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Theristus (Penzancia) anoxybioticus n. sp. (Nematoda: Xyalidae) from Sublittoral Methane Seepages in the Northern Kattegat, Denmark

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Abstract: The free-living marine nematode *Theristus (Penzancia) anoxybioticus* n. sp. is described from specimens collected in muddy sediment at 10–12 m water depth in the northern Kattegat, Denmark, where the benthic environment is influenced by methane seepages. Mean body length of the male is 1,121 μm and of the female 1,159 μm. *Theristus (Penzancia) anoxybioticus* n. sp. has one crown of 10 cephalic setae and a clavate tail tip without setae. The three caudal gland cells are prominent. The intestinal lumen is hexaradiate in cross section and the lining is devoid of microvilli. Reproductive adults have so far only been found in the uppermost centimeter of sediment, and their presence is restricted to April and May. Juveniles are found in deep anoxic sediment layers during other months of the year.

Key words: Kattegat, marine nematode, intestine, methane, mud, nematode, new species, seepages, *Theristus (Penzancia) anoxybioticus,* ultrastructure.

Sublittoral muddy bottoms in the northern Kattegat, near the Danish coast, have numerous methane seepages. The benthic fauna around these seepages consist almost entirely of microscopic animals (6). The reduction of the fauna at the methane seepages is ascribed to the continual stress of toxic hydrogen sulfide carried from deeper sediment layers in the gas stream, which also may intensify oxygen depletion in the uppermost centimeter of sediment. The metazoan found most deeply in the sediment is a nematode of which only juveniles had been found up to 1992, with a population maximum at 6–12 cm depth; these specimens were previously identified as *Daptonema* sp. (6). A few fourth-stage juveniles and young adults were found in the uppermost 2 cm of sediment in March 1992, and many adults were present in the uppermost few mm during April and May, 1993–94. This free-living marine nematode is described herein as *Theristus (Penzancia) anoxybioticus* n. sp. (Monhysterida, Xyalidae).

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MATERIALS AND METHODS

Sediment samples consisting of mud with varying amounts of fine sand were collected by SCUBA divers at 10–12 m water depth NW of the Hirsholm Islands in the northern Kattegat, Denmark. Tubes (30 cm long, 5.5-cm-d) were pushed 15–25 cm into the sediment and sealed with rubber stoppers at both ends. In the laboratory the cores were sliced into 0.5-, 1-, 2-, or 4-cm subsamples, and the subsamples fixed in Trump's fixative (10). The fauna was concentrated on a 45-μm-mesh sieve, and the nematodes were removed with the aid of a stereomicroscope. Specimens for light microscopy (LM) were mounted in glycerol on slides and examined with a differential interference microscope. Specimens for transmission electron microscopy (TEM) were postfixed in 1% OsO_4, dehydrated in a graded series of alcohol, stained in uranyl acetate in 70% alcohol, embedded in Epon, and sectioned on an LKB microtome. The sections were examined at 80 kV in a Zeiss EM 900 transmission electron microscope.

SYSTEMATICS

Theristus (Penzancia) anoxybioticus n. sp. (Figs. 1–4)

Holotype male: Body length 1,027 μm; greatest body width 38 μm; head width 15

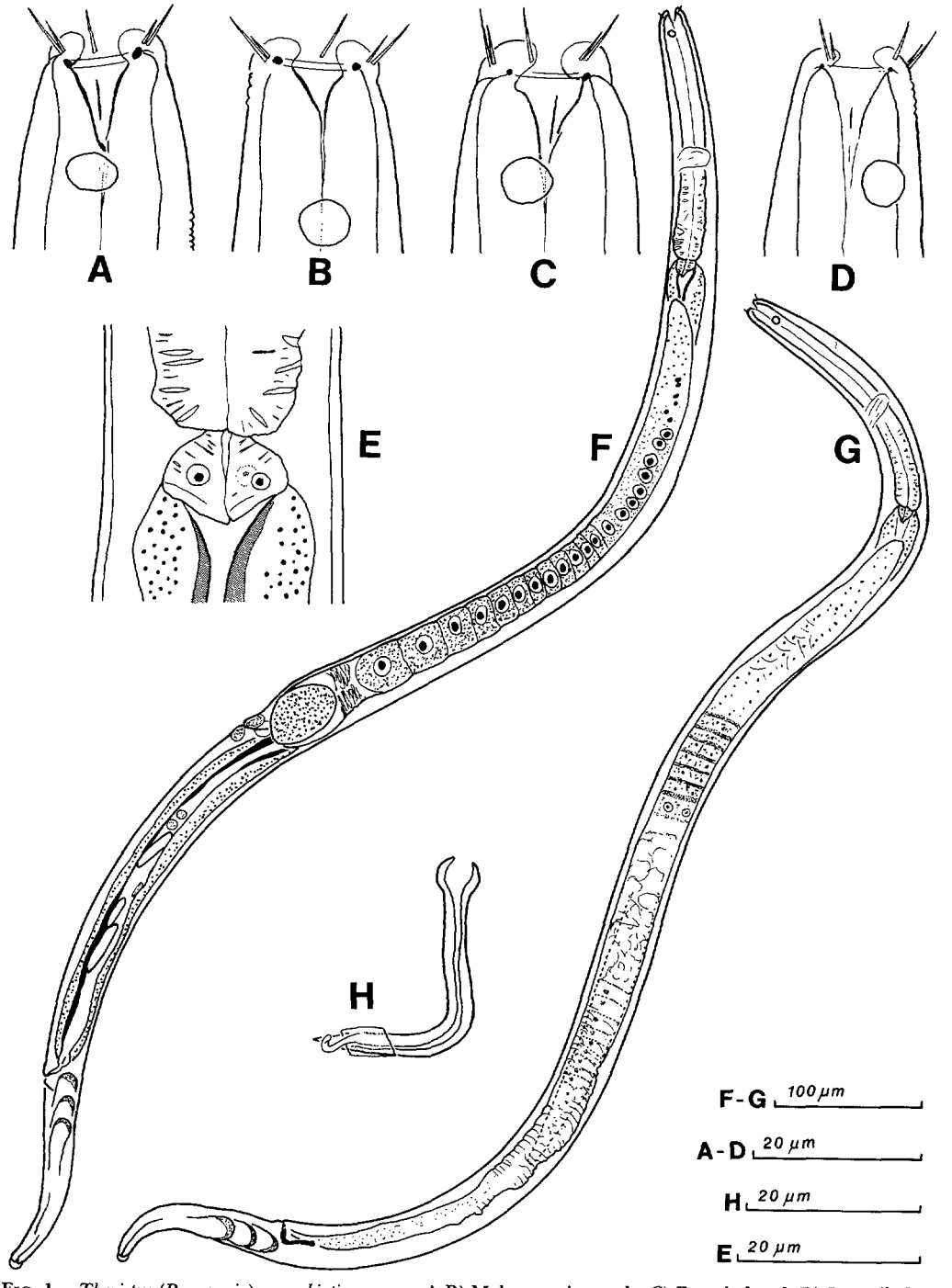

FIG. 1. *Theristus (Penzancia) anoxybioticus* n. sp. A,B) Male anterior ends. C) Female head. D) Juvenile head. E) Juvenile esophageal–intestinal junction. F) Female. G) Male. H) Male copulatory apparatus.

µm; distance from anterior end to nerve ring 115 µm; body width at nerve ring 30 µm; length of esophagus 179 µm; body width at posterior esophagus 30 µm; tail length 129 µm; body width at cloaca 29 µm; spicule length: chord 35 µm, tip to tip 26 µm; a = 27; b = 6; c = 8; c′ = 4.4.

Males: Measurements of 11 males in Ta-

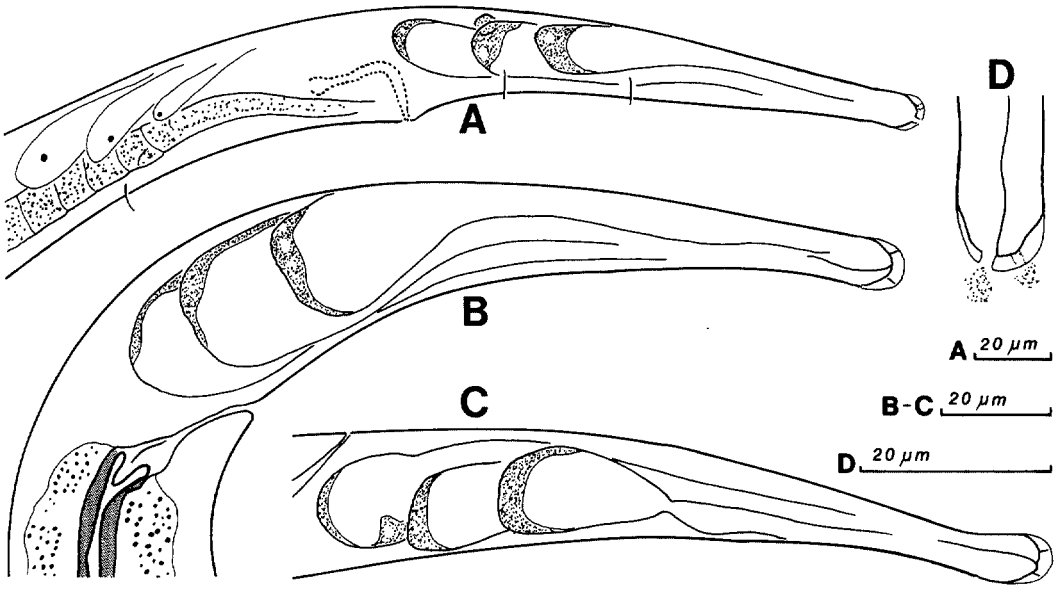

FIG. 2. *Theristus (Penzancia) anoxybioticus* n. sp. A) Male posterior end. B) Female tail. C) Juvenile tail. D) Male tail tip.

ble 1. Body cylindrical, tapering at both ends (Fig. 1G) with slightly clavate tail tip (Fig. 2D). Cuticle annulated except on head and tail tip. Somatic setae 5–6 µm long, sparse, in esophageal and tail regions. Amphid circular, 6–7 µm in diameter or transversely oval, 5 µm × 7 µm, situated 15–19 µm behind anterior end (Fig. 1A,B). Head with two crowns of cephalic sense organs: six labial papillae and a posterior crown of six setae 6 µm long and four setae 4 µm long (Fig. 1A,B). Buccal cavity funnel-shaped, widest at lip base, walls weakly sclerotized, without teeth (Fig. 1A,B). Esophageal musculature surrounding buccal cavity and attached to a sclerotized lining at junction of base of lips and buccal cavity. Esophagus cylindrical with few plasmatic interruptions. Cardia short, separated from esophagus. Ventral gland cell not observed. Intestinal lumen cylindrical, lined with hyaline layer up to 4 µm thick (Fig. 3B). Intestinal lumen hexaradiate with two variants: one arm to each lateral sector and one pair of arms to each median sector on dorsal and ventral body halves (Fig. 4A,B), or one pair of arms to each sublateral sector on left and right of the body, and one arm to dorsal and ventral body halves (Fig. 4C,D). Intestinal lining 0.25 µm–0.30 µm thick (Fig. 4). No microvilli observed. Testes opposed, outstretched, anterior testis to left and posterior testis to right of intestine (Fig. 1G). Three to four ejaculatory gland cells along dorsal body on each side of vas deferens. Spicules strongly sclerotized, proximally

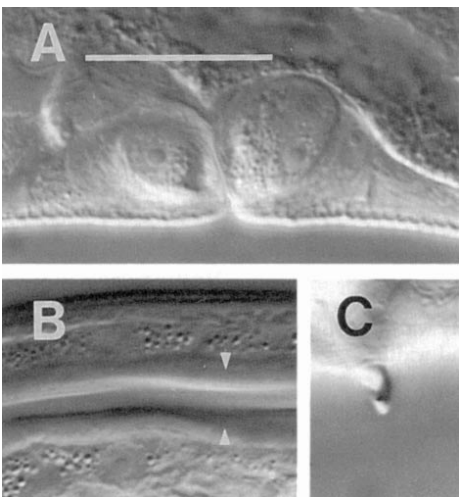

FIG. 3. Photomicrographs of *Theristus (Penzancia) anoxybioticus* n. sp. A) Vaginal gland cells. B) Longitudinal view of intestine with lining indicated (arrowheads). C) Outermost tip of lateral guiding piece of copulatory apparatus. Scale bar = 20 µm.

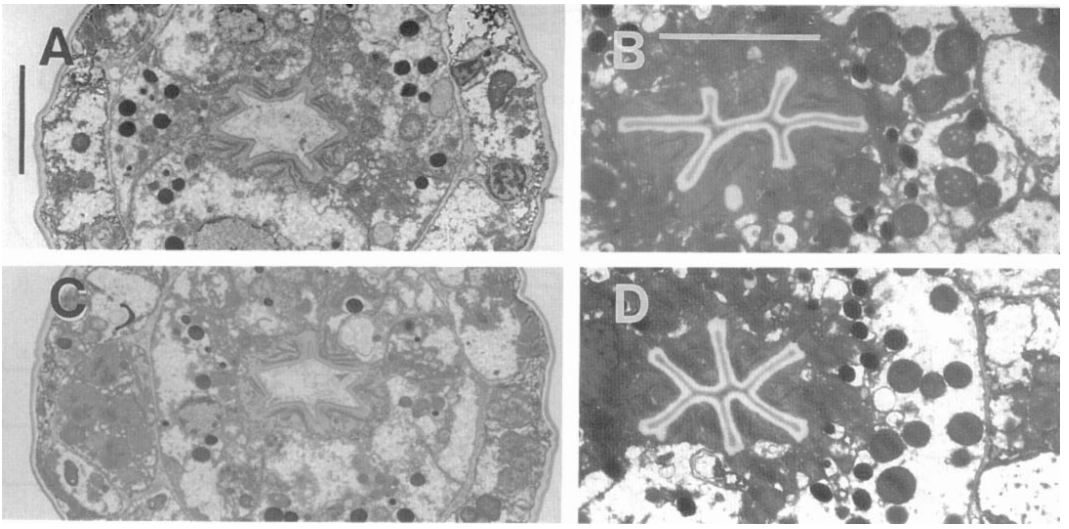

FIG. 4. Transmission electron micrographs of cross sections of *Theristus* (*Penzancia*) *anoxybioticus* n. sp. A,B) Open and collapsed hexaradiate intestine with paired dorsal and ventral arms. C,D) Open and collapsed hexaradiate intestine with single dorsal and ventral arms. Scale bars: A,C = 5 μm, B,D = 5 μm.

cephalated, distally tapering, bent almost 90°, proximal part slightly curved. Gubernaculum weakly sclerotized, surrounding spicules distally, a lateral curved tube with bent tip distally (Figs. 1H,3C). Three prominent caudal gland cells with separate outlets (Fig. 2A). No subterminal or terminal setae, but mucus threads at openings of caudal gland cells sometimes mimicking setae.

Allotype female: Body length 1,078 μm; body width at vulva 39 μm; head width 17 μm; distance from anterior end to nerve ring 106 μm; body width at nerve ring 29 μm; length of esophagus 182 μm; body width at posterior esophagus 32 μm; tail length 145 μm; body width at anus 31 μm; a = 27; b = 6; c = 7; c′ = 4.7; V = 62%.

Females: Measurements of 16 females in Table 2. Similar to males in most respects (Figs. 1C,F; 2B). Vaginal gland cells present (Figs. 1F; 3A). One ovary, anteriorly directed, outstretched and left of intestine (Fig. 1F).

Juveniles: Body shape and general organ systems as in adults (Figs. 1D,E; 2C).

TABLE 1. Morphometrics of males of *Theristus* (*Penzancia*) *anoxybioticus* n. sp. (*n* = 11).

Character	Mean	SD	Range
	Measurements in μm		
Body length	1,121	88	900–1,232
Greatest width	36	5	32–51
Head width	16	1	15–18
Esophagus length	190	14	153–205
Esophagus width	31	2	27–34
Tail length	134	11	118–150
Body width at cloaca	30	2	28–34
Spicule length (chord)	37	2	35–42
Spicule length (tip–tip)	28	2	25–30
	Ratios		
a	31	5	21–37
b	6	0	6
c	8	0.5	8–9
c′	4.4	0.4	3.6–4.9

TABLE 2. Morphometrics of females of *Theristus* (*Penzancia*) *anoxybioticus* n. sp. (*n* = 16).

Character	Mean	SD	Range
	Measurements in μm		
Body length	1,159	55	1,068–1,268
Body width at vulva	42	3	38–48
Head width	18	1	15–19
Esophagus length	199	12	181–217
Esophagus width	34	2	32–39
Tail length	146	9	131–159
Body width at anus	29	3	23–33
	Ratios and percentage		
a	28	2	24–32
b	6	0.4	5–6
c	8	0.6	7–9
c′	5.3	0.5	4.3–6.3
V	62	1	60–64

Type locality

Northwest of the Hirsholm Islands in northern Kattegat, Denmark, in the "bubbling reef" submarine landscape at 10–12 m water depth, in muddy sediment with methane seepages (6).

Type specimens

Ten adults isolated from uppermost centimeter of oxidized sediment during April–May, 1993 and 1994; five juveniles from deep, anoxic sulfide-containing layers during other months of 1989–93. All type specimens deposited in the nematode collection at the Alfred-Wegener-Institute for Polar and Marine Research, Bremerhaven, Germany (NSIMB): holotype male (571.1), allotype female (571.2), four paratype males (571.3–6), four paratype females (571.7–10), and five paratype juveniles (J4-female, 571.11; four J3, 571.12–15).

Diagnosis

Theristus (*Penzancia*) *anoxybioticus* n. sp. differs from all other species of the subgenus *Penzancia* by the combination of 10 cephalic setae, lack of somatic setae except for a few in the esophageal and tail regions, and a clavate tail tip.

Relationships

Theristus (*Penzancia*) *anoxybioticus* n. sp. (=*Daptonema* sp. [6]) is tentatively placed in the subgenus *Penzancia* De Man, 1889. Species of *Penzancia* are characterized by the lack of terminal setae on the tail, opposed to *Daptonema* species, which have terminal setae on the tail (8). On the other hand, most *Daptonema* species have a clavate tail tip, which is absent in *Theristus* species. *Theristus* (*Penzancia*) *anoxybioticus* n. sp. lacks terminal setae but has a clavate tail tip (Fig. 2A,B,C). I give the former differentiating character a higher priority ("presence–absence" character) than the latter one, which may show intermediate forms.

Riemann (11) tentatively placed *T. meyli* Riemann, 1966 in *Penzancia* because of the lack of terminal setae, although the tail tip was slightly clavate. *Theristus* (*Penzancia*) *anoxybioticus* n. sp. differs from *T. meyli* by the lack of a supplementary lateral cephalic seta and absence of most somatic setae, except a few in the esophageal region and on the tail. The body dimensions of *T. anoxybioticus* n. sp. are larger than those of *T. meyli*, except the lengths of the cephalic setae, which are longer in *T. meyli*. *Theristus* (*Penzancia*) *anoxybioticus* n. sp. also resembles *Daptonema biggi* Gerlach, 1951 in body shape, configuration and size of cephalic setae, and location and size of amphid (1, 4,7). All three descriptions of *D. biggi* depict, however, three terminal setae on the tail. Body dimensions and size of the copulatory apparatus of *D. biggi* are also much less than those of *T. anoxybioticus* n. sp. Finally, the two species inhabit different substrates: *T. anoxybioticus* found in muddy sediment, whereas *D. biggi* inhabits sandy sediments.

Remarks

The intestine of a nematode is normally described as a simple tube lined with microvilli (2,9). Three species, all belonging to Monhysteridae, have been described with an atypically shaped intestinal lumen: square in *Diplolaimella* sp. (3), tetraradiate in *D. dievengatensis* Jacobs et al., 1988 (12), and hexaradiate in *Geomonhystera disjuncta* (Bastian, 1865) (12). All three species have an intestinal lining of microvilli. The hexaradiate intestinal lumen in *Theristus* (*Penzancia*) *anoxybioticus* n. sp. appears similar to the shape found in *D. dievengatensis;* the major difference is the lack of microvilli in *T. anoxybioticus*. Several specimens of *T. anoxybioticus* n. sp. sectioned both longitudinally and transversely gave the same results; thus, the unique intestinal structure cannot be ascribed to fixation of processing artifacts. The intestinal lining of *Southerniella nojii* Jensen, 1991 (Monhysterida, Diplopeltidae) was described as a distinctly sclerotized layer in the anterior region (5), apparently similar to that of *T. anoxybioticus* n. sp. (Fig. 3B). Observations (unpubl.) of the intestine of many Xyalidae show a similar structure,

indicating that a hexaradiate intestine probably is more widespread among free-living marine nematodes. A 10- to 15-fold discrepancy between LM and TEM estimates of intestinal lining thickness (4 μm vs. 0.25–0.30 μm) may be explained by the fact that the length of an arm erroneously is taken as the thickness of the lining itself when observed by LM in longitudinal sections.

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