

A new free-living marine nematode species of the genus *Pseudosteineria* (Monhysterida: Xyalidae) from a subtidal zone of the East Sea, Korea

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Abstract: A new free-living marine nematode species of the genus *Pseudosteineria* Wieser, 1956 affiliated with the family Xyalidae is described based on specimens collected from the sediment of a subtidal benthic environmental habitat in the East Sea, Korea. *Pseudosteineria varisetis* sp. nov differed from its congeners by the combination of the following characteristics: a relatively long body (1,628–1,691 μm long in males), a circular amphideal fovea situated behind the subcephalic setae, the presence of lateral cuticular alae starting from behind the nerve ring, the presence of eight groups of long subcephalic setae, the presence of irregularly distributed variable lengths of somatic setae on the body, solid spicules (43.2–43.9 μm long) with a cephalated proximal end, a long tubular shaped gubernaculum with dorsal swelling, and a conico-cylindrical tail with two to four terminal setae. In this report, we provide a taxonomic description and illustrations of a new species of the genus *Pseudosteineria* by differential interference contrast microscopy.

Keywords: taxonomy, marine nematode, *Pseudosteineria*, East Sea, Korea

INTRODUCTION

The family Xyalidae Chitwood, 1951 consisting of 47 genera, are characterized by striated cuticle, funnel-shaped buccal cavity, six outer labial setae and four cephalic setae arranged in one circle, commonly presence of additional cephalic setae, and infrequently presence of eight groups of subcephalic setae. Most of the species in this family are reported to live in apparent cosmopolitan distribution of marine environments, but some species were discovered from the inland waters and the terrestrial habitats (Fonseca and Bezerra 2014; Venekey *et al.* 2014). The genus *Pseudosteineria* was firstly established by Wiser (1956) based on *P. anteferens* Wieser, 1956 as a type species of the genus, and

classified within the family Xyalidae. The genus *Pseudosteineria* Wiser, 1956 is characterized by the presence of striated cuticle, the presence of conical buccal cavity, six to eight groups of distinctly long subcephalic setae arranged at the around of the amphideal fovea, and the presence of circular amphideal fovea and conico-cylindrical tail with terminal setae (Wieser 1959; Tchesunov 2000; Huang and Li 2010; Cidreira *et al.* 2020). Since Tchesunov (2000) first revised the genus *Pseudosteineria* to 11 valid species, four species of the genus (*P. sinica* Huang and Li, 2010, *P. zhangii* Huang and Li, 2010, *P. anteramphida* Sun, Huang, Tang, Zang, Xiao and Tang, 2019, and *P. longisetis* Cidreira, Venekey, De Souza Alves and Kelmo, 2020) have been continuously reported. Consequently, 15 valid species are currently rec-

ognized in the genus *Pseudosteineria* (Cidreira *et al.* 2020). Of these, five species of the genus *Pseudosteineria* have been reported around the Korean waters, i.e. *P. innaequispiculata* (Platanova 1971) and *P. sagittispiculata* Fadeeva, 1986 from the adjacent Russian waters of the East Sea (Sea of Japan), *P. sinica* and *P. zhangi* from the Yellow sea, China, and *P. anteramphida* from the Bohai sea, China. However, there is no record of morphological analysis or description on the genus *Pseudosteineria* in Korea yet.

During a continuous ecological survey on the biodiversity of the free-living marine nematodes around the East Sea, Korea, a new *Pseudosteineria* nematode species was recovered from the washings of shallow subtidal sediment of the benthic environment. Present paper deals with morphological study of a new species of the genus *Pseudosteineria* using a differential interference contrast (DIC) microscope. We provide description and illustrations of *P. varisetis* sp. nov., and we also examined its taxonomic relationships with other valid species of the genus.

MATERIALS AND METHODS

Samples were collected from shallow subtidal zone around discharge channel of Uljin nuclear power plant in June 2020 using Smith McIntyre grab. Benthic sediment was expressed silty sands, and was obtained from the water depth of 25 m. To separate the meiofauna from sediment, filtered it using a 67 µm sieve, and then fixed in 5% formalin (Kristensen 1989). Samples were brought back to the laboratory, nematodes were picked out from the mixed meiobenthos under a stereoscopic microscope (LEICA 205C; Wetzlar, Germany). The nematodes were transferred to 5% glycerin solution, and mounted on between two coverslips of the HS slide (Shirayama *et al.* 1993). The mounted nematodes were identified, measured, photographed using Olympus BX 53 microscope equipped with Olympus DP 26 digital camera and Olympus Cellsens corresponding imaging software (Olympus, Tokyo, Japan). The line drawings of the specimens were made on Olympus BX 53 microscope with drawing tube, and traced over using Adobe illustrator CC program. All the measurements are given in µm.

SYSTEMATIC ACCOUNTS

Phylum Nematoda Potts, 1932

Class Chromadorea Inglis, 1983
Order Monhysterida Filipjev, 1929
Family Xyalidae Chitwood, 1951
Genus *Pseudosteineria* Wieser, 1956

Pseudosteineria varisetis sp. nov. (Figs. 1–3, Table 1)

Type material. Holotype male (MABIK NA00156667), in glycerin on HS slide, was deposited in the nematode collection at the specimen conservation room of the Marine Biodiversity Institute of Korea (MABIK), Seochun, Korea. Three paratype males (KIOST NEM-1-2610, KIOST NEM-1-2611, KIOST NEM-1-2612) and two paratype females (KIOST NEM-1-2613, KIOST NEM-1-2614), mounted on HS slides, were deposited in the nematode collection at the specimen conservation room of the Bio-Resources Bank of Marine Nematodes (BRBMN), East Sea Research Institute, Korea Institute of Ocean Science & Technology (KIOST), Korea. All the specimens collected on 9 June 2020 from the type locality by H. S. Rho and H. Lee. All are mounted in anhydrous glycerin between two coverslips on HS slide, sealed with nail polish.

Type locality and habitat. Shallow subtidal zone around discharge channel of Uljin nuclear power plant (37°09' 36.72"N, 129°22'09.45"E), Buk-myeon, Uljin-gun, Gyeongsangbuk-do, Korea. The nematodes were extracted from subtidal sediments with tiny shell gravels and detritus collected at a depth of 25 m.

Etymology. The specific epithet of the species is derived from the various numbers and lengths of somatic setae of the species, which is one of the diagnostic key characters of *P. varisetis* sp. nov.

Measurements. See Table 1 for detailed measurements and morphometric ratios.

Diagnosis. Body relatively long (1,628–1,691 µm in males); presence of lateral cuticular alae started from behind nerve ring; circular amphideal fovea situated behind subcephalic setae; presence of eight groups of long subcephalic setae; variable lengths of somatic setae irregularly distribute on the body; solid spicules with a cephalated proximal end; long tubular shaped gubernaculum with dorsal swelling.

Description. Males. Body slender, 1,628–1,691 µm long, gradually tapering towards both extremities (Figs. 1A, 3A). Cuticle transversely annulated, beginning at base of buccal cavity and ending at tail tip. Perceptible lateral cuticular alae prolonged from level of behind nerve ring to anterior portion of tail (Fig. 3D). Labial region set off from remain-

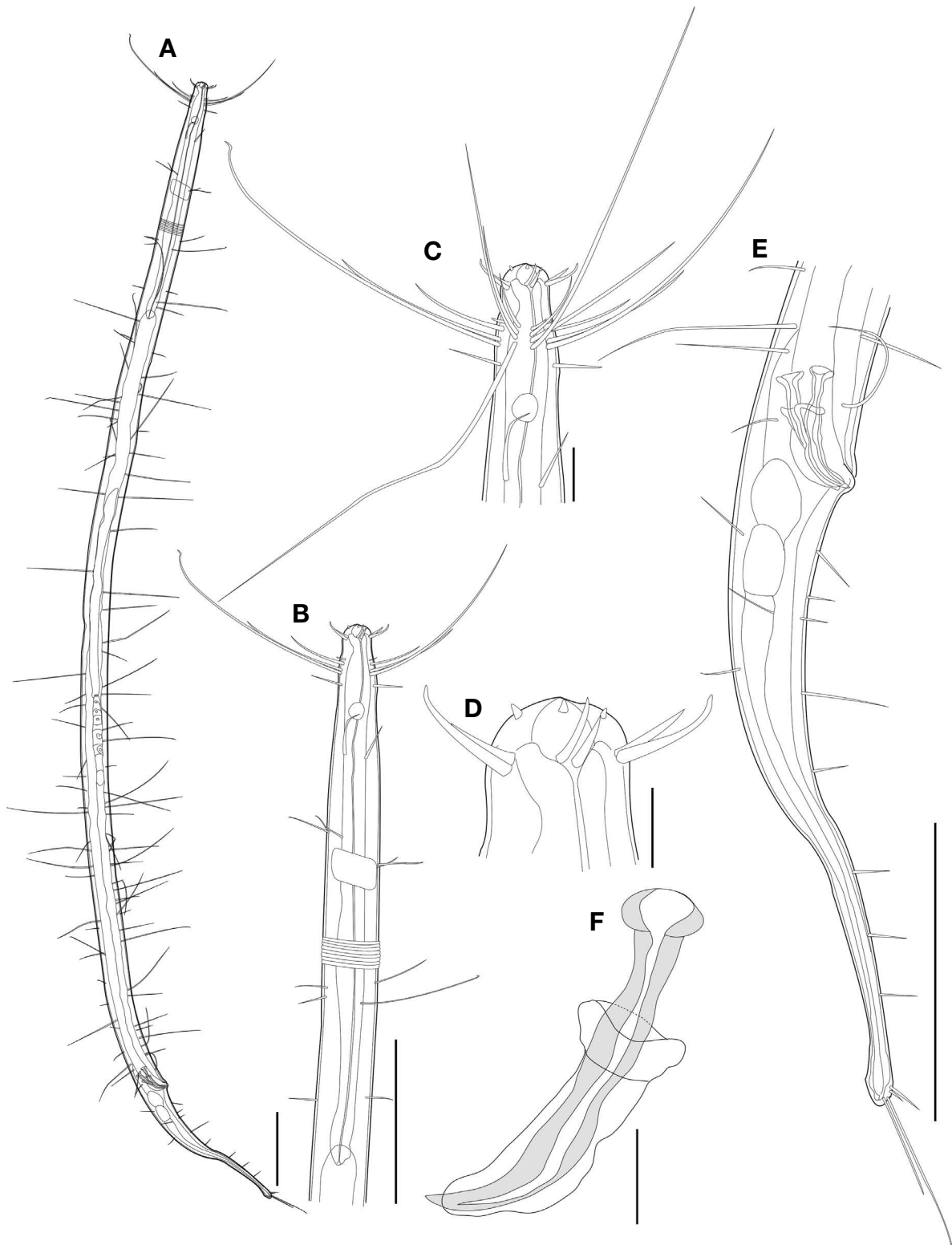


Fig. 1. *Pseudosteineria varisetis* sp. nov., holotype male in the lateral view: A, habitus; B, anterior region; C, head end including subcephalic setae and amphideal fovea; D, lip region including labial and cephalic setae; E, spicules and tail region; F, spicule and gubernaculum in a paratype male (scale bars: A, B, and E = 100 μ m; C = 20 μ m; and D, F = 10 μ m).

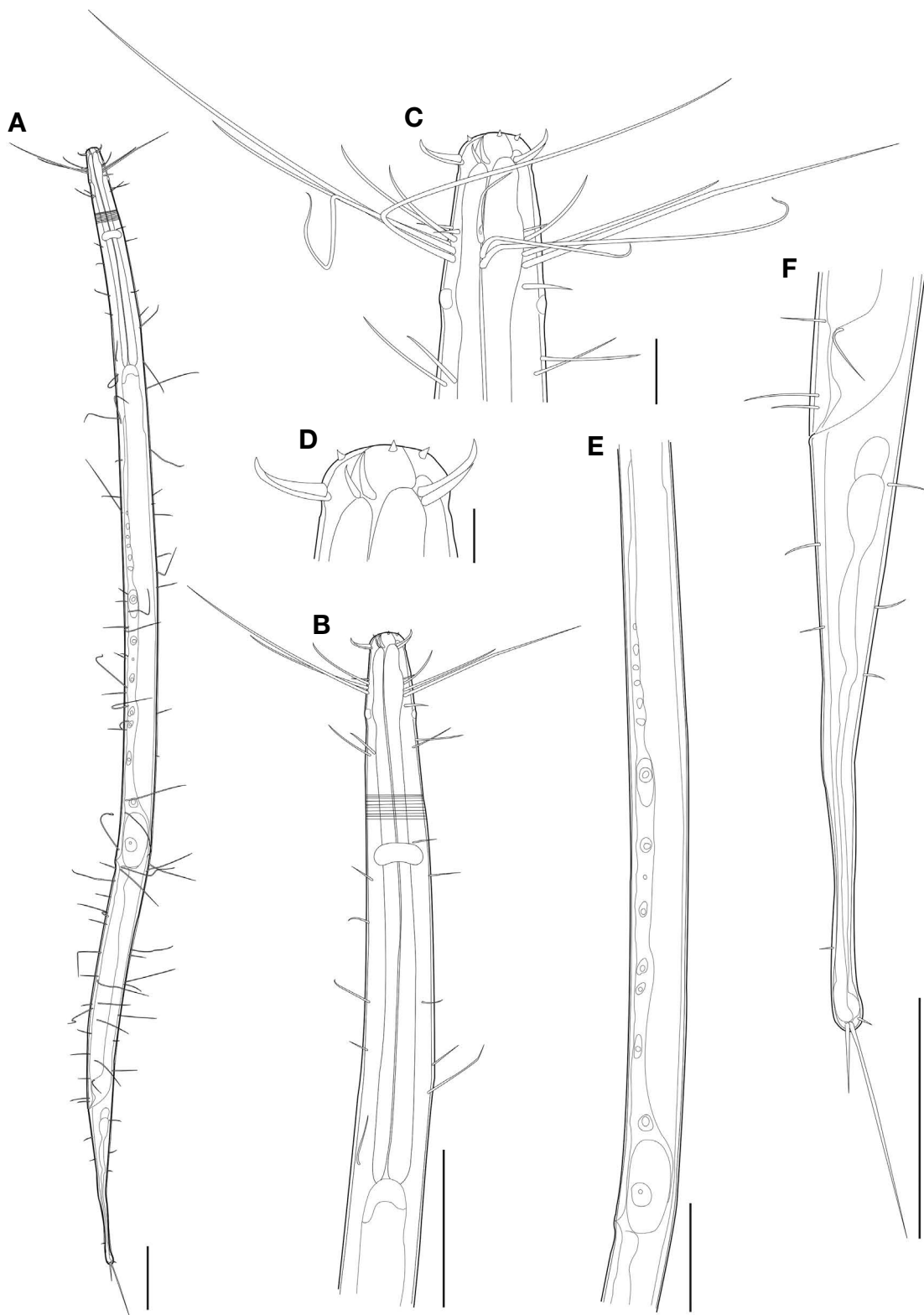


Fig. 2. *Pseudosteineria varisetis* sp. nov., paratype females in the lateral view: A, habitus; B, anterior region; C, head end including sub-cephalic setae and amphideal fovea; D, lip region including labial and cephalic setae; E, vulva region; F, tail region (scale bars: A, B, E, and F = 100 μ m; C = 20 μ m; and D = 10 μ m).

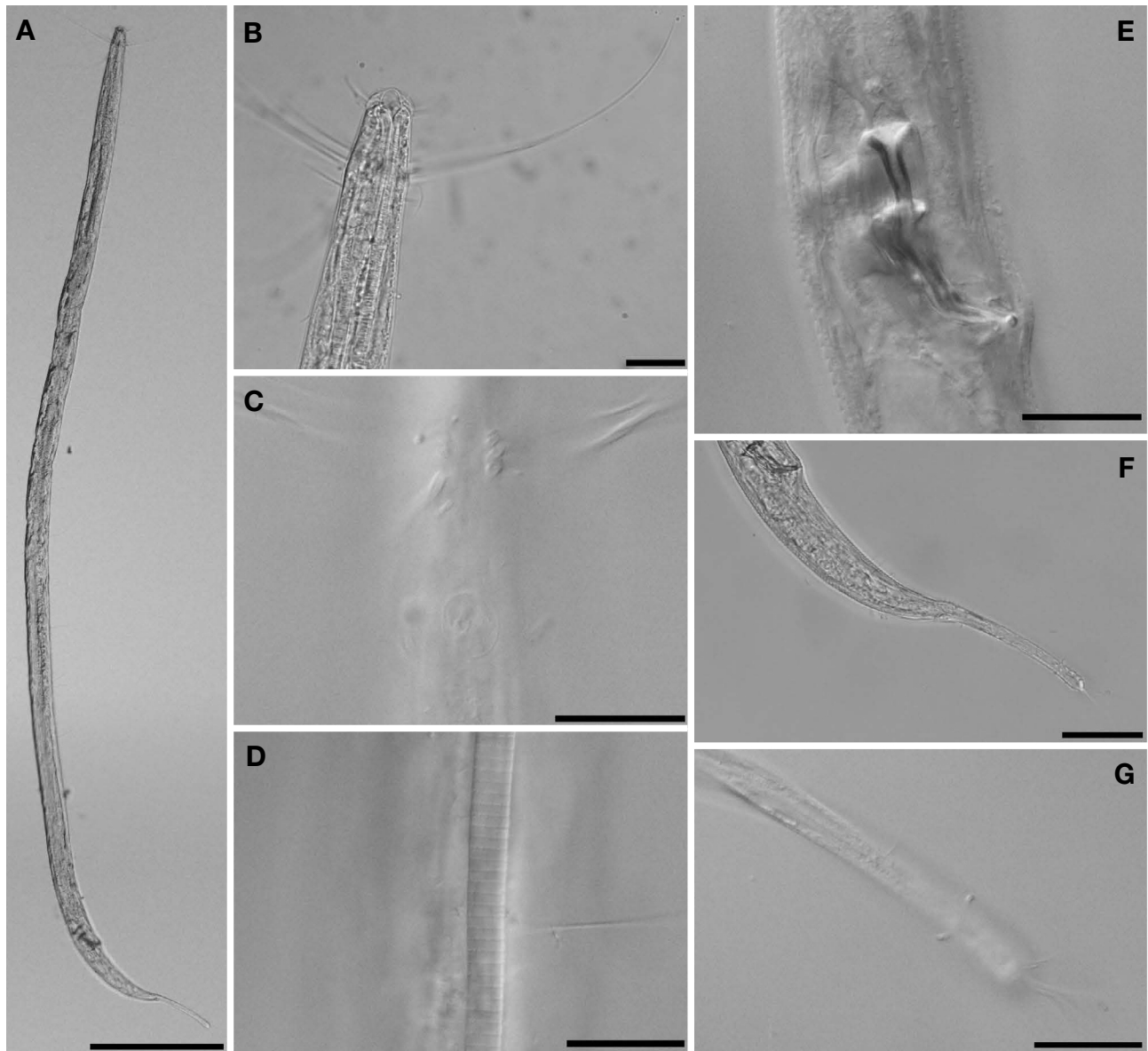


Fig. 3. *Pseudosteineria varisetis* sp. nov., DIC photomicrographs, holotype male in lateral view: A, habitus; B, head region; C, amphideal fovea; D, lateral cuticular alae; E, spicule and gubernaculum; F, tail region; and G, tail end including four terminal setae (scale bars: A = 200 μ m; B–E, G = 20 μ m; and F = 50 μ m).

ing body. Six lips slightly inflated. Labial sensillae arranged in two circles, anterior one with six inner labial sensillae and posterior with six outer sensillae. Six inner labial sensillae papilliform, 1.2–1.7 μ m long, and six outer sensillae setiform, 13.5–15.1 μ m long. Four cephalic setae 10 μ m long, and arranged in one circle with six outer labial sensillae (Fig. 1D). Subcephalic setae arranged closely in eight longitudinal rows of subdorsal, sublateral and subventral on both sides of body. Long and strong subcephalic setae

situated at behind cephalic setae, 20.5–23.3 μ m from anterior end. Each group with three to four subcephalic setae, which is increasing gradually from anterior to posterior setae in every row. Length of the shortest subcephalic setae 4.5–6.4 μ m long and the longest one 114.2–120.2 μ m long (Figs. 1C, 3B). Somatic setae variable in length, scattered throughout body, longest setae about 83.5–108 μ m long, about 2.3–3.1 times of corresponding body diameter (Fig. 1A). Buccal cavity funnel-shaped, without teeth.

Table 1. Morphometrics of *Pseudosteineria varisetis* sp. nov.(in μm)

Characters	Holotype Male	Paratypes	
		Males (n=3) mean \pm SD (range)	Females (n=2) mean \pm SD (range)
Total body length	1628	1658 \pm 25.0 (1631–1691)	1696 \pm 83.5 (1612–1779)
a	41.3	44.3 \pm 0.6 (43.5–44.8)	30.4 \pm 1.0 (29.3–31.4)
b	5.1	5.3 \pm 0.1 (5.3–5.5)	5.0 \pm 0.0 (5.0–5.0)
c	7.4	7.1 \pm 0.1 (7.0–7.1)	6.7 \pm 0.0 (6.7–6.8)
Head diameter at cephalic setae level	16.6	17.6 \pm 0.3 (17.2–17.8)	21.5 \pm 0.1 (21.4–21.5)
Body diameter at pharynx level	35.2	34.6 \pm 0.5 (34.0–34.9)	47.3 \pm 1.4 (45.9–48.7)
Maximum body diameter	39.4	37.4 \pm 1.0 (36.4–38.9)	55.8 \pm 0.8 (54.9–56.6)
Length of cephalic setae	10	10 \pm 0.0 (10–10)	15.8 \pm 0.9 (14.8–16.7)
Length of the longest subcephalic setae	120.2	118.2 \pm 3.3 (114.2–118.2)	144.9 \pm 1.1 (143.9–146.0)
Length of the shortest subcephalic setae	4.4	5.3 \pm 0.8 (4.5–6.4)	7.5 \pm 0.0 (7.5–7.5)
Amphideal fovea diameter	9.5	9.0 \pm 0.1 (8.8–9.1)	6.5 \pm 0.1 (6.4–6.7)
Amphids diameter as percentage of corresponding body diameter	41.9	36.9 \pm 1.6 (34.6–38.5)	21.6 \pm 0.8 (20.8–22.5)
Distance from anterior end to groups of subcephalic setae	21.4	21.6 \pm 1.2 (20.5–23.3)	25.7 \pm 0.2 (25.5–25.9)
Distance from anterior end to amphids	48.5	47.1 \pm 2.3 (43.9–48.6)	46.1 \pm 0.9 (45.2–47.0)
Distance from anterior end to nerve ring	136.9	136.7 \pm 2.8 (132.7–138.9)	139.2 \pm 1.9 (137.3–141.1)
Body diameter at amphids level	22.6	24.5 \pm 1.4 (22.9–24.5)	30.2 \pm 0.6 (29.6–30.7)
Body diameter at nerve ring level	33.4	32.0 \pm 2.0 (29.2–33.8)	39.6 \pm 0.5 (39.0–40.1)
Pharynx length	320.8	310.1 \pm 1.1 (308.6–310.3)	340.5 \pm 16.3 (324.2–356.8)
Length of the longest somatic setae	108.0	87.8 \pm 5.3 (83.5–87.8)	102.0 \pm 4.4 (97.5–106.4)
Body diameter at longest somatic setae level	35.3	34.9 \pm 1.4 (32.9–36.0)	51.1 \pm 1.9 (49.2–53.0)
Spicules length along the arc	43.2	46.3 \pm 2.7 (43.2–49.9)	–
Gubernaculum length	34.6	32.4 \pm 1.9 (31.1–35.0)	–
Distance from anterior end to vulva	–	–	1082 \pm 45.8 (1036–1128)
Body diameter at vulva level	–	–	52.5 \pm 1.9 (50.6–54.5)
V (%)	–	–	63.8 \pm 0.4 (63.4–64.2)
Anal body diameter	34.7	36.0 \pm 2.6 (33.2–39.4)	39.0 \pm 2.8 (36.1–41.8)
Tail length	220.5	233.1 \pm 3.9 (229.0–238.3)	251.5 \pm 13.0 (238.5–264.5)
Length of posterior cylindrical portion of tail as percentage of entire tail	41.9	40.0 \pm 2.5 (37.3–43.4)	43.2 \pm 0.1 (43.1–43.3)

(a, body length divided by maximum body diameter; b, body length divided by pharynx length; c, body length divided by tail length; V (%), vulva distance from anterior end as percentage of total body length).

Amphideal fovea circular, 8.8–9.5 μm in diameter, situated behind subcephalic setae, 43.9–48.5 μm from anterior end (Figs. 1C, 3C). Pharynx cylindrical, 309–321 μm long, about 18–20% of total body length. Pharyngo-intestinal junction with cardia. Nerve ring encircling pharynx, situated at 43–45% of pharynx length from anterior end (Fig. 1B). Tail conico-cylindrical, 220.5–238.3 μm long; length of posterior cylindrical portion of tail equal to 37.3–43.3% of entire tail. Tail tip bearing two to four terminal setae, the longest setae up to 36–55 μm long (Figs. 1E, 3F, 3G). Two testes, anterior one outstretched and situated on left side

of intestine, posterior one reflexed and on right side of intestine. Spicules paired, slightly curved, and 43.2–49.9 μm long arc. Spicules with well-developed sclerotized outer wall, proximally slightly rounded and pointed distally. Gubernaculum tubular shaped, encloses distal part of spicule, with dorsal swelling (Figs. 1F, 3E). Precloacal supplements absent.

Females. Similar to males in general appearance except slightly larger body diameter, longer subcephalic setae and smaller amphideal fovea. (Fig. 2A–D). Body length 1,612–1,779 μm long; cylindrical body distinctly tapered toward

anterior end. Maximum body diameter 54.9–56.6 μm wide (Fig. 2A). Reproductive system monodelphic, one anterior outstretched ovary, situated to left of intestine. Vulva 1,036–1,128 μm from anterior end, situated at 50.6–54.5% of total body length (Fig. 2E). Tail conico-cylindrical, 238.5–264.5 μm long, about 6.3–6.6 times of anal body diameter (Fig. 2F).

Differential diagnosis and relationships

The Genus *Pseudosteineria* was first erected by Wieser (1956) belongs to the family Xyalidae Chitwood, 1951. So far, 15 valid species in the genus *Pseudosteineria* have been reported from various localities of the intertidal and subtidal habitats in marine environment all over the world (Tchesunov 2000; Huang and Li 2010; Sun *et al.* 2019; Cidreira *et al.* 2020). *Pseudosteineria* can be artificially divided into three groups depending on the main diagnostic character of the genus, i.e. the position of the amphideal fovea. The first group consists of species in which the amphideal fovea is located anterior to or at the level of the subcephalic setae, and this group contains eight species in total: *P. anteferens* Wieser, 1956, *P. anteramphida* Sun, Huang, Tang, Zang, Xiao and Tang, 2019, *P. anticipans* Wieser, 1956, *P. longisetis* Cidreira, Venekey, De Souza Alves and Kelmo, 2020, *P. pavo* (Gerlach 1957), *P. sagittispiculata* Fadeeva, 1986, *P. ventropapillata* Tchesunov, 2000 and *P. zhangii* Huang and Li, 2010. The second group consists of species in which the amphideal fovea is located behind of subcephalic setae, and this group includes five species (*P. horrida* (Steiner 1916), *P. inaequispiculata* (Platonova 1971), *P. paramirabilis* (Gerlach 1955), *P. pulchra* (Mawson 1957), and *P. scopae* (Gerlach 1956)) with the present new species, *P. varisetis* sp. nov. Finally, the third group doesn't have amphideal fovea or was not observed, includes two species (*P. coronata* (Gerlach 1955) and *P. sinica* Huang and Li, 2010).

Pseudosteineria varisetis sp. nov. is characterized by the combination of the following features: (1) circular amphideal fovea situated behind subcephalic setae, (2) the presence of eight groups of long subcephalic setae; each group consists of 3 to 4 setae, (3) the presence of variable lengths of somatic setae, longest setae up to 2–3 times of anal body diameters, (4) the presence of lateral cuticular alae started from behind the nerve ring, (5) the absence of preloacal supplement, (6) spicules broad and solid with cephalated proximal end and tapered distal end, (7) long tubular shaped gubernaculum with dorsal swelling, and (8) conico-cylindrical tail with two to four terminal setae.

Among the species group having an amphideal fovea which is located behind of the subcephalic setae, *P. varisetis* sp. nov. is very similar to *P. horrida* and *P. paramirabilis* by the number of subcephalic setae, the absence of preloacal supplement, and the absence of distinct gubernaculum dorsal apophysis. However, *P. varisetis* sp. nov. can be easily distinguished from *P. horrida* by relatively thinner body diameter in males ($a = 41.3\text{--}44.8$ vs. $a = 20.8\text{--}26$), length of the longest subcephalic setae (114–122 μm vs. 58 μm), length of longer somatic setae (about 2–3 times of the corresponding body diameter vs. short), and the number of tail terminal setae (two to four vs. two). *Pseudosteineria varisetis* sp. nov. is also resemble to *P. paramirabilis* in the presence of long somatic setae, but differs by morphological characteristics: (1) relatively longer total body length in males (1,628–1,691 μm vs. 980–1,005 μm), (2) length of the longest subcephalic setae in males (114–122 μm vs. 68–78 μm), (3) longer spicules (43–44 vs. 23–24 μm), (4) the shape of gubernaculum (with one dorsal swelling posterior margin vs. with spiky posterior margin), and (5) the presence of lateral cuticular alae.

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