New species of *Pseudochromadora* Daday, 1899 (Nematoda: Desmodoridae) from Russky Island (the Sea of Japan)

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Summary. *Pseudochromadora rossica* sp. n. is described from shallow subtidal muddy sediments in Russky Island (the Sea of Japan). *Pseudochromadora rossica* sp. n. differs from other species of the genus through the combination of the following characters: the position of cephalic setae; the position of amphids and sexual dimorphism in the shape of the *fovea amphidialis* (loop-shaped in males, unispiral in females); six longitudinal rows of somatic setae; the presence of interdigitation of body annuli at level of lateral alae; no precloacal supplements; cephalic capsule with rounded labial region; buccal cavity with a large dorsal tooth and two small ventrosublateral teeth; and absence of spines on the cuticle. The sequences of D2-D3 region of the 28S rDNA are provided for identification based on the DNA analysis.

Key words: free-living nematodes, Desmodoridae, D2-D3, morphology, morphometrics, molecular taxonomy.

Up to now only two valid species of the family Desmodoridae were reported from the Sea of Japan: *Metachromadora itoi* Kito, 1978 and *Spirinia parasitifera* (Bastian, 1865) Gerlach, 1963 (Kito, 1978; personal observation). The presence of different desmodorids identified to genus level or as "working species" is often noted in ecological and faunal studies from the Sea of Japan. Representatives of the genus *Pseudochromadora* are found frequently in shallow subtidal muddy sediment of the Sea of Japan.

The genus *Pseudochromadora* was established by Daday in 1899 with *P. quadripapillata* as a type species. Representatives of the genus *Pseudochromadora* are found in all types of sediments and in different habitats, such as estuaries, mangroves and intertidal shores from tropical to cold seas. Therefore, the genus can be considered as cosmopolitan. Sporadically, it can even be found in brackish or freshwater (Verschelde *et al.*, 2006). So far, 10 valid species of *Pseudochromadora* are reported in the world.

The broad application of DNA barcoding to marine nematodes requires building a reference database of sequences and morphological vouchers from widespread localities, since most nematode sequences in the molecular database come from north Western Europe and the New World (Pereira *et al.*, 2010). At the same time, it has been shown that the D2-D3 domain of the 28S gene is suitable for both DNA barcoding and phylogenetic reconstruction of marine nematodes (Pereira *et al.*, 2010). Representatives of the family Desmodoridae are still poorly documented by DNA sequences data, indicating a clear necessity to generate sequence data from taxa that have been under represented (Armenteros *et al.*, 2014a, b). Until now, there are no published sequence data of ribosomal DNA genes from *Pseudochromadora*.

The aim of this study is to describe a new species of *Pseudochromadora* together with data on its partial sequences of the D2-D3 region of the 28S rDNA.

MATERIALS AND METHODS

Sediment samples containing nematodes were collected from the upper sublitoral zone on the coast of Russky Island, Peter the Great Bay, West (Russian) Coast of the Sea of Japan. Samples were placed in a beaker and an equal volume of 7% $MgCl_2$ solution was added. After 10 min, the sediments

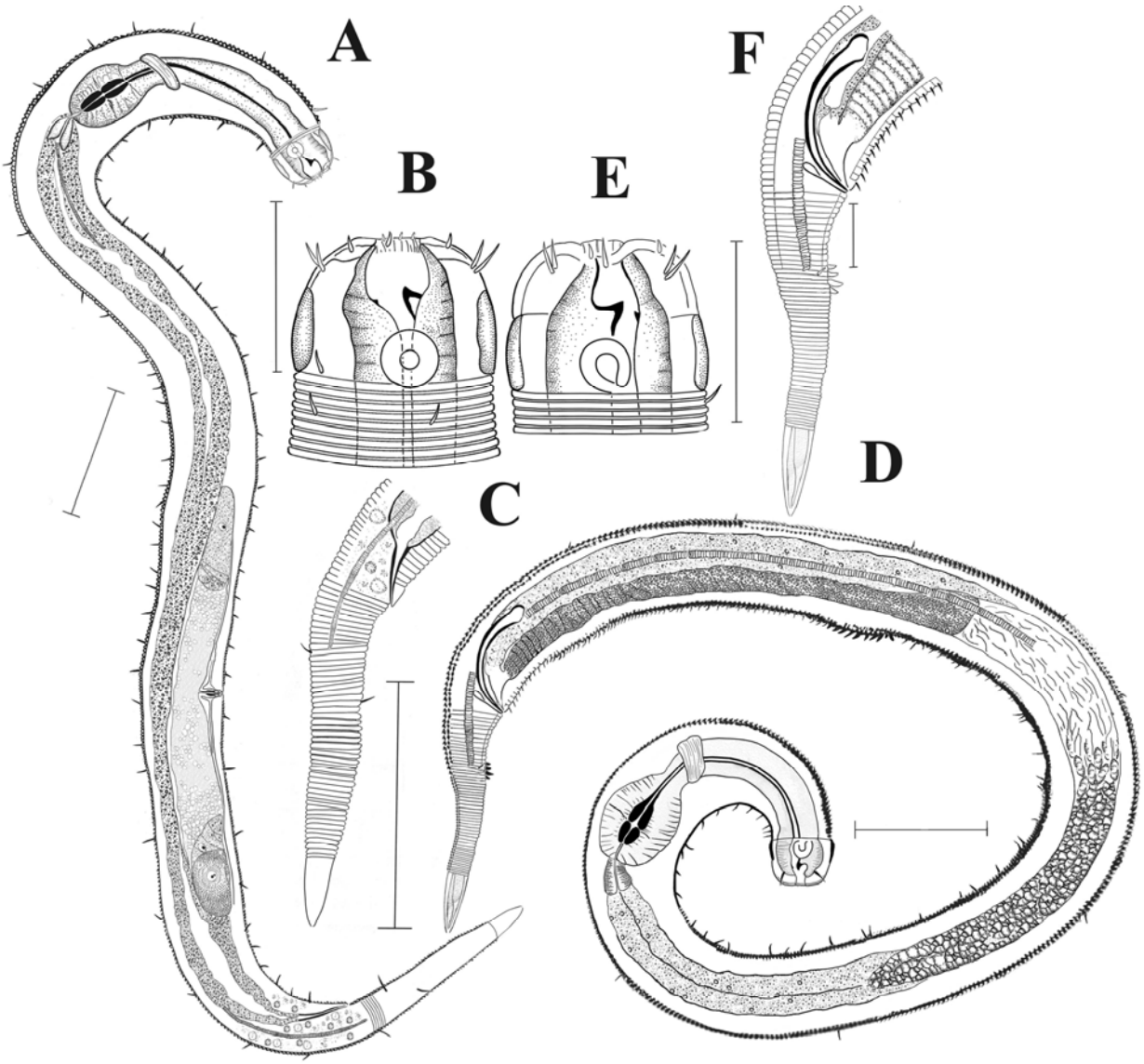

Fig. 1. *Pseudochromadora rossica* sp. n. A: female allotype, entire body, lateral view; B: head of female, lateral view; C: female tail region, lateral view; D: holotype male, entire body, lateral view; E: head of holotype male, lateral view; F: lateral view of tail region and copulatory apparatus. Scale bars: A, C = 100 μm; D = 50 μm; B, E, F = 20 μm.

were stirred thoroughly and the supernatant poured through a 40 μm mesh sieve and washed with filtered sea water. One half of the sample was fixed with 10% formalin; another half was fixed with 96% ethanol and then was stored in the laboratory at –20°C.

Nematodes were picked out from the samples under a stereoscopic microscope, transferred to glycerin using the Seinhorst's (1959) rapid method as modified by De Grisse (1969), and mounted on permanent slides. Drawings were made on an optical microscope Carl Zeiss Axioimager A1 with the aid of a drawing tube. DIC (differential interference contrast) photographs were taken with a Carl Zeiss Axioimager A1 light microscope furnished with a digital camera. For the scanning electron microscopy, specimens were gradually dehydrated in a series of baths of increasing ethanol content, dried in a critical-point dryer, sputter-coated with gold and observed and imaged with a Ziess Evo 40 scanning electron microscope (SEM). Type specimens are preserved in the collection of the Zoological Museum of Far Eastern Federal University, Vladivostok, Russia.

Abbreviations of the measured variables in the description are: a – body length divided by maximum body diameter; a. b. d. – anal body diameter (μm); amph. dist. – amphidial fovea

distance from the anterior end; am. w. – width of the amphidial fovea, as percentage of corresponding body diameter (%); amph. h. – height of the amphidial fovea (μm); amph. w. – width of the amphidial fovea (μm); b – body length divided by pharyngeal length; c' – tail length divided by corresponding body diameter at cloacal level; c – body length divided by tail length; c. b. d. – corresponding body diameter (μm); diam. c. s. – body diameter at the level of cephalic setae (μm); gub. l. – length of gubernaculum (μm); h. c. – length of head capsule (μm); L – body length (μm); l. c. s. – length of cephalic setae (μm); l. tail – tail length (μm); M – maximum body diameter (μm); ph. L – pharyngeal length (μm); ph. b. d. – pharyngeal bulb diameter (μm); spic. arch – length of spicule along the arch (μm); tmr – length of non-annulated tail end; V – distance of the vulva from the anterior end (μm); V (%) – distance of the vulva from the anterior end as percentage of body length (%).

For molecular analyses, five specimens of *Pseudochromadora rossica* sp. n. were picked out from the ethanol samples, transferred to glycerol, mounted on glycerin slides, examined under the light microscope and video-captured. Identified and photographed nematodes were transferred to individual Eppendorf tubes containing 96% ethanol. Subsequently, they were frozen at –20°C to break the cell walls.

Total genomic DNA was extracted from the samples using the Invitrogen (Invitrogen, Carlsbad, CA, USA) protocol. The DNA precipitate was resuspended in TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0) then stored in –20°C. The polymerase chain reaction (PCR) was used to amplify a fragment of 28S rDNA. The primers for amplification of the 28S fragment were forward D2A (5'-ACAACGTACCGTGAGGGAAAGTTG-3') and reverse D3B (5'-TCGGAAGGAACCAGCT ACTA-3') (De Ley *et al.*, 2005). PCR reaction for this fragment was run in a total volume of 10 μl with 5 μl Go Taq Green Master Mix (Promega, Madison, WI, USA), 0.5 μl of each primer (100 ng μl^{-1}), 3 μl nuclease-free water and 1 μl of total DNA. The PCR thermal regime consisted of one cycle of 3 min at 95°C; 35 cycles of 30 s at 94°C, 40 s at 55°C and 2 min at 72°C and a final cycle of 5 min at 72°C. Each fragment was purified using FastAP and Exo I (Fermentas) and cycle sequenced on an ABI 3130x (Applied Biosystems) automated sequencer using BigDye terminator v3.1 cycle kit methods. Forward and reverse sequences were aligned and manually edited in MEGA 6.06 (Tamura *et al.*, 2013).

SYSTEMATICS

Family Desmodoridae Filipjev, 1922
Subfamily Desmodorinae Micoletzky, 1924
Genus *Pseudochromadora* Daday, 1899

Diagnosis (after Verschelde *et al.*, 1998; Verschelde *et al.*, 2006; Decraemer & Smol, 2006; Tchesunov, 2014, emended). Short cylindrical body with short head capsule and short conical tail. Body annuli with distinct spaces in between. Lateral alae extending from posterior to the pharynx as far as the beginning of the tail. Short somatic setae arranged in six or eight longitudinal rows. Two (or three) parts in a head capsule: slender labial region, followed by main part of the head capsule, which has an extra-thick inner layer of the cuticle; a sutura can be present between the two (or three) regions of the head capsule. Four cephalic setae located either on the labial region or on the anterior rim of the main part of the head capsule. Unispiral amphids (at least in females in case of sexual dimorphism) located on main region of the head capsule. Short cylindrical pharynx with bipartite terminal bulb.

Males of most species have copulatory thorns and postcloacal thorns. Arched spicules; gubernaculum with capitulum.

Type species: *Pseudochromadora quadripapillata* Daday, 1899 (Syn. *Micromicron cephalatum* Cobb, 1920 and *Micromicron luticola* Timm, 1952).

Other species:

Pseudochromadora buccobulbosa Verschelde & Vincx, 1995

Pseudochromadora cazca (Gerlach, 1956) Gerlach, 1963 (Syn. *Desmodora cazca* Gerlach, 1956 and *Metachromadora benepapillata* Timm, 1961)

Pseudochromadora coomansi Verschelde & Vincx, 1995

Pseudochromadora galeata Verschelde, Nicholas & Vincx, 2006

Pseudochromadora incubans Gourlault & Vincx, 1990

Pseudochromadora interdigitatum Muthumbi, Verschelde & Vincx, 1995

Pseudochromadora parva Gagarin & Nguyen Vu Thanh, 2008

Pseudochromadora reathae Leduc & Wharton, 2010

Pseudochromadora rossica sp. n.

Pseudochromadora securis Verschelde, Nicholas & Vincx, 2006.

DESCRIPTION

Pseudochromadora rossica sp. n. (Figs 1-4)

Material examined. Five males (holotype and four paratypes) and five females (paratypes). The type species are deposited in the Zoological Museum of Far Eastern Federal University, Vladivostok, Russia (MN RI 14-5 Ps1). Paratypes are deposited in the Zoological Museum of Far Eastern Federal University, Vladivostok, Russia (MN RI 14-5 Ps2).

Measurements. See Table 1.

Males. Short cylindrical body with blunt head and slender conical tail. Cuticle annulated posterior to head capsule; lateral alae start from posterior to pharyngeal bulb extending to the level of postcloacal thorns on the tail. At the level of the lateral alae, body annuli split up into two (seldom three) narrow annuli and interdigitate. Annuli without spines. Six longitudinal (one dorsal, one ventral, two subdorsal and two subventral) rows of somatic setae running from head capsule to tail.

Well-developed head capsule, consisting of two regions: a shorter anterior lip region and a larger posterior main head region with an extra layer of thick cuticle. Main head region usually wider than lip region and ornamented with numerous tiny vacuoles (Fig. 2C & E); no additional setae. Six setiform internal labial sensilla, six larger external labial sensilla, and four cephalic setae located on labial region of cephalic capsule. Amphids situated laterally on main head region, characterised by the closed loop-shaped to cryptospiral *fovea amphidialis* with the open loop-shaped external part (Figs 2C, E, 3B & 4A).

Buccal cavity with one large dorsal tooth and two small ventrosublateral teeth. Muscular pharynx with large oval bipartite terminal bulb. Nerve ring at *ca.* 47-52 % of pharyngeal length.

Reproductive system monorchic with one anterior outstretched testis, situated to the right of intestine. Spicules arcuate with large capitula, gubernaculum 15-17 μm long or 1/3 spicule length. A group of ventral and subventral copulatory thorns (usually 8-10) is found at 91-134 μm anterior to cloaca, anterior to this group located a ventral row of nine to twelve thorns extending approximately to the level of the beginning of the alae. A ventral row of broad somatic setae located between precloacal group of copulatory thorns and cloaca (Figs 2F, 3F & I). On the tail, at 12-23 μm posterior to cloaca, a group (4-5) of medioventral thorns flanked by a pair of two broad setae (Fig. 3D, G & H). Tail is conical with a non-annulated tip and slender spineret.

Females. Similar to males, but with unispiral amphids located on the posterior part of the head capsule (Figs 2E & 4A). Reproductive system is didelphic, amphidelphic with reflexed ovaries. Thorns absent.

Locality. The Sea of Japan, Peter the Great Bay, Russky Island (42.999583°N, 131.9251°E), depth about 0.3 m, muddy sediment.

Etymology. The species name is an adjective derived from the type locality, Russky Island.

Diagnosis. *Pseudochromadora rossica* sp. n. is characterised by the combination of the following characters: a cephalic capsule separated into main head region and rounded labial region, sexual dimorphism in the shape of the *fovea amphidialis* (loop-shaped in males, unispiral in females), buccal cavity with a large dorsal tooth and two small ventrosublateral teeth, absence of spines on the cuticle, interdigitation of body annuli at the level of lateral alae, six rows of somatic setae, absence of precloacal supplements.

Relationships. *Pseudochromadora rossica* sp. n. is similar to the three congeners: *P. buccobulbosa*, *P. galeata* and *P. parva* in the position of amphids (laterally) and form of the *fovea amphidialis* of the male, number of the rows of the somatic setae (6), absence of the supplements. However, the described species differs from *P. buccobulbosa* in the absence of the buccal bulb and the absence of a ventral row of small thorns in females (five to ten located anterior to vulva in *P. buccobulbosa*). The new species differs from *P. galeata* in the form of the lip region of the head (rounded labial region *vs* hat-shaped region respectively), in the absence of the spine on the annuli, in the number of the ventral copulatory thorns in the precloacal group (10-14 *vs* 8) and location of the postcloacal thorns: in *P. galeata* postcloacal thorns situated in transverse row while in *P. rossica* sp. n. postcloacal thorns arranged in a longitudinal row. *Pseudochromadora rossica* sp. n. differs from *P. parva* in the larger body size (590-782 μm *vs* 399-531 μm), in the length of spicules (38-50 μm *vs* 28-31 μm), in the index c (7.1-9.0 *vs* 4.8-6.6), and in the form of the lip region of the head (rounded labial region *vs* hat-shaped).

Nucleotide sequences. GenBank accession numbers KT207832, KT207833, KT207834, KT207835 and KT207836 (D2-D3 region of 28S rDNA, *ca.* 500 bp), sequences for all individuals were completely identical.

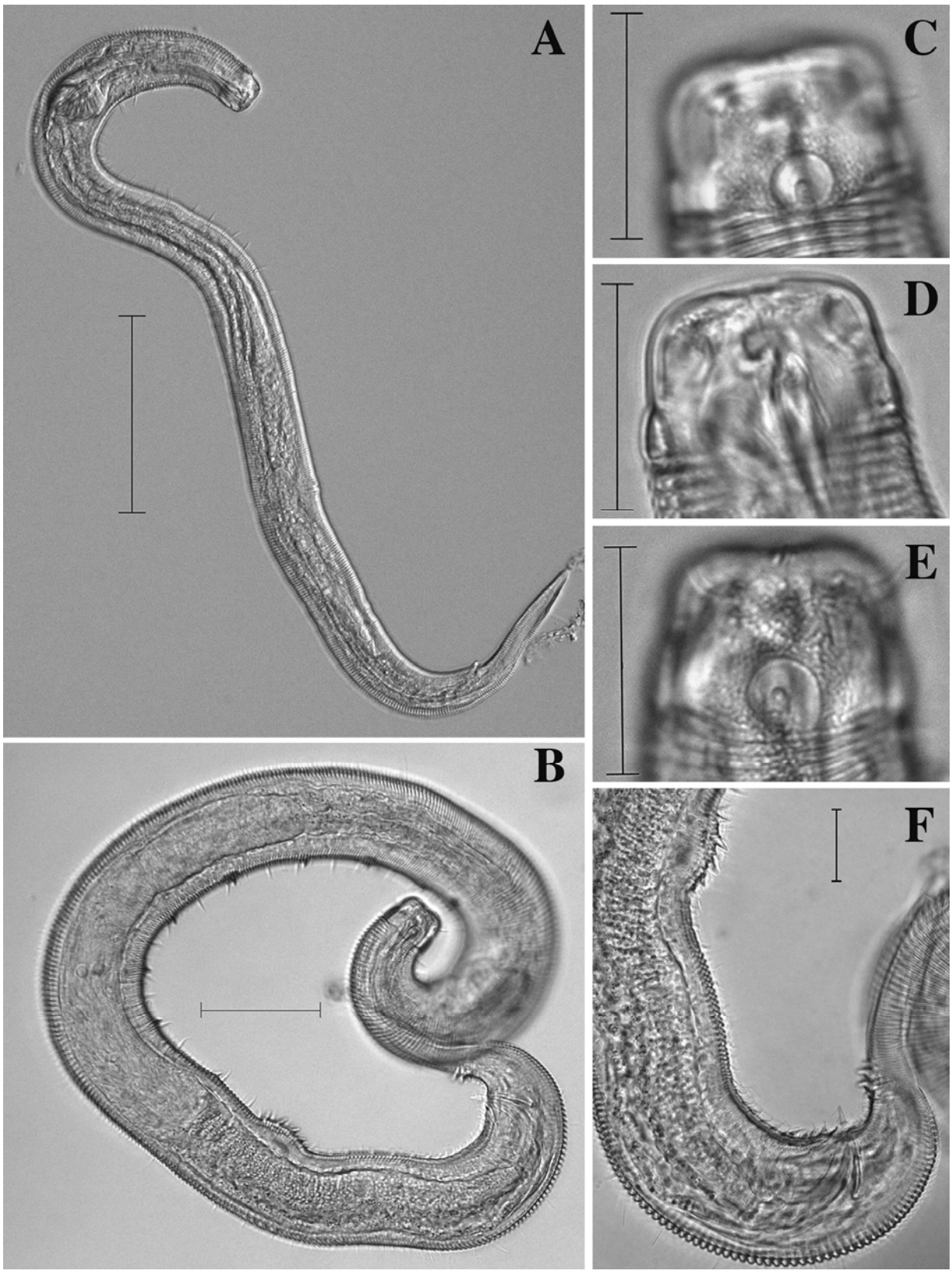

Fig. 2. *Pseudochromadora rossica* sp. n. Light microphotographs. A: entire female; B: entire male; C: head of female showing *fovea amphidialis*; D: head of female showing teeth; E: head of male showing *fovea amphidialis*; F: posterior body region of male. Scale bars: A = 100 μm; B = 50 μm; C-F = 20 μm.

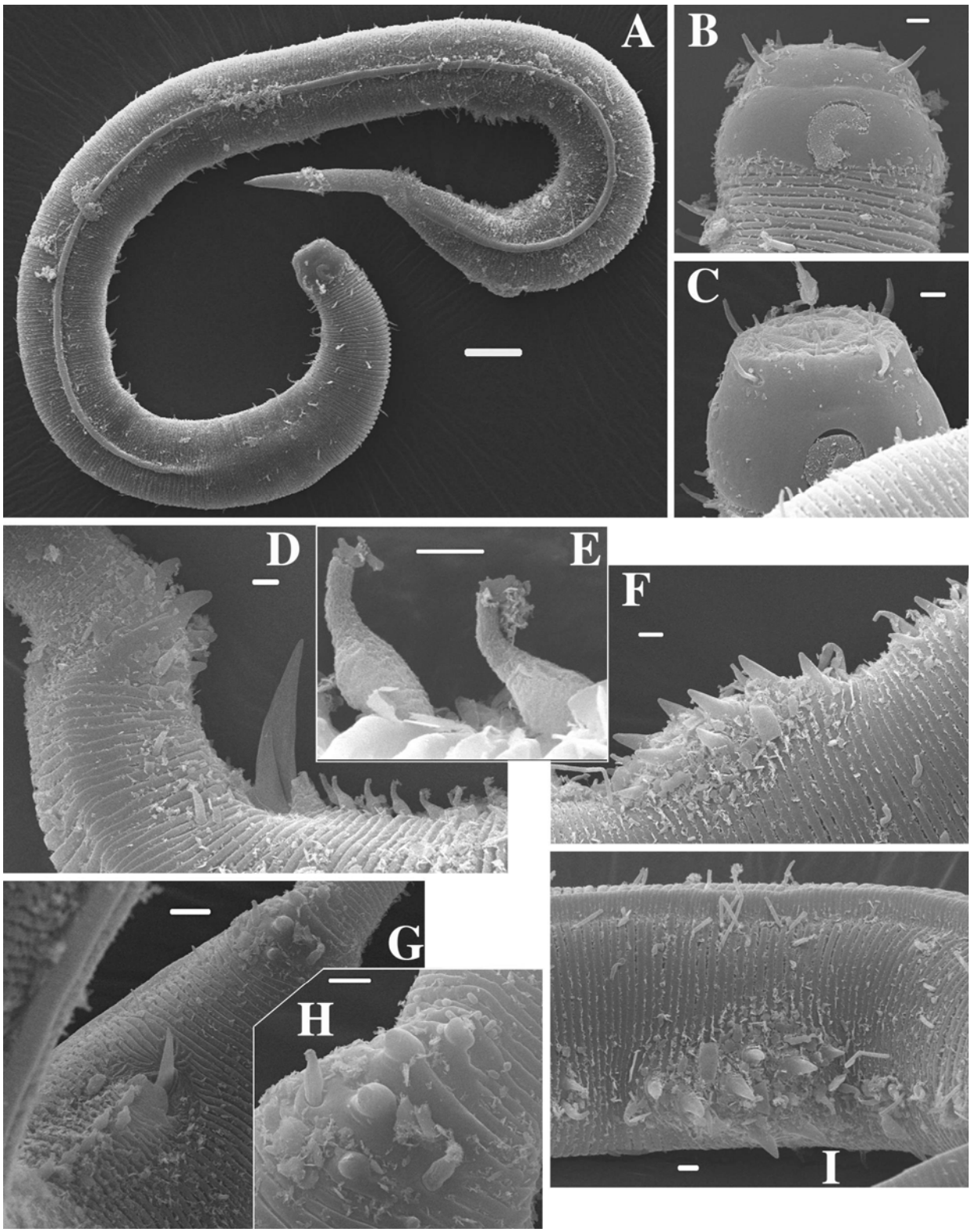

Fig. 3. *Pseudochromadora rossica* sp. n. SEM microphotographs. A: entire male, lateral view; B: head of male showing *apertura amphidialis*; C: head of male showing labial papillae and cephalic setae; D: lateral view of cloacal region of body; E: precloacal setae; F: lateral view of copulatory thorns; G: ventral view of cloacal region of body; H: postcloacal thorns; I: ventral view of copulatory thorns. Scale bars: A = 20 μm; B-D, F, H, I = 2 μm; E = 1 μm; G = 4 μm.

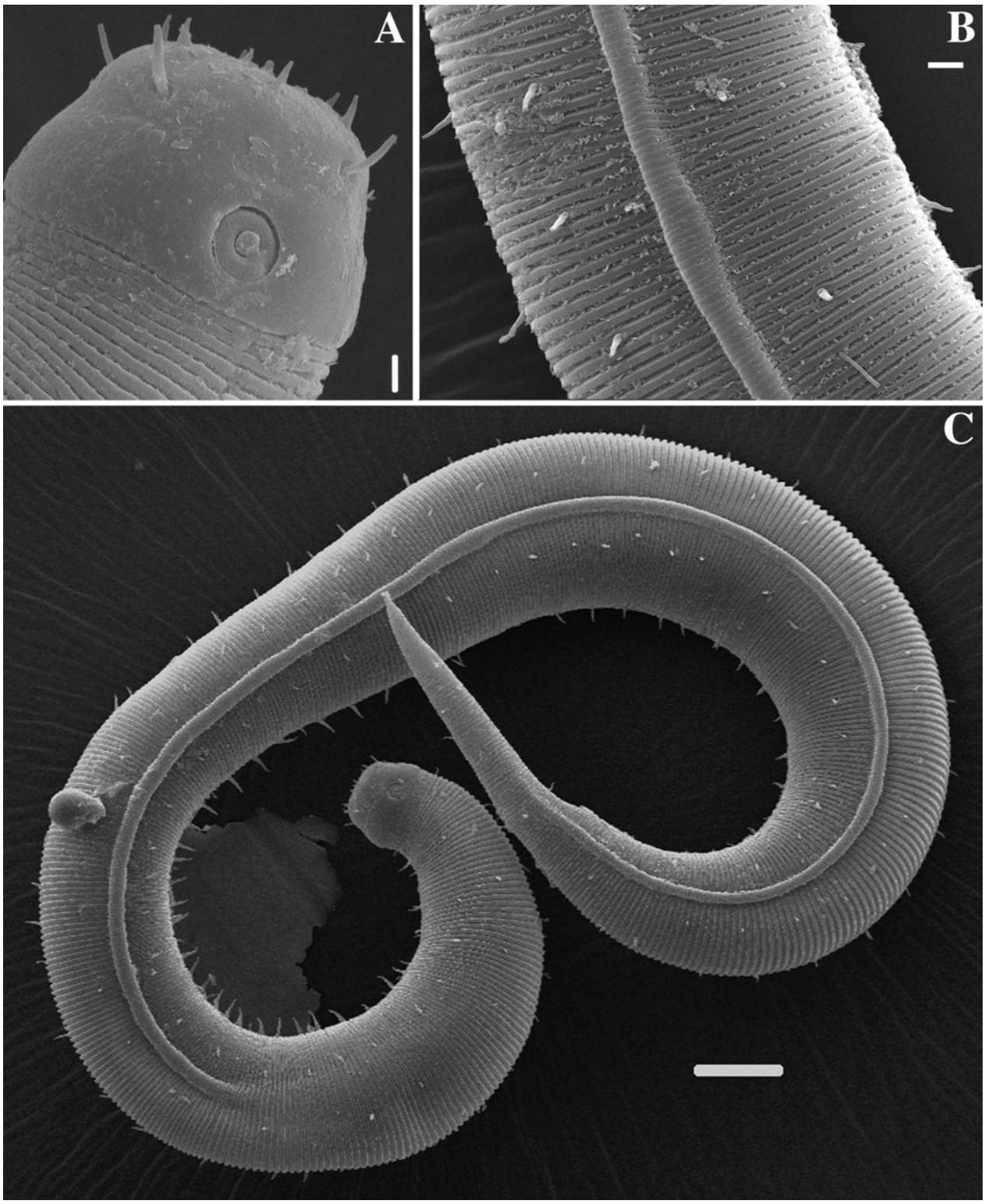

Fig. 4. *Pseudochromadora rossica* sp. n. A: head of female; B: detail of cuticle in mid-region of body; C: entire female. Scale bars: A = 2 μm; B = 3 μm; C = 20 μm.

Table 1. Morphometrics of *Pseudochromadora rossica* sp. n. (all measurements in μm)

Character	Holotype ♂♂	Paratype ♂♂ (n = 4)		Paratype♀♀ (n = 5)	
		Min	Max	Min	Max
L	752	712	782	590	751
V	–	–	–	350	446
M	35	35	40	30	42
ph. L	111	113	127	111	123
a. b. d.	23	24	28	14	17
diam. c. s.	18	17	19	19	20
l. tail	87	87	108	70	90
tmr	20	18	21	14	19
l. c. s.	4	4	4	3	4
h. c.	13	14	16	14	20
amph. dist.	7	8	10	8	11
amph. w.	25	18	27	19	25
am. w. (%)	6	5	6	4	5
amph. h	6	6	8	4	5
ph. b. d.	28	25	29	18	30
spic. arch	47	38	50	–	–
gub. l.	17	15	17	–	–
a	21.5	17.8	22.0	17.4	22.0
b	6.8	6.1	6.8	5.3	6.4
c	8.6	7.1	8.2	8.3	9.0
c’	3.8	3.5	4.3	4.5	5.6
V (%)	–	–	–	59	63

Key to *Pseudochromadora* species

1 – Body annuli do not split up at the level of the lateral alae; males without copulatory thorns, but with precloacal supplements …..…..…… 2

– Body annuli split up and interdigitate at the level of the lateral alae; males with copulatory thorns 3

2 (1) – Males with loop-shaped *fovea amphidialis*; precloacal supplements consist of 8-9 cone-shaped structures with a central projection flanked by two cuticularised pieces, central portion of precloacal supplements consists of a star-shaped structure with six minute pointed projections ……..…… *P. reathae*

– Males with unispiral *fovea amphidialis*, cup-shaped precloacal supplements .. *P. quadripapillata*

3 (1) – Hat-shaped labial region distinct from the main part of the head capsule 4

– Rounded labial region 6

4 (3) – Males with large unispiral *fovea amphidialis* ……..... *P. securis*

– Males with loop-shaped *fovea amphidialis* (sexual dimorphism) …..... 5

5 (4) – Buccal cavity with large dorsal tooth oriented opposite to the small subventral teeth; a group of four to five postcloacal thorns situated ventrally in transverse row ……...…… *P. galeata*

– Buccal cavity with large claw-shaped dorsal tooth, one or two smaller teeth, situated slightly below dorsal tooth; three postcloacal thorns situated ventrally in longitudinal row ..... *P. parva*

6 (3) – Amphids shifted towards a dorso-lateral position on the head capsule; fertilized females with protruded anterior lip of the vulva ……..…..... *P. incubans*

– Amphids located laterally (centrally) on the main part of the head capsule .....…..... 7

7 (6) – Males with loop-shaped *fovea amphidialis* (sexual dimorphism) ……..…….. 8

– No sexual dimorphism in amphidial shape … 9

8 (7) – Buccal cavity with a dorsal plug followed by the dorsal tooth in the pharyngostome; females with ventral row of five to ten small thorns located anterior to the vulva …..……. *P. buccobulbosa*

– Buccal cavity without dorsal plug anterior to

the dorsal tooth; females without thorns ..... *P. rossica* sp. n.

9 (7) – Cephalic setae located on the labial region of the head capsule 10

– Cephalic setae located at the transition (sutura) between the labial and main region of the head capsule ..... *P. coomansi*

10 (9) – Copulatory thorns clustered; spicules with rounded capitulum …..……..... *P. cazca*

– Copulatory thorns more dispersed longitudinally; spicules with funnel-shaped capitulum ……..... *P. interdigitatum*.

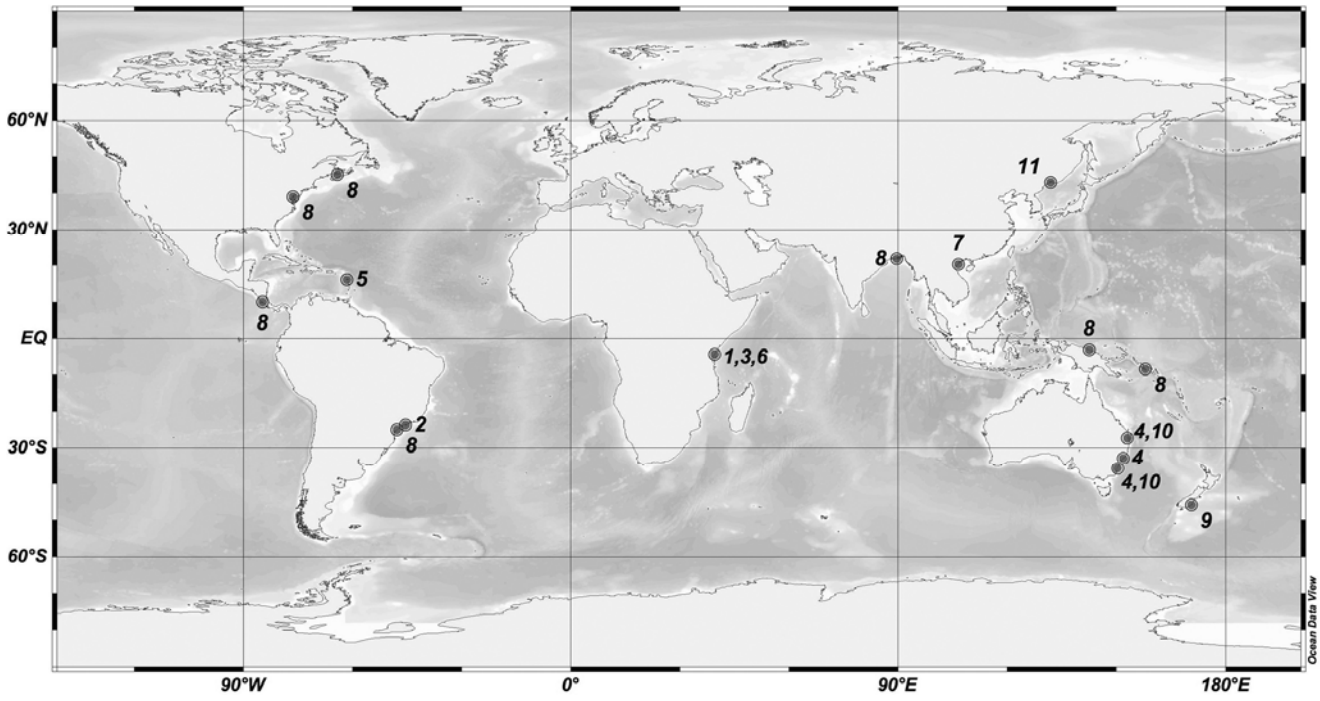

Fig. 5. Global map of the distribution of species of the genus *Pseudochromadora*. 1 – *P. buccobulbosa*; 2 – *P. cazca*; 3 – *P. coomansi*; 4 – *P. galeata*; 5 – *P. incubans*; 6 – *P. interdigitatum*; 7 – *P. parva*; 8 – *P. quadripapillata*; 9 – *P. reathae*; 10 – *P. secures*; 11 – *P. rossica* sp. n.

Genus and species distribution. In general, the biogeographical distribution of the meiobenthic species is poorly known (Fadeeva *et al.*, 2012; Moens *et al.*, 2014). Investigations of large-scale species distribution patterns of Nematoda came to the fore only in recent years. Many marine nematode genera are known to be widespread (Bik *et al.*, 2010; Tchesunov, 2006; Vanreusel *et al.*, 2010). Verschelde *et al.* (2006) mentioned that *Pseudochromadora* is a cosmopolitan genus. Representatives of the genus *Pseudochromadora* have never been previously reported from the Sea of Japan and the North-Eastern Pacific (Fig. 5).

Species of *Pseudochromadora* have been recorded in many oceans in sandy, as well as in muddy sediments in estuarine, mangrove, intertidal and upper subtidal areas from tropical to cold seas and have never been registered in deep-sea. Moreover, some species can be found in brackish or fresh water (Decraemer & Smol, 2006). For most species, restricted geographical distribution is typical. There is only one exception. *Pseudochromadora quadripapillata* is a cosmopolitan species, found in different areas and oceans: Berlinharbor, Seleo Island, New-Guinea (Daday, 1899, 1901), Punta Arena, Pacific coast of Costa Rica (Cobb, 1920), Brazilian mangroves at Cananeia (Gerlach, 1957); Chesapeake Bay, Maryland, USA (Timm, 1952), Mangla, Nil Kamal, Sundarbans (Timm, 1967); Nova Scotia, Canada (Hopper, 1969) and Solomon Islands (Coomans *et al.*, 1985).

At some areas more than one species was observed; for example, three different species were found in the Gazi, Kenya (Fig. 5). The high local species richness within the genus and high turnover of species between different regions suggests a high diversity of these marine nematodes.

Finally, in order to provide a complete review on biogeography of the *Pseudochromadora* genus, the distribution of the remaining undescribed species should be presented.

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Резюме. *Pseudochromadora rossica* sp. n. описан с мелководья острова Русского (Японское море). *Pseudochromadora rossica* sp. n. отличается от других видов рода комбинацией следующих признаков: положением головных щетинок, положением амфида и половым диморфизмом строения амфида (петлевидным у самцов и односпиральным у самок), шестью продольными рядами соматических щетинок, наличием разветвления колец кутикулы в районе латеральных гребней, отсутствием преклоакальных суплементов, головной капсулой с округлой губной частью, стомой с большим дорзальным и двумя маленькими субвентральными зубами, отсутствием микрошипов на кутикуле. Получены фрагменты D2-D3 участка 28S рДНК.