

A new marine nematode, *Catanema schiemeri* sp. nov. (Desmodoroidea) with multiple prokaryotic symbionts

Jörg OTT, Philipp PRÖTS & Florian SCHARHAUSER

A new species of Stilbonematinae (marine free-living nematodes with ectosymbiotic bacteria), *Catanema schiemeri* n. sp. is described from subtidal sand in the Northern Adriatic near Rovinj, Croatia. In addition to the obligate coat of ectosymbiotic Gammaproteobacteria large stalked sulfur bacteria are found attached to the anterior body region of about 25 % of the individuals. The new species is distinguished from its congeners by its size and a series of preloacal supplements in males.

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Eine neue Art der Stilbonematinae (freilebende Meeresnematoden mit ektsymbiotischen Bakterien) aus dem sublitoralen Sand der Nordadria nahe Rovinj (Kroatien) wird beschrieben. Neben den obligaten ektsymbiotischen Gammaproteobakterien tragen etwa 25 % der Individuen große gestielte Schwefelbakterien auf ihrem vorderen Körperabschnitt. Die neue Art unterscheidet sich durch ihre Größe und den Besitz von präkloakalen Hilfsorganen bei den Männchen von den bisher beschriebenen Arten der Gattung.

Keywords: meiofauna, symbiosis, sulfur bacteria, intersex, Mediterranean Sea, Northern Adriatic, subtidal.

Introduction

A diverse epigrowth by bacteria and protozoans is common among the marine superfamily of Desmodoroidea. All representatives of the Stilbonematinae – a taxon presently classified as a subfamily of the family Desmodoridae – live in an obligate association with species specific chemoautotrophic Gammaproteobacteria covering all or the better part of their body surface (ZIMMERMANN et al. 2016). The bacteria use the energy gained from oxidizing sulfide to fix inorganic carbon. The organic matter produced can be used as food by the host, which by migrating in the sediment provides its symbionts with the necessary access to electron donors and acceptors (SCHIEMER et al. 1990, OTT et al. 1991). Stilbonematinae occur worldwide in tropical to temperate shallow sands, and occasionally in deeper sandy habitats (TCHESUNOV et al. 2012, LEDUC 2013).

Early descriptions do not mention the symbionts or misinterpret the observations. COBB, who is the author of the majority of the stilbonematid genera and a keen observer, disregarded them entirely (COBB 1920). In the description of *Eubostriechus filiformis* (GREEFF 1869) the bacteria are thought to be an excretion of the worm. CHITWOOD (1936) believed them to be fungal spores, GERLACH (1950) and WIESER (1969) classified them as cyanobacteria. This opinion has prevailed until the identification as Gammaproteobacteria (POLZ et al. 1992).

Besides these obligate symbionts occasionally other organisms, such as suctorians, are found to colonize the cuticle of stilbonematine nematodes. Here we describe a new species of the genus *Catanema*, *C. schiemeri* sp. nov., which is remarkable because approximately 25 % of the population in the type locality, the Bay of Veštar (Rovinj, Croatia), carry sev-

eral giant sulfur bacteria attached to their anterior body portion that is not covered by the obligate symbiotic bacteria.

An emended diagnosis of the genus *Catanema* (COBB 1920) has been given by TCHESUNOV (2013). Applying the diagnostic characters listed there the genus presently comprises only 3 described species, *C. exile* (COBB 1920), *C. australis* (HOURSTON & WARWICK 2010) and *C. dambayensis* (TCHESUNOV 2013). Members of the genus, however, have been recorded repeatedly from all over the world and can be locally extremely abundant (RIERA et al. 2013) and species rich (own unpublished observations).

Material and Methods

Specimens of the new species were collected from subtidal medium sand in 2–3m depth at the Bay of Veštar south of Rovinj, Croatia (Fig. 1). Buckets of sediment were taken skin diving and live meiofauna was extracted in the laboratory by shaking and decanting through fine mesh sieves (64µm mesh widths) and sorted under a dissecting microscope. Specimens for light microscope whole mounts were relaxed in a MgCl₂ solution isotonic to sea water and fixed in 4% formaldehyde or PFA. Subsequently they were transferred to a mixture of 1 part glycerol and 9 parts distilled water, slowly evaporated and embedded in water-free pure glycerol. Drawings were made with the aid of a camera lucida on a Diavar microscope (Reichert, Vienna, Austria). Specimens for sequencing of barcoding genes were put in a drop of MgCl₂, mounted on non-permanent slides for photo vouchering, and subsequently fixed in 96% ethanol. Light micrographs were made on a Zeiss Axio Imager A1 (Carl Zeiss, Oberkochen, Germany).

DNA was extracted from single worm specimens with the DNeasy Blood and Tissue Kit (QUIAGEN, Hilden, Germany) following the manufacturer's instruction with two exceptions:

1. Digestion with proteinase K was extended to 14 hours.
2. Elution was done with 100 µl of elution buffer. A ≥ 1000 bp long fragment of the 18S rRNA gene was amplified with newly designed nematode specific primers 466f (CCACATCCAAGGAAGGCAG) and 1548r (ATTCCTTCAGTGTAGCGCG) (designed in Primer 3, UNTERGASSER et al. 2012)

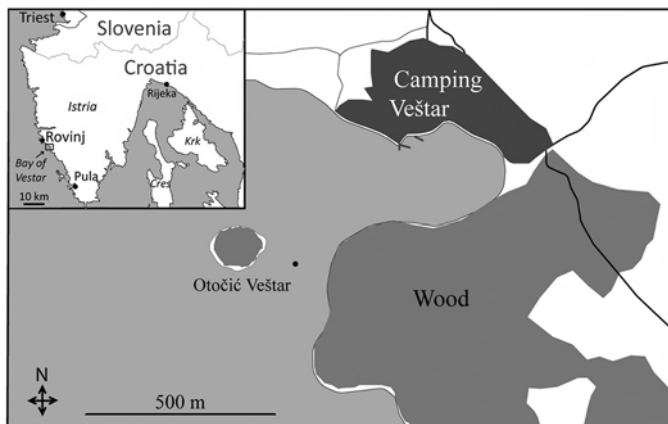


Fig. 1: Sampling location in the Northern Adriatic Sea, south of the town of Rovinj, Croatia. A black dot in the insert marks the exact sample site. – Abb. 1: Fundort in der Nordadria, südlich der Stadt Rovinj, Kroatien. Ein schwarzer Punkt in der Detailkarte zeigt die genaue Probenstelle.

using the following cycling conditions: 5 min at 95°C followed by 35 cycles of 30 s at 95°C, 45 s at 60.7°C and 1,2 min at 72°C, followed by a final extension of 10 min at 72°C. Quality and length of the PCR products were checked with gel electrophoresis and purified with ExoSAP-IT[™] PCR Product Cleanup (Applied Biosystems[™], Waltham, Massachusetts, USA). Purified products were sent to an external sequencing company (MicroSynth, Switzerland). ABI chromatograms of forward and reverse primer products were quality checked, trimmed and aligned in Geneious Prime 2020.1.2 (Biomatters, New Zealand).

Phylogenetic analyses were done using the 18S rRNA dataset by SCHARHAUSER et al. (2020) including two new partial 18S rRNA from *Catanema schiemeri* sp. nov. All sequences were aligned with MAFFT v.7 (KATOH & STANDLEY 2013) using the Q-insi option (KATOH & TOH 2008) which considers the secondary structure of the RNA. Alignments were trimmed and quality checked using Geneious Prime 2020.1.2 (Biomatter, New Zealand). The optimal substitution model for each alignment was assessed using ModelFinder (KALYAANAMOORTHY et al. 2017). The TIM3e + I + G4 model was the best fit for our 18S rRNA alignment. IQTREE (NGUYEN et al. 2015) was used for phylogenetic reconstruction using the Ultrafast Bootstrap Approximation UFBoot (MINH et al. 2013) to assess node stability (10,000 bootstrap runs). Additionally, support values were generated using approximate Bayes (aBayes) (ANISIMOVA et al. 2011) and SH-aLRT analyses (GUINDON et al. 2010). Final Tree was graphically optimized using FigTree v. 1.4.3 (RAMBAUT 2009) and Adobe Illustrator CC 22.1 (Adobe Inc.©, San Jose, USA).

Results

Class Chromadorea INGLIS, 1983
Subclass Chromadoria PEARSE, 1942
Order Desmodorida DE CONINCK, 1965
Suborder Desmodorina DE CONINCK, 1965
Superfamily Desmodoroidea FILIPJEV, 1922
Family Desmodoridae FILIPJEV, 1922
Subfamily Stilbonematinae CHITWOOD, 1936
Genus *Catanema* COBB, 1920

Catanema schiemeri sp. nov.

<http://zoobank.org/74B00479-92E9-43EF-8DF8-7377BF3E7D10>

Synonymy:

Syn. *Catanema* sp. in: KAMPFER et al. (1998), URBANCIK et al. (1996 a,b)
Nec *Catanema* sp. in: OTT & NOVAK (1989), SCHIEMER et al. (1990), OTT et al. (1991),
NEBELSICK et al. (1992), POLZ et al. (1992)

Type material:

Holotype male, paratypes male 3, paratypes female 3, paratypes juvenile 3; deposited in the Natural History Museum Vienna under the numbers Evertebrata Varia NHMW 5845 (holotype) and 5846–5854 (paratypes).

The sequences of the 18S rRNA gene are available from GENBank under the accession numbers V013-MW267952 and V015-MW267953.

Measurements: See table 1.

Additional material. Several specimens (males, females, juveniles, intersexes) in the first author's collection.

Type locality: Bay of Veštar (45°02'46" N, 13°40'49" E)

Table 1. *Catanema schiemeri* sp. nov. Measurements of holotype and paratypes. All lengths are in μm . a: body length/maximal diameter, b: body length/pharynx length, c: body length/tail length, c': tail length/anal diameter, n.a.: not applicable. – Tabelle 1. *Catanema schiemeri* sp. nov. Maße des Holotyps und der Paratypen. Alle Längenangaben in μm . a: Körperlänge/größter Durchmesser, b: Körperlänge/Pharynxlänge, c: Körperlänge/Schwanzlänge, c': Schwanzlänge/Analdurchmesser, n.a.: trifft nicht zu.

	Holotype Male	Paratypes Males (n=3)	Paratypes Females (n=3)	Males total (n=8)	Males mean \pm SD	Females total (n=7)	Females mean \pm SD
Body length	3935	3602–4135	4735–5928	3602–4622	4164 \pm 297	4409–5928	5045 \pm 504
a	112	103–118	118–169	103–122	112 \pm 6.5	110–169	132 \pm 20
b	28.5	26.7–28.9	30.6–39.5	26.7–31.2	29.2 \pm 1.4	30.6–39.5	34.8 \pm 3
c	32.8	29.5–35	36.4–49.3	29.5–37	32.9 \pm 2.6	36.4–49.3	43.2 \pm 4.6
max. diameter	35	35	35–40	35–41	37.1 \pm 2.4	35–40	38.4 \pm 2
Pharynx length	138	135–145	142–155	135–158	143 \pm 6.9	130–155	145 \pm 8.5
Tail length	120	118–140	110–150	118–140	130 \pm 8.6	98–150	118 \pm 16
c'	3.4	3.4–4.1	3.4–5	3.3–4.1	3.7 \pm 0.3	3.4–5	3.7 \pm 0.6
Head diam.	12	10–12	10–12	10–15	12.1 \pm 2	10–15	13 \pm 1.9
Cephalic setae length	16	18–19	20	15–20	17.4 \pm 1.7	15–20	17.8 \pm 2.7
Subcephalic setae length	10	7–12	11–13	7–13	9.6 \pm 1.7	8–13	10.4 \pm 1.6
Spicula arc/chord	40/35	40–50/30–40	n.a.	40–50/30–45	46 \pm 4.2 39 \pm 5.2	n.a.	n.a.
Gubernaculum	12	12–15	n.a.	12–17	14.6 \pm 1.7	n.a.	n.a.
Testis start %	52	46–47	n.a.	44–52	48 \pm 2.7	n.a.	n.a.
Vulva %	n.a.	n.a.	58–62	n.a.	n.a.	51–62	57.3 \pm 3.5
Supplements #	12	10–13	n.a.	10–17	12.6 \pm 2.1	n.a.	n.a.
Supplements start precloacal	442	445–537	n.a.	420–550	498 \pm 53	n.a.	n.a.
Bacterial coat start (μm)	190	205–215	177–215	190–235	206 \pm 15	177–230	209 \pm 16.4
Bacterial coat start (pharynx lengths)	1.4	1.4–1.5	1.4–1.5	1.4–1.5	1.4 \pm 0.05	1.4–1.8	1.5 \pm 0.2
Corpus %	21.7	22.5–27.6	21.3–25	21.7–28.6	24.7 \pm 2.5	19.2–25.4	22.3 \pm 2.1
Isthmus %	60.1	53.1–59.9	58.1–63.3	53.1–62	56.8 \pm 3.2	53.6–63.4	59.3 \pm 3.7
Bulbus %	18.1	17.6–20.7	14.6–20	15.7–20.7	18.4 \pm 1.8	14.6–21.6	18.4 \pm 2.6

Etymology:

Named in honor of Friedrich SCHIEMER, eminent freshwater biologist, nematologist, friend and good companion on many trips to sample meiofauna in pleasant locations.

Description (Figs. 2–4):

Body slender, cylindrical; tail conical. Body diameter decreases abruptly to accommodate the thickness of the coat of symbiotic bacteria. Cuticle finely transversely striated (annule width 0.4–0.5 μm), starting with a fingerprint-like pattern at the anterior end, but changing to a regular striation at the level of the subcephalic setae. Cuticula about 2 μm thick, in cephalic region reinforced by a doubling of the fiber layers (URBANCÍK et al. 1996a,b). Under the cuticle there are dark-brown areas (hypodermis?) of unknown function.

Inner labial sense organs are present as six finger-shaped papillae within the mouth opening. Of the circle of outer labial sense organs only four in the form of minute (< 1 μm long) setae in submedian position can be seen in *en face* view. The lateral position in this circle is occupied by the *fovea amphidialis*. Four cephalic setae close to the anterior end, followed by a circle of eight subcephalic setae, which is continued by eight rows of somatic setae extending over the pharyngeal region. Midbody with sparse, short somatic setae obscured by the bacterial coat. In the posterior region of the male the setation is denser ventrally, starting a short distance anterior to the cloaca and extending along the tail as two rows of stout setae on each side. Tubular structures as described by COBB (1920) for the type species *C. exile* not present.

The amphidal fovea is reduced to a pore on the apical part of the head extremely close to the mouth opening. In practically all specimens the *corpus gelatum* is protruding and can reach a length of several hundred μm .

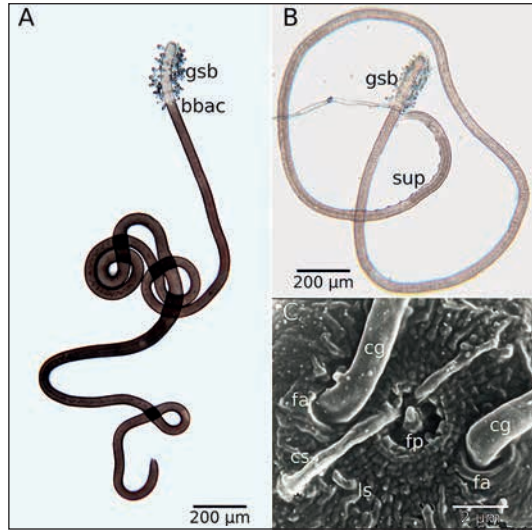


Fig. 2: *Catanema schiemeri* sp. nov. A. Total view of a female with giant sulfur bacteria attached to anterior body region. Coat of symbiotic Gammaproteobacteria appearing dark in transmitted light due to sulfur inclusions. B. Total view of a male with giant sulfur bacteria, showing preloocal supplements. Light micrographs of live specimens. C. *En face* view of anterior end, showing pore-shaped *foveae amphidiales* each with *corpus gelatum* emanating, fingerlike papillae in mouth opening and small labial sense organs. A bacterial cell is attached to the fingerlike papillae. SEM. Abbreviations: bbac – begin of the bacterial coat, cg – corpus gelatum, cs – cephalic setae, fa – fovea amphidialis, fp – finger-like papillae, gsb – giant sulfur bacteria, ls – labial sense organs, sup – supplement. – Abb. 2: *Catanema schiemeri* sp. nov. A. Totalansicht eines Weibchens mit Riesen-Schwefelbakterien am Vorderkörper. Der Überzug durch die symbiontischen Gammaproteobakterien erscheint im Durchlicht dunkel durch ihre Schwefel-einschlüsse. B. Totalansicht eines Männchens ebenfalls mit Riesen-Schwefelbakterien und einer Reihe präloakaler Hilfsorgane. Lichtmikroskopische Bilder lebender Tiere. C. Die *en face* Ansicht des Vorderendes zeigt die porenförmigen *foveae amphidiales*, aus denen jeweils das *corpus gelatum* austritt, die fingerförmigen Papillen in der Mundöffnung und die kleinen labialen Sinnesorgane. Eine Bakterienzelle klebt an den fingerförmigen Papillen. SEM. Abkürzungen: bbac – Beginn des bakteriellen Überzugs, cg – Corpus gelatum, cs – Cephalborste, fa – Fovea amphidialis, fp – fingerförmige Papille, gsb – Riesen-Schwefelbakterien, ls – labiales Sinnesorgan, sup – Hilfsorgan.

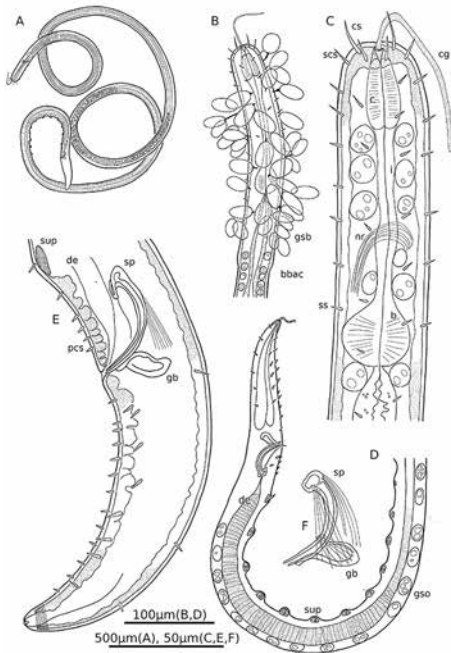


Fig. 3: *Catanema schiemeri* sp. nov. male. A. Total view. B. Anterior end bearing stalked giant sulfur bacteria. C. Pharyngeal region. D. Posterior body region with row of supplements. E. Cloacal region and tail, with spicular apparatus. F. Spicular apparatus of another male. Abbreviations: b – bulbus, bbac – begin of the bacterial coat, c – corpus, cg – corpus gelatum, cs – cephalic setae, de – ductus ejaculatorius, gb – gubernaculum, gsb – giant sulfur bacteria, gso – glandular sense organ, i – isthmus, nr – nerve ring, pcs – precloacal setae, sp – spiculum, sup – supplement. – Abb. 3: *Catanema schiemeri* sp. nov. Männchen. A. Totalansicht. B. Vorderende mit gestielten Riesenschwefelbakterien. C. Pharynxregion. D. Hinterende mit präkloakaler Reihe von Hilfsorganen. E. Kloakalregion und Schwanz, Spikularapparat. F. Spikularapparat eines anderen Männchens. Abkürzungen: b – Bulbus, bbac – Beginn des bakteriellen Überzugs, c – Corpus, cg – Corpus gelatum, cs – Cephalborste, de – Ejakulationskanal, gb – Gubernaculum, gsb – Riesen-Schwefelbakterien, gso – glanduläres Sinnesorgan, i – Isthmus, nr – Ringnerv, pcs – Präkloakalborste, sp – Spikularapparat, sup – Hilfsorgan.

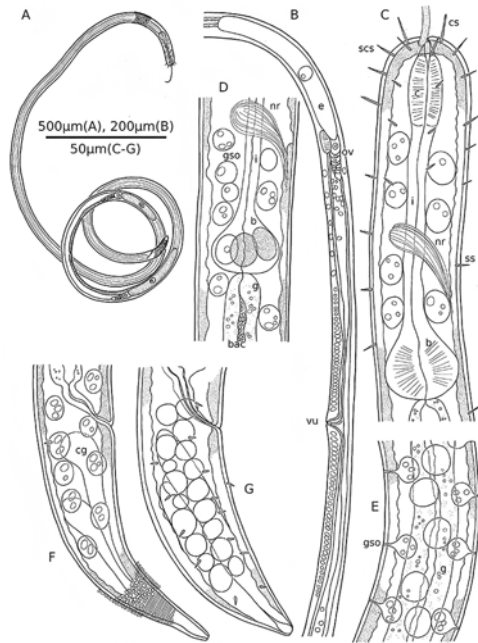


Fig. 4: *Catanema schiemeri* sp. nov. female. A. Total view. B. Vulva region with anterior ovary, uteri and enlarged glandular sense organs (gsos) anterior and posterior to the vulva. C. Pharyngeal region. D. Bulbus of another female with large gland cells, gut with bacteria in lumen. E. Midbody region showing lateral and median gsos, the latter opening through pores to the outside. F and G. Tails of two females. Abbreviations: b – bulbus, c – corpus, cg – caudal gland, cs – cephalic setae, e – egg, g – gut, gso – glandular sense organ, i – isthmus, nr – nerve ring, ov – ovary, scs – subcephalic setae, ss – somatic setae, vu – vulva. – Abb. 4: *Catanema schiemeri* sp. nov. Weibchen. A. Totalansicht. B. Vulva-Region mit vorderem Ovar, Uteri und vergrößerten Glandulären Sinnesorganen (GSOs) vor und hinter der Vulva. C. Pharynxregion. D. Bulbus eines anderen Weibchens mit großen Drüsenzellen, Darm mit Bakterien im Lumen. E. Mittelkörper mit lateralen und medianen GSOs, letztere münden durch Poren nach außen. F und G. Schwanzregion zweier Weibchen. Abkürzungen: b – Bulbus, c – Corpus, cg – Schwanzdrüse, cs – Kopfborste, e – Ei, g – Darm, gso – glanduläres Sinnesorgan, i – Isthmus, nr – Ringnerv, ov – Ovar, scs – Subcephalborste, ss – Körperborste, vu – Vulva.

A small conical buccal cavity leads into the pharynx, which is distinctly tripartite, beginning with a muscular corpus that is clearly set off from the thin isthmus, and a spherical bulb.

The nerve ring is located at about 2/3 of the pharynx length surrounding the isthmus, ventral gland not present. There are eight rows of glandular sense organs (gso) in submedian and sublateral position, respectively, opening to the outside via somatic setae or pores.

Males monorchic, testis beginning at 44–46% of body length. Spicula arcuate with a round to hook-shaped capitulum (depending on the angle of observation); gubernaculum with a dorso-caudally directed apophysis with a loop-shaped reinforcement. Pre-cloacal there is a row of 10 to 17 ventral bumps, cushion like dense structures having the same color as the dark areas under the cuticle underlie the bumps. Depending on the number of bumps the row begins 570 to 420µm and ends about 50µm anterior to the cloaca.

Females didelphic, amphidelphic, ovaries reflexed, uteri long, vagina at 55–62% of body length. Anterior and posterior to the vulva the gsos on the ventral side are enlarged and much denser than in the rest of the body.

Several females found were intersexes (Fig. 5) containing ovaries with large ripe eggs, uteri, vulva and enlarged gsos in the vulvar region. In addition, they possessed spicula rudiments and in some cases a few preanal bumps. One obviously male individual with testis and 14 precloacal bumps lacked spicula and gubernaculum.

The symbiotic Gammaproteobacteria (Fig. 6) that are characteristic for Stilbonematinae cover the body except for an anterior region of about 1.3 to 1.5 pharynx lengths. Here the diameter of the worms decreases abruptly as to accommodate the thickness of the bacterial coat. Only the very tip of the tail is free of bacteria again. The bacteria are corn-kernel shaped and divide perpendicular to the body surface.

Some specimens have very large stalked prokaryotes (25–30 × 10–13µm) (Fig. 7) attached to the anterior body region. These bacteria con-

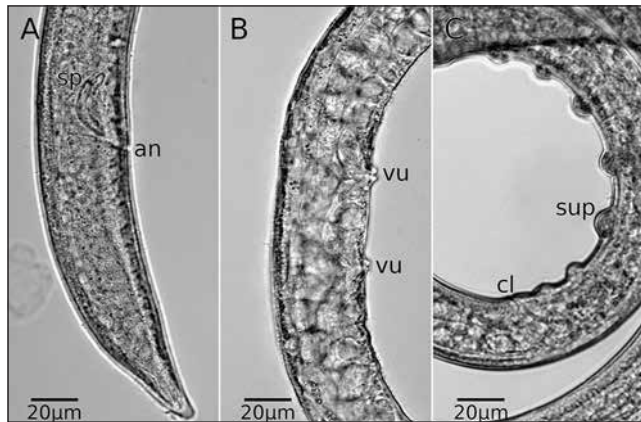


Fig. 5: *Catanema schiemi* sp. nov. A. Tail of a female intersex with spicula rudiments. B. Double vulva of another female intersex. C. Cloacal region of a male with supplements but no spicular apparatus. Light micrographs of preserved specimens. Abbreviations: an – anus, cl – cloaca, sp – spiculum, sup – supplement, vu – vulva. – Abb. 5: *Catanema schiemi* sp. nov. A. Schwanzregion eines weiblichen Intersexes mit Spicula-Rudimenten. B. Doppelte Vulva eines anderen weiblichen Intersexes. C. Kloakalregion eines Männchens mit Hilfsorganen jedoch ohne Spikular-Apparat. Lichtmikroskopische Bilder fixierter Tiere. Abkürzungen: an – Anus, cl – Kloake, sp – Spikularapparat, sup – Hilfsorgan, vu – Vulva.

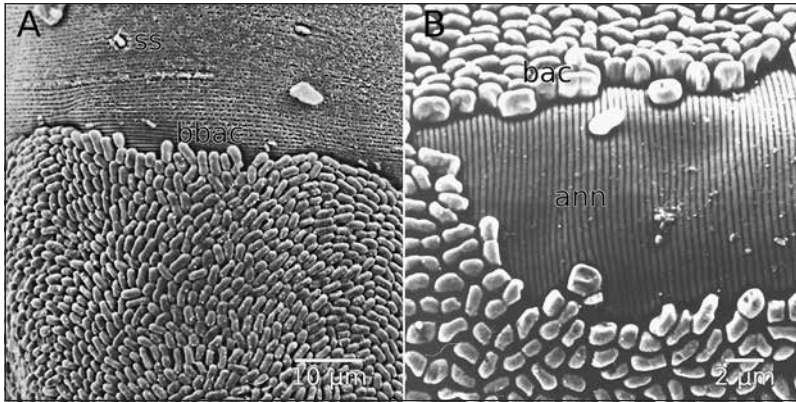


Fig. 6: *Catanema schieneri* sp. nov. Symbiotic bacteria. A. Begin of the coat of chemoautotrophic Gammaproteobacteria. B. Detail of the bacterial coat, showing corn-kernel shape of bacteria and annulated host cuticle. SEM. Abbreviations: ann – annulation, bac – bacteria, bbac – begin of the bacterial coat, ss – somatic setae. – Abb. 6: *Catanema schieneri* sp. nov. Symbiotische Bakterien. A. Beginn des Überzugs aus chemoautotrophen Gammaproteobakterien. B. Detail des bakteriellen Überzugs zeigt die Maiskorn-Form der Bakterien und die Ringelung der Wirtskutikula. SEM. Abkürzungen: ann – Ringelung der Kutikula, bac – Bakterien, bbac – Beginn des bakteriellen Überzugs, ss – Körperborste.

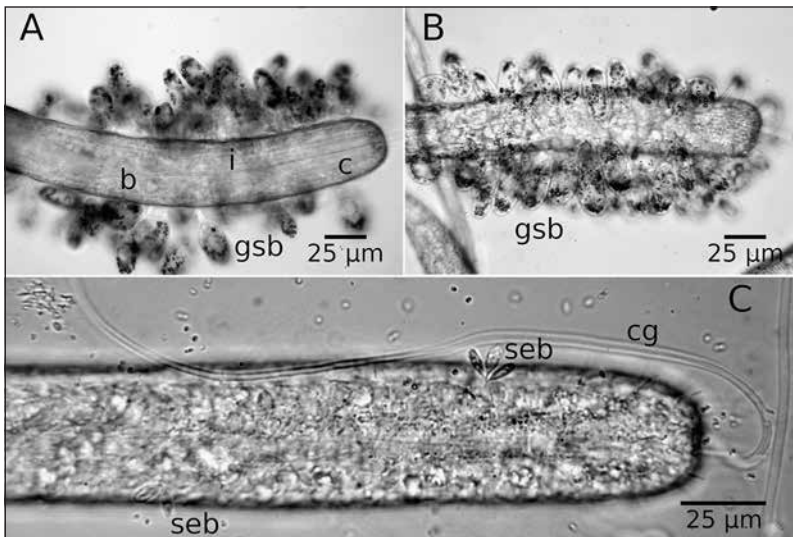


Fig. 7: *Catanema schieneri* sp. nov. A and B. Anterior body region of two individuals carrying giant sulfur bacteria in optical section and surface view, respectively. C. Anterior body region of another individual showing the long *corpus gelatum* and small stalked microorganisms attached to the cuticle. Light micrographs of live specimens. Abbreviations: b – bulbus, c – corpus, cg – corpus gelatum, gsb – giant sulfur bacteria, i – isthmus, seb – small ectobiotic bacteria. – Abb. 7: *Catanema schieneri* sp. nov. A und B. Vorderkörper zweier Individuen mit Riesen-Schwefelbakterien in unterschiedlichen Schärfeebenen. C. Vorderkörper eines Individuums mit langem *Corpus gelatum* und kleinen gestielten Mikroorganismen an der Kutikula angeheftet. Lichtmikroskopische Bilder lebender Tiere. Abkürzungen: b – Bulbus, c – Corpus, cg – Corpus gelatum, gsb – Riesen-Schwefelbakterien, i – Isthmus, seb – kleine ektobiotische Bakterien.

tain conspicuous granules appearing white in incident and dark in transmitted light resembling the sulfur granules found in filamentous or solitary giant sulfur bacteria (SALMAN et al. 2013).

Occasionally other stalked prokaryotes of much smaller size are found attached to the anterior body region.

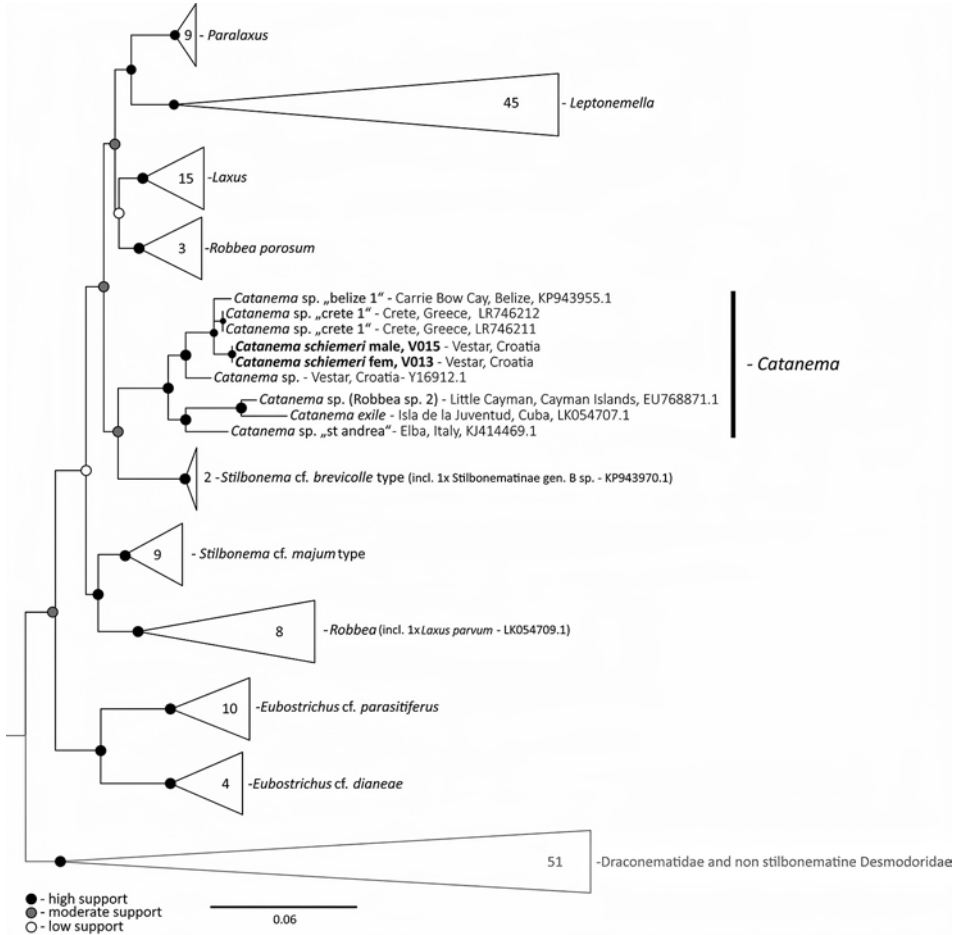


Fig. 8: *Catanema schiemeri* sp. nov. Phylogenetic relationship with other representatives of Stilbonematinae based on the partial 18S rRNA gene sequences. Support values for the nodes are given in black (high support: SH-aLRT support >80%/aBayes support >0.9/ultrafast bootstrap support >95%), grey (medium: one of the three values is slightly below the threshold) and white (low: two or more values are clearly below the threshold). Numbers in the collapsed clades indicate the numbers of 18S rRNA sequences within each clade. – Abb. 8: *Catanema schiemeri* sp. nov. Verwandtschaft mit anderen anderen Vertretern der Stilbonematinae basierend auf Sequenzen der partiellen 18S rRNA Gene. Die statistische Unterstützung der Verzweigungspunkte im Baum ist mit schwarz (stark: SH-aLRT support >80%/aBayes support >0.9/ultrafast bootstrap support >95%), grau (mittel: einer der drei Werte unterhalb des Grenzwertes) und weiß (niedrig: zwei oder mehr Werte unterhalb des Grenzwertes) dargestellt. Die Zahlen in den zusammengefassten Kladen zeigen die Anzahl der 18S rRNA Gen Sequenzen pro Klade an.

Phylogenetic Results:

The two partial 18S rRNA sequences from *Catanema schiemeri* sp. nov. form a well-supported clade within the 18S rRNA phylogeny of the Stilbonematinae in general, and a specific subclade in the bigger genus clade of *Catanema* (Fig. 8). A 18S rRNA sequence from an older study from the same locality (EU768871, KAMPFER et al. 1998) is similar to the ones of the new species but not in the exact same clade. It is probably a chimaera, because it was derived from a pool of about 50 specimens.

Discussion

Systematics

The genus *Catanema* was erected by COBB (1920) for the type species *Catanema exile*. COBB clearly depicts the swollen muscular corpus of the tripartite pharynx. Based on this feature several species were incorrectly assigned to this genus, e. g. *Catanema cobbi* INGLIS 1968 and *Catanema* sp. sensu OTT & NOVAK 1989, SCHIEMER et al. 1990, OTT et al. 1991, NEBELSICK et al. 1992, POLZ et al. 1992. Both species were later transferred to the genus *Laxus* COBB 1894 by OTT et al. (1995). HOPPER & CEFALU (1973) described *Catanema porosum* from Florida. Later e. PLATT & ZHANG (1982) synonymized the genus *Robbea* GERLACH 1956 – which is also characterized by a strongly enlarged and muscular corpus – with *Catanema* and added two species from Scotland, *C. smo* and *C. macintyreii*. In a taxonomic review TCHESUNOV (2013) redefined the genus on morphological grounds and separated it again from *Robbea* on the basis of the shape of the *fovea amphidialis*, which is spiral in *Robbea*, but reduced to just a porus in *Catanema*, adding a new species, *C. dambayensis* TCHESUNOV 2013. He was probably unaware of the description of *Catanema australis* by HOURSTON & WARWICK (2010), which also conforms to the emended diagnosis.

Molecular phylogenetic studies (KAMPFER et al. 1998, OTT et al. 2014, ZIMMERMANN et al. 2016) support the separation of those species with a porous amphidial fovea from those species with a swollen muscular corpus having a spiral fovea. The latter belong in part to the genus *Robbea* GERLACH 1956, in part will be transferred to a new genus, which is currently under description by the research group of the first author.

The present species is distinguished from the three so far described species by the presence of the praecloacal row of bumps in males. It lacks the subventral rows of conspicuous tubular setae of the type species, which are also absent from *C. dambayensis* and *C. australis*. With more than four mm in length it is also the largest of the four species.

Symbiosis

Live specimens always had a complete coat of symbiotic bacteria starting at about 1.5 pharynx lengths from the anterior end and covering the body except for the tip of the tail. The bacteria often detach during the fixation and preparation procedure and specimens in slides sometimes are only partly covered or completely bare. COBB (1920), although a keen observer, does not mention the bacteria (as also in the descriptions of other stilbonematid genera, such as *Laxus*, *Stilbonema* or *Leptonemella*). Both HOURSTON & WARWICK (2010) and TCHESUNOV (2013) report only a partial covering of the surface of the worms. None of the two papers mentions a reduction of body diameter at the onset of the bacterial coat, contrary to what is evident in all specimens of *C. schiemeri* sp. nov. A similar reduction of

body diameter has been reported for *Laxus oneistus* OTT et al. 1995 where the anterior region is likewise symbiont free, but not for *L. cosmopolitus* OTT et al. 1995 where the bacteria cover the whole body. We may therefore assume that in *C. australis* and *C. dambayensis* the bacteria cover the whole body in life.

A remarkable feature is the large stalked sulfur bacteria that are attached to the anterior body region in about 25 % of the specimens. The frequency of occurrence suggests a symbiotic relationship. Similar bacteria have been occasionally found on other stilbonematine genera (*Stilbonema*, *Leptonemella*) in other locations (unpublished personal observations). A mutualistic relationship seems unlikely, rather it can be considered a phoresis, where the giant bacteria take advantage of the movement of the worm similar to the symbiotic Gammaproteobacteria. Whether the small stalked prokaryotes occasionally present in the same body region of the new species represent different symbionts is presently unclear.

Intersexuality

Intersexuality has been described for marine free-living nematodes from the orders Chromadorida (GOURBAULT & VINCX 1990, MILJUTINA et al. 2013) and Desmodorida (Desmodoridae: MOURA et al. 2014) and specifically for Stilbonematinae (*Leptonemella*: RIEMANN et al. 2003; *Robbea*: OTT et al. 2014). In all cases, the intersexes have female gonads and rudimentary male sexual characters. Generally, they comprise only a very small percentage of the population. In *Robbea hypermnestra* OTT et al. 2014, however, all non-male adults are intersexes.

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