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A New Record From East Malaysia: *Sphaerolaimus macrocirculus* (Nematoda)

NORLIANA MOHD ROSLI*[1], SHABDIN MOHD LONG[2], CHENG-ANN CHEN[3]

[1]Department of Biology, Faculty of Science and Mathematics, Sultan Idris Education University, 35900 Tanjong Malim, Perak; [2]Department of Aquatic Science, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia; [3]Borneo Marine Research Institute, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia.

ABSTRACT

A new record of *Sphaerolaimus macrocirculus* (Filipjev 1918) in East Malaysian coastal water was described. The species was isolated from the muddy area of Muara Tebas, Sarawak, Malaysia. A total of 47 species of *Sphaerolaimus* have been reported worldwide. Samples of *Sphaerolaimus macrocirculus* were previously recorded in the sublittoral mud area of temperate country. *S. macrocirculus* possesses a stout body (1.1 – 1.6 mm; De Man ratios: a (body length/body width) = 16.8 – 27.5; b (body length/oesophagus length) = 2.3 – 4.3; c (body length/tail length) = 5.6 – 8.6). The circular amphid is very clear and situated at the base of the cephalic capsule. In male, the amphid ranged from 11.3 – 14.3 µm in diameter (mean = 12.7 ± 1.3) and 0.4 c.d. (corresponding diameter). In female, the amphid ranged from 7.2 – 9.4 µm in diameter (mean 7.8 ± 0.9) and 0.2 c.d. The species is characterised by the presence of eight groups of subcephalic setae at the head region and three longer terminal setae.

Keywords**:** East Malaysia, *Sphaerolaimus*, Nematoda

INTRODUCTION

Sarawak is the largest of Malaysia's thirteen states. The state has a long coastline stretches over 800 km along the northwest coast of Borneo.

However, research on free-living nematodes is still scarce not only in Sarawak but Malaysia. Recently, only a few researches had been conducted on free-living marine nematodes in Malaysia (Shabdin & Othman 2005; Chen & Shabdin 2009; Chen *et al*. 2012). Before that, the studies in Malaysia only covered the ecology and diversity of meiobenthos (Sasekumar 1994; Somerfield *et al*. 1998; Shabdin & Othman 2005; Sakri *et al.* 2006; Shabdin 2006). Preliminary studies in Sarawak related to marine nematodes only covered the density of higher meiofauna taxa (Bejie *et al*. 1999; Tengku Balkis & Shabdin 2001). Chen *et al.* (2012) lead the study of marine nematode in Sarawak. Free-living nematodes play an important role in marine ecosystem providing the food source for macro-consumers (Chinnadurai & Fernando 2007) and also as pollution indicator. The present study has been conducted to assess the species that presence in Muara Tebas, Sarawak, Malaysia together with their taxonomic structure. Muara Tebas was chosen as the sampling site due to its geographical location at Sarawak River mouth where the freshwater meet the saline that contains unique community structure.

One species, *Sphaerolaimus macrocirculus* (Filipjev 1918) had been documented in the study area. This species is a new record of free-living marine nematodes in East Malaysian coastal waters. So far, about 47 species in this genus have been reported worldwide. *Sphaerolaimus penicillus* was recorded in Sabah (Shabdin & Othman 2008) and none from Sarawak. Most of the *Sphaerolaimus* spp. are recorded in temperate country. Genus *Sphaerolaimus* was described by Bastian (1865) with characters such as having buccal cavity surrounded by solid, heavily sclerotized buccal capsule (Warwick *et al.* 1998). The inner lining of oesophagus strongly cuticularised and groups of subcephalic setae can be observed clearly. *Sphaerolaimus macrocirculus* is characterized by having a large buccal cavity, barrel shaped

*Corresponding author: *norliana.m@fsmt.upsi.edu.my*

and heavily cuticularised. They have faint transverse striations of cuticle which are not always visible. The circular amphid is situated at the base of cephalic capsule. Three longer terminal setae are the characterization in this species that differ from the others. *S. macrocirculus* was previously recorded in the sublittoral mud area. Many species from genus *Sphaerolaimus* are known to be carnivores, feeding particularly on other nematodes (Filipjev 1918).

MATERIALS & METHODS

A transparent corer of four cm diameter was used to collect the samples. Samples for nematode study were preserved in 5% formalin in the field and were brought back to the laboratory for further analysis and identification.

Nematodes were extracted using a 45 µm sieve. They were rinsed with tap water to remove the formalin and the residues that retained on the sieve were transferred to a Petri dish and sorted under stereomicroscope. Then, they were mounted on slide using standard method (Platt & Warwick 1983).

The nematode morphology and characteristic were observed and drawn using compound microscope equipped with Camera Lucida (Model Zeiss MC 80 DX). The identifications were based on several keys of identification (Platt & Warwick 1983, 1988; Warwick *et al*. 1998). The following abbreviations were used: a, b, c: de Man's ratios; a.b.d.: anal/cloacal body diameter; c.d.: corresponding diameter; spic arc: length of spicule along the arc; V %: position of vulva as percentage of total body length from anterior.

RESULTS

Type specimen

Four males and nine females were examined. Holotype: male (SML0188). Paratypes: three males (SML0199, 0201, 0214). Allotype: female (SML0131). Paratypes: eight females (SML0166, 0185, 0186, 0189, 0191, 0215, 0216, 0219).

Type locality and habitat: Intertidal zones, Muara Tebas, Sarawak, Malaysia; located near the river mouth of Sarawak River. Muddy area dominated with *Avicennia marina*.

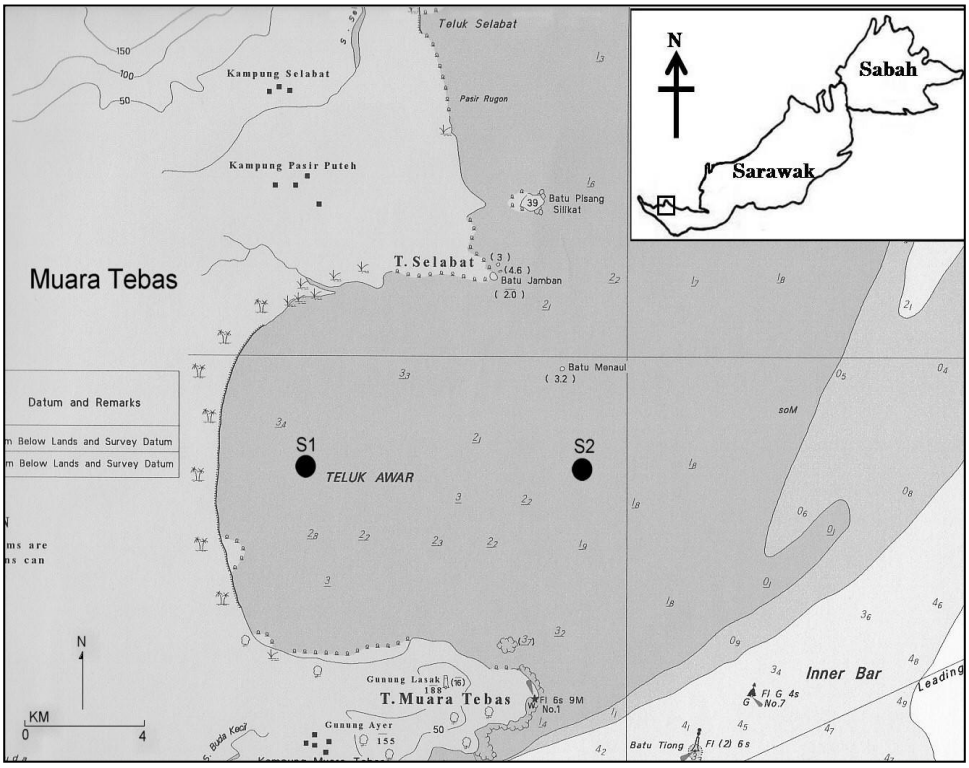

Figure 1. Site location (Muara Tebas, Sarawak).

Systematic

Class Adenophorea

Subclass Chromadoria

Order Monhysterida

Suborder Monhysterina

Superfamily Monhysteroidea

Family Sphaerolaimidae

Subfamily Sphaerolaiminae

Genus *Sphaerolaimus*

Species *macrocirculus*

Measurement

Holotype: L = 1238.6 µm, a = 23.9, b = 4.3, c = 7.4 (Table 1)

Males (n = 4, including holotype): L = (1.1 – 1.2 mm)

Allotype: L = 1532 µm, a = 27.5, b = 3.9, c = 8.3, V = 62.2% (Table 2)

Females (n = 9, including allotype): L = (1.2 – 1.6) µm, V = (62 – 77)%

Description

MALE (Holotype SML0188: range and/or average ± s.d. in parentheses): Body stout, 1.3 mm long (1.1 – 1.2, 1.2 ± 0.05) mm (Figure 2D). Maximum diameter 51.8 µm (51.8 – 66.9, 57.5 ± 6.5) µm, a = 23.9, b = 4.3, c = 7.4 (a = 17.3 – 23.9, 21.5 ± 2.9; b = 3.9 – 4.3, 4.1 ± 0.2; c = 6.9 – 7.6, 7.2 ± 0.4). Cuticle with faint and fine transverse striations, which mostly visible in all specimens. Somatic setae were short and scattered along the body.

Head diameter 26.4 µm (23.9 – 28.2, 26.0 ± 1.8) (Figure 2A) with six small labial papillae. Cephalic setae short (2.8 µm). Eight groups of subcephalic setae situated between amphids and cephalic setae with three to four setae in each groups (Figure 2A). Subcephalic setae long with 14.9 µm (14.1 – 16.9, 15.3 ± 1.2). Cervical setae length: long (7.2 – 13.8) µm and short (2.8 – 5.5) µm, arranged longitudinally in a row (eight or nine) from head region to the mid part of oesophagus. Clearly visible amphids situated at the base of the cephalic capsule. Amphid located at 25.3 µm (22.5 – 25.4, 24.3 ± 1.3) µm from anterior body end.

Table 1. Morphometrics of *Sphaerolaimus macrocirculus* (male).

Characters (µm)	Holotype	Paratype		
	SML0188	SML0199	SML0201	SML0214
Total body length	1238.6	1158.8	1229.3	1270.2
Head diameter	26.4	28.2	25.4	23.9
Amphids diameter	14.3	12.7	11.3	12.7
Amphids c.d.	0.5	0.4	0.4	0.5
Amphids ant.	25.3	25.4	23.9	22.5
Oesophagus length	289.7	284.1	295.3	328.7
Oesophagus c.d.	31.4	36.6	33.8	31.0
Max. diameter	51.8	66.9	55.7	55.7
Spicule arc	82.6	59.2	59.2	53.5
Tail length	167.1	167.1	178.3	167.1
Cloacal diameter	39.0	44.6	44.6	44.6
a.b.d	4.3	3.8	4.0	3.8
a	23.9	17.3	22.1	22.8
b	4.3	4.1	4.2	3.9
c	7.4	6.9	6.9	7.6

Notes: c.d.: corresponding diameter; a.b.d.: anal/cloacal body diameter; a, b, c: de Man's ratios; Spicule arc.: Length of spicule measured on arc; Amphids ant.: Amphids from anterior; Max. diameter: Maximum body diameter. All measurements in µm, except for de Man's ratios (a, b, c), c.d. and a.b.d.

Table 2. Morphometrics of *Sphaerolaimus macrocirculus* (female).

Characters (µm)	Allotype	Paratype							
	SML0131	SML0166	SML0185	SML0186	SML0189	SML0191	SML0215	SML0216	SML0219
Total body length	1532.0	1342.6	1665.7	1487.5	1214.5	1309.2	1292.5	1442.9	1387.2
Head diameter	35.8	37.5	30.3	33.6	30.9	32.0	34.2	29.8	31.4
Amphids diameter	7.2	8.3	7.7	6.6	7.2	8.3	8.8	9.4	18.2
Amphids c.d.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.5
Amphids ant.	28.1	23.1	31.4	33.1	26.4	29.8	28.7	27.5	29.8
Oesophagus length	395.5	573.8	468.0	456.8	356.5	417.8	323.1	378.8	434.5
Oesophagus c.d.	50.1	43.5	47.9	41.9	43.5	41.3	42.4	43.5	41.9
Max. diameter	55.7	61.3	89.1	83.6	72.4	61.3	55.7	78.0	55.7
Tail length	183.8	167.1	261.8	183.8	161.6	234.0	195.0	167.1	195.0
Anal diameter	39.0	39.0	50.1	44.6	44.6	55.7	39.0	50.1	39.0
a.b.d	4.7	4.3	5.2	4.1	3.6	4.2	5.0	3.3	5.0
Vulva (V %)	62.2	70.5	71.9	77.9	70.2	70.2	62.1	66.8	71.9
a	27.5	21.9	18.7	17.8	16.8	21.4	23.2	18.5	24.9
b	3.9	2.3	3.6	3.3	3.4	3.1	4.0	3.8	3.2
c	8.3	8.0	6.4	8.1	7.5	5.6	6.6	8.6	7.1

Notes: c.d.: corresponding diameter; a.b.d.: anal/cloacal body diameter; a, b, c: de Man's ratios; Amphids ant.: Amphids from anterior; Max. diameter: Maximum body diameter; V %: position of vulva as percentage of total body length from anterior. All measurements in µm, except for de Man's ratios (a, b, c), c.d. and a.b.d.

Amphid circular, 14.3 µm (11.3 – 14.3, 12.7 ± 1.3) µm in the diameter; 0.5 c.d. (0.4 – 0.5) c.d. Barrel-shaped buccal cavity; not completely surrounded by oesophageal tissue. Buccal cavity with alternating heavily and weakly punctuated areas. Two sclerotized rings at the posterior end of buccal cavity. Oesophagus widens posteriorly, but has no distinct bulb and strongly sclerotized. Oesophagus length 0.28 mm (0.28 – 0.32, 0.29 ± 0.02) mm. Cardia and nerve ring were not visible.

Single testes were noted. Spicule was long and slender from distal to proximal. Length of spicule form the arc was 82.6 µm (53.5 – 82.6, 63.6 ± 13.0) µm (Figure 2F). The gubernaculum were small. No precloacal supplements detected. Short caudal setae (2.2 – 3.3) µm scattered randomly at the end of tail. Two caudal gland noted in male. Tail 4.3 a.b.d. (3.8 – 4.3, 3.9 ± 0.3) a.b.d. The anterior tail was two thirds conical and the remainder is cylindrical. Tail length from anus, 0.16 mm (0.16 – 0.17, 0.16 ± 0.05) mm. Three long terminal setae (16 – 20.4) µm.

FEMALE (Allotype SML0131: range in parentheses). Similar to males in most features. Females body, 1.5 mm (1.2–1.6, 1.4 ± 0.1) mm longer than in males (1.1–1.2) mm (Figure 2C). Maximum body diameter 55.7 µm (55.7 – 89.1, 68.1 ± 13.0) µm, a = 27.5, b = 3.9, c = 8.3 (a = 16.8 – 27.5, 21.2 ± 3.6; b = 2.3 – 4.0, 3.4 ± 0.5; c = 5.6 – 8.6, 7.4 ± 1.0).

Head diameter 35.8 µm (29.8 – 37.5, 32.8 ± 2.6) (Figure 2B). Cephalic setae short, 3.9 µm (3.3 – 3.9, 3.2 ± 1.2) µm. Subcephalic setae long 19.3 µm (17.6 – 27.0, 19.8 ± 3.1), situated in eight group. Amphids located at a similar position in males, at the base of cephalic capsule, but smaller in diameter, 7.2 µm (7.2 – 9.4, 7.8 ± 0.9) µm. Amphid located at 28.1 µm (23.1 – 33.1, 28.7 ± 2.9) µm from anterior body end. Two sclerotized rings clearly noted at the posterior end buccal cavity of female. Oesophagus length in female longer than male, 0.39 mm (0.32 – 0.57, 0.42 ± 0.07) mm.

Females are monodelphic, with single anterior outstretched ovary, clearly can be seen in matured females. Ovary located right from the anterior intestine extended posteriorly to the vulva. Vulva located at 62% (62 – 77, 69.3 ± 5.0)% of body length. Vulva short, slit-like. One egg observed in uterus, oval shape. Caudal gland obscure in female. Caudal setae were short (5.5 – 7.2) µm but a little bit longer than male (2.2 – 3.3) µm. Tail length from anus 0.18 mm (0.16 – 0.26, 0.19 ± 0.03) (Figure 2E).

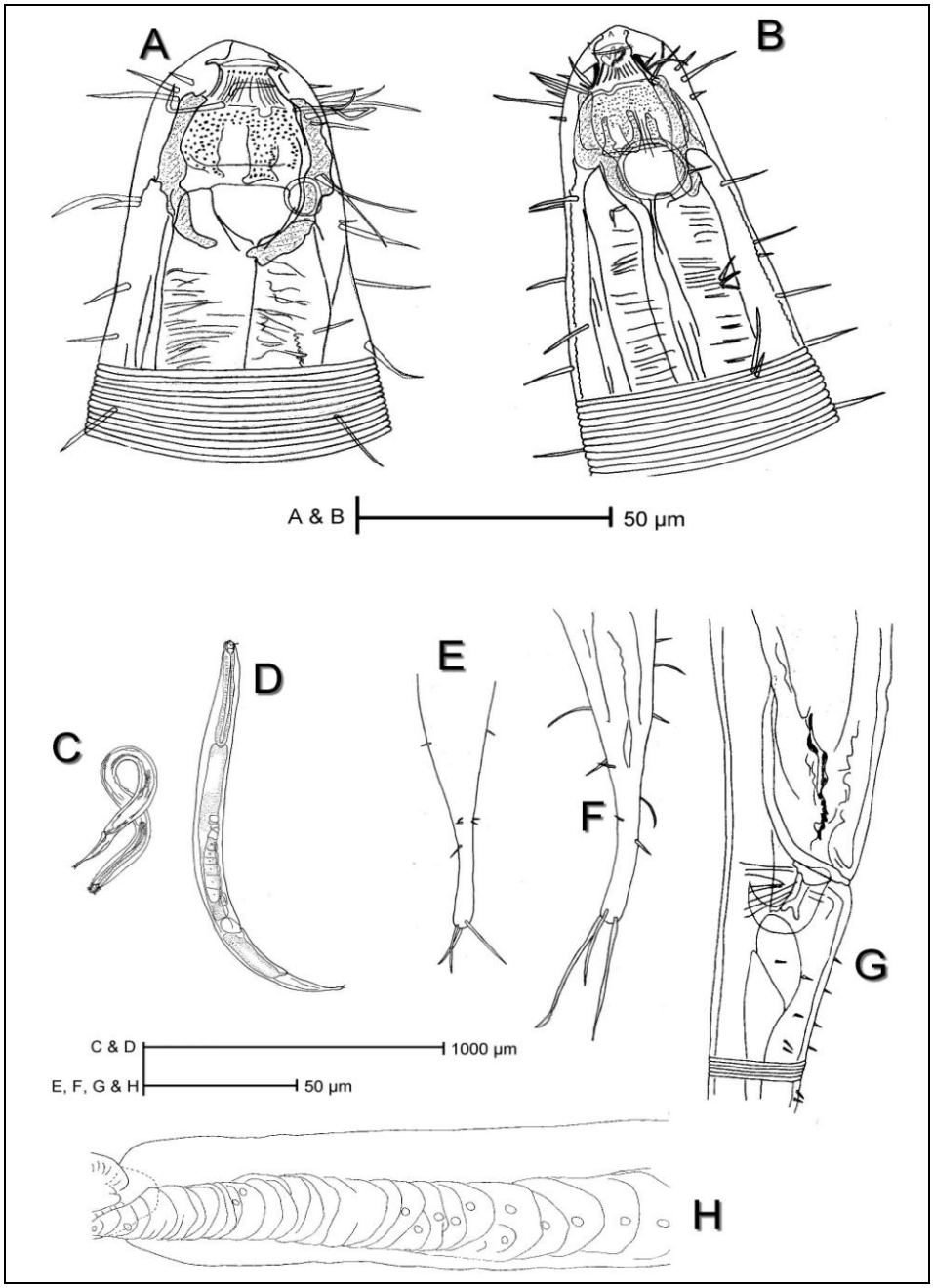

Figure 2. *Sphaerolaimus macrocirculus* Filipjev, 1918. Male (B, C, E, G, holotype SML0188): B. Head. C. Entire body. E. Tail. G. Cloacal region. Female (A, D, F, H, SML0131): A. Head. D. Entire body. F. Tail. H. Anterior, single ovary.

Distribution

Malaysia: Muara Tebas, Sarawak, Malaysia.

Global: Adriatic Sea (Travisi & Vidakovic 1997), Black Sea and Mediterranean Sea (Gerlach & Riemann 1973), European waters (De Smet *et al.* 2001), North Atlantic (Warwick *et al.* 1998), United Kingdom Exclusive Economic Zone (MEDIN 2011).

DISCUSSION

S. macrocirculus was first described by Filipjev, 1918. The present specimens examined conform well to the earlier description by Warwick *et al.* (1998) except for the smaller body size. The total length of the body in present specimen is shorter (1.2 – 1.6 mm) compared to the previous studies (1.9 – 2.2 mm) with smaller ratios. But, it is still

within the range that supported by the original population of Filipjev (1918) and Schuurmans Stekhoven (1950 in Deprez 2005) (1.8 mm; 1.2 – 2.1 mm respectively) compared to Warwick *et al.* (1998). Besides that, cephalic setae and amphids are also shorter and smaller compared to Warwick *et al*.'s (1998) population.

However, slight differences occurred in male specimen where the length of spicule differs markedly (160–167 µm in previous population) compared to 82.6 µm in the present. The precloacal supplements also were not visible in the present specimen, which they are visible in Warwick *et al.*'s samples (1998), by having seven precloacal supplements that contains raised pores (but they were not visible in all specimens).

These probably due to the differences in maturity of specimen since the precloacal supplements also absent in some of Filipjev's population. According to Gaugler & Bilgrami (2004), there is distinct adult stage for nematodes, separated adult from juvenile by a moult, where fully formed gonads and copulatory apparatus are only found in the adults (Warwick *et al.* 1998).

Vulva in female is located at 62% (with range 62 – 77%) of body length, differ with Warwick, Platt & Somerfield's population which at 75% of body length. Schuurmans Stekhoven (1950 in Deprez 2005) also discovered the vulva of female specimen at 70% body length which also differs with previous finding.

Little is known about variations in body-size which might occur from place to place (Warwick *et al.* 1998). Ansari *et al*. (2012), recorded several species found in tropical region have smaller size than species that were found in temperate region.

The locality of geographical areas can play a significant role in the variety of individual of nematodes species. These differences can also be regarded as individual variation and cannot alone be justified as two different species (Vitiello 1970 in Deprez 2005).

GENERAL CONCLUSIONS

In conclusion, this specimen is morphologically resembles *S. macrocirculus* except for the smaller body size. These differences (variations of the body size of *S. macrocirculus*) potentially contributed either by the differences in geographical locations (temperate region and tropical region) or the maturity stage.

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