker present 334 (310–363) from posterior end, 51.8 in diameter (n=1), rounded with thick wall (Fig. 2I). Spicules equal 236 (221–249) long, spike-liked, becoming wider at distal. Tail conical, 246 (221–275) long. Phasmid present dorsolateral from caudal papilla at the tip of tail (Fig. 2G).

\textit{Female} (n=10) – Total body length 10.6 (6.5–12.7) mm. Nerve ring 240 (210–320) and excretory pore 277 (210–290) from anterior end, respectively. Total length of oesophagus 703 (650–725) consist of pharynx 66 (60–70) long and bulb with diameter 122 (119–125) x 143 (140–146). Vulva open directly in front of middle of body 4.0 (3.7–4.5) from anterior end, associated with five cuticular processes. Uteri opposed. Tail tapering, 209 (187–220) long. Eggs oval, thick, smooth shell 42 (39–47) by 60 (57–62).

3.1.2. \textit{Remarks}. The general morphology and measurements of \textit{H. spumosa} in this study agree well with the previous studies [12, 13]. Ribas et al. [12] observed \textit{H. spumosa} from \textit{Cricetomys gambianus} from Senegal, while del Rosario Robles [13] studied that species from \textit{R. norvegicus} of Argentina. Both of studies observed \textit{H. spumosa} by SEM but they did not report the presence of phasmid at the dorsolateral of caudal papilla at the tip of tail. This study support data that \textit{H. spumosa} is a cosmopolitan nematode species that also can survive as the parasite in the endemic murine rodent.
Figure 2. *Heterakis spumosa*. A. Anterior portion with cervical alae (lateral view), B. End view. C. Cuticle striations at the middle body, D. Anterior portion (lateral view), E. Tail of male with cloacal sucker and cloaca (ventral view), F. Cloaca and caudal papillae (ventral view), G. Caudal papilla anad phasmid, H. Anterior part of male (lateral view), I. Cloacal sucker.

Bars: A: 200 µm; B: 20 µm; C: 10 µm; D, F: 25 µm, E, H: 100 µm

(Abb: ca= cervical alae, cp: cervical papilla, dl: dorsal lip, am: amphid, vl: ventral lip, cap: caudal papilla, clo: cloaca, ps: precloacal sucker, ph: phasmid.)
3.2. Discussion

Species of *Gongylonema* Molin, 1857 are parasites of birds and mammals [9, 10]. This genus was established for the first time by Molin (1857) from *Goerbillus musculi* [11]. This genus has potential zoonosis because nearly 50 cases of human infected by *Gongylonema* have been reported worldwide since 1864 in Italy, United States, Europe, Sri Lanka, China, the Middle East, Australia, New Zealand, and Japan [17]. One of the species belonging *Gongylonema* is *G.neoplasticum*. This species is cosmopolitan and has worldwide distribution and wide host range [5, 15]. In Indonesia, *G. neoplasticum* has been reported from *R. tanezumi* of Lampung, Sulawesi, and Java, *B. crysocomus* of Sulawesi, *Niviventer lepturus* of Lampung, and *R. rattus* from Halmahera [18].

The taxonomic study in this genus is difficult because of the big variation in the length of worms and the number of caudal papillae. It has been stated by various authors that many characters that usually are given for the taxonomic study in nematodes have no specific value in the case of the identification of *Gongylonema* spp. [5]. Many authors restrict the useful measurements to lengths of body, buccal capsule, spicule, gubernaculum, and tail, the distance of the vulva from the posterior end, and the eggs size [5]. However, another study stated that the more stable characters in this genus include the size of embryonated eggs, size, and structure of spicules, and gubernaculum [11].

*Heterakis spumosa* was first reported from the cecum of Berlin rats by Schneider in 1966. This nematode is cosmopolitan having distribution worldwide and has been already reported from various rat hosts [13, 18]. In Indonesia, *H. spumosa* was reported from *R. tiomanicus* of Lampung, *B. prolatus*, *B. crysocomus*, *R. hoffmanni*, *R. marmoxurus*, *R. xanthurus* of Central Sulawesi, and *R. hainaldi* of Flores [19]. This study provided the detailed morphology of *H. spumosa* from Indonesian specimens observed by SEM.

*Heterakis spumosa* are originating from Southern Asia and spread worldwide with the black rat *R. rattus* and the brown rat *R. norvegicus* [12]. Southern Asia is also for the originating of *G. neoplasticum*. However, their dispersal in Sulawesi has occurred with the human facilitation of the dispersal of these commensal rats. *Bunomys chrysocomus* is the endemic murine rodents in Sulawesi. This murine was infected by swollen the infective stage of the eggs that laid along with the faeces of *R. rattus* and *R. norvegicus*. Furthermore, *G. neoplasticum* and *H. spumosa* can live and adapt inside the body of *B. chrysocomus* and then are maintained in the body host.

4. Conclusion

*Gongylonema neoplasticum* and *Heterakis spumosa* are cosmopolite nematodes which have wide host range and worldwide distribution including as the parasite of endemic murine rodent (*Bunomys chrysocomus*) in Sulawesi.

5. References

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