A New Species of Free-living Marine Nematode, *Proplatycoma tsukubae* sp. nov. (Enoplida: Leptosomatidae), from Japan

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A new species of free-living marine nematode, *Proplatycoma tsukubae* sp. nov. (Enoplida: Leptosomatidae: Platycominae), is described from Japan. The specimens were obtained from sea-floor sediments collected at a depth of 117–202 m off the coast of Shimoda, Shizuoka Prefecture, Japan. *Proplatycoma tsukubae* sp. nov. is similar to *P. africana* (Gerlach, 1959) in the unilobed amphidial flaps. However, it differs from *P. africana* based on the much shorter amphidial flaps just reaching the anterior edge of the amphidial aperture, the accessory structure of the spicules being constricted at the middle and tapering toward both ends, the longer apophyses of the gubernaculum, and the blunt tip of the tail. The partial nucleotide sequences for the cytochrome *c* oxidase subunit I and the 18S rRNA regions in *P. tsukubae* sp. nov. are also presented for future applications in DNA barcoding or phylogenetic studies. Updated taxonomic keys to genera in the subfamily Platycominae and species in *Proplatycoma* are provided.

**Key Words:** 18S, meiobenthos, meiofauna, COI, morphology, Platycominae, Platycoma, Platycomopsis, Pseudoplatycoma, taxonomy.

**Introduction**

The subfamily Platycominae in the family Leptosomatidae hosts the genera *Platycoma* Cobb, 1894, *Platycomopsis* Ditlevens, 1926 (=*Dactylonema* Filipjev, 1927 =*Parabarbnema* Inglis, 1964 =*Pilosinema* Platonova, 1976), *Proplatycoma* Platonova, 1976, and *Pseudoplatycoma* Chen, 2015 (Chen 2015). *Proplatycoma* is a genus of free-living marine nematodes hitherto containing only four valid species. *Proplatycoma* was established by Platonova (1976) based on the type species *P. sudafricana* (Inglis, 1966), and two other species, *P. curiosa* (Gerlach, 1955) and *P. africana* (Gerlach, 1959), which were transferred from *Platycoma* by Gerlach (1962). *Proplatycoma curiosa* and *P. africana* were originally described as *Platycomopsis* and then transferred to *Platycoma* by Gerlach (1962). In addition, Hope (1988a) described a new species, *P. fleurdelis* Hope, 1988, following a review of *Proplatycoma* and *Platycoma*. The members of *Proplatycoma* are characterized by sexual dimorphism in the amphidial apertures with an expanded posterior edge forming a flap-like structures in males and simple elliptical shapes in females, and the presence of a pair of dorso-caudal apophyses of gubernaculum (Hope 1988a).

During a benthic survey of shallow waters around Izu Islands, northwestern Pacific Ocean, Japan, in 2017, five specimens of an undescribed species belonging to *Proplatycoma* were collected. Hitherto, all the known species of the genus have been found only from coastal sediments (Gerlach 1955, 1957, 1959, 1962; Inglis 1966; Gerlach 1967; Rao and Ganapati 1968a, b; Nagabhushanam and Rao 1969; Rao 1969, 1970; Hope 1988a; Smythe 2015). In ecological studies, Tsujino et al. (2002) and Tsujino and Uchida (2011) reported unidentified *Platycoma* spp. from Seto Inland Sea, western Japan; however, this is the first taxonomic report of nematodes in the subfamily Platycominae from Japanese waters.

**Materials and Methods**

 Specimens were collected on 12 October 2017, at a depth of 117–202 m off the coast of Shimoda, Shizuoka Prefecture, Japan (34°42.153’N 139°02.378’E–34°42.216’N 139°02.621’E), using a Satsuki-type mini dredge on board the research vessel Tsukuba II (Shimoda Marine Research Center, University of Tsukuba). The sorting, fixing and mounting methods, in addition to observations using a differential interference contrast microscope, were followed as described in Shimada and Kakui (2019). All the examined specimens were deposited in the Invertebrate Collection of Hokkaido University Museum (ICHUM), Sapporo, Japan.
DNA samples for polymerase chain reaction (PCR) were extracted from two DESS-fixed immature females using ISOHAIR (NIPPO GENE, Japan), according to the methods of Tanaka et al. (2012). Partial cytchrome c oxidase subunit I (COI; mitochondrial) and 18S rRNA (18S; nuclear) regions were amplified by PCR using the KOD One PCR Master Mix (TOYOBO, Japan). Primers used in the present study were JB3 and JB5 (Derycke et al. 2007) for COI, and EukA (Medlin et al. 1988), 9FX, 2FX, 26R, 13R, and 18P (De Ley et al. 2002) for 18S. The PCR program consisted of 35 cycles of 98°C for 10 s, 50°C for 5 s, and 68°C for 10 s. PCR products were purified using ISOSPIN (NIPPON GENE). DNA sequences were aligned using MEGAX (Kumar et al. 2018) and deposited in the DDBJ/EMBL/GenBank database (accession numbers: LC495489–LC495492).

Results

Family Leptosomatidae Filipjev, 1916
Subfamily Platycominae Platonova, 1976
Genus Proplatycoma Platonova, 1976
Proplatycoma tsukubae sp. nov. (Figs 1–4)

Material examined. Holotype, adult male (ICHUM 5965), whole mount slide, off Shimoda, Shizuoka Prefecture, Japan (34°42.153′N 139°02.621′E), 117–202 m deep, 12 October 2017. Two paratypes, adult male (ICHUM 5966) and immature female (ICHUM 5967), same collection data as holotype. Two nontype specimens, immature females, used for molecular studies, same collection data as holotype.

Etymology. The specific name tsukubae is a noun in the genitive case derived from R/V Tsukuba II.

Diagnosis. Proplatycoma tsukubae sp. nov. is characterized by the largest body size (longer than 20 mm) in the genus, short, unilobed amphidial flaps just reaching the anterior edge of the amphidial apertures, long (1.5 cloacal body diameter) spicules, uniquely shaped accessory structures of spicules constricted at the middle and tapering toward both ends, a gubernaculum with long dorso-caudal apophyses equal to the accessory structure length, two precloacal ventromedian supplements: anterior one with a papilliform sensillum, posterior one with a pair of ventrolateral setae, the presence of postcloacal ventrolateral supplements, and a blunt (not acute) tail tip.

Measurements. See Table 1.

Description of males. Body (Fig. 1A, B) longer than 20 mm, almost uniform in diameter except in cervical and caudal regions. Cuticle finely striated only anterior to nerve ring; each striation 0.8–1.2 µm wide. Papilliform somatic sensilla sparse. Numerous gland cells (largest 60×25 µm) surrounding intestine. Ocellus-like pigment spots absent. Head (Figs 1C, D, 2A) truncated at anterior end, 40–45% of maximum body diameter at level of cephalic setae, and constricted at level of amphids. Six inner labial sensilla papilliform. Six outer labial setae (0.5 cephalic diameter) and four cephalic setae (0.6 cephalic diameter) arranged in single circle. Subcephalic setae just behind amphids absent. Cervical setae (Fig. 1E) slightly shorter than outer labial setae, arranged in eight longitudinal rows, and present anterior to nerve ring. Cephalic capsule (Figs 1C, D, 2A) slightly shorter than anterior gonad; fovea cup-shaped, 10–12 µm deep. Buccal cavity minute and tooth-like structure absent. Buccal aperture surrounded by low three microlobia; each microlobium equal in shape and length. Excretory pore and ventral gland not observed. Nerve ring (Fig. 1E) located at 16–19% of pharyngeal length from anterior body end: aperture transversely elliptical, 0.4–0.5 cephalic diameter wide, with a short flap expanded from middle of posterior edge; flap unilobed, anterior tip reaching at anterior edge of aperture in holotype but shorter in paratype male; fovea cup-shaped, 10–12 µm deep. Buccal cavity minute and tooth-like structure absent. Buccal aperture surrounded by low three microlobia; each microlobium equal in shape and length.

Material examined. Holotype Paratype Paratype
Male Immature female

Table 1. Morphometrics of Proplatycoma tsukubae sp. nov. All measurements are in µm.

<table>
<thead>
<tr>
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<th>Holotype</th>
<th>Paratype</th>
<th>Paratype</th>
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<tr>
<td>L</td>
<td>21137</td>
<td>21868</td>
<td>17254</td>
</tr>
<tr>
<td>a</td>
<td>176.1</td>
<td>174.9</td>
<td>156.9</td>
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<tr>
<td>b</td>
<td>6.9</td>
<td>7.7</td>
<td>7.9</td>
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<tr>
<td>c</td>
<td>75.5</td>
<td>65.1</td>
<td>63.9</td>
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<tr>
<td>c’</td>
<td>2.4</td>
<td>2.7</td>
<td>2.9</td>
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<td>V</td>
<td>—</td>
<td>—</td>
<td>68.3</td>
</tr>
<tr>
<td>Cephalic diameter</td>
<td>54</td>
<td>52</td>
<td>45</td>
</tr>
<tr>
<td>Maximum body diameter</td>
<td>120</td>
<td>125</td>
<td>110</td>
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<tr>
<td>Cloacal/anal body diameter</td>
<td>115</td>
<td>123</td>
<td>94</td>
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<tr>
<td>Outer labial setae length</td>
<td>26–27</td>
<td>25–29</td>
<td>23–24</td>
</tr>
<tr>
<td>Cephalic setae length</td>
<td>30–31</td>
<td>30–32</td>
<td>24–25</td>
</tr>
<tr>
<td>Anterior body end to amphid</td>
<td>27</td>
<td>27</td>
<td>26</td>
</tr>
<tr>
<td>Amphid width</td>
<td>20</td>
<td>26</td>
<td>6.0</td>
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<tr>
<td>Cervical setae length</td>
<td>21–26</td>
<td>19–22</td>
<td>17–20</td>
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<tr>
<td>Anterior end to nerve ring</td>
<td>487</td>
<td>552</td>
<td>409</td>
</tr>
<tr>
<td>Pharyngeal length</td>
<td>3049</td>
<td>2834</td>
<td>2187</td>
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<tr>
<td>Tail length</td>
<td>280</td>
<td>336</td>
<td>270</td>
</tr>
<tr>
<td>Spicule length on arc (right, left)</td>
<td>183, 188</td>
<td>188, 195</td>
<td>—</td>
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<tr>
<td>Accessory structure length</td>
<td>87</td>
<td>74</td>
<td>—</td>
</tr>
<tr>
<td>Gubernaculum apophysis length (right, left)</td>
<td>85, 83</td>
<td>88, 88</td>
<td>—</td>
</tr>
<tr>
<td>Anterior end to gonad</td>
<td>13154</td>
<td>14062</td>
<td>10461</td>
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<tr>
<td>Testis length (anterior, posterior)</td>
<td>487, 466</td>
<td>327, 596</td>
<td>—</td>
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<tr>
<td>Anterior end to vulva</td>
<td>—</td>
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<td>11777</td>
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ends, 0.4–0.5 spicule length, finely striated at distal end. Gubernaculum (Figs 2D, 3C) with paired apophyses; each apophysis almost straight, directed dorso-caudally, gradually tapering toward caudal end, 0.75 cloacal body diameter or 45% of spicule length. Two precloacal ventromedian supplements (Figs 2E, 3C) present as papilliform elevation of body cuticle: anterior one larger, located at 2.7–3.0 cloacal body diameter from cloaca, with papilliform sensillum at top; posterior one at 1.2–1.6 cloacal body diameter from cloaca, with a pair of posterior ventrolateral setae. A postcloacal ventromedian supplement (Figs 2D, 3C) also present, similar to precloacal ventromedian supplements but smaller in size, located at 0.6–0.7 cloacal body diameter from cloaca, with a setiform sensillum at top. A row of smaller supplements (Figs 2D–F, 3C) present at each ventrolateral field: 31–39 supplements anterior to precloacal ventromedian supplements; 4–6 supplements situated between precloacal ventromedian supplements; 8–9 supplements posterior to postcloacal ventromedian supplement. Tail (Fig. 3C) conico-cylindrical, anterior part 80% tapering and posterior part slightly expanded with blunt tip. Caudal setae consisting of several short (ca. 10 µm) setae at distal end of conical part. At tail tip (Fig. 2G), three short setae present. Spinneret and caudal glands not observed.

**Description of immature female.** Body (Fig. 4A) similar to males except for following characters. Cervical setae in eight longitudinal rows slightly shorter than those in males. Cephalic capsule inconspicuous. Amphids (Figs 2H, 4B) smaller than those in males: aperture elliptical, 1/8 of cephalic diameter wide, without flap; fovea cup-shaped, 8 µm deep. Reproductive system (Fig. 4C) not developed. Anterior ovary present, beginning at 61% of body length; however posterior end inconspicuous. Posterior ovary and uteri not observed. Vulva pore-like, situated at 68% of body length, without gland cells. Posterior region of intestine twisted as illustrated in Fig. 4D, probably due to damage during sampling. Tail (Fig. 4D) conical in anterior part 70% and cylindrical in posterior part, with sparse caudal setae.
Sequence data. The partial COI and 18S sequences were determined from two immature females. Two COI sequences (342 bp: accession numbers LC495491 and LC495492) differed by 20 nucleotide substitutions, which represented the first COI sequences from any Proplatycoma species. The 18S sequences from two specimens (1706 bp: accession numbers LC495489 and LC495490) were identical and the most similar to the partial 18S sequence (1625 bp: accession number KR265047) of P. fleurdelis among the sequences in the DDBJ/EMBL/GenBank database.

Remarks. Our specimens are identified as a member of the genus Proplatycoma based on the presence of the amphidial flaps, the accessory structures of the spicules, and the dorso-caudal apophyses of the gubernaculum [amended from Smol et al. (2014)]. Proplatycoma tsukubae sp. nov. is clearly distinguishable from three congers, P. curiosa,
A new species of Proplatycoma

The genus Proplatycoma differs from all the other genera in Platycominae based on the amphidial apertures with an expanded posterior edge forming a flap-like structure in males and simple elliptical shape in females, in addition to the presence of a pair of accessory structures on ventrolateral side of each spicule and a pair of dorso-caudal apophyses of the gubernaculum (Platonova 1976; Hope 1988a; Chen 2015). Morphologically, Proplatycoma seems to closely resemble Platycoma, which is a monospecific genus with the type species Platycoma cephalata Cobb, 1894 characterized by amphidial apertures with two unique flat setae only in males (Cobb 1894; Platt and Warwick 1983). The original description of Platycoma cephalata by Cobb (1894) indicates that two flat setae are located on the anterior edge of each oval amphidial aperture; however, Platt and Warwick (1983) noted in the redescription that the two flat setae are located on the posterior edge of each slit-like aperture and amphidial fovea is oval. Hope (1988a, b) considered the flat setae homologues of the amphidial flaps in Proplatycoma and concluded that whether setae or flaps are present is not an appropriate diagnostic character to distinguish the two genera. Chen (2015) followed Platonova (1976), distinguishing Proplatycoma and Platycoma based on whether setae or flaps were present. However, according to Chen (2015), whether the setae were “flat” or not was not critical, and he transferred two Platycomopsis species, P. mesjatzevi (Filipjev, 1927) and P. dimorphica Mawson, 1956, to Platycoma because of the several normal setae they possessed, which were located posterior to (not on the posterior edge of) the amphidial apertures (cf. Filipjev 1927; Mawson 1956). Based on the findings from our literature survey, we concluded that P. mesjatzevi and P. dimorphica should be maintained in Platycomopsis based on the absence of “flat” setae.

Chen (2015) established Pseudoplatycoma as a monospecific genus based on Pseudoplatycoma malaysianis Chen, 2015. He differed Pseudoplatycoma from Platycomopsis based on the presence of the ocellus-like paired pigment spots throughout the body, and the bluntly rounded proximal end of spicules. However, Filipjev (1927) described...
the presence of similar pigment spots in *Dactylenoma mesjatzevi* Filipjev, 1927, which is currently classified as *Platycomopsis mesjatzevi* by Filipjev (1927); and the structure of the proximal end of spicules is unclear in several *Platycomopsis* species. We consider that *Pseudoplatycoma* should be distinguished from all the other genera in Platycominae based on its unique paired tubular precloacal ventromedian supplements and the dorsal apophyses of the gubernaculum.

The taxonomic key for the genera of Platycominae amended from Smol *et al.* (2014) and Chen (2015) is as follows:

**Key to genera of Platycominae**

1. Amphidial aperture with two flat setae on its posterior edge in males ........................................... *Platycoma*
   — Amphidial aperture with no setae on its posterior edge ...................................................... 2
2. Amphidial aperture with a flap on its posterior edge in males ............................................... *Proplatycoma*
   — Amphidial aperture without a flap ................... 3
3. Gubernaculum with dorsal apophyses, precloacal ventromedian supplements tubular .......... *Pseudoplatycoma*
   — Gubernaculum without apophyses, precloacal ventromedian supplements papilliform .......... *Platycomopsis*

Currently, *Proplatycoma* contains five valid species including *P. tsukubae* sp. nov. (Platonova 1976; Hope 1988a; Chen 2015). *Proplatycoma curiosa* is characterized by trilobed amphidial flaps with dorsal and ventral lobes branched numerousl. *Proplatycoma sudafricana* and *P. fleurdelis*, despite the great distance between their ranges (*P. fleurdelis* from Barbados and Belize vs. *P. sudafricana* from South Africa) (Inglis 1966; Hope 1988a; Smythe 2015), closely resemble each other based on the following characters: the body approximately 10 mm long, trilobed amphidial flaps without branching, an acute tail with a pair of ventral setae, an anterior precloacal ventromedian supplement with 3–4 pairs of setae, and having a gubernaculum with apophyses shorter than half of the spicule length. Hope (1988a) distinguished *P. sudafricana* and *P. fleurdelis* based on the shapes of the amphidial flaps (arrowhead-shaped in *P. sudafricana* vs. fleur-de-lis-shaped in *P. fleurdelis*), although Inglis (1966) reported the fleur-de-lis-shaped flaps as a variant (Inglis 1966: fig. 4). We consider that they should be distinguished based on the shape of the accessory structures of spicules [tapering toward the proximal end in *P. fleurdelis* (Hope 1988a) vs. expanded with a knob-like proximal head in *P. sudafricana* (Inglis 1966)]. *Proplatycoma tsukubae* sp. nov. and *P. africana* share unilobed flaps (Gerlach 1959; this study) but are distinguished based on the characters of the flaps, the shape of the accessory structures of spicules, the length of gubernaculum apophyses, and the shape of tail tip (see above).

The taxonomic key for the males of *Proplatycoma* amended from Hope (1988a) is as follows:

**Key to species of Proplatycoma**

1. Amphidial flaps trilobed ........................................... 2
   — Amphidial flaps unilobed ........................................... 4
2. Dorsal and ventral lobes on amphidial flaps with a number of branches ................................ *P. curiosa*
   — Lobes on amphidial flaps without branches .......... 3
3. Proximal end of spicule accessory structure with a knob-like head ............................... *P. sudafricana*
   — Proximal end of spicule accessory structure tapering ............................................... *P. fleurdelis*
4. Amphidial flap tip much anterior to edge of aperture .................................................. *P. africana*
   — Amphidial flap tip around anterior to edge of aperture ............................................ *P. tsukubae* sp. nov.

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A new species of Proplatycoma

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