Parasitic nematodes from turtles: New species and new record from Indonesia

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ABSTRACT

Objective: To describe the morphological characteristics of some species of parasitic nematodes found in freshwater turtles from Sumatera (Amyda cartilaginea) and Kalimantan (Notochelys platynota).

Methods: Specimens for light microscopy examination were fixed with warm 70% alcohol, cleared and mounted in lactophenol for stout specimens and glycerine for delicate specimens, prior to wet mounting. Drawings were made with the aid of a drawing tube attached to an Olympus compound microscope. Specimens for scanning electron microscope examination were fixed in cacodylate buffer and glutaraldehyde, dehydrated in alcohol concentration series, dried in vacuum dried and coated with gold in Eiko-IB2 Ion Coater. Measurements were given in micrometers as the mean followed by the range in parentheses, unless otherwise stated.

Results: Four species of parasitic nematodes were found, namely, Monhysterides jambiensis sp. n. (M. jambiensis), Spiroxys sumatraensis sp. n. (S. sumatraensis), Cissophyllus laverani and Cissophyllus roseus. M. jambiensis differed from Monhysterides lissemydis in ratio of right-to-left spicule (1:3 in M. jambiensis vs. 1:3.8–5.6 in M. lissemydis) and had no gubernaculum. M. jambiensis also differed from Monhysterides testudinicola in longer spicule (11.2%–12.7% in M. jambiensis vs. 5.6%–6% of body length), ratio right-to-left spicule (1:3 in M. jambiensis vs. 1:2) and the numbers of caudal papillae and differed from Monhysterides testudinicola because of the lateral alae began from the half posterior of body, but that in M. jambiensis began from anterior part of body, behind the anterior end. S. sumatraensis differed from Spiroxys annulatus in having tooth at median lobe and having no well mark cuticular collar behind the based of lips and constriction at the base of pseudolabium was not pronounced. S. sumatraensis differed from Spiroxys transversalata in having no tooth at median lobes of each pseudolabium and differed from Spiroxys chelodinae in the unpaired position of median caudal papillae since it was more anterior than that of Spiroxys jambiensis. Additionally there was no adanal papillae in the male of Spiroxys ankarafantsika in having double papillae on each pseudolabium, more anterior of deirid and longer spicule (9.5%–12.4% vs. 6% of body length in Spiroxys ankarafantsika). Two species of Cissophyllus morphologically were agreed with its previous species.

Conclusions: Monhysterides spp. are found in Oriental region turtle, suborder Cryptodira, and there is no report from others zoogeographical regions. S. sumatraensis belonging to Group 2 includes three other species. This group was found in Oriental, Australian and Ethiopian, Spiroxys presumably widespread amongst the species of turtle when Gondwanaland existed. Cissophyllus laverani is a new record in Indonesia.

1. Introduction

The population of turtle begin to decrease because they are utilized by some people from natural habitat for some purposes. Some species were traded for consumption and to be pets[1]. Parasitic nematodes of turtle may transmit to human because some species of nematodes are zoonoses[2]. Parasitic nematodes of turtle have been well known from many countries in the world, however the information of those are very limited in Indonesia. There is no report after describing Cissophyllus roseus (C. roseus) of Testudo sp. from Java, Indonesia[3]. During survey
on reptile trading in Kalimantan and Sumatera, some nematodes were collected from freshwater turtles. The species found were reported in this paper and the two of them were new species.

2. Materials and methods

The examined materials were collected from the 3 turtles [Amyda cartilaginea (A. cartilaginea) from Jambi, Notochelys platynota (N. platynota) from Berong Village, Central Kalimantan, Indonesia and one unknown species]. The nematodes were cleared in lactophenol for stout specimens and in glycerine for delicate specimens prior to wet mounting to observe under compound light microscope. Figures were made with drawing tube attached at compound microscope. Nematodes observed with scanning electron microscope were fixed with cacodylate buffer and glutaraldehyde, dehydrated with alcohol concentration series (70%-absolute), dried with freeze dryer and coated with gold in IB-Eiko sputter coater for 5 min (about 400 angstrom thickness). Measurements were given in micrometers as the mean followed by the range in parentheses, unless otherwise stated.

3. Results

Nematodes found from freshwater turtles are Monhysterides jambiensis sp. n. (M. jambiensis) and Spiroxys sumatraensis sp. n. (S. sumatraensis) of which both were found in the intestine of A. cartilaginea from Jambi, Sumatera. Cissophyllus laverani (C. laverani) was found in the intestine of N. platynota from Berong Village, Kalimantan and C. roseus from a unknown species and locality of turtle.

3.1. Descriptions

3.1.1. M. jambiensis sp. n. (Nematoda: Atractidae)

3.1.1.1. General

The body was small and delicate with striae along its length. Each striae was patterned as scales (Figure 1T). Thin lateral alae was presented starting from behind the anterior end (Figure 1S) to almost tip of tail. The body was attenuated anteriorly and posteriorly, ending in a thin, long processus in both sexes (Figures 2D and 2E). Posterior extremity was spirally coiled in male (Figure 2E). The body of the adult female was wider at a half-length, but not in larvae. Anterior extremity ended with six lips, each of median lips with 2 papillae, 2 lateral lips with amphid (Figure 2A). Oesophagus was tubular with shorter anterior muscular and longer posterior part, wider at its posterior end, but not forming a bulb and without valve. Nerve ring and excretory pore lay behind the oesophagus (Figure 2C).
wide at the maximum level. The oesophagus was 509 (481–530) µm long and was devided into anterior and posterior parts which were 170 (160–177) µm and 339 (321–353) µm long, respectively. The ratio of anterior to posterior esophagus was 1:2. The nerve ring and excretory pore were 226 (215–228) µm and 418 (400–443) µm from anterior end, respectively. The tail was abruptly narrower behind cloaca, ending in a long, thin and tapering structure which was 842 (704–960) µm long. Caudal papillae were small, arranged as follows: 2 precloacals, 1 adcloacal, 1 just posterior to cloaca, 2 pairs posterior, 2 double pairs ventral, 2 pairs subdorsal. The spicules were unequal and similar, tubular, wider at anterior end, with pointed posterior end (Figures 2E and 2F). The left spicule was 454 (415–475) µm long and the right spicule was 119 (103–158) µm long. The ratio of right to left spicule was 1:3. The gubernaculum was short and comma shaped (Figure 2G) and was 52 (45–60) µm long.

3.1.1.3. Female (based on 10 specimens)

The body was 3.49 (3.56–3.99) mm long and 196 (125–240) µm wide at maximum level. The oesophagus was 538 (490–580) µm long and was devided into anterior and posterior part which were 171 (131–210) µm and 367 (359–370) µm long, respectively. Its ratio was 1:1.7–2.7. The nerve ring and excretory pore were 238 (228–265) µm and 427 (380–480) µm from anterior end, respectively. The tail was abruptly narrower behind anus, ending in a long, thin and tapering structure and was 963 (938–1050) µm long. Vulva lay near anus at 1051 (973–1090) µm from posterior end, 88 (35–43) µm in front of anus.

Host: *A. cartilaginea*; Site: Stomach; Locality: Jambi, Sumatera, Indonesia; Specimens deposited: Museum Zoologicum Bogoriense-, Research Center for Biology-LIPI, Indonesia: MZBNa 665 (1 paratype male), MZBNa 677 (1 allotype female and 4 paratype male, 9 female); Etymology: this species was named after the island name of type locality.

3.1.2. *S. sumatraensis* sp. n. (Nematoda: Gnathostomatidae)

3.1.2.1. General

The body was stout, medium in sized with striae along its length. Anterior extremity was strongly curved ventrally (Figure 3I). The anterior end with two pseudolabia, the pseudolabium was wider than long (each of pseudolabium with three lobes, anterior end of each lobe with cuticular thickening) (Figure 3H and Figure 1U). A tooth was presented in the middle of each median lobe (Figure 3O). Cuticular collar was smooth and oesophagus was divided into 2 portions without constriction, shorter, thicker at anterior portion. The nerve ring lay at anterior portion of esophagus, deirid very thin, and lay behind the base of lips (Figure 3I).

3.1.2.2. Male (based on 2 specimens)

The body was 34.25 (33.8–34.7) mm long and 885 (875–895) µm wide at the maximum level. The pseudolabium was 92 (85–98) µm long and 158 (155–161) µm wide. The nerve ring, deirid, excretory pore were 700 µm, 420 µm and 1240 µm from anterior end, respectively. Oesophagus was 5830 µm long. Caudal papillae were 10 pairs, arranged as follows: 2 pairs in half of anterior length of caudal alae, 1 pair anterior to anus, 1 unpaired, anterior to third precloacal papillae, 1 pair ventral, just anterior and posterior to anus respectively, 1 pairs lateral to anus, 1 pair posterior to anus, 3 pairs, lateral, near the tip of tail (Figure 1W). Caudal alae was thick and it was united at anterior end (Figures 3I and 3K). The spicules were tubular, wider at anterior end and conical at the tip (Figures 3O and 3P). It was 2.073 (1.90–2.41) µm long. The tail was short with mucron at the tip, curved ventrally (Figure 3J) and it was 370 (350–390) µm long. Gubernaculum was small (Figure 3N) and it was 23 µm × 2.5 µm long.

3.1.2.3. Female (based on 3 specimens)

The body was 35.55 (34.7–6.4) mm long and 956 (780–1090) µm wide at the maximum level. The pseudolabia were 92 (80–110) µm long and 167 (162–181) µm wide. The nerve ring, deirid, excretory pore were 760, 420 and 5830 µm from anterior end respectively. The oesophagus was tubular and it was 6070 µm long. The vulva lay at anterior to anus and it was 18950 µm from anterior end, in one specimen. The tail was 860 µm long with conical tip. Eggs were elongated in uteri (Figure 3M), 99 (93–110) µm long and 48 (45–80) µm wide.

![Figure 3. Drawing images of *S. sumatraensis* sp.n. (H-P) and *C. laverani* (Q-R).](image-url)
The mouth was complex, extended from the dorso-ventral with triangular stoma. The three complex lips were consisted of large-trident-shaped dorsal lips and 2 lateral lips with 4 segmented plates, numerous lamellae on it towards the center of the mouth (Figure 1X). The cephalic papillae were consisted of 4 submedian and 2 double lateral. Osephyagus was devided into short anterior part, without musculature, followed by longer tube that was wider at posterior, then narrower ending in a bulb with valve.

3.1.3.2. Male (based on 8 specimens)

The body was 23.77 (23.3–24.4) mm long and 1 785 (1 700–1 875) μm wide at the maximum level. The nerve ring and excretory pore were 950 (880–1 025) μm and 3 065 (2 945–3 150) μm, respectively. The osephyagus was 3 848 (3 575–4 070) μm long. The tail was curved ventrally and abruptly narrower after cloaca and it was 483 (450–525) μm long. Precloacal sucker was presented (Figure 1Z), 3 00 (275–325) μm in diameter. Caudal papillae were pedunculated, arranged as follow: 8 pairs preanal, 7 was presented (Figure 1Z), 300 (275–325) μm wide at the maximum level. From anterior end to nerve ring and excretory pore were 955 (880–1 025) μm and 3 065 (2 945–3 150) μm, respectively. The osephyagus was 3 848 (3 575–4 070) μm long. The tail was straight, narrower behind anus with knob at posterior tip and it was 695 (540–800) μm long. The vulva lay at 7 609 (6 050–9 760) μm from posterior end. The eggs were elongated, thick-shelled, 118 (114–121) μm long and 66 (63–70) μm wide.

3.1.3.3. Female (based on 5 specimens)

The body was 24.38 (23.73–26.33) mm long and 1 833 (1 650–2 030) μm wide at the maximum level. From anterior end to nerve ring and excretory pore were 955 (880–1 025) μm and 3 065 (2 945–3 150) μm, respectively. The osephyagus was 3 848 (3 575–4 070) μm long. The tail was straight, narrower behind anus with knob at posterior tip and it was 695 (540–800) μm long. The vulva lay at 7 609 (6 050–9 760) μm from posterior end. Accessory piece was presented (Figure 3Q) and it was 465 μm long and 165 μm wide.

3.1.4. C. roseus (Nematoda: Kathlaniidae)

3.1.4.1. General

The body was cylindrical with straight-forward anterior end and attenuated posterior end, cuticle with striae. The mouth was complex with triangular stoma. The dorsal lips were large-trident-shaped and 2 lateral lips were 4 segmented plates, numerous lamellae on it, towards the center of the mouth (Figure 3Y). Cephalic papillae were consisted of 4 submedian and 2 double lateral. The osephyagus was short at anterior part without musculature, followed by longer tube, with small bulb at posterior end of osephyagus, follow by tubular part, ending in a bulb with valve.

3.1.4.2. Female

No male was found. The body of female was 21.05 (22.05–25.95) mm long and 1 785 (1 700–1 875) μm wide at the maximum level. The nerve ring and excretory pore were 915 (820–1 100) μm and 2 717 (2 550–2 900) μm from anterior end, respectively. The osephyagus was 3 800 (3 775–3 825) μm long. The tail was very short, straight, with small knob at the tip, 665 (675–700) μm long. The vulva lay at about 1/3 posterior of the body length, vagina vera extending posteriorly. No egg was found.

Host: N. platynota; Site: Intestine; Locality: Berong Village, Central Kalimantan (C. laverani) unknown locality (C. roseus); Specimens deposited: Museum Zoologicum Bogoriense, Research Center on Biology-LIPI, Indonesia; C. laverani: MZBNa 662, 663 (8 paratype male, 5 female), C. roseus: MZBNa 664 (6 paratype female).

4. Discussion

Monhysterides was described for the first time from fish, Barbus tor in India with Monhysterides piscicola Baylis Daubney, 1922 (M. piscicola) as species type[4]. Other species reported from fish (Piaractus brachypomus and Mayleus sp.) which came from Brazil was Monhysterides iheringi Solis & Moravec, 2002 from Vieja intermedia in Mexico[5,6]. Monhysterides iheringi was transferred to Klossinemella iheringi[7]. Monhysterides is very similar to Atractis, but both of those genus are distinguished due to the strong chinitized corpus of esophagus and longer anterior portion of esophagus than posterior part in Atractis[6,8]. Monhysterides is considered to be a synonym of Pseudatractis and this genera is established for Pseudatractis testudinicolina (Baylis, 1933) from Trionyx cartilagineus in Pahang, Malaya[9]. Another reported species was Monhysterides lissemysidis (M. lissemysidis) from Lissemys punctata punctata in India[10].

The nematode in this study agreed with morphological characteristics of Monhysterides and this species differs from M. piscicola in shorter left spicule [6% in M. piscicola vs. 12.7% of body length in M. jambienisis], number of precloacal papillae (4 in M. piscicola vs. 2) and shorter female tail (16% in M. piscicola vs. 30% of body length)[3]. It differs from Monhysterides testudinicolina (M. testudinicolina) in longer left spicule (5.6%–6% in M. testudinicolina vs. 11.2%–12.7% of body length), ratio right-to-left spicule (1:2 in M. testudinicolina vs. 1:3) and the numbers of caudal papillae[11,12]. M. testudinicolina is characterized by the lateral alae which begins from the half posterior of body and in Monhysterides presently study begins from anterior part of body, behind the anterior end. It also differs from M. lissemysidis in no lateral alae, no gubernaculum, almost equal length of anterior and posterior esophagus and more precloacal papillae (8 in M. lissemysidis vs. 2)[10]. Based on these differences, therefore, the Monhysterides in this study was considered as a new species and it was named as M. jambienisis.

Both Monhysterides from fish and turtle were found in Oriental region, while Atractis reported from almost all of zoogeographical region in some species of turtle, lizards, fish and mammals[5,6,12,13]. Since this genus was not found in others zoogeographical regions, presumably the diversity of Monhysterides is restricted in Oriental region. However, the additional data of Monhysterides from other regions and host species are needed to acquire the diversity accurately.
Australia and Spiroxys ankarafantsika Roca & Garcia, 2008 from Pelusios castanoides, Madagascar. The nematodes in this study fall into Group 2 and differs from S. annulatus in having no tooth at median lobes, well marked cuticular collar behind the base of lips, constriction at the base of pseudolabium pronounce[4]. It differs from S. transversalata in having no tooth at median lobes of each pseudolabium[15]. It differs from S. chelodinae firstly in unpaired of median caudal papillae since located more anterior than that of Spiroxys present study, secondly no adanal papillae in the male of S. chelodinae[15]. This new species differs from Spiroxys ankarafantsika (S. ankarafantsika) in having double papillae on each pseudolabium, more anterior of deirid and longer spicule (9.5%–12.4% in S. ankarafantsika vs. 6% of body length)[18]. These differences are justified the designation of the new species S. sumatraensis.

Spiroxys Group 2 is characterized by 1 tooth only at median lobes of pseudolabial[15]. However the Group 2 also contains species without teeth at median lobes (S. annulata, S. transversalata, S. ankarafantsika), so it is considered to be the Group 3 of Spiroxys as suggested by Roca and García[18]. Spiroxys Group 2 found in three zoogeographical regions (Oriental, Australian and Ethiopian). The genus Spiroxys may have origin in non-marine chelonians of the suborder Cryptodira in the Holarctic or Oriental region[15]. The host of S. sumatraensis belongs to suborder Cryptodira[11], including S. annulata, whereas S. chelodinae and S. ankarafantsika found in pleuropodran turtles. This genus is probably widespread amongst the species of turtle when Gondwanaland existed and after the land fragmented, the speciation was occurred. Furthermore, the study of molecular could differentiate Spiroxys spp. accurately[19]. We agree with Roca and García who proposed the Group 3 for Spiroxys having pseudolabia without tooth at each lobes[18]. This group contains S. annulata, S. transversalata and S. ankarafantsika. Cissophyllus (Nematode: Kathianidae) has been reported from land turtle and lizard in Asia and America[6]. Four species have been described, i.e. C. laverani Railliet & Henry, 1912 from Testudo emys in India and Malaya, C. roseus from Tesudo emys in Java, Indonesia, Cissophyllus leytenis Tubangui and Villaamil, 1933 from Hydroaurus pisulosus in Philippine and Cissophyllus penitus (Leydi, 1886) Barreto, 1917 from Chrysemys scripta in North America. Cissophyllus in this study, morphologically agreed with C. lavearni and C. roseus. The complex anterior end (mouth) of the two species of Cissophyllus apparently is not different, however both of them are distinguished by the the rectilinear body (spindle shaped) in C. roseus, while the body of C. laverani is strongly curved ventrally[3]. C. laverani is the new record for host species (Amyda and Notochelys) and for locality Sumatera and Kalimantan.

Conflict of interest statement

We declare that we have no conflict of interest.

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