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Setaphyes algarvensis sp. nov., the first description of an allomalorhagid mud dragon (Kinorhyncha: Allomalorhagida) from Portugal (Eastern Atlantic Ocean)

Alberto GONZÁLEZ-CASARRUBIOS[1,*], Diego CEPEDA[2], Fernando PARDOS[3],
Ricardo C. NEVES[4] & Nuria SÁNCHEZ[5]

[1,2,3,5] Universidad Complutense de Madrid (UCM), Faculty of Biological Sciences, Department of Biodiversity, Ecology and Evolution, C/ José Antonio Novais 12, 28040 Madrid, Spain.
[2] Institut français de Recherche pour l'Exploitation de la Mer (IFREMER), Laboratoire Environnement Profond, ZI de la Pointe du Diable, 29280 Plouzané, France.
[4] Department of Biology, August Krogh Building, University of Copenhagen, Copenhagen Ø, Denmark.

[*] Corresponding author: albert23@ucm.es
[2] Email: diego.cepeda.gomez@ifremer.fr
[3] Email: fpardos@ucm.es
[4] Email: ricardon.6@gmail.com
[5] Email: nurisanc@ucm.es

[1] urn:lsid:zoobank.org:author:E5637211-39C0-4403-8101-1D9BDFC50E41
[2] urn:lsid:zoobank.org:author:734A11DE-8E3F-44BB-A0FF-D7341CEE3A83
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[5] urn:lsid:zoobank.org:author:878029BD-0D80-4CC5-93ED-F6848744A6EC

Abstract. The kinorhynch fauna from Portugal has been explored, yielding a new species of the genus *Setaphyes* (Kinorhyncha: Allomalorhagida). This is the first description of an allomalorhagid species from Portugal. Specimens of the new species were collected at a subtidal muddy beach in Alvor, a village located in the southernmost region of Portugal. *Setaphyes algarvensis* sp. nov. may be distinguished from its congeners by a unique arrangement of the setae: paired paradorsal setae on segments 2–7 and 9, paradorsal seta on segment 8 unpaired, laterodorsal setae on segments 2–3 and 6–9 in males and 2–9 in females, paralateral setae on segment 1, lateroventral setae on segments 2–10 (two pairs on segment 5), ventrolateral setae on segment 1 in males and 1–3 in females, and ventromedial setae on segments 3–9 in males and 4–9 in females. The diagnostic features of *Setaphyes algarvensis* sp. nov. are discussed from a comparative perspective with the congener species. Additionally, morphometric analyses of selected features, namely the total trunk length and the relation between the total trunk length and the length of lateral terminal spines, turned out to be useful to distinguish between the new species and *Setaphyes kielensis* (its most similar congener).

Keywords. Kinorhynch, Iberian Peninsula, taxonomy, diversity, Europe.

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Introduction

The kinorhynch fauna of the Iberian Peninsula has been extensively sampled since the late 1990s. The first ever report of a mud dragon species in this area occurred in 1998, when two new species of the genus *Echinoderes* Claparède, 1863 were described from the Cantabrian coast (Pardos *et al*. 1998): *Echinoderes cantabricus* Pardos *et al*., 1998 and *E. hispanicus* Pardos *et al*., 1998. Ten years later, three new species of the same genus were also discovered from this northern coastline (G^{a}Ordóñez *et al.* 2008): *E. isabelae* G^{a}Ordóñez *et al.*, 2008; *E. neospinosus* G^{a}Ordóñez *et al.*, 2008 and *E. parrai* G^{a}Ordóñez *et al.*, 2008. The first Iberian allomalorhagid kinorhynch, *Paracentrophyes quadridentatus* (Zelinka, 1928), was also found a few years later in the Cantabrian Sea (Sørensen *et al.* 2010). The first descriptions of new allomalorhagid species were made by Sánchez *et al*. (2011), namely *Pycnophyes aulacodes* Sánchez *et al.*, 2011 and *Higginsium dolichurum* (Sánchez *et al.*, 2011), both species collected from the northwestern coast of Spain. Subsequently, *E. gerardi* Claperède, 1863 and a new cyclorhagid genus and species, *Meristoderes macracanthus* Herranz *et al.*, 2012, were found along the Mediterranean coasts of Spain (Sánchez-Tocino *et al*. 2011; Herranz *et al*. 2012; Sørensen *et al*. 2020). Moreover, the first Iberian dracoderid kinorhynch, *Dracoderes gallaicus* Sørensen *et al.*, 2012, was described from the north- and southwestern coasts of Spain (Sørensen *et al.* 2012).

However, it was not until 2012 that the first comprehensive campaign to assess the diversity of Kinorhyncha Reinhard, 1885 in the Iberian Peninsula was accomplished (Sánchez *et al*. 2012). The inspection of 81 sampling stations yielded approximately 2000 specimens, whose examination by light and scanning electron microscopy eventually led to the identification of 11 genera and 29 kinorhynch species. Interestingly, only 11 of the 29 species (i.e., less than 40%) had previously been recorded from Iberian waters. Since then, five additional species of kinorhynchs have been described, namely *Pycnophyes almansae* Sánchez *et al.*, 2014, *Setaphyes cimarensis* Sánchez *et al.*, 2018, *Leiocanthus lageria* (Sánchez *et al.*, 2014), *Echinoderes lusitanicus* Neves *et al.*, 2016 and *E. reicherti* Neves *et al.*, 2016 (Sánchez *et al.* 2014, 2018; Neves *et al*. 2016). The latter two species actually are the only kinorhynch species described from the Portuguese coast (Neves *et al.* 2016). As compared to the Spanish coastline, the knowledge on the Portuguese kinorhynch fauna is as yet extremely limited.

The aim of the present paper is thus to increase the knowledge on the Portuguese kinorhynch fauna and, hence, to provide more comprehensive data on the biogeography of Kinorhyncha in the Iberian Peninsula. In the present study, we describe a new intertidal species of *Setaphyes* Sánchez *et al.*, 2016 from Portuguese waters, one the most common and abundant genera of Pycnophyidae Zelinka, 1896 in Europe. Five out of the seven species belonging to this genus were described from or reported in European waters; namely *S. cimarensis* Sánchez *et al.*, 2018, *S. dentatus* (Reinhard, 1881), *S. elenae* Cepeda *et al.*, 2020, *S. flaveolatus* (Zelinka, 1908) and *S. kielensis* (Zelinka, 1928) (see Reinhard 1881; Zelinka 1908, 1928; Neuhaus 2013; Dal Zotto & Todaro 2016; Sánchez *et al*. 2018; Cepeda *et al.* 2020). This is the first description of an allomalorhagid kinorhynch for the southwestern region of the Iberian Peninsula.

Material and methods

Sampling and study of specimens

Kinorhynch specimens were collected in December 2012 from a muddy beach at Alvor (37°07.714′ N; 08°36.329′ W), a small fishing village located in the southernmost region of Portugal (Fig. 1). Samples

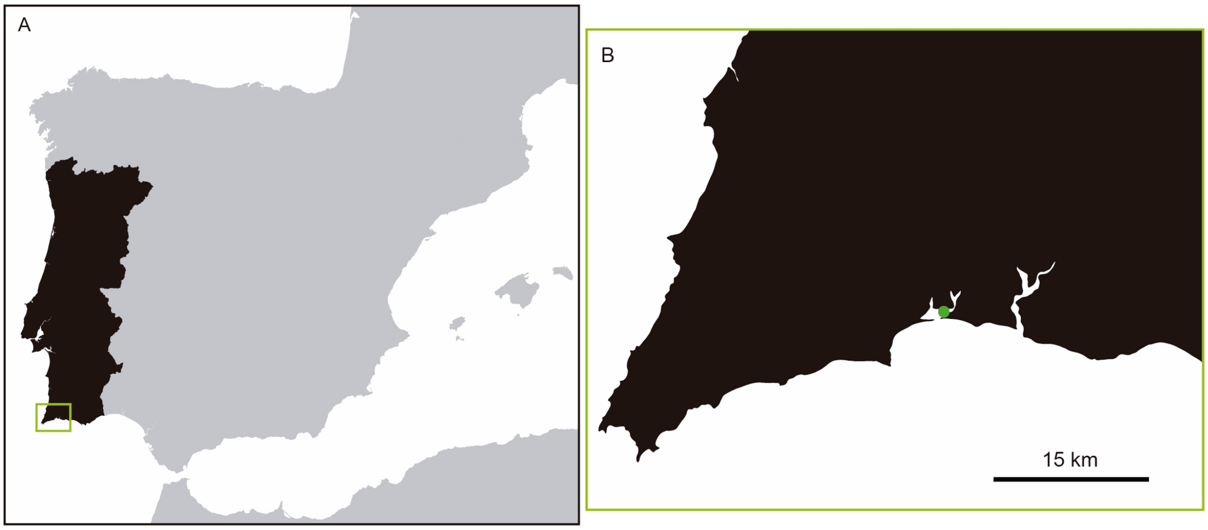

Fig. 1. A. Map showing the sampling area of Alvor (Faro, Portugal). **B**. Close-up of the sampling area. Green point marks the sampling site.

were taken by hand from an intertidal muddy area containing *Zostera* sp. Animals were extracted from the sediment following the bubble-and-blot method (Higgins & Thiel 1988; Sørensen & Pardos 2020), and subsequently preserved in 70% ethanol.

Kinorhynchs were sorted under a stereo microscope (ZEISS Stemi SV 6). For light microscopy (LM), specimens were dehydrated through a graded series of ethanol, transferred to 100% glycerine for 24 h, and then mounted on glass slides with Fluoromount G®. Mounted specimens were studied and photographed with an Olympus© BX51-P microscope with differential interference contrast (DIC) optics equipped with an Olympus© DP-70 camera.

For scanning electron microscopy (SEM), specimens were dehydrated through a graded series of ethanol and then transferred to acetone through a graded ethanol/acetone series, critical point dried, mounted on aluminum stubs and sputter-coated with a gold/palladium mixture for examination with a JEOL® JSM-6335F field emission scanning electron microscope at the ICTS Centro Nacional de Microscopía Electrónica (Universidad Complutense de Madrid, Spain). Line drawings and image plate compositions were made using Illustrator CC-2014 and Adobe® Photoshop software, respectively. The type material of the new species is deposited at the Natural History Museum of Denmark (NHMD).

Morphometric statistics

Differences in selected morphometric measures (i.e., total trunk length and relative proportion between total trunk length and length of lateral terminal spines) of the new species and the most similar species of *Setaphyes* were tested. For this, we selected 30 specimens of *S. kielensis* (15 ♂♂, 15 ♀♀: ZMB-12294 to 12313, ZMB-12359 to 12361, ZMB-12363, ZMB-12367 and ZMB-12394 to 12398) collected at Sylt (Germany), and stored at the Museum für Naturkunde Berlin (Germany), as well as 19 specimens (18 ♂♂ and 1 ♀) of the new species described here.

Saphiro-Wilk and Barlett tests were used to check the normality and the homoscedasticity of the variables, respectively. In addition, visual methods of density and quantile-quantile (Q-Q) plots were applied for the same purpose. To assess differences, a one-way analysis of variance (ANOVA) was used, except when the variable was heteroscedastic, in which case a Welch's ANOVA was applied. These statistical analyses were performed in R ver. 1.1.453 (R Core Team 2021) using the *stats*, *ggplot2* and *car* packages (Fox *et al*. 2019).

Results

Class Allomalorhagida Sørensen, Dal Zotto, Rho, Herranz, Sánchez, Pardos & Yamasaki, 2015
Family Pycnophyidae Zelinka, 1896
Genus *Setaphyes* Sánchez, Yamasaki, Pardos, Sørensen & Martínez, 2016

Setaphyes algarvensis sp. nov.
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Figs 2–4, Tables 1–2

Diagnosis

Setaphyes with middorsal elevations on segments 1–6, superficially covered by tufts of elongated, thick hairs whose tips sometimes surpass the posterior margin of segment, and middorsal processes on segments 7–9. Paired paradorsal setae on segments 2–7 and 9; seta on segment 8 unpaired. Laterodorsal setae on segments 2–3 and 6–9 in males and 2–9 in females. Paralateral setae on segment 1. Lateroventral setae on segments 2–10 (two pairs on segment 5). Ventrolateral setae on segment 1 in males and 1–3 in females. Ventromedial setae on segments 3–9 in males and 4–9 in females. Small and abundant cuticular scars (likely outlets of glandular cells) scattered throughout the trunk. Lateral terminal spines present, short, slender.

Etymology

The species name, *algarvensis*, refers to the Algarve, the southern region of Portugal where the new species was found.

Material examined

Holotype

PORTUGAL • adult ♂, mounted in Fluoromount G® on a glass slide; Alvor; 37°07.714′ N, 08°36.329′ W; 16 Dec. 2012; intertidal mud with *Zostera* sp.; NHMD-921475.

Paratypes

PORTUGAL • 13 adult ♂♂, 1 adult ♀, mounted as the holotype; same collection data as for holotype; NHMD-921477–921489 (♂♂), NHMD-921476 (♀).

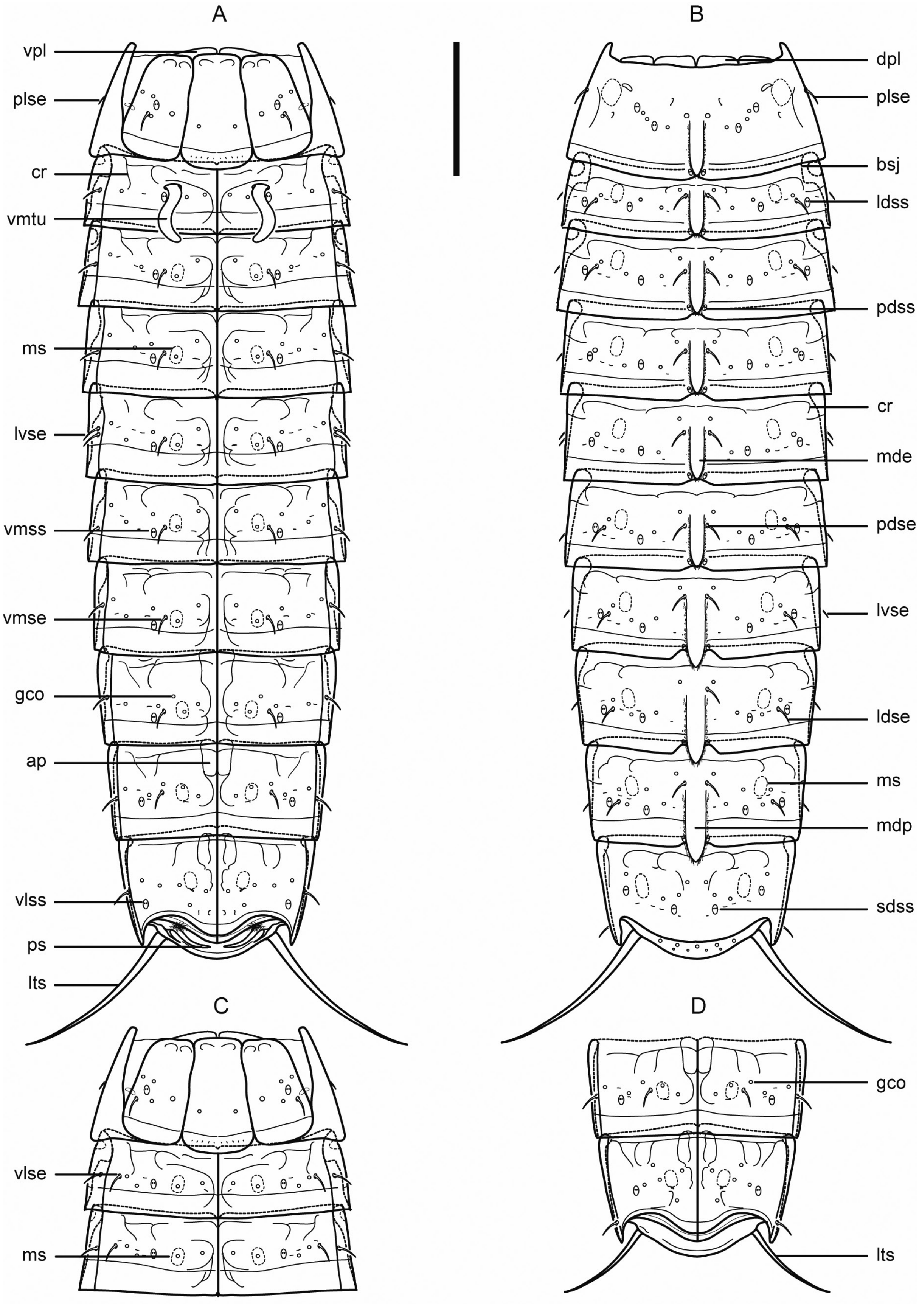
Additional material

PORTUGAL • 8 adult ♂♂, five of them mounted as the holotype and three mounted for SEM; same collection data as for holotype; UCM Meiofauna Collection.

Description

See Table 1 for measurements and dimensions and Table 2 for a summary of the middorsal cuticular specialization, seta, tube, nephridiopore and sensory spot locations.

HEAD. With retractable mouth cone and introvert. Although two of the examined specimens had the head everted, their structures tend to collapse when mounted for LM; hence, only some details on the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C
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ms
D
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Table 1. Measurements of 19 adult specimens of *Setaphyes algarvensis* sp. nov. (18 ♂♂, 1 ♀). Abbreviations: LTS = lateral terminal spine length; MSW = maximum sternal width (measured at segment 7); S = segment length (followed by number of corresponding segment); SD = standard deviation; SSW = standard sternal width (measured at segment 10); TL = total length.

Character	Range ♂	Mean (SD; n) ♂	Value ♀	Total range	Total mean (SD; n)
TL (µm)	541.67–680.40	621.42 (40.49; 18)	665.64	541.67–680.40	623.75 (40.64; 19)
MSW (µm)	148.81–169.25	156.71 (4.45; 17)	163.97	148.81–169.25	157.12 (4.64; 18)
MSW/TL (%)	22.96–28.88	25.27 (1.69; 17)	24.63	22.96–28.88	25.23 (1.64; 18)
SSW (µm)	126.42–142.28	135.18 (4.59; 17)	145.95	126.42–145.95	135.78 (5.12; 18)
SSW/TL (%)	20.19–25.74	21.80 (1.56; 17)	21.93	20.19–25.74	21.81 (1.51; 18)
S1 (µm)	77.38–87.60	81.59 (2.76; 17)	91.45	77.38–91.45	82.13 (3.54; 18)
S2 (µm)	48.03–64.09	56.91 (4.80; 17)	59.57	48.03–64.09	57.06 (4.70; 18)
S3 (µm)	51.36–71.96	61.61 (5.50; 17)	64.68	51.36–71.96	61.78 (5.39; 18)
S4 (µm)	51.15–76.47	67.89 (7.30; 17)	68.70	51.15–76.47	67.93 (7.08; 18)
S5 (µm)	56.86–80.76	73.28 (6.37; 17)	76.18	56.86–80.76	73.45 (6.22; 18)
S6 (µm)	64.60–89.43	77.51 (6.85; 17)	84.08	64.60–89.43	77.88 (6.83; 18)
S7 (µm)	66.94–93.53	81.83 (6.19; 17)	85.16	66.94–93.53	82.02 (6.05; 18)
S8 (µm)	69.72–92.92	84.38 (5.34; 17)	86.23	69.72–92.92	84.49 (5.20; 18)
S9 (µm)	84.40–107.40	94.19 (6.37; 17)	92.38	84.40–107.40	94.09 (6.19; 18)
S10 (µm)	63.76–84.37	73.57 (5.36; 17)	84.13	63.76–84.37	74.16 (5.77; 18)
S11 (µm)	28.00–51.54	36.06 (5.91; 17)	36.52	28.00–51.54	36.09 (5.74; 18)
LTS (µm)	123.47–139.78	132.61 (4.88; 8)	67.96	67.96–139.78	125.42 (22.03; 9)
LTS/TL (%)	19.42–22.24	20.60 (0.82; 8)	10.21	10.21–22.24	19.45 (3.55; 9)

morphology of oral styles and scalids can be provided. Internal part of mouth cone with several rings of inner oral styles; exact number, arrangement and morphology not determined. External part of mouth cone with single ring of nine equally-sized outer oral styles, arranged as one anterior to each introvert sector, except for middorsal sector 6 where style is missing. Each outer oral style composed of single, flexible unit, wider at base, bearing fringed sheath, progressively tapering toward distally pointed tip. Introvert with six transverse rings of scalids and 10 longitudinal sectors defined by arrangement of primary spinoscalids. Primary spinoscalids larger than remaining scalids, each one composed of basal, rectangular, wide sheath and distal, elongated, distally pointed end-piece. Scalids from remaining rings regular-sized, similar in morphology to primary spinoscalids but smaller, also composed of a longer distal end-piece and a shorter basal sheath. Exact number, arrangement and detailed morphology of scalids not determined.

Fig. 2 (preceding page). Line illustrations of adult *Setaphyes algarvensis* sp. nov. **A**. ♂, ventral overview. **B**. ♂, dorsal overview. **C**. ♀, segments 1–3, ventral view. **D**. ♀, segments 9–11, ventral view. Abbreviations: ap = apodeme; bsj = ball-and-socket joint; cr = cuticular ridge; dpl = dorsal placid; gco = glandular cell outlet; ldse = laterodorsal seta; ldss = laterodorsal sensory spot; lts = lateral terminal spine; lvse = lateroventral seta; mde = middorsal elevation; mdp = middorsal process; ms = muscular scar; pdse = paradorsal seta; pdss = paradorsal sensory spot; plse = paralateral seta; ps = penil spine; sdss = subdorsal sensory spot; vlse = ventrolateral seta; vlss = ventrolateral sensory spot; vmse = ventromedial seta; vmss = ventromedial sensory spot; vmtu = ventromedial tube; vpl = ventral placid. Scale bar: 100 µm.

Neck. With four dorsal and two ventral sclerotized placids (Fig. 2A–B). Dorsal placids rectangular, with a slightly convex anterior margin; mesial ones broader (ca 31–33 µm wide at base) than lateral ones (ca 28–30 µm wide at base) (Fig. 2B). Ventral placids (ca 22–23 µm wide at base) morphologically similar to dorsal ones but much more elongated, getting thinner towards lateral sides (Fig. 2A, C).

Trunk habitus. With eleven segments (Figs 2A–D, 3A, 4A, G). Segment 1 with one tergal, two episternal and one trapezoidal, midsternal plate; remaining segments with one tergal and two sternal

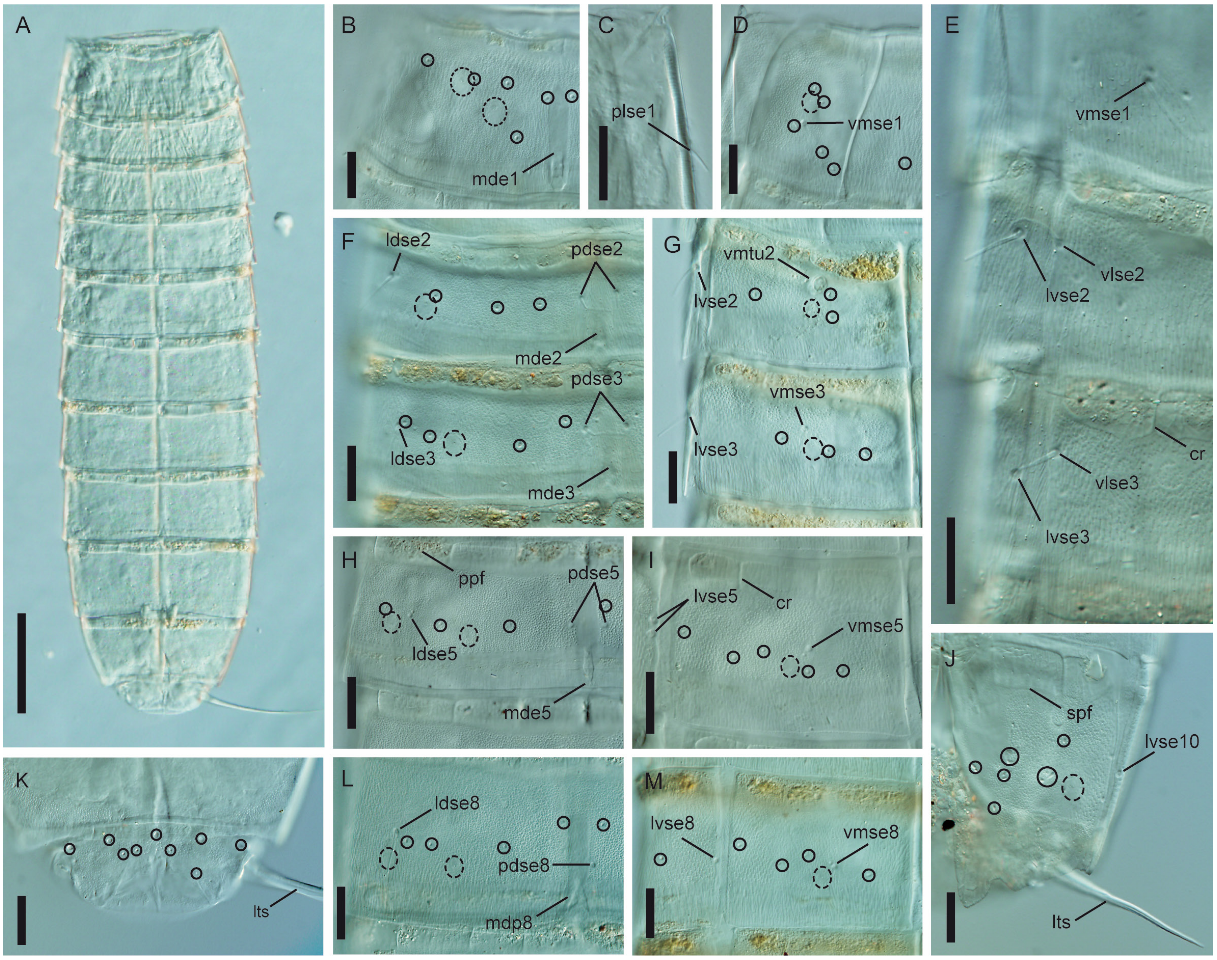

Fig. 3. Light micrographs of holotype, ♂ (NHMD-921475) (A–D, H–I, K–L), paratypes, ♂ (F–G, M) (F: NHMD-921479, G: NHMD-921489, M: NHMD-921484), and paratype, ♀ (E, J) (NHMD-921485), showing trunk overviews and details of cuticular trunk characters of adult *Setaphyes algarvensis* sp. nov. **A**. Dorsal overview. **B**. Dorsal view of left half of segment 1. **C**. Detail of paralateral seta of segment 1. **D**. Ventral view of right half of segment 1. **E**. Ventral view of right half of segments 1–3. **F**. Dorsal view of left half of segments 2–3. **G**. Ventral view of right half of segments 2–3. **H**. Dorsal view of left half of segment 5. **I**. Ventral view of right half of segment 5. **J**. Ventral view of left half of segment 10. **K**. Dorsal view of segment 11. **L**. Dorsal view of left half of segment 8. **M**. Ventral view of right half of segment 8. Abbreviations: cr = cuticular ridge; ldse = laterodorsal seta; lts = lateral terminal spine; lvse = lateroventral seta; mde = middorsal elevation; mdp = middorsal process; pdse = paradorsal seta; plse = paralateral seta; ppf = primary pectinate fringe; spf = secondary pectinate fringe; vlse = ventrolateral seta; vmse = ventromedial seta; vmtu = ventromedial tube; numbers after abbreviations indicate corresponding segment; sensory spots are marked as dashed circles and glandular cell outlets as continuous circles. Scale bars: A = 100 µm; B–M = 20 µm.

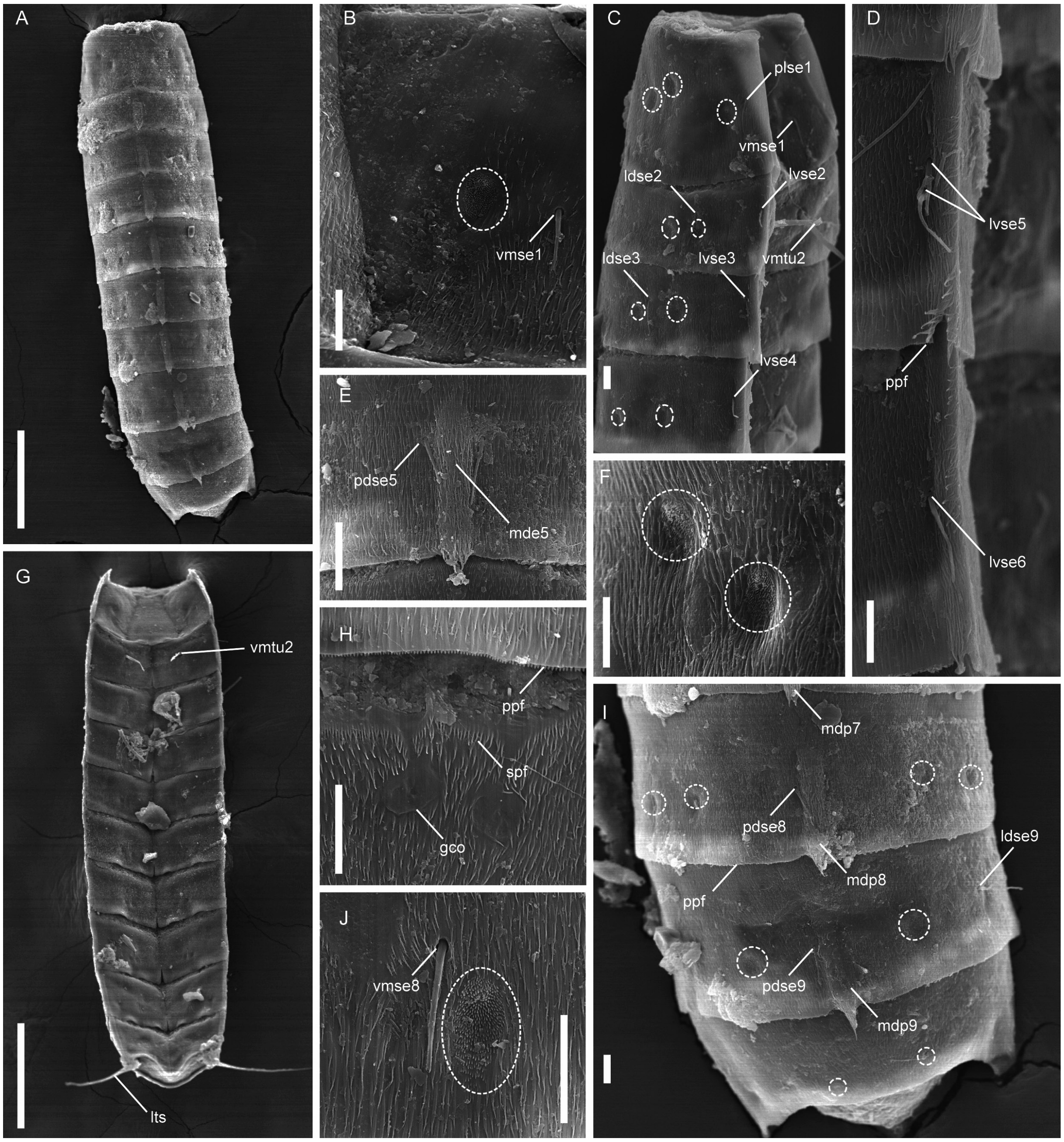

Fig. 4. Scanning electron micrographs showing general overview and details of the cuticular trunk morphology of three adult males, additional specimens of *Setaphyes algarvensis* sp. nov. (UCM Meiofauna Collection) **A**. Dorsal overview. **B**. Ventral view of left half segment 1. **C**. Lateral view of segments 1–4. **D**. Ventral view of segments 5–6. **E**. Middorsal elevation of segment 5. **F**. Detail of dorsal sensory spots on segment 1. **G**. Ventral overview. **H**. Detail of pectinate fringe and glandular cell outlets of segment 8. **I**. Dorsal view of segments 8–11. **J**. Detail of ventral seta and sensory spot on segment 8. Abbreviations: gco = glandular cell outlet; ldse = laterodorsal seta; lts = lateral terminal spine; lvse = lateroventral seta; mde = middorsal elevation; mdp = middorsal process; pdse = paradorsal seta; plse = paralateral seta; ppf = primary pectinate fringe; spf = secondary pectinate fringe; vmse = ventromedial seta; vmtu = ventromedial tube; numbers after abbreviations indicate corresponding segment; sensory spots are marked as dashed circles. Scale bars: A, G = 100 µm; B–F, H–J = 10 µm.

Table 2. Summary of nature and arrangement of cuticular elevations, processes, spines, tubes, setae, sensory spots and nephridiopores in *Setaphyes algarvensis* sp. nov. Abbreviations: ce = cuticular elevation; cp = cuticular process; LD = laterodorsal; lts = lateral terminal spine; LV = lateroventral; MD = middorsal; ne = nephridiopore; PD = paradorsal; PL = paralateral; ps = penile spine; SD = subdorsal; se = seta; ss = sensory spot; tu = tube; VL = ventrolateral; VM = ventromedial; * = unpaired structures; ♂/♀ indicate sexually dimorphic structures.

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

cuticular plates (Figs 2A–D, 3A, 4A, G). Tergal cuticular plates slightly bulging middorsally (Fig. 4A). Sternal plates reach maximum width at segment 7, but almost constant in width across trunk. Sternal cuticular plates relatively narrow in ratio maximum width to total trunk length (MSW:TL average ratio = 25.23%), giving the animal a relatively slender appearance. Middorsal elevations on segments 1–6, rectangular, narrow, distally blunted, not projecting beyond posterior margin of segments (Figs 2B, 3B, F, H, 4A, E). Middorsal elevations covered by tufts of elongated, thick cuticular hairs whose tips may surpass posterior margin of segment (Figs 2B, 4A, E). Middorsal processes on segments 7–9, exceeding posterior margin of segment, also covered by tufts of elongated, thick cuticular hairs (Figs 2B, 3L, 4A, I). Middorsal processes progressively longer towards posterior trunk, reaching maximum length on segment 9 (Figs 2B, 4A, I). Paired, paradorsal, intracuticular butterfly- to trident-like atria associated with middorsal structures (Figs 3B, F, H). Glandular cell outlets as minute, dot-shaped, rounded to oval perforations throughout cuticle on segments 1–11 (Figs 2A–D, 3B, D, F–M, 4H); number and position of these structures vary greatly among examined specimens, not showing any specific pattern. Up to three pairs of conspicuous laterodorsal and ventromedial cuticular ridges on segments 2–10 (Figs 2A–D, 3E, H–J). Cuticular hairs acicular, non-bracteate, distributed across trunk on segments 1–10, not following any particular pattern (Fig. 4F, H, J). Pachycycli and ball-and-socket joints conspicuous on segments 2–9, reduced on segments 10 and 11 (Fig. 2A–B). Apodemes on segments 9–10 (Fig. 2A). Primary pectinate fringes finely serrated (Figs 2A–D, 3L, 4A, C–E, G–I); secondary pectinate fringes as wavy, quite inconspicuous single line (Figs 2A–D, 3J, 4H). Muscular scars as conspicuous, rounded to oval, hairless areas in laterodorsal and ventrolateral positions on segments 1–10 (Fig. 2A–D).

SEGMENT 1. Middorsal elevation not projecting beyond posterior margin of segment (Figs 2B, 3B, 4A). Anterolateral margins of tergal plate as triangular, short, wide, distally rounded extensions (Figs 2A–C, 3C–D, 4G). Paired setae in paralateral and ventromedial positions (Figs 2A–C, 3C–E, 4B–C). Two pairs of sensory spots in subdorsal positions; and one pair in paradorsal and ventromedial positions (Figs 2A–C, 3B, D, 4B–C, F). Sensory spots on this and following segments as oval areas with several rows of cuticular micropapillae surrounding a single pore (Figs 2A–D, 3B, D, F–J, L–M, 4B–C, F, I–J).

Segment 2. Middorsal elevation as on preceding segment (Figs 2B, 3F, 4A). Paired setae in paradorsal, laterodorsal and lateroventral position; females with additional, sexually dimorphic pair in ventrolateral position (Figs 2A–C, 3E–G, 4C). Males with sexually dimorphic tubes in ventromedial position (Figs 2A, 3G, 4C, G). Paired sensory spots in paradorsal, subdorsal, laterodorsal and ventromedial positions (Figs 2A–C, 3F–G, 4C).

Segment 3. Middorsal elevation as on preceding segments (Figs 2B, 3F, 4A). Paired setae in paradorsal, laterodorsal, lateroventral and ventral positions. Additional paired setae in ventromedial position in males and in ventrolateral position in females (Figs 2A–C, 3E–G, 4C). Paired sensory spots in paradorsal, subdorsal, laterodorsal and ventromedial positions (Figs 2A–C, 3F–G, 4C).

Segment 4. Middorsal elevation as on preceding segments (Figs 2B, 4A). Paired setae in paradorsal (except for a single specimen, NHMD-921488, with unpaired paradorsal seta), lateroventral and ventromedial positions; females with additional, sexually dimorphic pair in laterodorsal position (Figs 2A–B, 4C). Paired sensory spots in paradorsal, subdorsal, laterodorsal and ventromedial positions (Figs 2A–B, 4C).

Segment 5. Middorsal elevation as on preceding segments (Figs 2B, 3H, 4A, E). One pair of setae in paradorsal (except for one additional male specimen, with unpaired paradorsal seta) and ventromedial positions, and two pairs in lateroventral position; females with additional, sexually dimorphic pair in laterodorsal position (Figs 2A–B, 3H–I, 4D–E). Paired sensory spots in paradorsal, subdorsal, laterodorsal and ventromedial positions (Figs 2A–B, 3H–I).

Segment 6. Middorsal elevation as on preceding segments (Figs 2B, 4A). Paired setae in paradorsal, laterodorsal, lateroventral and ventromedial positions (except for single specimen, NHMD-921489, with unpaired paradorsal seta) (Figs 2A–B, 4D). Paired sensory spots in paradorsal, subdorsal, laterodorsal and ventromedial positions (Fig. 2A–B); two specimens with deviation in pattern of sensory spots in this segment: one (NHMD-921475) with two ventral sensory spots on right half of segment (one ventromedial and one ventrolateral) and only ventromedial pair on left half, and another (NHMD-921486) with one ventromedial sensory spot on left half of segment and without ventral sensory spot on right half of segment.

Segment 7. Middorsal process extending beyond posterior margin of segment (Figs 2B, 4A, I). Paired setae in paradorsal (except for single specimen, NHMD-921487, with unpaired paradorsal seta), laterodorsal, lateroventral and ventromedial positions (Fig. 2A–B). Paired sensory spots in paradorsal, subdorsal, laterodorsal and ventromedial positions (Fig. 2A–B).

Segment 8. Middorsal process as on preceding segment, slightly longer (Figs 2B, 3L, 4A, I). Unpaired seta in paradorsal position; paired setae in laterodorsal, lateroventral and ventromedial positions (Figs 2A–B, 3L–M, 4I–J). Paired sensory spots in paradorsal, subdorsal, laterodorsal and ventromedial positions (Figs 2A–B, 3L–M, 4I–J).

Segment 9. Middorsal process as on preceding segment, slightly longer (Figs 2B, 4A, I). Paired setae in paradorsal, laterodorsal, lateroventral and ventromedial positions (Figs 2A–B, D, 4I). Paired sensory spots in paradorsal, subdorsal, laterodorsal and ventrolateral positions (Figs 2A–B, D, 4I). Nephridiopore in lateroventral position.

Segment 10. Without middorsal cuticular specialization. Paired setae in lateroventral position (Figs 2A–B, D, 3J). Paired sensory spots in subdorsal, laterodorsal and ventrolateral positions (Figs 2A–B, D, 3J, 4I).

Segment 11. Without middorsal cuticular specialization. Tergal plate triangular, with concave and distally pointed posterior margin; sternal plates with pair of ventral extensions distally rounded (Figs 2A–B, D, 3J–K, 4A, G, I). Males with two sexually dimorphic pairs of stout, thick penile spines (Fig. 2A). Short lateral terminal spines, sexually dimorphic in length (LTS/TL average ratio ca 20% in males and ca 10% in females) (2A–B, D, 3A, J–K, 4G).

Statistical analysis (Fig. 5, Table 1)

The comparison of the total trunk length (TL) between *Setaphyes algarvensis* sp. nov. and *S. kielensis* revealed statistically significant differences ($p < 0.01$; Fig. 5A). In contrast, significant differences were not found in the relationship between the total trunk length and the length of lateral terminal spines (LTS/TL) of both species ($p = 0.86$; Fig. 5B). However, due to the broad range observed in the LTS/TL of *S. kielensis* (Fig. 5B), the ratio LTS/TL was compared between males and females in order to find sexually dimorphic differences. The statistical analysis revealed highly significant gender-based

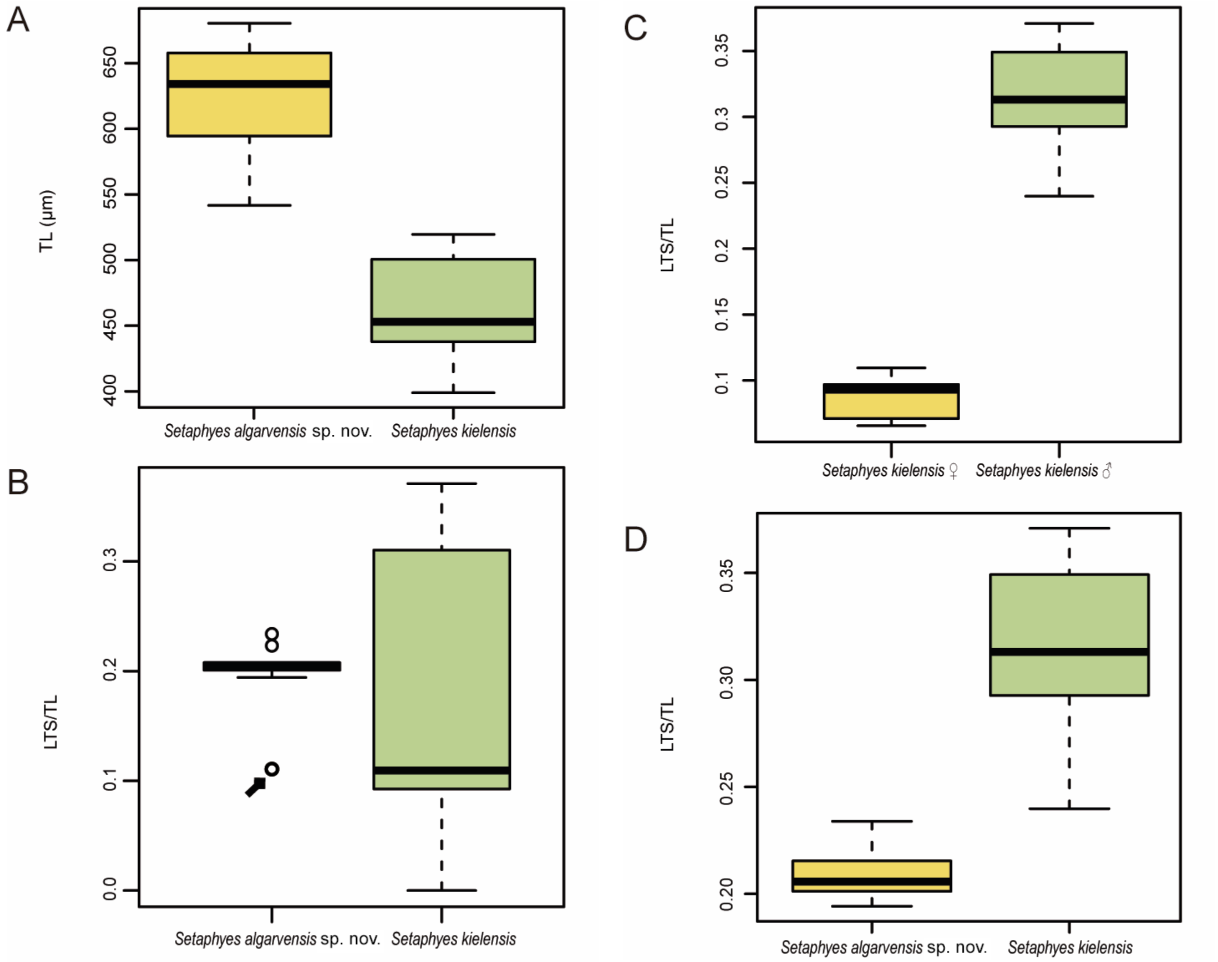

Fig. 5. Boxplots represent the ranges of different body measurements of *Setaphyes algarvensis* sp. nov., and *S. kielensis* (Zelinka, 1928). **A**. Total trunk length. **B**. Relationship between total trunk length and lateral terminal spine length; arrow points out the female of *S. algarvensis* sp. nov. **C**. Relationship between total trunk length and lateral terminal spine length among males and females of *S. kielensis*. **D**. Relationship between total trunk length and lateral terminal spine length among the males of both species.

differences within this species ($p < 0.01$; Fig. 5C). The LTS/TL seems to present sexual dimorphism in *S. algarvensis* sp. nov. as well (Fig. 5B, the arrow points out the single female value), but this fact could not be statistically verified due to the fact that only a single female of this species was collected. Therefore, the LTS/TL ratio was only tested between males of both species, which revealed significant differences ($p < 0.01$; Fig. 5D).

Discussion

Taxonomical remarks

The new species described here clearly belongs to *Setaphyes* according to the diagnostic features of the genus provided in Sánchez *et al*. (2016). *Setaphyes algarvensis* sp. nov. possesses a combination of morphological features present in *Setaphyes* only, e.g., the absence of ventrolateral setae on segment 5, the presence of paradorsal setae on segments 2–9 and lateroventral setae on segments 2–10, as well as the presence of scattered, dot-shaped muscular scars (likely outlets of glandular cells) on both tergal and sternal plates.

Currently, the genus accommodates seven species, namely *Setaphyes australensis* (Lemburg, 2002), *S. cimarensis*, *S. dentatu*s, *S. elenae*, *S. flaveolatus*, *S. iniorhaptus* (Higgins, 1983) and *S. kielensi*s. The new species can be unequivocally distinguished from its congeners by the unique arrangement of dorsal setae and the absence of cuticular ornamentations. The lack of any characteristic cuticular ornamentation on segments 1 or 10 in *S. algarvensis* sp. nov. is remarkable, as most of the remaining congeners have a certain kind of ornamentation: *S. dentatus* and *S. cimarensis* possess longitudinal, parallel, folded cuticular thickenings in laterodorsal and ventrolateral positions on segment 10 (Sánchez *et al.* 2018); *S. cimarensis* additionally has a net-like structure forming rounded, isolated depressions near the anterior margin of the tergal plate of segment 1, whereas *S. dentatus* has a continuous, reticular ornamentation covering the entire anterior surface of the tergal plate of segment 1 (Sánchez *et al.* 2018); both *S. elenae* and *S. flaveolatus* have the ornamentation present solely in the middle anterior region of the tergal plate segment 1 (Zelinka 1908; Sánchez *et al.* 2016, 2018; Cepeda *et al.* 2020); *Setaphyes australensis*, *S. iniorhaptus* and *S. kielensis* lack cuticular ornamentations, but they can easily be distinguished from the new species by the number of paired lateroventral setae on segment 5, as also occurs for the remaining congeners (two pairs in *S. algarvensis* sp. nov. vs one pair in the remaining species of *Setaphyes*) (Higgins 1983; Lemburg 2002; Sánchez *et al.* 2016, 2018).

A species that can potentially be problematic to compare with its congeners is *S. iniorhaptus*. Indeed, the presence of a dorsal series of setae in this species is difficult to verify due to the badly preserved condition of the type material, which prevents accurate observation of these morphological features (Sánchez *et al*. 2016). Nevertheless, *S. iniorhaptus* is easily distinguished from the new species by the presence of a single pair of lateroventral setae on segment 5, and furthermore by the absence of setae on the episternal plates of segment 1 (present ventromedially in *S. algarvensis* sp. nov.).

Regarding the setal arrangement, *S. algarvensis* sp. nov. is most similar to *S. kielensis*, as both species share the same paradorsal setal arrangement (Zelinka 1928; Sánchez *et al.* 2016), but again the presence of two pairs of lateroventral setae on segment 5 and a single pair of paralateral and ventromedial setae on segment 1 in the new species allows its differentiation from *S. kielensis*.

Morphometrical remarks

The morphometry of selected morphological measures in *Setaphyes* has turned out to be useful to distinguish between congeners (Sánchez *et al*. 2018; Cepeda *et al*. 2020). Specifically, in the present study, these analyses help to find more differences between *S. algarviensis* sp. nov. and *S. kielensis*, the most similar congener in terms of setal arrangement. The total trunk length could be used to differentiate both species, as *S. algarviensis* sp. nov. is substantially longer than *S. kielensis*. The ratio between the total trunk length and the length of the lateral terminal spines is also useful when comparing males, being significantly higher in *S. kielensis*. We expect the LTS/TL ratio to be different in females as well; however, this cannot be confirmed with the investigation of only one female and further analyses with more female specimens are needed.

Biological remarks

Knowledge about kinorhynch sex ratios is largely scarce for all ocean bathymetries. Only a few studies limited to the littoral zone have addressed this topic, showing a 1:1 ratio of females to males in *Cristaphyes carinatus* (Zelinka, 1928), *Echinoderes coulli* Higgins, 1977, and *Pycnophyes communis* Zelinka, 1908 (Zelinka 1908, 1928; Higgins 1977). More recently, Hoffman *et al.* (2021) found similar proportions for several shallow water species of *Echinoderes* and *Leiocanthus* along the Alabama coast (Hoffman *et al.* 2021). By contrast, the ratio was biased toward females in the shallow water species *Echinoderes dujardinii* Claparède, 1863 (female:male 3:1) (Zelinka 1928; Higgins & Fleeger 1980), and in six deep-sea echinoderid species found in the Clarion-Clipperton Zone (Sánchez *et al.* 2019, in press). This bias may be explained as a reproductive strategy to enable minimization of energetic investment, as hypothesized for other meiofaunal groups (Hicks & Coull 1983; Shimanaga *et al*. 2009).

Our results show, for the first time, the opposite pattern, with a much higher proportion of males (female:male 1:20). Although most ecological and biological aspects for kinorhynchs are still poorly known, including sex ratio, mating system, and factors driving their spatial-temporal variations (Neuhaus 2013), the observed bias may be related to sex differential mortality after mating or disparities in the ability to move, triggered by external factors, e.g., females burrow more actively into sediment than males under dehydration/insulation conditions at low tide. However, the total number of collected specimens is too low to reach a clear conclusion, and more sampling along the studied area, throughout several years, and controlling time exposition to low tide would be needed in order to know whether our observations are merely fortuitous or define a consistent pattern for this species.

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