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## 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 lisemydis* in ratio of right-to-left spicule (1:3 in *M. jambiensis* vs. 1:3.8–5.6 in *M. lisemydis*) 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 chelodynae*. *S. sumatraensis* also differed from *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

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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 drier 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

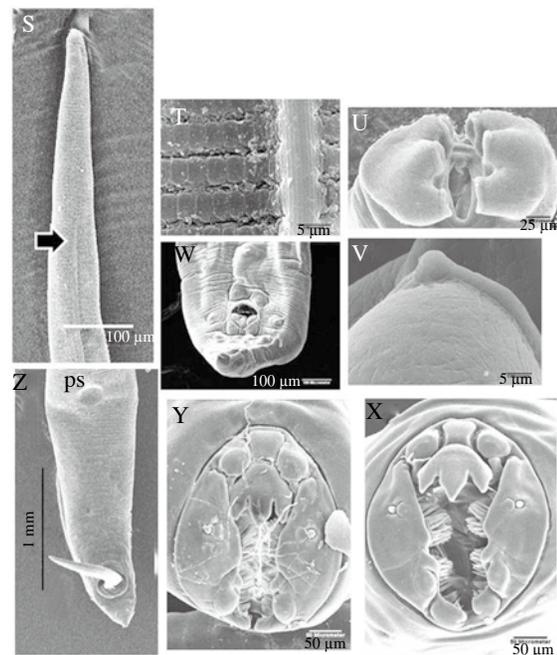
Nematodes found from freshwater turtles are *Monhyserides jambiensis* sp. n. (*M. jambiensis*) and *Spiroxys sumatraensis* sp. n. (*S. sumatraensis*) of which both were found in the intestine of *A. cartilaginea* from Jambi, Sumatera. *Cissophyllus laverani* (*C. laverani*) was found in the intestine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

### 3.1. Descriptions

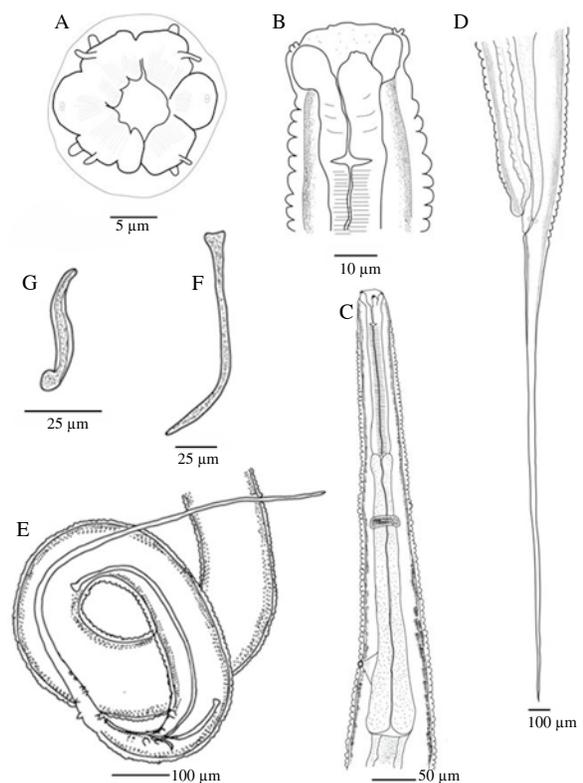
#### 3.1.1. *M. jambiensis* sp. n. (Nematoda: Atractidae)

##### 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).



**Figure 1.** Scanning electron microscope images of *M. jambiensis* sp.n. (S-T), *S. sumatraensis* sp.n. (U-W), *C. laverani* (X) and *C. roseus* (Y). S: Anterior part of male; T: Striae with scales; U: Pseudolips; V: Median lips showing teeth at the middle; W: Tail of male; X: Mouth of male; Y: Mouth of female.



**Figure 2.** Drawing image of *M. jambiensis* sp. n. (*M. jambiensis*).

A: Mouth of male, face view; B: Anterior end of male, lateral view; C: Anterior part of male, lateral view; D: Posterior end of male, lateral view; E: Posterior extremity of male, lateral view; F: Right spicule, lateral view; G: Gubernaculum, lateral view.

#### 3.1.1.2. Male (based on 5 specimens)

The body was 4.04 (3.75–4.23) mm long and 143 (138–150)  $\mu$ m

wide at the maximum level. The oesophagus was 509 (481–530)  $\mu\text{m}$  long and was divided into anterior and posterior parts which were 170 (160–177)  $\mu\text{m}$  and 339 (321–353)  $\mu\text{m}$  long, respectively. The ratio of anterior to posterior esophagus was 1:2. The nerve ring and excretory pore were 226 (215–228)  $\mu\text{m}$  and 418 (400–443)  $\mu\text{m}$  from anterior end, respectively. The tail was abruptly narrower behind cloaca, ending in a long, thin and tapering structure which was 842 (704–960)  $\mu\text{m}$  long. Caudal papillae were small, arranged as follows: 2 preloacals, 1 adloacal, 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)  $\mu\text{m}$  long and the right spicule was 119 (103–158)  $\mu\text{m}$  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)  $\mu\text{m}$  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)  $\mu\text{m}$  wide at maximum level. The oesophagus was 538 (490–580)  $\mu\text{m}$  long and was divided into anterior and posterior part which were 171 (131–210)  $\mu\text{m}$  and 367 (359–370) long, respectively. Its ratio was 1:1.7–2.7. The nerve ring and excretory pore were 238 (228–265)  $\mu\text{m}$  and 427 (380–480)  $\mu\text{m}$  from anterior end, respectively. The tail was abruptly narrower behind anus, ending in a long, thin and tapering structure and was 963 (938–1050)  $\mu\text{m}$  long. Vulva lay near anus at 1051 (973–1090)  $\mu\text{m}$  from posterior end, 88 (35–43)  $\mu\text{m}$  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 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 1V). 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)  $\mu\text{m}$  wide at the maximum level. The pseudolabium was 92 (85–98)  $\mu\text{m}$  long and 158 (155–161)  $\mu\text{m}$  wide. The nerve ring, deirid and excretory pore were 700  $\mu\text{m}$ , 420  $\mu\text{m}$  and 1240  $\mu\text{m}$  from anterior end, respectively. Oesophagus was 5830  $\mu\text{m}$  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 preloacal 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 3J 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)  $\mu\text{m}$  long. The tail was short with mucron at the tip, curved ventrally (Figure 3J) and it was 370 (350–390)  $\mu\text{m}$  long. Gubernaculum was small (Figure 3N) and it was 23  $\mu\text{m} \times 2.5$   $\mu\text{m}$  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)  $\mu\text{m}$  wide at the maximum level. The pseudolabia were 92 (80–110)  $\mu\text{m}$  long and 167 (162–181)  $\mu\text{m}$  wide. The nerve ring, deirid, excretory pore were 760, 420 and 5830  $\mu\text{m}$  from anterior end respectively. The oesophagus was tubular and it was 6070  $\mu\text{m}$  long. The vulva lay at anterior to anus and it was 18950  $\mu\text{m}$  from anterior end, in one specimen. The tail was 860  $\mu\text{m}$  long with conical tip. Eggs were elongated in uteri (Figure 3M), 99 (93–110)  $\mu\text{m}$  long and 48 (45–80)  $\mu\text{m}$  wide.

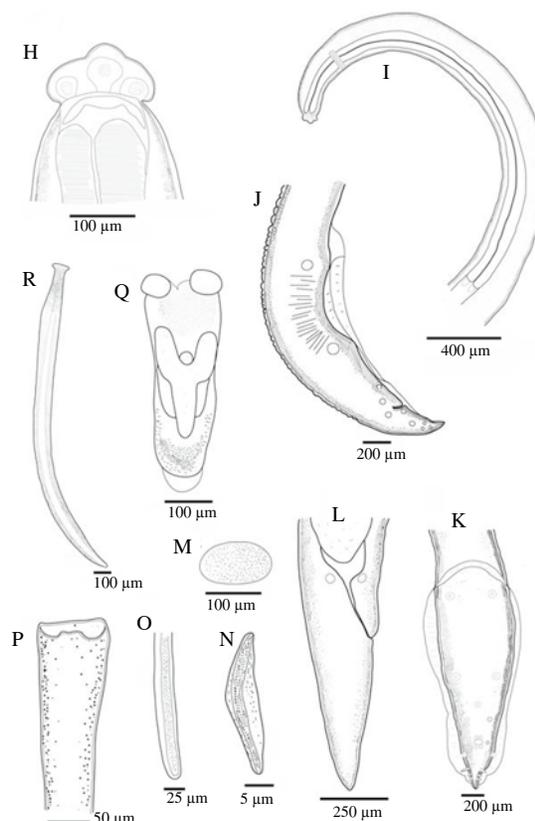


Figure 3. Drawing images of *S. sumatraensis* sp.n. (H-P) and *C. laverani* (Q-R).

H: Anterior end of male, lateral view; I: Anterior part of male, lateral view; J: Tail of male, lateral view; K: Tail of male, ventral view; L: Tail of female, ventral view; M: Egg; N: Gubernaculum, lateral view; O: Posterior end of spicule, ventro-lateral view; P: Anterior end of spicule, ventral view; Q: Accessory piece, ventral view; R: Spicule, lateral view.

Host: *A. cartilaginea*; Site: Intestine; Locality: Jambi, Sumatera, Indonesia; Specimens deposited: Museum Zoologicum Bogoriense-, Research Center for Biology-LIPI, Indonesia: MZBNa 666 (1 paratype male), MZBNa 678 (1 allotype female, 1 paratype male, 2 female); Etymology: this species was named after the island name of type locality.

### 3.1.3. *C. laverani* (Nematoda: Kathlaniidae)

#### 3.1.3.1. General

The body was cylindrical with striae along its length. The anterior end was tilted ventrally and posterior end was attenuated.

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. Oesophagus was divided into short anterior part, without musculature, followed by longer tubule 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)  $\mu\text{m}$  wide at the maximum level. The nerve ring and excretory pore were 950  $\mu\text{m}$  and 2 688 (2 650–2 725)  $\mu\text{m}$  from anterior end, respectively. The oesophagus was 3 930 (3 700–4 200)  $\mu\text{m}$  long. The tail was curved ventrally and abruptly narrower after cloaca and it was 483 (450–525)  $\mu\text{m}$  long. Preloacal sucker was presented (Figure 1Z), 300 (275–325)  $\mu\text{m}$  in diameter. Caudal papillae were pedunculated, arranged as follow: 8 pairs preanal, 7 pairs postanal. The spicules were, torch shaped (Figure 3R), stout, curved ventrally, alate until tip, similar and equal and it was 1 905 (1 760–2 075)  $\mu\text{m}$  long. Accessory piece was presented (Figure 3Q) and it was 465  $\mu\text{m}$  long and 165  $\mu\text{m}$  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)  $\mu\text{m}$  wide at the maximum level. From anterior end to nerve ring and excretory pore were 955 (880–1 025)  $\mu\text{m}$  and 3 065 (2 945–3 150)  $\mu\text{m}$ , respectively. The oesophagus was 3 848 (3 575–4 070)  $\mu\text{m}$  long. The tail was straight, narrower behind anus with knob at posterior tip and it was 695 (540–800)  $\mu\text{m}$  long. The vulva lay at 7 609 (6 050–9 760)  $\mu\text{m}$  from posterior end. The eggs were elongated, thick-shelled, 118 (114–121)  $\mu\text{m}$  long and 66 (63–70)  $\mu\text{m}$  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 oesophagus was short at anterior part without musculature, followed by longer tubule, with small bulb at posterior end of esophagus, 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)  $\mu\text{m}$  wide at the maximum level. The nerve ring and excretory pore were 915 (820–1 100)  $\mu\text{m}$  and 2 717 (2 550–2 900)  $\mu\text{m}$  from anterior end, respectively. The oesophagus was 3 800 (3 775–3 825)  $\mu\text{m}$  long. The tail was very short, straight, with small knob at the tip, 666 (675–700)  $\mu\text{m}$  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 brachipomus* 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 chitinized 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 testudinicola* (Baylis, 1933) from *Trionyx cartilaginea* in Pahang, Malaya[6,9]. Another reported species was *Monhysterides lissemeydis* (*M. lissemeydis*) 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. jambiensis*], number of preloacal papillae (4 in *M. piscicola* vs. 2) and shorter female tail (16% in *M. piscicola* vs. 30% of body length)[2]. It differs from *Monhysterides testudinicola* (*M. testudinicola*) in longer left spicule (5.6%–6% in *M. testudinicola* vs. 11.2%–12.7% of body length), ratio right-to-left spicule (1:2 in *M. testudinicola* vs. 1:3) and the numbers of caudal papillae[11,12]. *M. testudinicola* 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. lissemeydis* in no lateral alae, no gubernaculum, almost equal length of anterior and posterior esophagus and more preloacal papillae (8 in *M. lissemeydis* 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. jambiensis*.

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.

*Spiroxys* Schneider, 1922, is the genera described from *Emys orbicularis* and *Spiroxys contorta* as a species type[14]. Some species were reported from stomach and duodenum of non-marine turtle, frogs, salamander, and colubrid snakes from India, Malaya, Japan, China, Europa, Algeria and the US[15,16]. The genera was divided into 2 groups. The Group 1 was characterized by the presence of teeth at the each lobes of pseudolabia and the Group 2 was with teeth only at medium lobe[17]. The Group 2 contains *Spiroxys annulata* Baylis & Daubney, 1922 (*S. annulata*) from *Chitra indica*, India; *Spiroxys transversalata* Belous, 1963 (*S. transversalata*) from *Amyda sinensis*, Far East and *Spiroxys chelodinae* Berry, 1985 (*S. chelodinae*) from *Chelodynae* spp.,

Australia and *Spiroxys ankarafantstika* 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 pseudolabia[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. ankarafantstika* found in pleurodiran 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. ankarafantstika*.

*Cissophyllus* (Nematode: Kathlaniidae) 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 leytensis* Tubangui and Villaamil, 1933 from *Hydrosaurus 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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