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Two new oxystominid species (Nematoda: Enoplida) from an abyssal plain in the southern Philippine Sea

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Two new species of free-living marine nematodes, Litinium dispariseta *sp. nov. and* Wieseria minor *sp. nov. are described from an abyssal plain with a water depth of 4117–5035 m near the Southern Kyushu-Palau Ridge in the tropical Western Pacific Ocean.* Litinium dispariseta *sp. nov. is characterized by having distinct inner labial setae two times longer than the outer labial setae, wrench-like amphideal foveas, a single preanal midventral supplementary seta and a short cylindrical tail with bluntly rounded tip. It differs from congeners by its distinctly long inner labial setae relative to the outer labial setae (two times longer* vs *shorter or equal in length) and peculiar wrench-like amphideal foveas (*vs *horseshoe shaped or ovoid with round to oval anterior aperture).* Wieseria minor *sp. nov. has a clavate tail, a character found only in the four congeners* W. glandulosa *(Kreis, 1929),* W. longiseta *(Allgén, 1947),* W. clavata *Gerlach, 1956 and* W. inaequalis *Gerlach, 1956. However,* Wieseria minor *sp. nov. differs from these congeners by its much smaller body size (1045* μ*m* vs *2120–3125* μ*m) and oblong amphideal foveas with double contour (*vs *a single oblong or ovoid loop). An emended diagnosis for* Wieseria *and pictorial keys for* Litinium *and* Wieseria *are provided.*

Keywords: deep sea, nematode, taxonomy, *Litinium dispariseta* sp. nov., *Wieseria minor* sp. nov.

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INTRODUCTION

Oxystominidae Chitwood, 1935 is a common family widely distributed from marine to freshwater environments (Smol *et al.*, 2014). Members of the family are often numerically dominant or subdominant in nematode samples, occupying about 4–16% of the total nematode abundance (Tietjen, 1991; Vopel & Thiel, 2001; Lambshead *et al.*, 2003; Miljutina *et al.*, 2010; Pastor de Ward *et al.*, 2015). The family now contains nine genera and over 160 species (Smol *et al.*, 2014; Tchesunov *et al.*, 2014).

So far, only a few taxonomic studies have been performed for deep-sea species of the family, for various reasons (Miljutina *et al.*, 2010; Tchesunov *et al.*, 2014). First, in spite of their high species diversity, deep-sea nematodes are mostly in rather low abundance, making the collection of sufficient specimens for description rather difficult. Second, deep-sea nematodes including those of Oxystominidae are usually several times smaller in body size than those in shallow-water habitats (Widbom, 1984; John, 1989; Vanhove *et al.*, 2004). Third, members of the family Oxystominidae have usually a much slenderer body, making features for distinguishing species indistinct. Thus, Oxystominidae has been indicated as one of the most 'underinvestigated' families of deep-sea nematodes (Miljutin *et al.*, 2010).

Both *Litinium* Cobb, 1920 and *Wieseria* Gerlach, 1956 are small genera in the family Oxystominidae. Species of them are usually rare, accounting for merely a small proportion of the marine nematode assemblages (Gerlach, 1958a; Tietjen, 1971; Soetaert *et al.*, 1995; Mokievsky *et al.*, 2011). For instance, individuals of *Litinium* and *Wieseria* constituted only about 0.87 and 2.91%, respectively, of the total nematode abundance in our samples collected from the southern Philippine Sea, as described below. Most species of *Litinium* and *Wieseria* were described on the basis of a single specimen. Even worse, about 18% of *Litinium* species and 40% of *Wieseria* species have been erected on the basis of only a single female specimen. Nevertheless, species of the two genera are generally well-defined and present few problems in species separation (Tchesunov *et al.*, 2014).

During our investigation of meiofauna in the Philippine Sea near the Southern Kyushu-Palau Ridge in the tropical Western Pacific Ocean with a water depth of 4117–5035 m, two new Oxystominidae species belonging to the genera *Litinium* and *Wieseria* were discovered from the sediment and are described herein.

MATERIALS AND METHODS

Sediment samples were collected at the stations D47, D48, D93 and D12 (Figure 1), from an abyssal plain of the

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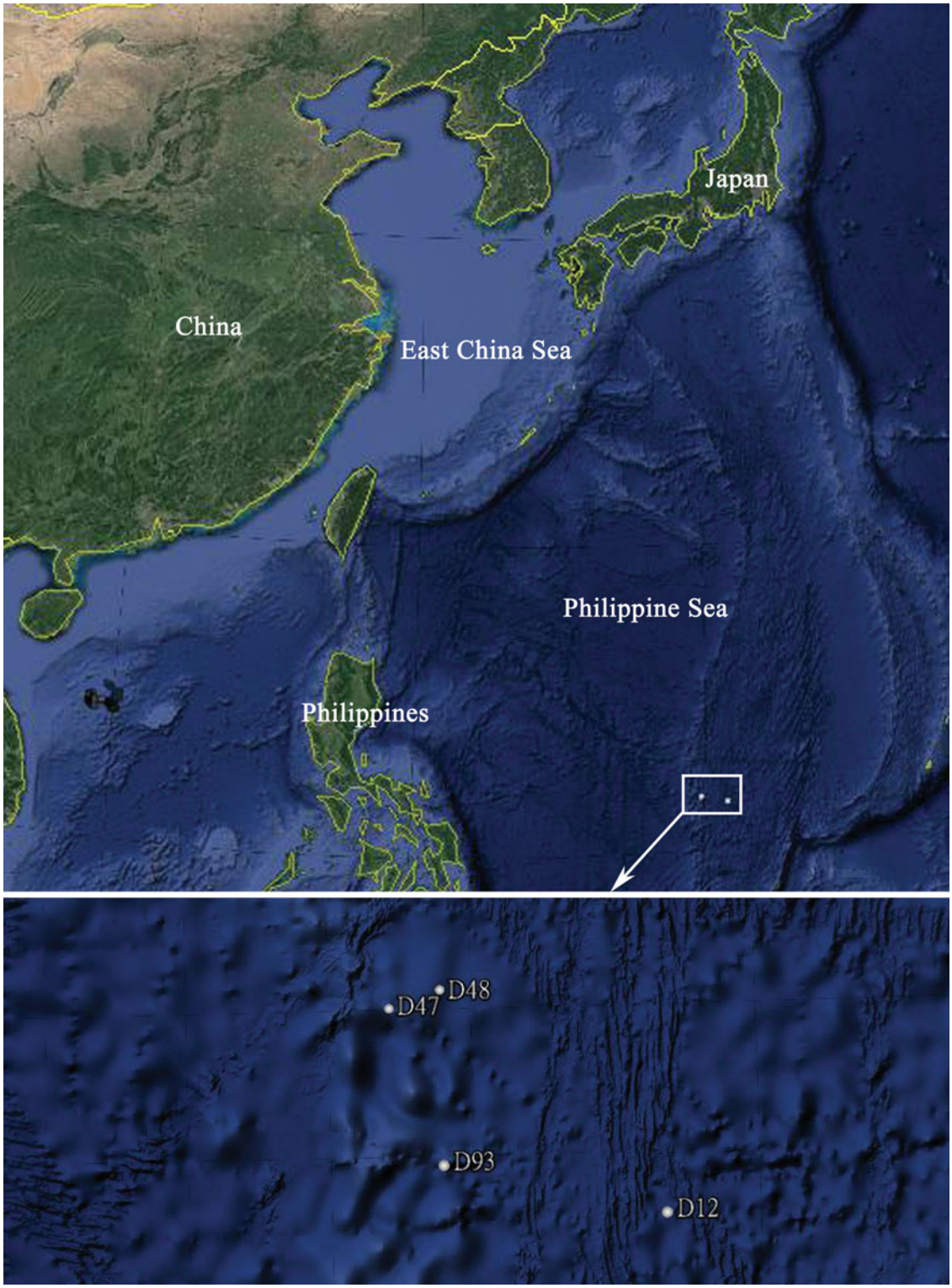

Fig. 1. Sampling stations (dots) in the southern Philippine Sea.

Philippine Sea near the Southern Kyushu-Palau Ridge in the Western Pacific Ocean in June 2014 by R/V KEXUE, using a 0.25 m^2 Gray-O'Hara box corer. For stations D47 and D48, meiofauna samples were taken from the core samples by inserting tubes of 9.5 cm inner diameter. Each tube samples were sliced into 0–1, 1–2, 2–4, 4–6 and 6–10 cm subsamples. For stations D93 and D12, due to disturbance, qualitative meiofauna samples were taken from the 0–5 cm layer only. All samples were preserved with formalin (5% final concentration) onboard. In the lab, the fixed samples were stained with 0.1% Rose Bengal for 12 h, washed on a 300 μm sieve to remove large particles and a 31 μm sieve to retain meiofauna. Ludox HS 40 was used to extract meiofauna from the remaining sediments by centrifugation. The

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extracted samples were sorted out under a dissecting microscope. Nematodes were transferred into a 9:1 (v/v) solution of 50% alcohol-glycerol in a cavity block to slowly evaporate to pure glycerol, and then mounted into permanent slides (Huang & Zhang, 2005).

The descriptions were made from glycerine mounts (Platt & Warwick, 1983) using a differential interference contrast (DIC) microscope (Nikon E80i). Line drawings were made with the aid of a drawing device. All measurements are in μm, and all curved structures are measured along the arch.

Abbreviations are as follows: a, body length divided by maximum body diameter; b, body length divided by pharynx length; c, body length divided by tail length; c', tail length divided by anal body diameter; a.b.d., anal body diameter; c.b.d., corresponding body diameter; V, distance of vulva from the anterior body end; V%, position of vulva from anterior end expressed as a percentage of total body length.

SYSTEMATICS

Order ENOPLIDA Filipjev, 1929
Family OXYSTOMINIDAE Chitwood, 1951
Subfamily OXYSTOMININAE Chitwood, 1935
Genus *Litinium* Cobb, 1920
Litinium dispariseta sp. nov.
(Figures 2A–E, 3A–D, 4; Table 1).

DIAGNOSIS

Body length 659–775 μm. Inner labial setae 5–8 μm long and outer labial setae 3–5 μm long. Amphideal foveas wrench-like with short handle. Cephalic setae at the same level of short handles of amphideal foveas. A single cloacal midventral supplementary seta, located at about 3 μm anterior to cloacal vent. A short cylindrical tail with bluntly rounded tip.

TYPE MATERIAL

Two males and two females. Male 1 (Holotype) on slide KP-20140629-D48-(0–1)-II-3. Male 2 on slide KP-20140628-D93-3. Female 1 on slide KP-20140629-D48-(2–4)-I. Female 2 on slide KP-20140622-D12-1. All type specimens have been deposited in the Marine Biological Museum of Chinese Academy of Sciences, Qingdao, China.

TYPE LOCALITY AND HABITATS

Muddy sediment at stations D48 (14°9′N 134°51′E), D93 (13°19′N 134°51′E) and D12 (13°4′N 135°56′E) in the Philippine Sea near the Southern Kyushu-Palau Ridge in the tropical Western Pacific Ocean. Station D48, water depth 4692 m, surface 0–1 cm sediment layer, median particle diameter 4.9 μm, and organic matter content 0.50%. Station D93, water depth 4117 m, surface 0–5 cm sediment layer. Station D12, water depth 5035 m, surface 0–5 cm sediment layer.

ETYMOLOGY

Composite of the Latin adjective *dispar* (unequal) and the Latin noun *seta* (bristle), refers to a main feature of the species, viz., the distinctly unequal length of the inner and outer labial setae.

DESCRIPTION

Males: Body slender, nearly cylindrical, with two terminals slightly narrower than body trunk. Cuticle smooth and evenly thick (about 1 μm) over entire body. Only one somatic seta observed, distributed in pharyngeal region, 3–4 μm long (Figure 2A; Table 1). A circle of six inner labial setae close to the circle of six outer labial setae. All labial setae slender, inner labial setae 5–7 μm long, situated about 1 μm from the anterior end; outer labial setae 3–4 μm long. Amphideal foveas large, wrench-like with short handle. Four slender cephalic setae, each 4–5 μm long, situated at the same level of short handles of amphideal foveas, about 20 μm posterior to the circle of outer labial setae (Figure 2C).

Mouth opening tiny. Internal lining of anterior pharynx thickened and cuticularized, forming a narrow buccal capsule about 11 μm long and 1 μm wide. Pharynx cylindrical and slightly widened at base, with clear internal cuticular lining and weak radial muscular striation, occupying about one fifth of total body length. Cardia small, cordate, surrounded by intestine tissue. Nerve ring located at middle of pharynx. Ventral gland and excretory pore not visible.

Single anterior outstretched testis on the right of intestine, 39–51 μm long. Arcuate and cuticularized spicules, 0.9–1.2 a.b.d. long, with a small proximal capitulum and pointed distal end. Elongate droplet-shaped gubernaculum, parallel to spicules, about 4 μm long. A midventral setae about 3 μm long, located 3 μm anterior to the cloacal opening present. Tail cylindrical with bluntly rounded tip, 2.4–2.8 a.b.d. long, terminal cuticle thickened; with a terminal pore and ducts of caudal glands. Caudal glands barely distinguishable (Figure 2E).

Females: Similar to males but with a slightly smaller body (659–696 μm *vs* 758–775 μm) and much smaller amphideal foveas (8–9 × 6 μm *vs* 18–19 × 8–9 μm; Table 1). One or two somatic setae observed, irregularly arranged in pharyngeal region, 4–6 μm long. Single posterior reflexed ovary on the right of intestine, 28–31 μm long. Vulva located at about two-fifths of body length from anterior end (Figure 2B, D). Three caudal glands, confined within the tail or slightly extending precaudally.

SPECIES COMPARISONS

Litinium dispariseta sp. nov. differs from all congeners by the relative length of inner labial setae to outer labial setae and the peculiar shape of amphideal foveas. In *L. dispariseta* the inner labial setae are almost two times longer than the outer labial setae, while in the other *Litinium* species the inner labial setae are either equal to or shorter than the outer labial setae. The amphideal foveas in *L. dispariseta* are wrench-like with short handle, while in all other congeners the amphideal foveas are either horseshoe shaped or ovoid with round to oval anterior apertures. Moreover, *L. dispariseta* has only a single midventral preanal supplementary seta, a feature found only in two congeners: *L. abyssorum* Tchesunov, Nguyen Dinh Tu & Nguyen Vu Thanh, 2014 and *L. quangi* Tchesunov, Nguyen Dinh Tu & Nguyen Vu Thanh, 2014. However, *L. dispariseta* differs from L. *abyssorum*, a species found in deep-sea sediment from the South-east Atlantic Ocean, also by its spicules equal in length (*vs* unequal), and a shorter tail relative to body length (ratio of body length to tail length about 25 *vs* 13–16). It differs from *L. quangi* also by its much smaller body size (659–775 μm *vs* 2523–2918 μm long) but much longer tail relative to body

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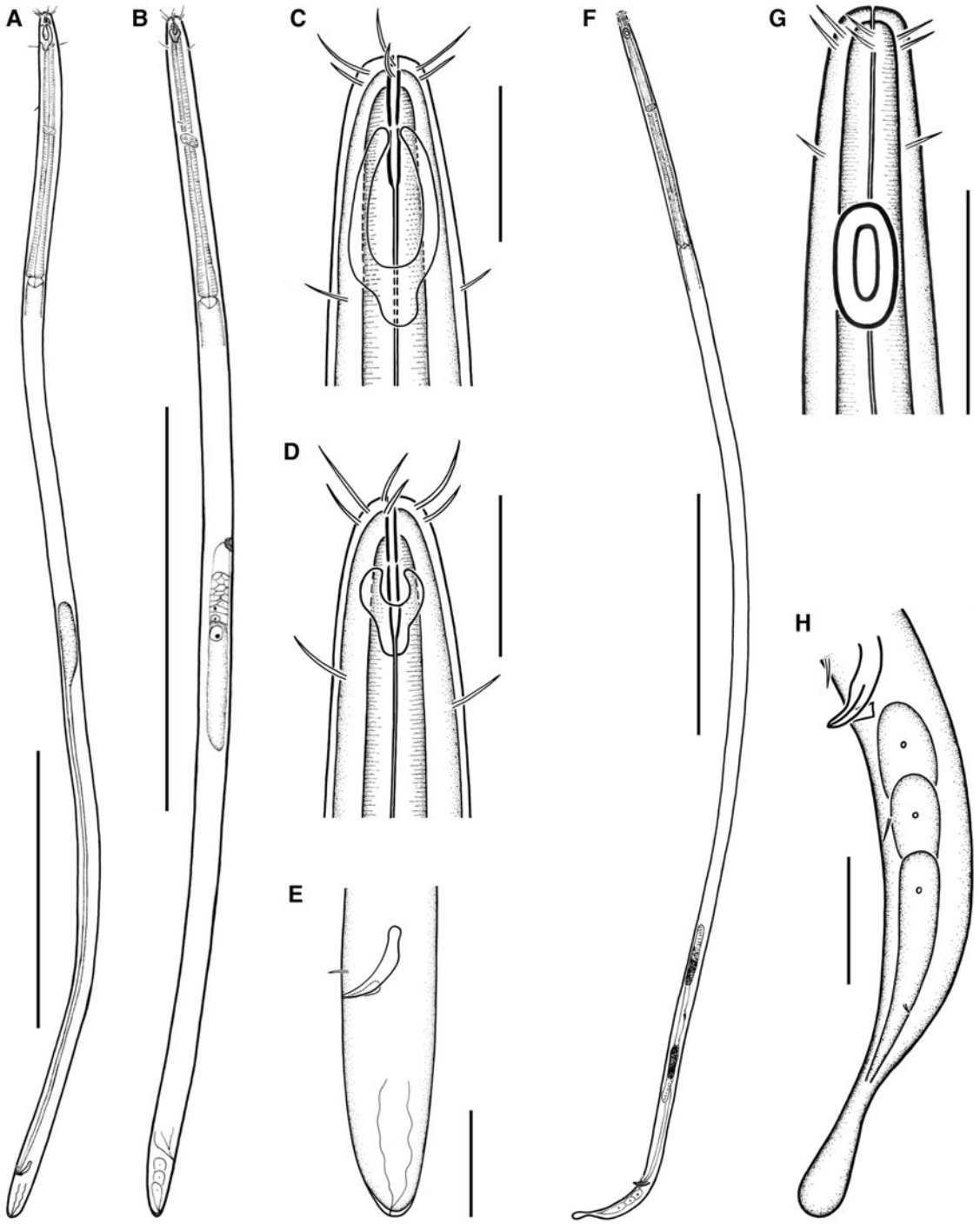

Fig. 2. *Litinium dispariseta* sp. nov. (A–E; A, C, E, male holotype; B, D, female 1) and *Wieseria minor* sp. nov. (F–H; male holotype): (A, B, F) overall view, showing the testes or ovary; (C, D, G) lateral view of anterior end, showing the anterior setae, buccal cavity and amphideal fovea; (E, H) lateral view of posterior end, showing the spicule, gubernaculum and precloacal supplementary seta. Scale bars: A, B, F, 200 μm; C–E, G, H, 15 μm.

length (ratio of body length to tail length about 25 *vs* 72–97), distinctly shorter spicules (12 μm *vs* 30–33 μm) and much shorter distance of the midventral preanal supplementary seta to cloaca (3 μm *vs* 32–35 μm).

Up to now, the genus *Litinium* contains 11 valid species including the new species *L. dispariseta*. Based on the review of *Litinium* by Tchesunov *et al.* (2014) and the description of *L. dispariseta* sp. nov., we propose a pictorial key to species of *Litinium* (Figure 4).

Genus *Wieseria* Gerlach, 1956

Wieseria minor sp. nov.

(Figures 2F–H, 3E, F, 5; Table 1)

DIAGNOSIS

Body length 1045 μm. Anterior setae 3 μm long, arranged in three circles: a circle of six inner labial setae close to a circle of six outer labial setae, and a circle of four cephalic setae situated

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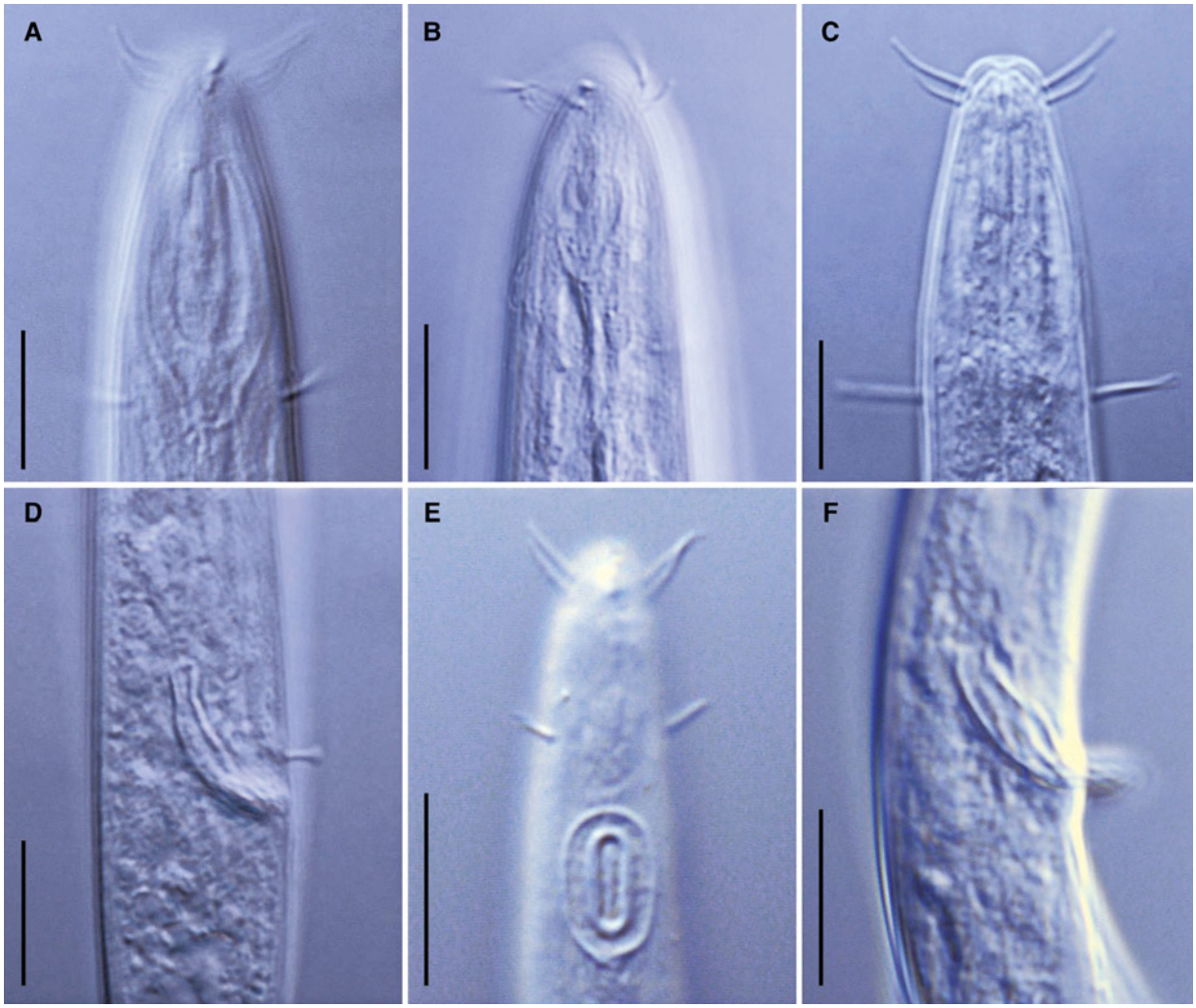

Fig. 3. *Litinium dispariseta* sp. nov. (A–D; A, C, D, male holotype; B, female 1) and *Wieseria minor* sp. nov. (E, F; male holotype): (A, B, E) lateral view of anterior end, showing the anterior setae and amphideal fovea; (C) lateral view of anterior end, showing the anterior setae and buccal cavity. Note that *Litinium dispariseta* has distinct longer inner labial setae relative to outer labial setae; (D, F) lateral view of male cloacal region, showing the spicule and precloacal supplementary seta. Scale bars: 10 μm.

about 7 μm posterior to outer labial setae. Amphideal foveas oblong with double contour, 4 μm posterior to cephalic setae. Tail clavate.

TYPE MATERIAL

Holotype male on slide KP-20140628-D47-(2–4)-II. The holotype has been deposited in the Marine Biological Museum of Chinese Academy of Sciences, Qingdao, China.

TYPE LOCALITY AND HABITAT

Muddy sediment at station D47 (14°4′N 134°36′E) from an abyssal plain near the Southern Kyushu-Palau Ridge in the tropical Western Pacific Ocean. Water depth 4430 m, surface 2–4 cm sediment layer, median particle diameter 5.0 μm, and organic matter content 0.49%.

ETYMOLOGY

This species is named for its smaller body relative to similar congeners.

DESCRIPTION

Male/Holotype: Body slender, nearly cylindrical, with two terminals slightly narrower than body trunk. Cuticle smooth. Somatic setae not observed (Figure 2F; Table 1). A circle of six inner labial setae situated about 2 μm from the anterior end and close to a circle of six outer labial setae. A circle of four cephalic setae situated about 7 μm posterior to outer labial setae. All labial and cephalic setae slender, about 3 μm long. Amphideal foveas oblong with double contour, 14 μm from anterior body end and 4 μm posterior to cephalic setae (Figure 2G).

Buccal cavity minute, slit-like. Pharynx cylindrical and slightly widened at base, with clear internal cuticular lining and weak radial muscular striation, occupying about 18.9% of total body length. Cardia small, cordate, and surrounded by intestine tissue. Nerve ring located at about two-fifths of pharynx length from anterior end. Excretory pore about 41 μm anterior to nerve ring.

Two opposed testes, outstretched, anterior branch on the left of intestine and posterior branch on the right (Figure 2F). Arcuate and cuticularized spicules, blunt in the proximal end and pointed in the distal end, with an internal

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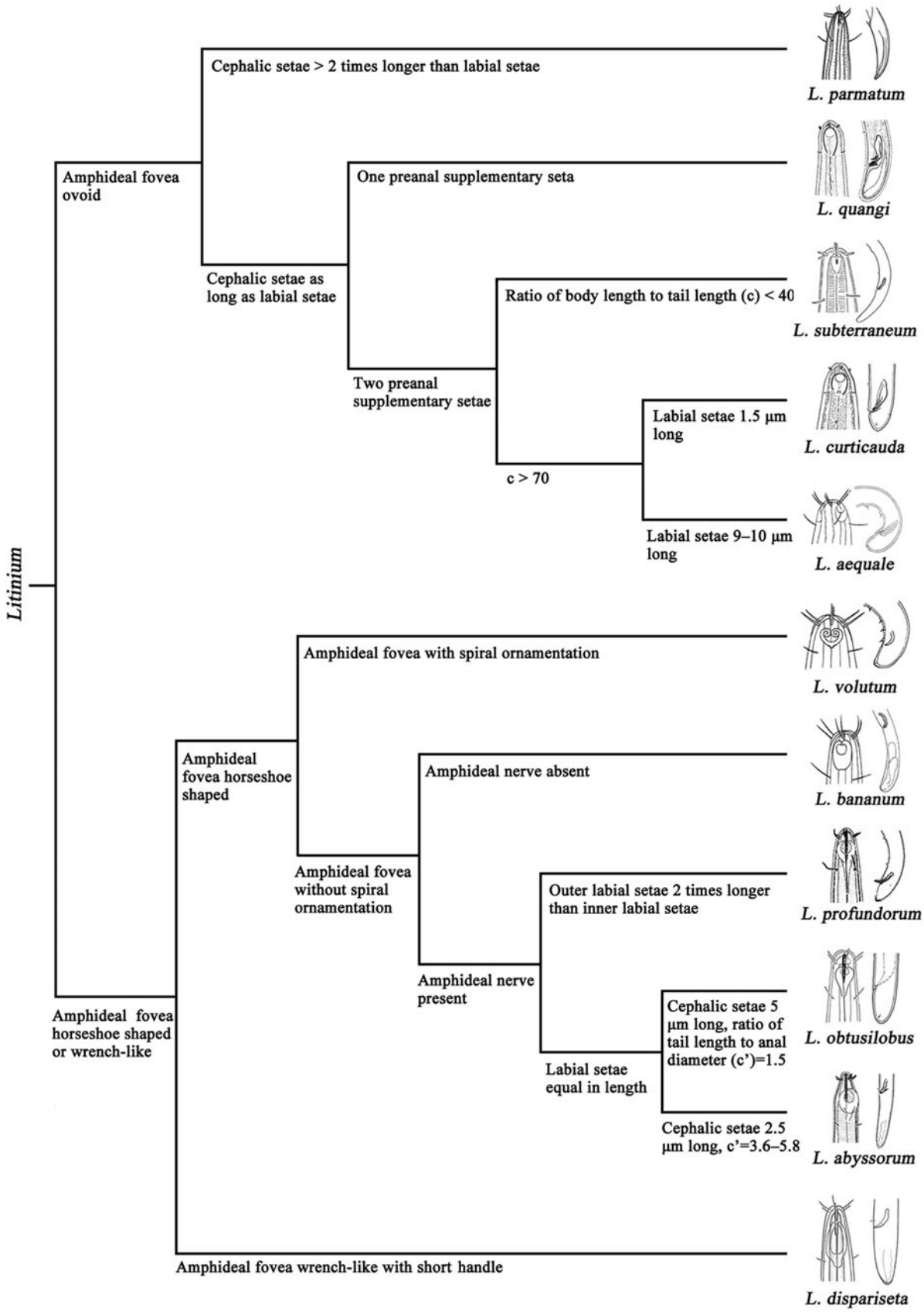

Fig. 4. Pictorial key to species of *Litinium*. Images of species taken from published descriptions: *abyssorum* – Tchesunov *et al.*, 2014; *aequale* – Gerlach, 1958b; *bananum* – Gerlach, 1956; *curticauda* – Tchesunov *et al.*, 2014; *dispariseta* – orig.; *obtusilobus* – Bussau, 1993; *parmatum* – Wieser, 1954; *profundorum* – Tchesunov *et al.*, 2014; *quangi* – Tchesunov *et al.*, 2014; *subterraneum* – Tchesunov *et al.*, 2014; *volutum* – Gerlach, 1962.

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Table 1. Morphometric data and individual measurements (in μm) of *Litinium dispariseta* sp. nov. and *Wieseria minor* sp. nov.

Characters	*Litinium dispariseta*				*Wieseria minor*
	Male 1 (Holotype)	Male 2	Female 1	Female 2	Male (Holotype)
Body length	775	758	659	696	1045
a	51.7	50.5	36.6	46.4	80.4
b	4.6	4.6	4.3	4.4	5.3
c	25.0	24.5	22.0	24.9	16.6
Maximum body diameter	15	15	18	15	13
Labial setae c.b.d.	7	7	6	6	5
Length of inner labial setae	7	5	8	8	3
Length of outer labial setae	4	3	5	5	3
Length of cephalic setae	5	4	7	8	3
Amphideal fovea length	19	18	8	9	8
Amphideal fovea width	9	8	6	6	5
Amphideal fovea width/c.b.d.(%)	70.9%	80.0%	58.3%	66.7%	54.5%
Amphideal foveas from anterior end	7	7	7	6	14
Nerve ring from anterior end	81	76	64	74	80
Nerve ring c.b.d.	15	15	15	12	13
Pharynx length	167	164	153	158	197
Pharynx c.b.d. at base	14	14	15	13	13
Anal body diameter	13	11	15	12	11
Tail length	31	31	30	28	63
c'	2.4	2.8	2.0	2.3	5.7
Spicule length as arc	12	13	–	–	13
Spicule length/a.b.d.	0.9	1.2	–	–	1.2
Length of testes	39	51	–	–	45–50
Vulva from anterior end	–	–	287	272	–
Vulva c.b.d.	–	–	17	13	–
V%	–	–	43.6%	39.0%	–
Length of ovary	–	–	28	31	–

stripe and an apophysis in the middle, 1.2 a.b.d. long. Small cuneate gubernaculum, about 4 μm long. A midventral setae about 3 μm long, positioned 6 μm anterior to the cloacal opening, in a backward orientation and thus difficult to recognize under light microscope. Clavate tail, 5.7 a.b.d. long. Spinneret and terminal duct not visible. Three caudal glands, confined within the tail (Figure 2H).

SPECIES COMPARISONS

Wieseria minor sp. nov. belongs to a subgroup of the genus *Wieseria* with a swollen tail tip. These include *W. glandulosa* (Kreis, 1929), *W. longiseta* (Allgén, 1947), *W. clavata* Gerlach, 1956 and *W. inaequalis* Gerlach, 1956. *Wieseria minor* sp. nov. differs from congeners with a swollen tail tip by its much smaller body (1045 μm *vs* 2120–3125 μm) and oblong amphideal foveas with double contour (*vs* a single oblong or ovoid loop). Moreover, it differs from *W. glandulosa* and *W. longiseta* also by the presence of four cephalic setae (*vs* absent); and from *W. clavata* and *W. inaequalis* by the position of cephalic setae relative to the amphideal foveas (4 μm anterior to *vs* at the same level of the amphideal foveas) and much shorter anterior setae (3 μm *vs* 10–24 μm). Besides, *Wieseria minor* sp. nov. differs from *W. glandulosa* also by the much shorter labial setae (3 μm *vs* 7.8–9.1 μm), and from *W. clavata* by the presence of gubernaculum (*vs* absent).

DISCUSSION

Within the family Oxystominidae, the shape of tail has been considered as a main feature distinguishing similar genera. For instance, *Nemanema* Cobb, 1920 differs from *Oxystomina* Filipjev, 1921 only by its conical or cylindrical tail with a rounded tip (*vs* clavate tail with a swollen tip), and *Litinium* Cobb, 1920 differs from *Thalassoalaimus* de Man, 1893 mainly by its short, cylindrical or occasionally conical tail with a rounded tip (*vs* mostly conical tail with a caudal capsule).

The genus *Wieseria* Gerlach, 1956 is most similar to *Litinium* and *Thalassoalaimus* in having three circles of anterior setae, but differs by the shape of tail and the location of amphideal foveas relative to cephalic setae. *Wieseria* has a conico-cylindrical tail with swollen, pointed or bifurcate tip, while *Litinium* has a short, cylindrical or occasionally conical tail with rounded tip. Most *Thalassoalaimus* species have a conical tail with a terminal structural elaboration, forming a so-called tail capsule equipped with distinctly thickened inner cuticle (Tchesunov *et al.*, 2014). The amphideal foveas in *Wieseria* are situated at the same level of or posterior to cephalic setae, while in *Litinium* and *Thalassoalaimus* the amphideal foveas are situated anterior to cephalic setae.

The most recent generic diagnosis of *Wieseria* was given by Smol *et al.* (2014). However, several important features used to distinguish species of *Wieseria* are either absent or incompletely stated in the generic diagnosis. For instance, Smol *et al.* (2014) defined all species of *Wieseria* as having a sharp tail tip. However, half of the known species of *Wieseria* have a conico-cylindrical tail with swollen tip (clavate), while *W. scotlandica* Zhang, 1983 and *W. hispida* Vitiello, 1972 have a bifurcate tail tip. The location of amphideal foveas relative to cephalic setae is another important feature separating *Wieseria* from *Litinium* and *Thalassoalaimus*. However, this feature is also lacking in the generic diagnosis. Last but not least, Smol

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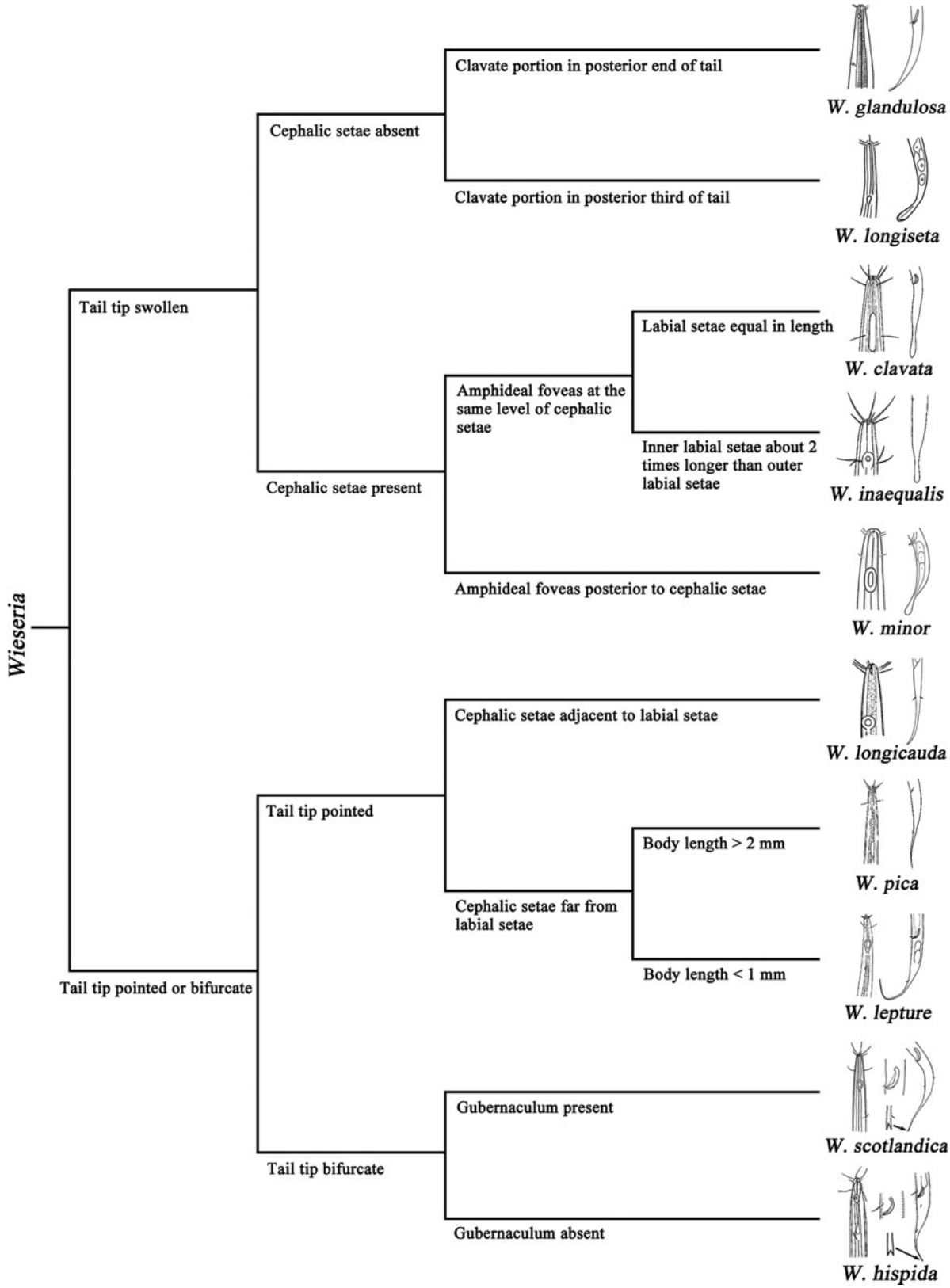

Fig. 5. Pictorial key to species of *Wieseria*. Images of species taken from published descriptions: *clavata* – Gerlach, 1956; *glandulosa* – redrawn from Kreis, 1929; *hispida* – Vitiello, 1972; *inaequalis* – Gerlach, 1956; *lepture* – Vitiello, 1972; *longicauda* – Timm, 1961; *longiseta* – redrawn from Allgén, 1947; *minor* – orig.; *pic* – Gerlach, 1956; *scotlandica* – Zhang, 1983.

et al. (2014) defined the genus *Wieseria* as having four cephalic setae in backward position. However, the known species *W. longicauda* Timm, 1961 has four cephalic setae in forward position (just behind the labial setae), and *W. glandulosa* (Kreis, 1929) and *W. longiseta* (Allgén, 1947) have no cephalic setae at all.

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Based on the generic diagnosis of Smol *et al.* (2014) and the features discussed above, we provide an emended diagnosis for *Wieseria*: Marine Oxystominidae. Inner and outer labial sensilla setiform; four cephalic setae if present. Amphideal foveas in various shapes mainly with double contour, situated at the same level of or posterior to cephalic setae. A precloacal supplementary seta present in males. Tail conico-cylindrical with a swollen, pointed or bifurcate tip.

Up to now, the genus *Wieseria* contains 10 valid species. Based on the features discussed above and the description of *Wieseria minor* sp. nov., we propose a pictorial key to species of *Wieseria* (Figure 5).

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