

The genus *Setaphyes* (Kinorhyncha, Pycnophyidae) in European waters: Redescription of *Setaphyes dentatus* (Reinhard, 1881) and *Setaphyes kielensis* (Zelinka, 1928), including notes on morphometrics, sexually dimorphic features and reproduction of the genus

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ABSTRACT

Six of the eight species of the genus *Setaphyes* have been described from Europe. In the present contribution, the European *Setaphyes* species are revised and two of them, *S. dentatus* (Reinhard, 1881) and *S. kielensis* (Zelinka, 1928), are redescribed following the current approaches of Kinorhyncha taxonomy. For this purpose, material from 17 localities throughout the Atlantic Ocean and the Mediterranean and Black Seas was investigated. The redescriptions of two of the most common pycnophyid species in Europe include detailed information on intraspecific variation among populations. *Setaphyes dentatus* is characterized by the presence of middorsal elevations on segments 2–6, and middorsal processes on segments 1, 7–9, unpaired paradorsal setae on segments 2–9; laterodorsal setae on segments 3, 5 and 7; ventromedial setae on segments 4–5, 7–8 in males and 3–5, 7–9 in females; anterior cuticular, reticulate ridges on the tergal plate of segment 1 and conspicuous patch of laterodorsal and ventrolateral longitudinal, cuticular ridges on segment 10. *Setaphyes kielensis* is characterized by middorsal elevations on segments 1–9; paired paradorsal setae on segments 2–9 (unpaired on segment 8); laterodorsal setae on segments 2–9; ventromedial setae on segments 3–9. Furthermore, morphometric features of the European *Setaphyes* are analysed. Specifically, the morphometric analyses revealed sex-specific variability in the LTS/TL proportion, with females of all the European species of *Setaphyes* showing smaller LTS/TL. Thus, the LTS/TL ratio unveils a conspicuous sexual dimorphism in the genus.

1. Introduction

The European coast is currently considered one of the most well-explored marine areas in terms of Kinorhyncha diversity. After the discovery of the phylum (Dujardin, 1851), the Black Sea and the waters surrounding the Italian Peninsula were the main working areas of W. Reinhard and C. Zelinka, respectively, resulting in vast contributions of species descriptions (Reinhard, 1881, 1885; Zelinka, 1928). Over the last century, surveys in marine environments across the European territory significantly increased our knowledge on kinorhynch taxonomy, up to the current about 60 Kinorhyncha species (Neuhaus, 2013). Some of these species were discovered or reported in several regions by

isolated samplings throughout the Atlantic coast and in the Mediterranean and Black Sea (see Adrianov and Malakhov, 1999; Neuhaus, 2013), and the number of known species increased considerably with the extensive campaigns conducted along the Iberian (Pardos et al., 1998; Sánchez et al., 2011, 2012, 2014, 2018; Herranz et al., 2012; González-Casarrubios et al., 2022) and Italian Peninsulas (Dal Zotto, 2015; Dal Zotto and Todaro, 2016; Dal Zotto et al., 2016, 2019; Yamasaki and Dal Zotto, 2019).

More recently, research on kinorhynch taxonomy in the eastern deep-sea Mediterranean and the Anatolian Peninsula to some extent balanced out the current sampling bias towards the middle and western Mediterranean (Sönmez et al., 2016; Ürkmez et al., 2016; Yıldız et al.,

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2016; Yamasaki et al., 2018a,b; Yamasaki and Durucan, 2018; Sørensen et al., 2020, 2021). Regarding records of allomalorhagids in this region, four species have been found: *Setaphyes dentatus* (Reinhard, 1881), *Setaphyes kielensis* (Zelinka, 1928) *Fujuriphyes ponticus* (Reinhard, 1881), and *Pycnophyes paraneapolitanus* (Sheremetevskij, 1974). However, all these records dated back to Reinhard times (end of 19th century) in the Anatolian Peninsula and the remaining coastline of the Black Sea (or from the second half of the 20th century). On top of this, no type material was designated for these species, namely *S. dentatus*, *S. kielensis* *F. ponticus*, and *P. paraneapolitanus*, and subsequently not deposited in a reference collection (Neuhaus, 2022). Curiously, two of the species, *S. dentatus* and *S. kielensis*, are among the most common Pycnophyidae species throughout European waters (Sánchez et al., 2012; Neuhaus, 2013), but the absence of modern descriptions and type material hampers the proper identification of the specimens. Moreover, these two species belong to a genus of predominantly European distribution (six out of the eight species are currently restricted to it), which was stressed after the recent discovery of three new congeners: *Setaphyes algarvensis* González-Casarrubios et al., 2022 (Atlantic coast of Portugal), *Setaphyes cimarensis* Sánchez et al., 2018 (Mediterranean coast of Spain) and

Setaphyes elenae Cepeda et al., 2020 (North Sea). These recent descriptions also highlighted the potential usefulness of morphometry to distinguish between *Setaphyes* species.

The main goal of the present contribution is to redescribe both *S. dentatus* and *S. kielensis* based on material collected at several localities from the North Sea (North Frisia), via the Cantabrian Sea and East Atlantic (Iberian Peninsula), to the Mediterranean Sea (Italian, Iberian and Anatolian Peninsulas). The inclusion of Anatolian specimens allows at the same time to increase our knowledge on allomalorhagid Kinorhyncha from the South-Eastern border of Europe to Asia. Moreover, we explore whether selected morphometric measurements of the body can be used as dimorphic characters or diagnostic features of the genus.

2. Material and methods

2.1. Sampling sites and study of selected specimens for the redescrptions

Kinorhynch specimens were collected in four marine areas along the European coast (North-Eastern Atlantic Ocean, Mediterranean, Cantabric and North Seas) and the Black Sea (Fig. 1).

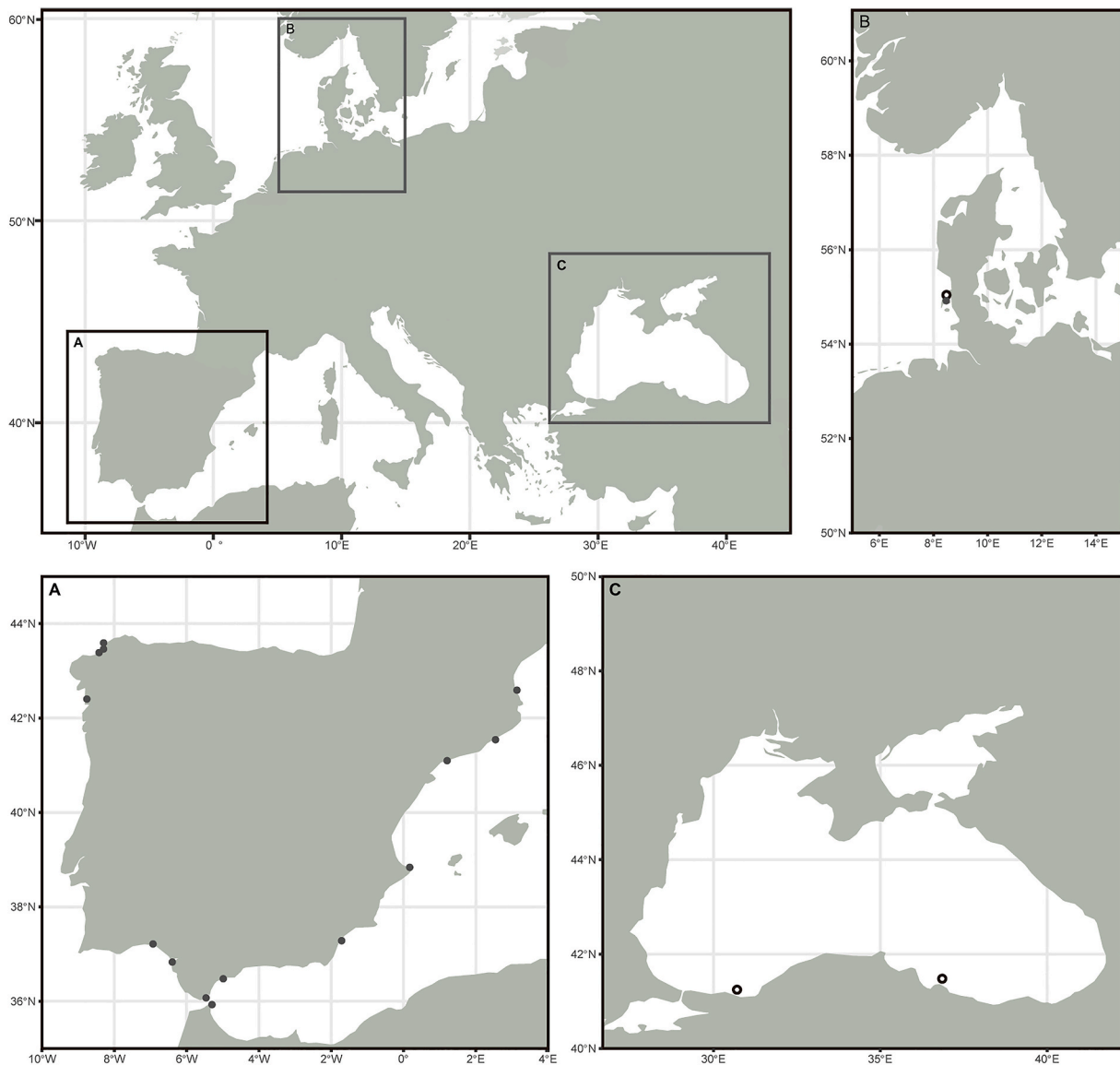


Fig. 1. Map of the sampling localities. Black circles indicate localities in which specimens of *Setaphyes dentatus* were found. White circles indicate localities in which specimens of *Setaphyes kielensis* were found. A: enlarged map of the Iberian Peninsula; B: enlarged map of the North Frisian region; C: enlarged map of the Anatolian region.

Morphological information for the redescription of *S. dentatus* was gathered from 136 specimens (53 males and 83 females) belonging to 17 populations collected in eight marine ecoregions following the classifications of Spalding et al. (2007, 2012) and García-Herrero et al. (2021): Anatolian Peninsula (Southern Black Sea, Black Sea ecoregion, four specimens: Türkiye), Italian Peninsula (Western Mediterranean ecoregion, one specimen: Naples, Italy), eight Mediterranean areas in the Iberian Peninsula (Western Mediterranean ecoregion, 30 specimens: Banyuls-sur-Mer, France, one specimen; Blanes and Tarragona, Spain, with four and 25 specimens, respectively; Alboran Sea ecoregion, 30 specimens: Dénia, Garrucha, Málaga, Ceuta and Algeciras, Spain, with one, seven, six, five and 11 specimens, respectively); three Atlantic areas in the Iberian Peninsula (Gulf of Cádiz ecoregion, 30 specimens: Cádiz and Huelva, Spain, with seven and 23 specimens, respectively; Portugal ecoregion, 13 specimens: Pontevedra, Spain); three Cantabric areas in the Iberian Peninsula (Gulf of Biscay ecoregion, 17 specimens: La Coruña, Ares and Ferrol, Spain, with eight, six and three specimens, respectively); and the North Sea (North Sea ecoregion, 11 specimens: Sylt, Germany) (Fig. 1). The redescription of *S. kielensis* is based on the information gathered from 34 specimens (15 males and 19 females) of two populations inhabiting in two marine ecoregions: Anatolian Peninsula (Southern Black Sea, Black Sea ecoregion: Türkiye, four specimens) and the North Sea (North Sea ecoregion, 26 specimens: Sylt, Germany) (Fig. 1). Detailed information of the sampling localities of each specimen included in the study can be found in the Supplementary Table I. Moreover, data on the additional *Setaphyes* species included in the morphometric analysis (see 2.2 Morphometric analyses and statistics) are gathered in the Supplementary Table II.

Soft-bottom qualitative samples from the Iberian Peninsula were obtained in several sampling campaigns from 1997 to 2021 using a Higgins Meiobenthic Dredge (Fleeger et al., 1988; Sørensen and Pardos, 2020) or taken by hand (intertidal samples of Combarro, Pontevedra, Spain). North Frisian samples were taken by hand in the North Sea, German Bight, on the island of Sylt, either at Königshafen (*S. kielensis*) or at the east coast of Kampen (*S. dentatus* and *S. kielensis*). Meiofauna was extracted from the sediment following the bubble-and-blot method (Higgins, 1988; Sørensen and Pardos, 2020), and subsequently fixed and/or preserved in 5–7 % neutralized formalin or 70–96 % ethanol. Samples around the Anatolian Peninsula were collected using a Van Veen Grab, and push corer was used to obtain subsamples. The samples were washed through sieves of 500 µm, 250 µm and 63 µm mesh sizes and preserved in 75 % ethanol.

Kinorhynch specimens for light microscopy were passed through a graded series of ethanol/glycerine and kept in 100 % glycerine during 24 h. The Iberian Peninsula specimens were mounted in Fluoromount G®, the Sylt *S. dentatus* specimens in Berlese's Medium, and the Sylt *S. kielensis* specimens in Euparal Green, Chroma. All specimens were studied and photographed with an Olympus® BX51-P microscope with differential interference contrast optics equipped with an Olympus® DP-23 camera. Measurements of the studied specimens were made using Olympus® CellD software. Kinorhynch specimens for scanning electron microscopy studied in Spain were transferred to acetone through a graded series of ethanol/acetone and critical point dried. Specimens were finally mounted on aluminium stubs and sputter-coated with gold/palladium for examination with a JEOL® JSM-6335 F field emission SEM at the ICTS Centro Nacional de Microscopía Electrónica (Universidad Complutense de Madrid, Spain). Kinorhynch specimens for scanning electron microscopy studied in Germany were dehydrated through an ethanol series, critical point dried with a Leica EM CPD 300, sputter-coated with a Quorum Q150 RS, and studied with a Zeiss EVO LS10 SEM. Line art illustrations and image compositions were made using Adobe® Illustrator CC-2014 and Photoshop software.

2.2. Morphometric analyses and statistics

Previous studies (Sánchez et al., 2018; Cepeda et al., 2020;

González-Casarrubios et al., 2022) suggested some morphometric differences between European *Setaphyes* species and sexes in the same species. These differences were found in the total trunk length and the ratio between total trunk length and length of lateral terminal spines.

To furthermore assess discrepancies in these two measurements, we took advantage of the high number of *S. dentatus* and *S. kielensis* specimens measured in the present study, together with previously available morphometric data of the remaining European congeners (*S. algarvensis*, *S. cimarensis*, *S. elenae* and *Setaphyes flaveolatus* (Zelinka, 1908)). Information on the localities and measurements of these congeners may be found in Supplementary Table II. Moreover, additional material of *S. algarvensis* (one specimen from Peniche, Portugal), *S. cimarensis* (18 specimens from Tarragona, Spain) and *S. flaveolatus* (10 specimens from Alicante, Spain; 18 specimens from Naples, Italy) was included in the analyses.

Normality and homoscedasticity of the variables were checked by Saphiro-Wilk and Barlett tests, respectively. To assess differences between normal datasets, a one-way analysis of variance (ANOVA) was used, except for heteroscedastic variables, for which a Welch's ANOVA was applied. On the contrary, when the dataset was not normal, a Kruskal–Wallis test was conducted. Pairwise comparisons using Wilcoxon rank sum test were performed to compare between more than two groups of specimens. All statistical analyses were performed in R v.1.1.453 (R Core Team 2021) using the *stats*, *ggplot2* and *car* packages (Fox and Weisberg, 2019).

3. Results

Class Allomalorhagida (Sørensen et al., 2015) *sensu* Herranz et al., 2022.

Family Pycnophyidae (Zelinka, 1928) *sensu* Sánchez et al., 2016.

Genus *Setaphyes* Sánchez et al., 2016

3.1. Redescription of *Setaphyes dentatus* (Reinhard, 1881)

Figs 2–8, Tables 1–2.

3.1.1. Diagnosis

Setaphyes with anterior margin of the tergal plate on segment 1 finely denticulated, posteriorly followed by a transverse area of minute cuticular, net-like ridges. Middorsal elevations on segments 2–6, and middorsal processes on segments 1 and 7–9. Posterior end of middorsal structures covered by tufts of elongated, thick hairs whose tips usually surpass the posterior segment margin. Unpaired paradorsal setae on segments 2–9, alternating left and right position along the trunk and showing intraspecific variability but without any specific pattern. Laterodorsal setae on segments 3, 5 and 7. Lateroventral setae on segments 2–10. Ventromedial setae on segments 4–5 and 7–8 in males and 3–5 and 7–9 in females. Patch of conspicuous longitudinal cuticular ridges on segment 10, from laterodorsal to ventrolateral positions. Lateral terminal spines short, slender (males LTS:TL average ratio 21.2 %; females LTS:TL average ratio 15.7 %; males and females average ratio 17.8 %).

3.1.2. Examined material

For light microscopy, 136 adult specimens, 53 males and 83 females, were studied from 17 populations belonging to eight marine ecoregions (ZMB 12408–12418, 12616–12740; see Supplementary Table I). Of those, four females from the Anatolian Peninsula are new records for the region. 11 additional adult specimens were examined with SEM: one adult specimen from the Anatolian Peninsula (indeterminate sex: ZMB 12745; station YSL09R2; 13 July 2019, 41°24.738'N, 036°39.210'E, 76 m depth; collector: Derya Ürkmez), 10 from the Iberian Peninsula, collected by F. Pardos, M. Herranz and N. Sánchez (one female ZMB 12746, one male ZMB 12747, and three adults ZMB 12748–12750 of indeterminate sex from Algeciras, 8 February 2011, 36°10.741'N, 005°23.243'W, 8 depth; two females ZMB 12754 and 12755 from

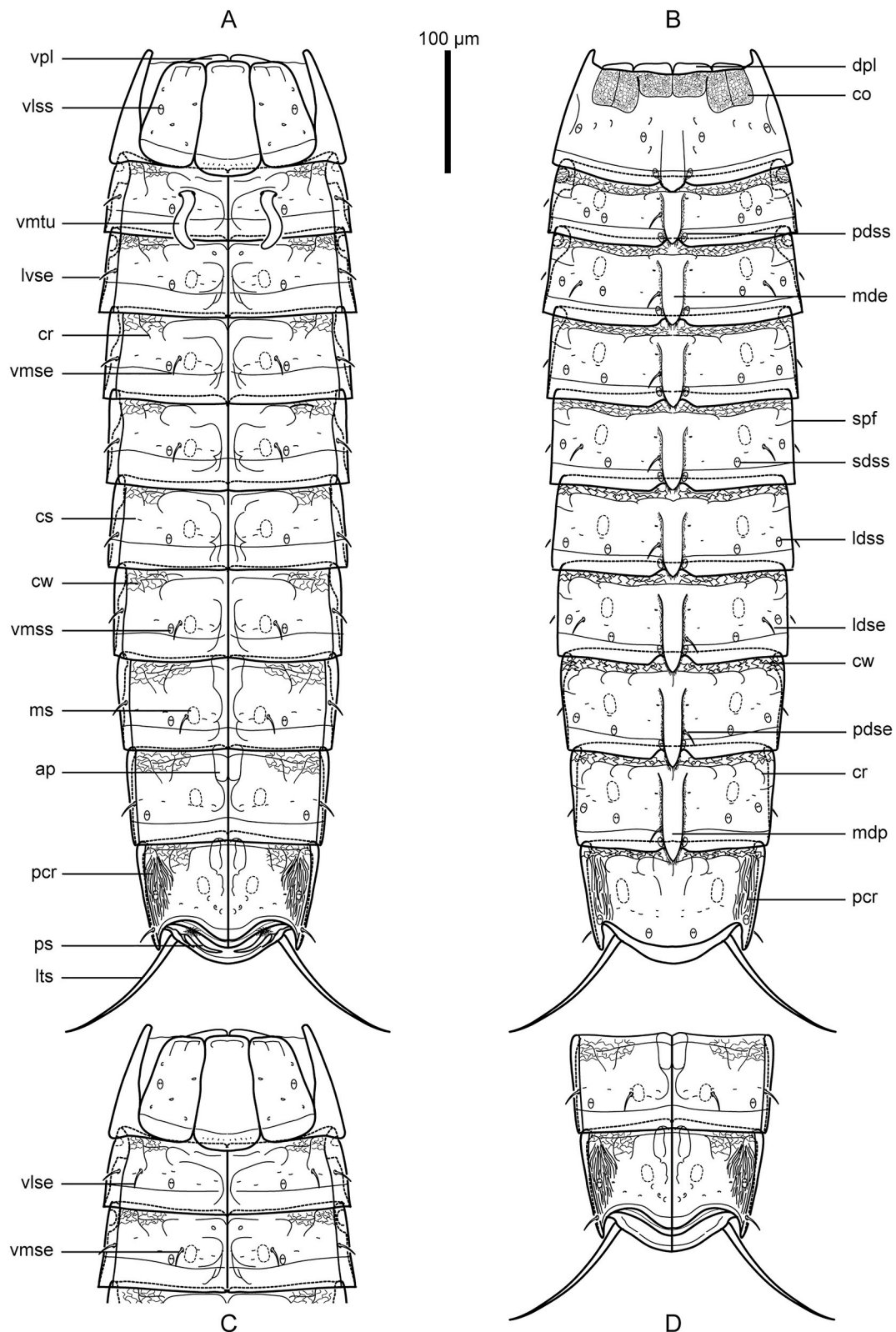


Fig. 2. Line art illustration of *Setaphyes dentatus*. A: Male, ventral view; B: Male, dorsal view; C: Female, segments 1–3, ventral view; D: female, segments 9–11, ventral view. Scale bar: 100 µm. Abbreviations: ap, apodeme; co, cuticular ornamentation, reticular, net-like structure; cr, cuticular ridge; cs, cuticular scar; cw, cuticular wrinkles; dpl, dorsal placid; ica, intracuticular atria; ldse, laterodorsal setae; ldss, laterodorsal sensory spot; lts, lateral terminal spine; lvse, lateroventral setae; mde, middorsal elevation; mdp, middorsal process; ms, muscular scar; ne, nephridiopore; pcr, patch of cuticular ridges; pdse, paradorsal setae; pdss, paradorsal sensory spot; ps, penile spine; sdss, subdorsal sensory spot; spf, secondary pectinate fringe; vlse, ventrolateral setae; vlss, ventrolateral sensory spot; vmse, ventromedial setae; vmtu, ventromedial tube; vmss, ventromedial sensory spot; vpl, ventral placid.

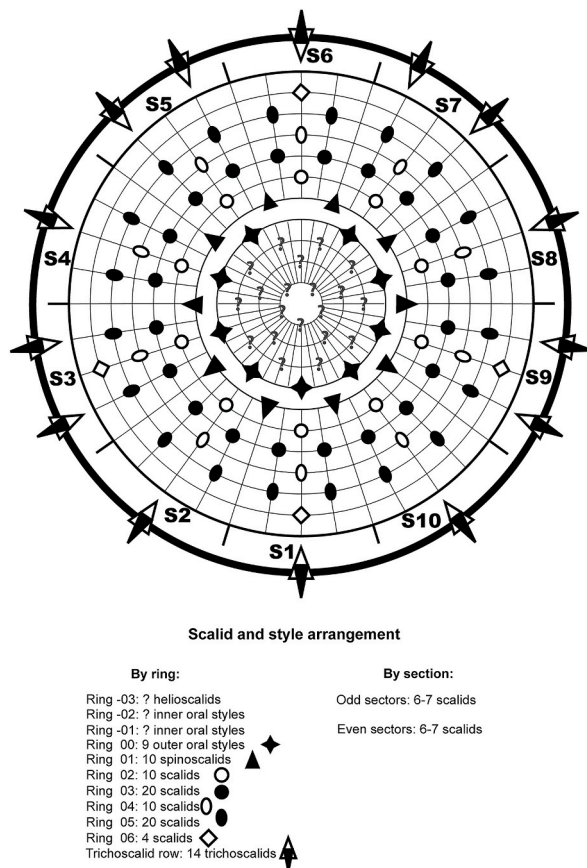


Fig. 3. Diagram of mouth cone and introvert in *Setaphyes dentatus* showing the distribution and type of scalids by ring and sector. Abbreviations: ? position of inner oral styles not revealed; S, sector followed by number of sector.

Huelva, 12 April 2011, 37°08.324'N, 007°20.308'W, 15 m depth; one additional female ZMB 12753, and two indeterminate sex, ZMB 12751 and ZMB 12752, from Huelva, 11 April 2011, 37°10.963'N, 007°16.549'W, 11 m depth).

3.1.3. Description

See Table 1 and Supplementary Table III for measurements and dimensions and Table 2 for location of middorsal cuticular specializations, setae, tubes, nephridiopores and sensory spots.

Ring 00 of mouth cone with nine, equally sized outer oral styles composed of a single, flexible unit, wider at base, with a fringed sheath, tapering progressively toward the distal, pointed tip (Figs 3; 4D). Outer oral styles located anterior to each introvert sector, except in the middorsal position (sector 6) where a style is missing (Fig. 3). Inner rings of mouth cone not observed, herein details on the morphology and distribution of inner oral styles are not provided.

Introvert with six rings of spinoscalids and 10 longitudinal sectors, each limited by the position of two adjacent primary spinoscalids (Figs 3; 4A; 5A–D). Ring 01 with 10 primary spinoscalids, conspicuously larger than the other ones; primary spinoscalids composed of a basal, rectangular, wide sheath and a distal, elongated, flexible, distally pointed end-piece; basal sheath equipped with a row of elongated fringes, longer in the middle region of the spinoscalid than on the lateral margins (Figs 3; 4A; 5A–C). Ring 02 with 10 spinoscalids, arranged medially in each sector; spinoscalids on this and following rings are also composed of a basal, rectangular, wide sheath and a distal, elongated, flexible, distally pointed end-piece; basal sheath appears hairy at its proximal end and terminates into a short fringe (Figs 3; 4A; 5A–B). Ring 03 with 20 spinoscalids, arranged as two in each sector (Figs 3; 4A; 5A–B). Ring 04 with 10 spinoscalids, arranged medially in each sector

(Figs 3; 4A; 5A–B). Ring 05 with 20 spinoscalids, arranged as two in each sector (Figs 3; 4A; 5A–B). Ring 06 with four, smaller spinoscalids, arranged medially in sectors 1, 3, 6 and 9 (Figs 3; 4A; 5A–B). The location of spinoscalids throughout rings 02–06 follows a strict pattern around the introvert: sectors 1, 3, 6 and 9 bear seven spinoscalids, while the remaining sectors carry six spinoscalids (Figs 3; 4A; 5A–B).

A ring of 14 hairy trichoscalids present posterior to the spinoscalid rings, arranged as two in the odd-numbered sectors (except sector 1 with a single trichoscalid) and one in the even-numbered sectors of the introvert (Figs 3; 4A; 5A–B, D).

Neck with four dorsal and two ventral sclerotized placids (Figs 2A–B; 4B–C; 5D). Dorsal placids rectangular, mesial ones broader (ca. 24 µm wide at the base) than lateral ones (ca. 22 µm wide at the base) (Figs 2B; 4B; 5D). Ventral placids (ca. 22–23 µm wide at the base) similar to the dorsal ones but much more elongated, becoming thinner towards the lateral sides (Figs 2A, C; 4C).

Trunk with eleven segments (Figs 2AB; 6AF). Segment 1 with one tergal, two episternal and one trapezoidal, midsternal plate; remaining segments with one tergal and two sternal cuticular plates (Figs 2AD; 4B–C, F–G; 6A–F). Sternal plates reaching their maximum width at segment 7, almost constant in width throughout the trunk, progressively tapering at the last trunk segments. Sternal cuticular plates relatively narrow (MSW-5:TL average ratio = 24 %). Middorsal elevations on segments 2–6, rectangular, narrow, distally blunted, not projecting beyond the posterior margin of segments (Figs 2B; 4B, F; 6A, C; 7C–D; 8B). Middorsal processes on segments 1 and 7–9, similar in morphology but exceeding the posterior margin of segment (Figs 2B; 4B, F; 6A, E; 7B, F–G; 8H). Paradorsal butterfly to trident-like intracuticular atria associated to middorsal structures (Figs 2B; 4B, F). Lateral margin of middorsal structures surrounded by short, thick cuticular hairs; and posterior ends covered by tufts of elongated, thick hairs whose tips usually surpass the posterior margin of segment (Figs 2B; 7C–D, F–G; 8B) (those of segment 1 remarkably shorter). Middorsal processes progressively longer towards the posterior segments, reaching their maximum length on segment 9 (Figs 2B; 4F; 6E; 7F–G; 8H). Cuticular scars (likely glandular cell outlets) as minute, dot-shaped, rounded to oval perforations throughout the cuticle on segments 1–11 (Figs 2A–D; 4B–C, F–G), also present at the base of the middorsal processes and elevations; the number and arrangement of these structures vary greatly among the specimens, not showing any clear pattern. Up to two pairs of cuticular ridges in subdorsal (one pair) and laterodorsal (one pair) positions on segments 2–4; an unpaired middorsal cuticular ridge and up to three pairs in subdorsal (one pair) and laterodorsal (two pairs) positions on segments 5–10; one pair of ventrolateral cuticular ridges on segments 2–10, with adjacent, minute glandular cell outlets (Fig. 2A–D). Conspicuous reticulate pattern of cuticular wrinkles, as a net-like ornamentation, across the most anterior dorsal and ventrolateral margins, at the overlapping area between following segments, on segments 2–10 (Figs 2A–D; 4B–C, F–G). Cuticular hairs acicular, non-bracteate, scattered throughout the trunk on segments 1–10, except in ventromedial position, denser at the tergosternal junctions, not following any particular pattern. Pachycycli and ball-and-socket joints conspicuous on segments 2–8, reduced on most posterior segments (Fig. 2A–D). Apodemes on segments 9–10 (Figs 2A–B, D; 4F–G). Primary pectinate fringes finely serrated (Figs 6A–F; 8B–C, E, G–H); secondary pectinate fringes as a wavy, quite inconspicuous single line in laterodorsal and ventrolateral positions at the anterior most region of the segments (Figs 2A; 8E). Muscular scars as rounded to oval, hairless areas in laterodorsal and ventromedial positions on segments 1–10 (Fig. 2A–D).

Segment 1 with anterior dorsal margin finely denticulated, posteriorly followed by a transverse area of cuticular wrinkles forming a net-like band, broader towards the lateral than in the middle region (Figs 2B; 4B; 5D; 7A–B). Anterolateral margins of the tergal plate as short, wide, distally rounded extensions (Figs 2A–C; 6B). Middorsal process with paradorsal, butterfly to trident-like atria of associated paradorsal sensory spots located near the posterior margin of the segment (Figs 2B;

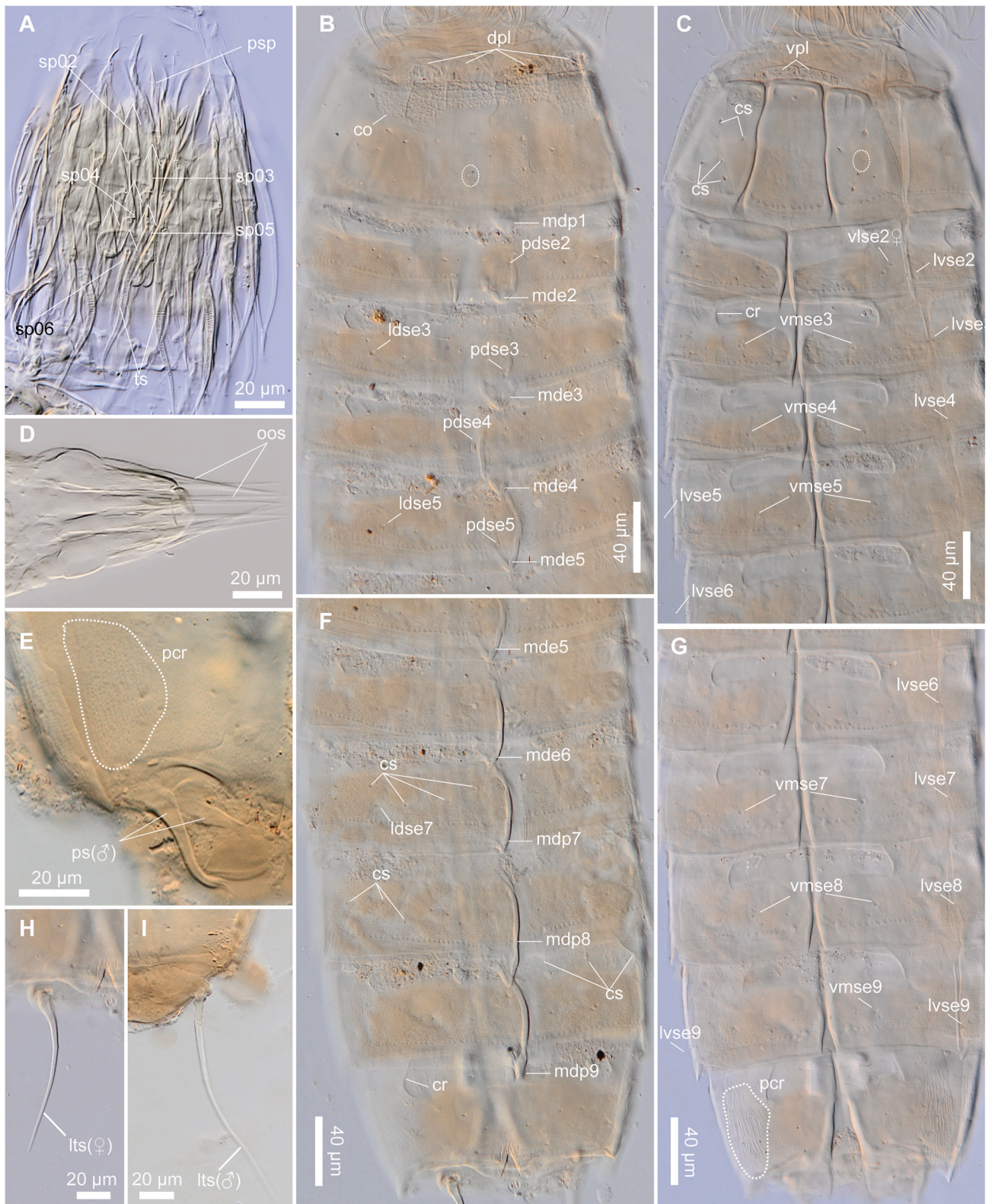


Fig. 4. Light micrographs of females (ZMB 12617: A, D; ZMB 12409: B–C, F–H) and male (ZMB 12418: E, I) of *Setaphyes dentatus* from Sylt. A: Sector 1 (midventral) of the introvert; B: Dorsal view on segments 1–5; C: Ventral view on segments 1–6; D: Mouth cone, outer oral styles (ring –01); E: Cuticular ornamentation on segment 10. Note that punctuate appearance represents preservation artefact. F: Dorsal view on segments 5–11; G: Ventral view on segments 6–11. H: Female LTS; I: Male LTS. Lambda symbols (Λ) mark anterior attachment points of spinoscalids on introvert (A). Abbreviations: co, cuticular ornamentation; cs, cuticular scars; dpl, dorsal placid; ldse, laterodorsal setae; lts, lateral terminal spine; lvse, lateroventral setae; mde, middorsal elevation; mdp, middorsal process; oos, outer oral style; pcr, patch of cuticular ridges; pdse, paradorsal setae; ps, penile spine; psp, primary spinoscalid; sp, spinoscalid (followed by the number of corresponding ring); ts, trichoscalid; vlse, ventrolateral setae; vmse, ventromedial setae; vpl, ventral placid. Numbers after abbreviations indicate the corresponding segment.

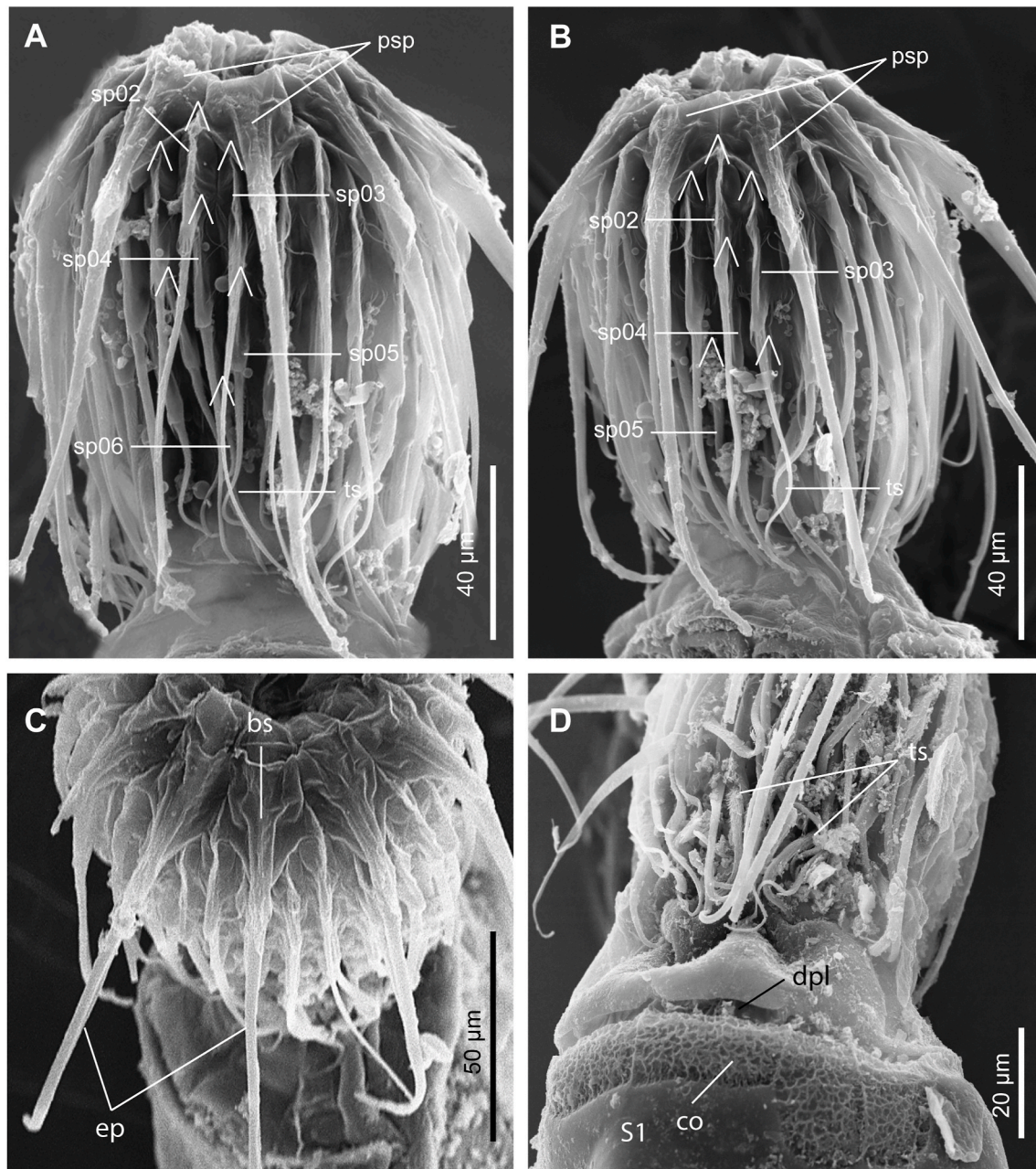


Fig. 5. SEM photographs of the head of *Setaphyes dentatus* (ZMB 12751). A: Introvert sector 1 (midventral); B: introvert sector 10 (ventrolateral); C: detail of introvert ring 01 showing the primary spinoscalid morphology; D: detail of neck (dorsal view) and anterior half of segment 1 tergal plate with focus on its cuticular ornamentation. Abbreviations: bs, basal sheath; co, cuticular ornamentation; dpl, dorsal placid; ep, end-piece of spinoscalid; psp, primary spinoscalid; S1, segment 1; sp, spinoscalid (followed by number of corresponding ring); ts, trichoscalid. Lambda symbols (Λ) mark attachment points of scalids.

4B). Anterior margin of episternal plates with a pair of ridges forming small, quadrangular depressions; anterior margin of midsternal plate with a single, large rectangular depression (Figs 2A, C; 4C). Episternal plates with usually five, scattered, minute, dot-shaped glandular cell outlets forming a quincunx (Figs 2A, C; 4C). Trapezoidal midsternal plate, wider at the base (ca. 34 μm wide at the most anterior margin, ca. 56 μm wide at the most posterior margin; average ratio = 61 %), with wavy lateral margins at the middle region (Figs 2A, C; 4C). Sensory spots present in paradorsal, subdorsal, laterodorsal and ventrolateral positions (Figs 2A–C; 6A–B). Sensory spots on this and following segments as oval areas with an oval area of cuticular micropapillae surrounding a single pore (Figs 7E; 8D–E).

Segment 2 with middorsal elevation with paradorsal, butterfly to trident-like atria of associated paradorsal sensory spots (near the posterior

margin) (Figs 2B; 4B; 6A; 7 C), surrounded by tufts of elongated, thick hairs with tips usually surpassing the posterior segment margin (Figs 6A; 7C). Unpaired seta in paradorsal position, and paired setae in lateroventral position; females with sexual dimorphism, an additional, pair of setae in ventrolateral position lacking in males. Males with sexually dimorphic tubes in ventromedial position lacking in females (Figs 2A, C; 8A). Sensory spots in paradorsal (near the posterior margin), subdorsal, laterodorsal and ventromedial positions (Figs 2A–C; 4B–C; 6A–B; 7C).

Segment 3 with middorsal elevation as on the preceding segment (Figs 2B; 4B; 6A; 7D). Unpaired seta in paradorsal position, and paired setae in laterodorsal and lateroventral positions. Females with an additional, pair of setae in ventromedial position, more mesial than the ventromedial sensory spots. Sensory spots in paradorsal (near the posterior margin), subdorsal, laterodorsal and ventromedial positions;

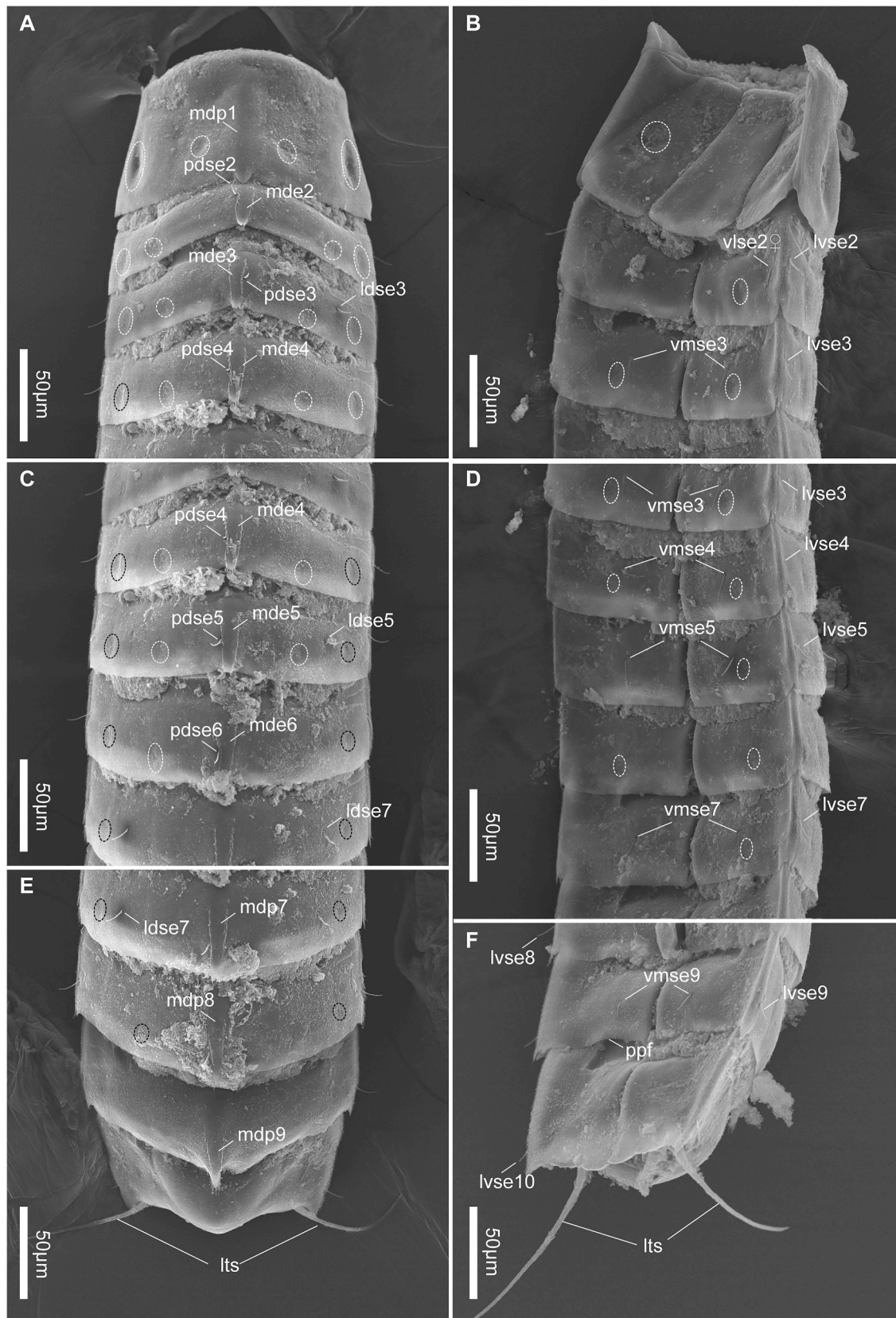


Fig. 6. SEM photographs of female (ZMB 12746: B, D, F) and adult of indeterminate sex (ZMB 12750: A, C, E) of *Setaphyes dentatus* from the Mediterranean coast of the Iberian Peninsula, Algeciras population. A: dorsal view of segments 1–4; B: ventral view of segments 1–3; C: dorsal view of segments 4–7; D: ventral view of segments 3–7; E: dorsal view of segments 7–10; F: ventral view of segments 8–10. Abbreviations: ldse, laterodorsal setae; lts, lateral terminal spine; lvse, lateroventral setae; mde, middorsal elevation; mdp, middorsal process; pdse, paradorsal setae; ppf, primary pectinate fringe; vlse, ventrolateral setae; vmse, ventromedial setae. Numbers after abbreviations indicate the number of the corresponding segment. Dashed circles mark sensory spots.

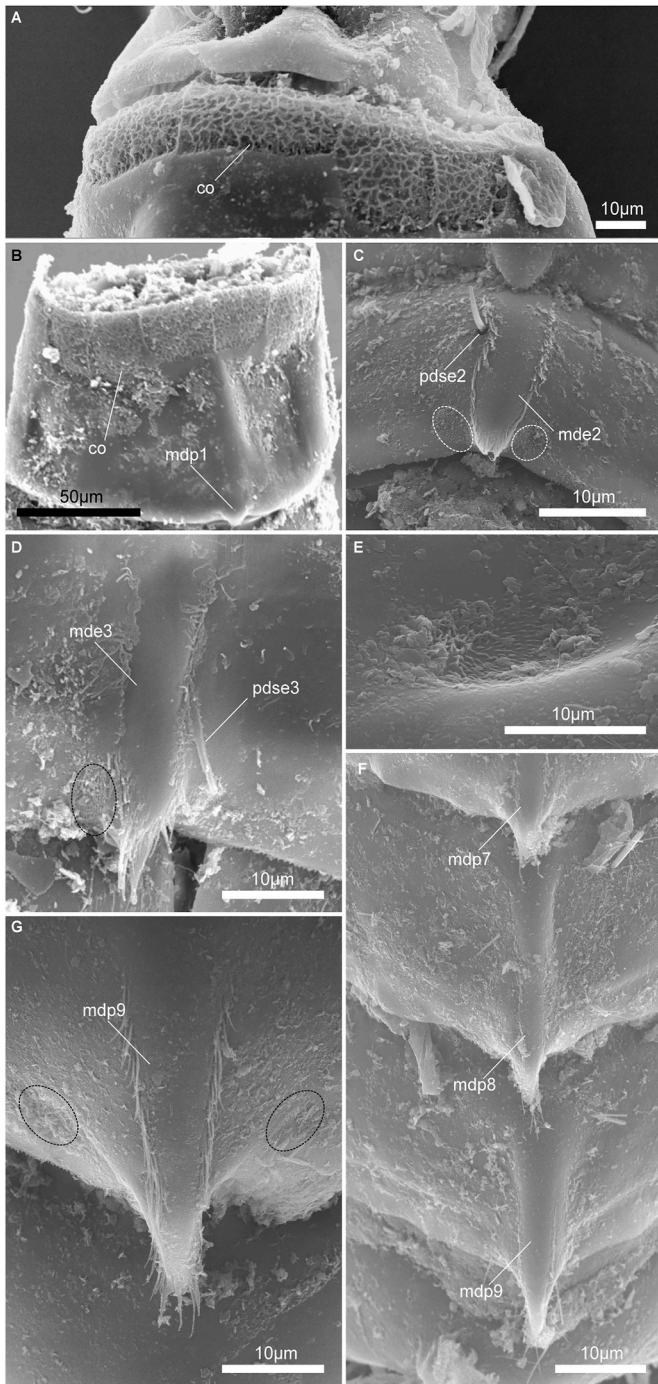


Fig. 7. SEM photographs of two females (110412 Huelva_sp1: A; 110412 Huelva_sp2: B, D; specimens damaged during study and not deposited) from Huelva and adults of indeterminate sex of *Setaphyes dentatus* from Huelva (ZMB 12751: E; ZMB 12752: F), and Algeciras (ZMB 12750: C; G) populations. A: detail of the reticular, net-like ornamentation on the dorsal, anterior margin of segment 1; B: middorsal to lateroventral view of segment 1; C: middorsal elevation of segment 2; D: middorsal elevation of segment 3; E: detail of laterodorsal sensory spot of segment 1; F: middorsal view of segments 8–9; G: middorsal process of segment 9. Abbreviations: co, cuticular ornamentation; mde, middorsal elevation; mdp, middorsal process; pdse, paradorsal setae. Numbers after abbreviations indicate the corresponding segment. Dashed circles mark sensory spots.

ventromedial sensory spots present in females only (Figs 2A–C; 4B–C; 6A–B, D; 7D).

Segment 4 with middorsal elevation as on the preceding segments

(Figs 2B; 4A; 6A, C). Unpaired seta in paradorsal position, and paired setae in lateroventral and ventromedial positions. Paired sensory spots in paradorsal (near the posterior margin), subdorsal, laterodorsal and ventromedial positions (Figs 2A–B; 4B–C; 6A, C–D), the latter more lateral than the ventromedial setae.

Segment 5 with middorsal elevation as on the preceding segments (Figs 2B; 4B, F; 6C; 8B). Unpaired seta in paradorsal position, and paired setae in laterodorsal, lateroventral and ventromedial positions; laterodorsal setae longitudinally aligned with those of segment 3. Laterodorsal setae longitudinally aligned with those of segment 3. Paired sensory spots in paradorsal (near the posterior margin), subdorsal, laterodorsal and ventromedial positions (Figs 2A–B; 4B–C; 6C–D; 8B–D), the latter more lateral than the ventromedial setae.

Segment 6 with middorsal elevation as on the preceding segments (Figs 2B; 4F; 6C; 8B). Unpaired seta in paradorsal position, and paired in lateroventral position. Paired sensory spots in paradorsal (near the posterior margin), subdorsal, laterodorsal and ventromedial positions (Figs 2A–B; 4C, F–G; 6C–D; 8B–C, F).

Segment 7 with middorsal process extending beyond the posterior margin of segment (Figs 2B; 7F). Similar to segment 5 in the arrangement of setae and sensory spots (Figs 2A–B; 4F–G; 6C–E; 8C, E).

Segment 8 with middorsal process longer than that of the preceding segment (Figs 2B; 4F; 7F). Similar to segment 4 in the arrangement of setae and sensory spots (Figs 2A–B; 4F–G; 6E–F).

Segment 9 with middorsal process longer than that of the preceding segment (Figs 2B; 4F; 6E; 7F–G; 8H). Unpaired seta in paradorsal position, and paired in lateroventral position; females with an additional pair of setae in ventromedial position lacking in males (Figs 2A–B, D; 4G; 6F). Sensory spots in paradorsal (near the posterior margin), subdorsal, laterodorsal, and ventrolateral positions (Fig. 2A–B, D). Nephridiopore as small opening surrounded by short tubes in lateroventral position.

Segment 10 without middorsal cuticular specialization. Setae in lateroventral position (Figs 2A, D; 6F; 8H). Sensory spots in subdorsal, laterodorsal and ventrolateral positions, the two latter between patches of conspicuous, parallel cuticular ridges, extending throughout the laterodorsal to ventrolateral areas (Figs 2A, D; 4E, G; 8G–H). Tergal plate with rounded posterior margin; sternal plates distally straight in females, more pointed in males.

Segment 11 without middorsal cuticular specialization. Males with two pairs of stout, thick penile spines (Figs 2A; 4E). Short lateral terminal spines, longer in males than in females (males LTS:TL average ratio 21.21 %; females LTS:TL average ratio 15.66 %) (Figs 2A–B, D; 4H–I; 6E–F; 8G).

3.1.4. Intraspecific variation

Due to the preservation conditions of the studied material, the pattern of sensory spots throughout the trunk could not be fully confirmed in all the LM specimens. The remaining cuticular characters of taxonomic relevance for pycnophyids (i.e. setae, middorsal cuticular specializations, spines, glandular cell outlets, and ornamentation) could be examined in detail in the Anatolian population (four females, one adult specimen for SEM), North Frisian (three females, two males), Italian (one adult specimen) and Iberian (Pontevedra, one female, four males; Algeciras, one male, one female, three adult specimens mounted for SEM; Huelva, one adult specimen mounted for SEM; and Tarragona, four females, three males) populations.

Anatolian population: All specimens lack the ventromedial setae on segment 8.

North Frisian population: ventromedial seta on segment 2 absent on one sternal plate in one male (ZMB 12418); ventromedial setae on segment 6 present on one sternal plate in one female (ZMB 12413); ventromedial setae on segment 9 absent in one female (ZMB 12408).

Iberian population: laterodorsal seta on one side of the tergal plate on segment 2 present in one male (ZMB 12715); laterodorsal seta on segment 3 absent on one side of the tergal plate in one male (ZMB 12640) and in one SEM specimen (ZMB 12751); laterodorsal seta on one

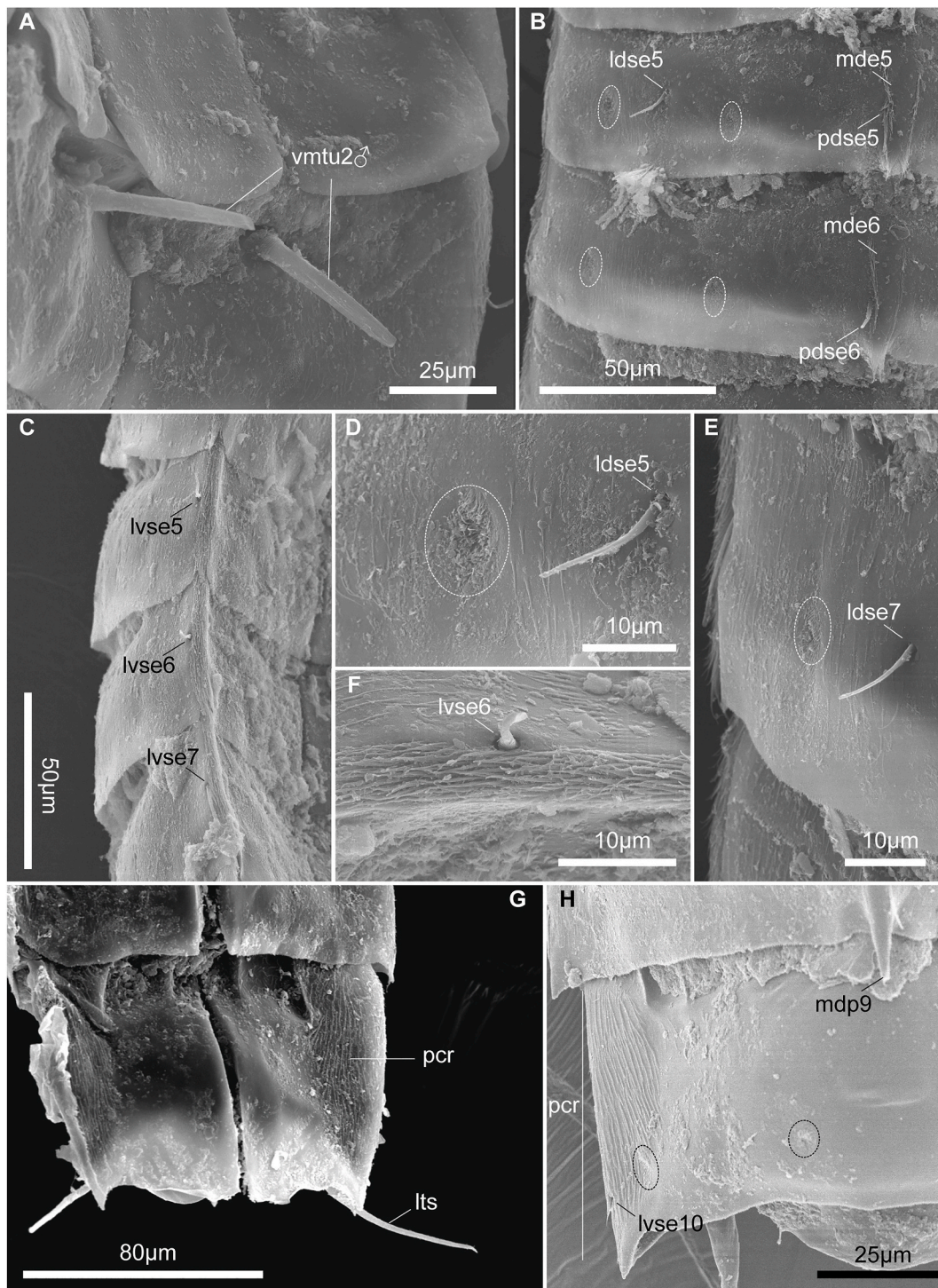


Fig. 8. SEM photographs of female (ZMB 12746: G), male (ZMB 12747: A, C, F) and adults of indeterminate sex (ZMB 12748: B, D; ZMB 12750: E; ZMB 12745: H) of *Setaphyes dentatus* from the Atlantic and the Mediterranean coasts of the Iberian Peninsula and from the Black Sea coast of Türkiye, Huelva (G), Algeciras (A–F), Türkiye (H) populations. A: ventral view of segments 1–2; B: left side of the tergal plate, segments 5–6; C: right side of segments 5–7; D: left side of the tergal plate, segment 5, laterodorsal setae and sensory spot; E: laterodorsal side of the tergal plate, segment 7; F: right lateroventral seta of segment 6; G: ventral view of segment 10; H: left side of the tergal plate, segment 10. Abbreviations: ldse, laterodorsal setae; lts, lateral terminal spine; lvse, lateroventral setae; mde, middorsal elevation; mdp, middorsal process; pcr, patch of cuticular ridges; pdse, paradorsal setae; vmtu, ventromedial tube. Numbers after abbreviations indicate the corresponding segment. Dashed circles mark sensory spots.

Table 1

Measurements (μm) and proportions (%) of *Setaphyes dentatus*, including total values from the different studied populations split by sex (values per specimen and population can be found in [Supplementary Table 3](#). Abbreviations: LTS, lateral terminal spine length; MSW5, maximum sternal width (measured at segment 5); n, number of measured specimens; S, segment length (followed by number of corresponding segment); Sd, standard deviation; SW10, standard sternal width (measured at segment 10); TL, total trunk length.

Character	n	Mean, Sd ♀	Range ♀	Mean, Sd ♂	Range ♂	Mean, Sd ♀+♂	Range ♀+♂
TL	83♀/53♂	652, 55.9	528–780	630, 51.2	518–735	643, 55	518–780
MSW5	82♀/53♂	157, 8.1	128–179	154, 10.2	131–175	156, 9	128–179
MSW5/TL	82♀/53♂	24, 0.04	20–49	25, 0.03	18–31	24, 0.04	20–49
SW10	82♀/53♂	135, 9.7	105–158	130, 10.2	110–157	133, 10.1	105–158
SW10/TL	82♀/53♂	21, 0.03	15–39	21, 0.02	18–28	21, 0.03	15–39
S1	83♀/52♂	101, 8.3	68–117	96, 7	78–112	99, 8.2	68–117
S2	83♀/52♂	63, 7.2	43–85	61, 7.3	37–75	62, 7.2	37–85
S3	83♀/52♂	66, 7.1	53–84	63, 7.6	39–78	65, 7.5	39–84
S4	83♀/52♂	70, 7.7	53–92	66, 7.4	51–80	69, 7.8	51–92
S5	83♀/52♂	73, 7.3	57–96	70, 7.4	58–86	72, 7.5	57–96
S6	83♀/52♂	76, 7	61–96	73, 7.3	55–89	75, 7.2	55–96
S7	83♀/52♂	78, 7.2	61–98	75, 7	61–92	77, 7.2	61–98
S8	83♀/52♂	78, 7	65–99	77, 7	62–95	78, 7	62–99
S9	83♀/52♂	78, 6.3	62–94	76, 6.2	64–92	77, 6.3	62–94
S10	83♀/52♂	94, 12.5	83–114	86, 14.2	68–110	91, 13.4	68–114
S11	82♀/52♂	33, 6.6	19–53	29, 6.2	17–43	31, 6.6	17–53
LTS	74♀/48♂	100, 13.2	74–140	133, 13.4	103–166	113, 21	74–166
LTS/TL	74♀/48♂	16, 0.03	11–32	21, 0.02	14–28	18, 0.04	11–32

Table 2

Summary of nature and arrangement of cuticular elevations, processes, spines, tubes, setae, sensory spots and nephridiopores in *Setaphyes dentatus*. Abbreviations: ce, cuticular elevation; cp, cuticular process; LD, laterodorsal; lts, lateral terminal spine; LV, lateroventral; MD, middorsal; ne, nephridiopore; PD, paradorsal; ps, penile spine; SD, subdorsal; se, seta; ss, sensory spot; tu, tube; VL, ventrolateral; VM, ventromedial; * indicates unpaired structures; ♂ and ♀ indicates sexually dimorphic characters.

Segment	MD	PD	SD	LD	LV	VL	VM
1	cp*	ss	ss	ss		Ss	
2	ce*	se*, ss	ss	ss	se	se (♀)	tu (♂), ss
3	ce*	se*, ss	ss	se, ss	se		se (♀), ss
4	ce*	se*, ss	ss	ss	se		se, ss
5	ce*	se*, ss	ss	se, ss	se		se, ss
6	ce*	se*, ss	ss	ss	se		ss
7	cp*	se*, ss	ss	se, ss	se		se, ss
8	cp*	se*, ss	ss	ss	se		se, ss
9	cp*	se*, ss	ss	ss	se, ne	ss	se (♀)
10			ss	ss	se	ss	
11					lts	ps x2 (♂)	

side of the tergal plate on segment 4 present in one female (ZMB 12716); ventromedial seta on one sternal plate on segment 4 not detected in one male (ZMB 12640); ventromedial setae on segment 6 present in one male and one female (ZMB 12638 and ZMB 12714) and on the lateral half of the sternal plate in one female (ZMB 12716); ventromedial setae on segment 8 absent in one female (ZMB 12643).

3.2. Redescription of *Setaphyes kielensis* (Zelinka, 1928)

[Figs 9–14, Tables 3–4.](#)

3.2.1. Diagnosis

Setaphyes with middorsal elevations on segments 1–9, superficially covered by tufts of elongated, thick hairs. Paradorsal setae on segments 2–9 (that of segment 8 unpaired). Laterodorsal setae on segments 2–9. Lateroventral setae on segments 2–10. Ventromedial setae on segments 3–9. Lateral terminal spines short, slender (males LTS:TL average ratio 31.4 %; females LTS:TL average ratio 9 %; males and females average ratio 19.1 %).

3.2.2. Material examined

59 specimens collected from two different populations located in the Anatolian Peninsula and North Frisia. Material from Anatolia are new records for the region, which includes four females mounted for LM, and one female studied with SEM (ZMB 12745, station YSL09R2, 13 July 2019, 41°24.738'N, 036°39.210'E, 76 m depth; collector: Derya

Ürkmez). Material from Germany (Sylt) included 17 males and 15 females mounted for LM (ZMB 12364–12365 were studied only for the intraspecific variability, same sampling locality as for the remaining LM specimens included in the [Supplementary Table I](#)); and 22 adults for SEM (22 June 2016, 54°57'15"N, 008°21'34"E, precision 50 m, collectors: Birger Neuhaus and Hiroshi Yamasaki) (10 males: ZMB 12455, 12457, 12458, 12461–12463, 12468, 12 470, 12472, 12477; 12 females: ZMB 12454, 12456, 12460, 12464, 12465, 12467, 12469, 12471, 12474–12476, 12478). Detailed information of the sampling localities of each LM specimen is provided in the [Supplementary Table I](#).

3.2.3. Description

See [Table 3](#) and [Supplementary Table III](#) for measurements and dimensions and [Table 4](#) for summary of middorsal cuticular specialization, seta, tube, nephridiopore and sensory spot locations.

Nine, equally sized outer oral styles in Ring 00 of mouth cone. Each outer oral style formed by a single, flexible unit, wider at base, and showing a sheath with up to 13 fringed rows broader at the proximal region and becoming narrower towards the pointed tip ([Figs 3; 11A](#)). Trapezoidal, superficially smooth, cuticular thickenings in between the outer oral styles, with two basal, short, fringed rows ([Fig. 11A](#)). Outer oral styles arranged one by each introvert sector, except in sector 6 where a style is missing ([Fig. 3](#)). Inner rings of mouth cone not observed and, hence, detailed information on the morphology and distribution of inner styles is not provided.

Introvert with six rings of spinoscalids and 10 longitudinal sectors

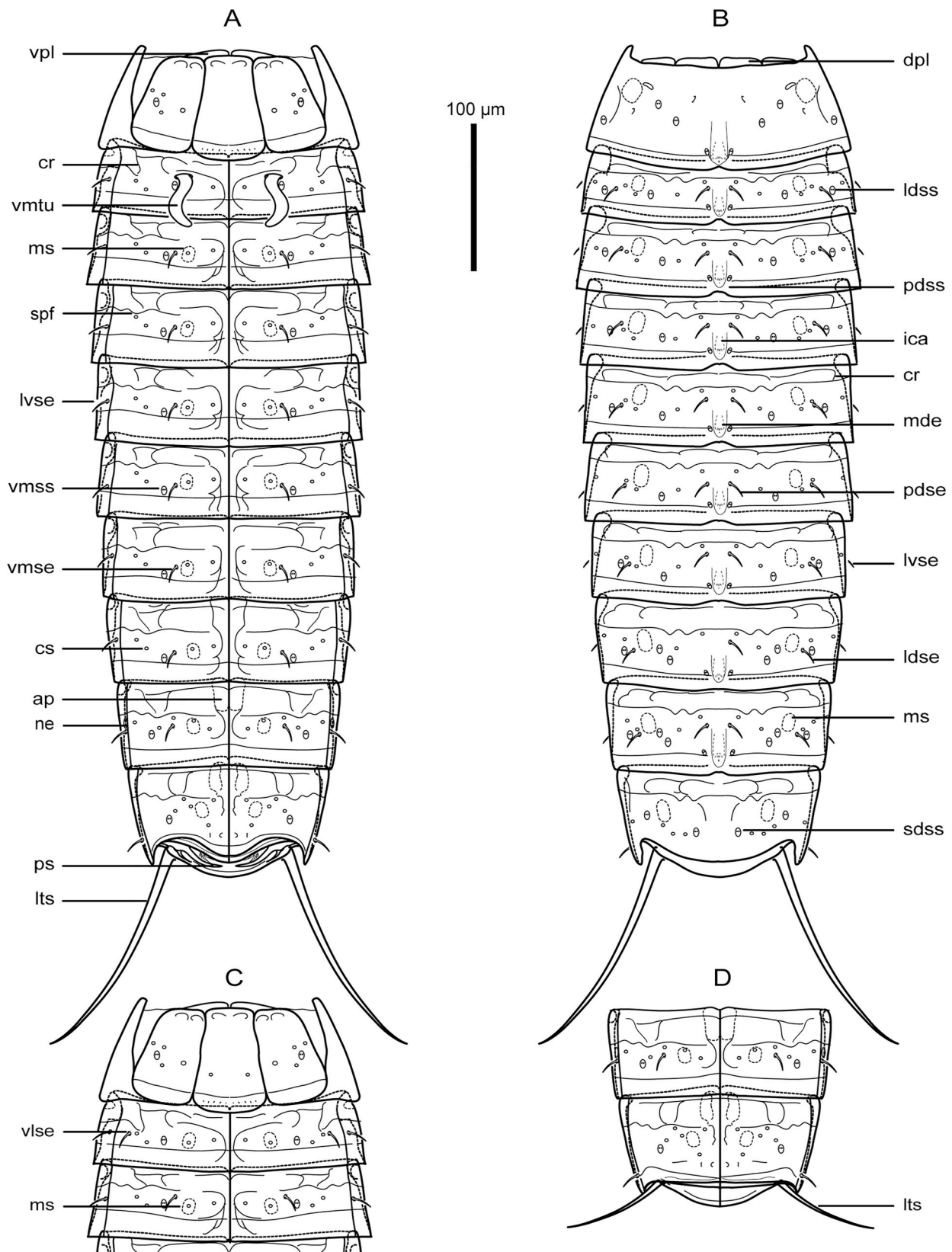


Fig. 9. Line art illustration of *Setaphyes kielenensis*. A: Male, ventral view; B: Male, dorsal view; C: Female, segments 1–3, ventral view; D: female, segments 9–11, ventral view. Scale bar: 100 µm. Abbreviations: ap, apodeme; cr, cuticular ridge; cs, cuticular scar; dpl, dorsal placid; ica, intracuticular atria; ldse, laterodorsal setae; ldss, laterodorsal sensory spot; lts, lateral terminal spine; lvse, lateroventral setae; mde, middorsal elevation; ms, muscular scar; ne, nephridiopore; pdse, paradorsal setae; pdss, paradorsal sensory spot; ps, penile spine; sdss, subdorsal sensory spot; spf, secondary pectinate fringe; vlse, ventrolateral setae; vmse, ventromedial setae; vmss, ventromedial sensory spot; vmtu, ventromedial tube; vpl, ventral placid.

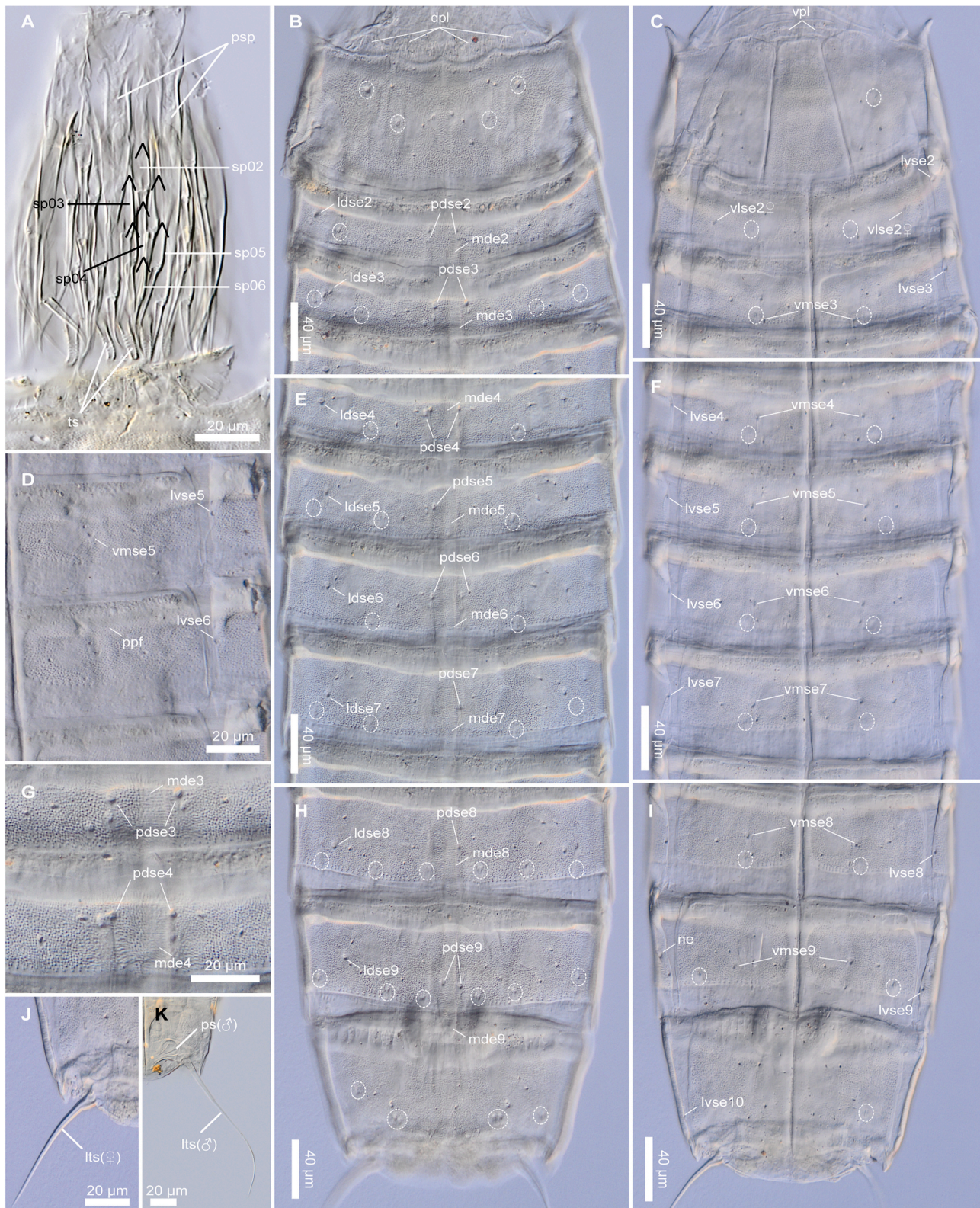


Fig. 10. Light micrographs of females (ZMB 12307: A; ZMB 12742: B–C, E–J; ZMB 12744: D), and male (ZMB 12294: K) of *Setaphyes kielensis*. A: Sector 6 (middorsal) of the introvert; B: Dorsal view of segments 1–3; C: Ventral view of segments 1–3; D: Ventral view on left side of the sternal plate of segments 5–6; E: Dorsal view of segments 4–7; F: Ventral view of segments 4–7; G: Dorsal view on the central part of the tergal plate of segments 3–4; H: Dorsal view of segments 8–11; I: Ventral view of segments 8–11; J: Female LTS; K: Male LTS. Abbreviations: ldse, laterodorsal setae; lts, lateral terminal spine; lvse, lateroventral setae; mde, middorsal elevation; ne, nephridiopore; pdse, paradorsal setae; ppf, primary pectinate fringe; ps, penile spines; psp, primary spinoscalid; sp, spinoscalid (followed by the number of corresponding ring); ts, trichoscalids; vlse, ventrolateral setae; vmse, ventromedial setae. Lambda symbols (Λ) mark attachment points of scalids (A). Numbers after abbreviations indicate the number of the corresponding segment. Dashed circles mark sensory spots.

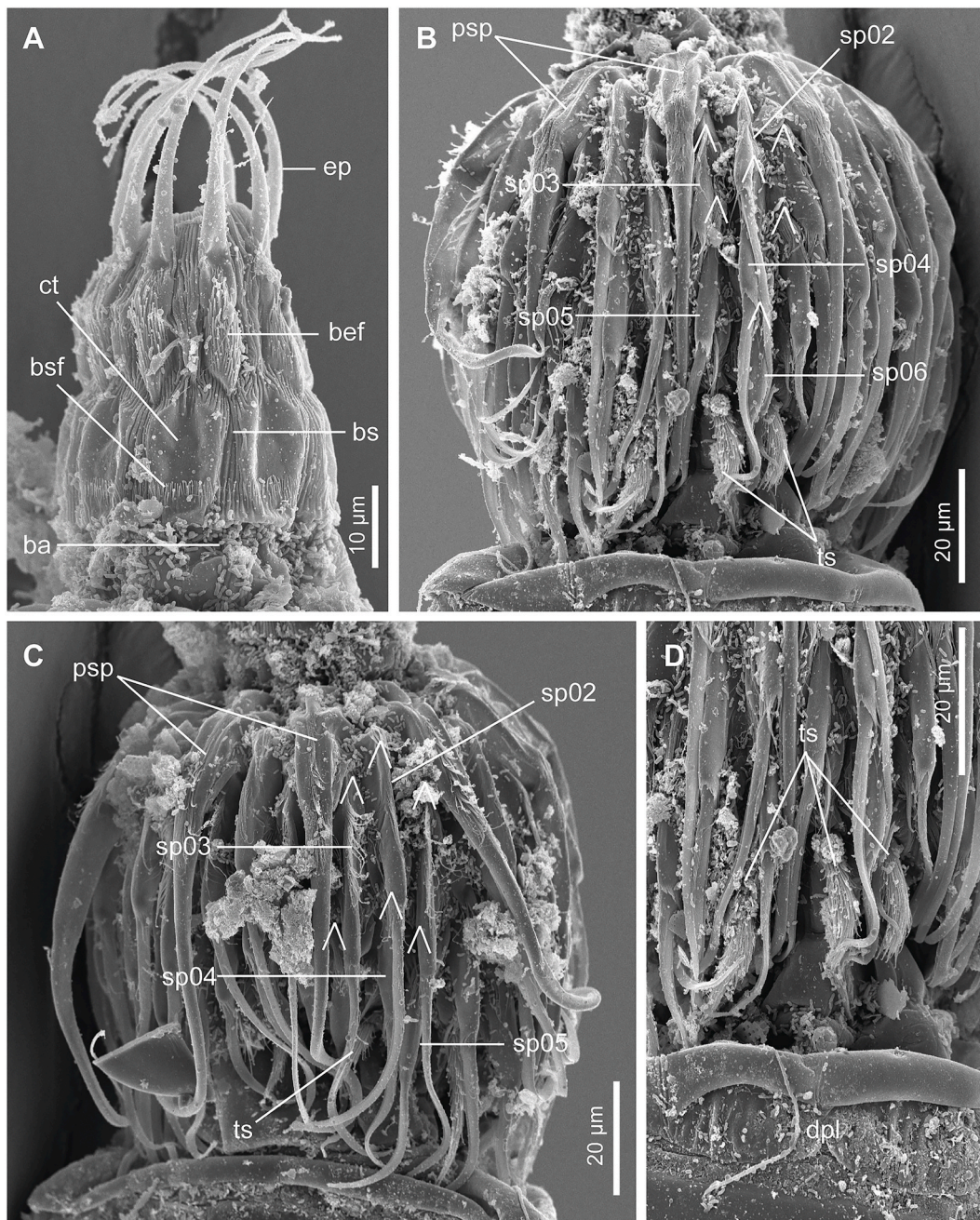


Fig. 11. SEM photographs of the head of *Setaphyes kielensis* (male ZMB 12455: A–D). A: Detail of mouth cone ring 00 showing the outer oral style morphology; B: introvert sector 4 (laterodorsal); C: introvert sector 2 (ventrolateral); D: detail of neck (dorsal view) showing the trichoscalids and the placids. Abbreviations: ba, bacteria; bef, basal elongated fringe; bs, basal sheath; bsf, basal short fringe; ct, cuticular thickening; dpl, dorsal placid; ep, end-piece of spinoscalid; psp, primary spinoscalid; sp, spinoscalid (followed by number of corresponding ring); ts, trichoscalid. Lambda symbols (Λ) mark attachment points of scalids.

defined by the disposition of the primary spinoscalids. Scalid and trichoscalid arrangement and morphology as described for *S. dentatus* (Figs 3; 10A; 11A–D).

Neck with four dorsal and two ventral, sclerotized placids (Figs 9A–C; 10B–C; 11D). Dorsal placids rectangular, with a slightly convex anterior margin; mesial ones broader (ca. 32 µm wide at base) than lateral ones (ca. 23 µm wide at base) (Figs 9B; 10B; 11D). Ventral placids (ca. 21 µm wide at base) morphologically similar to the dorsal ones but much more elongated, getting thinner towards the lateral sides (Figs 9A, C; 10C).

Trunk with eleven segments (Figs 9A–B; 12A, F; 13A, D). Segment 1 with one tergal, two episternal and one trapezoidal, midsternal plate; remaining ones with one tergal and two sternal cuticular plates (Figs 9A–D; 10B–C, E–F, H–I; 12A–C, E–H; 13A–F; 14A–C, F). Sternal plates

reaching their maximum width at segment 5, almost constant in width throughout the trunk (Figs 9A–B; 12A, F; 13A, D; 14A, F). Sternal cuticular plates relatively narrow (MSW-5:TL average ratio = 30 %) (Figs 9A–B; 12A, F). Middorsal elevations on segments 1–9, quite inconspicuous, rectangular, narrow, distally blunted, not projecting beyond the posterior margin of segments (Figs 9B; 10B, E, G–H). Paired, paradorsal, butterfly to trident-like intracuticular atria associated to the middorsal structures (Fig. 10G). Cuticular scars (possibly glandular cell outlets) as minute, dot-shaped, rounded to oval perforations throughout the cuticle on segments 1–11 (Figs 9A–D; 10B–I); the number and position of these structures vary greatly among the analysed specimens, with no specific pattern. Up to three pairs of conspicuous laterodorsal and ventromedial cuticular ridges on segments 2–10 (Fig. 9A–D).

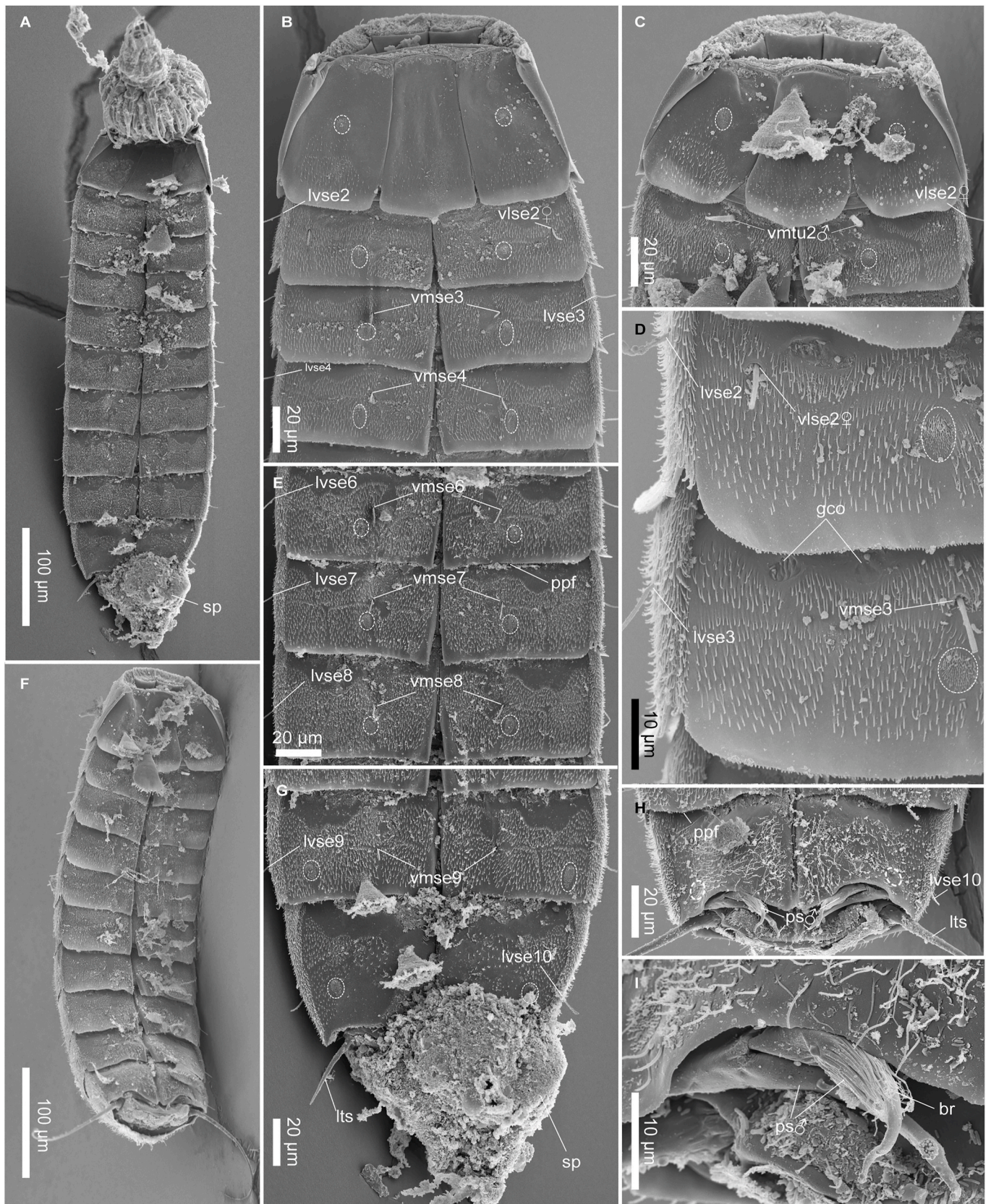


Fig. 12. SEM photographs of females (ZMB 12456: A, E, G; ZMB 12467: B; ZMB 12471 D), males (ZMB 12477: C, H–I; ZMB 12463: F) of *Setaphyes kielensis* from Sylt. A: ventral overview; B: ventral view of segments 1–4; C: ventral view of segments 1–2; D: right sternal plates of segments 2–3; E: ventral view of segments 6–8; F: ventral overview; G: ventral view of segments 9–10; H: ventral view of segments 10–11; I: detail of the penile spines and bristles. Abbreviations: br, bristle; gco, glandular cell outlets; lts, lateral terminal spine; lvse, lateroventral setae; ppf, primary pectinate fringe; ps, penile spines; sp, spermatophore; vlse, ventrolateral setae; vmse, ventromedial setae; vmtu, ventromedial tube. Numbers after abbreviations indicate the corresponding segment. Dashed circles mark sensory spots.

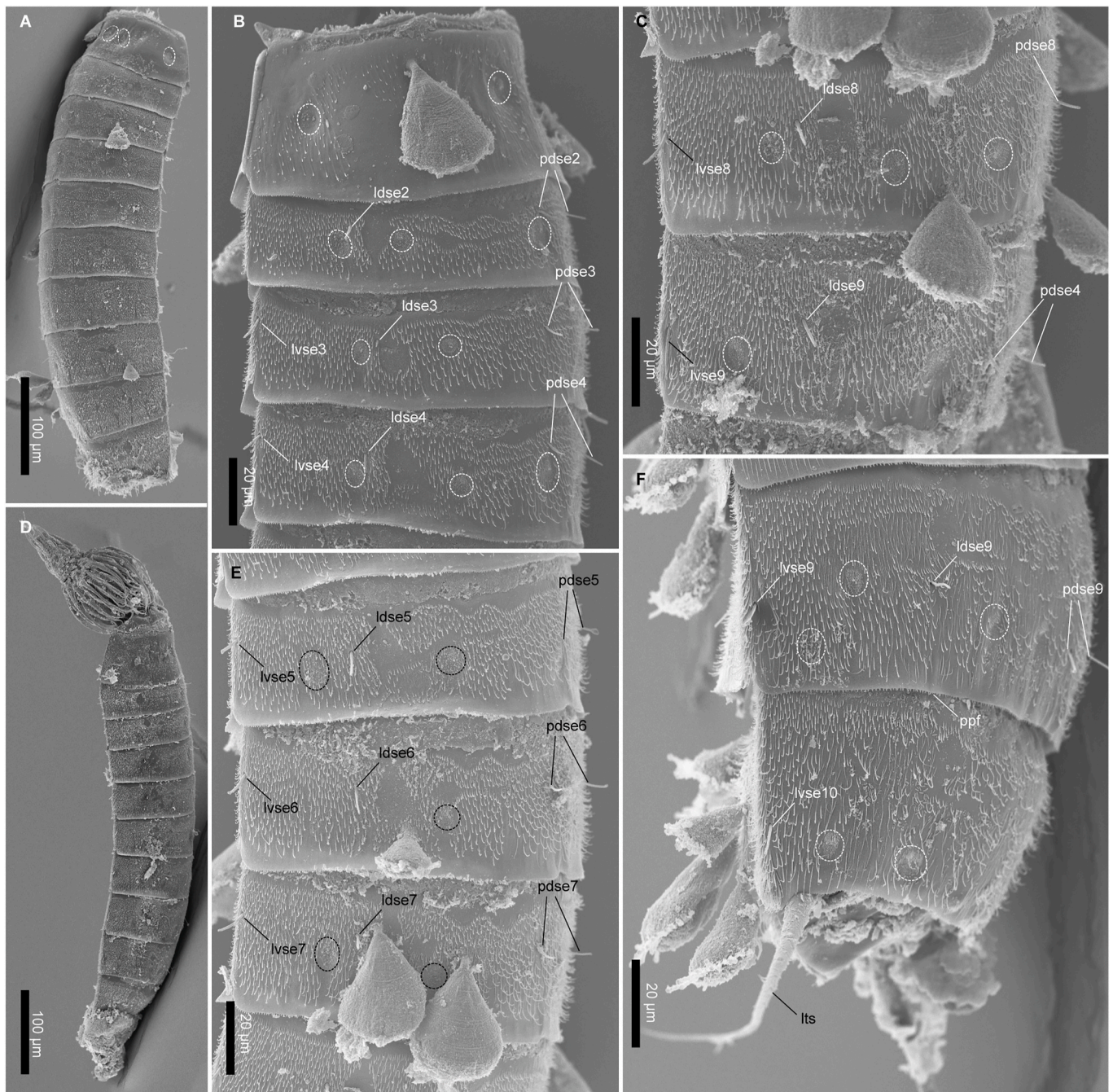


Fig. 13. SEM photographs of females (ZMB 12476: A; ZMB 12474: B–C, E; ZMB 12456: D), male (ZMB 12470: F) of *Setaphyes kielensis* from Sylt. A: dorsolateral overview, right side of the trunk; B: lateral view of the left side of segments 1–4; C: lateral view of the left side of segments 8–9; D: dorsolateral overview, left side of the trunk; E: lateral view of the left side of segments 5–7; F: lateral view of the left side of segments 9–11. Abbreviations: ldse, laterodorsal setae; lts, lateral terminal spine; lvse, lateroventral setae; pdse, paradorsal setae; ppf, primary pectinate fringe. Numbers after abbreviations indicate the number of the corresponding segment. Dashed circles mark sensory spots.

Cuticular hairs acicular, non-bracteate, distributed throughout the trunk on segments 1–10 not following any particular pattern and giving the animal a furry appearance (Figs 12A–I; 13A–F; 14A–D). Pachycycli and ball-and-socket joints conspicuous on segments 2–9, reduced on most posterior segments, and also depending on maturation of specimens (Fig. 9A–D). Apodemes on segments 9–10 (Figs 9A, D; 10H–I). Primary pectinate fringes finely serrated (Figs 10D; 12H; 13F); secondary pectinate fringes as a wavy, single line along the entire dorsal side with two subdorsal indentations pointing backwards. Ventral secondary pectinate fringes as a wavy, single line in ventromedial or paraventral positions (Fig. 9A–D). Muscular scars as conspicuous, rounded to oval,

hairless areas in laterodorsal and ventrolateral positions on segments 1–10 (Figs 9A–D; 13B, E; 14B–D).

Segment 1 with middorsal elevation not projecting beyond the posterior margin of the segment, with paradorsal, butterfly to trident-like atria of associated paradorsal sensory spots (Figs 9B; 10B). Antero-lateral margins of the tergal plate as triangular, short, wide, distally rounded extensions (Figs 9A–C; 10B–C; 13B). Episternal plates with usually four, scattered, minute, dot-shaped glandular cell outlets (Fig. 9A, C). Trapezoidal midsternal plate, wider at the base (ca. 29 µm wide at the most anterior margin, ca. 56 µm wide at the most posterior margin; average ratio = 52 %), with parallel lateral margins (Figs 9A, C;

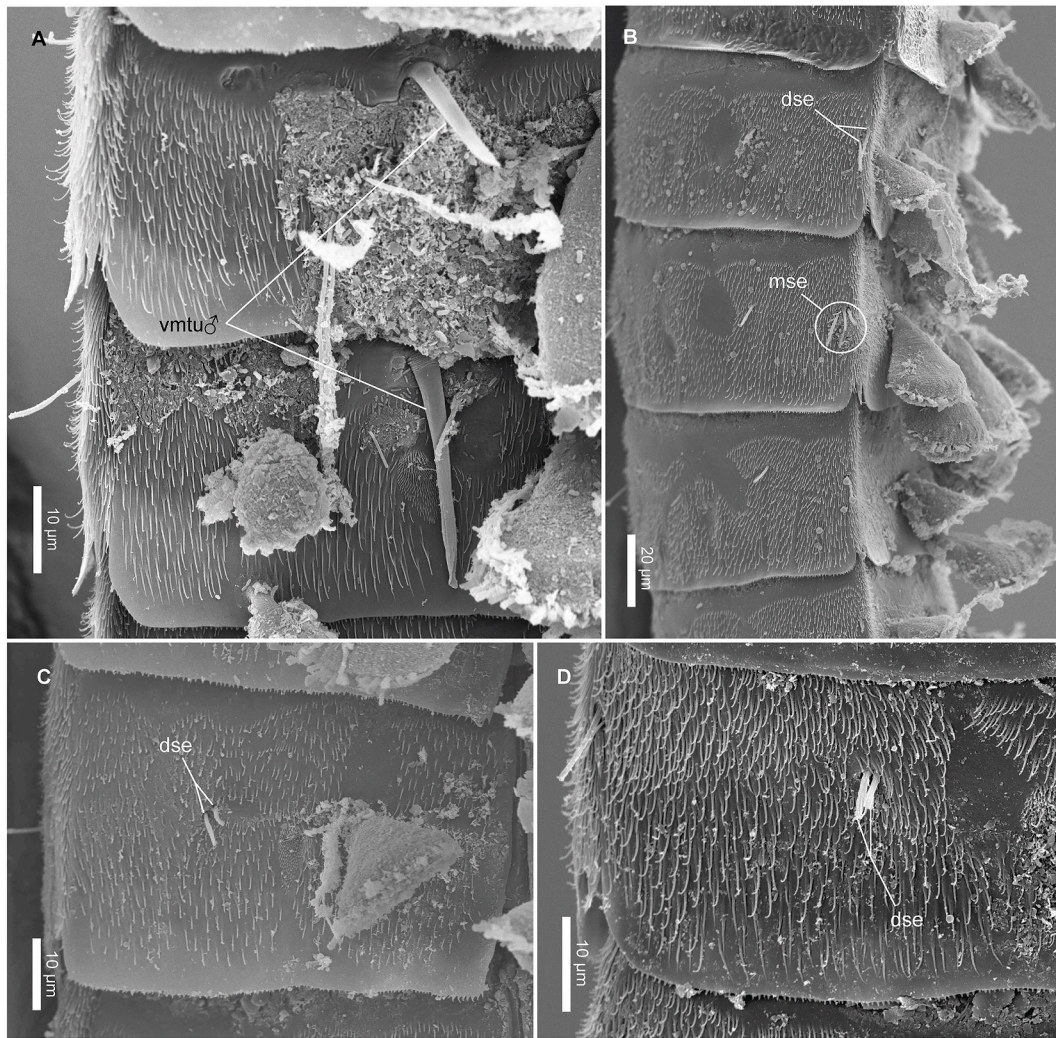


Fig. 14. SEM photographs of females (ZMB 12475: B; ZMB 12454: D), and males (ZMB 12458: A; ZMB 12470: C) of *Setaphyes kielensis* from Sylt. A: ventral view of the right sternal plates of segments 2–3 with an extra ventromedial tube on only one sternal plate of segment 3; B: lateral view of the right side of segments 2–4; C: ventral view of the right sternal plate of segment 5; D: dorsolateral view of the left side of the tergal plate of segment 2. Abbreviations: dse, double setae; mse, multiple setae; vmtu, ventromedial tube.

Table 3

Measurements (μm) and proportions (%) of *Setaphyes kielensis*, including total values from the different studied populations split by sex (values per specimen and population can be found in [Supplementary Table 3](#). Abbreviations: LTS, lateral terminal spine length; MSW5, maximum sternal width (measured at segment 5); n, number of measured specimens; S, segment length (followed by number of corresponding segment); Sd, standard deviation; SW10, standard sternal width (measured at segment 10); TL, total trunk length.

Character	n	Mean, Sd ♀	Range ♀	Mean, Sd ♂	Range ♂	Mean, Sd ♀+♂	Range ♀+♂
MSW5	19♀/15♂	152, 16.7	130–200	132, 13.2	99–145	143, 18.3	99–200
MSW5/TL	19♀/15♂	30, 0.03	30–31	30, 0.04	20–36	30, 0.03	20–36
SW10	19♀/15♂	131, 15.7	103–166	110, 12.8	84–127	122, 17.7	84–166
SW10/TL	19♀/15♂	26, 0.02	26–27	25, 0.03	19–28	26, 0.03	19–31
S1	19♀/15♂	78, 8.5	64–96	64, 6.5	54–79	72, 10.3	54–96
S2	19♀/15♂	49, 5.9	41–63	43, 4.8	36–51	47, 6.1	36–63
S3	19♀/15♂	53, 7.1	42–72	46, 3.8	40–54	50, 6.7	40–72
S4	19♀/15♂	59, 8.9	47–84	51, 3.5	43–58	55, 8.2	43–84
S5	19♀/15♂	62, 6.6	55–78	51, 2.8	46–57	57, 7.7	46–78
S6	19♀/15♂	64, 7.8	53–85	52, 4.5	46–61	58, 8.8	46–85
S7	19♀/15♂	67, 7	55–82	56, 3.5	50–63	62, 7.8	50–82
S8	19♀/15♂	69, 7.5	58–88	58, 3.1	50–62	64, 8.3	50–88
S9	19♀/15♂	73, 9.2	62–99	61, 3.8	55–68	68, 9.3	55–99
S10	19♀/15♂	77, 9.3	52–92	60, 5.6	54–74	70, 11.5	52–92
S11	19♀/15♂	32, 4.9	25–41	35, 5.8	22–43	33, 5.5	22–43
LTS	17♀/14♂	46, 10.3	31–67	137, 14.2	104–162	87, 47.8	31–162
LTS/TL	17♀/14♂	9, 0.02	7–11	31, 0.04	24–37	19, 0.1	7–37

Table 4

Summary of nature and arrangement of cuticular elevations, spines, tubes, setae, sensory spots and nephridiopores in *Setaphyes kielensis*. Abbreviations: ce, cuticular elevation; LD, laterodorsal; lts, lateral terminal spine; LV, lateroventral; MD, middorsal; ne, nephridiopore; PD, paradorsal; ps, penile spine; SD, subdorsal; se, seta; ss, sensory spot; tu, tube; VL, ventrolateral; VM, ventromedial; * indicates unpaired structures; ♂ and ♀ indicates sexually dimorphic characters.

Segment	MD	PD	SD	LD	LV	VL	VM
1	ce*	ss	ss x2	ss			
2	ce*	se, ss	ss	se, ss	se	se (♀)	tu (♂), ss
3	ce*	se, ss	ss	se, ss	se		se, ss
4	ce*	se, ss	ss	se, ss	se		se, ss
5	ce*	se, ss	ss	se, ss	se		se, ss
6	ce*	se, ss	ss	se	se		se, ss
7	ce*	se, ss	ss	se, ss	se		se, ss
8	ce*	se*, ss	ss x2	se, ss	se		se, ss
9	ce*	se, ss	ss x2	se, ss	se, ne		se, ss
10			ss x2		se		
11				lts		ps x2 (♂)	

10C). Two pairs of sensory spots in subdorsal position, and one pair in laterodorsal and paradorsal positions, the latter towards the middle region of the segment (Figs 9B; 10B; 13A–B). Sensory spots on this and following segments as oval areas with several rows of cuticular micropapillae surrounding a single pore (Fig. 12D).

Segment 2 with middorsal elevation as on the preceding segment (Figs 9B; 10B). Setae in paradorsal, laterodorsal and lateroventral positions; females with additional pair of sexually dimorphic ventrolateral setae (Figs 9A–C; 10B–C; 12B–D; 13B; 14B, D). Males with tubes in ventromedial position (Figs 9A; 12C; 14A). Sensory spots in paradorsal (not near the posterior margin), subdorsal, laterodorsal and ventromedial positions (Figs 9A–C; 10B–C; 12B–C; 13B).

Segment 3 with middorsal elevation as on the preceding segments (Fig. 9B, G). Setae in paradorsal, laterodorsal, lateroventral and ventromedial positions. Sensory spots in paradorsal (not near the posterior margin), subdorsal, laterodorsal and ventromedial positions (Figs 9A–C; 10B–C, G; 12B, D; 13B; 14B).

Segments 4 to 7 similar to segment 3 in the arrangement of cuticular elevation, setae and sensory spots (Figs 9A–B; 10D–G; 12B, E; 13B, E; 14B–C).

Segment 8 with middorsal elevation as on the preceding segments (Figs 9B; 10H). Unpaired seta in paradorsal position, at the right side of the middorsal elevation; paired setae in laterodorsal, lateroventral and ventromedial positions. Sensory spots in paradorsal (not near the posterior margin), subdorsal (two pairs), laterodorsal and ventromedial positions (Figs 9A–B; 10H–I; 12E; 13C).

Segment 9 with middorsal elevation as on the preceding segments (Figs 2B; 10H). Setae in paradorsal, laterodorsal, lateroventral and ventromedial positions. Sensory spots in paradorsal (not near the posterior margin), subdorsal (two pairs), laterodorsal, and ventrolateral positions (Figs 9A–B, D; 10H–I; 12G; 13F). Nephridiopores as small opening surrounded by short tubes in lateroventral position (Fig. 10I).

Segment 10 without middorsal cuticular specialization. Setae in lateroventral position. Two pairs of sensory spots in subdorsal position (Figs 9A–B, D; 10I; 12G–H; 13F). Tergal plate with rounded posterior margin; posterior margins of sternal plates straight in females, more pointed in males.

Segment 11 without middorsal cuticular specialization. Males with two lateral pairs of stout, thick penile spines and with one pair of collapsed tubes terminating in penile bristles (Figs 9A; 10K; 12I). Short lateral terminal spines, longer in males than in females (males LTS:TL average ratio 31.43 %; females LTS:TL average ratio 9.0 1 %) (Figs 9A–B, D; 10J–K; 12F–H).

3.2.4. Intraspecific variation

Due to the preservation conditions of the material, the arrangement

of sensory spots throughout the trunk could not be fully confirmed in all the examined specimens from Sylt (North Frisia). The remaining cuticular characters of taxonomic relevance for pycnophyids (i.e. setae, middorsal cuticular specializations, spines, glandular cell outlets, and ornamentation) could be examined in detail both in the Anatolian (four females) and the North Frisian (14 males and 12 females; ZMB 12364–12365 were only studied for this purpose) populations.

Anatolian population: Deviations from the common pattern of paradorsal setae distribution in segment 5 were observed in one female from Yesilirmak River mouth, with a single paradorsal seta present on one side of the middorsal elevation, only one seta of the laterodorsal pair commonly present on segment 5, and an unpaired paradorsal seta on segment 7 (ZMB 12741). Segment 8 in two female specimens off Sakarya river mouth (ZMB 12741, 12744) with the paradorsal seta on the left side of the middorsal elevation. The laterodorsal pair of setae on segment 9 could not be confirmed in two females (ZMB 12743, 12744).

North Frisian population: Deviations from the described bilateral pattern were observed in several specimens, both for seta and sensory spot distribution. Segment 2 with double laterodorsal setae on one side in two females (ZMB 12454, Fig. 14D; ZMB 12475, Fig. 14B) and absent on one side in one male (ZMB 12463); lateroventral seta absent on one side in one female (ZMB 12467) and one female (ZMB 12474); ventrolateral setae absent in one female (ZMB 12454); both ventromedial tube and seta present on one sternal plate in one male (ZMB 12457). Segment 3 without the laterodorsal seta on one side in five males (ZMB 12457, 12461, 12463, 12468, 12472) and six females (ZMB 12454, 12460, 12467, 12471, 12476, 12478); with minute laterodorsal seta on one side in four males (ZMB 12458, 12462, 12470, 12477) and four females (ZMB 12456, 12464, 12465, 12469); with triple laterodorsal seta in one female (ZMB 12475, Fig. 14B); ventromedial seta on one sternal plate absent in one female (ZMB 12460); with minute ventromedial setae on both sternal plates in one male (ZMB 12461); with minute ventromedial seta on one sternal plate in four males (ZMB 12462, 12463, 12468, 12470); typical male tube of the segment 2 present on one sternal plate in one male (ZMB 12458; Fig. 14A); laterodorsal sensory spot absent on one side in three males (ZMB 12455, 12457, 12472) and one female (ZMB 12475). Segment 4 without laterodorsal seta on one side in one male (ZMB 12477); lateroventral seta absent on one side in one female (ZMB 12476); laterodorsal sensory spot of one side located more dorsally than the laterodorsal seta in one male (ZMB 12457). Segment 5 without the paradorsal seta on one side of the middorsal elevation in two females (ZMB 12474, 12476); laterodorsal seta absent on one side in one male (ZMB 12457); with two pairs of lateroventral setae on one side in one female (ZMB 12475); with ventrolateral seta on one side instead of the common lateroventral seta in one female (ZMB 12456); with two pairs of lateroventral setae in one female (ZMB 12464); with double ventromedial setae on one sternal plate in one male (ZMB 12470; Fig. 14C); with a single paradorsal sensory spot on the middorsal elevation in one male (ZMB 12477) and one female (ZMB 12474); without one subdorsal sensory spot in one female (ZMB 12467); without one laterodorsal sensory spot in seven males (ZMB 12455, 12458, 12461, 12468, 12470, 12472, 12477) and eight females (ZMB 12456, 12465, 12467, 12469, 12471, 12474, 12476, 12478). Segment 6 without laterodorsal seta on one side in one male (ZMB 12457); lateroventral seta on one side absent in one male (ZMB 12457) and one female (ZMB 1469); with three pairs of lateroventral setae on one side in one female (ZMB 12475); ventrolateral seta on one sternal plate in one male (ZMB 12457); subdorsal sensory spot on one side absent in one male (ZMB 12457). Segment 7 with only an unpaired paradorsal seta on one side of the middorsal elevation in two males (ZMB 12365, ZMB 12367) and one female (ZMB 12464); with a single paradorsal sensory spot on one side in one female (ZMB 12464); without the paradorsal pair of sensory spots in one female (ZMB 12474); without laterodorsal sensory spot on one side in two males (ZMB 12462, 12472) and one female (ZMB 12478). Segment 8 with the paradorsal seta on the left side of the middorsal elevation in four males (ZMB 12367, 12463, 12470, 12472) and five females (ZMB 12465, 12469, 12474–12476);

lateroventral seta absent on one side in one male (ZMB 12458) and two females (ZMB 12454, 12471); paradorsal sensory spots absent in one female (ZMB 12476); laterodorsal sensory spot on one side absent in one male (ZMB 12462) and two females (ZMB 12454, 12475). Segment 9 with a single paradorsal seta present on one side of the middorsal elevation in one female (ZMB 12464); with minute laterodorsal seta on one side in two males (ZMB 12461, 12472) and one female (ZMB 12476); laterodorsal pair absent in one male (ZMB 12462); laterodorsal seta absent on one side in two females (ZMB 12464, 12465); paradorsal sensory spots absent in one female (ZMB 12476); without laterodorsal sensory spot on one side in one female (ZMB 12475); without the ventrolateral sensory spot on one sternal plate but with a ventromedial sensory spot in one male (ZMB 12470). Segment 10 with a ventrolateral seta on one sternal plate and without the lateroventral one in one female (ZMB 12475).

3.3. Statistics

The comparison in the total trunk length (TL) between *Setaphyes* species revealed statistically significant differences between species, except between *S. elenae* and *S. dentatus* (and only marginally between *S. elenae* and *S. algarvensis*) (see Table 5; Fig. 15A). Despite the statistical significance, measurements overlap between most of the species, except for *S. cimarensis* (Fig. 15A). Analyses discriminating between sexes of each species only found differences in TL between males and females of *S. dentatus* and *S. kielensis* (and marginal significance for *S. elenae*) (Table 6; Fig. 15C). However, each species shows a clear overlap in the TL of males and females (Fig. 15C).

Statistically significant differences were also found in the ratio between the total trunk length and the length of lateral terminal spines (LTS/TL) (see Table 5; Fig. 15B). However, these differences can be affected by an unbalanced number of representatives of each sex. Indeed, the statistic comparison of the proportion LTS/TL between males and females of the same species revealed highly significant sexual dimorphic differences for all the analysed species of *Setaphyes* (Table 6; Fig. 15D). Therefore, the analyses comparing the LTS/TL ratio between species were carried out on separated sexes, which revealed significant differences between males (except between *S. dentatus* and *S. algarvensis* and *S. flaveolatus*) and females (except between *S. dentatus* and *S. flaveolatus*; and *S. algarvensis* with all its congeners, except for *S. elenae*) of several but not all the species (Table 5; Fig. 15D).

4. Discussion

4.1. Comparison with previous descriptions of *Setaphyes dentatus*

The species was originally described as *Echinoderes dentatus* Reinhard, 1881 (see Reinhard, 1881), subsequently assigned to *Pycnophyes* by Sheremetevskij (1974), and recently reassigned to *Setaphyes* by Sánchez et al. (2016). No type material is known to exist. We decided not to designate a neotype, because our samples were not close enough to the type locality.

Our observations agree with those of Reinhard (1881, 1885, 1887), Sheremetevskij (1974) and Zelinka (1928) about specimens possessing middorsal (spinose) processes on segments 1–10, ventromedial tubes on segment 2 of males only, apodemes in segments 9 and 10, a tendency towards shorter lateral terminal spines in females than in males, an anterior reticulate area dorsally on segment 1 (only reported by Sheremetevskij 1974 and Zelinka 1928), and longitudinal cuticular ridges laterodorsally to ventrolaterally on segment 10 (only reported by Zelinka 1928); specimens of Reinhard are slightly smaller (510 µm) than reported by us (518–780 µm). Our observations differ from those of Reinhard (1885, 1887) and of Sheremetevskij (1974) regarding the number of ventral placids, viz, four versus two (our specimens). Other characters like the four dorsal placids, four penile spines, ventromedial tubes on segment 2 of males only, and the principal occurrence of lateral setae are too general to identify the species. More characters were not

mentioned by Reinhard (1881, 1885, 1887) and Sheremetevskij (1974).

Our findings also fit with those of Neuhaus (1993) inclusive the general distribution pattern of sensory spots and setae, apodemes in segments 9 and 10, a tendency towards shorter lateral terminal spines in females (68–108 µm versus 74–140 µm in our study) than in males (79–130 µm versus 103–166 µm), and a size (481–753 µm) close to the range reported by us (518–780 µm). However, our observations disagree with Neuhaus (1993) in that ventrolateral structures are now interpreted as lateroventral. We could also not confirm the presence of ventromedial setae on segment 6 in females (but found such a seta only in one specimen on one side), laterodorsal setae on even segments plus segment 9, and setae on the medial tergal plate of segment 10. The 11 specimens from Sylt studied here were part of the 37 specimens studied previously by Neuhaus, who stated that the specimens of this species “often show reduced setae or their anlagen with extremely shortened peduncle and diminished diameter”, and this referred especially to the laterodorsal setae on segments 2, 4, 6 and 9 (see *Intraspecific variation* of the species above, and Neuhaus, 1993, p. 168, fig. 8G, O). It should also be stressed that the drawings illustrated the maximum number of characters found (Neuhaus, 1993, p. 175, fig. 8G, O), which agrees with the high intraspecific variability observed by the authors herein.

4.2. Diagnosis of *Setaphyes dentatus*

At first sight, the congeners that most closely resemble *S. dentatus* are *S. elenae*, *S. cimarensis* and *S. flaveolatus* since the three species have conspicuous, reticulate ridges on the dorsal anterior margin of segment 1, which is absent in the remaining congeners (Zelinka, 1928; Higgins, 1983; Lemburg, 2002; Sánchez et al., 2018; Cepeda et al., 2020; González-Casarrubios et al., 2022). However, the ornamentation extends through the entire anterior margin of the segment only in *S. dentatus*, *S. elenae* and *S. cimarensis*; (Zelinka, 1928; Neuhaus, 1993, fig. 12A; Sánchez et al., 2018; Cepeda et al., 2020). The ornamentation on segment 1 in *S. cimarensis* consists of rounded depressions, while in *S. dentatus* and *S. elenae* it resembles a net-like structure. Regarding the distribution of setae, only three out of the eight *Setaphyes* species have laterodorsal setae only on segments 3, 5, and 7, and lack ventromedial setae at least on one of segments 3–9, namely *S. dentatus*, *S. elenae* and *S. flaveolatus*. However, neither *S. elenae* nor *S. flaveolatus* share the

Table 5

Output of the one-way classic ANOVA, Welch's ANOVA or Kruskal–Wallis test and pairwise comparison test to determine differences in total trunk length and ratio between total trunk length and length of the lateral terminal spines between the European *Setaphyes* species. Analyses were also conducted discriminating between sexes of each species. Probability level: **p < 0.01; *p < 0.05; italics, marginally significant; absence of asterisk, not significant.

TL: p-value < 2.2e-16					
	<i>S. algarvensis</i>	<i>S. cimarensis</i>	<i>S. dentatus</i>	<i>S. elenae</i>	<i>S. flaveolatus</i>
<i>S. cimarensis</i>	<2e-16**				
<i>S. dentatus</i>	0.048*	<2e-16**			
<i>S. elenae</i>	0.076	<2e-16**	0.488		
<i>S. flaveolatus</i>	<8.7e-05**	<2e-16**	5.3e-14**	1.5e-06**	
<i>S. kielensis</i>	<2e-16**	<2e-16**	<2e-16**	<2e-16**	7.8e-11**
LTS/TL: p-value=1.506e-05					
<i>S. cimarensis</i>	0.00231*				
<i>S. dentatus</i>	0.23527	0.00036**			
<i>S. elenae</i>	0.00063**	0.00011**	6.4e-05**		
<i>S. flaveolatus</i>	0.37389	0.20831	0.53695	0.00017**	
<i>S. kielensis</i>	0.78388	0.77553	0.53695	0.59184	0.70554
♂LTS/TL: p-value=2.675e-11					
<i>S. cimarensis</i>	0.00063**				
<i>S. dentatus</i>	0.56159	2.4e-05**			
<i>S. elenae</i>	0.01121*	0.00602**	0.00571**		
<i>S. flaveolatus</i>	0.01884*	0.00319**	0.19302	0.01812*	
<i>S. kielensis</i>	0.00055**	8.0e-05**	5.1e-07**	0.02891*	0.00319**
♀LTS/TL: p-value=2.839e-13					
<i>S. cimarensis</i>	1.00000				
<i>S. dentatus</i>	0.40473	3.8e-05**			
<i>S. elenae</i>	0.04459*	0.00295**	0.00068**		
<i>S. flaveolatus</i>	0.83294	0.00161**	0.31128	0.00272**	
<i>S. kielensis</i>	0.17732	0.00069**	2.5e-09**	0.00161**	3.8e-05**

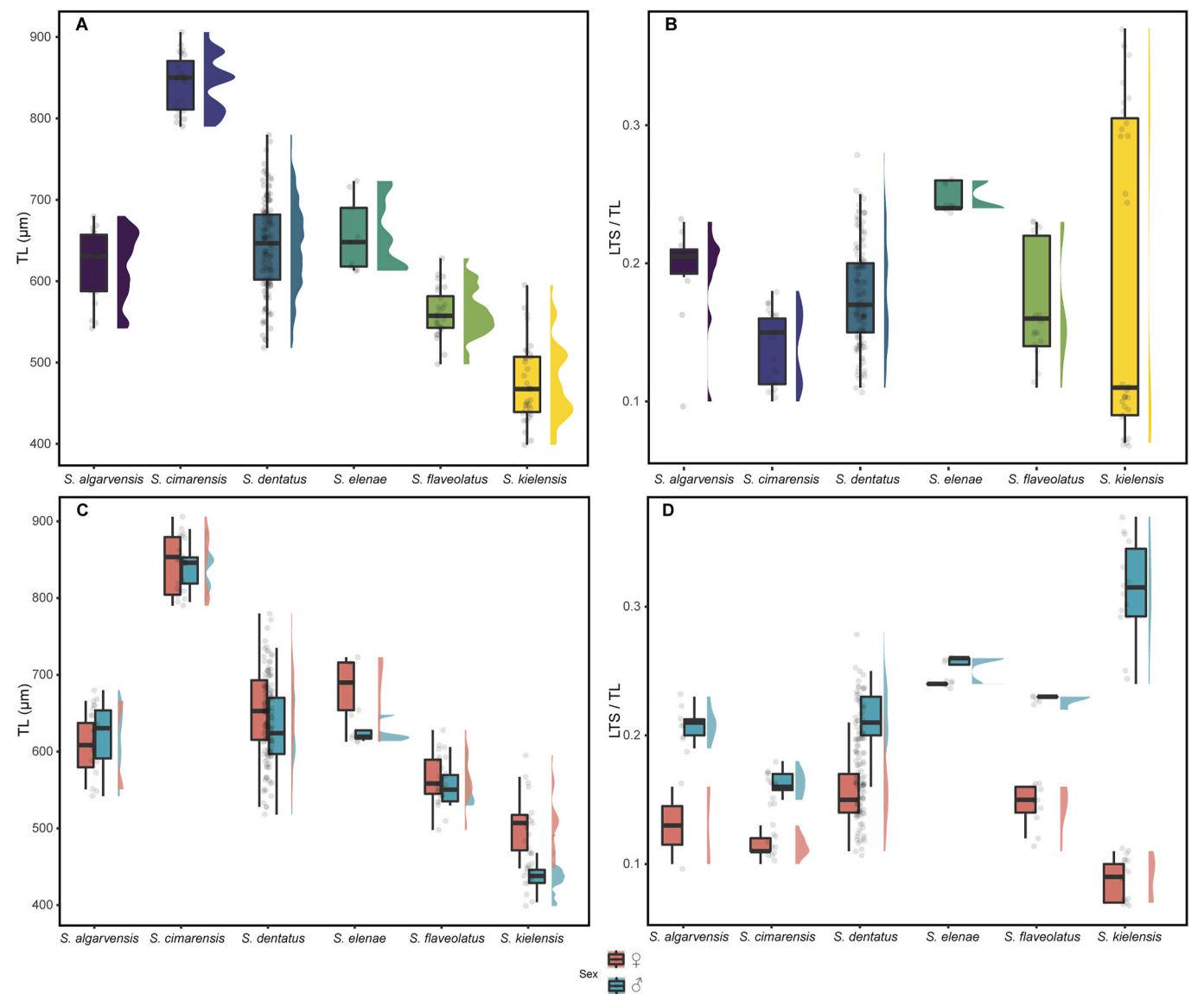


Fig. 15. Variation in selected measurements of *Setaphyes algarvensis* (18♂♂, 2♀♀), *S. cimarensis* (13♂♂, 10♀♀), *S. dentatus* (53♂♂, 83♀♀), *S. elenae* (4♂♂, 5♀♀), *S. flaveolatus* (10♂♂, 18♀♀) and *S. kielensis* (15♂♂, 19♀♀). Jittered points mark the values, boxplots mark the median and quantiles and density plots summarize the data distribution. A: Total trunk length; B: Ratio between lateral terminal spines length and total trunk length; C: Total trunk length separated by sex; D: Ratio between lateral terminal spines length and total trunk length separated by sex. Abbreviations: LTS, lateral terminal spines; TL, total trunk length.

Table 6
Output of the one-way classic ANOVA, Welch's ANOVA or Kruskal–Wallis test to assess sexual differences in total trunk length and ratio between total trunk length and length of the lateral terminal spines in the European *Setaphyes* species. Probability level: **p < 0.01; *p < 0.05; italics, marginally significant; absence of asterisk, not significant.

	♂/♀	
	TL	LTS/TL
<i>S. dentatus</i>	0.0257*	6.721e-16**
<i>S. algarvensis</i>	0.715	0.03389*
<i>S. elenae</i>	0.0612	0.02535*
<i>S. cimarensis</i>	0.777	6.046e-05**
<i>S. flaveolatus</i>	0.565	0.00129**
<i>S. kielensis</i>	1.539e-06**	1.539e-06**

presence of patches of thick, longitudinal, parallel ridges on segment 10 with *S. dentatus* (Zelinka, 1928; Cepeda et al., 2020). These cuticular features enable easy discrimination of *S. dentatus* from its congeners.

4.3. Comparison with previous descriptions of *Setaphyes kielensis*

The species was originally described as *Pycnophyes kielensis* Zelinka, 1928 and lately reassigned to *Setaphyes* by Sánchez et al. (2016). No type material is known to exist. We chose not to designate a neotype, because our samples were not close enough to the type locality.

Our findings agree with Zelinka (1928) that specimens possess no middorsal (spinose) processes on segments 1–10, a scalloped anterior margin on segment 1, apodemes in segment 10, and a size (600 μm) close to the range reported by us (399–595 μm). Our results disagree with Zelinka about ventromedial tubes on segment 2 missing in the single male of Zelinka but present in our specimens, about the size of the lateral terminal spines being much longer in Zelinka's male specimen (211–233 μm) than in our males (104–162 μm) and about the existence of four ventral placids *versus* only two placids in our material. The occurrence of four dorsal placids and of four penile spines represent general characters of Pycnophyidae. More characters were not mentioned by Zelinka (1928).

Our observations generally also agree with those of Neuhaus (1993),

except for laterodorsal setae that are on segment 3 (reported as lacking by Neuhaus (1993)). It should also be noted that the drawings illustrated the maximum number of characters found (Neuhaus, 1993, p. 175, fig. 1G, O), which agrees with the high intraspecific variability observed by the authors in the present contribution.

4.4. Diagnosis of *S. kielensis*

S. kielensis is easily differentiated from its congeners by its unique setae pattern. The only species that can be potentially problematic is *S. algarvensis*, as both species share the pattern of paradorsal, lateroventral and ventromedial setae (González-Casarrubios et al. 2022). However, *S. algarvensis* possesses pairs of setae in paralateral and ventromedial positions on segment 1, which are lacking in *S. kielensis*, and two pairs of lateroventral setae on segment 5, one of which is absent in *S. kielensis*. These differences, in addition to slight differences in the laterodorsal setae pattern, make *S. kielensis* a species easily distinguishable from *S. algarvensis* and the remaining species of the genus.

4.5. Morphometric remarks

Morphometric analyses on the total trunk length (TL), might be helpful in discriminating between the European congeners of *Setaphyes* (Sánchez et al., 2018; Cepeda et al., 2020; González-Casarrubios et al., 2022). However, other morphological measurements, including the standard sternal width do not turn out as relevant characters for this purpose (Cepeda et al., 2020). Our results showed significant differences between the TL of most of the species (see Table 5 and section 3.4.), but the individual values overlap between the species (Fig. 15A). Therefore, the TL are informative regarding the average size of the species, which is indeed different between some of the congeners, but is not as useful when comparing individual values. Similarly, TL is not a dimorphic sexual character for the entire genus, since male and female values overlap between species (same sex, different species) and sexes (same species, different sex).

On the other hand, our morphometric study revealed sex-based differences in the comparison of the LTS/TL proportion for all the European species of *Setaphyes* showing smaller LTS/TL in females than in males. Even though *Setaphyes australensis* and *Setaphyes iniorhaptus* were not included in the analysis, the available information in LTS/TL by sexes in the original descriptions also seems to follow this pattern. Males of *S. australensis* have values of LTS/TL between 13.5 % and 19.5 % (mean of 17 %), while females reach values between 14.3 % and 16.1 % (mean of 15.4 %) (Lemburg, 2002). Similarly, males of *S. iniorhaptus* show ranges between 24.4 % and 29.3 % (mean of 27.1 %), whereas considerably smaller in females, between 16.0 % and 17.7 % (mean of 16.9 %) (Higgins, 1983). Therefore, the LTS/TL ratio seems to turn out as a sexual dimorphic characteristic most likely suitable for the whole *Setaphyes*.

4.6. Remarks about reproductive characters

Information about reproductive features of male Pycnophyidae is scarce, except that penile spines and penile bristles, but no tubes connected with the bristles were reported previously (e. g., Zelinka, 1928; Higgins and Kristensen, 1988; Sánchez et al., 2013; Cepeda et al., 2019). However, an SEM image of *Cristaphyes cristatus* Sánchez et al. (2013) does show a tube (Sánchez et al., 2013, fig 10H) similar to our findings for *S. kielensis* (Fig. 10H–I). For male *Paracentrophyes praedictus* Higgins, 1983, collapsed tubes without terminal bristles were documented and suggested to possibly “represent the cuticle-lined part of the gonoducts, which is everted in order to aid in the transfer of sperm to the female” (Neuhaus, 2013, p. 260, fig. 5.1.35A, B). Surprisingly, the tubes appeared laterodorsally between segments 10 and 11 in *P. praedictus*, whereas the tubes of *C. cristatus* and *S. kielensis* occurred ventrolaterally close to the border of segments 10 and 11. The finding of collapsable

tubes in *P. praedictus*, *C. cristatus*, and *S. kielensis* may support the hypothesis suggested above, but the different position either dorsally or ventrally raises some doubt whether or not such tubes may serve the same function and may have evolved only once.

Additional files

One additional set of files show images taken by SEM of *S. kielensis* and is available under a CC BY NC SA licence via the data publisher Museum für Naturkunde Berlin (MfN) - Leibniz Institute for Evolution and Biodiversity Science, <https://doi.org/10.7479/dt3j-v667>.

Each file name of a SEM image indicates a continuous image number, the species name, the catalogue number, the segments photographed from which side (d, dorsal; v, ventral; le, left; ri, right side or combinations thereof), and the magnification. SEM images were taken with 2048 × 1536 pixels at a resolution of 72 dpi and stored with a depth of grey levels of both 8 bit (with image metadata) and 16 bit (for technical reasons of the SEM without image metadata) as uncompressed TIF files, altogether 714 files with 3.4 GB of data. Metadata of the 8 bit files include scale in micrometers, magnification as multiples of 1,000x (Mag K), voltage of the beam (EHT), working distance (WD), photo number (Photo No.), and date. Usually, noise reduction at the SEM was done in the mode “pixel averaging”, but various amounts of “line integration” (LineInteg) were applied, if charging of a specimen occurred. Last not least, best results were obtained in those cases with pixel averaging after sputtering the specimens again.

Additional set of files “MfN_ZMB_CollectionVermes_SEM”. Overview and detailed SEM images of several segments from dorsal, lateral, and ventral side if available, non-types, ZMB “Vermes” 12454–12478 of *S. kielensis* (Kinorhyncha) from Sylt, Germany.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Data availability

A link to the SEM photos is provided in the Additional files section. Remaining data are in the maintext and in Supplementary Tables.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jcz.2022.12.004>.

References

- Adrianov, A.V., Malakhov, V.V., 1999. *Cephalorhyncha of the World Ocean*. KMK Scientific Press, Moscow, p. 328.
- Cepeda, D., Sánchez, N., Pardos, F., 2019. First extensive account of the phylum Kinorhyncha from Haiti and the Dominican Republic (Caribbean Sea), with the description of four new species. *Mar. Biodivers.* 49 (5), 2281–2309. <https://doi.org/10.1007/s12526-019-00963-x>.
- Cepeda, D., González-Casarrubios, A., Sánchez, N., Pardos, F., 2020. *Setaphyes elenae* sp. nov., a new species of mud dragon (Kinorhyncha: Allomalorhagida) from Skagerrak (north-eastern Atlantic Ocean). *Eur. J. Taxon.* 637, 1–15. <https://doi.org/10.5852/ejt.2020.637>.
- Dal Zotto, M., 2015. *Antygonomas caeciliae*, a new kinorhynch from the Mediterranean Sea, with report of mitochondrial genetic data for the phylum. *Mar. Biol. Res.* 11, 689–702. <https://doi.org/10.1080/17451000.2015.1007872>.
- Dal Zotto, M., Neuhaus, B., Yamasaki, H., Todaro, M.A., 2019. The genus *Condyloeres* (Kinorhyncha: Cyclorhagida) in the Mediterranean Sea, including the description of two new species with novel characters. *Zool. Anz.* 282, 206–231. <https://doi.org/10.1016/j.jcz.2019.05.006>.
- Dal Zotto, M., Santulli, A., Simonini, R., Todaro, M.A., 2016. Organic enrichment effects on a marine meiofauna community, with focus on Kinorhyncha. *Zool. Anz.* 265, 127–140. <https://doi.org/10.1016/j.jcz.2016.03.013>.
- Dal Zotto, M., Todaro, M.A., 2016. Kinorhyncha from Italy, a revision of the current checklist and an account of the recent investigations. *Zool. Anz.* 265, 90–107. <https://doi.org/10.1016/j.jcz.2016.01.004>.
- Dujardin, F., 1851. Observations zoologique. I. Sur un petit animal marin, l'Echinodère, formant un type intermédiaire entre les crustacés et les vers. *Ann. Sci. Nat. Sér. 3* (15), 158–160.
- Fleeger, J.W., Thistle, D., Thiel, H., 1988. Sampling equipment. In: Higgins, R.P., Thiel, H. (Eds.), *Introduction to the Study of Meiofauna*. Smithsonian Institution Press, Washington D.C., pp. 115–125.
- Fox, J., Weisberg, S., 2019. *An R Companion to Applied Regression*, third ed. Sage, Thousand Oaks CA.
- García-Herrero, A., Martínez, A., García-Gómez, G., Sánchez, N., Bird, G.J., Fontaneto, D., Pardos, F., 2021. A dataset of Tanaidacea from the Iberian Peninsula and surrounding areas. *Biogeographia* 36, a008. <https://doi.org/10.21426/B636050361>.
- González-Casarrubios, A., Cepeda, D., Pardos, F., Neves, R.C., Sánchez, N., 2022. *Setaphyes algarvensis* sp. nov., the first description of an allomalorhagid mud dragon (Kinorhyncha: Allomalorhagida) from Portugal (Eastern Atlantic Ocean). *Eur. J. Taxon.* 821, 150–165. <https://doi.org/10.5852/ejt.2022.821.1803>.
- Herranz, M., Stiller, J., Worsaae, K., Sørensen, M.V., 2022. Phylogenomic analyses of mud dragons (Kinorhyncha). *Mol. Phylogenet. Evol.* 168, 107375 <https://doi.org/10.1016/j.ympev.2021.107375>.
- Herranz, M., Thormar, J., Benito, J., Sánchez, N., Pardos, F., 2012. *Meristoderes* gen. nov., a new kinorhynch genus, with the description of two new species and their implications for echinoderid phylogeny (Kinorhyncha: Cyclorhagida, Echinoderidae). *Zool. Anz.* 251 (3), 161–179. <https://doi.org/10.1016/j.jcz.2011.08.004>.
- Higgins, R.P., 1983. The atlantic barrier reef ecosystem at Carrie Bow Cay, Belize, II. Kinorhyncha. *Smithsonian Contributions to the Marine Sciences* 18, 1–131. <https://doi.org/10.5479/si.01960768.18>.
- Higgins, R.P., 1988. Kinorhyncha. In: Higgins, R.P., Thiel, H. (Eds.), *Introduction to the Study of Meiofauna*. Smithsonian Institution Press, Washington D.C., pp. 328–331.
- Higgins, R.P., Kristensen, R.M., 1988. Kinorhyncha from Disko Island, west Greenland. *Smithsonian Contrib. Zool.* 458, 1–56.
- Lemburg, C., 2002. A new kinorhynch *Pycnophyes australensis* sp. n. (Kinorhyncha: Homalorhagida: Pycnophyidae) from magnetic island, Australia. *Zool. Anz.* 241 (2), 173–189.
- Neuhaus, B., 1993. Postembryonic development of *Pycnophyes kielsensis* and *P. dentatus* (Kinorhyncha) from the North Sea. *Microfauna Mar.* 8, 163–193.
- Neuhaus, B., 2013. Kinorhyncha (= Echinodera). In: Schmidt-Rhaesa, A. (Ed.), *Handbook of Zoology, Gastrotricha, Cycloneuralia and Gnathifera*, Volume 1: Nematomorpha, Priapulida, Kinorhyncha, Loricifera. Walter de Gruyter, Berlin, pp. 181–348. <https://doi.org/10.1515/9783110272536.181>.
- Neuhaus, B., 2022. How repeatable are scientific studies of Kinorhyncha? An analysis of specimen-based location and deposition data in WoRMS from 1863–2020. *Zool. Anz.* 301, 163–173. <https://doi.org/10.1016/j.jcz.2022.08.006>.
- Pardos, F., Higgins, R.P., Benito, J., 1998. Two new *Echinoderes* (Kinorhyncha, Cyclorhagida) from Spain, including a reevaluation of kinorhynch taxonomic characters. *Zool. Anz.* 237, 195–208.
- Reinhard, W., 1881. Über *Echinoderes* und *Desmoscolex* der Umgebung von Odessa. *Zool. Anz.* 4 (97), 588–592.
- Reinhard, W., 1885. Kinorhyncha (*Echinoderes*), leur structure anatomique et leur place dans le système, vol. 19. *Travaux de la Société des Naturalistes à l'Université Impériale de Kharkow*, pp. 205–305.
- Reinhard, W., 1887. Kinorhyncha (*Echinoderes*), ihr anatomischer Bau und ihre Stellung im System. *Zeitschrift für wissenschaftliche Zoologie* 45, 401–467.
- Sánchez, N., García-Herrero, Á., García-Gómez, G., Pardos, F., 2018. A new species of the recently established genus *Setaphyes* (Kinorhyncha, Allomalorhagida) from the Mediterranean with an identification key. *Mar. Biodivers.* 48, 249–258. <https://doi.org/10.1007/s12526-017-0651-1>.
- Sánchez, N., Herranz, M., Benito, J., Pardos, F., 2012. Kinorhyncha from the Iberian Peninsula: new data from the first intensive sampling campaigns. *Zootaxa* 3402, 24–44. <https://doi.org/10.11646/ZOOTAXA.3402.1.2>.
- Sánchez, N., Herranz, M., Benito, J., Pardos, F., 2014. *Pycnophyes almansae* sp. nov. and *Pycnophyes lageria* sp. nov., two new homalorhagid kinorhynchs (Kinorhyncha: Homalorhagida) from the Iberian Peninsula, with special focus on the introvert features. *Mar. Biol. Res.* 10, 17–36. <https://doi.org/10.1080/17451000.2013.793804>.
- Sánchez, N., Pardos, F., Herranz, M., Benito, J., 2011. *Pycnophyes dolichurus* sp. nov. and *P. aulacodes* sp. nov. (Kinorhyncha, Homalorhagida, Pycnophyidae), two new kinorhynchs from Spain with a reevaluation of homalorhagid taxonomic characters. *Helgol. Mar. Res.* 65, 319–334. <https://doi.org/10.1007/s10152-010-0226-z>.
- Sánchez, N., Rho, H.S., Min, W.G., Kim, D., Sørensen, M.V., 2013. Four new species of *Pycnophyes* (Kinorhyncha: Homalorhagida) from Korea and the east China sea. *Sci. Mar.* 77 (2), 353–380. <https://doi.org/10.3989/scimar.03769.15a>.
- Sánchez, N., Yamasaki, H., Pardos, F., Sørensen, M.V., Martínez, A., 2016. Morphology disentangles the systematics of a ubiquitous but elusive meiofaunal group (Kinorhyncha: Pycnophyidae). *Cladistics* 32 (5), 479–505. <https://doi.org/10.1111/clc.12143>.
- Sheremetevskij, A.M., 1974. Kinorhyncha of the Black Sea. *Zool. Zhurnal* 53 (7), 974–987.
- Sönmez, S., Köroğlu, N.Ö., Karaytuğ, S., 2016. First record of family Echinoderidae Zelinka, 1894 (Kinorhyncha: Cyclorhagida) from Turkish marine waters. *Biharean Biol* 10, 8–11.
- Sørensen, M.V., Herranz, M., Pardos, F., Durucan, F., 2021. Kinorhynchs from sandy coastal habitats in Turkey, with the description of a new pan-Mediterranean species of *Echinoderes* (Cyclorhagida: Echinoderidae). *Turk. Zool. Derg.* 45 (7:3) <https://doi.org/10.3906/zoo-2108-20>.
- Sørensen, M.V., Goetz, F.E., Herranz, M., Chang, C.Y., Chatterjee, T., Durucan, F., Neves, R.C., Yıldız, N.O., Norenburg, J., Yamasaki, H., 2020. Description, redescription and revision of sixteen putatively closely related species of *Echinoderes* (Kinorhyncha: Cyclorhagida), with the proposition of a new species group - the *Echinoderes dujardinii* group. *Eur. J. Taxon.* 730 (1), 1–101. <https://doi.org/10.5852/ejt.2020.730.1197>.
- Sørensen, M.V., Dal Zotto, M., Rho, H.S., Herranz, M., Sánchez, N., Pardos, F., Yamasaki, H., 2015. Towards a phylogeny of Kinorhyncha, based on morphology and two molecular loci. *PLoS One* 10, e0133440. <https://doi.org/10.1111/clc.12143>.
- Sørensen, M.V., Pardos, F., 2020. Kinorhyncha. In: Schmidt-Rhaesa, A. (Ed.), *Guide to the Identification of Marine Meiofauna*. Verlag Dr. Friedrich Pfeil, Munich, pp. 391–414.
- Spalding, M.D., Agostini, V.N., Rice, J., Grant, S.M., 2012. Pelagic provinces of the world: a biogeographic classification of the world's surface pelagic waters. *Ocean Coast Manag.* 60, 19–30. <https://doi.org/10.1016/j.ocecoaman.2011.12.016>.
- Spalding, M.D., Fox, H.E., Allen, G.R., Davidson, N., Ferdaña, Z.A., Finlayson, M., et al., 2007. Marine ecoregions of the world: a bioregionalization of coastal and shelf areas. *Bioscience* 57, 573–583. <https://doi.org/10.1641/B570707>.
- Ürkmez, D., Pardos, F., Sezgin, M., Karaçuha, M.E., Öksüz, İ., 2016. *Echinoderes dujardinii* Claparède, 1863 (Kinorhyncha, Cyclorhagida): a new record for the kinorhynch fauna of Turkey. *Ecol. Montenegrina* 6, 1–8.
- Yamasaki, H., Dal Zotto, M., 2019. Investigation of echinoderid kinorhynchs described 90 years ago: redescription of *Echinoderes capitatus* (Zelinka, 1928) and *Echinoderes ferrugineus* Zelinka, 1928. *Zool. Anz.* 282, 189–205. <https://doi.org/10.1016/j.jcz.2019.05.013>.
- Yamasaki, H., Durucan, F., 2018. *Echinoderes antalyensis* sp. nov. (Cyclorhagida: Kinorhyncha) from antalya, Turkey, Levantine Sea, eastern Mediterranean Sea. *Species Divers.* 23, 193–207. <https://doi.org/10.12782/specdiv.23.193>.
- Yamasaki, H., Grzelak, K., Sørensen, M.V., Neuhaus, B., George, K.H., 2018a. *Echinoderes pterus* sp. n. showing a geographically and bathymetrically wide distribution pattern on seamounts and on the deep-sea floor in the Arctic Ocean, Atlantic Ocean, and the Mediterranean Sea (Kinorhyncha, Cyclorhagida). *ZooKeys* 771, 15–40. <https://doi.org/10.3897/zookeys.771.25534>.
- Yamasaki, H., Neuhaus, B., George, K.H., 2018b. New species of *Echinoderes* (Kinorhyncha: Cyclorhagida) from Mediterranean seamounts and from the deep-sea floor in the Northeast Atlantic Ocean, including notes on two undescribed species. *Zootaxa* 4387, 541–566. <https://doi.org/10.11646/zootaxa.4387.3.8>.
- Yıldız, N.Ö., Sørensen, M.V., Karaytuğ, S., 2016. A new species of *Cephalorhyncha* Adrianov, 1999 (Kinorhyncha: Cyclorhagida) from the aegean coast of Turkey. *Helgol. Mar. Res.* 70, 24. <https://doi.org/10.1186/s10152-016-0476-5>.
- Zelinka, C., 1908. Zur Anatomie der Echinoderen. *Zool. Anz.* 33 (19/20), 629–647.
- Zelinka, K., 1928. *Monographie der Echinodera*. Verlag von Wilhelm Engelmann, Leipzig, pp. 1–396.