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RESEARCH ARTICLE

Synopsis of the genus *Greeffiella* (Nematoda: Desmoscolecida), with the description of two new species from the Sea of Japan and the Black Sea

Обзор рода *Greeffiella* (Nematoda: Desmoscolecida) с описаниями двух новых видов из Японского и Черного морей

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Abstract. The genus *Greeffiella* Cobb, 1922 includes twelve species inhabiting all oceans of the world. Updated brief and illustrated diagnoses, an identification key and data on the geographical and bathymetrical distribution of all *Greeffiella* species are given. Two new species are described and illustrated: *G. japonica* **sp. nov.** from the Sea of Japan and *G. pontica* **sp. nov.** from the Black Sea. The genus is recorded from the Sea of Japan for the first time, and *G. japonica* **sp. nov.** completes the list of the free-living nematode species inhabiting the shelf of the Far Eastern seas of Russia. This species is characterised by 70 annules dorsally and 62 ones ventrally, the ocelli located on the annules 19 and 29, somatic setae 11–25 μm long, six pairs of subdorsal tubular setae on the body, the vulva surrounded by dense fine hair-like setae, located between the annulus 37 and 38, the tubular anus, and the terminal tube 10 μm long. *Greeffiella pontica* **sp. nov.** mentioned by the authors in 2021 as *Greeffiella* sp. was collected in the northwestern shelf of Crimea (the Black Sea) at a depth of 56 m and is characterised by the following characters: 67–70 annules dorsally and 67–68 ones ventrally, the absence of ocelli, somatic setae 12–42 μm long, seven pairs of subdorsal tubular setae on the body, the vulva surrounded by short sparse setae, not visible anus, and the presence of three thin terminal tubes 13 μm long.

Резюме. Род *Greeffiella* Cobb, 1922 включает 12 видов, обитающих во всех океанах мира. Приведены и проиллюстрированы краткие уточненные диагнозы, ключ и данные о географическом и батиметрическом распространении видов *Greeffiella*. Описаны и проиллюстрированы два новых вида: *G. japonica* **sp. nov.** из Японского моря и *G. pontica* **sp. nov.** из Чёрного моря. Род впервые отмечен в Японское море, *G. japonica* **sp. nov.** дополняет список видов свободноживущих нематод шельфа дальневосточных морей России. Этот вид характеризуется наличием на теле 70-и дорсальных и 62-х вентральных кутикулярных колец, положением глазков на 19-и и 29-м кольцах кутикулы, соматическими щетинками длиной 11–25 мкм, наличием 6-и пар субдорсальных трубчатых щетинок на теле, вульвой, окруженной густыми тонкими волосовидными щетинками (опушение), расположенной между 37 и 38 кольцами кутикулы, трубчатым анусом и терминальной трубкой длиной 10 мкм. *Greeffiella pontica* **sp. nov.** (упомянутый авторами в 2021 г. как *Greeffiella* sp.) обнаружен на глубине 56 м северо-западного шельфа Крыма (Черное море). Этот вид характеризуется наличием 67–70 дорсальных и 67–68 вентральных кутикулярных колец, отсутствием глазков, соматическими щетинками длиной 12–42 мкм, 7-ю парами субдорсальных трубчатых щетинок, вульвой, окруженной короткими редкими щетинками, непросматривающимся анусом и тремя тонкими терминальными трубками длиной 13 мкм.

Key words: free-living nematodes, taxonomy, key, Vladimir Bay, Sea of Japan, Black Sea, *Greeffiella*, new species

Ключевые слова: свободноживущие нематоды, систематика, определительные ключи, Залив Владимира, Японское море, Черное море, *Greeffiella*, новые виды

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Introduction

Checklist of the free-living marine nematodes from the Russian Far Eastern Seas includes approximately 250 species (Fadeeva & Dashchenko, 2003; Pavlyuk et al., 2003, 2007, 2009; Fadeeva, 2005; Pavlyuk & Trebukhova, 2007; Trebukhova & Pavlyuk, 2006; Shoshin, 2013; Trebukhova et al., 2013; Belogurova & Maslennikov, 2016). According to Mordukhovich (2007), 233 nematode species have been described exclusively from the estuary of the Amur River, the marine part of which belongs to the Sea of Japan.

As a result of the study of meiobenthos in the northwestern part of the Sea of Japan, we found a nematode of the genus *Greeffiella* Cobb, 1922. The free-living nematode from this genus is recorded for the first time for the fauna of the Far Eastern seas of Russia. According to Nemys (2022), only the species *G. antarctica* Timm, 1978 was previously recorded on the coast of Japan (Wajima). In addition, representatives of *Greeffiella* were recorded in the Black Sea for the first time during study of the meiobenthos in 2001. Two mature females were collected in the northwestern shelf of Crimea in strongly silted fine sand with detritus at 56 m water depth. These two species from the two seas (Fig. 1) were identified by us as new to science.

This study aims to describe two new species, to distinguish its morphometric and morphological characteristics from those of females of the known species of this genus, to present an illustrated key for the species of *Greeffiella*, and to reveal their geographical and bathymetrical distribution.

Material and methods

During the 64th cruise of the R/V "Academician Oparin" (June–July 2021), 34 samples of bottom sediments were collected in the depth range of 0.3–86 m at 17 stations to study the meiobenthos inhabiting the Russian coast of the Sea of Japan (Fig.1). Sediment columns were collected *in situ* as two replicates using a tubular sampler with a mouth area of 10 cm^2 and a height of 5 cm. Sediments were either obtained by a diver or by subsampling through the surface of monoliths brought on the R/V via a Van Veen Grab (0.1 m^2).

In the Black Sea, samples of bottom sediments were obtained on 15 December 2001 on the Crimean shelf (Fig.1) during the 56th cruise of the R/V "Professor Vodyanitskiy" in the framework of the National Program of Ukraine (Sergeeva & Revkova, 2021). The sediment columns with a height of 5 cm and an area of 18cm^2 were collected in triplicate from the surface monoliths of bottom sediments brought on the R/V and obtained with the box-corer "Ocean-50" (0.5 m^2).

All samples were fixed in 75% alcohol. Based on our previous experiences, this percentage of alcohol preserves morphological structures without distortion. We avoided prior fixation in formalin in order not to damage calcareous taxa. The sediment was washed in a laboratory using a sieve set with mesh sizes of 1 mm, 63 μm and 32 μm. The fractions retained on the sieves were stained with Rose bengal dye before being sorted in water under a stereomicroscope LOMO MSP-2; then the "live" (stained) individuals were picked out, counted and identified to the level of higher taxa or to the species level. A random sampling of 150–200 nematode specimens was used for each sample to determine the species composition of the community. Morpho-anatomical analysis and measurements of all specimens were carried out under a magnification of 1000–1300× using an Olympus CX41 and Olympus BX65 compound microscopes. Microphotographs were obtained using a Nikon compound microscope equipped with an E200 digital camera.

Type specimens are kept in the collection of A.O. Kovalevsky Institute of Biology of the Southern Seas, Sevastopol, Republic of Crimea (IBSS).

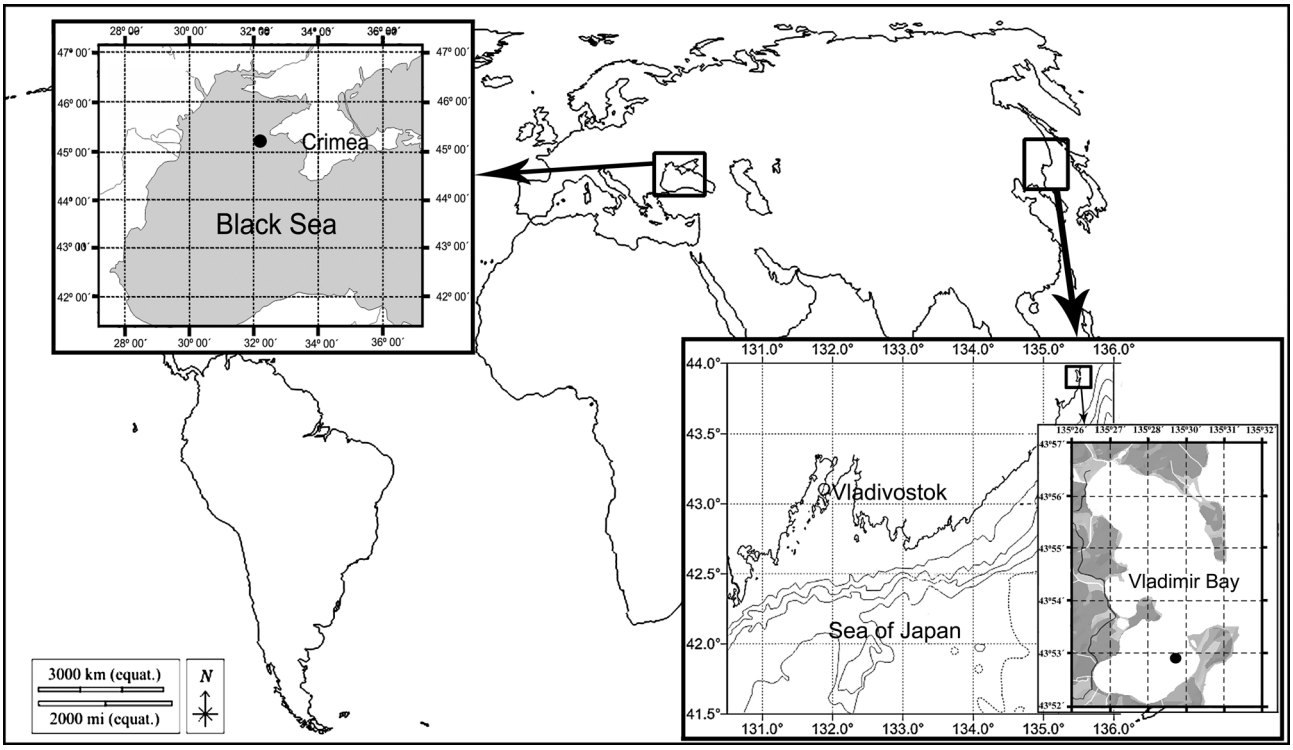

Fig 1. Map showing collection localities of *G. japonica* **sp. nov.** and *G. pontica* **sp. nov.** in the Sea of Japan (Vladimir Bay) and the Black Sea (Crimea), respectively.

Taxonomy

Phylum **Nematoda**

Order **Desmoscolecida** Filipjev, 1929

Family **Desmoscolecidae** Shipley, 1896

Tribe **Greeffiellini** Filipjev, 1929

Genus ***Greeffiella*** Cobb, 1922

Diagnosis (updated from Decraemer & Rho, 2014). Body cuticle annulated, annules with long hair-like spines; posterior spines with thickened base; numerous hair-like spines arranged either in circlets or dorso- or ventrolateral groups. Four cephalic setae between hair-like ornamentation. Somatic setae with desmoscolecoid arrangement; subventral setae absent in female. Ocelli present or absent. Male monorchic. Paired spicules without gubernaculum. Males with or without subventral pre- and postanal papilla shaped as transformed somatic setae inserted on teat-like base. Last ring elongated, with or without elongated terminal tube in some species.

Type species: *Trichoderma oxycaudatum* Greeff, 1869, designated by Cobb (1922).

Composition: *Greeffiella alleni* Schrage et Gerlach, 1975, *G. antarctica* Timm, 1978 *G. australis* Schrage et Gerlach, 1975, *G. beatlei* Lorenzen, 1969, *G. dasyura* Cobb, 1922, *G. longiseta* Bussau, 1993, *G. loxa* (Steiner, 1916) Stammer, 1935, *G. macrotricha* (Steiner, 1916), *G. moppa* Schrage, 1972, *G. myponga* Schrage et Gerlach, 1975, *G. oxycaudata* (Greeff, 1869), and *G. pierri* Schrage et Gerlach, 1975.

Note. Greeffiella comosa Lorenzen, 1969 was used as a type species to erect the monotypic genus *Greeffiellopsis* Schrage et Gerlach, 1975, based on rod-shaped structures located between the body annules and the presence of four setiform appendages on the tail tip.

Greeffiella japonica sp. nov.

(Figs 2–3)

Holotype. Female (IBSS, Meib.26.N.h.), **Sea of Japan**, **Russia**, *Primorskiy Terr.*, Vladimir Bay, 43°53′15″N, 135°29′48″E, station No. 42, depth 24 m, pelitic black silt, cruise No. 64 of R/V "Academician Oparin", Van Veen grab (0.1 m^2), 28.VI.2021, coll. T.N. Revkova.

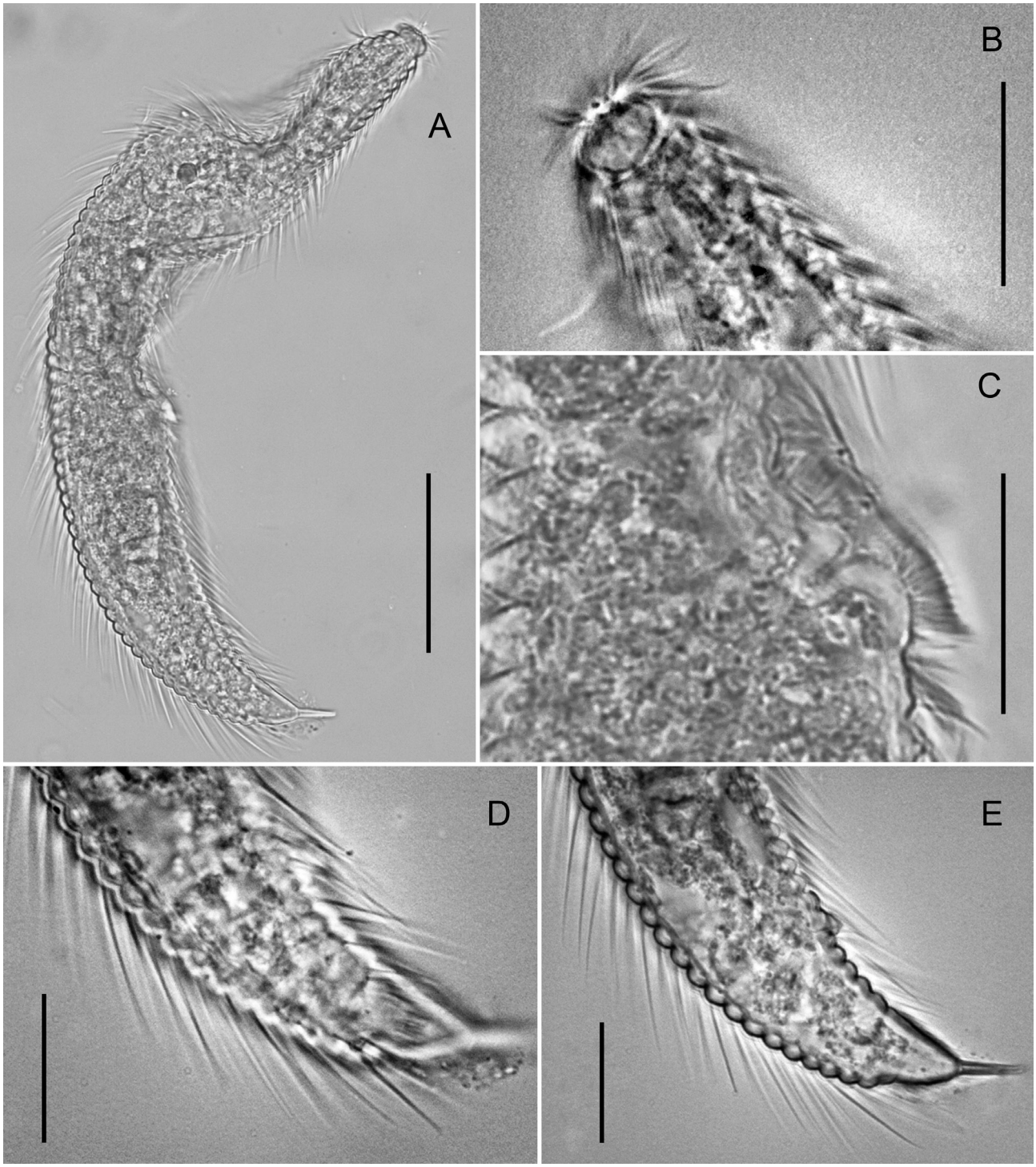

Fig. 2. *Greeffiella japonica* **sp. nov.**, female. **A**, general appearance; **B**, amphidial fovea; **C**, vulval region; **D**, somatic setae in the tail region; **E**, tail region and anus. Scale bars: 50 μm (A), 20 μm (D, E), and 10 μm (B, C).

Description. Body 260 μm long, cylindrical, tapering posteriorly (a = 6.8, b = 10, c = 6.3), consisting of 70 annules dorsally and 62 annules ventrally (annulus 3–6 μm wide). Cuticle along entire length of body transparent, with large numbers of hair-like spines of different lengths. Hair-like spines located strictly in transverse rows from head to base of tail.

Head (4 × 8 μm) short, rounded, narrower than first annulus, bearing large, elliptical thick-

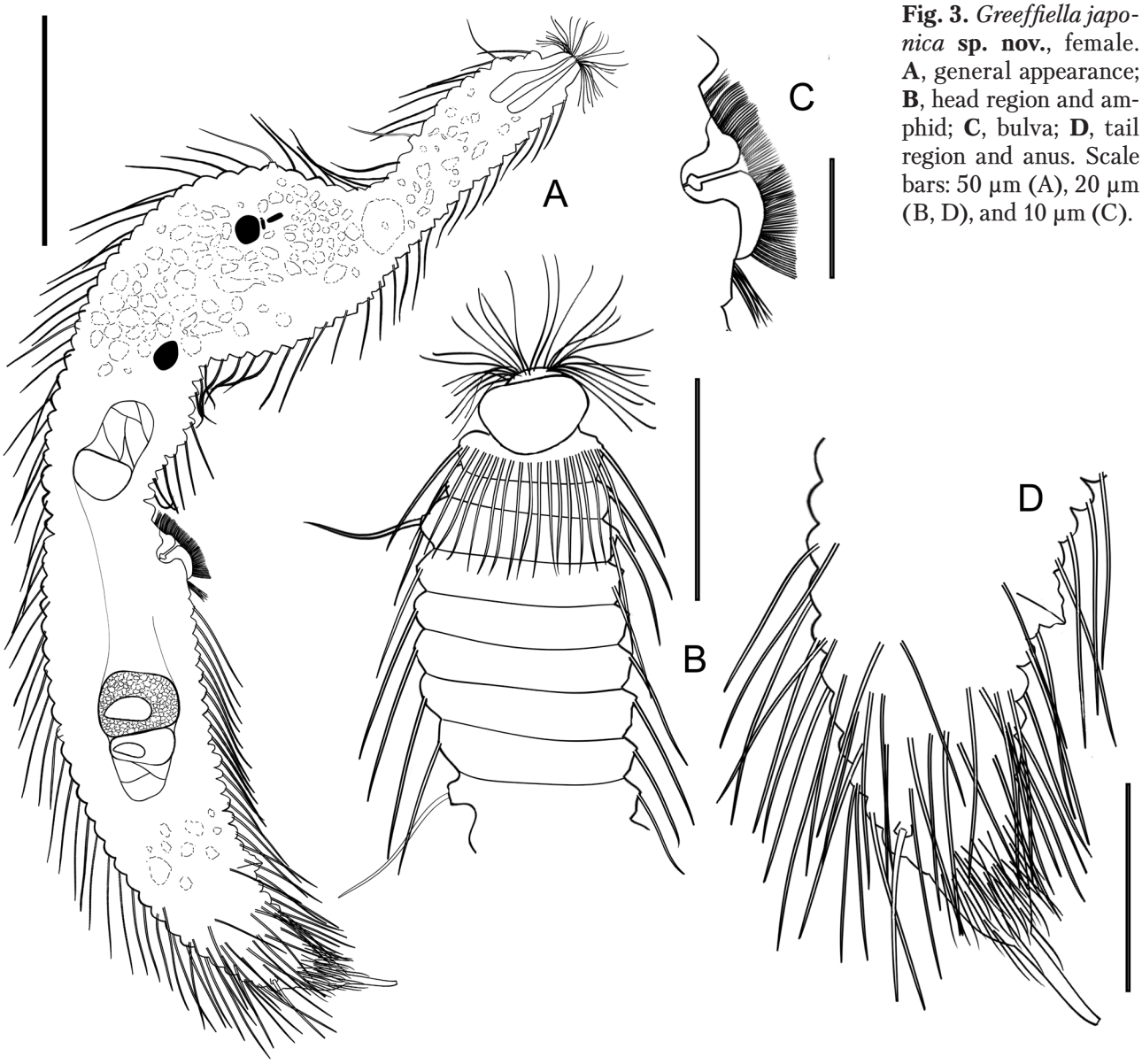

Fig. 3. *Greeffiella japonica* **sp. nov.**, female. **A**, general appearance; **B**, head region and amphid; **C**, bulva; **D**, tail region and anus. Scale bars: 50 μm (A), 20 μm (B, D), and 10 μm (C).

rimmed amphids, those being much broader than head. Thin hair-like (about 11–12 μm long) ornamentation anterior to amphids. Four cephalic setae not visible. A pair of dark yellowish, oval ocelli situated at level of annules 19 and 29. Pharynx short, 26 μm, posteriorly extending to the level of annulus 6. Maximal body diametre 38 μm, diametre in vulval region 33 μm. Each annulus of body bearing a row of hair-like spines with length increasing from anterior part (11–15 μm) towards middle (15–23 μm) and posterior part (15–25 μm) of body. Six pairs of subdorsal tubular somatic setae on small peduncles devoid of hair-like setae, located on annules 3, 9, 17, 30, 50, and 68. These tubular setae with diverse lengths (10–15 μm), looking slightly thicker than other setae. Reproductive system didelphic, amphidelphic, with equal genital branches. Vulva strongly protruding, located between annules 37 and 38 (55% from anterior end of body). Cuticle around vulva strongly thickened, densely surrounded by fine hair-like setae (Figs 2C, 3C). Anus tubular, located between 55–57 annules. Tail 41 μm long, consisting of six ventral annules and ten dorsal annules (including terminal ring). Terminal ring (11 × 10 μm) wide, smooth, shaped as cone, with rows of short, thin hair-like setae; terminal tube 2 μm wide and 10 μm long.

Diagnosis. Greeffiella japonica **sp. nov.** is characterised by having of 70 annules dorsally and 62 ones ventrally, ocelli located on annules 19 and 29, length (11–25 μm) of somatic setae increasing from head to tail, six pairs of subdorsal tubular setae, pubescent vulva surrounded by dense fine hair-like setae, located between annules 37 and 38, tubular anus, wide cone-shaped terminal ring with rows of short thin hair-like setae, and terminal tube 10 μm long.

Comparison. In the number of subdorsal setae, the female of *G. japonica* **sp. nov.** is similar to females of the following species: *G. australis* Schrage et Gerlach 1975, *G. dasyura* Cobb 1922 and *G. pierri* Schrage et Gerlach 1975, but differs from them in the position of vulva: 36–37 annules (55%) *vs.* 41–42 annules (57%) in *G. australis*, 28–29 annules (38.5%) in *G. dasyura*, and 32–36 annules (51–55%) in *G. pierri*. The new species is most similar to *G. australis*, but differs from it in the shorter body (260 μm *vs.* 330 μm), relatively longer anterior hair-like spines on the annules (11–15 μm *vs.* 3–10 μm), the longer terminal tube (10 μm *vs.* 6 μm), a longer hair-like ornamentation of the head (11–12 μm *vs.* 3–8 μm), and the position of ocelli (on 19 and 29 *vs.* 13–14 body annules). It differs from *G. dasyura* in the number of the body annules (62–70 *vs.* 55–59 and 60–61), the shorter tail (c = 6.3 *vs.* 4.2), thinner body (a = 6.8 *vs* 3.7), and relatively longer anterior hair-like spines (11–15 μm *vs.* 4–11 μm). The female of the new species differs from females of *G. pierri* in having the shorter tail (c = 6.3 *vs.* 4.2–4.9), shorter terminal tube (10 μm *vs.* 15 μm), the number of the body annules (62–70 *vs.* 54–61), the position of ocelli (on 19 and 29 *vs.* 9–16 body annules), and the shape of terminal ring (wide *vs.* elongated). The female of *G. japonica* **sp. nov.** is similar to that of *G. beatlei* Lorenzen, 1969 only in the pubescence of vulva (Fig. 6), but differs from this species in the shorter tail (c = 6.3 *vs.* 2.9), the number of subdorsal setae (6 *vs.* 7), and longer anterior (11–15 μm *vs.* 4–5 μm) and posterior hair-like spines (15–25 μm *vs.* 4–13 μm). *Greeffiella japonica* **sp. nov.** differs from *G. pontica* **sp. nov.** in the thinner body (a = 6.8 *vs.* 3.8–4.4), the body length (260 μm *vs.* 378–398 μm), the number of subdorsal setae (6 *vs.* 7), and the position of vulva (37–38 annules [55%] *vs.* 41–44 [49.5–52%]).

Note. Most authors have not sufficiently illustrated the vulval region. There are two types of pubescence around the vulva: (1) dense, with fine short hair-like setae as in females of *G. beatlei* and *G. japonica* **sp. nov.** (Fig. 2) and (2) sparse, with small setae as in *G. myponga* (Fig. 7) and *G. pontica* **sp. nov.** (Figs 4) (Sergeeva & Revkova, 2021).

Etymology. The specific name is a Latin adjective meaning "Japanese".

Greeffiella pontica sp. nov.

(Fig. 4)

Holotype. Female (IBSS, Meib.1.N.h.), **Black Sea**, *Crimea*, shelf, 45°11′ N, 32°12′ E, station No. 5549, depth 56 m, strongly silted fine sand with detritus, cruise No. 56 of R/V "Professor Vodyanitskiy", 15.XII.2001, coll. N.G. Sergeeva.

Paratype. Female (IBSS, Meib.2.N.p.), same data as for holotype.

This species has been mentioned by Sergeeva & Revkova (2021) as *Greeffiella* sp. and is described below with some new characteristics.

Description. Body 380–398 μm long (a = 3.8–4.4, b = 15.7–16.6, c = 6.5–9.3), consisting of 67–70 annules dorsally and 67–68 annules ventrally (annulus 3–6 μm wide). Body smoothly widening from head to middle and then gradually tapering to tail.

Cuticle along entire length of body transparent, with large numbers of hair-like spines of different lengths. Hair-like spines located strictly in transverse rows from head to base of tail. Head (7 × 10 μm) short rounded, narrower than first annulus, bearing large elliptical thick-rimmed amphids, those being much broader than head. Thin hair-like (about 13–15 μm long) ornamentation anterior to amphids. Cephalic setae and ocelli not visible.

Pharynx short, 24 μm, posteriorly extending to level of annulus 5, devoid of bulb, with a pair of salivary glands at base. Cardia (valve) shaped as special elongated area situated at salivary glands and posteriormost margin of pharynx and bordering anterior part of intestine. Maximal body diametre 87–105 μm, diametre in vulval region 82–102 μm. Each annulus of body bearing a row of hair-like spines with length increasing from anterior part (12–24 μm) towards middle (21–35 μm) and posterior (21–42 μm) part of body. Seven pairs of subdorsal tubular setae on small

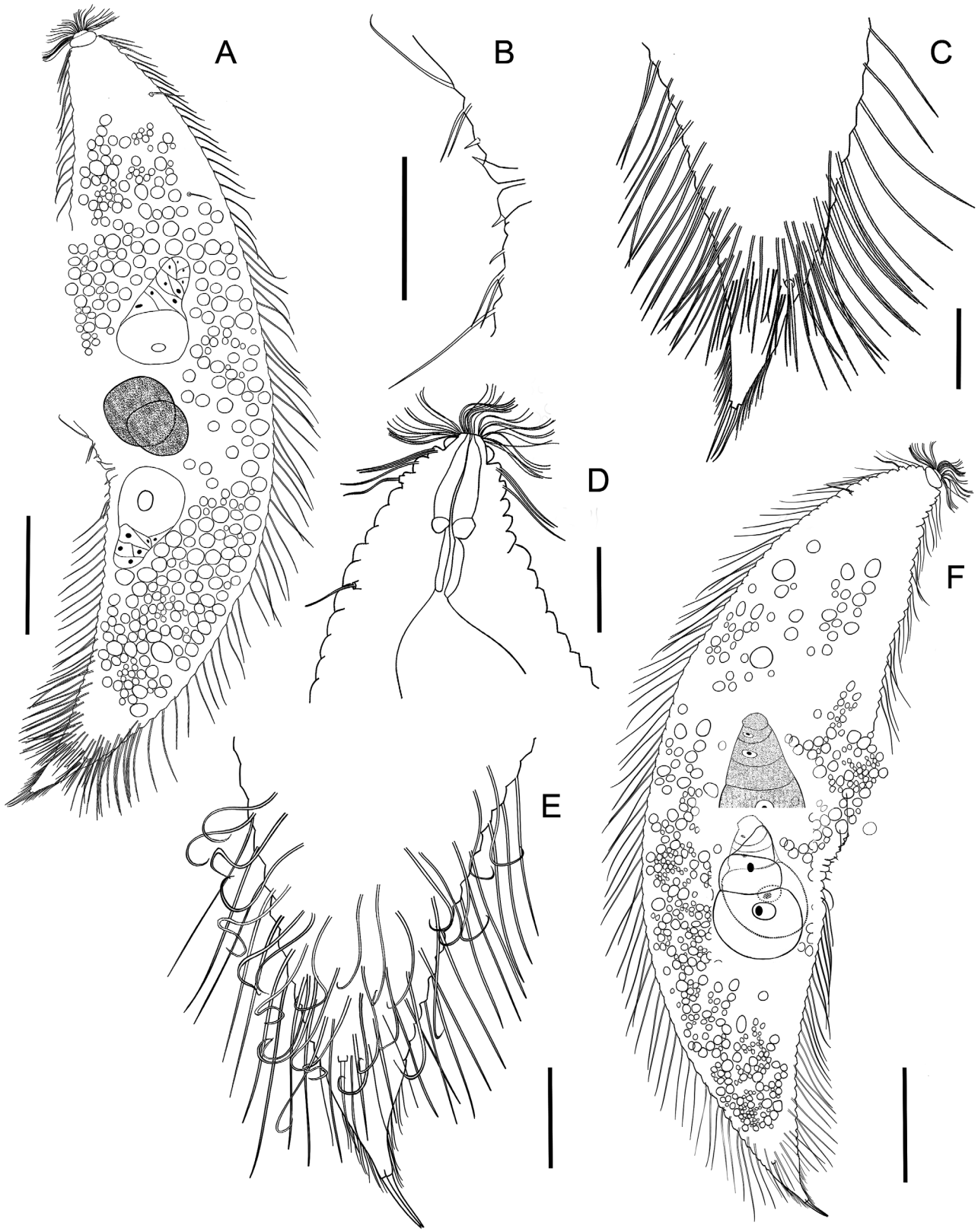

Fig. 4. *Greeffiella pontica* **sp. nov.**, females. **A**, holotype, general appearance; **B**, holotype, vulva; **C**, holotype, tail region; **D**, paratype, head region; **E**, paratype, tail region; **F**, paratype, general appearance. Scale bars: 50 μm (A, F) and 20 μm (B, C, D, E).

peduncles devoid of hair-like setae, located on annules 3, 8, 18, 27–30, 41, 55–53, and 69–68. These tubular setae of diverse lengths (11–20 μm), looking slightly thicker than other setae.

Reproductive system didelphic, amphidelphic, with equal genital branches. Vulva not protruding, located between annules 41 and 43 in holotype and 42 and 44 in paratype, surrounded by short, sparse setae (2 μm long) (Fig. 4B). Mature egg 30 × 32 μm.

Anus not visible. Tail ca 43–58 μm long, consisting of 6–8 ventral annules and 6–7 dorsal annules (including terminal ring). Terminal ring (23–28 × 14–18 μm) wide, smooth, shaped as triangle, with pointed apex and rows of short hair-like setae. Tail tip with three long thin terminal tubes (13 μm long) (Fig. 4C).

Diagnosis. Greeffiella pontica **sp. nov.** is characterised by having of 67–70 dorsal and 67–68 ventral annules, not visible ocelli, somatic setae 12–42 μm long, increasing from head to tail, seven pairs of subdorsal tubular setae, vulva surrounded by short sparse setae (2 μm long) and situated between 41–43 and 42–44 annules, not distinct anus, cone-shaped terminal ring with rows of short thin hair-like setae, and three thin terminal tubes 13 μm long.

Comparison. Females of *G. pontica* **sp. nov.** differ from all known females of *Greeffiella* in having three thin terminal tubes. In the number of cuticular annules, the new species is similar to *G. antartica*, *G. japonica* **sp. nov.**, *G. australis*, and *G. moppa*, but differs from them by having the thicker body (a = 3.8–4.4 *vs.* 5.8–6.6 in *G. moppa*, 6.3 in *G. australis*, 6.8 in *G. japonica* **sp. nov.**, and 7.1–7.4 in *G. antarctica*) and the absence of ocelli. The new species differs from *G. antartica* in the number of tail annules (6–8 *vs.* 11–14), the position of vulva (41–44 *vs.* 38 annules), and the shape of terminal ring (conical and wide *vs.* elongate and narrow). The new species differs from *G. australis* in the longer body (378–398 μm *vs.* 240–330 μm), the number of subdorsal setae (7 *vs.* 6), longer somatic setae (12–42 μm *vs.* 3–22 μm), and the longer terminal tube (13 μm *vs.* 6 μm). It differs from *G. moppa* in the longer body (378–398 μm *vs.* 202–275 μm), the shape of terminal ring (conical and wide *vs.* elongate and narrow), and the position of vulva (41–44 *vs.* 37–38 annules). *Greeffiella pontica* **sp. nov.** differs from *G. japonica* **sp. nov.** in the body length (378–398 μm *vs.* 260 μm), the number of subdorsal setae (7 *vs.* 6), and the position of vulva (41–44 [49.5–52%] *vs.* 37–38 annules [55%]).

Etymology. The specific name is a Latin adjective meaning "pertaining to the Pontus, Euxine, or Black Sea".

Geographical and bathymetrical distribution

The species of the genus *Greeffiella* are found in all oceans of the world (Fig. 5). Of twelve species, four are known from Europe (Lorenzen, 1969; Schrage, 1972; Schrage & Gerlach, 1975), three from Africa (Steiner, 1916; Schrage, 1972), one from Asia (Nemys, 2022), three from South and North America (Cobb, 1922; Schrage & Gerlach, 1975; Bussau, 1993), one from the Antarctic region (Timm, 1978), and three from the Australian region (Timm, 1970; Schrage & Gerlach, 1975). *Greeffiella pontica* **sp. nov.** inhabits the Black Sea, *G. antarctica* (Nemys, 2022) and *G. japonica* **sp. nov.**, the Sea of Japan.

The species of *Greeffiella* not only have a wide geographical distribution, but they also inhabit shallow bathyal and abyssal biotopes. Not all records of *Greeffiella* species contain indications of the depth habitat of particular species. We summarise the estimated bathymetric distribution of some species based on available data from publications and the Nemys database (Steiner, 1916; Timm, 1978; Bussau, 1993; Nemys, 2022): *G. loxa* and *G. macrotricha* were found in the upper subtidal zones (8–9 m); *G. oxycaudata* is adapted to a wide range of habitat depths from the upper subtidal (40 m) to the abyssal (4000 m) zones; *G. moppa* and *G. antarctica* also have a wide bathymetric range from the upper subtidal (29–220 m) to the bathyal (310–3000 m) zones; *G. beatlei* is limited to the bathyal zone (500 m), while *G. longiseta* is found only in the deepest abyssal zone (4000 m).

Diagnoses and notes on the descriptions of *Greeffiella* species

Morphometric data and illustrations of the species of *Greeffiella* are given in Table 1 and in Figs 2–4, 6 and 7.

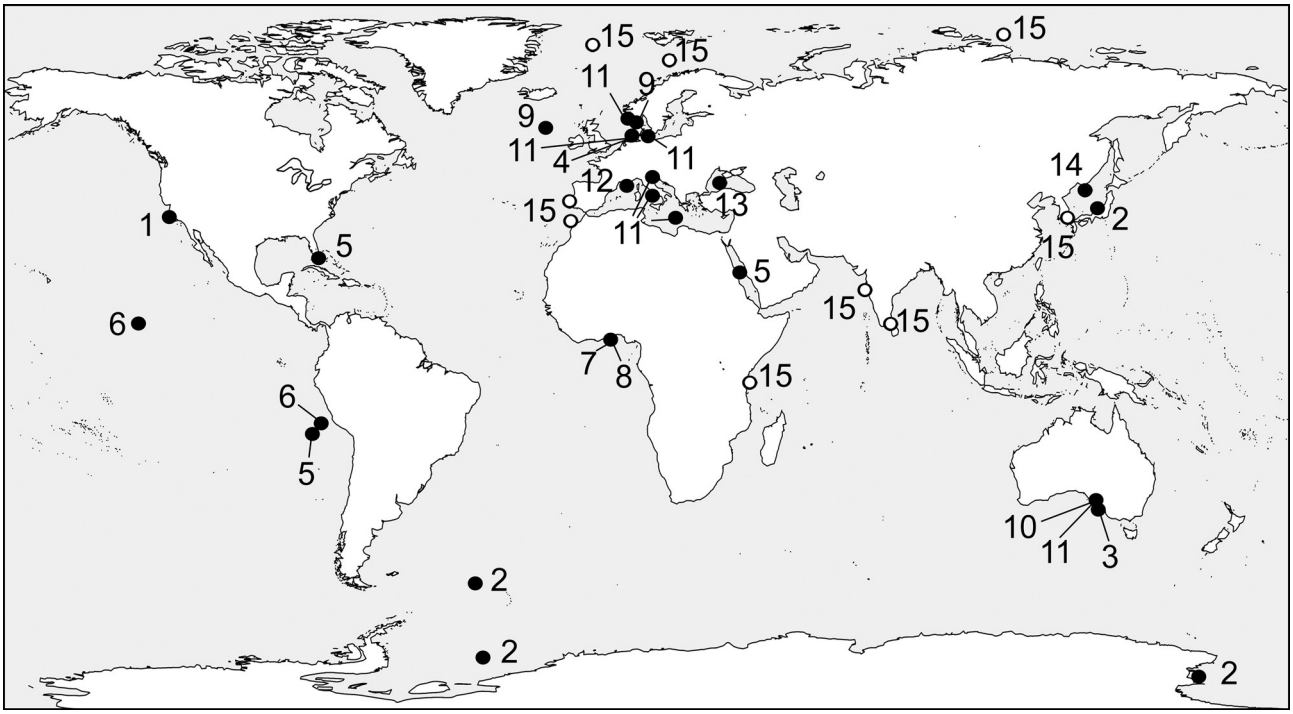

Fig 5. Map of geographical distribution of *Greeffiella* species. **1**, *G. alleni*; **2**, *G. antarctica*; **3**, *G. australis*; **4**, *G. beatlei*; **5**, *G. dasyura*; **6**, *G. longiseta*; **7**, *G. loxa*; **8**, *G. macrotricha*; **9**, *G. moppa*; **10**, *G. myponga*; **11**, *G. oxycaudata*; **12**, *G. pierri*; **13**, *G. pontica* **sp. nov.**; **14**, *G. japonica* **sp. nov.**; **15**, *Greeffiella* sp. (Fonseca & Soltwedel, 2009; Hwang et al., 2009; Nanajkar et al., 2011; Nemys, 2022; OBIS, 2022).

Greeffiella alleni Steiner, 1916 (Fig. 6A, B). The species was described from one male. Cephalic setae are about the same length as the hair-like ornamentation. Spines of the cuticular annules are accompanied by shorter hair-like setae from the head to the tail. The terminal ring is very short; the length of terminal tube is equal to twice the width of an annulus. Male supplements are absent. Subventral setae are situated on the following body annules: 6/7, 17/18, 24/23, 40, 48, 54, and 56.

Greeffiella antarctica Timm, 1978 (Fig. 7A, B). Somatic setae are fine, their length increases from the head to the tail. Timm (1978) indicated only the maximal length of anterior (26 μm long) and posterior (51–55 μm long) setae. Subdorsal and subventral setae were not measured. In the female, anus is indistinct. The terminal ring is 46–48 μm long, consisting of the narrowly triangular anterior part and the spike-shaped terminal tube. Male supplements are absent.

Greeffiella australis Schrage et Gerlach, 1975 (Fig. 6C, D). Cephalic setae are much shorter than the hair-like ornamentation of the head. The length of spines increases from the head to the tail. Hair-like setae are visible between spines on all the cuticular annules of body (1.5–6 μm long). Male supplements are absent. Subventral setae are situated on the following body annules: 3/5, 6/7, 16, 26, 42, 51/52, and 54/57.

Greeffiella beatlei Lorenzen, 1969 (Fig. 6E–H). The maximal length of setae on the anterior cuticular annules is 4–5 μm, they increase continuously (12–13 μm long) to the posterior body end, their minimal length is 4–5 μm throughout the body. The male has preanal hair-like setae and two postanal saber-shaped setae on the anus, and two pairs of pre- and postcloacal papillae. The author of the original description does not indicate the location of 7–8 pairs of subventral setae. According to Schrage & Gerlach (1975), subventral setae are situated on the following body annules: 1, 12, 19, 27, 35/34, 41/40, 48/47, 50, and 56/57.

Greeffiella dasyura Cobb, 1922 (Fig. 6I–K). Among the somatic setae, there are several relatively large hollow open setae. The length and thickness of spines increases from the head to the tail. Cobb (1922) suggested that males have the preanal papillae, the ventral submedian papilla-like organs on the annules 5 and 11 anterior to the

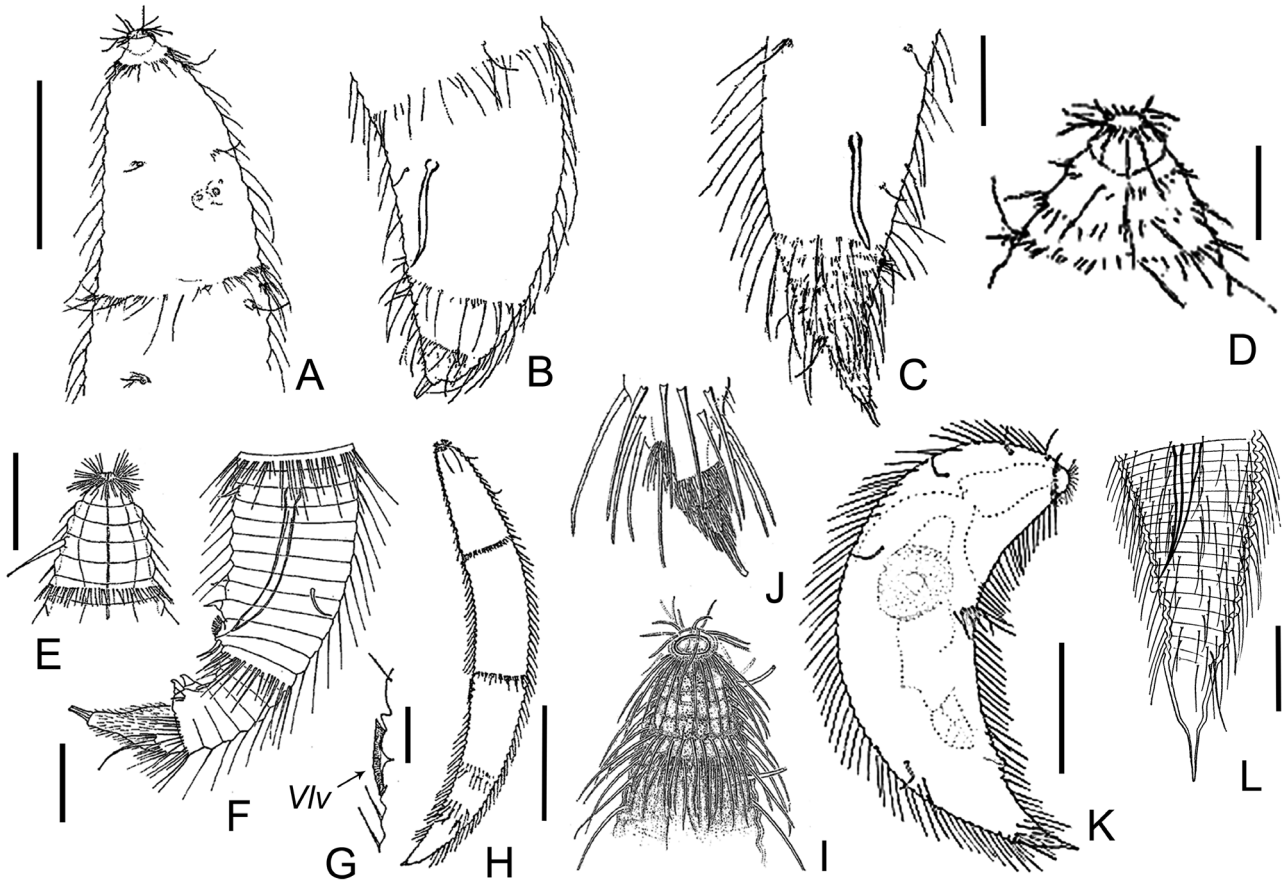

Fig. 6. Details of structure of *Greeffiella* species. **A**, **B**, *G. alleni*; **C**, **D**, *G. australis*; **E**–**H**, *G. beatlei*; **I**–**K**, *G dasyura*; **L**, *G. loxa*. Head (A, D, E, I); male caudal region (B, C, F, L); vulval region (G); general appearance (H, K); tail region (J). Abbreviation: *vlv* – vulva. Scale bars: 10 μm (D, G), 20 μm (C, E, F, L), and 50 μm (A, B, H, K). By Schrage & Gerlach (1975) (A–D), Lorenzen (1969) (E–H), Cobb (1922) (I, J), Schrage (1972) (K), and Steiner (1916) (L).

anus, which are marked by a cluster of minute setae near nerve ending, about ten setae on the annulus 5 and a smaller number on the annulus 11, and a bundle of fine setae on the lateral margin of body near its middle. Schrage & Gerlach (1975) note five pairs of subventral setae in males, more often preanal.

Greeffiella longiseta Bussau, 1993 (Fig. 7C–F). Each cuticular annulus is covered with spines of various lengths (5–50 μm). The anus in males is small, tubular, surrounded by short setae. The anus in females is not distinct, therefore the *c*-value is not defined. Subventral setae are situated on the following body annules: 12–13, 22–23, 33–34, 40–41, 46–47, and 52–53.

Greeffiella loxa (Steiner, 1916) Stammer, 1935 (Fig. 6L). A poorly described species; the figure shows only the tail of the male. Somatic setae increase from the head to the tail. There is a pair of uniformly thick subdorsal setae 18–20 μm long anterior to the terminal ring. The terminal ring is cone-shaped, without setae.

Greeffiella macrotricha Steiner 1916 (Fig. 7G). This species was described from one female, and only the tail tip was illustrated. Spines of the same length (up to 29 μm) along the body are arranged in 10–12 longitudinal rows. The terminal ring is thin, elongate, with the long spike-shaped terminal tube.

Greeffiella moppa Schrage, 1972 (Fig. 7H, I). The length and thickness of spines increase from the head to the tail. Cephalic setae are about the same length as the hair-like ornamentation. The author determined the location of the vulva only approximately. Males have two types of subventral setae: (1) tiny on the anterior five annules of

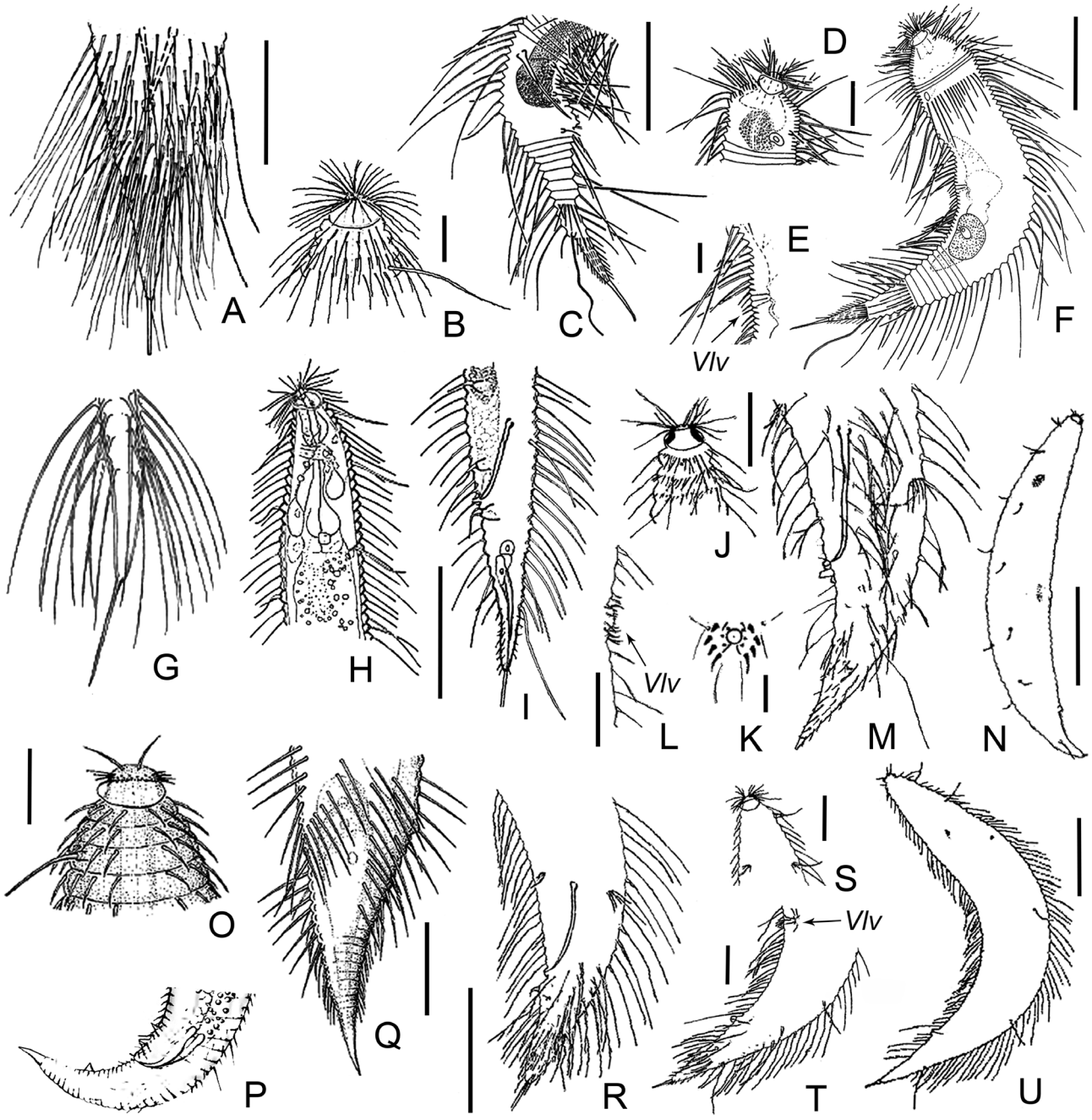

Fig. 7. Details of structure of *Greeffiella* species (continued). **A**, **B**, *G. antarctica*; **C**–**F**, *G. longiseta*; **G**, *G. macrotricha*; **H**, **I**, *G. moppa*; **J**–**N**, *G. myponga*; **O**–**Q**, *G. oxycaudata*; **R**–**U**, *G. pierri*. Tail region (A); head (B, D, J, O, S); male caudal region (D, I, M, R), vulval region (E, L); general appearance (F, N, U); tail tip (G); anterior part of body (H); anal papilla (K); tail region of male (P); tail region of female (Q); posterior part of female (T). Abbreviation: *vlv* – vulva. Scale bars: 10 μm (E, K, O), 12 μm (B), 20 μm (D, J, L, Q, S, T), and 50 μm (A, C, F, H, I, N, R, U). By Timm (1978) (A, B), Bussau (1993) (D–F), Steiner (1916) (G), Schrage (1972) (H–I), Schrage & Gerlach (1975) (J–N, R–U), Greeff (1869) (P), and Timm (1970) (O, Q).

the body and (2) eight pairs of whip-like curved, standing on strong papillary tubercles; the anterior of these pairs is very small and indistinct. Schrage (1972) confused subventral setae with the pre- and postanal papillae which transform into body setae located on the papilla-shaped tubercles. Subventral setae are situated on the following body annules: 5, 13, 21, 29, 37, 45, 53, and 57.

Table 1. Distinctive morphological and morphometric characters of the species of *Greeffiella*.

Characters	*G. alleni*	*G. antarctica*	*G. australis*	*G. beatlei*	*G. dasyura*	*G. longiseta*	*G. loxa*	*G. macrotricha*	*G. moppa*	*G. myponga*	*G. oxycaudata*	*G. pierri*	*G. japonica* **sp.n.**	*G. pontica* **sp.n.**
Body length, μm	355♂	275–430♂ 365–476♀	240 ♂ 330 ♀	240–323♂ 260–320♂♀	210♀ 310–340	240–260♂ 245–255♀ 165 juv	123–185♂ 137–227♀	216♀	235–292 ♂ 202–275 ♀	218–330♂ 255–375♀	300♂ 375♀	255–315♂ 260–325♀	260♀	378–398♀
a	7.1	7.1–7.4	6♂ 6.3♀	5.1–7.8♂ 7.1–8.2	3.7	7.1–7.3♂ 6.5–7.2♀ 7.5 juv	4.6–5.1♂ 4–5.5♀	7.4	6.4–7.7♂ 5.8–6.6♀	7.7♂ 5.6–7.2♀	5.8	5.3–7.4	6.8	3.8–4.4
b	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	3.7–5.3♂ 4–6.9	4.6	n.a.	n.a.	n.a.	n.a.	10	15.7–16.6
c	8.9	4.8–4.9♀ 3.5♂	6♂ 6.3♀	2.9♂	4.2	2.7–3.3♂	3.7–5.6♂ 3.8–5.7♀	3.5	2.9–3.7♂	5.2♂ 6–7♀	5.1	4.2–4.9	6.3	6.5–9.3◄
Number of annules	64d 61–62v	62–64♂ 67–70♀	64♂ 68–69♀	59d ♂ 58v 62 ♀ 58–64	55–59 60–61	63♂ 65–67♀ 49juv	n.a.	n.a.	65♂ 66–68♀	52♂ 58–66d ♂ 56–64v 54–60♀ 60–61d ♀ 59–60v	90d 86v 88–89♀ 75–77♀	56d ♂ 55v 54–58d ♂ 57–61d ♀	70d 62v	67–70 d 67–68v
Subdorsal tubular setae	6–7	n.a.	6	7	6	4	n.a.	n.a.	7	6–7	4♀ and 7	6	6	7
Subventral setae	7	n.a.	7	7–9	5	6	n.a.	n.a.	8	5–6	7♀	5–6	n.a.	n.a.
Location of ocelli	8	10–15	13–14	abs	8–9*	abs	n.a.	n.a.	10–16	12–13♂ 10–14♀	15	12–16♂ 9–16♀	19 and 29	abs
Anterior somatic setae length, μm	2–24	26**	3–10	4–5	4–11	5–50	7–9	up to 29	9–24♂ 17–23♀	5–13 6–11♂ 16–18♀	3	3–17	11–15	12–24
Middle somatic setae length, μm	2–21	n.a.	10–20	4–13	n.a.	5–50	n.a.	up to 29	11–29♂ 20–26♀	13–24	n.a.	10–41	15–23	21–35
Posterior somatic setae length, μm	2–24	51–55***	12–22	4–13	10–18	5–50	13.8–20	up to 29	15–50♂ 25–45♀	15–27 17–18♂ 18–33♀	16	7–40	15–25	21–42
Spicule length, μm	33 (0.8 abd)	32	29 (1.2 abd)	39–56	n.a.	38	n.a.	n.a.	25.7–33 (1–1.4 abd)	30–37 (1.6 abd)	n.a.	34–41 (1.4–1.6 abd)	n.a.	n.a.
Pre- and postanal papilla in male	abs	abs	abs	Pair of pre- and postanal	2 or 3 preanal?	abs	abs?*	n.a.	pre- and postanal whip-shaped, curved	a pair of pre- and postanal	large pre- and postanal	one preanal in one male; one postanal in holotype and other specimens	n.a.	n.a.
Number of annules in tail	4–5	7–10♂ 11–14♀	7♂ 7–8♀	7*	7–8	12	6?	n.a.	8	5♀ 5–6♂	19–20	5–6	10d 6v	6–8
Tail terminal tube length, μm	6.5	22*	6	6*	9	17	n.a.	n.a.	12.8–6.2♂ 11–15.7♀	5	n.a.	15	10	13
Location of vulva (number of annules and %)	n.a.	38	41–42 (57%)	35–36 (54–58%)	28–29 (38.5%)*	38–39 and 34–35 (40.8–47.5%)	n.a.	n.a.	37–38	31–34 (49–50%)	38 and 43	32–36 (51–55%)	37–38 (55%)	41–44 (49.5–52%)

Note. * measurements from illustrations; ** length of first somatic setae; *** maximal length; ◄ approximate measurements; *a*, body length / maximum body diametre; *b*, body length / pharynx length; *c*, body length / tail length; *c'*, tail length / anal body diametre. *v*, ventral; *d*, dorsal; *n.a.*, not available; *juv.*, juvenile; *abs*, absent.

Greeffiella myponga Schrage et Gerlach, 1975 (Fig. 7J–N). Head is sclerotised. The length and thickness of spines increase from the head to the tail. The base of spines on the tail is strongly thickened (2 μm in wide). Short hair-like setae among spines are visible only on the anterior four annules.

The specimen of "*G. dasyura*" from Australia (Port Noarlunga) was redescribed by Timm (1970) and assigned to *G. myponga* by Schrage and Gerlach (1975). It has two circles of anal papillae around the anus. The inner circle consists of two cone-shaped papillae (4 μm long), there are three papillae of equal length (1–2 μm) on each side of it, and two setae-like papillae (5 μm long) are locater posterior to the circle. The outer circle has three thick cone-shaped papillae (5 μm long) on the sides of anus, as well as one strong spine. Subventral setae are situated on the following body annules: 1, 7/8, 17/16, 25/26, 39/40, and 45.

Greeffiella oxycaudata (Greeff, 1869) (Fig. 7O–Q). The head bears numerous hair-like ornamentations and four sublateral setae. Spines length increases from the anterior to posterior body parts (3–16 μm long); first subdorsal setae are 12 μm long (Timm, 1970). In a female from Australia, Timm (1970) indicated seven subventral pairs of setae on the annules 3, 11, 21, 31, 40, 76, and 80 in addition to four subdorsal setae, without indication of their location. However, this is not consistent with the diagnosis of the genus *Greeffiella* (Decraemer & Rho, 2014) and requires a re-examination of the female from Australia (Timm, 1970). Subventral setae are situated on the following body annules: 3, 10, 19, 29, 38, 63, and 80 (Schrage & Gerlach, 1975).

Greeffiella pierri Schrage et Gerlach, 1975 (Fig. 7R–U). The length and thickness of spines increase from the head to the tail. There are hair-like setae on the anterior 12–13 annules between spines, which gradually disappears. Males have one triangular postanal papilla with the curved tip, but only one male has one preanal papilla. Diametre of the base (9 μm) of the postanal papillae and its length (3 μm) approximately corresponds to the width of the two annules to which these papillae are attached. Thin subdorsal setae from the first to fifth pairs are 14–20 μm long, the sixth pair on the tail is the longest (27 μm). Subventral setae situated on the following body annules: 6–8, 15–17, 25–27 or 25/27, 40–44, 46–48, 50–53 and 7, 17/18, 28/29, 40/39, and 46–47.

Key to the species of *Greeffiella*

1. Number of cuticular annules more than 70 . . . . . ***G. oxycaudata***
– Number of cuticular annules no more than 70 2
2. Terminal ring thin, elongate 3
– Terminal ring wide, conical 6
3. Spines of equal length . . . . . ***G. macrotricha***
– Spines of different length 4
4. Male supplement present . . . . . ***G. moppa***
– Male supplement absent 5
5. Spines strong, with length varying from head to tail. . . . . ***G. longiseta***
– Spines no strong, with length increasing from head to tail . . . . . ***G. antarctica***
6. Terminal ring without setae . . . . . ***G. loxa***
– Terminal ring with setae. 7
7. Short and thin setae between spines absent 8
– Short and thin setae between spines present. 10
8. Tail with three terminal tubes . . ***G. pontica* sp. nov.**
– Tail with one terminal tube 9
9. Spines not thickened; vulva surrounded by thin dense setae. . . . . ***G. japonica* sp. nov.**
– Spines thickness increasing to tail; vulva surrounded by short sparse setae . . . . . ***G. dasyura***
10. Spines from head to tail not thickened; male supplements absent 11
– Spines thickness increasing from head to tail; male supplements present. 12
11. Number of tail annules: 4–5, c = 8.9. . . . . **G. *alleni***
– Number of tail annules: 7–8, c = 6–6.3 . . . . . ***G. australis***
12. From six to eight conical papillae around anus; head strongly sclerotised . . . . . ***G. myponga***
– From one to four triangular anal papillae with a claw-like apex; head not sclerotised 13
13. Three or four cuticular annules between postanal papilla and terminal ring; six pairs of subdorsal setae . . . . . ***G. pierri***
– Cuticular annules between postanal papilla and terminal ring absent; seven pairs of subdorsal setae . . . . . ***G. beatlei***

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