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Two New Records of Free-living Marine Nematodes (Nematoda: Enoplida: Ironidae and Plectida: Leptolaimidae) from India

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Abstract

We report two new records of free-living Marine Nematodes from India, viz. *Trissonchulus provulvatus* Orselli and Vinciguerra, 1997 and *Antomicron quindecimpapillatus* Holovachov, 2012. Sediment samples were collected from Coastal regions of Puducherry. Morphological data along with description and distribution for both the species are provided here. Both the species reported here have been recorded previously only from their type locality: *T. provulvatus* Orselli and Vinciguerra, 1997 from the Mouth of River Simeto, Catania, Italy, while, *A. quindecimpapillatus* Holovachov, 2012 from Gullmarn Fjord near Fiskebackskil, Sweden.

Keywords: *Trissonchulus provulvatus, Antomicron quindecimpapillatus,* new records, nematoda.

Introduction

Free-living marine nematodes belonging to the phylum Nematoda are highly abundant to the extent that four out of five-bottom dwelling metazoans are nematodes (Bongers and Ferris, 1999). Considering both the terrestrial and the marine nematodes, about 25% of them are free-living marine forms (Du *et al.*, 2014). The order Enoplida Filipjev, 1929, consist of 7 suborders, amongst which the suborder Ironina Siddiqi, 1983, consist of one superfamily Ironoidea de Man, 1876, which further comprises of 3 families; Ironidae de Man, 1876, Leptosomatidae Filipjev, 1916 and Oxystominidae Chitwood, 1935. The genus *Trissonchulus* Cobb, 1920 belongs to the family Ironidae de Man, 1876. Furthermore, there are 16 valid species belonging to genus *Trissonchulus* Cobb, 1920. The species *Trissonchulus provulvatus* Orselli and Vinciguerra, 1997 is being reported for the first time from India. The order Plectida Malakhov, Ryzhikov and Sonin, 1982, consists of 2 suborders, namely Ceramonematina Cobb, 1933 and Plectina Malakhov, Ryzhikov and Sonin, 1982. Suborder Plectina consists of superfamily Leptolaimoidea Örley, 1880 and has two families Aphanolaimidae Chitwood, 1936 and Leptolaimidae Örley, 1880. Genus *Antomicron* belongs to the family Leptolaimidae Örley, 1880 and comprises 10 valid species. *Antomicron quindecimpapillatus* Holovachov, 2012 is being reported here for the first time from India.

Material and Methods

Sediment samples were collected from Pondy Marina (11°54'25.32"N; 79°49'41.39"E) and Kodikkarai (10°16'31.83"N; 79°49'9.49"E), both located in Puducherry, India. After collection, the sediment samples were fixed with 4% Formalin in filtered sea water. Rose Bengal stain was used to stain the samples after fixation (Williams and Williams, 1974). The stained samples were brought to the laboratory and were sieved using 2 mm Coarse sieve with the purpose to remove the larger particles and then passed in 53 µm mesh sieve to retain the meiofauna. The sieved residue was then examined under SZX-16 Olympus Microscope and free-living marine nematode specimens were picked out in cavity blocks containing Glycerin-Alcohol. The specimens were kept in desiccator containing anhydrous Calcium chloride for dehydration, following which permanent slides were prepared and identified under BX-53 DIC Olympus research

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microscope. The specimens were photomicrographed using DP27 camera.

Abbreviations used: *a*= Body Length/ maximum body diameter; *b*= Body length/ pharynx length; *c*= Body length/ tail length; c'= Tail length/anal body diameter

Systematic Accounts

Phylum NEMATODA Cobb 1932

Class ENOPLEA Inglis, 1983

Order ENOPLIDA Filipjev, 1929

Suborder IRONINA Siddiqi, 1983

Superfamily IRONOIDEA de Man, 1876

Family IRONIDAE de Man, 1876

Genus ***Trissonchulus*** Cobb, 1920

Trissonchulus provulvatus Orselli and Vinciguerra, 1997

Class CHROMADOREA Inglis, 1983

Subclass CHROMADORIA Pearse, 1942

Order PLECTIDA Malakhov, Ryzhikov and Sonin, 1982

Suborder PLECTINA Malakhov, Ryzhikov and Sonin, 1982

Superfamily LEPTOLAIMOIDEA Örley, 1880

Family LEPTOLAIMIDAE Örley, 1880

Subfamily LEPTOLAIMINAE Örley, 1880

Genus ***Antomicron*** Cobb, 1920

Antomicron quindecimpapillatus Holovachov, 2012

Results and Discussion

Trissonchulus provulvatus Orselli and Vinciguerra, 1997

(Figure 1; Table 1)

Materials examined: 2 Females and 3 Males: India, Puducherry, Pondy Marina, 11°54'25.32" N, 79°49'41.39" E, 16-ix-2021, coll. R. Datta (Reg. No. ZSI–HQ/NZC/WN.3574).

Description: *Male:* Body long and slender, the total body length varies from 3250.04-3296.87 µm. Smooth cuticle without any striations. The maximum body length varies from 47.54-51.5 µm. The head and the lip region are slightly set off. Labial and cephalic setae are absent, however six labials and ten cephalic papillae observed. Pocket-shaped amphids present, situated at a distance of 11.98-15.25 µm from the anterior most end and just posterior to the head region. The buccal cavity is in the form of a sclerotized tube, the anterior most region of which consist of three solid teeth. The two subventral teeth are curved and larger than the dorsal one. The pharynx without any prominent anterior or posterior bulb, however the posterior end is slightly thickened. Prominent heart shaped cardia present. Nerve ring is present, almost at a position half the length of the pharynx. The reproductive system in males is composed of paired and opposed testes. Spicules (Fig 1e) are 42.01-48.82 µm in length, curved ventrally and are cephalate at the proximal end, small sized gubernaculum present, 20.52-23.71 µm. Tail not very long, the terminal end is blunt and with the presence of ventrally opening caudal glands. Post cloacal papillae, six pairs in number have been observed (Fig 1g).

Female: Body is long and slender shaped, varying from 2781.5-3391.6 µm and with a maximum body width of 54.2-70.85 µm. Cuticle smooth. The head and the lip region are set off by a constriction and the internal surface of the lip region constitutes of minute denticles. Six labial and ten cephalic papillae are present. The region posterior to the lips constitutes of pocket-shaped amphid. Sclerotized tube-shaped buccal cavity present, (Fig 1d). Three solid teeth are present at the anterior end of the tube, the two sub ventral teeth (Fig 1b) are equal in shape and are bent, the dorsal tooth is smaller in size. Pharynx without any prominent bulb in anterior or posterior end, however the posterior end is slightly broadened, heart shaped cardia present beneath the posterior end of the pharynx (Fig 1c). Nerve ring is situated at almost a position half the length of the pharynx. Reproductive system is didelphic and opposed in females. Vulva situated at 1333.5-2485.3 µm from anterior end (Fig 1f). In comparison to males, females possess more rounded shaped and slightly longer tail (Fig 1h), Presence of ventrally opening caudal glands.

Remarks: The genus *Trissonchulus* under the family Ironidae was first erected by Cobb in 1920. The head sensilla is mostly papilliform, in case it is setiform it is very short, these characters differentiate this genus from the other genus under the family Ironidae. Presently 16 valid species represent this genus (Website Nemys: World Database of Nematodes; Bezerra *et al.*, 2022). The valid species are *T. acuticaudatus* Gagarin, Nguyen Vu Thanh, Nguyen Dinh Tu and Nguyen

Xuan Phuong, 2012; *T. acutus* (Gerlach, 1953) Gerlach and Riemann, 1974; *T. benepapillosus* (Schulz, 1935) Gerlach and Riemann, 1974; *T. dubius* Orselli and Vinciguerra, 1997; *T. janetae* Inglis, 1961; *T. latispiculum* Chen and Guo, 2015; *T. latus* (Wieser, 1953) Inglis, 1961; *T. littoralis* Yeates, 1967; *T. mangrovi* Nguyen Vu Thanh and Gagarin, 2015; *T. minor* Gagarin *et al.*, 2012; *T. obtusus* (Schuurmans Stekhoven, 1935) Inglis, 1961; *T. oceanus* Cobb, 1920;*T. provulvatus* Orselli and Vinciguerra, 1997; *T. quinquepapillatus* Yeates, 1967; *T. raskii* (Chitwood, 1960) Inglis, 1961; *T. reversus* Chitwood, 1951.

T. provulvatus was first described by Orselli and Vinciguerra, 1997 from Mouth of River Simeto (Catania, Italy) on the basis of reduced anterior female genital tract region and the anterior vulva. The species also differed from the other species in the shape of spicules and gubernaculum and also it did not consist of any precloacal papillae. The species described here conforms well with the first description of the species, almost same amphid and buccal cavity width. However, the body length of the species reported here is slightly smaller than the original description (2781.5-3391.6 µm vs 3570-3750 µm) with slightly greater tail length (74.06-92.09 µm vs 57.5- 65 µm).

Antomicron quindecimpapillatus Holovachov, 2012

(Figure 2; Table 2)

Materials examined: 3 Males: India, Kodikkarai, Puducherry; 10°16'31.83" N, 79°49'9.49" E, 19-ix-2021, coll. R. Datta (Reg. No. ZSI–HQ/NZC/ WN.3416). 1 Females and 2 Males: India, Pondy Marina, Puducherry; 11°54'25.32" N, 79°49'41.39" E, 16-ix-2021, coll. R. Datta (Reg. No. ZSI–HQ/NZC/ WN.3575).

Description: Male: The male body is slightly tapering in the anterior region and appears curved after fixation (Fig 2a). The male body size varies from 921.4- 1173.95 µm. The maximum body diameter is 25.13- 35.42 µm in the mid body region. Cuticle is completely annulated. The head and the labial region are not set off and the anteriormost end is slightly rounded. The anterior most annule starts from the base of the amphid (Fig 2b). Labial setae (2.47-3.59 µm) observed and smaller than the prominent cephalic setae, 8.82- 11.94 µm (Fig 2c). At the anterior most part of the isthmus, nerve ring is present. The secretory-excretory system is present. Buccal cavity is minute and tubular with undifferentiated cheilostom and gymnostom. The pharynx is heavily muscular, the anterior part is cylindrical without any bulb and the posterior region is with distinct oval basal bulb (Fig 2d). Anterior part of the pharynx is divided into procorpus, metacorpus, both cylindrical and a narrow isthmus. Cardia is small. The reproductive system in males composed of two testes. Spicules short (30.15- 38.07 µm) and symmetrical, the shaft is arcuate subcylindrical and the manubrium is ovoid in shape (Fig 2e). Plate-like gubernaculum present, 17.91-20.82 µm. A total of fifteen midventral tubular supplements are present anterior to the spicule. Two pairs of precloacal setae (Fig 2f) and four pairs of caudal setae have been observed. Tail is small and elongate-conoid shaped. The tail tip is unstriated and do not possess swollen tip (Fig 2h). Three caudal glands present.

Female: Body short and tapering in the anterior region. The whole body is completely annulated except in the anterior most part and tail tip region. In the anterior end, annules begin just posterior to the amphid. The head is not set off, labial setae is small 3.2 µm. The cephalic setae larger in size than the labial setae, 6.99 µm. Amphid present almost at the anterior end. Nerve ring is present at 79.8 µm from anterior end. The anterior most part of the pharynx is without bulb and is divided into procorpus, metacorpus and isthmus, the posterior end consists of a basal ovoid bulb. The bulb is 45.6 µm in length. Small cardia present beneath the pharyngeal bulb. The reproductive system is didelphic (Fig 2g). The tail is not very long, elongate-conoid in shape, and lack annulations towards the tip.

Remarks: The genus *Antomicron* under the family Leptolaimidae was first erected by Cobb, 1920. Presently a total of 10 species are considered valid under this genus (Website Nemys: World Database of Nematodes; Bezerra *et al.*, 2022), namely *A. alveolatum* Villares and Pastor de Ward, 2011; *A. chinensis* Zhai, Wang and Huang, 2020; *A. elegans* (de Man, 1922) De Coninck, 1965; *A. holovachovi* Zhai, Wang and Huang, 2020; *A. intermedius* Gagarin and Nguyen Vu Thanh, 2005; *A. lorenzeni* Holovachov, 2012; *A. pellucidum* Cobb, 1920; *A. pratense* Lorenzen, 1966; *A. profundum* Vitiello, 1971; *A. quindecimpapillatus* Holovachov, 2012.

A. quindecimpapillatus Holovachov, 2012 was described as a new species from, Gullmarn Fjord near Fiskebackskil, Sweden, on the basis of its difference in body length and number of tubular supplements. This species consists of 15 tubular supplements whereas the other consists of a smaller number of supplements, 2-10 in number. The species being reported here for the first time from India, conforms well with the original description, except in having slightly

smaller body length and pharynx length (869.37-1173.95 vs 1390-1459 µm and 108.81- 137.21 µm vs 150-151 µm respectively). All the male specimens consist of 15 tubular supplements.

Discussion

The two species *Trissonchulus provulvatus* Orselli and Vinciguerra, 1997 and *Antomicron quindecimpapillatus* Holovachov, 2012 are being reported here or the first time from India. Both the species have been collected from Puducherry. Both the species have been recorded previously only from their type locality: *T. provulvatus* Orselli and Vinciguerra, 1997 from the Mouth of River Simeto, Catania, Italy, while, *A. quindecimpapillatus* Holovachov, 2012 from Gullmarn Fjord near Fiskebackskil, Sweden.

Table 1: Morphometrics of *Trissonchulus provulvatus* Orselli and Vinciguerra, 1997(measurements in µm). Mean values in parenthesis.

Characters	Male	Female
n	**2**	**3**
Body Length	3250.04-3296.87 (3273.45)	2781.5-3391.6 (3021.5)
Maximum body diameter	47.54-51.5 (49.52)	54.2-70.85 (60.19)
Head diameter	20.6-24.2 (22.4)	22.99-23.89 (23.36)
Amphid from anterior end	11.98-15.25 (13.61)	9.76-12.62 (10.93)
Amphid diameter	9.55-11.89 (10.72)	7.54-10.5 (9.38)
Amphid length	4.93-5.54 (5.235)	5.56-8.04 (6.41)
Amphid cbd	17.87-30.03 (23.95)	25-27.1 (25.9)
Buccal cavity length	43.67-48.01 (45.84)	50.09-54.09 (52.03)
Nerve ring from anterior end	132.28-149.95 (141.11)	143.94-162.6 (152.55)
Nerve ring cbd	37.04-45.81 (41.42)	43.2-55.56 (49.21)
Pharynx length	345.03-366 (355.81)	335-382.6 (351.42)
Pharynx cbd	38.52-47.14 (42.83)	48.09-61.06 (53.94)
Anal body diameter	38.54-46.16 (42.35)	41.9-46.6 (44.14)
Tail length	74.06-82.52 (78.29)	84.9-92.09 (90.85)
Spicule length as chord	45.03-53.71 (49.37)	-
Spicule length as arc	42.01-48.82 (45.415)	-
Gubernaculum length	20.52-23.71 (22.11)	-
Vulva from anterior end	-	1333.5-2485.3 (1799.63)
a	64.01-68.36 (66.19)	47.87-53.34 (50.43)
b	8.99-9.41 (9.21)	8.26-8.86 (8.59)
c	39.95-43.88 (41.92)	29.10-34.05 (30.89)
c’	1.78-1.92 (1.85)	2.02-2.46 (2.22)

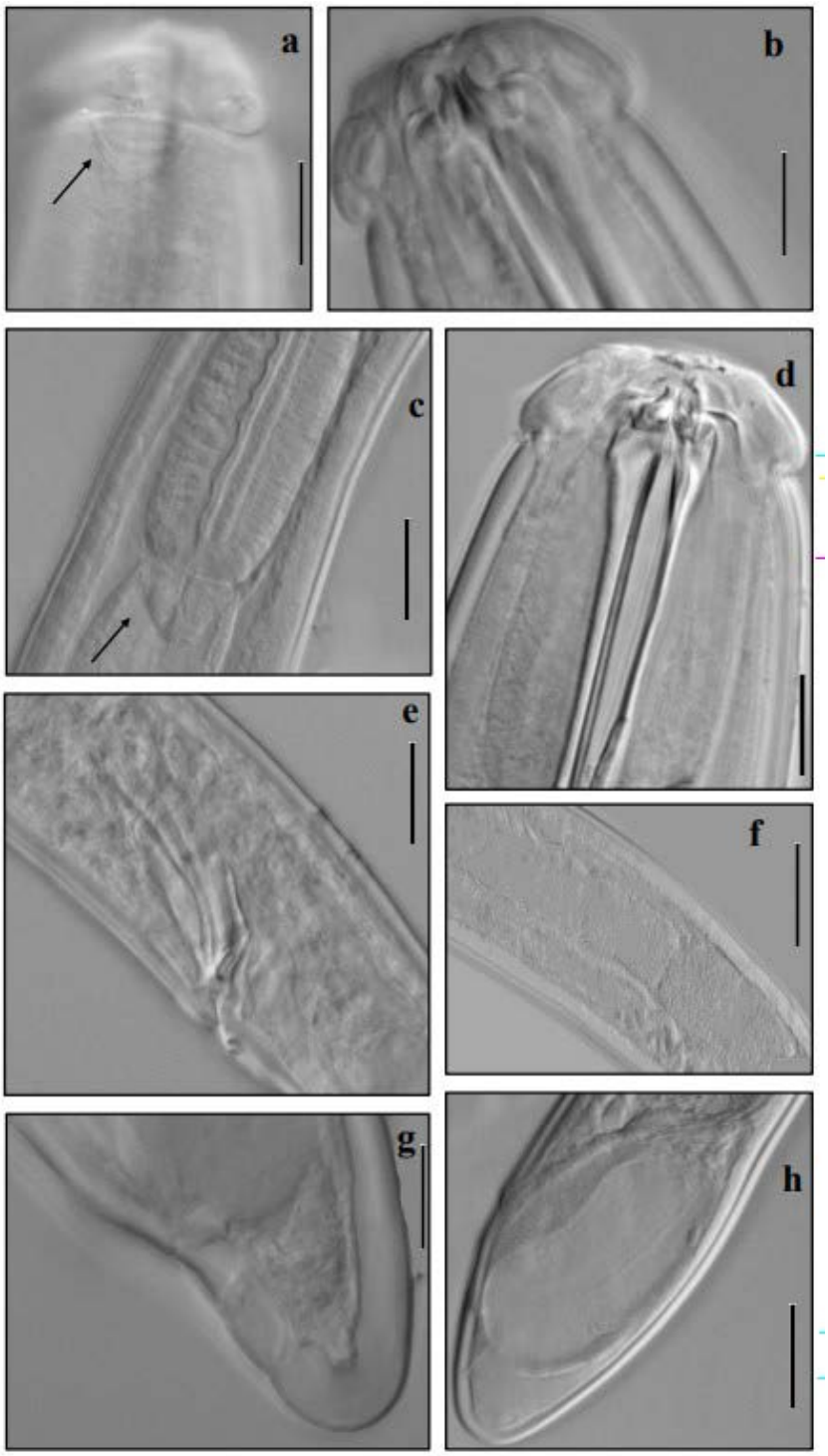

Figure 1: *Trissonchulus provulvatus* Orselli and Vinciguerra, 1997 (WN.3574 *Trissonchulus provulvatus*) (LM photographs) a: Female, amphid. b: Female, head and sub ventral teeth. c: Female, cardia. d: Female, buccal cavity e: Male, spicule. f: Female, genitalia. g: Male, post cloacal papillae. h: Female, tail. Scale bars: a, b, c, d, e, f, g and h: 5 µm.

Table 2: Morphometrics of *Antomicron quindecimpapillatus* Holovachov, 2012 (measurements in µm). Mean values in parenthesis.

Characters	Male	Female
n	**5**	**1**
Body length	921.4- 1173.95 (1016.72)	869.37
a	26.01- 39.01 (34.52)	27.68694268
b	7.21- 8.9 (7.95)	7.989798732
c	10.68- 13.02 (12.08)	9.249601021
c’	2.51- 4.04 (3.37)	3.356785714
Body diameter	25.13- 35.42 (29.86)	31.4
Labial setae	2.47-3.59 (3.09)	3.2
Pharynx length	115.33- 137.21 (127.88)	108.81
Tail length	73.75- 92.02 (84.21)	93.99
Anal or cloacal body diameter	21-29.8 (25.48)	28
Labial region diameter	8.06-16.84 (12.02)	12.5
Cephalic setae length	8.82- 11.94 (10.37)	6.99
Amphid length	5.23- 6.58 (6.29)	8.11
Amphid width	5.33- 7.39 (6.73)	11.61
Procorpus length	23.85 -30.06 (25.61)	21.9
Metacorpus length	22.1- 26.2 (24.26)	22.87
Isthmus length	37.35- 60.88 (48.25)	43.2
Bulbus length	39.41- 49.41 (43.99)	45.6
Nerve ring from anterior end	46.49- 82.4 (58.366)	79.8
Excretory pore from anterior end	79.1- 87.6 (81.62)	81.2
Spicule length	30.15- 38.07 (34.46)	-
Gubernaculum length	17.91- 20.82 (19.14)	-
Tubular supplements	15	-

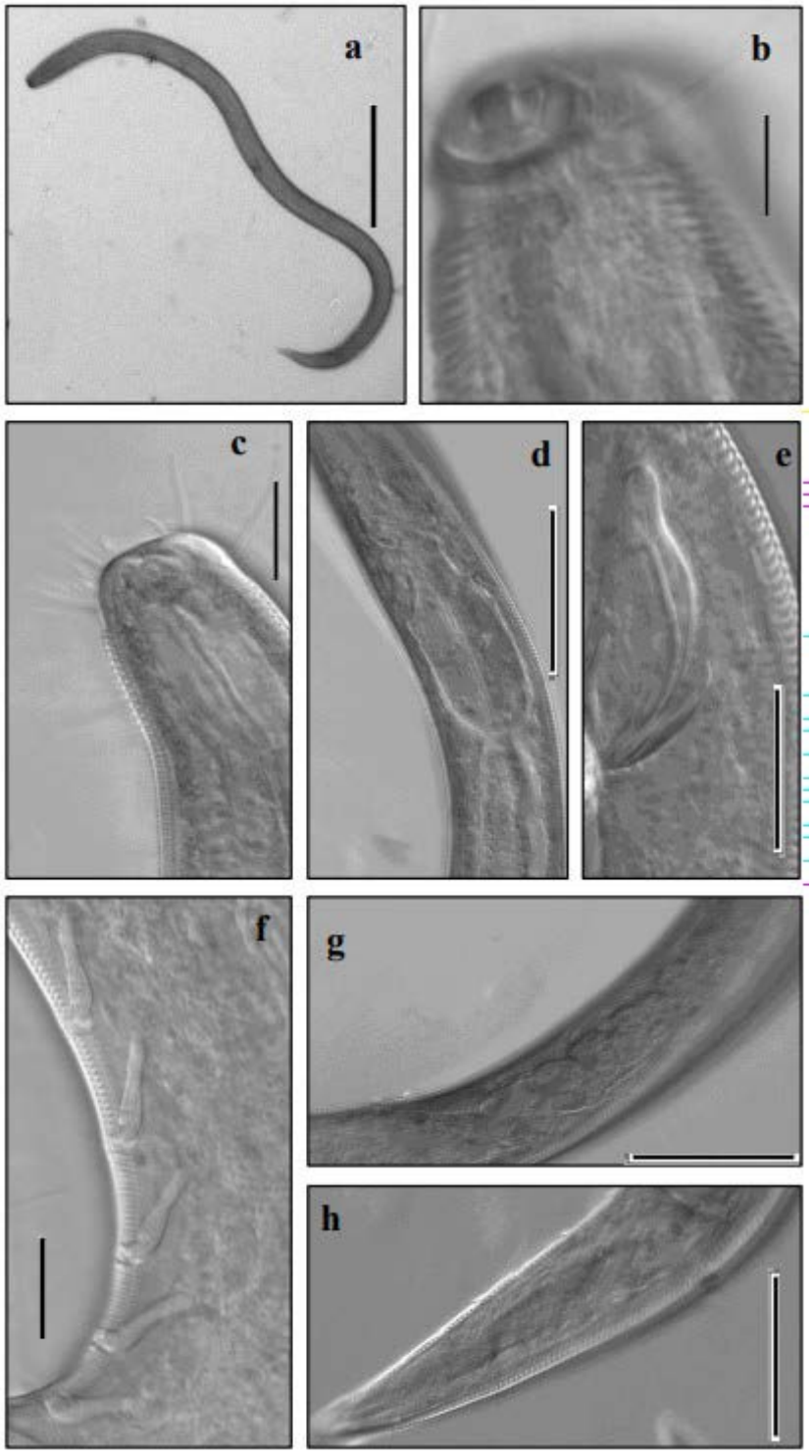

Figure 2: *Antomicron quindecimpapillatus* Holovachov, 2012. a: Male, whole body b: Male, amphid. c: Male, labial and cephalic setae. d: Male, posterior pharyngeal bulb. e: Male, spicule. f: Male, precloacal supplements. g: Female, genitalia. h: Male, tail. Scale bars: a= 200 µm; d and g= 50 µm; b, c, e, f and h= 5 µm.

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