

# Seven new and four known species of the genus *Acantholaimus* (Nematoda: Chromadoridae) from the abyssal manganese nodule field (Clarion-Clipperton Fracture Zone, North-Eastern Tropical Pacific)

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**Abstract** The descriptions of seven new and supplemental descriptions of four known species of the genus *Acantholaimus* (Nematoda: Chromadoridae) from about 5,000 m depth in the abyssal manganese nodule field of the French Claim of the Clarion–Clipperton Fracture Zone (north-eastern tropical Pacific) are given. *A. arthrochaeta* sp. n. differs from other *Acantholaimus* species in having jointed cephalic setae. *A. barbatus* sp. n. is characterized by long cephalic setae and the presence of numerous somatic setae at the level of the pharynx. *A. cornutus* sp. n. possesses strong onchia and rugae and short cephalic setae. *A. robustus* sp. n. is characterized by a very large body size, two very large onchia, strongly developed rugae, and cervical setae grouped in threes. *A. sieglerae* sp. n. is a comparatively small species, though with very large onchia. *A. tchesunovi* sp. n. can be differentiated from the other species by the lateral differentiation of the body cuticle, consisting of 6–7 longitudinal rows of pores. *A. veitkoehlerae* sp. n. has a narrow elongate anterior end, two cervical setae, and strong onchia. *A. angustus* and *A. occultus* were found about 5,200 km from their type localities in the Peru Basin, south-east Pacific. *A. iubilus* and *A. maks* were previously found in different parts of the Atlantic and in the Peru Basin.

**Keywords** *Acantholaimus* · Biodiversity · Deep sea · Marine nematode · New species · Taxonomy

## Introduction

We present results from studying the nematode collection of the NODINAUT cruise, conducted in 2004, to the French claim of the Clarion–Clipperton Fracture Zone (north-eastern tropical Pacific). This region is considered to be one of the most commercially important nodule areas of the World Ocean (Thiel 2001). The main goal of the cruise was the investigation of the mega-, macro-, and meiofauna of nodule fields. The study of the nematode species community of this area and descriptions of several species from the families Microlaimidae and Benthimermithidae have been published previously (Miljutin and Miljutina 2009a, b; Miljutina et al. 2010, Miljutin et al. 2011).

In the present study, nematodes of the genus *Acantholaimus* Allgén 1933 were examined. This genus is reported to be very species-rich and abundant in different parts of the deep ocean (Tietjen 1984; Bussau 1993; Vanreusel et al. 2000; Lamshead et al. 2003; De Mesel et al. 2006; Sebastian et al. 2007). *Acantholaimus* was also one of the most abundant and species-rich genera in the area studied. Of 23 samples examined, *Acantholaimus* dominated in 4 samples, ranked second in 13 samples, and ranked third in 4. In the samples, the relative abundance of this genus varied from 7.3 to 23.4% (mean value 14.8%), and its density ranged from 7.5 to 33.5 individuals per 10 cm<sup>2</sup> (mean 17.3 inds/10 cm<sup>2</sup>). In total, among 2,513 nematodes examined, 382 individuals (15.2% of all individuals) belonging to 27 putative *Acantholaimus* species were found. However, most of these species were in juvenile stages, or the number of specimens was insufficient for a new species description. In the end, only 7 putative species, the most abundant ones in the samples, were selected for further description. In addition, we found 4 known species, which were discovered far from the type localities.

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## Materials and methods

### Study area

The scientific cruise “Nodinaut” was conducted by the French RV “L’Atalante” (IFREMER) in the area of French mining claims in the Clarion–Clipperton Fracture Zone (CCFZ) (9–14°N, 130–150°W, ~5,000 m depth) in the summer of 2004 (Table 1).

The seabed in the sampling area is characterized by abyssal hills 100–300 m in height and spaced 5–10 km apart. Most of the area (about 90%) between the seabed hills and their slopes is covered by ferromanganese nodules (2 cm to 15+ cm in diameter). The sediments between the nodules are very fine-grained (<2 μm) silicate oozes (radiolarian and diatomaceous) (Khripounoff et al. 2006).

### Nematode sampling and treatment

Most samples were collected using the 8-tube multicorer (tube diameter 100 mm). Two samples were obtained by means of the manipulator of the “Nautile” submersible. On board, the samples were fixed with 10% formalin in seawater.

Nematodes were sorted out, processed into glycerin using Seinhorst’s (1959) method of slow evaporation, and permanently mounted on glycerin–paraffin slides.

The nematodes were examined with the aid of LEICA DMR and LEICA DM2500 microscopes equipped with a drawing apparatus. Initially, all nematodes were identified to family and genus level. Then, species of the genus *Acantholaimus* with a sufficiently large number of specimens of both sexes were selected for further study and description. Photographs were taken with a LEICA

DM2500 microscope equipped with a LEICA EC3 digital camera.

In about half of the *Acantholaimus* specimens examined, the tails were broken in their thin, cylindrical part. For this reason, in order to minimize errors in the body indices that are traditionally used in taxonomical descriptions of free-living aquatic nematode taxa ( $a$ ,  $b$ , and  $V$ ), additional indices  $a'$ ,  $b'$ , and  $V'$  were added (see the “List of abbreviations”). In addition, in order to clarify how length of onchia, esophastoma, and male spicules was measured, schemes of these measurements are given in Fig. 1.

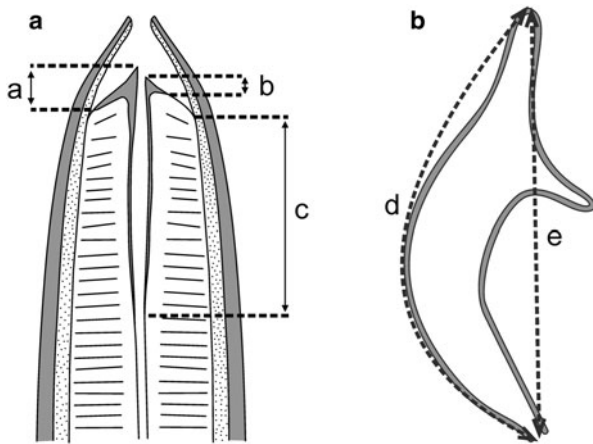
All type specimens were deposited in the Muséum National d’Histoire Naturelle, Paris (MNHN).

### List of abbreviations

- Parameter absent;
- $a$  Ratio “body length/maximum body diameter”;
- $a'$  Ratio “body length without tail/maximum body diameter”;
- $b$  Ratio “body length/length of pharynx”;
- $b'$  Ratio “body length without tail/length of pharynx”;
- $c$  Ratio “body length/length of tail”;
- $c'$  Ratio “length of tail / body diameter at anus”;
- c.b.d. Corresponding body diameter;
- $f$  Female;
- $i$  Intersex;
- $L$  Total body length;
- $L'$  Body length without tail;
- $m$  Male;
- n.a. Not available for measuring because of poor position or condition of specimen, broken tail or inverted head;

**Table 1** Positions of study stations with sample dates, depths, and gear type

Station	Date	Latitude (N)	Longitude (W)	Depth (m)	Device
MTB-1	26.05.2004	14°03.98'	130°06.96'	4,983	Multicorer
MTB-3	28.05.2004	14°02.74'	130°05.11'	5,046	Multicorer
MTB-4	28.05.2004	14°02.93'	130°04.74'	5,029	Multicorer
MTB-6	30.05.2004	14°02.86'	130°05.35'	5,040	Multicorer
MTB-7	30.05.2004	14°02.88'	130°04.97'	5,042	Multicorer
MTB-8	31.05.2004	14°02.55'	130°05.35'	5,035	Multicorer
MTB-10	01.06.2004	14°02.80'	130°05.00'	5,035	Multicorer
MTB-11	01.06.2004	14°02.94'	130°04.73'	5,035	Multicorer
MTB-15	03.06.2004	14°02.69'	130°07.84'	4,947	Multicorer
MTB-16	03.06.2004	14°02.70'	130°07.73'	4,950	Multicorer
MTB-17	07.06.2004	14°02.20'	130°06.60'	5,000	Multicorer
MTB-18	07.06.2004	14°02.52'	130°07.98'	4,950	Multicorer
PL-1597/5	28.05.2004	13°55.63'	130°12.20'	4,800	Manipulator of the “Nautile” submersible
PL-1599/7	31.05.2004	13°55.63'	130°12.20'	4,800	Manipulator of the “Nautile” submersible



**Fig. 1** Scheme of measurements of onchia and esophastoma (a), and male spicules (b) in specimens of the genus *Acantholaimus*. Abbreviations: *a*, *b* length of onchia, *c* length of esophastoma, *d* length of spicule in arc, *e* length of spicule in chord

- V* Ratio “distance from anterior end to vulva / total body length” (%);  
*V'* Ratio “distance from anterior end to vulva / body length without tail” (%)

### Taxonomic descriptions

*Acantholaimus angustus* Bussau 1993 (Figs. 2, 3, 4; Table 2)

**Material examined** 4 males, 5 females, and 1 intersex possessing well-developed female reproductive system and male spicules with gubernaculum but without testis and *vas deferens* (Table 1).

**Locality** Tables 1, 2.

### Description

Main measurements:  $L = 627\text{--}910\ \mu\text{m}$ ;  $L' = 473\text{--}604\ \mu\text{m}$ ;  $a = 23.2\text{--}47.9$ ;  $a' = 17.5\text{--}28.2$ ;  $b = 6.5\text{--}9.2$ ;  $b' = 4.7\text{--}6.7$ ;  $c = 2.3\text{--}4.1$ ;  $c' = 8.9\text{--}24.6$ ;  $V = 31.0\text{--}47.0\%$ ;  $V' = 41.6\text{--}62.4\%$  (Table 2).

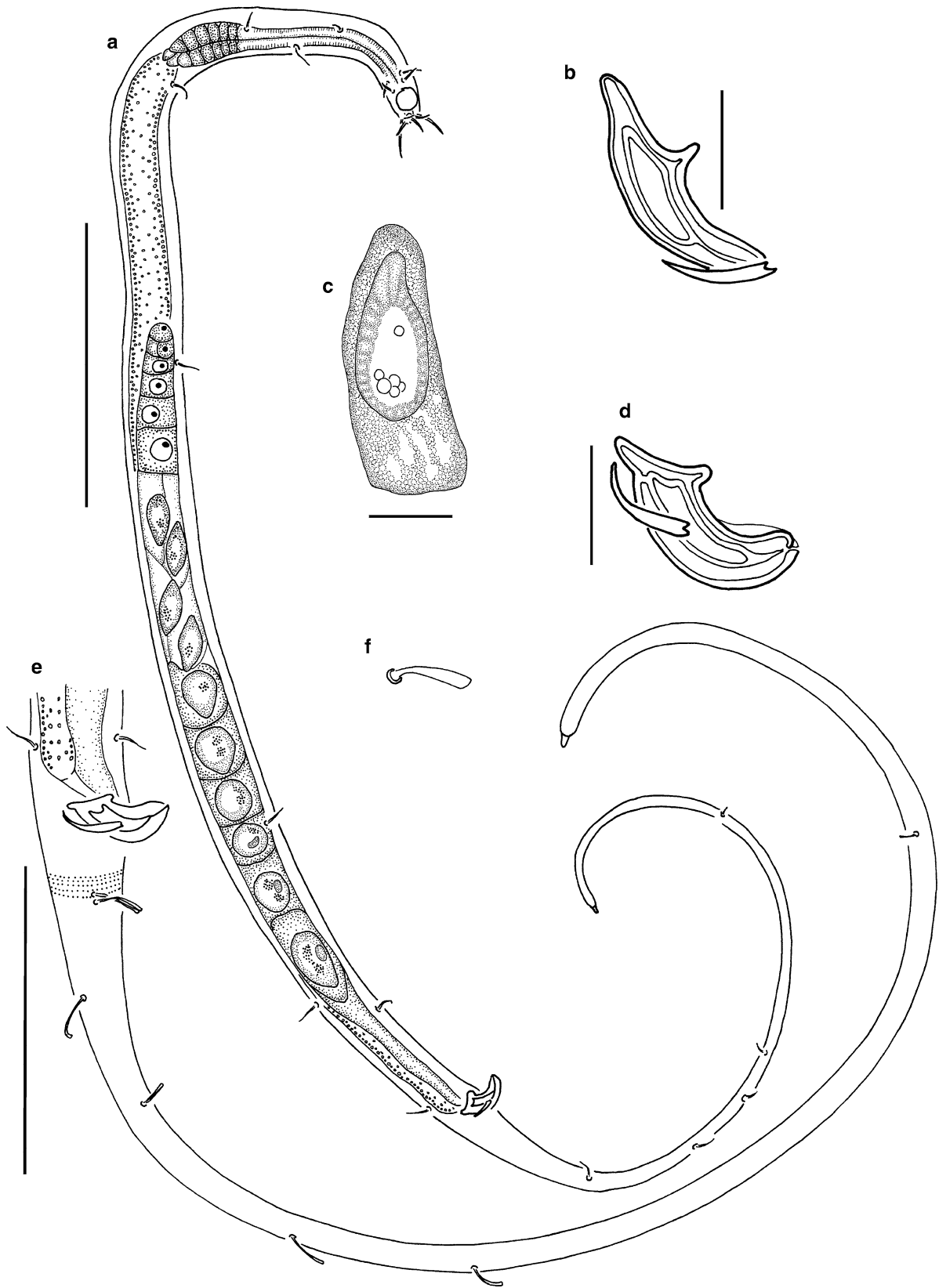
Body slightly spindle-shaped, with narrowed anterior end and filiform posterior end. Somatic setae 6–8- $\mu\text{m}$ -long, numerous, situated along entire body in 4 submedian rows. In postanal region, somatic setae clavate, i.e., with widened distal end; on rest of the body, setae of ordinary form, i.e., edged. Cuticle densely dotted (dots arranged in transverse rows), with lateral fields consisting of much larger dots. Lateral fields beginning at posterior border of amphideal fovea and extending along entire body length except caudal

part. In optical cross-section of the cuticle, the dots are discernible as tiny radial struts. Cuticle about 1.0–1.5- $\mu\text{m}$  thick along entire body length except at level of cephalic setae, where it is thinner (less than 1  $\mu\text{m}$ ). Lips not visible. 3 rings of head sensilla visible: inner labial papilloid sensilla ca. 1  $\mu\text{m}$  long (these sensilla not visible in most specimens), 6 outer labial setae 4–7  $\mu\text{m}$  long, and 4 submedian cephalic setae 9–12  $\mu\text{m}$  long. Outer labial and cephalic setae were cylindrical. Amphideal fovea large (about 1 c.b.d. in width in males), ventrally coiled, single-spiral, with longitudinally oriented oval shape (in males) or round (in females), 9–12  $\mu\text{m}$  width in males and 6–9  $\mu\text{m}$  in females, situated in 0.4–1.0 c.b.d. from anterior end. Two pairs (latero-subdorsal and latero-subventral) of cervical setae 4–10  $\mu\text{m}$  long, located close to posterior part of amphideal fovea. In latero-subventral pair of cervical setae, the distance between anterior and posterior setae is ca. twice the distance between setae of latero-subdorsal pair. Stoma consisting of wide, barrel-shaped cheilostoma and narrow esophastoma. At least two small sclerotized onchia ca. 0.2–0.4  $\mu\text{m}$  long (presumably, dorsal and subventral) visible at anterior end of esophastoma. Esophastoma marked very feebly, walls of esophastomal internal lumen only slightly thicker than in remainder of pharynx. Pharynx muscular, largely cylindrical but widened in its posterior quarter to an oval bulb. This bulb possesses large plasmatic inclusions regularly alternating with muscle cells. Renetta not visible. cardia small, rounded, surrounded by intestine. Tail consisting of proximal conical part and long terminal filiform cylindrical part that constitutes 75–90% of entire tail length. Diameter of cylindrical part of tail in its posterior end ca. 2–3  $\mu\text{m}$ .

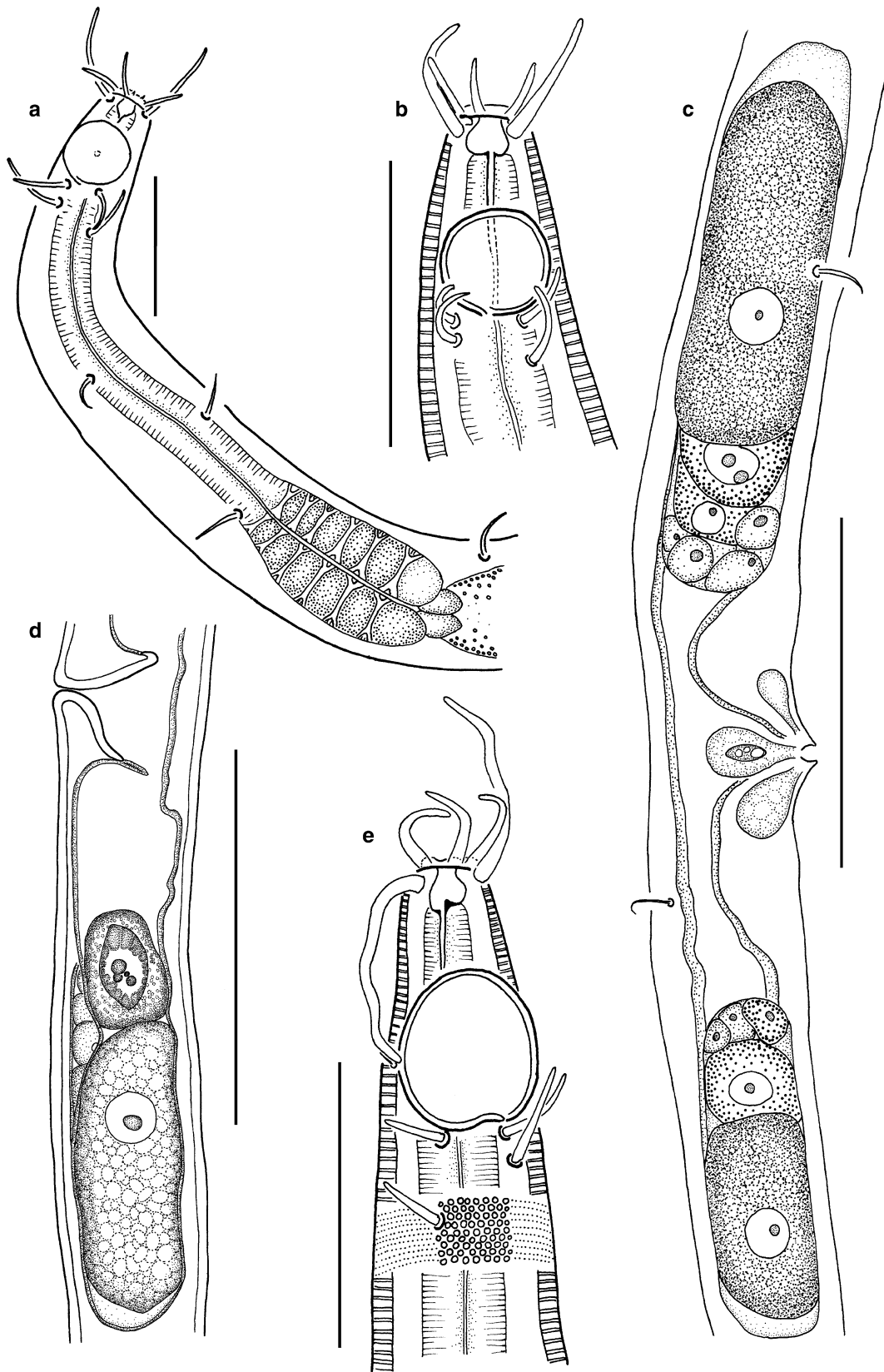
Male reproductive system monorchic. Testis directed anteriorly, outstretched, lying to the right of intestine, occupying about 40% of total body length. Spermatids oval, large (ca. 35  $\times$  10  $\mu\text{m}$ ), with clearly visible, oblong nuclei. Curved spicules possessing complex cuticular sculpture with thickened cuticular areas in form of longitudinal ridges. Gubernaculum shaped as curved triangular stick with edged proximal end and bifurcated distal end. Supplementary organs not found.

Female reproductive system consisting of two antiodromous ovaries (anterior ovary lying to right of intestine, and posterior one lying to left of intestine). Each ovary containing one mature oocyte, from 40  $\times$  15 to 60  $\times$  20  $\mu\text{m}$  in size. One, nearly round spermatozoon ca. 17  $\mu\text{m}$  in diameter visible in uterus of specimen No. 6. Three pairs of vulvar glands seen in some females.

Specimen No. 5 is an intersex with a well developed female reproductive system. However, the male reproductive system is incompletely developed with only spicules present, and the testis and *vas deferens* are lacking.

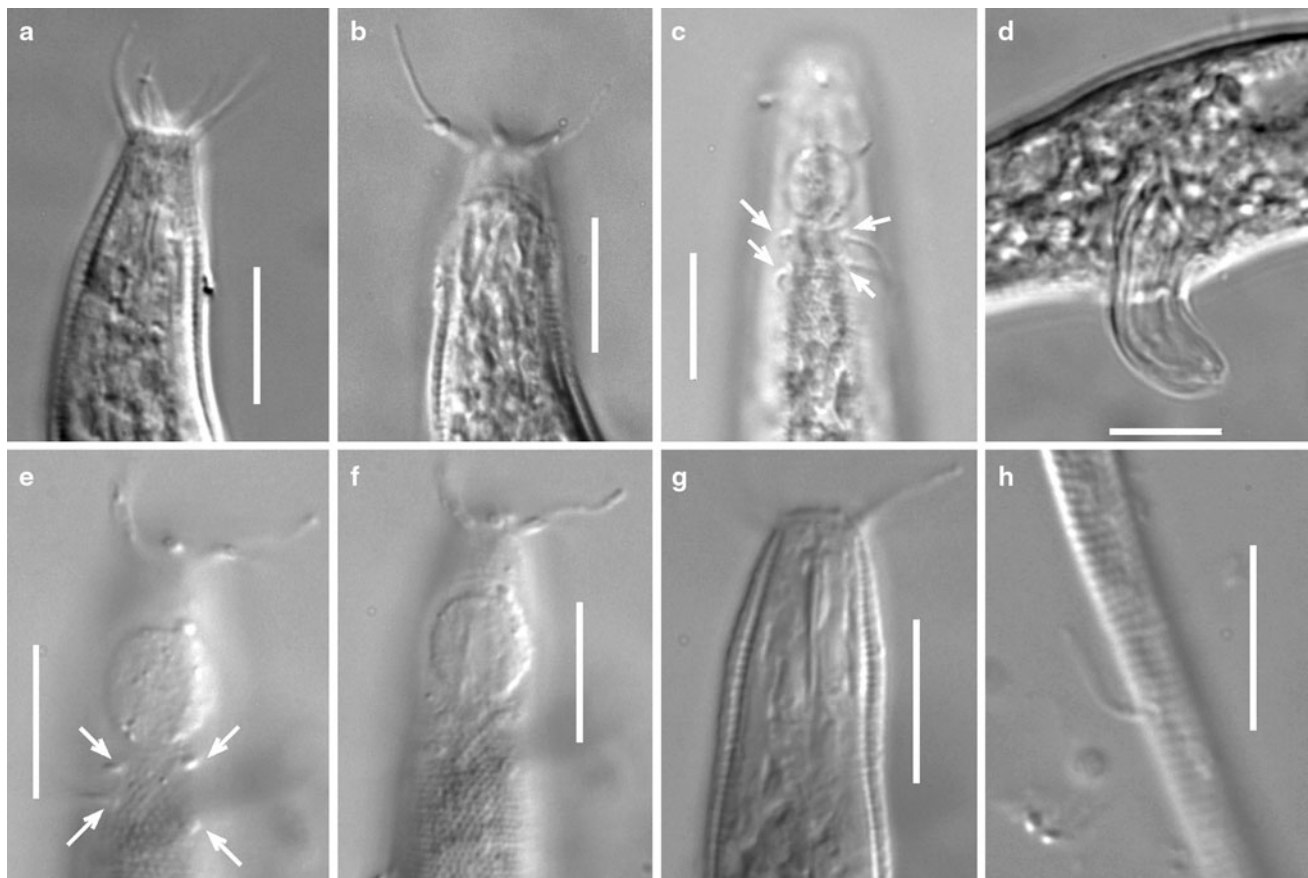


**Fig. 2** *Acantholaimus angustus*, males. **a** specimen No. 2, total view; **b** specimen No. 1, spicule; **c** specimen No. 1, spermatid; **d** specimen No. 2, spicule; **e** specimen No. 2, posterior end; **f** specimen No. 2, postcloacal somatic seta. Scale bars **a** = 100  $\mu\text{m}$ ; **b**, **c**, **d** = 10  $\mu\text{m}$ ; **e** = 50  $\mu\text{m}$



**Fig. 3** *Acantholaimus angustus*. **a** male, specimen No. 2, anterior end; **b** female, specimen No. 8, head region; **c** female, specimen No. 7, reproductive system; **d** female, specimen No. 6, posterior half of

reproductive system; **e** male, specimen No. 1, head region. Scale bars: **a, b, e** = 20  $\mu\text{m}$ ; **c, d** = 50  $\mu\text{m}$



**Fig. 4** *Acantholaimus angustus*, micrographs. **a** female, specimen No. 8, head region; **b** male, specimen No. 2, head region; **c** female, specimen No. 10, head region; **d** male, specimen No. 2, region of

spicule; **e–g** female, specimen No. 7, different optical sections of head region; **h** specimen No. 7, somatic seta on filiform part of tail. Scale bars = 10  $\mu\text{m}$ . Arrows mark location of cervical setae

#### Abundance

The density of this species varied from 0 to 7.3 inds/10  $\text{cm}^2$  (mean value 2.1 inds/10  $\text{cm}^2$ ). In relative abundance within the nematode community, this species ranked fourth (from 0 to 6.3% in different samples, mean 1.7%).

#### Remarks

*Acantholaimus angustus* was initially described by Bussau (1993) in his doctoral thesis. In this work, the author described in total 110 species (97 of them as new) and 11 new genera from the deep-sea nodule-bearing area (Peru Basin, CE Pacific, depth 4,160 m). Unfortunately, the collection of the holotypes was never deposited in a repository institution, and therefore some nematologists consider these species as invalid. Only a few of the species were formally described later (Bussau 1995; Bussau and Vopel 1999). Nevertheless, the value of this doctoral work is very high because of its completeness and care in preparation, and most of the genera and species described therein are easily recognized. Many of Bussau's species

and genera were rediscovered in other regions of the World Ocean (see e.g., Vanreusel et al. 1997; Vanhove et al. 1999; Fonseca et al. 2006, Miljutin and Miljutina 2009b; Miljutina et al. 2010).

The type location of *A. angustus* is about 5,200 km distant from the CCFZ, where the new specimens of this species were found. The new specimens closely resemble those described by Bussau (1993) in their appearance (location and size of amphideal foveas, length and arrangement of head and cervical setae, presence and location of lateral fields, presence of clavate somatic setae in the postanal region, shape of spicule). However, our individuals are larger and possess shorter pharynxes and longer tails than the type specimens [ $L = 627\text{--}910 \mu\text{m}$ ,  $b = 6.5\text{--}9.2$ , and  $c = 2.3\text{--}4.1$  vs.  $575\text{--}725 \mu\text{m}$ ,  $5.0\text{--}6.2$ , and  $4.7\text{--}5.5$ , respectively, in Bussau (1993)]. In addition, the gubernacula in the males studied here are less strongly curved than in the type male. In part, these differences may be explained by intra-specific variation (we were able to study more specimens) or by interpopulation differences. Additionally, in nematodes, smaller specimens tend to possess a relatively longer pharynx than larger ones (see e.g., Roggen 1970).

**Table 2** *Acantholaimus angustus* Bussau, 1993

Specimen No.	1	2	3	4	5	6	7	8	9	10
Station	MTB-7	MTB-1	MTB-1	MTB-17	MTB-6	MTB-1	MTB-8	PL-1599/7	MTB-18	MTB-11
Slide number	1-88	1-32	1-26	2-41	2-95	1-31	3-5	5-60	1-64	1-60
Sex	m	m	m	m	i	f	f	f	f	f
<i>L</i>	n.a.	775	715	910	719	818	768	627	747	809
<i>L'</i>	513	485	528	517	534	592	499	473	506	604
Amphidial width	10.0	9.0	10.5	8.5	9.0	6.0	8.5	7.5	8.0	8.0
Amphidial length	11.0	10.0	11.5	9.5	10.5	6.0	8.5	7.5	8.0	8.0
Length of inner labial setae	n.a.	1.0	n.a.	n.a.	1.0	n.a.	1.0	n.a.	n.a.	n.a.
Length of outer labial setae	9	6	7	9	9	n.a.	7	5	5	4
Length of cephalic setae	16	12	15	16	12	n.a.	12	9	12	11
Length of ventral pair of cervical setae	5–8	8–9	6–7	9–10	6–7	8–9	6–8	4–6	5–6	8–9
Length of somatic setae	7	8	7	7–10	8	7	6–8	6–7	–	8
Length of spicule in chord	18	18	20	23	20	–	–	–	–	–
Length of spicule in arc	25	25	26	32	26	–	–	–	–	–
Length of gubernaculum	12	10	13	14	10	–	–	–	–	–
Distance from anterior end to amphid	9	10	9	7	9	9	6	8	4	7
Diameter at level of cephalic setae	5.0	6.0	4.5	6.0	5.5	5.0	6.0	6.0	6.0	6.5
Diameter at level of first ring of cephalic setae	4.0	4.5	4.0	5.0	5.0	3.5	5	5	4	5
Diameter at level of middle of amphid	11	10	11	11	10	9	10	10	10	9
Diameter at level of cardia	20	17	19	17	20	17	18	19	18	18
Diameter at level of anus	17	16	16	16	18	14	13	15	13	16
Maximum body diameter	26	21	20	19	26	21	22	27	20	23
<i>a</i>	n.a.	36.9	35.8	47.9	27.7	39.0	34.9	23.2	37.4	35.2
<i>b</i>	n.a.	7.8	6.7	9.0	6.7	9.2	7.5	6.5	6.9	7.4
<i>c</i>	n.a.	2.7	3.8	2.3	3.9	3.6	2.9	4.1	3.1	3.9
<i>V</i>	–	–	–	–	45.8	37.2	38.8	47.0	41.0	31.0
<i>a'</i>	19.7	23.1	26.4	27.2	20.5	28.2	22.7	17.5	25.3	26.3
<i>b'</i>	5.0	4.9	5.0	5.1	4.9	6.7	4.8	4.9	4.7	5.5
<i>c'</i>	n.a.	18.1	11.7	24.6	10.3	16.1	20.7	10.3	18.5	8.9
<i>V'</i>	–	–	–	–	61.6	51.4	59.7	62.4	60.5	41.6
Notes	Broken tail									

Number of stations where specimens were found, measurements (in  $\mu\text{m}$ ) and body indices

The  $V'$  ratio varies widely in the new specimens (from 41 to 61%). A similarly wide variation has not been found in other *Acantholaimus* species. Nevertheless, the appearance of the head end, the arrangement and the shape of the head and cervical sensilla, and the structure of the stoma of all specimens examined appear identical. All the specimens examined can, therefore, be assigned to the same species.

#### Distribution

South-eastern Pacific, Peru Basin, nodule fields, 4,157, 4,159 m (Bussau 1993); north-eastern tropical Pacific, Clarion–Clipperton Fracture Zone, nodule fields, abyssal plain without nodules, 4,800–5,042 m, ooze (present report) (Fig. 5).

*Acantholaimus arthrochaeta* sp. n. (Figs. 6, 7, 8; Table 3)

**Type material** Collection number MNHN-BN498. Holotype: male. Paratypes: 2 males and 4 females (Table 3).

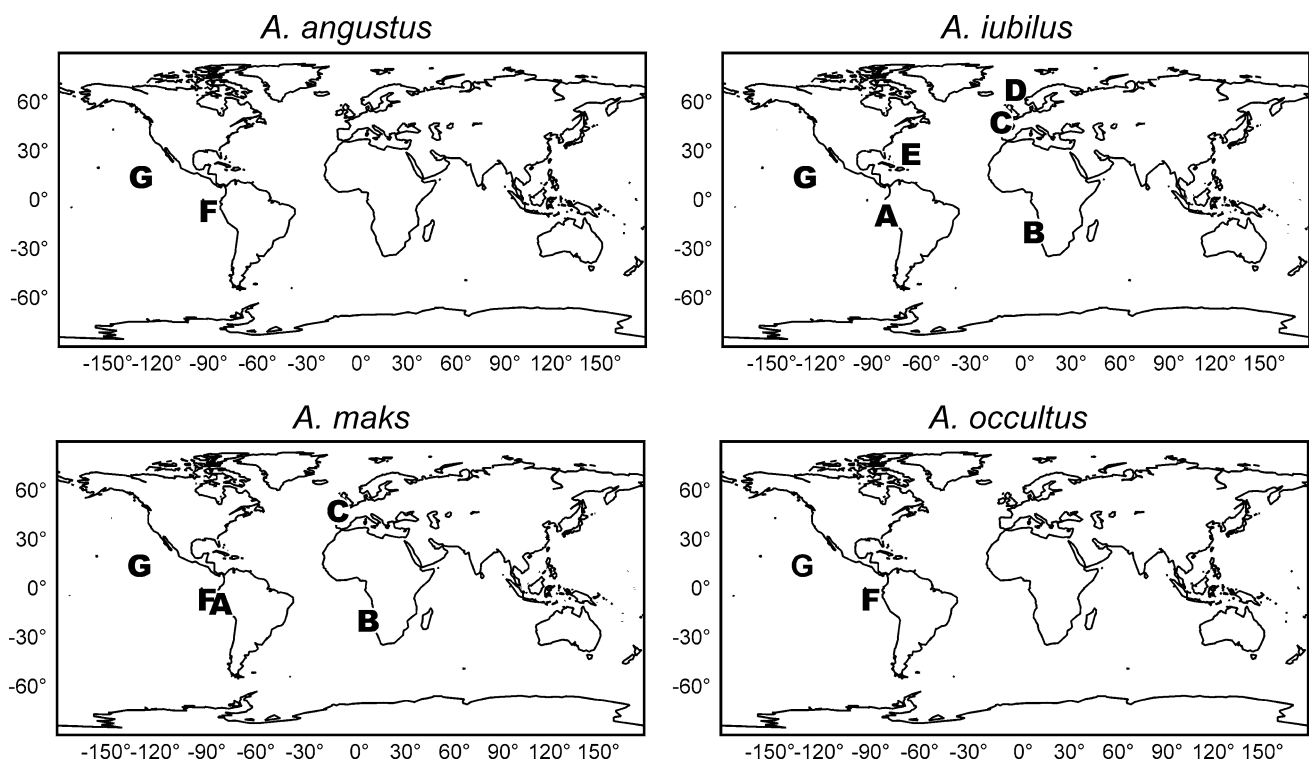
**Locality** Tables 1, 3.

**Etymology** Greek *arthrochaeta* (= bearing jointed setae).

#### Description

Main measurements:  $L = 1,098\text{--}1,545\ \mu\text{m}$ ;  $L' = 839\text{--}1,055\ \mu\text{m}$ ;  $a = 20.4\text{--}39$ ;  $a' = 15.0\text{--}31.0$ ;  $b = 8.5\text{--}10.1$ ;  $b' = 6.6\text{--}7.8$ ;  $c = 3.2\text{--}4.2$ ;  $c' = 8.6\text{--}13.6$ ;  $V = 55.0$ ;  $V' = 68.1\text{--}72.2$  (Table 3).

Body cylindrical, with slightly narrowed anterior end and long filiform tail. Somatic setae cylindrical or clavate,



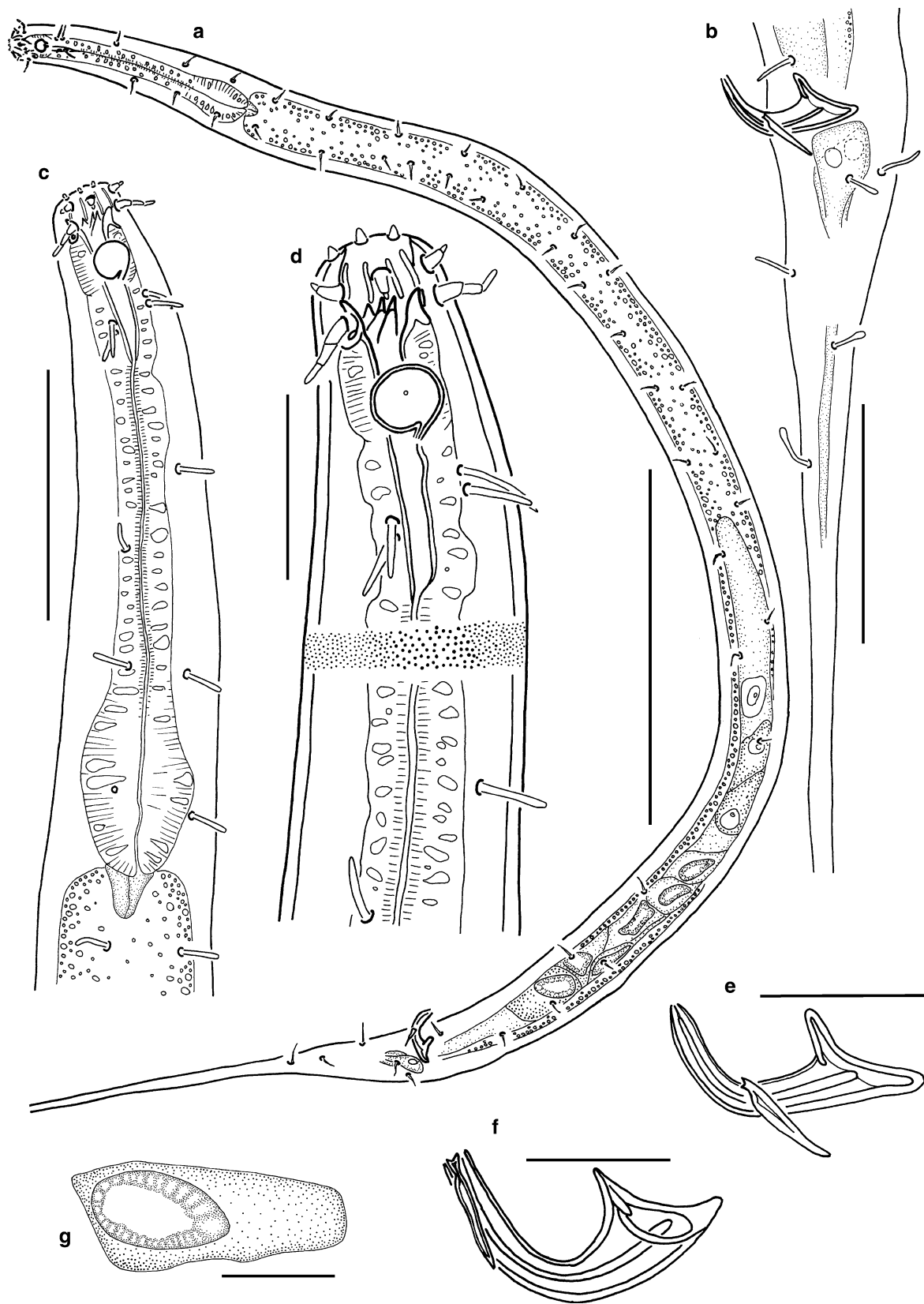
**Fig. 5** Maps showing the records of known *Acantholaimus* species found in the present study. **Bold letters** mark locations where the species was reported. Abbreviations mean reference sources:

A = Gerlach et al. 1979; B = Gourbault and Vincx 1985; C = Vivier 1985; D = Jensen 1988; E = Tietjen 1989; F = Bussau 1993; G = present study

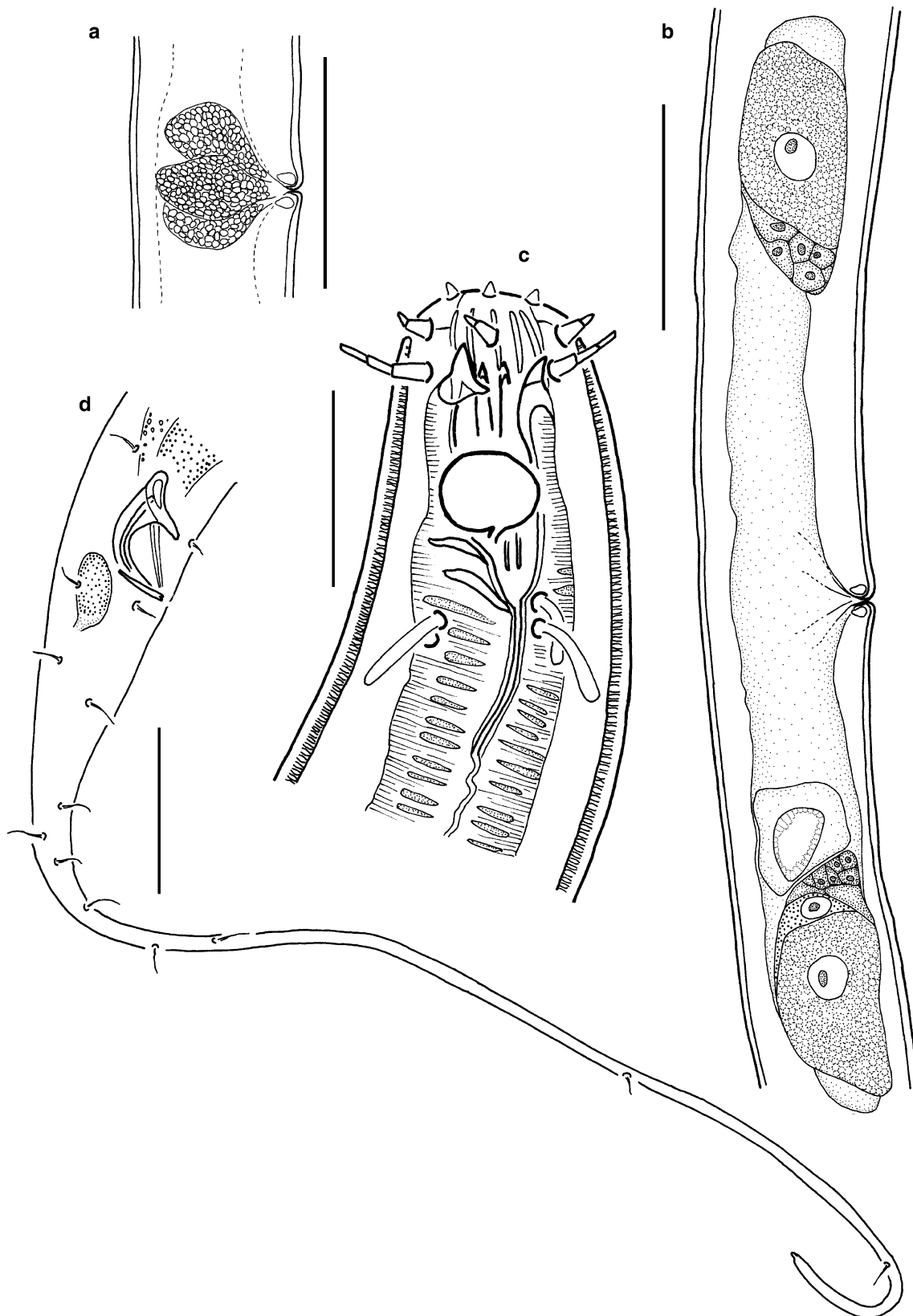
6–9  $\mu\text{m}$  long, numerous, situated along entire body in 4 submedian rows except filiform part of tail where they are irregularly arranged. Numerous pores also visible between somatic setae in these 4 submedian rows. Cuticle densely dotted in lateral fields, beginning posterior to amphideal fovea and extending along entire body except filiform part of tail. Lateral fields with larger dots arranged more sparsely than median dots, which are smaller and more densely arranged. Dots arranged irregularly at level of pharynx (length of body part with irregularly arranged dots varies in different specimens), whereas they are arranged in transverse rows along rest of body. In optical cross-section of cuticle, the dots are discernible as tiny radial struts. Cuticle ca. 1–1.5- $\mu\text{m}$  thick along entire body, except thinner at level of cephalic setae (less than 1  $\mu\text{m}$ ), and thicker at level of pharynx and beginning of intestine (ca. 2  $\mu\text{m}$ ). Lips not visible. Sensilla of cephalic end arranged in 3 rings: 6 short inner labial papilliform setae 2–3  $\mu\text{m}$  long; 6 short, jointed, bipartite, outer labial setae 4–8  $\mu\text{m}$  long; and 4 longer, jointed, tripartite, cephalic setae 7–8  $\mu\text{m}$  long. In about half of specimens, anterior part of head together with cephalic setae retracted. Amphideal fovea ventrally coiled, single-spiral, round or shaped as transversely oriented oval, 8–9  $\mu\text{m}$  wide in males and 6–8  $\mu\text{m}$  wide in females, with fine but distinct cuticular edging. Very fine concentric striation and more or less defined central spot visible in

amphideal fovea. Anterior end of amphideal fovea situated ca. 0.58–0.72 c.b.d. from anterior end. Two pairs (latero-subdorsal and latero-subventral) of cervical setae 5–10  $\mu\text{m}$  long, located posterior to each amphideal fovea. In all individuals, length of cervical setae related to lengths of head setae (they are longer in individuals with longer head setae, and shorter in individuals with shorter head setae). Dorsolateral pair of cervical setae situated at about 0.3 amphideal c.b.d. from amphideal fovea, whereas ventrolateral pair of cervical setae situated farther from amphideal fovea, at about 0.5 amphideal c.b.d. Stoma large, consisting of wide, cup-shaped cheilostoma and narrow, funnel-shaped esophastoma. Cheilostoma ca. 10  $\mu\text{m}$  long, possesses 12 cylindrical rugae. Esophastoma ca. 35  $\mu\text{m}$  long, with thick cuticular walls, containing 5 sclerotized onchia. Basal parts of onchia situated in anterior part of esophastoma, and their apical parts intruding into cheilostoma. One large dorsal or subdorsal onchium ca. 5–6  $\mu\text{m}$  long, one large subventral onchium ca. 5  $\mu\text{m}$  long, one small subventral and two small subdorsal onchia (these latter onchia often appearing as one bifurcated onchium) ca. 1.5–2.0  $\mu\text{m}$  long visible in most specimens. Some small onchia not distinguishable in several specimens. Pharynx narrow in its anterior part, then gradually widening to its posterior end where it forms a poorly developed bulb. Anteriormost part of pharynx ca. 10–15  $\mu\text{m}$  long (evidently





**Fig. 6** *Acantholaimus arthrochaeta* sp. n., males. **a** holotype, total view; **b** holotype, posterior part; **c** holotype, anterior end; **d** holotype, head region; **e** holotype, spicule; **f** paratype No. 1, spicule; **g** holotype, spermatid. Scale bars: **a** = 200  $\mu$ m; **b**, **d–g** = 20  $\mu$ m; **c** = 50  $\mu$ m



**Fig. 7** *Acantholaimus arthrochaeta* sp. n. **a** female, paratype No. 3, vulvar region; **b** female, paratype No. 3, female reproductive system; **c** female, paratype No. 5, head region; **d** male, paratype No. 1, posterior end. Scale bars: **a, b, d** = 50  $\mu$ m; **c** = 15  $\mu$ m

consisting of muscles operating onchia) appreciably detached from rest of pharynx by small constriction, possessing another pattern of muscle striation, and almost devoid of cytoplasmic inclusions. In remainder of pharynx, muscle cells alternate with cytoplasmic inclusions along its entire length. Nerve ring not visible. Renetta not visible. Cardia small, triangular. Tail conical in its anterior part and possessing long filiform, cylindrical terminal part constituting 60–80% of entire tail length. Diameter of cylindrical part of tail in its posterior end ca. 2.5–3.0  $\mu\text{m}$ . Cellular bodies of caudal glands visible posterior to anal region. Tails broken in about half of specimens.

Male reproductive system monorchic. Testis directed anteriorly, outstretched, lying to right of intestine, occupying about 0.4–0.5 of pre-anal body length. Curved funnel-shaped paired spicules possessing cuticular sculpture with thickened cuticular areas in shape of longitudinal or bounding ridges. Gubernaculum appearing stick-like, with slightly narrowed proximal end and narrow bifurcated distal end. Supplementary organs not visible.

Female reproductive system consisting of two antidiromous, uniformly sized ovaries (anterior ovary lying to right of intestine, and posterior one lying to left of intestine). Each ovary containing one mature oocyte, ca. 40  $\times$  20  $\mu\text{m}$ . One spermatozoon ca. 18  $\mu\text{m}$  in diameter visible in uterus in paratype No. 5. Three pairs of vulvar glands with granular content surrounding short vagina.

#### Abundance

*Acantholaimus arthrochaeta* sp. n. was mentioned as *Acantholaimus* sp. 27 in the description of nematode assemblages inhabiting deep-sea polymetallic nodule fields (Miljutina et al. 2010). Its density varied from 0 to 8.4 inds/10  $\text{cm}^2$  (mean 1.8 inds/10  $\text{cm}^2$ ), and its relative abundance within the nematode community was 0–6.2% (mean 1.4%). This species was among the five most abundant species in this area.

#### Differential diagnosis

*Acantholaimus arthrochaeta* sp. n. differs from all other *Acantholaimus* species by the structure of the cephalic setae. There is no other described congener with jointed setae. However, this feature could be difficult to detect in investigations conducted with the help of old or low-power microscopes. Therefore, we will not use this characteristic in comparing species.

The new species shares with *A. akvavitus* Gerlach et al. 1979, *A. barbatus* sp. n., *A. quintus* Gerlach et al. 1979, *A. septimus* Gerlach et al. 1979, and *A. veitkoehlerae* sp. n. the mid-sized body length (about 1,000–1,500  $\mu\text{m}$ ), the relatively small amphideal fovea (40–65% c.b.d.), and the

position of the amphideal fovea relative to the apical part of the body (about 1 c.b.d.).

*Acantholaimus arthrochaeta* sp. n. differs from *A. barbatus* sp. n. by the longer cephalic setae (7–8  $\mu\text{m}$  vs. 13–32  $\mu\text{m}$ ); the position of the cervical setae (situated 8–10  $\mu\text{m}$  behind the amphideal fovea vs. situated at the level of the amphideal fovea); the shape of the anterior end (truncate vs. conical); and the longer esophastoma (ca. 35  $\mu\text{m}$  vs. 15–20  $\mu\text{m}$ ).

*Acantholaimus arthrochaeta* sp. n. differs from *A. veitkoehlerae* sp. n. by their body shape (cylindrical with weakly narrowed anterior end vs. spindle-shaped body with strongly narrowed anterior end); the number of cervical setae (4 vs. 2); the shape of the amphideal fovea (oval vs. circular); and the shape of the spicules (thin vs. thick).

*Acantholaimus arthrochaeta* sp. n. differs from *A. quintus* by the shape of the anterior end of the body (truncate vs. conical); by the quantity and position of the cervical setae (4 vs. 6, situated far from the amphideal fovea vs. situated at the level of the amphideal fovea); the smaller number of somatic setae; the longer esophastoma (ca. 35  $\mu\text{m}$  vs. 10–15  $\mu\text{m}$ ); and the absence of preanal papillae.

*Acantholaimus arthrochaeta* sp. n. most closely resembles *A. akvavitus* and *A. septimus*. It differs from *A. akvavitus* by the body shape (cylindrical vs. spindle-shaped); the shape of the anterior end (truncate without well developed lips vs. conical with well developed lips); and the number of cervical setae (4 vs. 3).

*Acantholaimus arthrochaeta* sp. n. differs from *A. septimus* by the shape and size of the amphideal fovea (transversely oriented oval vs. longitudinally oriented oval, 37–53% vs. 67% of c.b.d.); the position of the cervical setae relative to each other (both pairs of setae are situated very close to one another vs. relatively far); the shape of the spicules (thin vs. thick); and the absence of preanal papillae.

*Acantholaimus barbatus* sp. n. (Figs. 9, 10, 11, 12; Table 4)

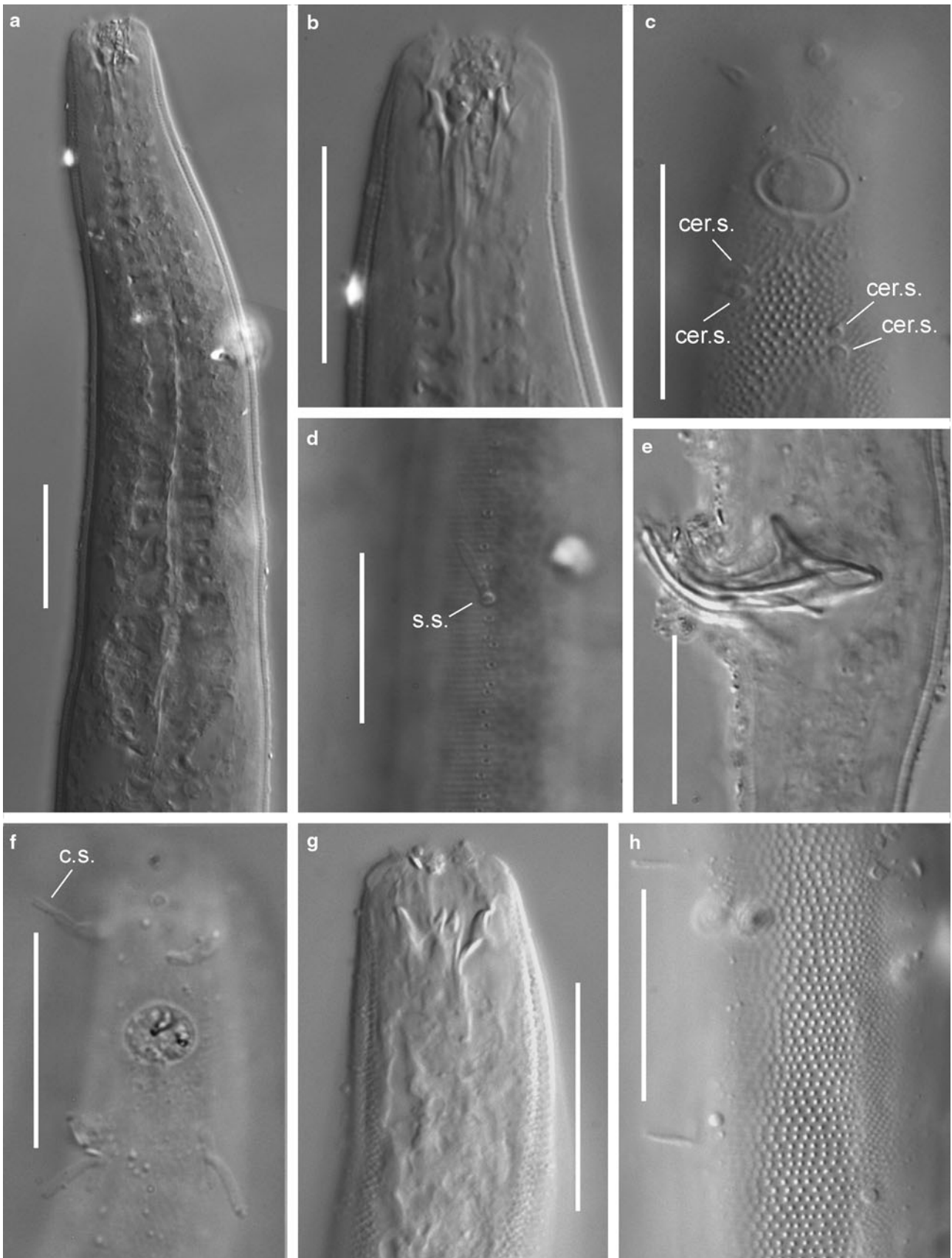
**Type material** Collection number MNHN-BN499. Holotype: male. Paratypes: 5 males, 4 females, and 1 intersex [specimen possessing well developed female reproductive system and male copulatory apparatus (spicules with gubernaculum) without testis and *vas deferens*] (Table 4).

**Locality** Tables 1, 4.

**Etymology** Latin *barbatus* (= barbate).

#### Description

Main measurements:  $L = 1,163\text{--}1,340 \mu\text{m}$ ;  $L' = 867\text{--}1,095 \mu\text{m}$ ;  $a = 33\text{--}40$ ;  $a' = 24.5\text{--}31.5$ ;  $b = 8.8\text{--}11.5$ ;



**Table 3** *Acantholaimus arthrochaeta* sp. n.

Collection status	Holotype	Paratype No. 1	Paratype No. 2	Paratype No. 3	Paratype No. 4	Paratype No. 5	Paratype No. 6
Station	MTB-15	MTB-18	MTB-18	MTB-6	MTB-18	MTB-15	MTB-15
Slide number	3-26(10)	1-12(7)	1-12(8)	2-29	1-12(3)	3-26(2)	3-26(4)
Sex	m	m	m	f	f	f	f
<i>L</i>	n.a.	1,545	1,244	1,098	n.a.	1,250	n.a.
<i>L'</i>	951	1,055	917	839	1,015	942	1,055
Amphidial width	8	9	9	6	7	8	n.a.
Amphidial length	6	6	7	5	6	6	n.a.
Length of inner labial setae	2	n.a.	2	n.a.	3	2	2
Length of outer labial setae	4	n.a.	5	n.a.	8	4	4
Length of cephalic setae	8	n.a.	8	n.a.	n.a.	7	8
Length of cervical setae	8	9	10	7	5	6	8
Length of somatic setae	7	9	9	7	7	6	6
Length of spicule in chord	31	41	31	–	–	–	–
Length of spicule in arc	41	56	43	–	–	–	–
Length of gubernaculum	12	17	10	–	–	–	–
Diameter at level of cephalic setae	15	n.a.	15	14	15	15	14
Distance from anterior end to amphid	13	n.a.	11	10	n.a.	13	11
Diameter at level of middle of amphid	18	21	19	16	19	19	19
Diameter at level of cardia	36	49	40	34	40	34	34
Diameter at level of anus	29	36	38	20	30	26	24
Maximum body diameter	37	61	61	38	47	40	34
<i>a</i>	n.a.	25.3	20.4	28.9	n.a.	31.3	n.a.
<i>b</i>	n.a.	10.0	8.9	10.1	n.a.	9.5	n.a.
<i>c</i>	n.a.	3.2	3.8	4.2	n.a.	4.1	n.a.
<i>V</i>	–	–	–	55.0	n.a.	53.4	n.a.
<i>a'</i>	25.7	17.3	15.0	22.1	21.6	23.6	31.0
<i>b'</i>	6.8	6.8	6.6	7.7	6.8	7.2	7.8
<i>c'</i>	n.a.	13.6	8.6	13.0	n.a.	11.8	n.a.
<i>V'</i>	–	–	–	72.0	72.2	70.9	68.1
Notes	Broken tail			Broken tail			Broken tail

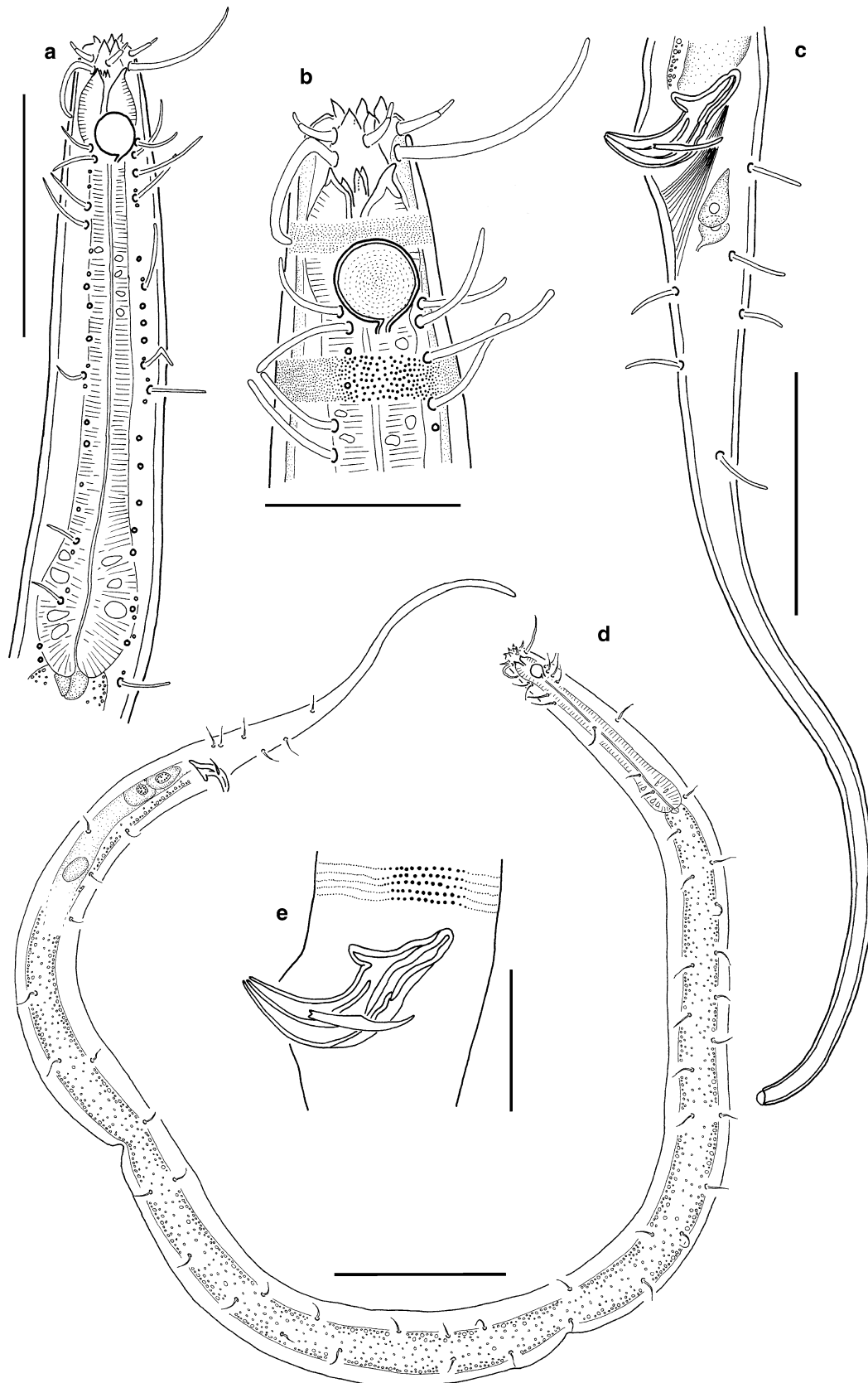
Number of stations where specimens were found, their collection status, measurements (in  $\mu\text{m}$ ), and body indices

$b' = 6.8\text{--}9.0$ ;  $c = 3.7\text{--}6.1$ ;  $c' = 7.1\text{--}15.7$ ;  $V = 55.6\text{--}58.9$ ;  $V' = 70.3\text{--}75.1$  (Table 4).

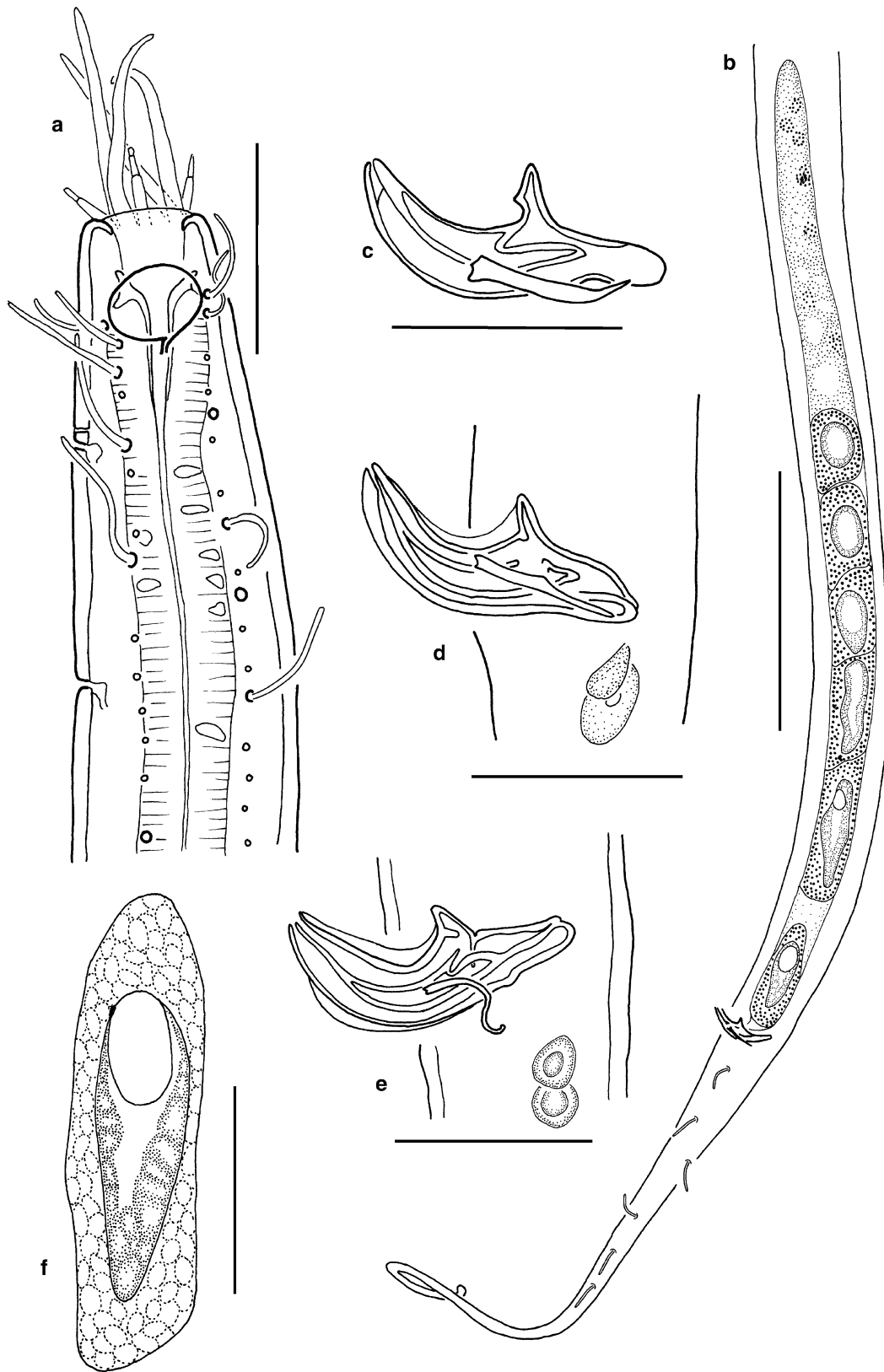
Body slightly spindle-shaped, with narrowed anterior end and filiform posterior end. Somatic setae cylindrical or clavate, 7–11  $\mu\text{m}$  long, numerous, situated along entire body in 4 submedian rows, except filiform part of tail. Numerous pores also visible between somatic setae in these 4 submedian rows. Some of these pores represent distinct

outlets of hypodermal glands. Large pores also visible on ventral side at level of pharynx, these pores also connected to hypodermal glands. Cuticle densely dotted, with lateral fields, beginning posterior to amphideal fovea and continuing along entire body length, except filiform part of tail. Dots of lateral fields larger and arranged more sparsely than dots outside lateral fields. Dots arranged irregularly at level of pharynx (length of body part with irregularly situated dots varying in different specimens), whereas they are arranged in transverse rows along remainder of body. In optical cross-section of cuticle, dots discernible as tiny radial struts. Cuticle about 1.0–1.5  $\mu\text{m}$  along entire body length, except thinner at level of cephalic setae (less than 1  $\mu\text{m}$ ). Six triangular lips with edged anterior tips present. Three rings of head sensilla visible: very short inner labial setae, 1.5–2.0  $\mu\text{m}$  long; 6 outer labial 2-jointed setae,

◀ **Fig. 8** *Acantholaimus arthrochaeta* sp. n., micrographs. **a** male, paratype No. 2, anterior end; **b, c** male, paratype No. 2, head region; **d** male, paratype No. 2, medio-lateral longitudinal row of small cuticular pores in pharyngeal region; **e** male, holotype, spicule region; **f** female, paratype No. 5, head region; **g** male, holotype, head region; **h** male, holotype, lateral field with larger irregularly arranged dots in region of mid-body. Scale bars = 20  $\mu\text{m}$ . Abbreviations: *cer.s.* = cervical seta, *c.s.* = cephalic seta, *s.s.* = somatic seta

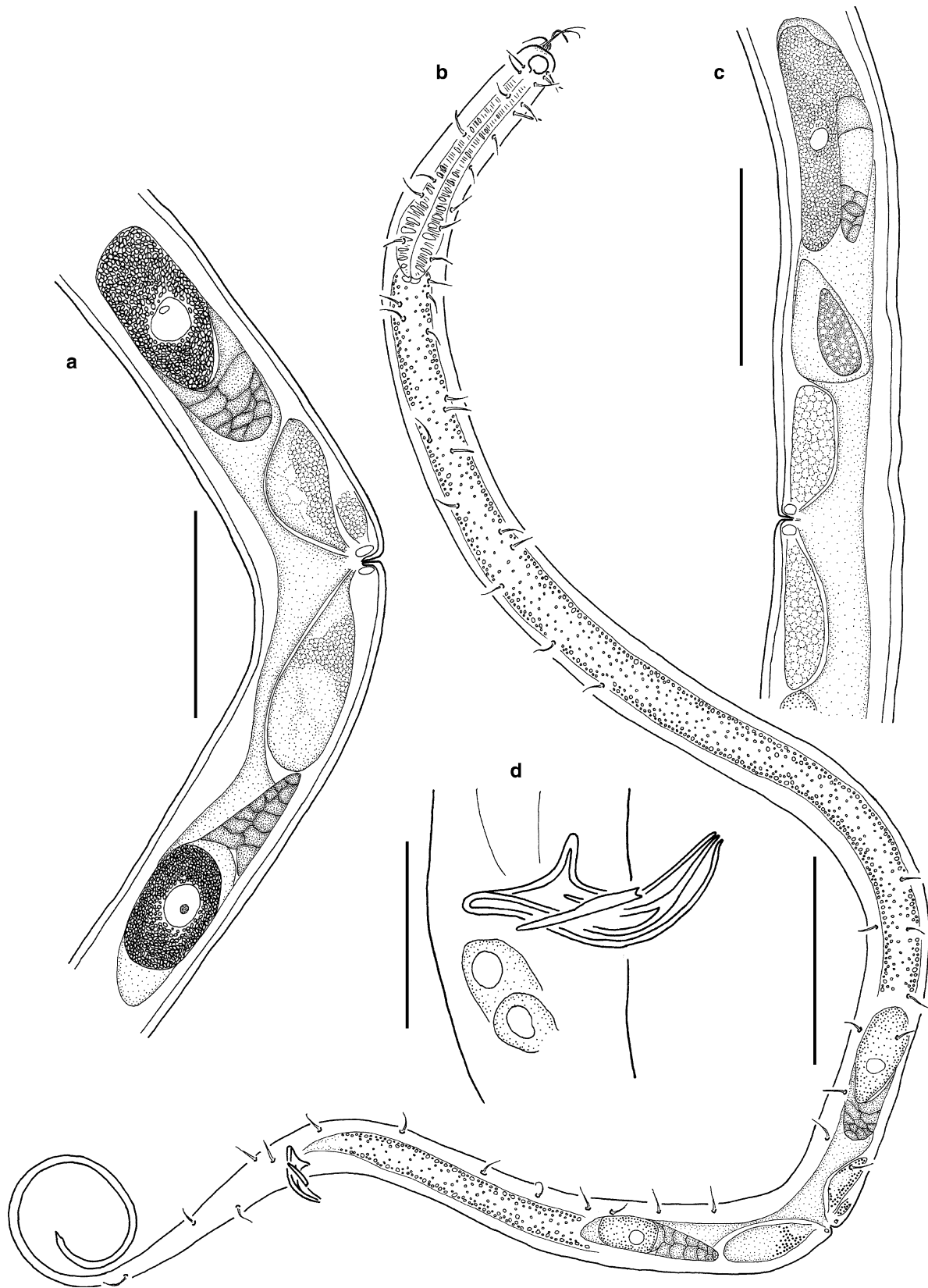


**Fig. 9** *Acantholaimus barbatus* sp. n., male, holotype. **a** anterior end; **b** head region; **c** posterior end; **d** total view; **e** spicule region. Scale bars: **a**, **c** = 50  $\mu$ m; **b**, **e** = 20  $\mu$ m; **d** = 100  $\mu$ m



**Fig. 10** *Acantholaimus barbatus* sp. n., males. **a** paratype No. 3, head region with retracted anterior part; **b** paratype No. 1, posterior end with reproductive system; **c** paratype No. 3, spicule; **d** paratype No. 2,

spicule region; **e** paratype No. 5, spicule region; **f** spermatid. Scale bars: **a, c–f** = 20  $\mu$ m; **b** = 100  $\mu$ m



**Fig. 11** *Acantholaimus barbatus* sp. n. **a** paratype No. 6 (intersex), female reproductive system; **b** paratype No. 6 (intersex), total view; **c** paratype No. 7 (female), anterior branch of reproductive system

with spermatozoon; **d** paratype No. 6 (intersex), spicule region. Scale bars: **a**, **c** = 50  $\mu$ m; **b** = 100  $\mu$ m; **d** = 20  $\mu$ m



5–6  $\mu\text{m}$  long; and 4 longer submedian cephalic setae, 13–32  $\mu\text{m}$  long. In about half of specimens, lips and anterior part of head, together with cephalic setae, retracted. Amphideal fovea situated at level of esophastoma, at 0.7–1.0 c.b.d. from the anterior end, ventrally coiled, single-spiral, round or shaped as transversely oriented oval, 8–10  $\mu\text{m}$  wide in males and 7–8  $\mu\text{m}$  wide in females, with fine but distinct cuticular edging. Very fine concentric striation and more or less distinct central spot visible in amphidial fovea. Two pairs (latero-subdorsal and latero-subventral pair) of cervical setae, 8–12  $\mu\text{m}$  long, located close to posterior part of amphideal fovea at same level, dorsally and ventrally. Also, several somatic setae (usually 2–3 setae in each of 4 latero-median rows) 11–14  $\mu\text{m}$  long, located posterior to cervical setae, these somatic setae longer and set more closely than other somatic setae. Cheilostoma cup-shaped. Esophastoma narrow, funnel-shaped, 15–20  $\mu\text{m}$  long, with thick cuticular walls and 5 sclerotized onchia ca. 2–3  $\mu\text{m}$  long (their arrangement not ascertained clearly). Basal parts of onchia situated in anterior part of esophastoma, and their apical parts intruding into cheilostoma. Some onchia not distinguishable in most specimens. Pharynx narrow, muscular, with few, irregularly located plasmatic inclusions. Pharynx enlarged at level of esophastoma (evidently, this swelling consisting of muscles operating onchia, plasmatic inclusions absent in this part) and gradually widening to its posterior end, where it forms a poorly developed bulb. Nerve ring little visible, lying 52–60  $\mu\text{m}$  from anterior end. Renetta not visible. Cardia small, triangular, surrounded by intestine. Tail consisting of proximal conical part, and long terminal filiform cylindrical part constituting 50–70% of entire tail length. Diameter of cylindrical part of tail in its posterior end ca. 2.5–3.0  $\mu\text{m}$ .

Male reproductive system monorchic. Testis directed anteriorly, outstretched, lying to right of intestine, occupying about 30% of body length. Spermatids oval, large (ca. 40  $\times$  15  $\mu\text{m}$ ), with clearly visible, long-oblong nuclei. Curved spicules possessing complex cuticular sculpture with thickened cuticular areas forming longitudinal ridges. In most specimens, gubernaculum in form of slightly curved stick with edged proximal end and bifurcated distal end. In paratype No. 5, gubernaculum curved, hook-like. Supplementary organs not visible.

Female reproductive system possessing two antidromous ovaries (anterior ovary lying to right of intestine, and posterior one lying to left of intestine). Each ovary containing one mature oocyte 42  $\times$  17  $\mu\text{m}$ . One pair of very large vulvar glands and small glands with granular content seen.

Paratype No. 6 is an intersex with a well developed female reproductive system, and an incompletely developed male reproductive system (only spicules are present, and the testis and *vas deferens* are lacking).

## Abundance

*Acantholaimus barbatus* sp. n. was mentioned as *Acantholaimus* sp. 19 in the description of nematode assemblages inhabiting deep-sea fields of polymetallic nodules (Miljutina et al. 2010). The density of *A. barbatus* sp. n. in the samples varied from 0 to 4.1 inds/10  $\text{cm}^2$  (mean 1.6 inds/10  $\text{cm}^2$ ). This species ranked fifth in mean relative abundance within the nematode community (mean 1.6%, from 0 to 8.6% in different samples).

## Differential diagnosis

The new species shares with *A. gigantasetosus* Vivier 1985, *A. invaginitus* Muthumbi and Vincx 1997 and *A. maks* Gerlach et al. 1979, the relatively small amphideal fovea (40–65% c.b.d.), the position of the amphideal fovea relative to the apical part of the body (about 1 amphideal c.b.d.), and the length of the cephalic setae (about 20–30  $\mu\text{m}$ ).

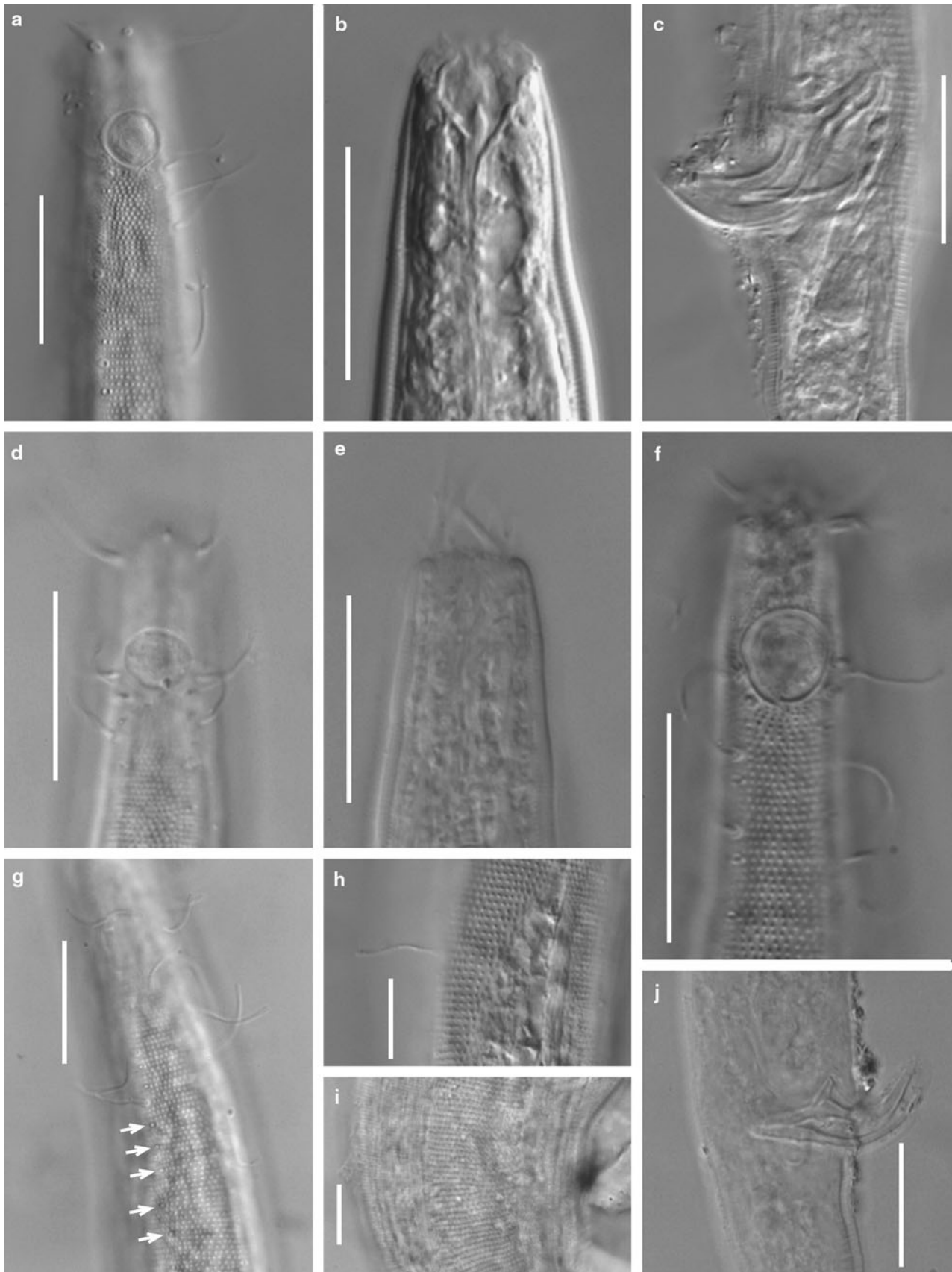
*Acantholaimus barbatus* sp. n. differs from *A. gigantasetosus* by the longer body (1,163–1,340  $\mu\text{m}$  long vs. 596–793  $\mu\text{m}$  long); the length of the cephalic setae relative to c.b.d. (1.9 c.b.d. vs. 4.2 c.b.d.); the number of cervical setae (4 vs. 3); and the shape of the spicules.

*Acantholaimus barbatus* sp. n. differs from *A. invaginitus* by the longer body (1,163–1,340  $\mu\text{m}$  long vs. 711–1,019  $\mu\text{m}$  long); the length of the cephalic setae (1.9 c.b.d. vs. 1.3–1.4 c.b.d.); the presence of a gubernaculum; and the position of the vulva ( $V = 55$ –59 vs.  $V = 49$ –52).

*Acantholaimus barbatus* sp. n. differs from *A. maks* by the shorter body (1,163–1,340  $\mu\text{m}$  long vs. 1,635–2,057  $\mu\text{m}$  long); the length of the cephalic setae relative to c.b.d. (1.9 c.b.d. vs. 1.2 c.b.d.); the presence of numerous somatic setae at the level of the pharynx; and the shape of the anterior end (gradually narrowing to the tip vs. sharply narrowing).

*Acantholaimus barbatus* sp. n. shares with *A. arthrochaeta* sp. n. the middle-sized body, the size and position of the amphideal fovea, and the number of cervical setae. The new species differs from *A. arthrochaeta* sp. n. by the shorter cephalic setae (7–8  $\mu\text{m}$  long vs. 13–32  $\mu\text{m}$  long); the position of the cervical setae (situated at the level of the amphideal fovea vs. situated far from the amphideal fovea); the shape of the anterior end (conical vs. truncate); and the shorter esophastoma (ca. 20  $\mu\text{m}$  long vs. ca. 35  $\mu\text{m}$  long).

*Acantholaimus barbatus* sp. n. is closer to *A. quintus* Gerlach et al. 1979. The new species shares with *A. quintus* such characteristics as the middle-sized body, the shape of the anterior end, the size and position of the amphideal fovea, the size of the spicules, and the presence of numerous somatic setae at the level of the pharynx. It differs from *A. quintus* in having longer cephalic and somatic setae (13–32  $\mu\text{m}$  vs. 11  $\mu\text{m}$  and 11–14  $\mu\text{m}$  vs.



◀ **Fig. 12** *Acantholaimus barbatus* sp. n., micrographs. **a** male, holotype, head region in surface view; **b** male, holotype, head region; **c** male, holotype, spicule region; **d** female, paratype No. 8, head region; **e** male, paratype No. 3, retracted head; **f** male, paratype No. 1, head region; **g** female, paratype No. 10, cervical region; **h** male, holotype, somatic seta at region of mid-body; **i** female, paratype No. 10, lateral field with larger, regularly arranged dots in region of mid-body; **j** intersex, paratype No. 5, spicule region. Scale bars: **a–g**, **j** = 20  $\mu\text{m}$ ; **h**, **i** = 10  $\mu\text{m}$ . Arrows mark location of cuticular pores arranged in medio-lateral rows

5–9  $\mu\text{m}$ , respectively), and by the number of cervical setae (4 vs. 6).

*Acantholaimus cornutus* sp. n. (Figs. 13, 14, 15; Table 5)

**Type material** Collection number MNHN-BN500. Holotype: male. Paratypes: 2 males and 1 female (Table 5).

**Locality** Tables 1, 5.

**Etymology** Latin *cornutus* (= horned).

#### Description

Main measurements:  $L = 1,932\text{--}2,243 \mu\text{m}$ ;  $L' = 1,513\text{--}1,844 \mu\text{m}$ ;  $a = 25.5\text{--}37.2$ ;  $a' = 18.4\text{--}30.3$ ;  $b = 6.4\text{--}7.5$ ;  $b' = 5.1\text{--}5.5$ ;  $c = 3.5\text{--}5.6$ ;  $c' = 7.8\text{--}14.9$ ;  $V = 55.3$ ;  $V' = 76.5$  (Table 5).

Body cylindrical, with truncate anterior end and tail consisting of proximal conical and distal cylindrical filiform parts. Four sublateral rows of round pores ca. 1.5–2.0  $\mu\text{m}$  in diameter with thick cuticular rims beginning posterior to amphideal fovea. Some of these pores bear cylindrical somatic setae 7–15  $\mu\text{m}$  long. Cuticle densely dotted, with short lateral fields beginning posterior to amphideal fovea and ending at posterior third of pharynx. Dots of lateral fields arranged more sparsely and irregularly than dots outside lateral fields, which are arranged in clearly visible, transverse rows and are arranged more densely. In optical cross-section of cuticle, dots are discernible as tiny radial struts. Oval pores with narrow cuticular rims (ca. 2–2.5  $\mu\text{m}$  in diameter at level of anterior third of pharynx, and ca. 1.5  $\mu\text{m}$  along rest of body) distributed irregularly along body. Cuticle ca. 2.5–3  $\mu\text{m}$  thick. Six lips visible. Labial region distinctly set off from remainder of body; its cuticle appreciably thinner (less than 0.5  $\mu\text{m}$  thick). Sensilla of cephalic end arranged in 3 rings: 6 short inner labial conical sensilla, ca. 2–5  $\mu\text{m}$  long; 6 outer labial conical setae, 4–6  $\mu\text{m}$  long; and 4 longer, cylindrical, cephalic setae, 4–7  $\mu\text{m}$  long. Amphideal fovea circular, 14–16  $\mu\text{m}$  in diameter, nearly indiscernible in most specimens because of lack of distinct cuticular rim. Anterior end of amphideal fovea situated ca. 0.2 c.b.d.

from anterior end of body. Two pairs (latero-subdorsal and latero-subventral pair) of cervical setae located posterior to amphideal fovea. Latero-subdorsal pair located closer to amphideal fovea than latero-subventral pair; in both pairs, seta situated more distal from amphid being slightly longer than more proximal seta. In holotype and paratype No. 1, pairs of cervical setae located very close to amphideal fovea (ca. 1  $\mu\text{m}$  from posterior end of amphideal fovea); proximal and distal setae in all pairs being 10–11  $\mu\text{m}$  and ca. 13  $\mu\text{m}$  long, respectively. In paratype Nos. 3 and 4, latero-subdorsal and latero-subventral pairs of cervical setae situated ca. 3–4  $\mu\text{m}$  and 5–6  $\mu\text{m}$  posterior to amphideal fovea, respectively; proximal and distal setae in all pairs ca. 5  $\mu\text{m}$  and ca. 7  $\mu\text{m}$  long, respectively. Stoma consisting of wide, cup-shaped cheilostoma and narrow, funnel-shaped esophastoma. Cheilostoma possessing 12 fang-shaped rugae, these protruding ahead of mouth opening in some specimens. Esophastoma 40–45  $\mu\text{m}$  long, in shape of narrow funnel, containing 6 sclerotized onchia: one large dorsal or subdorsal onchium 13–17  $\mu\text{m}$  long, one large subventral onchium 13–17  $\mu\text{m}$  long, and four smaller onchia 5–10  $\mu\text{m}$  long (their exact arrangement unclear). Basal parts of onchia situated in anterior part of esophastoma, and their apical parts intruding into cheilostoma. Longitudinal bands connected with onchia visible in anterior part of pharynx. Pharynx almost cylindrical, with thickened anterior end at level of esophastoma and with slightly widened distal end. Nerve ring and renetta not visible. Cardia round. Tail conical in its anterior part and possessing thin cylindrical terminal part constituting ca. 65–80% of entire tail length.

Male reproductive system monorchic. Testis directed anteriorly, outstretched, lying to right of intestine, occupying ca. 0.4 of pre-anal body length in holotype, and ca. 0.2 in young paratype No. 1. Size of mature spermatids ca. 65  $\times$  40  $\mu\text{m}$ . Curved funnel-shaped paired spicules possessing cuticular sculpture with thickened cuticular ridges. Gubernaculum blade-shaped, with bifurcated distal end. Supplementary organs not found.

Female reproductive system consisting of two antiodomous, uniformly sized ovaries (anterior ovary lying to right of intestine, and posterior one lying to left of intestine). Ovaries very short. In single female examined, 3 large spermatozoons ca. 130  $\times$  65  $\mu\text{m}$  present. Vulvar glands with granular content surrounding vulva.

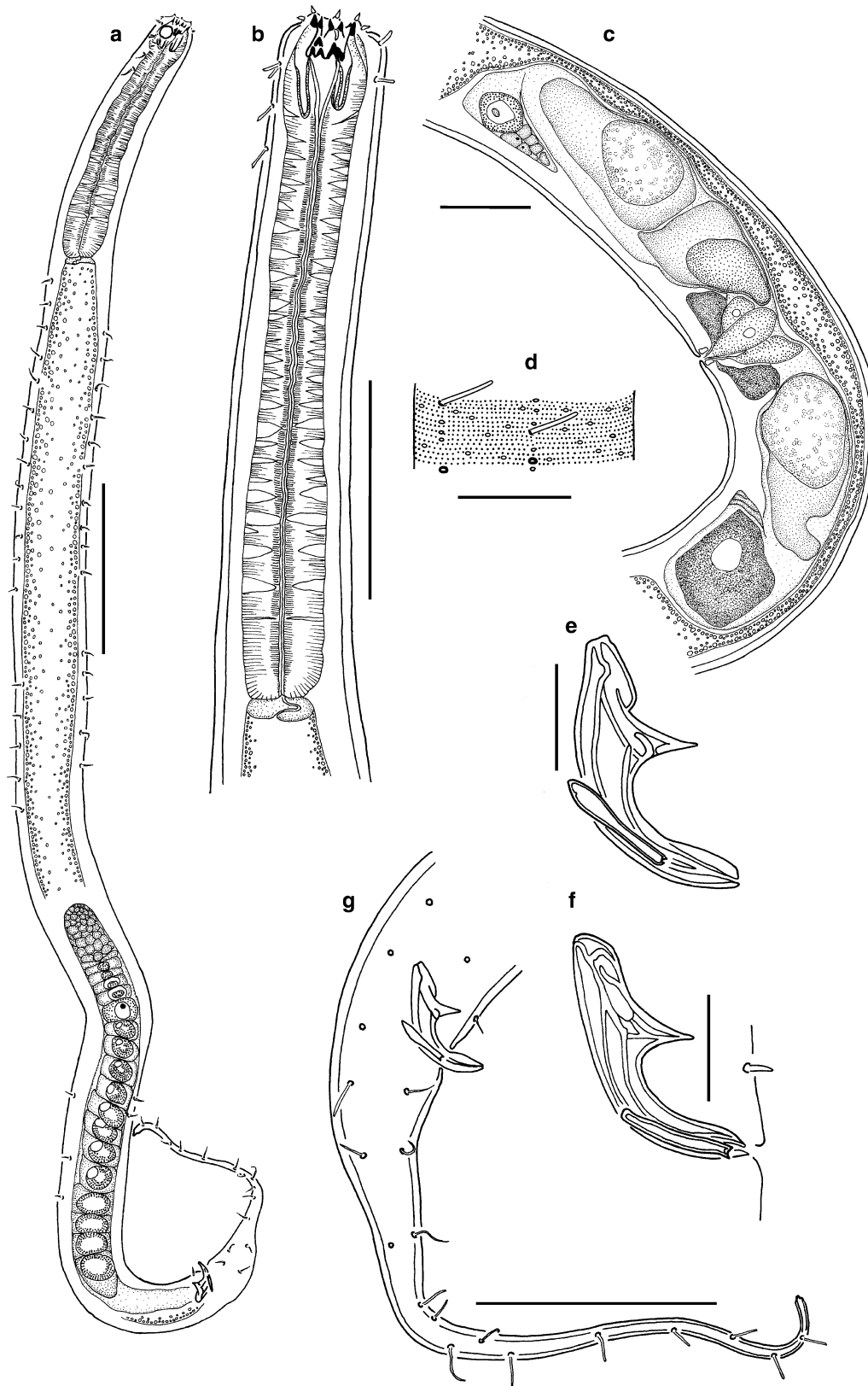
#### Abundance

The density of *A. cornutus* sp. n. varied from 0 to 1.7 inds/10  $\text{cm}^2$  (0.3 inds/10  $\text{cm}^2$  on average), and its relative abundance within the nematode community was 0–1.3% (mean 0.2%).

**Table 4** *Acantholaimus barbatus* sp. n.

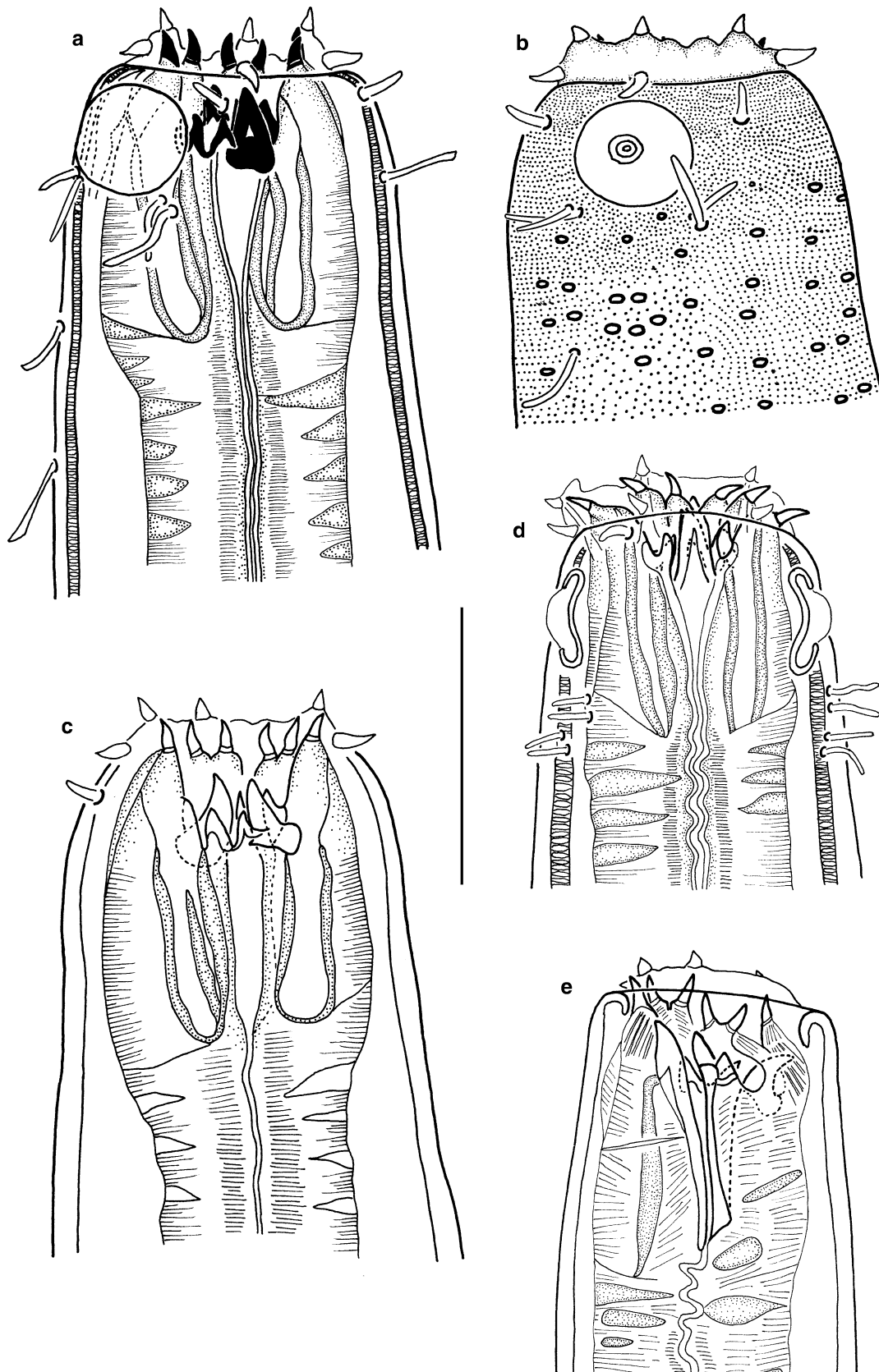
Collection status	Holotype	Paratype No. 1	Paratype No. 2	Paratype No. 3	Paratype No. 4	Paratype No. 5	Paratype No. 6	Paratype No. 7	Paratype No. 8	Paratype No. 9	Paratype No. 10
Station	MTB-18	MTB-18	MTB-16	MTB-16	MTB-18	MTB-10	MTB-17	MTB-16	MTB-16	MTB-15	MTB-18
Slide number	1-63	1-65(7)	1-1(6)	1-1(10)	2-4(7)	3-15	2-40	1-1(5)	1-1(8)	2-73	2-4(5)
Sex	m	m	m	m	m	m	i	f	f	f	f
<i>L</i>	1,286	1,340	1,205	1,200	1,233	1,270	1,163	1,186	1,217	n.a.	1,200
<i>L'</i>	1,076	1,095	928	938	903	980	867	946	950	956	960
Amphidial diameter	8	8	9	8	10	10	8	7	8	8	8
Length of inner labial setae	1.7	1.5	2.0	1.5	n.a.	n.a.	n.a.	2.0	1.5	1.5	1.5
Length of outer labial setae	6	6	5	5	n.a.	5	n.a.	5	5	5	5
Length of cephalic setae	23	17	13	18	n.a.	27	32	20	18	17	26
Length of cervical setae	10	10	9	10	12	11	9	9	9	8	10
Length of somatic setae close to posterior cervical setae	13	14	10	12	12	11	11–12	14	14	14	11
Length of somatic setae	9	11	10	11	8	8	10	10	8	7	10
Length of spicule in chord	29	29	31	28	25	29	29	–	–	–	–
Length of spicule in arc	42	38	36	32	35	35	38	–	–	–	–
Length of gubernaculum	12	12	14	14	13	14	n.a.	–	–	–	–
Distance from anterior end to amphid	14	12	13	n.a.	n.a.	11	n.a.	12	12	14	n.a.
Diameter at level of cephalic setae	11	10	9	n.a.	n.a.	11	n.a.	10	10	11	11
Diameter at level of middle of amphid	14	14	13	14	18	17	13	17	14	16	16
Diameter at level of cardia	27	28	24	25	28	31	27	25	27	29	27
Diameter at level of anus	25	23	18	23	21	24	21	21	20	22	20
Maximum body diameter	39	35	32	32	31	34	30	30	33	39	34
<i>a</i>	33.0	38.3	37.7	37.5	39.8	37.3	38.9	39.5	36.9	n.a.	35.3
<i>b</i>	9.8	10.9	10.8	11.5	9.7	8.8	10.3	10.6	10.3	n.a.	9.8
<i>c</i>	6.1	5.5	4.4	4.6	3.7	4.4	3.9	4.9	4.6	n.a.	5.0
<i>V</i>	–	–	–	–	–	–	56.0	56.1	55.6	n.a.	58.9
<i>a'</i>	27.6	31.3	29.0	29.3	29.1	28.8	28.9	31.5	28.8	24.5	28.2
<i>b'</i>	8.2	8.9	8.3	9.0	7.1	6.8	7.7	8.4	8.1	8.5	7.8
<i>c'</i>	8.4	10.7	15.4	11.4	15.7	8.5	14.1	11.4	13.4	n.a.	7.1
<i>V'</i>	–	–	–	–	–	–	75.1	70.3	71.3	72.6	73.6
Notes			Retracted head	Retracted head	Retracted head		Retracted head			Broken tail	

Number of stations where specimens were found, their collection status, measurements (in  $\mu\text{m}$ ), and body indices



**Fig. 13** *Acantholaimus cornutus* sp. n. **a** male, holotype, total view; **b** male, holotype, anterior end; **c** female, paratype No. 3, reproductive system; **d** holotype, fragment of cuticle surface at mid-body; **e** male,

holotype, spicule; **f** male, paratype No. 2, spicule; **g** male, holotype, posterior part. Scale bars: **a** = 200  $\mu$ m; **b**, **g** = 100  $\mu$ m; **c**, **d** = 50  $\mu$ m; **e**, **f** = 20  $\mu$ m



**Fig. 14** *Acantholaimus cornutus* sp. n., heads. **a, b, c** male, holotype, different optical levels; **d** female, paratype No. 3; **e** male, paratype No. 1. Scale bars = 40  $\mu$ m

## Differential diagnosis

*Acantholaimus cornutus* sp. n. differs considerably from most other *Acantholaimus* species by the shape of the anterior end and the stoma, the position of the amphideal fovea, and the length of the cephalic setae. There is only one similar species in the genus, *A. cyathibuca* Vivier 1985. The new species is very close to *A. cyathibuca* and shares with it the shape of the body and anterior end, the position and the size of the amphideal fovea, the arming of the stoma, and the length and position of the outer labial and cephalic setae. *A. cornutus* sp. n. differs from *A. cyathibuca* by the body length (1,932–2,243  $\mu\text{m}$  vs. 876  $\mu\text{m}$ ); the size and shape of the rugae (fang-shaped vs. rod-shaped); the number of cervical setae (4 vs. 5 plus one pore); the number of longitudinal rows of pores and setae (4 vs. 8); and the length of the somatic setae (7–15  $\mu\text{m}$  vs. 4  $\mu\text{m}$ ).

*Acantholaimus iubilus* Gerlach, Schrage, Riemann 1979 (Figs. 16, 17; Table 6)

**Material examined** Two females (Table 6).

**Locality** Tables 1, 6.

## Description

Body cylindrical, with narrowed anterior end and very long filiform tail. Somatic setae cylindrical or clavate, 5–9  $\mu\text{m}$  long, arranged in 4 submedian longitudinal rows (except filiform part of tail). Cuticle dotted. Dots arranged in transverse rows; they are significantly larger in tail region than on rest of body. Lateral differentiation of cuticle feebly expressed (dots arranged slightly less regularly and less densely here). Small, irregularly arranged pores along entire body length. Cuticle ca. 3  $\mu\text{m}$  thick at level of mid-body. Lips strongly developed, each lip with pair of large rod-shaped cuticular rugae. Only 4 cephalic setae, 11–14  $\mu\text{m}$  long, visible. These setae taper slightly to their tips, and are straight in all specimens examined. Amphids single-spiral, round (if head not inverted), with fine concentric striation, ca. 14  $\mu\text{m}$  wide. Anterior end of amphideal fovea situated at ca. 1 c.b.d. from the anterior end. Two pairs (latero-subdorsal and latero-subventral) of cervical setae ca. 10  $\mu\text{m}$  long located just posterior to each amphideal fovea. Cervical setae of each pair situated very close to each other. Stoma consisting of wide, cup-shaped cheilostoma and narrow, funnel-shaped esophastoma. Cheilostoma containing 6 pairs of large, well discernible cylindrical rugae. Esophastoma possessing three long, very robust sclerotized onchia: one dorsal and two subventral. Dorsal onchium and right subventral onchium ca. 15–20  $\mu\text{m}$  long, right subventral onchium ca. 8–10  $\mu\text{m}$  long. Basal parts of onchia situated in anterior part of

esophastoma, and their apical parts intruding to cheilostoma or even protruding outside mouth opening. In addition, 2 lateral, flat, cuticular laminae with almost right-angled distal ends visible at level of mouth opening. Borders of esophastoma faintly distinguishable, except its anterior part where its thick walls form a prolongation of onchia at a distance of 15–20  $\mu\text{m}$ . Pharynx thin at its anterior part, gradually widening to its posterior end, with large plasmatic interruptions along its entire length. Cardia flattened in direction of main body axis. Nerve ring not visible. Renetta not visible. Tail very long, representing about half of total body length (46–47%), gradually narrowed into filiform, cylindrical terminal part. Cellular bodies of caudal glands visible.

Female reproductive system consisting of two antiodomous, uniformly sized ovaries (anterior ovary lying to right of intestine, and posterior one lying to left of intestine), occupying 22–23% of pre-anal body length. Vulvar glands with granular content surrounding short vagina seen.

## Abundance

The density of *A. iubilus* was very low (only 2 specimens were found in the samples).

## Remarks

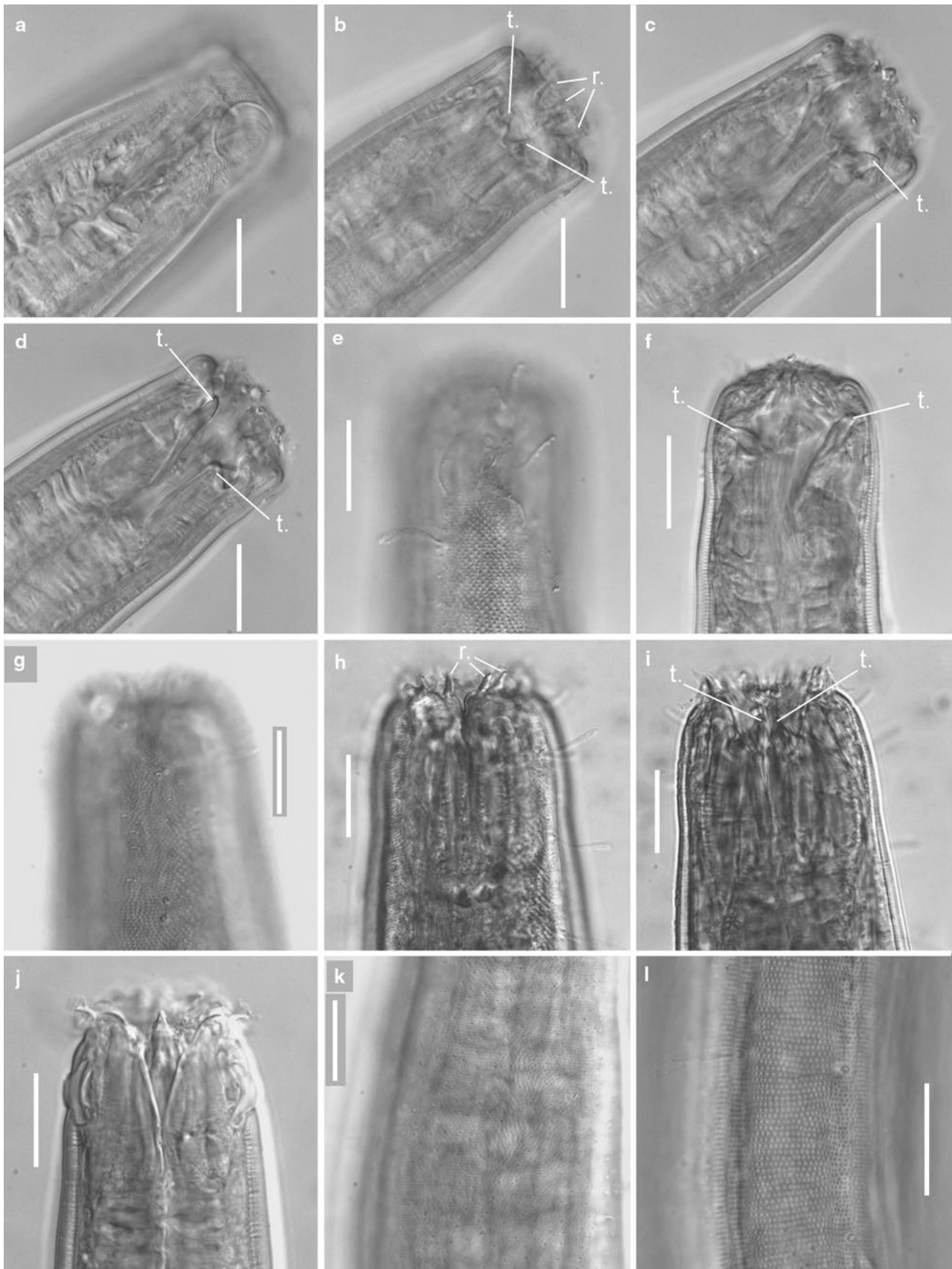
Our individuals closely resemble the type specimens in their appearance (a large strong body with a very long tail); in the size and position of the amphidial fovea; in the number and position of the cervical setae; and in the shape, length, and position of the cephalic setae.

Our individuals differ from the type specimens by their larger body (body length is 2,703–3,366  $\mu\text{m}$  vs. 1,950–2,320  $\mu\text{m}$  in the type specimens, and the maximum body diameter is 66–95  $\mu\text{m}$  vs. 57–60  $\mu\text{m}$ ); and by the number of onchia (3 vs. 5). Differences in body shape can be explained by interpopulation variability.

Various authors have described different numbers of onchia in this species. Gerlach et al. (1979) found, presumably, 5 onchia. Gourbault and Vincx (1985) described 3 onchia and 2 lateral right-angled cuticular plates. This structure was also found in our specimens. Possibly, Gerlach et al. (1979) described these cuticular plates as 2 additional onchia.

## Distribution

South-eastern Pacific, Peru Basin, 3,086–6,313 m, fine silt (Gerlach et al. 1979); North-eastern Atlantic, Bay of Biscay, 4,725 m (Vivier 1985); South-eastern Atlantic, 2,063–4,308 m (Gourbault and Vincx 1985); North





**Table 5** *Acantholaimus cornutus* sp. n.

Collection status	Holotype	Paratype No. 1	Paratype No. 2	Paratype No. 3
Station	MTB-8	MTB-11	MTB-4	MTB-8
Slide number	2-78(1)	1-55	3-55	3-11
Sex	m	m	m	f
<i>L</i>	2,243	2,108	1,932	2,194
<i>L'</i>	1,844	1,513	1,574	1,586
Amphidial diameter	16	14	16	15
Length of inner labial setae	5	n.a.	n.a.	2
Length of outer labial setae	6	n.a.	4	4
Length of cephalic setae	7	6	7	4
Length of somatic setae	15	10	12	7
Length of spicule in chord	58	54	54	–
Length of spicule in arc	77	70	75	–
Length of gubernaculum	24	21	18	–
Distance from anterior end to amphid	n.a.	n.a.	n.a.	12
Diameter at level of cephalic setae	40	36	33	31
Diameter at level of middle of amphid	46	34	32	42
Diameter at level of cardia	71	61	48	78
Diameter at level of mid-body	75	59	52	83
Diameter at level of anus	51	40	38	43
Maximum body diameter	81	61	52	86
<i>a</i>	27.7	34.6	37.2	25.5
<i>b</i>	6.6	7.1	6.4	7.5
<i>c</i>	5.6	3.5	5.4	3.6
<i>V</i>	–	–	–	55.3
<i>a'</i>	22.8	24.8	30.3	18.4
<i>b'</i>	5.5	5.1	5.2	5.4
<i>c'</i>	7.8	14.9	9.4	14.1
<i>V'</i>	–	–	–	76.5
Note	Slightly retracted head	Retracted head	Slightly retracted head, senile male with reduced testis	Slightly everted head

Number of stations where specimens were found, their collection status, measurements (in  $\mu\text{m}$ ), and body indices

Atlantic, Norway Sea, 970–3,294 m (Jensen 1988); Central western Atlantic, Hatteras plain, 5,411 m, Puerto Rico Trench, 7,460–8,380 m (Tietjen 1989); North-eastern tropical Pacific, Clarion–Clipperton Fracture Zone, nodule fields and area without nodules, 5,000–5,035 m (present report) (Fig. 5).

*Acantholaimus maks* Gerlach, Schrage, Riemann 1979 (Figs. 18, 19; Table 7)

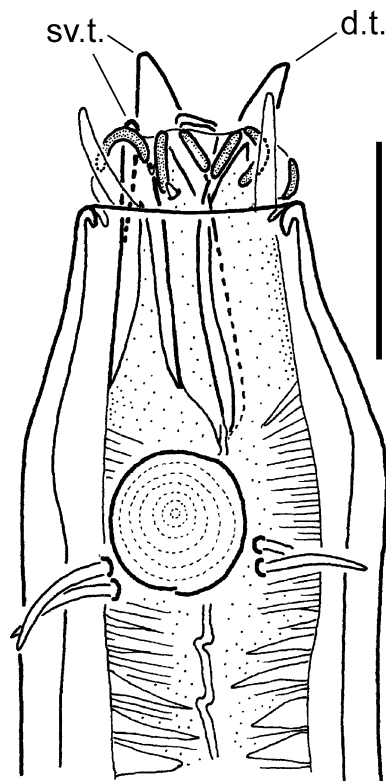
◀ **Fig. 15** *Acantholaimus cornutus* sp. n., micrographs. **a–d** male, paratype No. 1, head region; **e, f** male, paratype No. 2, head region at different optical levels; **g–i** male, holotype, head region at different optical levels; **j** female, paratype No. 4, head region; **k** female, paratype No. 4, fragment of cuticle in mid-body region, with numerous small, irregularly arranged cuticular pores; **l** male, paratype No. 2, lateral cuticular field with rows of larger dots. Scale bars = 20  $\mu\text{m}$ . Abbreviations: *r.* = ruga, *t.* = onchium

**Material examined** One male, one female, one juvenile (Table 7).

**Locality** Tables 1, 7.

#### Description

Body cylindrical, with slightly narrowed anterior end and long filiform tail. Somatic setae few, cylindrical or clavate, 9–12  $\mu\text{m}$  long, arranged in 4 submedian longitudinal rows (except filiform part of tail). Cuticle dotted. Dots arranged in transverse rows. Lateral fields not seen. Small, irregularly situated pores present at level of pharynx. Cuticle ca. 2–2.5  $\mu\text{m}$  thick at level of mid-body. Lips not visible. 3 rings of sensilla of cephalic end visible: 6 short inner labial setae ca. 5  $\mu\text{m}$  long; 6 short outer labial setae ca. 8  $\mu\text{m}$  long (some of them appearing 2-jointed); and 4 longer cephalic setae ca.



**Fig. 16** *Acantholaimus iubilus*, specimen No. 2, female, head region. Scale bar = 20  $\mu\text{m}$ . Abbreviations: d.t. = dorsal onchium, sv.t. = subventral onchium

23  $\mu\text{m}$  long. Amphids single-spiral, round (if head not inverted), ca. 16  $\mu\text{m}$  wide. Anterior end of amphideal fovea situated ca. 1.0 c.b.d. from the anterior end. Two pairs (latero-subdorsal and latero-subventral) of cervical setae 14–15  $\mu\text{m}$  long, located just posterior to each amphideal fovea. Stoma consisting of wide, cup-shaped cheilostoma and narrow, funnel-shaped esophastoma. Cheilostoma containing 12 large, well discernible cylindrical rugae. Esophastoma possessing 5 (presumably) sclerotized onchia: one large subventral onchium ca. 6  $\mu\text{m}$  long, and four other onchia 3–4  $\mu\text{m}$  long, one of them appearing bifurcated. Arrangement of small onchia not clear. Basal parts of onchia situated in anterior part of esophastoma, and their apical parts intruding into cheilostoma. Borders of esophastoma feebly distinguishable, except its anterior part where its thick walls form a prolongation of onchia at a distance of ca. 20  $\mu\text{m}$ . Pharynx thin at its anterior part, gradually widening to its posterior end, with numerous plasmatic interruptions. Cardia oblate. Nerve ring not visible. Renetta not visible. Tail conical in its anterior part and possessing long filiform, cylindrical terminal part constituting ca. 60–70% of entire tail length. Three cellular bodies of caudal glands visible in conical part of tail.

Male reproductive system monorchic. Testis directed anteriorly, outstretched, lying to right of intestine,

occupying 40% of pre-anal body length. Curved funnel-shaped paired spicules possessing cuticular sculpture with thickened cuticular areas, forming longitudinal or bounding ridges. Gubernaculum wide, with slightly narrowed proximal end and narrow bifurcated distal end. Size of mature spermatids ca. 70  $\times$  40  $\mu\text{m}$ . Supplementary organs not visible.

Female reproductive system consisting of two antidi-omous, uniformly sized ovaries (anterior ovary lying to right of intestine, and posterior one lying to left of intestine), occupying ca. 25% of pre-anal body length. Large vulvar glands with granular content surrounding short vagina.

#### Abundance

The density of *A. maks* was very low: only 3 specimens were found in the samples.

#### Remarks

Our individuals closely resemble the type specimens in most parameters, such as body size, position and shape of amphideal fovea, number of cervical setae, and shape of spicules.

Thiel (2003) suggested that this species inhabits only crevices inside polymetallic nodules. In our investigation, *A. maks* was found in the 26-year-old track after the experimental nodule dredging, which was therefore devoid of nodules (Miljutin et al. 2011).

#### Distribution

South-eastern Pacific, Peru Basin, 3,364–6,313 m, silt (Gerlach et al. 1979); North-eastern Atlantic, Bay of Biscay, 2,690 m (Vivier 1985); South-eastern Atlantic, 2,992–4,308 m (Gourbault and Vincx 1985); South-eastern Pacific, Peru Basin, nodule field, internal cavity of manganese nodules, 4,142–4,150 m (Bussau 1993); North-eastern tropical Pacific, Clarion–Clipperton Fracture Zone, track remaining after experimental nodule dredging (present report) (Fig. 5).

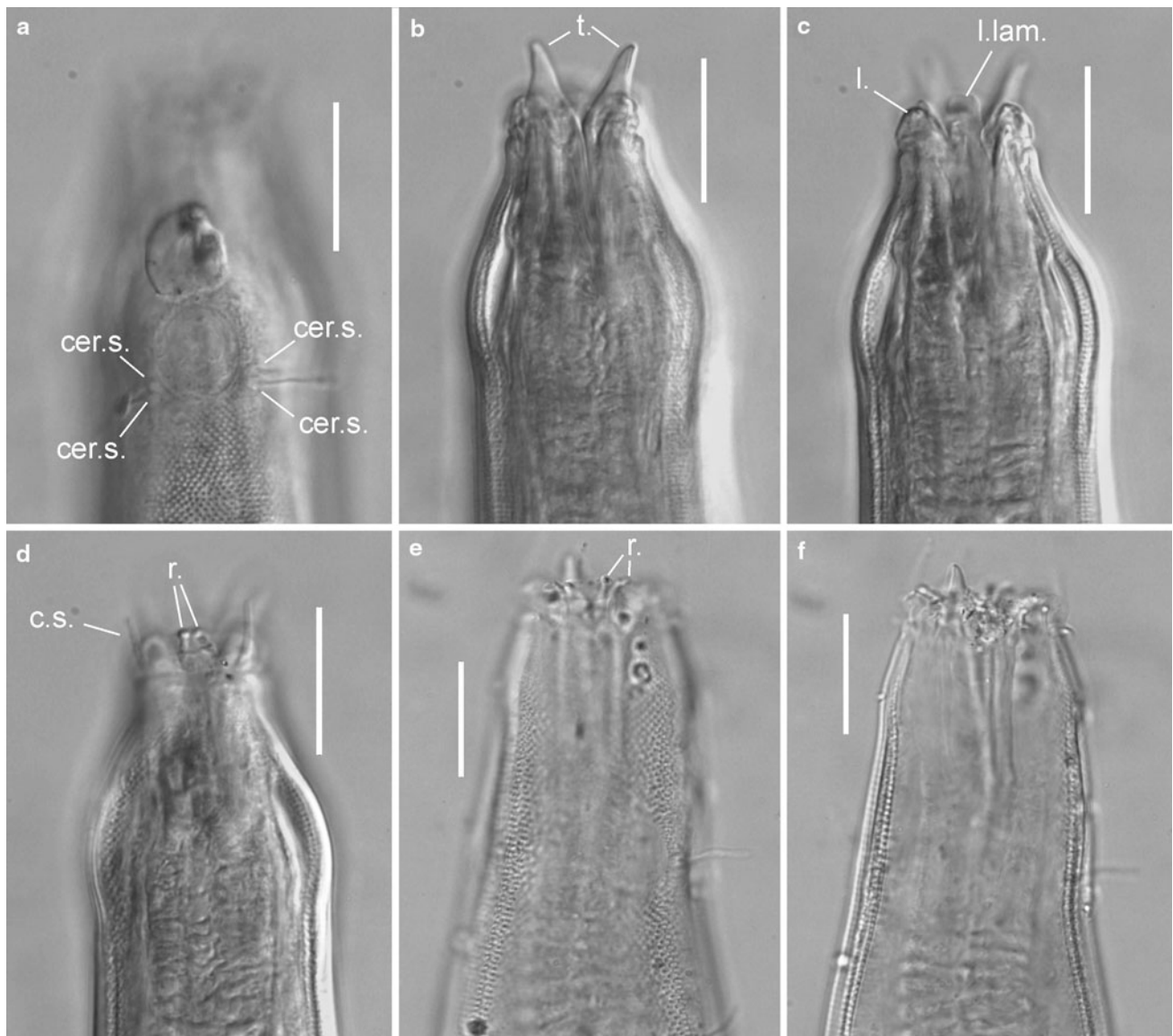
*Acantholaimus occultus* Bussau 1993 (Figs. 20, 21; Table 8)

*Material examined* Two males, one female (Table 8).

*Locality* Tables 1, 8.

#### Description

Body slender (in comparison with many other *Acantholaimus* species), slightly spindle-shaped, with slightly narrowing anterior end and very long filiform tail (length of tail more



**Fig. 17** *Acantholaimus iubilis*, micrographs. **a–d** female, specimen No. 2, head region at different optical levels; **e, f** female, specimen No. 1, head region at different optical levels. Scale bars = 20  $\mu\text{m}$ .

Abbreviations: *cer.s.* = cervical seta, *c.s.* = cephalic seta, *l.* = lip, *l.lam.* = lateral cuticular lamina, *t.* = onchium

than half of total body length). Somatic setae cylindrical, 6–7  $\mu\text{m}$  long, situated along entire body. Cuticle punctuated; punctuation feeble in males. Dots arranged in transverse rows. Lateral differentiation of cuticle present, lateral field quite narrow. Dots of lateral fields larger. Cuticle ca. 1  $\mu\text{m}$  thick along entire body, except thinner at level of cephalic setae (less than 1  $\mu\text{m}$ ). Lips large and well discernible. Two rings of sensilla of cephalic end discernible: 6 shorter clavate outer labial setae, 5–6  $\mu\text{m}$  long; and 4 longer, thicker, conical with slightly clavate tips, cephalic setae, 14–17  $\mu\text{m}$  long. Amphideal fovea ventrally coiled, single-spiral; in shape of longitudinally oriented oval and ca.  $9 \times 15 \mu\text{m}$  in males; ca. 8  $\mu\text{m}$  in diameter and round in female. Anterior end of amphideal fovea situated ca. 1 amphidial length from

anterior end of body. Two pairs (latero-subdorsal and latero-subventral) of cervical clavate or cylindrical setae located just posterior to each amphideal fovea. Anterior cervical seta of each pair ca. 5  $\mu\text{m}$  long; posterior seta ca. 7  $\mu\text{m}$  long. Cheilostoma containing 3 sclerotized onchia 3–4  $\mu\text{m}$  long: 1 dorsal and 2 subventral. Basal parts of onchia situated in anterior part of esophastoma, and their apical parts intruding into cheilostoma. Esophastoma long (20–32  $\mu\text{m}$  long, one-third of total length of pharynx), its internal lumen with thicker cuticular wall. Muscle envelope of esophastoma appreciably detached from rest of pharynx by small constriction, and differing from other part of pharyngeal muscle envelope by lack of plasmatic inclusions and more distinct muscle striation. In its posterior two-thirds, pharynx

**Table 6** *Acantholaimus iubilus*

Specimen No.	1	2
Station	MTB-10	MTB-17
Slide number	3-23	2-38
Sex	f	f
<i>L</i>	n.a.	n.a.
<i>L'</i>	1,813	1,419
Amphidial diameter	14	14
Length of cephalic setae	14	11
Length of cervical setae	10	10
Length of somatic setae	n.a.	9
Diameter at level of cephalic setae	26	19
Distance from anterior end to amphid	38	29
Diameter at level of middle of amphid	38	33
Diameter at level of cardia	80	52
Diameter at level of anus	39	32
Maximum body diameter	95	66
<i>a'</i>	19.1	21.5
<i>b'</i>	5.7	5.6
<i>V'</i>	51.4	53.8
Note	Broken tail	Broken tail

Number of stations where specimens were found, measurements (in  $\mu\text{m}$ ) and body indices

containing large and numerous, regularly altering plasmatic interruptions. Cardia cordate. Nerve ring not visible. Renetta not visible. Tail very long, conical in its anterior part and possessing long filiform, cylindrical terminal part constituting ca. 85–90% of total tail length.

Male reproductive system monorchic. Testis directed anteriorly, outstretched, lying to right of intestine, occupying about 0.5 of pre-anal body length. Spicule thick, shaped as curved funnel, with thick cuticular walls. Gubernaculum blade-shaped, with narrowed proximal end and bifurcated distal end. Mature spermatids ca.  $30 \times 13 \mu\text{m}$ . Supplementary organs not found.

Female reproductive system consisting of two antiodrous, uniformly sized ovaries (anterior one lying to right of intestine, and posterior one lying to left of intestine).

#### Abundance

The relative abundance of *A. occultus* within the nematode community was 0–2.3% (mean 0.4%). The density varied from 0 to 0.7 inds/10  $\text{cm}^2$  (0.1 inds/10  $\text{cm}^2$  on average).

#### Remarks

The new individuals closely resemble the type specimens in their appearance and many body parameters. The shape and construction of the spicule are also very similar.

The new individuals have a smaller and differently shaped amphideal fovea ( $9 \times 15 \mu\text{m}$  and oval in the males vs.  $18 \mu\text{m}$  in diameter and round in the type males). The amphids of the new specimens are situated closer to the anterior end (at 10–11  $\mu\text{m}$  vs.  $16 \mu\text{m}$  in the type specimens).

#### Distribution

South-eastern Pacific, Peru Basin, nodule field, 4,147 m, sediment from nodule field (Bussau 1993); North-eastern tropical Pacific, Clarion–Clipperton Fracture Zone, nodule field, 4950, ooze (present report) (Fig. 5).

*Acantholaimus robustus* sp. n. (Figs. 22, 23, 24; Table 9)

*Type material* Collection number MNHN-BN501. Holotype: male. Paratypes: 2 males, 2 females, and 1 juvenile (Table 9).

*Locality* Tables 1, 9.

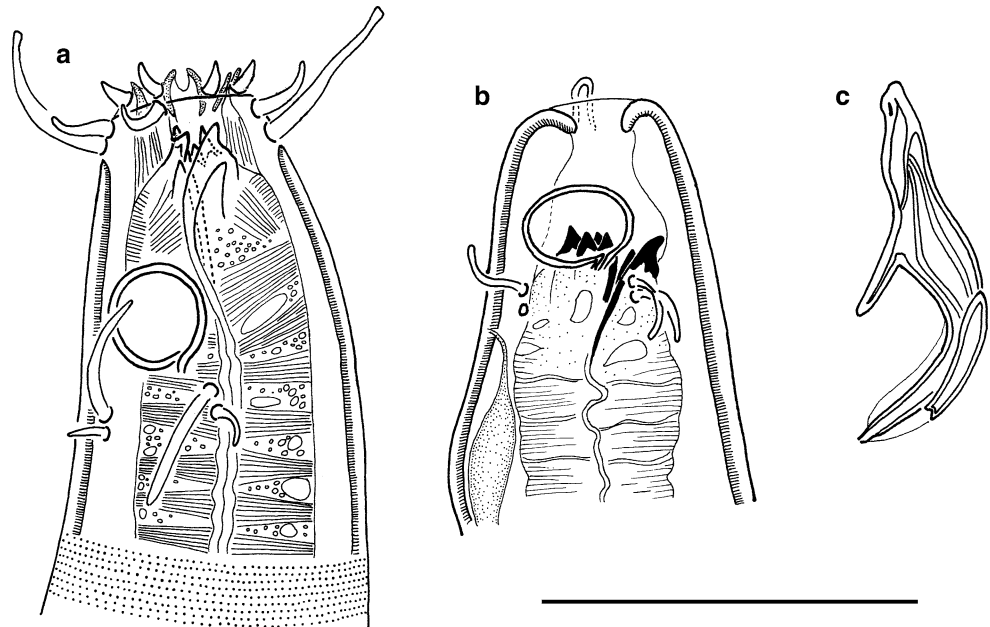
*Etymology* Latin *robustus* (= robust, stout).

#### Description

Main measurements: *L* = 1,500–2,487  $\mu\text{m}$ ; *L'* = 1,226–1,928  $\mu\text{m}$ ; *a* = 30.6–40.2; *a'* = 20.0–28.7; *b* = 6.6–10.6; *b'* = 5.3–7.6; *c* = 3.2–5.5; *c'* = 8.8–19.6; *V* = 45.6–53.8; *V'* = 65.4–69.4 (Table 9).

Body cylindrical, with narrowed anterior end and long filiform tail. Somatic setae numerous, cylindrical, 8–12  $\mu\text{m}$  long, situated in 4 submedian longitudinal rows (except filiform part of tail). Cuticle dotted. Dots arranged in transverse rows. No lateral differentiation of cuticle observed. Small cuticular pores arranged irregularly along entire body length. Cuticle ca. 3.0–3.5  $\mu\text{m}$  thick. Six lips visible. Each lip possessing a pair of large rod-shaped cuticular rugae connecting in pairs at their distal ends. Labial sensilla papilloid; 4 cephalic setae cylindrical, 12–17  $\mu\text{m}$  long. Amphideal fovea ventrally coiled, single-spiral, round, 11–14  $\mu\text{m}$  wide. Anterior end of amphideal fovea situated ca. 1 c.b.d. from the anterior end. Two rows (latero-subdorsal and latero-subventral) of 3 cervical setae, 7–12  $\mu\text{m}$  long, located posterior to each amphideal fovea. Stoma consisting of cup-shaped cheilostoma and narrow esophastoma with thick cuticular walls. Cheilostoma containing 6 pairs of large, well discernible cylindrical rugae. Esophastoma 42–48  $\mu\text{m}$  long, possessing 2 very large sclerotized onchia, 1 dorsal and 1 presumably subventral. Basal parts of onchia situated in anterior part of esophastoma, and their apical parts intruding into cheilostoma. Dorsal onchium 17–19  $\mu\text{m}$  long, subventral one 22–24  $\mu\text{m}$  long. Pharynx cylindrical, narrowing in the first third. Numerous large plasmatic interruptions visible posterior to esophastomal region. Cardia small, cordate.

**Fig. 18** *Acantholaimus maks*. **a** juvenile, head region; **b** male, head with retracted anterior part; **c** male, spicule. Scale bar = 50  $\mu\text{m}$



Nerve ring not visible. Renetta not visible. A small intestinal cecum directed anteriorly and situated at level of posterior part of pharynx, visible in all specimens examined. Tail gradually narrowed into filiform, cylindrical terminal part constituting ca. 60% of entire tail length. Cellular bodies of caudal glands visible.

Male reproductive system monorchic. Testis directed anteriorly, outstretched, lying to right of intestine, occupying about 30% of pre-anal body length. Size of mature spermatids ca. 50–60 to 30–40  $\mu\text{m}$ . Curved funnel-shaped paired spicules, quite thick and possessing complicated cuticular sculpture with thickened cuticular areas in shape of longitudinal or transverse ridges. Gubernaculum blade-shaped, with narrow proximal end and wide bifurcated distal end. Supplementary organs not found.

Female reproductive system consisting of two antiodrous, uniformly sized ovaries (anterior ovary lying to right of intestine, and posterior one lying to left of intestine), occupying ca. 20% of pre-anal body length. Numerous, well developed vulvar glands with granular content surrounding short vagina seen.

#### Abundance

The density of *A. robustus* sp. n. varied from 0 to 3.6 inds/10  $\text{cm}^2$  (mean 0.8 inds/10  $\text{cm}^2$ ), and its relative abundance within the nematode community was 0 to 2.1% (mean 0.5%).

#### Differential diagnosis

*Acantholaimus robustus* sp. n. is characterized by its very large body size, two very large onchia, and thick, strongly

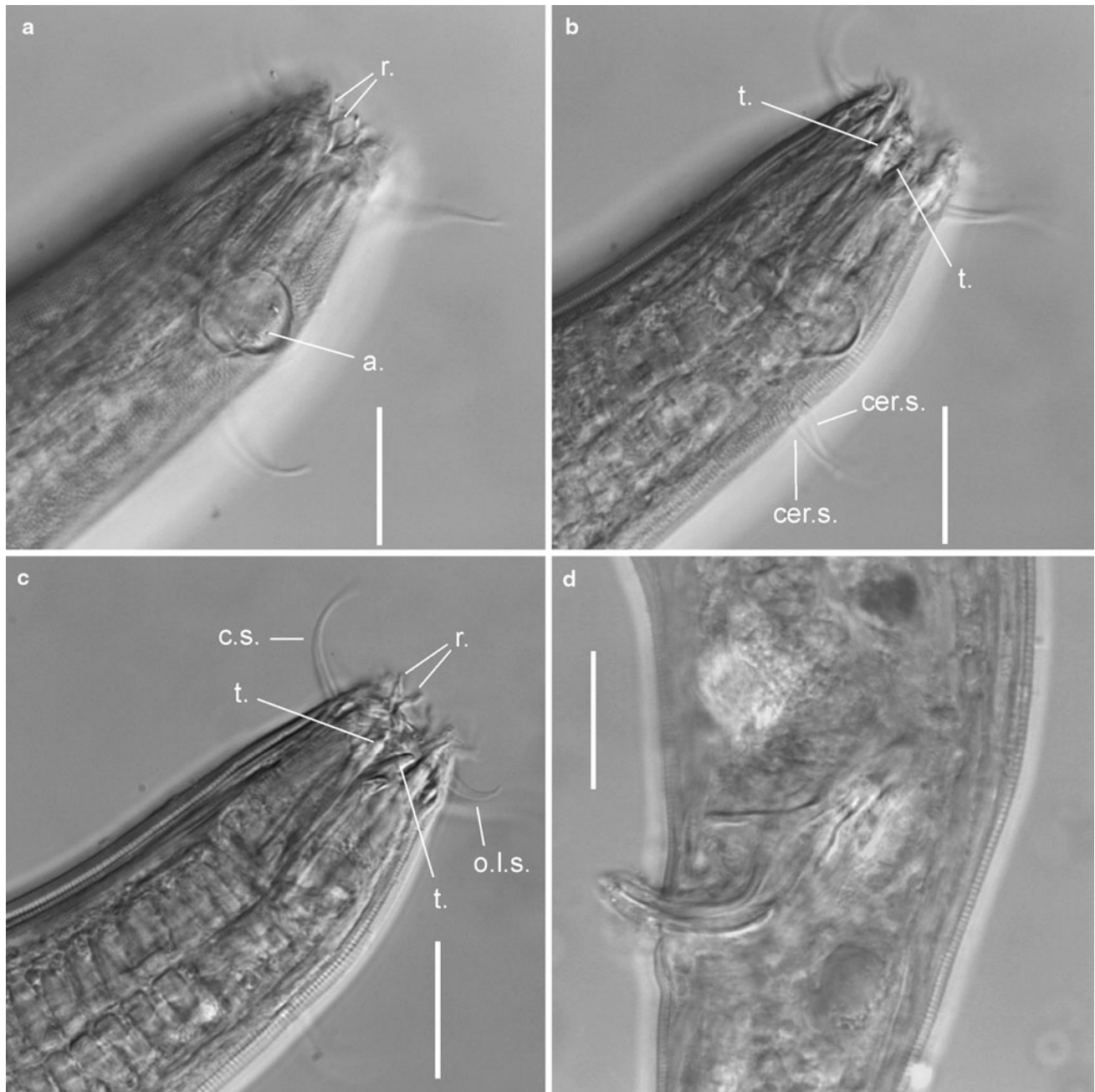
developed rugae. The cervical setae of the new species are grouped in threes.

There are only four equally large *Acantholaimus* species known: *A. arminius* Gerlach et al. 1979; *A. iubilus* Gerlach et al. 1979; *A. maks* Gerlach et al. 1979; and *A. quadridentatus* Jensen 1985.

*Acantholaimus robustus* sp. n. differs from *A. arminius* by its thicker body ( $a = 30\text{--}40$  vs. 53); the absence of lateral differentiation of the cuticle; the shorter cephalic setae (13–17  $\mu\text{m}$  vs. 21  $\mu\text{m}$ ); the arrangement and number of cervical setae (2 groups of 3 setae posterior to each amphideal fovea vs. 2 single setae in *A. arminius*); and the longer esophastoma (ca. 47–50  $\mu\text{m}$  vs. ca. 20  $\mu\text{m}$ ).

*Acantholaimus robustus* sp. n. differs from *A. quadridentatus* in many parameters, of which the most significant are: the amphid is farther from the anterior end (located ca. 1–1.5 amphidial c.b.d. from the anterior end vs. 0.2 c.b.d. in *A. quadridentatus*); the cephalic setae are shorter (12–17  $\mu\text{m}$  vs. 25  $\mu\text{m}$ ); the different shape of the outer labial sensilla (short and papilliform vs. long and setiform); and the much thicker spicules.

*Acantholaimus robustus* sp. n. is close to *A. maks* in the location of the amphids and in the length of the setae of the cephalic end. The new species differs from *A. maks* by the number of onchia (2 vs. 5–6 in *A. maks*); the much longer esophastoma (47–50  $\mu\text{m}$  vs. ca. 15  $\mu\text{m}$ ); the much thicker cuticular wall of the internal lumen of the esophastoma (this thick wall is easily distinguishable from the much thinner wall of the other, posterior part of the pharynx in *A. robustus*, whereas it does not differ from the other part of the pharynx in *A. maks*); the arrangement of the cervical setae (2 groups of 3 sparsely located setae posterior to each amphideal fovea



**Fig. 19** *Acantholaimus maks*, micrographs. **a–c** juvenile, head region at different optical levels; **d** male, spicule region. Scale bars = 20  $\mu$ m. Abbreviations: *cer.s.* = cervical seta, *c.s.* = cephalic seta, *i.l.s.* = inner labial seta, *o.l.s.* = outer labial seta, *r.* = ruga, *t.* = onchium

in *A. robustus* vs. 2 groups of 2 closely grouped setae in *A. maks*); and the shape of the spicules (thick vs. thin).

The new species shows the most resemblance to *A. iubilus* sp. n. in the size and location of the amphids, the size of the onchia, its very long esophastoma, and the very well developed rugae. The new species differs from *A. iubilus* by the length of the esophastoma (ca. 47–50  $\mu$ m vs. ca. 25  $\mu$ m in *A. iubilus*); the arrangement of the cervical setae (2 groups of 3 widely set setae posterior to each amphideal fovea in *A. robustus* sp. n. vs. 2 groups of 2 closely set

setae in *A. iubilus*); the number of onchia (2 onchia vs. 3 onchia supplemented by a pair of lateral cuticular laminae); and the shorter tail (22–31% of the entire body length vs. ca. 50%).

*Acantholaimus sieglerae* sp. n. (Figs. 25, 26; Table 10)

**Type material** Collection number MNHN-BN502. Holotype: male. Paratypes: 2 males and 2 females (Table 10).

**Locality** Tables 1, 10.

**Table 7** *Acantholaimus maks*

Specimen No.	1	2	3
Station	PL-1599/7	PL-1599/7	MTB-11
Slide number	5-29	5-29	1-60
Sex	m	f	j
<i>L</i>	2,028	n.a.	1,589
<i>L'</i>	1,744	1,767	1,204
Amphidial width	16	16	13
Amphidial length	13	12	13
Length of inner labial setae	n.a.	n.a.	5
Length of outer labial setae	n.a.	n.a.	9
Length of cephalic setae	n.a.	n.a.	22
Length of cervical setae	14	15	10–18
Length of somatic setae	12	12	10–13
Length of spicule in chord	46	–	–
Length of spicule in arc	53	–	–
Length of gubernaculum	19	–	–
Distance from anterior end to amphid	12	10	20
Diameter at level of cephalic setae	23	27	22
Diameter at level of middle of amphid	26	33	34
Diameter at level of cardia	63	68	53
Diameter at level of anus	44	42	35
Maximum body diameter	75	90	72
<i>a</i>	27.0	n.a.	22.0
<i>b</i>	10.1	n.a.	7.6
<i>c</i>	7.1	n.a.	4.1
<i>V</i>	–	n.a.	–
<i>a'</i>	23.3	19.6	16.7
<i>b'</i>	8.7	8.7	5.8
<i>c'</i>	6.5	n.a.	11.0
<i>V'</i>	–	81.4	–
Notes	Retracted head	Broken tail, retracted head	

Number of stations where specimens were found, measurements (in  $\mu\text{m}$ ) and body indices

**Etymology** In honor of Viola Siegler (German Centre for Marine Biodiversity Research, Senckenberg am Meer, Wilhelmshaven, Germany).

### Description

Main measurements:  $L = 457\text{--}668 \mu\text{m}$ ;  $L' = 360\text{--}443 \mu\text{m}$ ;  $a = 19.9\text{--}32.7$ ;  $a' = 15.7\text{--}25.2$ ;  $b = 5.6\text{--}6.1$ ;  $b' = 3.9\text{--}4.7$ ;  $c = 2.8\text{--}4.7$ ;  $c' = 7.5\text{--}13.9$ ;  $V = 52.8\text{--}54.3$ ;  $V' = 67.8\text{--}68.9$  (Table 10).

Body cylindrical, with slightly tapered anterior end and gradually narrowed tail. Maximum body width at posterior

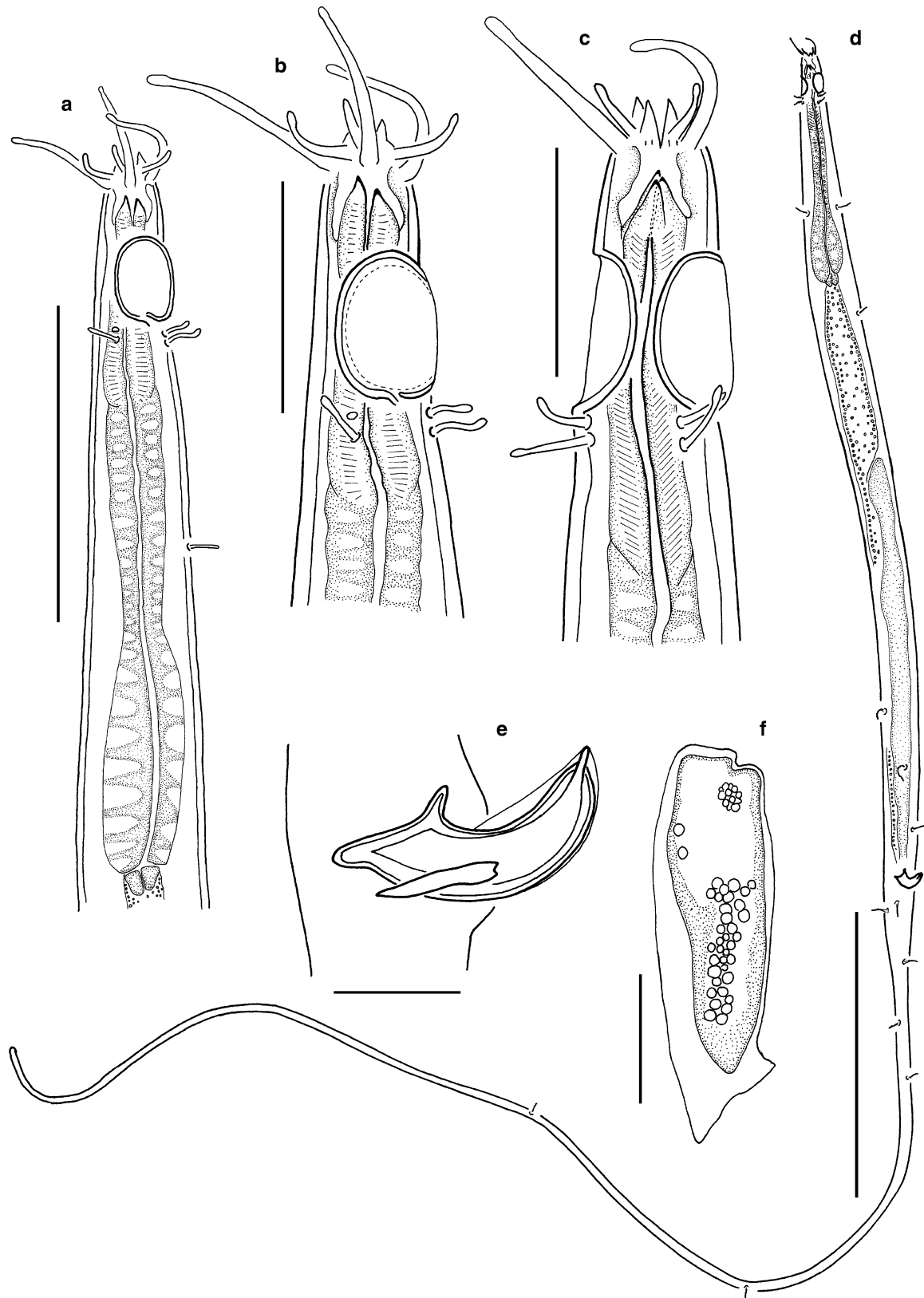
end of pharynx. Somatic setae numerous, arranged in 4 submedian longitudinal rows (except on filiform part of tail), 8–10  $\mu\text{m}$  long. These setae cylindrical, sometimes with slightly widened tips. Cuticle densely dotted. Dots arranged in regular transverse rows. Lateral field narrow; dots appearing larger there but also arranged in transverse rows. Numerous irregularly arranged pores visible at level of cervical region. Cuticle ca. 1.5–1.7  $\mu\text{m}$  thick. Lips not visible. Sensilla of cephalic end arranged in 3 rings: 6 short inner labial papilloid sensilla ca. 0.7  $\mu\text{m}$  long; 6 short, outer labial setae 1.5–3.5  $\mu\text{m}$  long; and 4 longer, cylindrical cephalic setae 7–10  $\mu\text{m}$  long. In several specimens, outer labial setae appearing jointed, consisting of two segments. Amphideal fovea ventrally coiled, single-spiral, in shape of transversely oriented oval, 5–9  $\mu\text{m}$  wide, with distinct cuticular edging and internal fine concentric striation. In females, amphideal fovea smaller in diameter than in males. Anterior end of amphideal fovea situated ca. 0.7–1 c.b.d. from the anterior end. Two pairs (latero-subdorsal and latero-subventral) of cervical setae 7–8  $\mu\text{m}$  long, situated just behind each amphideal fovea. Stoma large. Cheilostoma barrel-shaped, bearing 12 indistinct cuticular rugae. Esophastoma narrow, ca. 8  $\mu\text{m}$  long, with thick cuticular walls, possessing six sclerotized onchia. Right subventral and dorsal onchia are larger and more robust (ca. 2–3  $\mu\text{m}$  long) and easily distinguishable. Basal parts of onchia situated in anterior part of esophastoma, and their apical parts intruding into cheilostoma. Pharynx cylindrical, with slight bulb-like widening at its distal end. Numerous cytoplasmic inclusions visible along entire length of pharynx. Nerve ring not visible. Renetta not visible. Cardia triangular or oval. Tail elongated-conical in its anterior part and gradually narrowing into thin cylindrical terminal part constituting ca. 50–70% of entire tail length.

Male reproductive system monorchic. Testis directed anteriorly, outstretched, lying to right of intestine, occupying 37–51% of pre-anal body length. Size of mature spermatids ca. 30  $\times$  11  $\mu\text{m}$ . Curved funnel-shaped paired spicules possessing cuticular sculpture with thickened cuticular areas in shape of longitudinal or transverse ridges. Gubernaculum a thin, slightly curved rod, with bifurcated distal end. Supplementary organs not found.

Female reproductive system consisting of two antiodromous, uniformly sized ovaries (anterior ovary lying to right of intestine, and posterior one lying to left of intestine), occupying 33–38% of pre-anal body length.

### Abundance

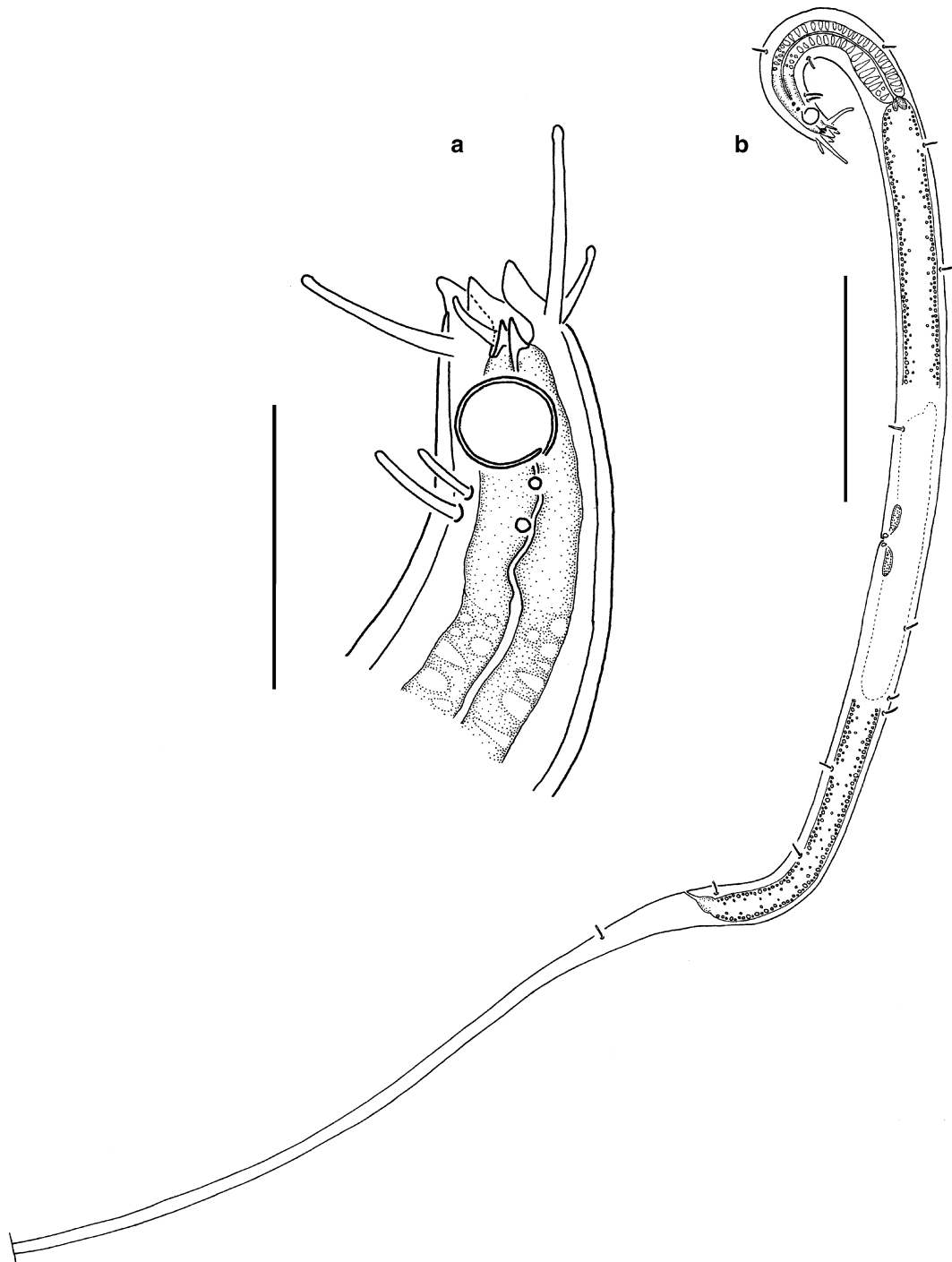
The density of *A. sieglerae* sp. n. was 0–2.0 inds/10  $\text{cm}^2$  (0.1 inds/10  $\text{cm}^2$  on average), and its relative abundance within the nematode community was 0–2.5% (mean 0.2%).



**Fig. 20** *Acantholaimus occultus*, males. **a** specimen No. 1, anterior end; **b** specimen No. 1, head region; **c** specimen No. 2, head region; **d** specimen No. 2, total view; **e** specimen No. 1, cloacal region with

spicule; **f** specimen No. 1, spermatid. Scale bars: **a** = 50  $\mu$ m; **b**, **c** = 20  $\mu$ m; **d** = 200  $\mu$ m; **e**, **f** = 10  $\mu$ m





**Fig. 21** *Acantholaimus occultus*, female. **a** head region; **b** total view. Scale bars: **a** = 20  $\mu\text{m}$ ; **b** = 100  $\mu\text{m}$

#### Differential diagnosis

*Acantholaimus sieglerae* sp. n. is a comparatively small species, and at the same time it possesses very large onchia. The latter feature is not characteristic for small *Acantholaimus* species.

The new species shows the most resemblance to *A. gathumai* Muthumbi and Vincx 1997 and to *A. incomptus*

Vivier 1985 in the body length and maximum diameter, the length of the cephalic setae, and the location of the amphids (ca. 1 amphidial c.b.d. from the anterior end).

*Acantholaimus sieglerae* sp. n. differs from *A. gathumai* by the shape of the cephalic end (cylindrical, with a sharply truncated end, vs. triangular, with a gradually narrowing end in *A. gathumai*), and by its larger onchia.

**Table 8** *Acantholaimus occultus*

Specimen No.	1	2	3
Station	MTB-16	MTB-16	MTB-16
Slide number	1-3	1-7	2-54
Sex	m	m	f
$L$	n.a.	1,546	n.a.
$L'$	560	592	532
Amphidial width	8	9	7
Amphidial length	14	15	9
Length of outer labial setae	6	5	5
Length of cephalic setae	17	16	14
Length of cervical setae	6	6	7
Length of somatic setae	6	6	7
Length of spicule in chord	25	22	–
Length of spicule in arc	31	31	–
Length of gubernaculum	11	10	–
Distance from anterior end to amphid	11	10	10
Diameter at level of cephalic setae	7	8	7
Diameter at level of middle of amphid	11	12	11
Diameter at level of cardia	20	23	20
Diameter at level of anus	14	16	14
Maximum body diameter	22	24	23
$a$	n.a.	64.4	n.a.
$b$	n.a.	11.0	n.a.
$c$	n.a.	1.6	n.a.
$V$	–	–	n.a.
$a'$	25.5	24.7	23.1
$b'$	4.8	4.2	4.9
$c'$	36.1	59.6	n.a.
Number of stations where specimens were found, measurements (in $\mu\text{m}$ ) and body indices	$V'$	–	60.3
Notes	Broken tail		Broken tail

*Acantholaimus sieglerae* sp. n. differs from *A. incomptus* by its larger amphids (diameter of amphids ca. 50–69% of amphidial c.b.d. vs. 45–51% in *A. incomptus*); the shape of the tail (gradually narrowing tail vs. tail with sharply distinguishable conical and flagelliform parts in *A. incomptus*); and its larger onchia.

*Acantholaimus tchesinovi* sp. n. (Figs. 27, 28, 29; Table 11)

**Type material** Collection number MNHN-BN503. Holotype: male. Paratypes: 1 male and 1 female (Table 11).

**Locality** Tables 1, 11.

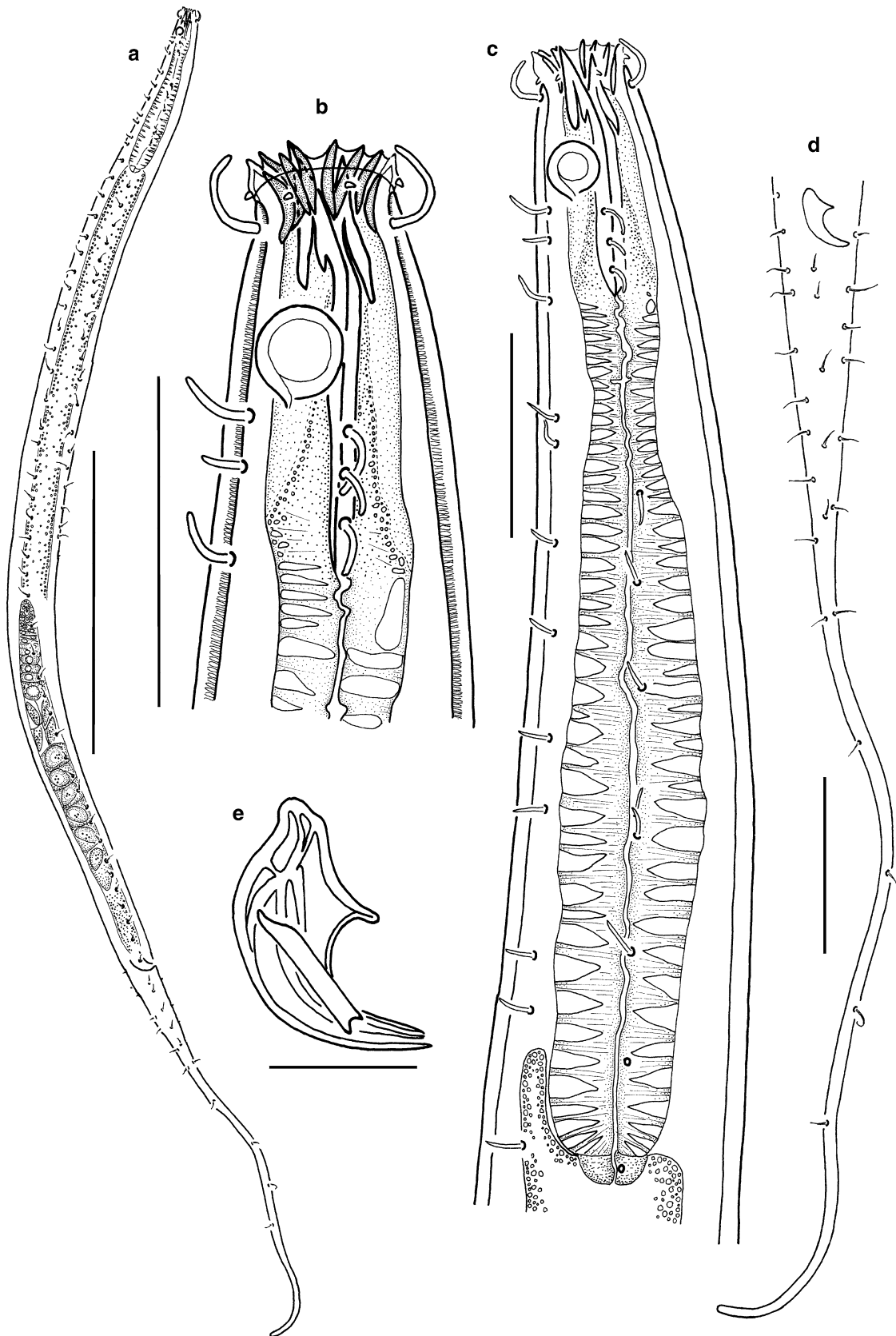
**Etymology** In honor of Prof. Dr. A. V. Tchesunov (Lomonosov's Moscow State University, Moscow, Russia).

#### Description

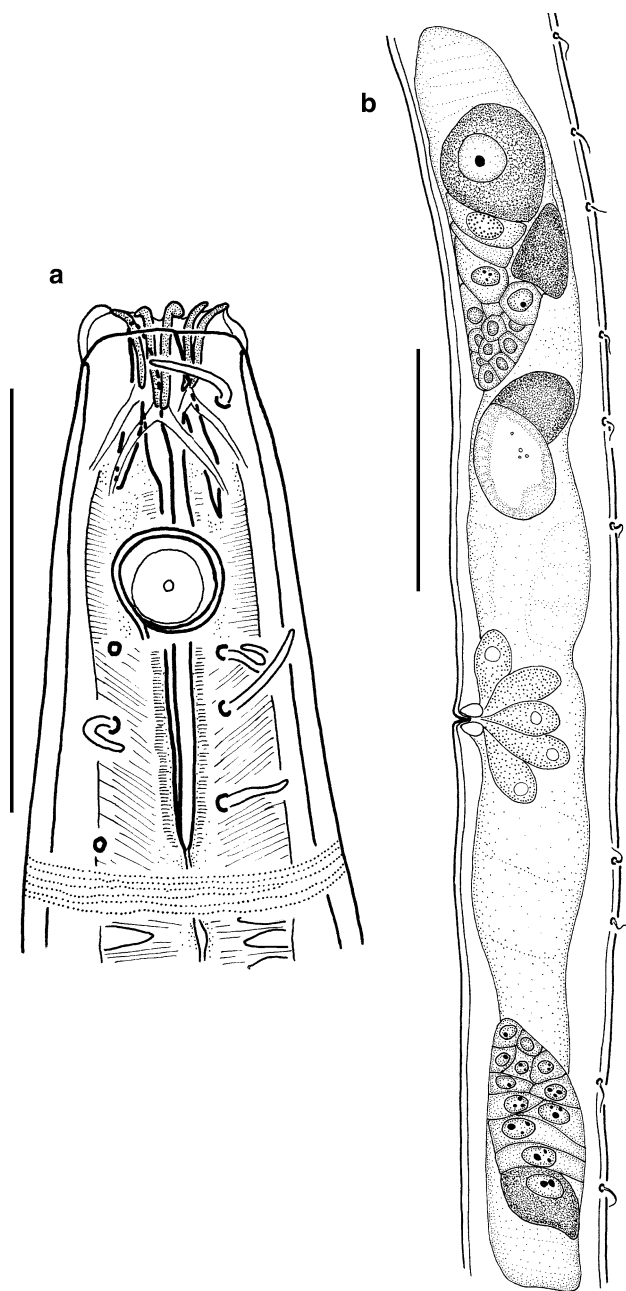
Main measurements:  $L = 707\text{--}750\ \mu\text{m}$ ;  $L' = 474\text{--}536\ \mu\text{m}$ ;  $a = 21.4\text{--}26.2$ ;  $a' = 15.3\text{--}19.2$ ;  $b = 7.1\text{--}7.4$ ;

$b' = 5.0\text{--}5.1$ ;  $c = 3.0\text{--}3.5$ ;  $c' = 10.7\text{--}12.9$ ;  $V = 52.0$ ;  $V' = 72.8$  (Table 11).

Body cylindrical, with slightly and gradually narrowed anterior end, and tail consisting of proximal conical and long, thin cylindrical parts. Somatic setae numerous, arranged in 4 submedian longitudinal rows (except filiform part of tail where they are arranged irregularly), 6–8  $\mu\text{m}$  long at level of pharynx, 4–6  $\mu\text{m}$  long at mid-body, and 6–8  $\mu\text{m}$  long at caudal part. These setae cylindrical, sometimes with slightly widened tips, on entire pre-anal part of body, but clavate on caudal part. Somatic setae thicker at level of pharynx than on remainder of body. Numerous cuticular pores alternating irregularly with somatic setae in 4 longitudinal rows. Cuticle densely dotted, with lateral fields, beginning posterior to amphideal fovea and extending along entire body except filiform part of tail. Dots of lateral fields appearing larger and distributed more sparsely than median ones; lateral dots arranged in clearly visible, longitudinal rows. Four such rows located closely posterior to amphideal fovea; one additional, slightly posterior row; and 1 or 2 additional rows at level of



**Fig. 22** *Acantholaimus robustus* sp. n., males. **a** holotype, total view; **b** holotype, head region; **c** holotype, anterior end; **d** holotype, tail region; **e** paratype No. 1, spicule. Scale bars: **a** = 500  $\mu$ m; **b**, **c** = 50  $\mu$ m; **d** = 100  $\mu$ m; **e** = 20  $\mu$ m



**Fig. 23** *Acantholaimus robustus* sp. n., paratype No. 3, female. **a**: head region; **b**: reproductive system. Scale bars: **a** = 50  $\mu$ m; **b** = 100  $\mu$ m

cardia, for a total of 6–7 longitudinal lateral rows extending from beginning of intestine to approximately middle of conical part of tail. Median dots smaller, arranged more densely and in transverse rows. In optical cross-section of cuticle, dots discernible as tiny radial struts. Cuticle ca. 2–3  $\mu$ m thick at mid-body level and 1–1.5  $\mu$ m thick at pharyngeal and caudal levels. Lips not visible. Sensilla of cephalic end arranged in 3 rings: 6 short inner labial papilloid sensilla ca. 1  $\mu$ m long; 6 short, 2-jointed, outer labial setae 3–4  $\mu$ m long; and 4 longer, clavate, cephalic setae ca. 12  $\mu$ m

long. Amphideal fovea ventrally coiled, single-spiral, round, 6–8  $\mu$ m wide, with distinct cuticular edging, situated ca. 1.2 c.b.d. from anterior end. Two pairs (latero-subdorsal and latero-subventral) of cervical setae 6–7  $\mu$ m long, located at level of posterior part of amphideal fovea. Cheilostoma barrel-shaped, containing 12 cylindrical rugae; esophastoma narrow, 5–8  $\mu$ m long, with thickened cuticular walls of internal pharyngeal lumen, and possessing at least 4 sclerotized onchia. Basal parts of onchia situated in anterior part of esophastoma, and their apical parts intruding into cheilostoma. One larger dorsal and one larger subventral onchia ca. 1.5  $\mu$ m long, more distinguishable than remaining onchia. Pharynx gradually widening to its posterior end, with numerous cytoplasmic inclusions along its entire length. Nerve ring not visible. Renetta not visible. Cardia round. Tail conical in its anterior part, and with thin cylindrical terminal part constituting ca. 70–75% of total tail length.

Male reproductive system monorchic. Testis directed anteriorly, outstretched, lying to right of intestine, occupying about 0.4–0.55 of pre-anal body length. Size of mature spermatids ca. 38  $\times$  16  $\mu$ m. Curved funnel-shaped paired spicules possessing cuticular sculpture with thickened cuticular areas forming longitudinal or transverse ridges. Gubernaculum blade-shaped, with narrow proximal end and wide bifurcated distal end. Supplementary organs not found.

Female reproductive system consisting of two antidromous, uniformly sized ovaries (anterior ovary lying to right of intestine, and posterior ovary lying to left of intestine). In single female examined, one large spermatozoon ca. 40  $\times$  25  $\mu$ m in anterior part of uterus, and presumably outgoing fertilized egg at early stage of division in posterior part of uterus present. Vulvar glands surrounding vulva.

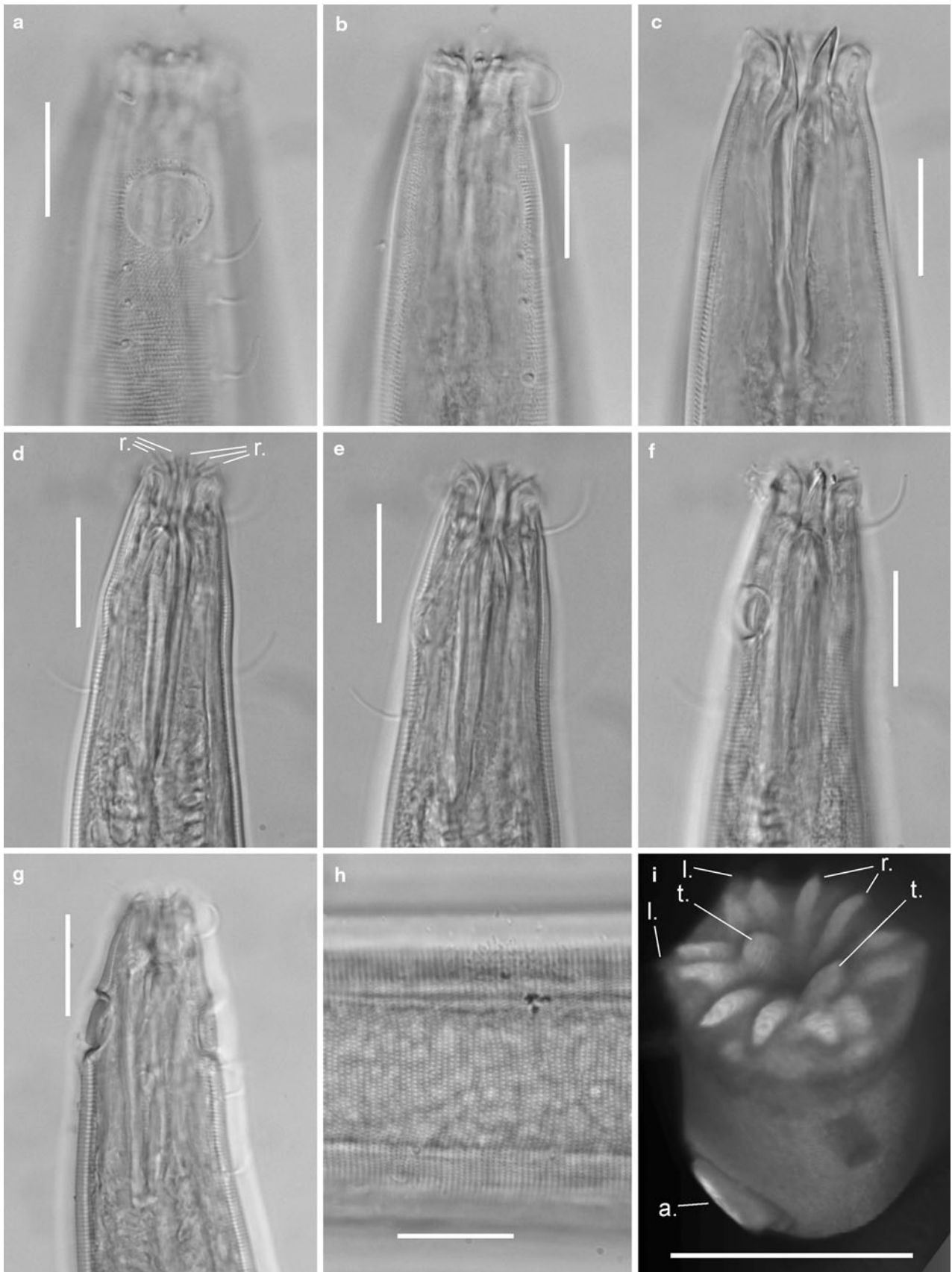
#### Abundance

The density of *A. tchesunovi* sp. n. was 0–2.5 inds/10  $\text{cm}^2$  (0.3 inds/10  $\text{cm}^2$  on average), and its relative abundance in the nematode community was 0–2.3% (mean 0.3%).

#### Differential diagnosis

The new species shares with *A. calathus* Gerlach et al. 1979; *A. ewensis* Platt and Zhang 1982; *A. polydentatus* Gerlach 1951; and *A. pilosus* Kreis 1963 the lateral differentiation of the cuticle in the form of regular longitudinal rows of larger punctuations. However, all these

**Fig. 24** *Acantholaimus robustus* sp. n. **a–c** male, paratype, head region at different optical levels; **d–f** juvenile, paratype No. 5, head region at different optical levels; **g** male, paratype No. 2, head region; **h** female, paratype No. 3, head; **i** male, paratype, head region, reconstruction based on micrographs made using a confocal microscope. Scale bars = 20  $\mu$ m. Abbreviations: *a.* = amphid, *cer.s.* = cervical seta, *l.* = lip, *r.* = ruga, *t.* = onchium



**Table 9** *Acantholaimus robustus* sp. n.

Collection status	Holotype	Paratype No. 1	Paratype No. 2	Paratype No. 3	Paratype No. 4	Paratype No. 5
Station	MTB-7	PL-1597/05	MTB-7	MTB-7	MTB-7	MTB-7
Slide number	1-90(1)	4-24	11-96	1-80	11-31	1-79
Sex	m	m	m	f	f	j
<i>L</i>	2,230	n.a.	2,332	2,487	2,298	1,500
<i>L'</i>	1,537	1,280	1,667	1,928	1,603	1,226
Amphidial diameter	13	11	14	12	14	10
Length of outer labial setae	1	n.a.	2	n.a.	n.a.	2
Length of cephalic setae	17	17	13	n.a.	12	13
Length of cervical setae	12	10	11	12	7	11
Length of somatic setae	10	8	10	12	10	11
Length of spicule in chord	n.a.	53	n.a.	–	–	–
Length of spicule in arc	n.a.	68	n.a.	–	–	–
Length of gubernaculum	n.a.	23	n.a.	–	–	–
Distance from anterior end to amphid	20	21	20	25	20	18
Diameter at level of cephalic setae	21	19	17	20	17	20
Diameter at level of middle of amphid	27	27	26	29	28	24
Diameter at level of cardia	58	60	51	68	58	43
Diameter at level of anus	48	40	34	48	42	31
Maximum body diameter	69	64	58	75	63	49
<i>a</i>	32.3	n.a.	40.2	33.2	36.5	30.6
<i>b</i>	8.4	n.a.	10.6	8.3	9.7	6.6
<i>c</i>	3.2	n.a.	3.5	4.4	3.3	5.5
<i>V</i>	–	–	–	53.8	45.6	–
<i>a'</i>	22.3	20.0	28.7	25.7	25.4	25.0
<i>b'</i>	5.8	5.3	7.6	6.4	6.7	5.4
<i>c'</i>	14.4	n.a.	19.6	11.6	16.5	8.8
<i>V'</i>	–	–	–	69.4	65.4	–
Note		Broken tail				

Number of stations where specimens were found, their collection status, measurements (in  $\mu\text{m}$ ), and body indices

species possess 2 rows of larger dots vs. 4–6 rows in *A. tchesunovi* sp. n. In addition, the lateral differentiation begins at the level of the bulb in *A. calathus*, *A. ewensis*, and *A. polydentatus*. In *A. pilosus* the rows of dots begin just behind the cephalic setae. In contrast, in the new species the lateral differentiation begins behind the amphideal fovea.

Besides these differences, *A. tchesunovi* sp. n. differs from *A. polydentatus* and *A. pilosus* by the position of the amphideal fovea (about 1.8 head diameter from the anterior end vs. 0.5 head diameter from the anterior end, just behind the cephalic setae); the presence of outer labial setae (vs. papillae); and the length of the somatic setae (5–7  $\mu\text{m}$  vs. 9–13.5  $\mu\text{m}$  in *A. pilosus* and up to 30  $\mu\text{m}$  in *A. polydentatus*).

*Acantholaimus tchesunovi* sp. n. differs from *A. ewensis* by the position of the amphideal fovea (about 1.8 head diameter from the anterior end vs. less than 0.5

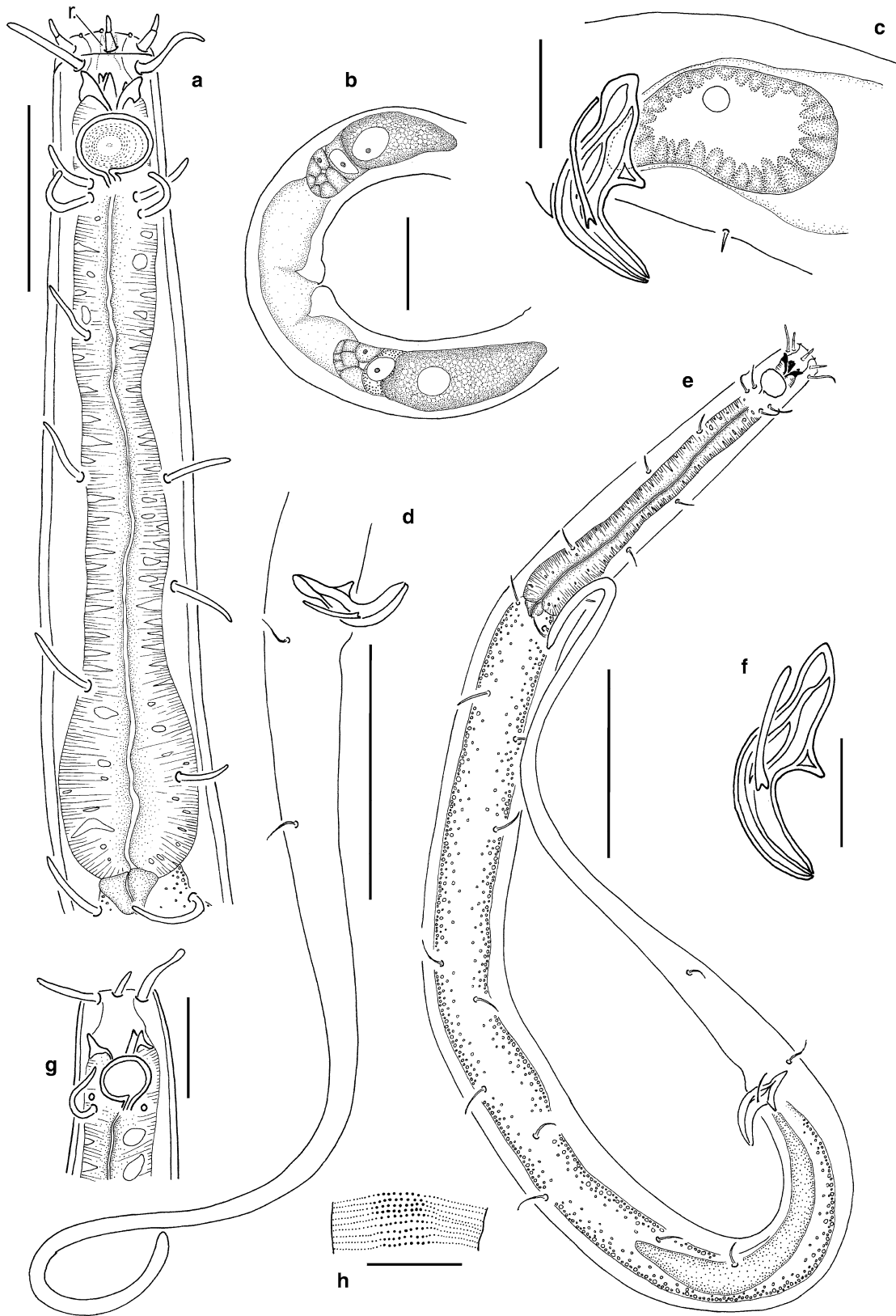
head diameter from the anterior end, just behind the cephalic setae); by the shorter cephalic setae (12  $\mu\text{m}$  vs. 30–33  $\mu\text{m}$ ); and the shorter somatic setae (5–7  $\mu\text{m}$  vs. 15–40  $\mu\text{m}$ ).

The new species is closer to *A. calathus* in the body size and shape, the tail shape, the position and size of the amphideal fovea, and the length of the cephalic setae. *A. tchesunovi* sp. n. differs from *A. calathus* by the thicker body ( $a = 21$ –26 vs.  $a = 35$ ), the longer pharynx ( $b = 7$ –7.4 vs.  $b = 8$ –9), and the shorter somatic setae (5–7  $\mu\text{m}$  vs. 4–27  $\mu\text{m}$ ).

*Acantholaimus veitkoehlerae* sp. n. (Figs. 30, 31, 32, 33; Table 12)

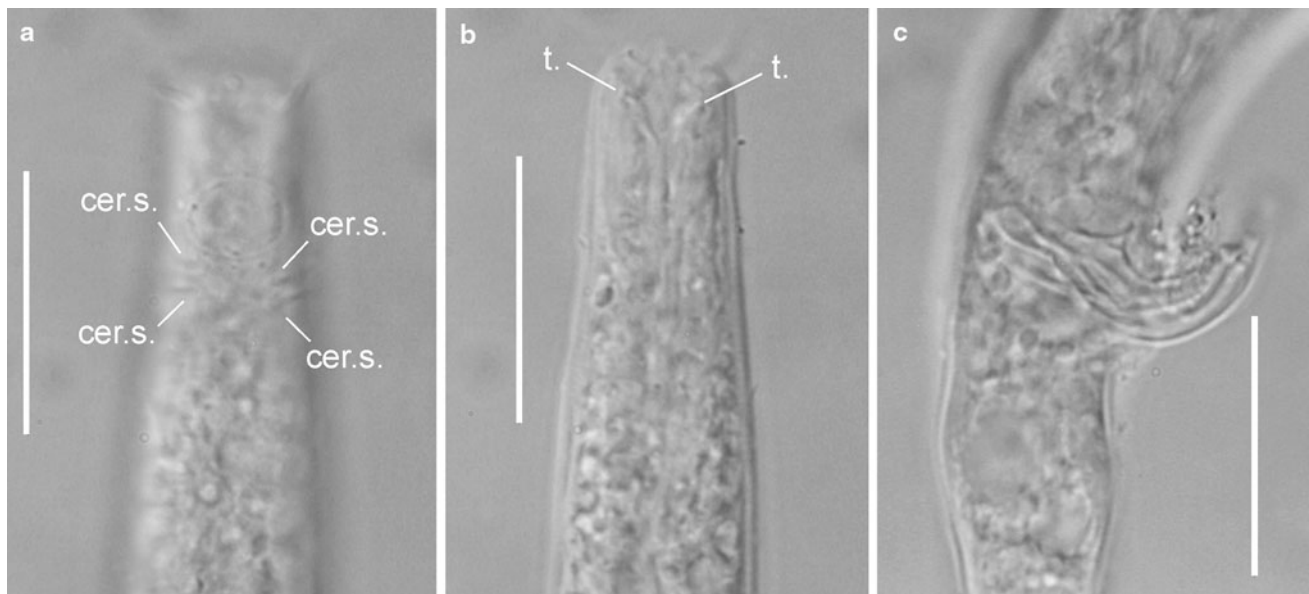
**Type material** Collection number MNHN-BN504. Holotype: male. Paratypes: 4 males and 7 females (Table 12).

**Locality** Tables 1, 12.



**Fig. 25** *Acantholaimus sieglerae* sp. n. **a** male, holotype, anterior end; **b** female, paratype No. 4, reproductive system; **c** male, paratype No. 2, spicule region; **d** holotype, tail region; **e** holotype, total view; **f** holotype, spicule; **g** paratype No. 4, head region; **h** paratype No. 4,

lateral differentiation of cuticular punctation. Scale bars: **a**, **b** = 20  $\mu$ m; **c**, **f**, **g**, **h** = 10  $\mu$ m; **d**, **e** = 50  $\mu$ m. Abbreviation: *r.* = a pair of rugae



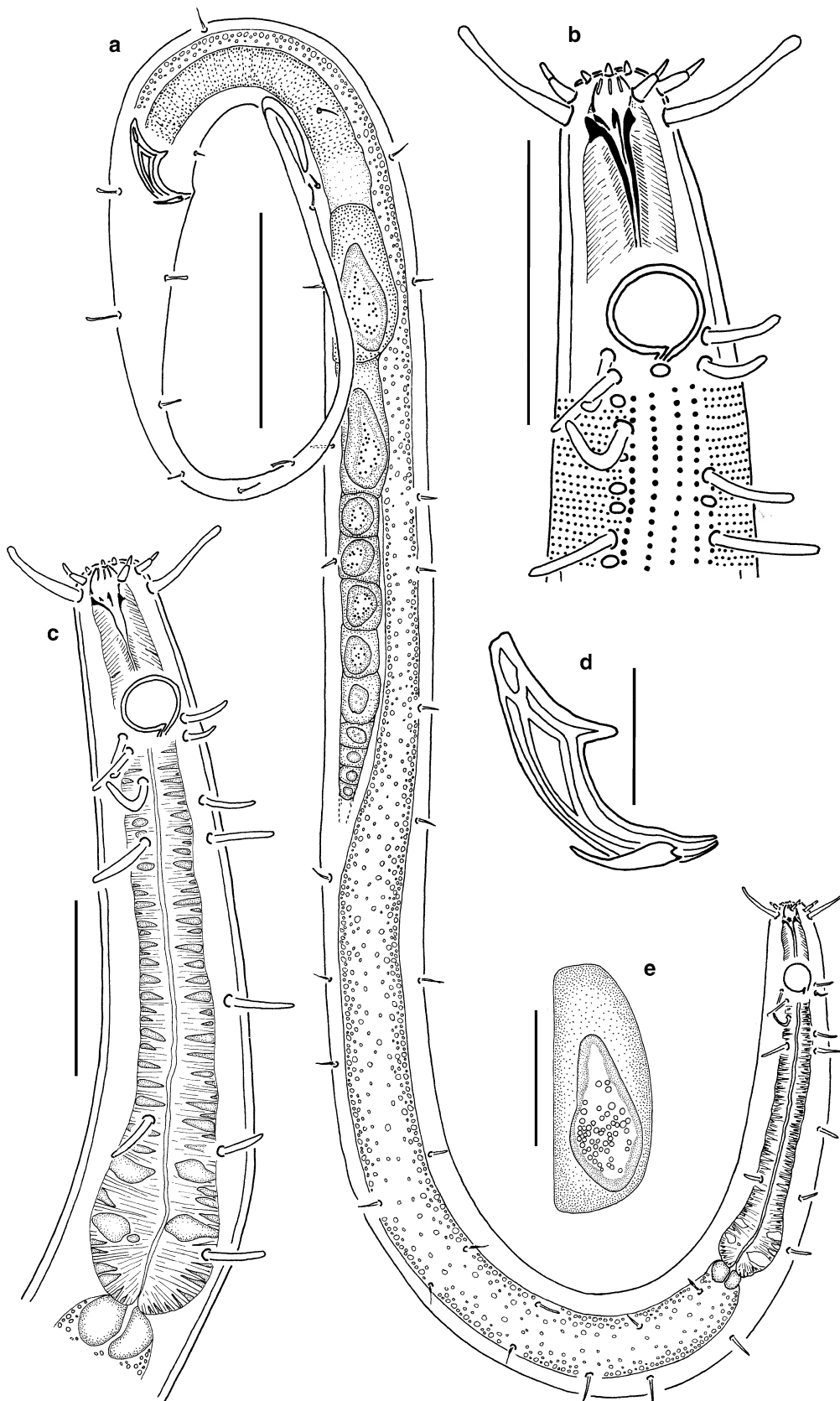
**Fig. 26** *Acantholaimus sieglerae* sp. n., male, holotype, micrographs. **a, b** head region at different optical levels; **c** spicule region. Scale bars = 20  $\mu$ m. Abbreviations: *cer.s.* = cervical seta, *t.* = onchium

**Table 10** *Acantholaimus sieglerae* sp. n.

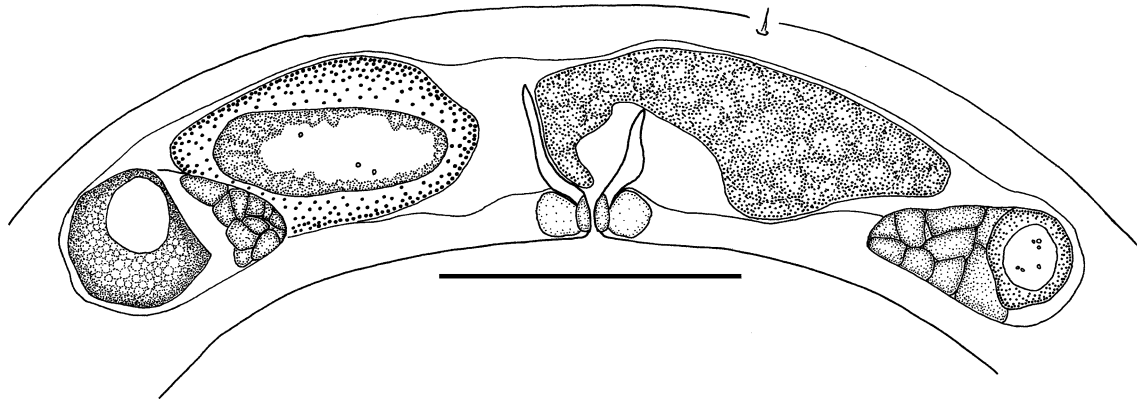
Collection status	Holotype	Paratype No.1	Paratype No.2	Paratype No.3	Paratype No.4
Station	MTB-1	PL-1599/7	PL-1599/7	PL-1599/7	PL-1599/7
Slide number	1-34	5-33	5-50	6-36	6-17
Sex	m	m	m	f	f
<i>L</i>	592	556	668	457	542
<i>L'</i>	384	429	443	360	422
Amphidial width	8	6	9	6	5
Amphidial length	7	4	8	4	4
Length of inner labial setae	0.7	0.7	n.a.	n.a.	n.a.
Length of outer labial setae	3.5	2	n.a.	n.a.	1.5
Length of cephalic setae	10	8	n.a.	n.a.	7
Length of cervical setae	8	8	n.a.	n.a.	7
Length of somatic setae	8	8	10	8	9
Length of spicule in chord	23	15	22	–	–
Length of spicule in arc	28	21	30	–	–
Length of gubernaculum	9	n.a.	9	–	–
Distance from anterior end to amphid	9	11	n.a.	7	7
Diameter at level of cephalic setae	10	10	10	8	8
Diameter at level of middle of amphid	12	11	13	10	10
Diameter at level of cardia	18	16	22	16	16
Diameter at level of anus	16	12	20	13	11
Maximum body diameter	23	17	26	23	21
<i>a</i>	25.7	32.7	25.7	19.9	25.8
<i>b</i>	6.0	6.1	6.1	5.6	6.0
<i>c</i>	2.8	4.4	3.0	4.7	4.5
<i>V</i>	–	–	–	54.3	52.8
<i>a'</i>	16.7	25.2	17.0	15.7	20.1
<i>b'</i>	3.9	4.7	4.0	4.4	4.7
<i>c'</i>	13.0	10.6	11.3	7.5	10.9
<i>V'</i>	–	–	–	68.9	67.8

Number of stations where specimens were found, their collection status, measurements (in  $\mu$ m), and body indices

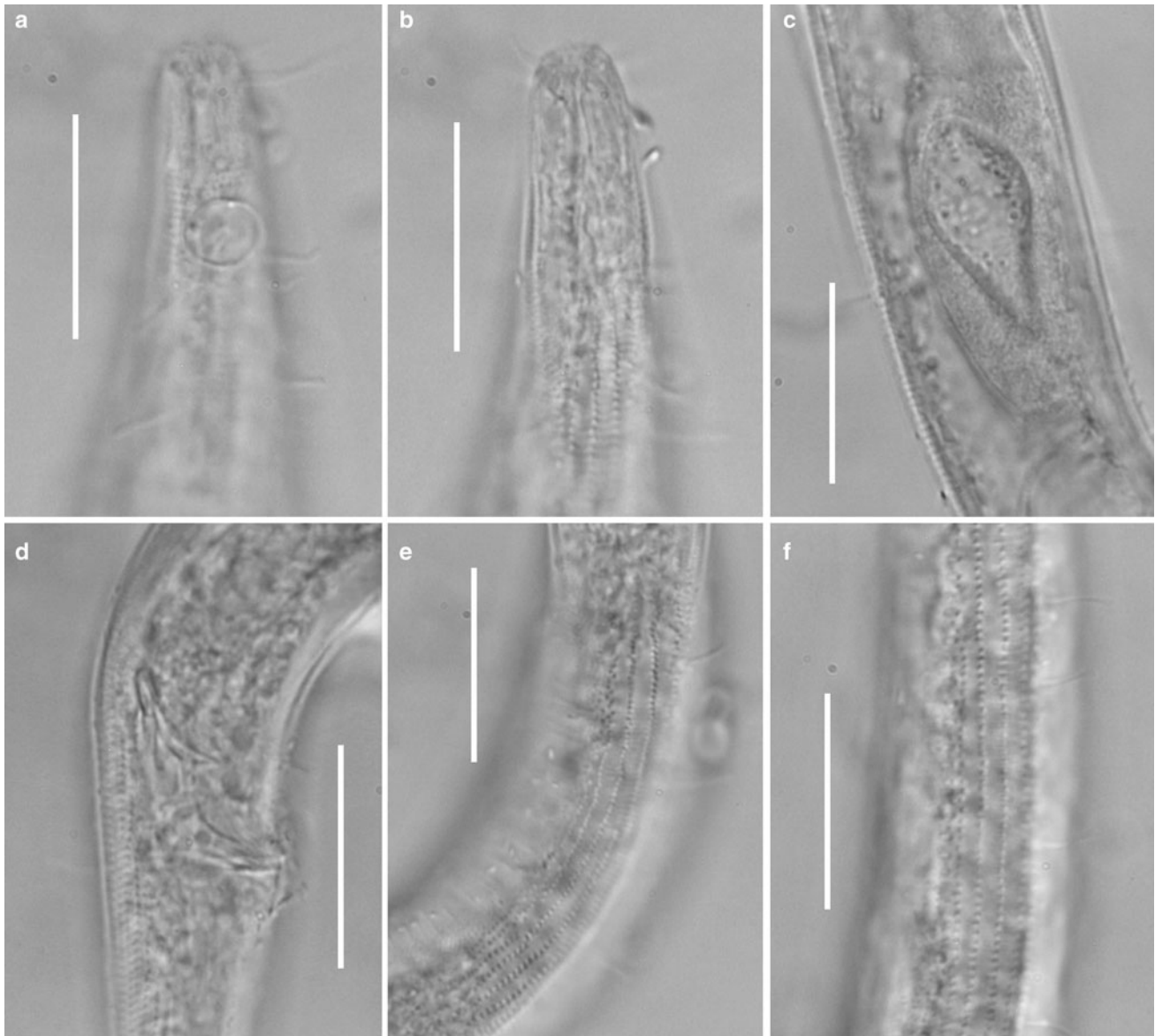




**Fig. 27** *Acantholaimus tchesunovi* sp. n., holotype. **a** total view; **b** head region; **c** anterior end; **d** spicule; **e** spermatid. Scale bars: **a** = 50  $\mu$ m; **b**, **c**, **e** = 20  $\mu$ m; **d** = 10  $\mu$ m



**Fig. 28** *Acantholaimus tchesunovi* sp. n., paratype No. 2, female reproductive system. Scale bar = 40  $\mu$ m



**Fig. 29** *Acantholaimus tchesunovi* sp. n., micrographs. **a, b** male, holotype, head region at different optical levels; **c** holotype, spermatid; **d** holotype, spicule region; **e** holotype, lateral differentiation of

cuticle at mid-body; **f** male, paratype No. 1, lateral differentiation of cuticle at mid-body. Scale bars = 20  $\mu$ m

**Table 11** *Acantholaimus tchesunovi* sp. n.

Collection status	Holotype	Paratype No. 1	Paratype No. 2
Station	MTB-8	MTB-3	MTB-6
Slide number	2-81	2-25	2-33
Sex	m	m	f
<i>L</i>	707	n.a.	750
<i>L'</i>	474	518	536
Amphidial diameter	8	7	6
Length of inner labial setae	1	1	n.a.
Length of outer labial setae	4	3	n.a.
Length of cephalic setae	12	12	n.a.
Length of cervical setae	7	7	n.a.
Length of somatic setae	5–7	6–7	n.a.
Length of spicule in chord	23	n.a.	–
Length of spicule in arc	28	32	–
Length of gubernaculum	9	n.a.	–
Diameter at level of cephalic setae	8	7	n.a.
Distance from anterior end to amphid	16	14	n.a.
Diameter at level of middle of amphid	13	12	12
Diameter at level of cardia	22	22	24
Diameter at level of anus	18	18	20
Maximum body diameter	27	27	35
<i>a</i>	26.2	n.a.	21.4
<i>b</i>	7.4	n.a.	7.1
<i>c</i>	3.0	n.a.	3.5
<i>V</i>	–	–	52.0
<i>a'</i>	17.6	19.2	15.3
<i>b'</i>	5.0	5.1	5.1
<i>c'</i>	12.9	n.a.	10.7
<i>V'</i>	–	–	72.8
Note		Broken tail	Retracted head

Number of stations where specimens were found, their collection status, measurements (in  $\mu\text{m}$ ), and body indices

**Etymology** In honor of Dr. Gritta Veit-Köhler [Senckenberg am Meer, German Centre for Marine Biodiversity Research (DZMB), Wilhelmshaven, Germany].

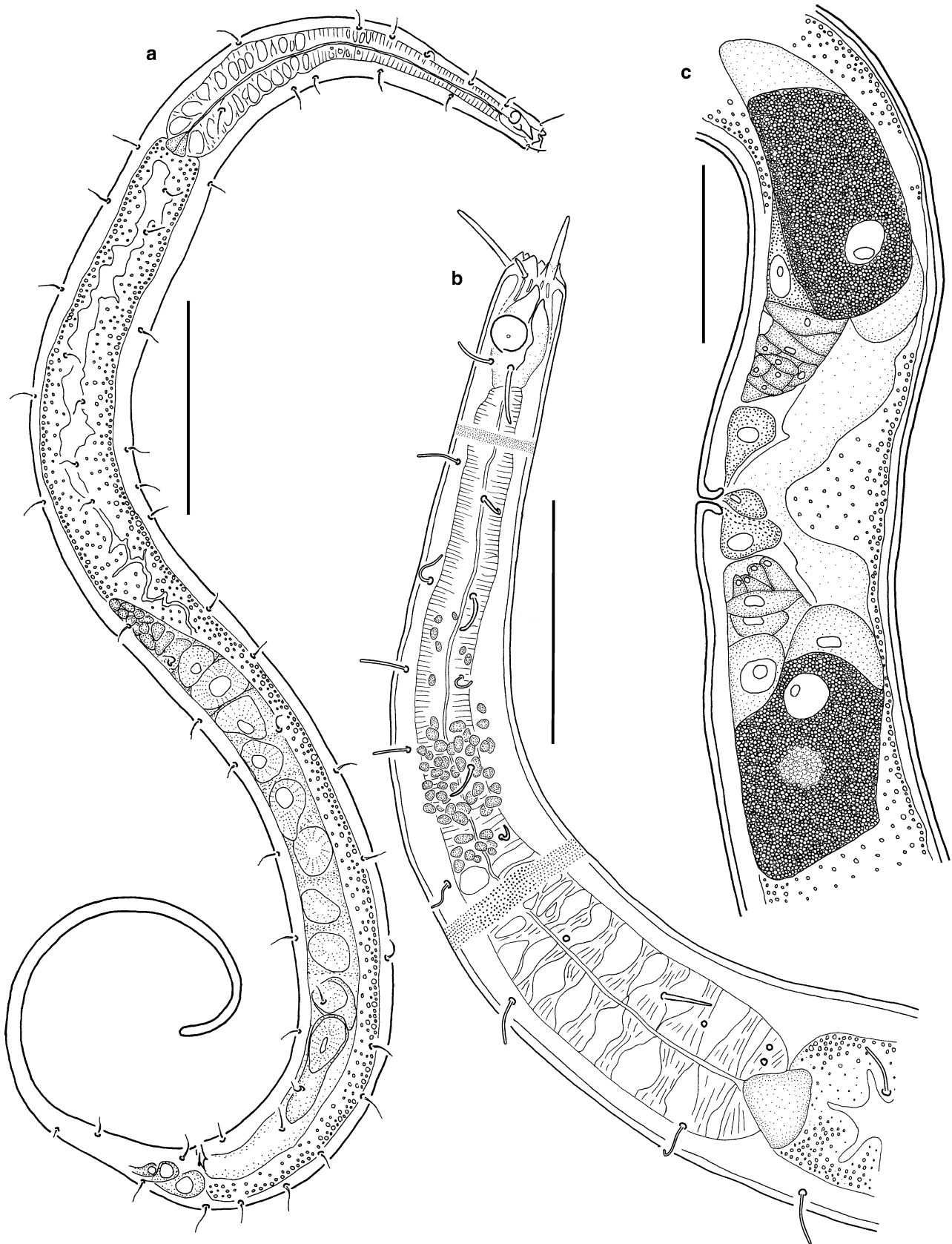
### Description

Main measurements:  $L = 1,069\text{--}1,541\ \mu\text{m}$ ;  $L' = 746\text{--}1,252\ \mu\text{m}$ ;  $a = 20.7\text{--}31.4$ ;  $a' = 13.3\text{--}24.1$ ;  $b = 4.6\text{--}6.6$ ;  $b' = 3.5\text{--}5.4$ ;  $c = 2.3\text{--}5.3$ ;  $c' = 7.1\text{--}19.0$ ;  $V = 39.6\text{--}50.2$ ;  $V' = 60.1\text{--}69.9$  (Table 12).

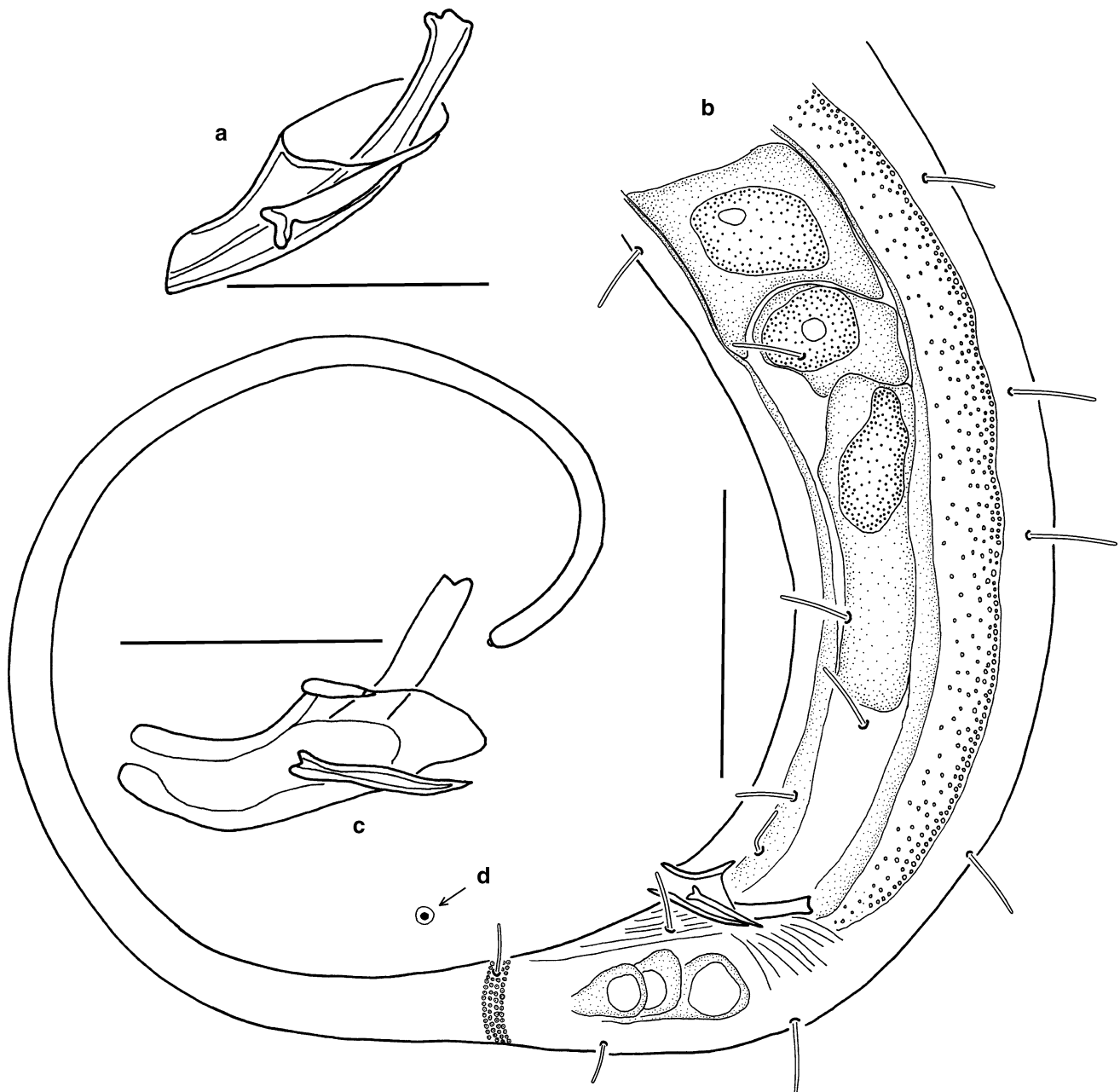
Body spindle-shaped, with sharply narrowed anterior end and filiform posterior end. Somatic setae cylindrical,

6–13  $\mu\text{m}$  long, present along entire body except filiform part of tail; these setae arranged in 4 submedian rows. Cuticle densely dotted, with indistinct lateral differentiation. Dots in lateral fields larger and spaced slightly farther apart than dots in median fields. Dots of cuticle outside lateral fields smaller, except in postanal part, where they are larger than dots of lateral fields in midbody region and appear toroidal. Dots usually arranged more or less in transverse rows. In optical cross-section of cuticle, dots discernible as tiny radial struts. Small cuticular pores irregularly situated along entire body length. Cuticle thickness ca. 1.5  $\mu\text{m}$  along entire body length except cervical part where it is thinner (ca. 0.8  $\mu\text{m}$ ). There are 6 triangular lips with edged anterior tips. Inner labial sensilla not visible. 6 outer labial setae 3–6  $\mu\text{m}$  long and 4 submedian cephalic setae 9–17  $\mu\text{m}$  long, appearing confluent and lying at same level. Amphideal fovea ventrally coiled, single-spiral, round or shaped as transversely oriented oval, with fine but distinct cuticular edging, situated at level of middle of esophastoma, at 0.5–0.7 c.b.d. posterior to the anterior end. Very fine concentric striation and more or less distinct central spot visible in amphidial fovea. One pair of cervical setae 7–12  $\mu\text{m}$  long (latero-subdorsal and latero-subventral) located at a short distance posterior to each amphideal fovea. Stoma consisting of cup-shaped cheilostoma and narrow, funnel-shaped esophastoma. Cheilostoma possessing 6 pairs of long, thick, slightly undulating, cuticular rugae (each pair located posterior to lip), esophastoma with 5 sclerotized onchia: 1 dorsal, 2 subventral, and 2 small lateral (1 on each side), Basal parts of onchia situated in anterior part of esophastoma, and their apical parts intruding into cheilostoma. Dorsal and one subventral onchia 7–8  $\mu\text{m}$  long, three other smaller onchia 4–5  $\mu\text{m}$  long. Esophastoma 15–20  $\mu\text{m}$  in length, with thick cuticular walls, its tissues distinctly separated from tissues of pharynx. Pharynx regularly muscular, narrow in its anterior half and gradually widening to its posterior half, without formation of distinct bulb. Posterior part of pharynx containing numerous large plasmatic interruptions. Nerve ring not visible, but numerous neuron bodies present at level of middle of pharynx. Renetta not visible. Cardia large, triangular, surrounded by intestine. In about half of specimens, anterior part of pharynx everted and protruding outside, including onchia. Some specimens with partly retracted lips. Tail consisting of proximal conical part and long terminal filiform cylindrical part constituting 50–75% of entire tail length. Diameter of cylindrical part of tail at its posterior end 3–5  $\mu\text{m}$ . Bodies of three caudal glands seen posterior to anus.

Male reproductive system. Single testis directed anteriorly, outstretched, lying to right of intestine. Moderately curved spicules consisting of two (wide distal and narrow proximal) parts. Gubernaculum in shape of slightly curved stick with edged proximal end and bifurcated distal end. Supplementary organs not found.



**Fig. 30** *Acantholaimus veitkoehlerae* sp. n. **a** male, holotype, total view; **b** male, holotype, anterior end; **c** female, paratype No. 5, reproductive system. Scale bars: **a** = 100  $\mu$ m; **b**, **c** = 50  $\mu$ m



**Fig. 31** *Acantholaimus veitkoehlerae* sp. n., males. **a** holotype, spicule; **b** paratype No. 1, posterior end; **c** paratype No. 1, spicule; **d** paratype No. 1, cuticle punctation pattern at postcloacal region. Scale bars: **a**, **c** = 20  $\mu$ m; **b** = 50  $\mu$ m

Female reproductive system consisting of two antitridomous ovaries (anterior ovary lying to right of intestine, and posterior one lying to left of intestine), and short oviducts. Uterus not defined. Each ovary containing one mature ovocyte. Three pairs of vulvar glands with granular content surrounding short vagina.

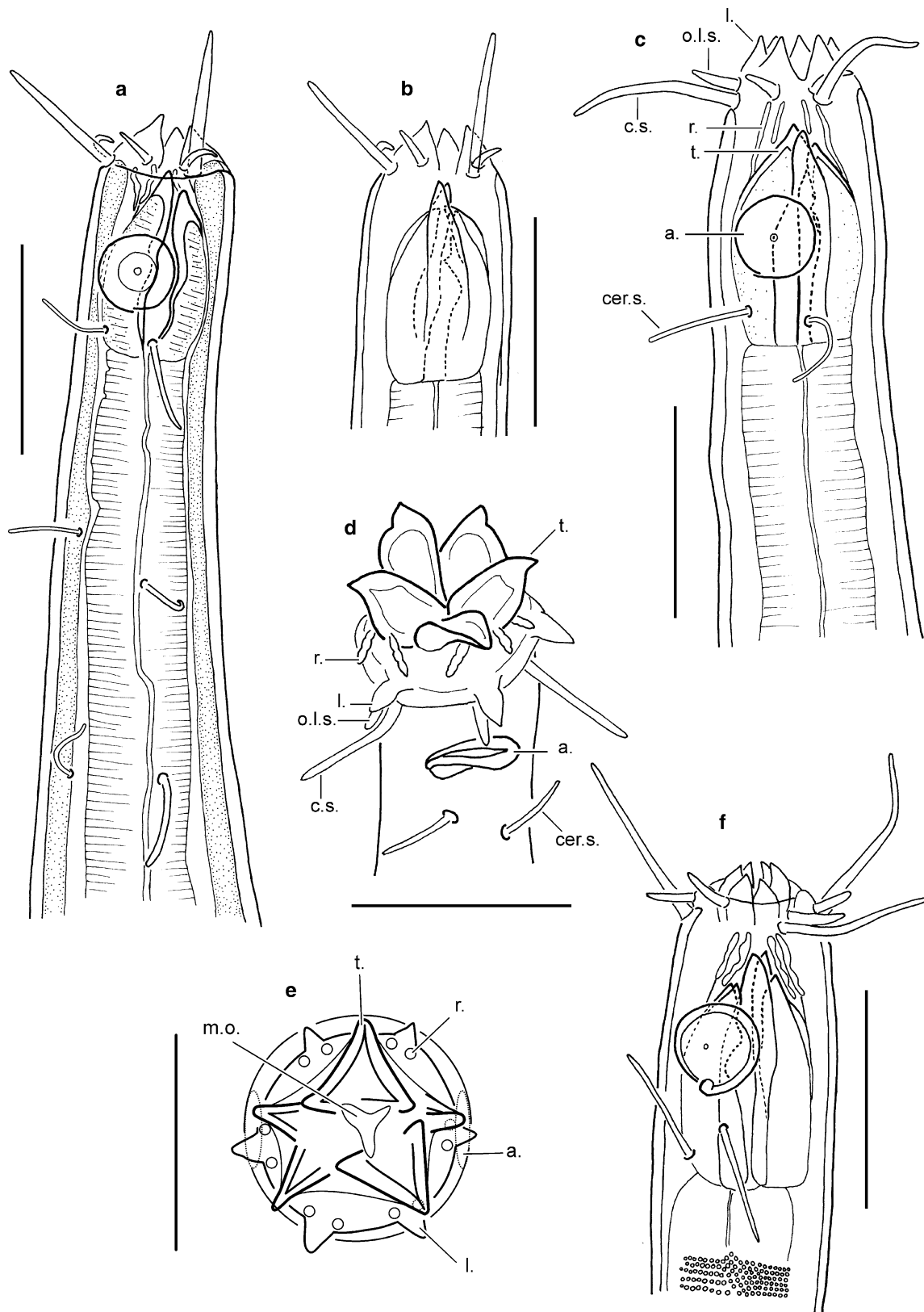
#### Abundance

*Acantholaimus veitkoehlerae* sp. n. was mentioned as *Acantholaimus* sp. 6 in the description of nematode

assemblages inhabiting deep-sea polymetallic nodule fields (Miljutina et al. 2010). The density of *A. veitkoehlerae* sp. n. varied from 0 to 8.4 inds/10 cm<sup>2</sup> (mean 2.6 inds/10 cm<sup>2</sup>). This species ranked third in relative abundance within the nematode community (from 0 to 4.8% in different samples, mean 1.9%).

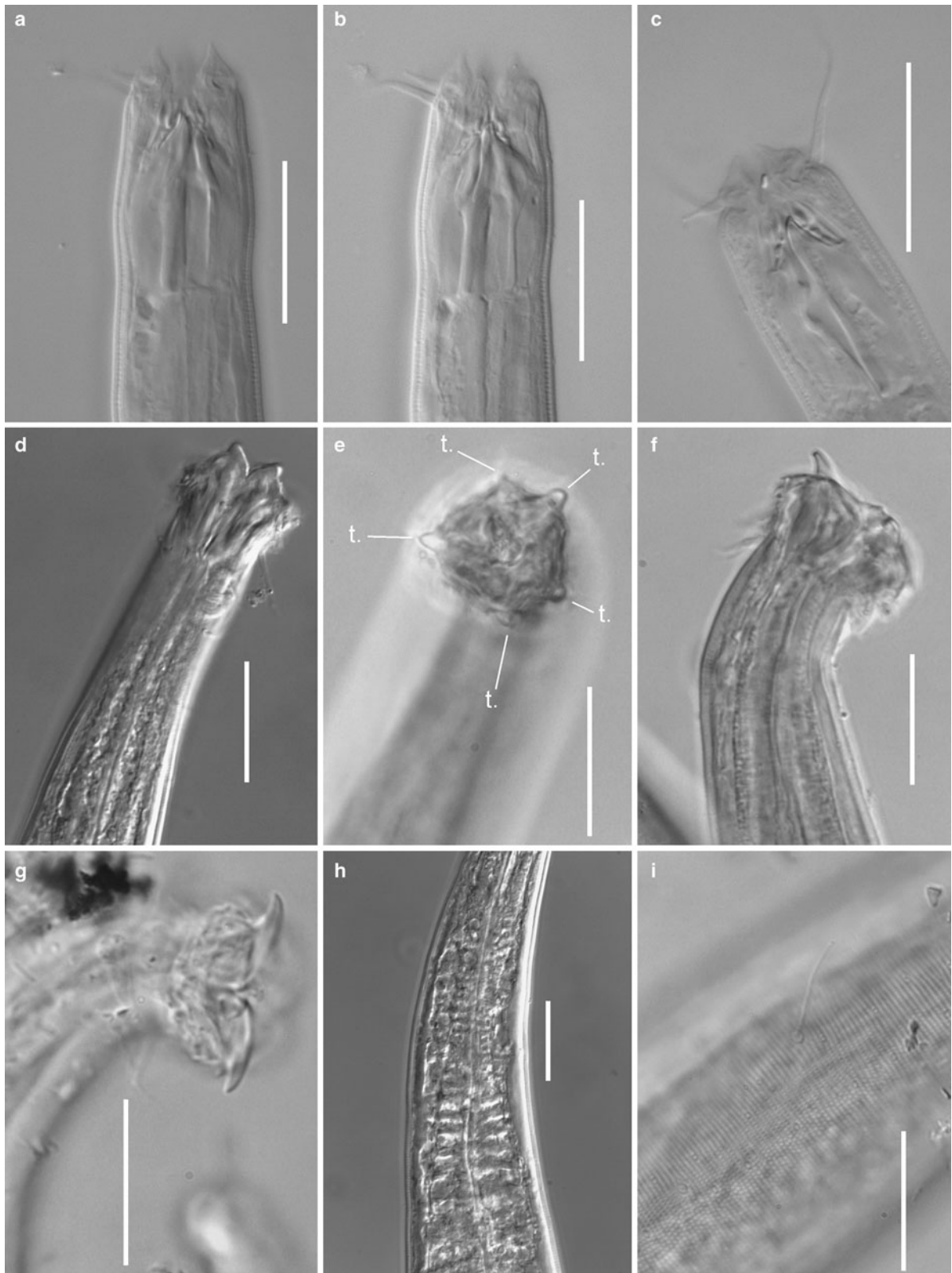
#### Differential diagnosis

The new species shares with *A. arthrochaeta* sp. n., *A. akavitus* Gerlach et al. 1979, *A. quintus* Gerlach et al. 1979,



**Fig. 32** *Acantholaimus veitkoehlerae* sp. n., heads. **a**, **b** male, holotype; **c** male, paratype No. 3; **d** male, paratype No. 2 (everted stoma); **e** female, paratype No. 8 (everted stoma, front view); **f** female, paratype No. 6 (retracted anterior part). Scale

bars = 20  $\mu$ m. Abbreviations: *a* = amphid, *c.s.* = cephalic seta, *cer.s.* = cervical seta, *l.* = labium, *m.o.* = mouth opening, *o.l.s.* = outer labial seta, *r.* = ruga, *t.* = onchium



**Fig. 33** *Acantholaimus veitkoehlerae* sp. n., micrographs. **a, b** female, paratype No. 7, head region at different optical levels; **c** female, paratype No. 6, head region; **d** male, paratype No. 2, head region with everted stoma; **e** female, paratype No. 8, head with everted stoma, apical view; **f** male, paratype No. 3, head region with everted stoma; **g** female,

paratype No. 11, head region with everted head; **h** male, paratype No. 2, pharyngeal region; **i** female, paratype No. 6, lateral view of cuticle surface (lateral differentiation of cuticular punctation not discernible, numerous small pores seen). Scale bars = 20 μm. Abbreviation: *t.* = onchium

**Table 12** *Acantholaimus veikkoehlerae* sp. n.

Collection status	Holotype	Paratype No. 1	Paratype No. 2	Paratype No. 3	Paratype No. 4	Paratype No. 5	Paratype No. 6	Paratype No. 7	Paratype No. 8	Paratype No. 9	Paratype No. 10	Paratype No. 11
Station	PL-1599/ 7	MTB-16	MTB-1	MTB-6	MTB-15	MTB-7	MTB-8	MTB-6	PL-1599/7	PL-1599/7	MTB-11	MTB-7
Slide number	5-3	2-59	1-31	2-91	3-26	1-76	2-76	2-91	1-49	1-48	1-60	1-89
Sex	m	m	m	m	m	f	f	f	f	f	f	f
<i>L</i>	1,120	1,300	1,183	1,541	1,166	1,362	1,138	1,522	1,152	1,156	1,340	1,069
<i>L'</i>	782	912	828	1,252	880	772	903	1,105	799	808	967	746
Length of outer labial setae	4	n.a.	5	n.a.	n.a.	n.a.	6	6	3	n.a.	n.a.	n.a.
Length of cephalic setae	13	n.a.	13	n.a.	n.a.	n.a.	16	17	9	n.a.	n.a.	n.a.
Length of cervical setae	8	n.a.	7	n.a.	n.a.	n.a.	7	12	n.a.	n.a.	n.a.	n.a.
Length of somatic setae	11	12	12	n.a.	n.a.	10	13	6	11	11	n.a.	n.a.
Length of spicule in chord	19	26	24	n.a.	28	-	-	-	-	-	-	-
Length of spicule in arc	21	32	26	n.a.	36	-	-	-	-	-	-	-
Length of gubernaculum	14	13	10	n.a.	13	-	-	-	-	-	-	-
Amphidial diameter	6	n.a.	n.a.	9	n.a.	n.a.	9	8	n.a.	n.a.	n.a.	n.a.
Distance from anterior end to amphid	8	n.a.	n.a.	20	n.a.	n.a.	12	11	n.a.	n.a.	n.a.	n.a.
Diameter at level of cephalic setae	14	n.a.	15	18	n.a.	15	15	14	n.a.	n.a.	n.a.	n.a.
Diameter at level of middle of amphid	17	n.a.	n.a.	20	n.a.	n.a.	17	17	n.a.	n.a.	n.a.	n.a.
Diameter at level of cardia	40	40	41	45	43	51	47	50	42	39	38	28
Diameter at level of anus	28	29	26	30	28	31	33	25	26	27	23	20
Maximum body diameter	46	46	52	52	43	58	55	60	48	50	44	34
<i>a</i>	24.3	28.3	22.8	29.6	27.1	23.5	20.7	25.4	24.0	23.1	30.5	31.4
<i>b</i>	5.3	5.2	5.5	6.6	4.6	6.2	4.9	5.8	5.3	5.2	6.1	6.1
<i>c</i>	3.3	3.4	3.3	5.3	4.1	2.3	4.8	3.6	3.3	3.3	3.6	3.3
<i>V</i>	-	-	-	-	-	39.6	50.2	47.0	46.9	42.0	46.3	45.3
<i>a'</i>	17.0	19.8	15.9	24.1	20.5	13.3	16.4	18.4	16.6	16.2	22.0	21.9
<i>b'</i>	3.7	3.7	3.9	5.4	3.5	3.5	3.9	4.2	3.6	3.6	4.4	4.2
<i>c'</i>	12.1	13.4	13.7	9.6	10.2	19.0	7.1	16.7	13.6	12.9	16.2	16.2
<i>V'</i>	-	-	-	-	-	69.9	63.2	64.8	67.6	60.1	64.1	64.9
Notes		Everted head	Everted head	Everted head	Everted head	Everted head	Everted head	Everted head	Everted head	Everted head	Everted head	Everted head

Number of stations where specimens were found, their collection status, measurements (in  $\mu\text{m}$ ), and body indices



and *A. septimus* Gerlach et al. 1979 the middle-sized body length (about 1,000–1,500  $\mu\text{m}$ ), relatively small amphideal fovea (40–65% c.b.d.), the position of the amphideal fovea relative to the apical part of the body (about 1 amphideal c.b.d.) and the length of the cephalic setae (about 10–15  $\mu\text{m}$ ). *Acantholaimus veitkoehlerae* sp. n. shares with *A. elegans* Jensen 1988 the spindle-shaped body; the long narrow eversible anterior end; and the length and shape of the pharynx ( $b = 4\text{--}6$ , consisting of two parts, a long narrow anterior muscular part and a thick posterior part with numerous plasmatic interruptions).

*Acantholaimus veitkoehlerae* sp. n. differs from *A. elegans* in the body length ( $L = 1,069\text{--}1,541 \mu\text{m}$  vs.  $L = 500\text{--}700 \mu\text{m}$ ); the size (35–53% c.b.d. vs 75%); the position of the amphideal fovea (0.5–0.7 c.b.d. posterior to the anterior end vs. 1.3–1.7 c.b.d.); and the presence of lateral differentiation.

*Acantholaimus veitkoehlerae* sp. n. distinctly differs from *A. quintus* by their body shape (spindle-shaped body with strongly narrowed anterior end vs. cylindrical body with slightly narrowed anterior end); the absence of preanal papillae; the number of cervical setae (2 vs. 6); and the absence of the numerous somatic setae at the level of the pharynx that are present in *A. quintus*.

*Acantholaimus veitkoehlerae* sp. n. differs from *A. arthrochaeta* by the spindle-shaped body with strongly narrowed anterior end vs. the cylindrical body with weakly narrowed anterior end; the absence of jointed outer labial and cephalic setae; the shape of the amphideal fovea (circular vs oval); the number of cervical setae (2 vs. 4); and the shape of the spicules (thick vs. thin).

*Acantholaimus veitkoehlerae* sp. n. is closer to *A. septimus* and *A. akvavitus*. The new species differs from *A. septimus* by the shape and thickness of the body (spindle-shaped vs. cylindrical;  $a = 21\text{--}31$  vs.  $a = 31\text{--}60$ ); the absence of preanal papillae; the number of cervical setae (2 vs. 4); the shape of the amphideal fovea (circular vs. circular-oval); the length of the outer labial setae (3–6  $\mu\text{m}$  vs. 8  $\mu\text{m}$ ); and the length of the inflated anterior part of the pharynx (1.2 vs. 3 cephalic diameters).

*Acantholaimus veitkoehlerae* sp. n. differs from *A. akvavitus* by the number of cervical setae (2 vs. 3); and by the length of the outer labial and cervical setae: the outer labial setae are three times longer than the cephalic setae in the new species, 3–6  $\mu\text{m}$  and 9–17  $\mu\text{m}$ , respectively, vs. the corresponding setae of almost identical length, 7  $\mu\text{m}$  and 11  $\mu\text{m}$ , respectively, in *A. akvavitus*.

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