

Two new free-living marine nematode species from an intertidal sandy-rocky shore on Pulau Ubin, Singapore with a key to the valid species of the genera *Prooncholaimus* and *Acanthonchus*

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Abstract. Marine nematodes belonging to two genera *Prooncholaimus* and *Acanthonchus* are described from Tanjung Tajam on the westernmost end of Pulau Ubin in the East Johor Strait. *Prooncholaimus tani* new species is characterised by having short cervical setae, a large buccal cavity, pocket-shaped amphid, thin gubernaculum and curved, L-shaped spicules. *Acanthonchus singaporensis* new species has a cuticle striated with transverse rows of minute punctations but is irregular laterally at the posterior region; cervical setae are present posterior to the amphid, the latter being multispiral with a total of 5.5 turns. Spicules are paired, cuticularised. The gubernaculum is also paired and almost equal length to the spicules. Six tubular supplements are present with the most anterior large and heavily cuticularised with a hook near the posterior end of the supplement.

Key words. Marine nematodes, intertidal, rocky, taxonomy, Singapore

INTRODUCTION

Studies on marine nematodes in the Southeast Asian region have been carried out recently in the following countries: Philippines (Rho & Kim, 2005); Thailand (Hope & Aryuthaka, 2009); Vietnam (Nguyen et al., 2008; 2011; Nguyen & Gagarin, 2011); Malaysia (Sasekumar, 1994; Shabdin & Othman, 1999; Chen et al., 2012a, b; Norliana et al., 2013; Shabdin et al., 2013; Chen & Shabdin, 2015). However, there is currently no information available concerning the marine nematode fauna in Singapore. This pioneer study aims to document the marine nematode species from the intertidal rocky shore of Tanjung Tajam in Singapore, and to provide a taxonomic key to the valid species of two genera occurring in this region.

MATERIAL AND METHODS

Samples were collected from a small intertidal sandy-rocky beach at Tanjung Tajam (SW 102–1°25.408'N 103°55.589'E) located on the westernmost corner of Pulau Ubin in the East Johor Strait. Sediment samples were collected to a depth of 5 cm with perspex cores (diameter=2.5 cm), sieved using a 45 µm mesh size sieve and were preserved in 5% formaldehyde. Each sample was washed into a lined Petri dish and nematodes were sorted out under a stereoscopic

microscope (Model Zeiss Stemi SV 6). Nematodes were later transferred to a cavity block containing 90% freshwater, 5% glycerol, and 5% ethanol prior to mounting on a microscopic slide with anhydrous glycerol (Platt & Warwick, 1988). The descriptions were made from glycerin mounts using a differential interference contrast microscope (Olympus BX 51) and the drawings were produced with the aid of camera lucida and Zeiss AxiosKop II plus. The following abbreviations are used throughout the text and figures: a, body length/maximum body diameter; b, body length/oesophagus length; c, body length/tail length; a.b.d., anal body diameter; h.d., head diameter; L, body length. Holotype samples are deposited in the Lee Kong Chian Natural History Museum, National University of Singapore with catalogue numbers ZRC.NEM 0001 and ZRC.NEM 0002 while the paratype samples are deposited in the Museum of Borneo Marine Research Institute under the catalogue numbers of BMRI.NEM 0001 and BMRI.NEM 0002.

SYSTEMATICS

Family Oncholaimidae Filipjev, 1916

Genus *Prooncholaimus* Micoletzky, 1924

Diagnosis (modified from Platt & Warwick, 1988 and Micoletzky, 1924). Buccal cavity with large sub ventral tooth; amphid pocket-shaped; spicule usually long; the most distinct characteristic to differentiate this genus from the other genera under the same family is the presence of large bubble-like cells in body cavity between intestine and longitudinal chords; gubernaculum present.

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Table 1. Measurements of *Prooncholaimus tani* new species (all measurements in μm)

Characteristic	Holotype (♂)	Paratype (♂)
Body length	1827	2119
a	26.94	27.33
b	5.18	6.47
c	18.58	21.46
c'	2.66	2.32
Head diameter	25.99	26.08
Buccal cavity length	38.63	43.51
Amphid width	7.3	8.79
Amphid / c.b.d % (relation of amphid diameter to labial body diameter, %)	28.08	33.76
Excretory pore from anterior body end	59.22	68.83
Oesophagus length	352.72	327.74
Maximum body diameter	67.85	77.54
Spicule length	78.50	72.90
Cloacal body diameter	36.97	42.58
Tail length	98.37	98.77

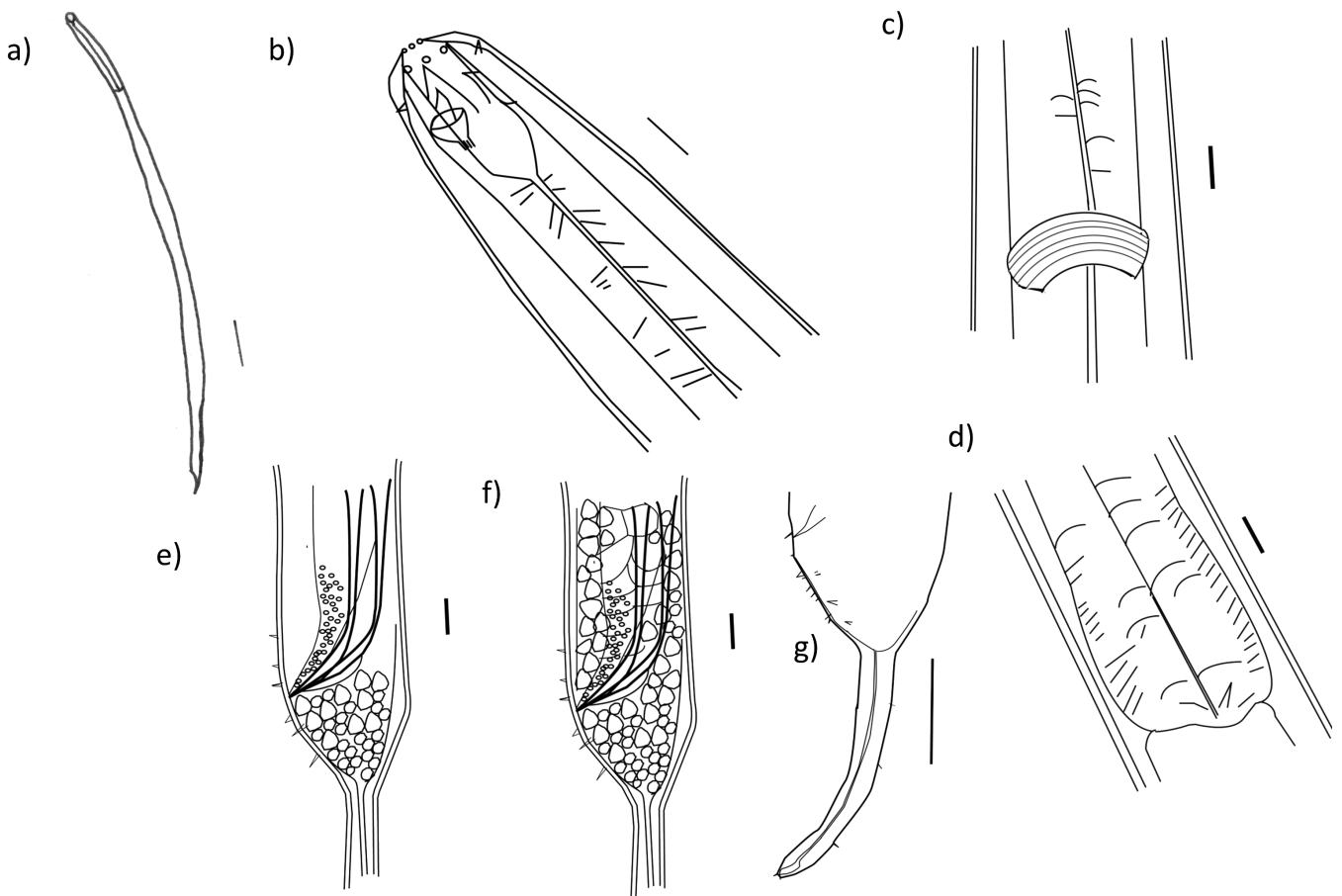


Fig. 1. *Prooncholaimus tani* new species. Holotype: a, total; b, head region; c, nerve ring; d, posterior end of oesophagus; e, spicules; f, large bubble-like cells in body cavity; g, tail region. Scale bars = 200 μm [a]; 10 μm [b–g].

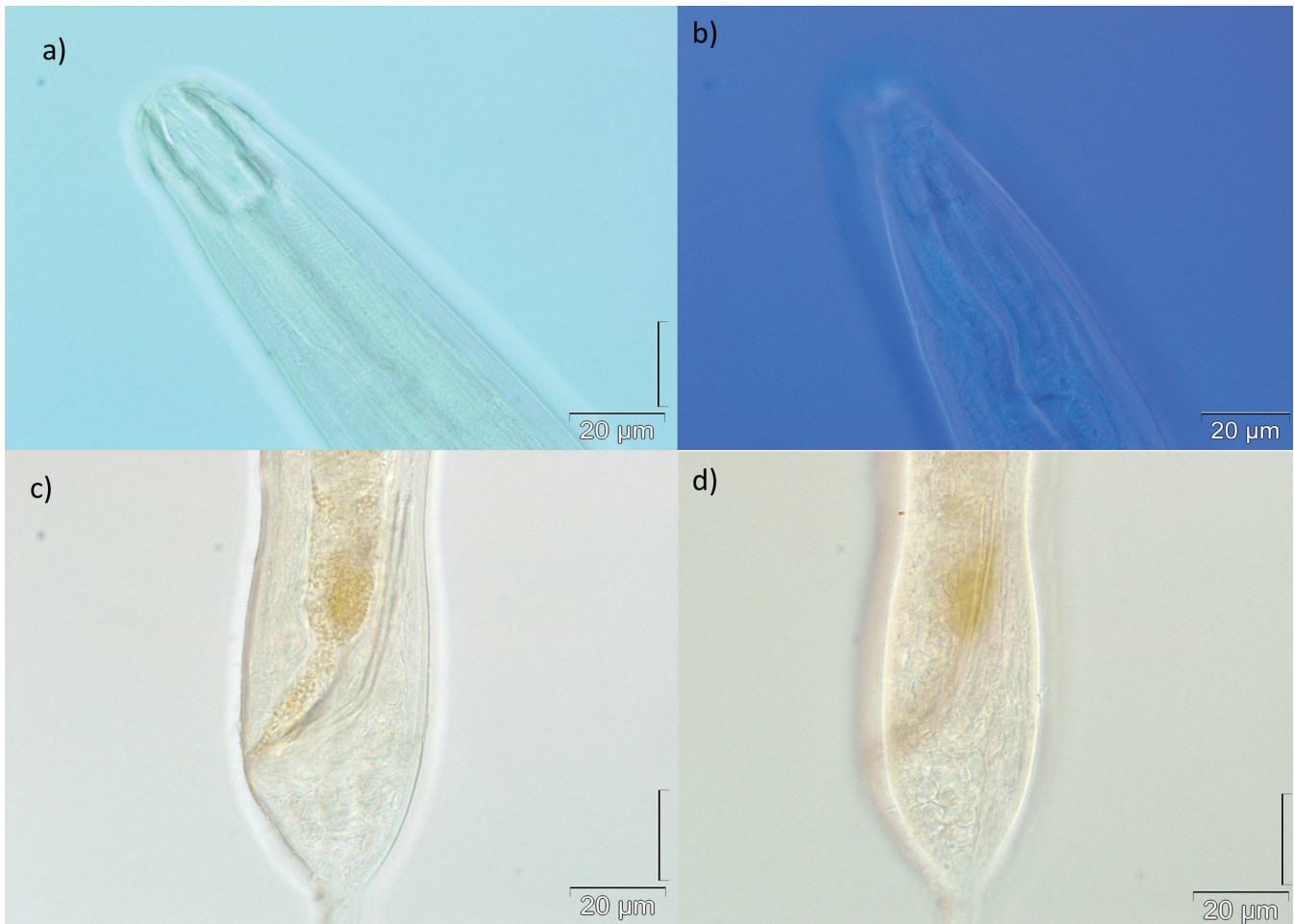


Fig. 2. *Prooncholaimus tani* new species. Holotype: a, head region; b, amphid; c, spicule; d, large bubble-like cells in body cavity.

***Prooncholaimus tani* new species**

(Figs. 1, 2)

Measurements. Refer to Table 1.

Holotype. Male: Body length 1827 µm. Six inner labial papillae followed by six outer labial papillae located at the anterior part of head region and followed by four extreme short cephalic setae in papillae form. Short cervical setae present. Head blunt (width = 29.0 µm). Buccal cavity large, 38.6 µm and surrounded by six prominent lip-lobes. Buccal cavity length to width, 2.60. Left subventral tooth large while the other two equal in size. Amphid pocket-shaped (7.3 µm width; 7.0 µm length) located at the same level of the smaller tooth. Cuticle smooth and cuticle pores were observed to be scattered throughout the body region. Excretory pore 63.6 µm from anterior end (approximately 3.2% of total body length). Oesophagus slightly expands along its entire length (352.7 µm) forming a swollen posterior end but not a true bulb (width of the oesophagus base 37.4 µm). Nerve ring located half way along oesophagus of total length (164.5 µm from anterior).

Testes diorchic and in tandem. Spicules are simple, paired, equal in length, cuticularised, curved, L-shaped, 78.5 µm (2.12 a.b.d). Barb absent. Spicule length in relation to cloacal body diameter, 2.12. Gubernaculum small and thin

(approximately 13.4 µm). Vas deferens with sperm cells. Large bubble-like cells present in body cavity between intestine and longitudinal chords (see Figs. 1f, 2d) but arranged in an irregular form. Pre- and post-cloacal setae present together with some short setae along the tail region. Caudal gland not observed. Two short terminal setae been observed. Tail length in relation to cloacal body diameter, 2.61. Tail funnel-shaped (98.37 µm; 2.66 a.b.d) and spinneret present at the tail tip.

Etymology. The species is named based on the family name of Tan Koh Siang from Tropical Marine Science Institute (National University of Singapore) for his great efforts in successfully organising the Comprehensive Marine Biodiversity Survey.

Diagnosis. In 1924, Micoletzky created the genus *Prooncholaimus* to allocate two described species: *Oncholaimus megastoma* Eberth 1863 and *Metoncholaimus eberthi* Filipjev 1918 and the latest species only been described in 1971 by Inglis. A total of 12 species had been found so far: *P. aransas* Chitwood, 1951; *P. armiger* Gerlach, 1955; *P. banyulensis* Inglis, 1962; *P. eberthi* Filipjev, 1918; *P. hastatus* Wieser & Hopper, 1966; *P. keiensis* Kreis, 1932; *P. longisetosus* Kreis, 1932; *P. mawsonae* Inglis 1971; *P. mediterraneus* Stekhoven 1943; *P. megastoma* Eberth, 1863; *P. obtusicaudatus* Kreis, 1932; *P. ornatus* Kreis, 1932. The

present *P. tani* new species, is similar to *P. banyulensis* based on the elongate-conical, gradually narrowed tail. In addition, the cephalic setae of *P. tani* new species are papillate compared to the others which have only short setae. The arrangement of the bubble-like cells is irregular as compared to its congeners. The character that distinguishes *P. tani* new species, from the other known species is the curved, L-shaped spicules. All the other species are known to possess straight spicules. A species identification key for the genus is provided below. Three species (*P. keiensis* Kreis, 1932; *P. longisetosus* Kreis, 1932 and *P. obtusicaudatus* Kreis, 1932) are excluded from the key as only juveniles and female specimen had been used for descriptions, hence we have not included them in this present identification key.

Type material. Holotype: ZRC.NEM 0001; Paratype: IPMB. NEM 0001. Collected by Chen Cheng-Ann from Tanjung Tajam (SW 102) Rocky area on 27 October 2012.

Key to all known species of *Prooncholaimus* (except *P. keiensis*, *P. longisetosus* and *P. obtusicaudatus*).

1. Tail short; cephalic setae long; spicule straight without barb. *P. armiger* Gerlach, 1955
 - elongate-conical, gradually narrowed 2
2. Spicule straight 3
 - Spicule curved and L-shaped spicules; without barb; cephalic setae short; bubble-liked cells arranged irregularly *P. tani* new species
3. Spicule without distinct set off barb 4
 - Spicule with distinct set off barb 5
 - Spicule without barb; pre and post cloacal setae present 6
 - Spicule without barb; pre and post cloacal setae absent *P. ornatus* Kreis, 1932
4. Posterior spicule blunt *P. eberthi* Filipjev, 1918
 - Posterior spicule sharply pointed; pre and post cloacal setae present *P. mawsonae* Inglis, 1971
 - Posterior spicule sharply pointed; pre and post cloacal setae absent *P. megastoma* Eberth, 1863

5. Three long terminal setae *P. banyulensis* Inglis, 1962
 - One short terminal setae *P. hastatus* Wieser & Hopper, 1966
6. Two widely separated subventral hair and a subventral apical setae *P. mediterraneus* Stekhoven 1943
 - Without any subventral hair and subventral apical setae *P. aransas* Chitwood, 1951

Family Cyatholaimidae Filipjev, 1918

Genus *Acanthonchus* Cobb, 1920

Diagnosis. (modified from Cobb, 1920; Platt & Warwick, 1988) Buccal cavity cyathiform and shallow; amphid multispiral; cuticle striated and punctated; precloacal supplements tubular, anterior-most larger; gubernaculum simple; ovaries reflexed; viviparous.

***Acanthonchus singaporensis* new species**
(Figs. 3, 4)

Measurements, Refer to Table 2

Holotype. Male: Body slender, curved ventrally, 1255 µm long. Anterior blunt and posterior gradually tapering forming a conical tail. Cuticle striated with transverse rows of minute punctations but irregular laterally at the posterior region. Head diameter 18.1 µm. Six inner labial papillae followed by six outer labial papillae. Four cephalic setae (the longest 9.1 µm). Cervical setae present and short. Cervical setae posterior to the amphid present. Amphid multispiral with 5.5 turns (9.2 µm in width) and 41.1% of corresponding body diameter. Buccal cavity shallow with a hollow dorsal tooth. Oesophagus cylindrical. Nerve ring 78.1 µm from anterior. Excretory pore not observed. Pore-liked structure present on the cuticle along the body region. Ocellus absent.

Testes paired and opposed. Spicule paired, equal in length, curved and cuticularised (37.5 µm long). Central cuticularised

Table 2. Measurements of *Acanthonchus singaporensis* new species (all measurements in µm)

Characteristic	Holotype (♂)	Paratype (♂)
Body length	1255	1209
a	26.48	25.62
b	6.57	5.11
c	14.23	14.05
Head diameter	18.14	19.65
Amphid diameter	9.23	8.74
Amphideal turns	5.5	5.25
Amphid / c.b.d % (relation of amphid diameter to labial body diameter, %)	41.13	44.48
Oesophagus length	191.22	236.44
Distance of nerve ring from anterior body end	78.05	-
Maximum body diameter	47.42	47.19
Spicule length	37.47	37.78
Gubernaculum length	36.35	37.61
Number of supplements	6	6
Most anterior supplement from cloaca	105.51	106.24
a.b.d (Cloacal body diameter)	34.66	36.94
Tail length	88.25	86.09

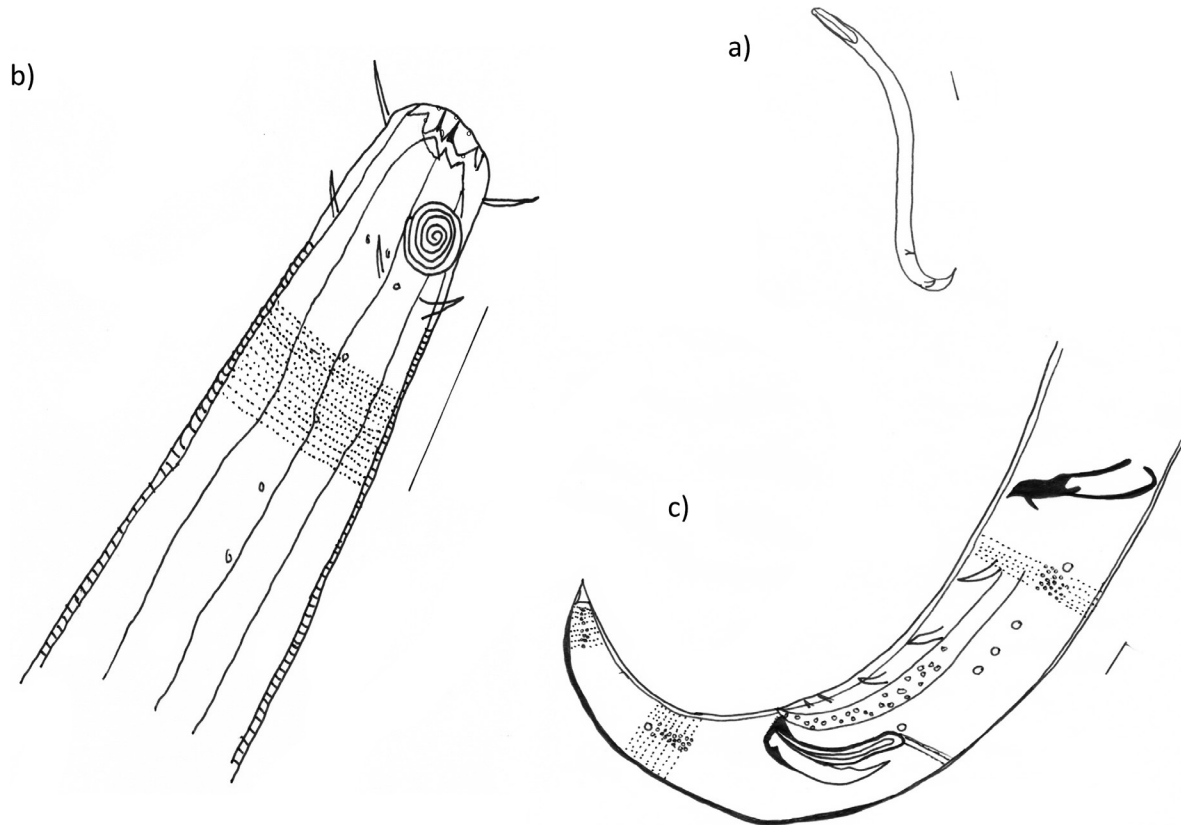


Fig. 3. *Acanthonchus singaporensis* new species. Holotype: a, total; b, head region; c, tail region. Scale bars = 200 µm [a]; 20 µm [b]; 10 µm [c].

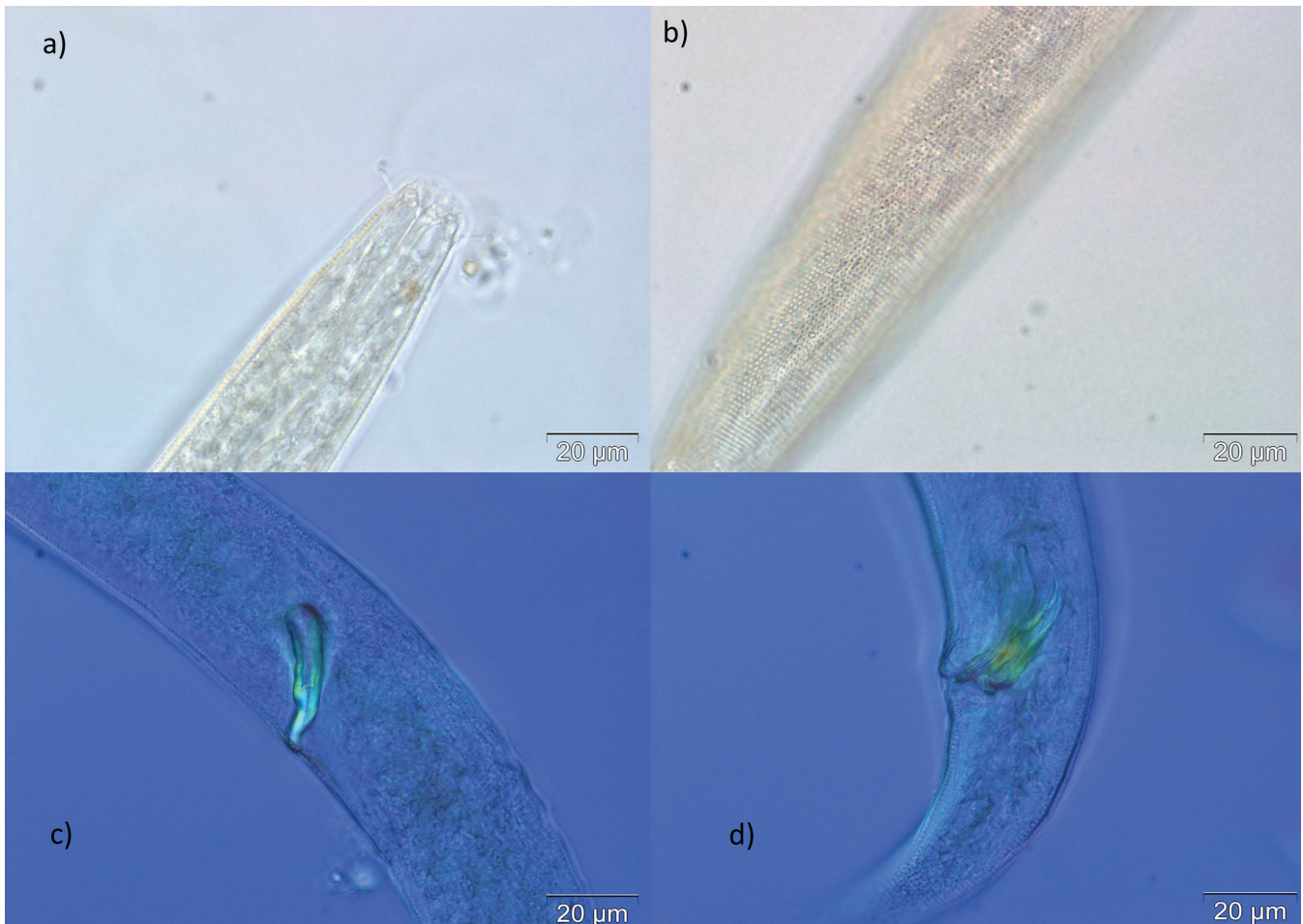


Fig. 4. *Acanthonchus singaporensis* new species. Holotype: a, head region; b, cuticle; c, most anterior precloacal supplement; d, spicules.

strip present at the spicules. Gubernaculum paired and almost as long as the length of spicules (36.4 µm) which expanding into a triangular plate at the distal end. Sperm cells observed. Six precloacal tubular supplements. Anterior-most supplement located 105.5 µm from cloacal was big and heavily cuticularised with a hook located near to the distal end. Two small posterior tubuli close to each other near to the cloacal where hardly can be seen. Caudal gland not observed. Tail conical (88.25 µm), 2.55 a.b.d.

Etymology. The species is named for the name of the country where the species was first collected – Singapore.

Diagnosis. The most recent addition to the genus *Acanthonchus*, *A. tridentatus*, was described from Japan in 1976 (Kito, 1976). A total of 10 species is assigned to this genus, but not all the recorded species indicated the presence of two minute supplements anterior to the cloacal region except *A. cobbi* Chitwood, 1951; *A. duplicatus* Wieser, 1959; *A. pugionatus* Vitiello, 1970; *A. rostratus* Wieser, 1959 and *A. tridentatus* Kito, 1976. *Acanthonchus singaporensis* new species closely resemble *A. tridentatus* in body length and the ratio between length spicules and gubernaculum (approximately 1:1), but differs in the unique high number of amphideal turns (5.5 vs 3.5 in *A. tridentatus*) and the absence of pre- or post-cloacal setae. Apart from these differences, a hook-like structure was observed near the posterior end of the most anterior tubular supplement. This has not been reported in any of the described species in this genus.

A key to identification to all the valid species is provided below. However, we encountered difficulties producing a consistent identification key as the descriptions of certain species are incomplete: *A. cobbi* Chitwood, 1951 (no drawing and no detailed descriptions were given on the tail and reproductive region); *A. duplex* Allgen, 1951 (no drawing and no description on the amphideal fovea turns); *A. californica* Allgen, 1951 (no drawing of the head region but a description was provided). The present key is based on the limited information available until such time more precise re-descriptions become available for those species mentioned above.

Type material. Holotype: ZRC.NEM 0002; Paratype: IPMB. NEM 0002. Collected by Chen Cheng-Ann from Tanjung Tajam rocky area (SW 102), Pulau Ubin, East Johor Strait, Singapore on 27 October 2012.

Key to all known species of *Acanthonchus*.

1. Four precloacal supplements; ocellus present
.....*A. gracilis* Ditlevsen, 1918
 - Four precloacal supplements; ocellus absent.....2
 - Five precloacal supplements; ocellus absent.....3
 - Six precloacal supplements; ocellus present.....4
 - Six precloacal supplements; ocellus absent.....5
2. Two most anterior supplements almost even size
.....*A. duplex* Allgen 1947
 - Most anterior supplement huge with a “swollen bump” on the cuticle of the supplement; amphideal fovea 2 turns
.....*A. californicus* Allgen 1947

- Most anterior supplement slightly bigger than the second and distally forked; amphideal fovea 3.75 turns
.....*A. cobbi* Chitwood, 1951
- 3. Precloacal setae present*A. viviparous* Cobb, 1920
 - Precloacal setae absent.....*A. setoi* Wieser 1955
- 4. Two post cloacal setae; amphideal fovea < 3 turns.....
.....*A. rostratus* Wiser, 1959
 - Four post cloacal setae; amphideal fovea 3.75 turns.....
.....*A. pugionatus* Vitiello, 1970
- 5. Most anterior supplement with hook at the posterior end; amphideal fovea 3.25; pre and post cloacal setae present
.....*A. tridentatus* Kito, 1976
 - Most anterior supplement with hook near the posterior end; amphideal fovea 5.5 turns; pre and post cloacal setae absent
.....*A. singaporensis* new species
 - Most anterior supplement without hook-liked structure; amphideal fovea 4.25 turns; precloacal setae absent but post cloacal setae present.....
.....*A. duplicatus* Wieser, 1959

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