

ASTOMONEMA SOUTHWARDORUM SP. NOV., A GUTLESS NEMATODE DOMINANT IN A METHANE SEEP AREA IN THE NORTH SEA

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A new species of mouthless and gutless free-living marine nematode, *Astomonema southwardorum* sp. nov., the dominant species from a methane seep pockmark in the North Sea, is described and illustrated. The new species is distinguished by its combination of short cephalic and cervical setae, with the latter being sparsely and irregularly distributed, and also in male specimens by its arrangement of precloacal and caudal setae and its postcloacal papillae. The genus *Astomonema* Ott, Rieger, Rieger & Enderes, 1982, is rediagnosed. *Astomonema brevicauda* (Vitiello, 1971) Vidakovik & Boucher, 1987, is considered to be a *species inquirenda*.

INTRODUCTION

This paper describes a new species of *Astomonema*, a mouthless and gutless nematode which was dominant in meiobenthic samples collected from a methane seep pockmark in the northern North Sea (Dando *et al.*, 1991).

METHODS AND MATERIAL STUDIED

Nematodes were collected during a cruise on the MV 'Resolution' on 11-12 July 1989 in a pockmark area at 58°16.95'N 00°59.20'E (Dando *et al.*, 1991). The pockmark is a shallow depression about 600x300 m with a maximum depth of 18.5 m below the surrounding sea floor which lies at 150 m below sea level. Nematodes were extracted by flotation in Ludox TM from samples taken from the base and sides of the pockmark, where the substrate was a stiff clay covered with a layer of poorly consolidated brown silt on the sides (Dando *et al.*, 1991). Formalin-fixed specimens were mounted in glycerol and examined under differential interference contrast illumination on a Nikon Optiphot-2 microscope. Further specimens were washed in sea-water, post-fixed using 2% osmic acid in sea-water, dehydrated through an acetone series, and either critical-point dried and gold coated for scanning electron microscopy, or embedded in epoxy resin (Spurr, 1969) for thin sectioning and transmission electron microscopy. Thin sections were cut on a Reichert Ultracut or OmU3 ultramicrotome using a diamond knife and contrasted using aqueous uranyl acetate followed by lead citrate solution (Reynolds, 1963). Specimens are described using a modification of Filipjev's (1918) standard formula described

by Platt (1973). The abbreviations M and V designate the midpoint of the body for male specimens, and the position of the vulva on the body as a percentage of the total body length for female specimens, respectively. Type material has been deposited at the Natural History Museum, London.

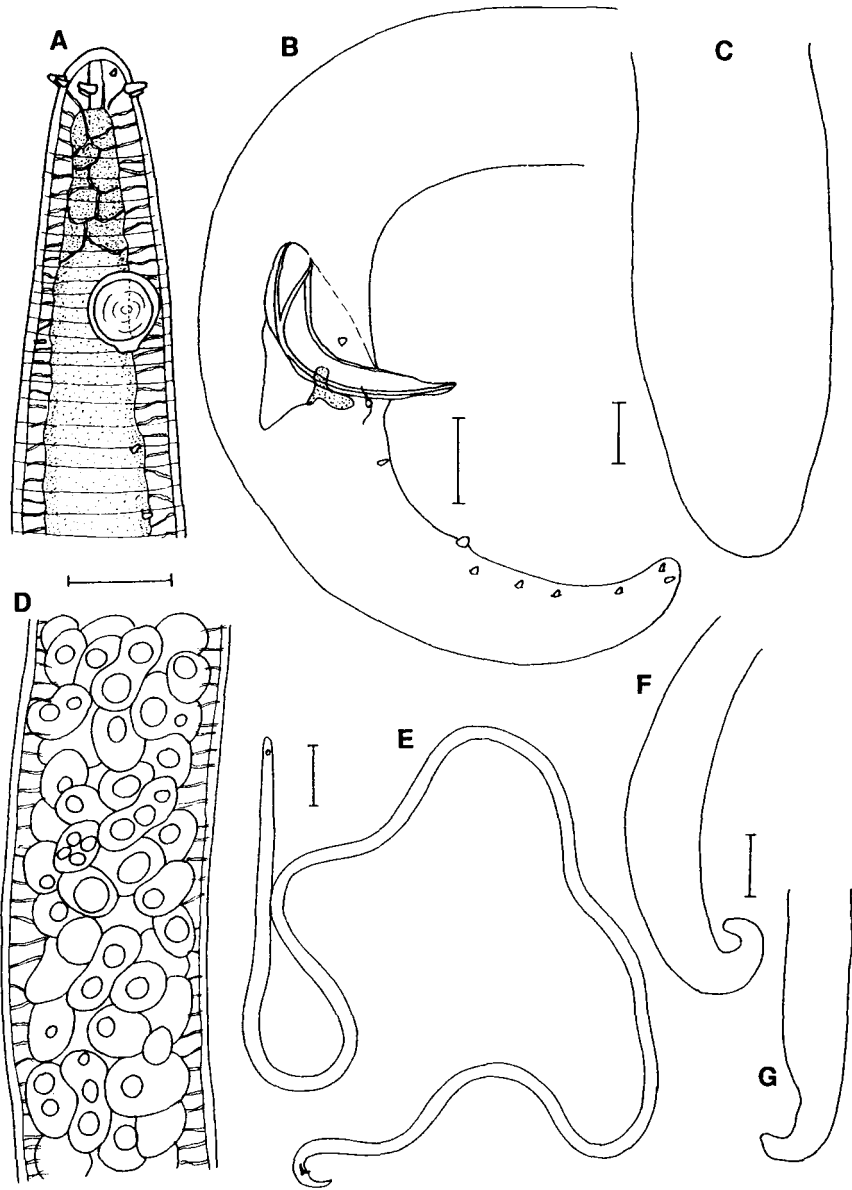


Figure 1. *Astomonema southwardorum* sp. nov. (A) Lateral view of head end of holotype male. (B) Lateral view of tail end of holotype male. (C) Lateral view of posterior end of allotype female. (D) Micro-organisms with vacuolated appearance in mid-body region of holotype male. (E) Total view of holotype male. (F) and (G) posterior ends of two juveniles. Scale bars: (A,D) 10 μ m; (B) 10 μ m; (C) 10 μ m; (E) 100 μ m; (F,G) 10 μ m.

Astomonema southwardorum sp. nov.

(Figures 1 & 2, Table 1)

Material studied

Three males, two females, three juveniles. Holotype male 1, allotype female 1, paratype males 2, 3, paratype female 2, BMNH Registered no. 1993.2.5; juveniles 1-3 BMNH Registered no. 1993.2.3.

Locality

Stiff clay sediment at 150-170 m depth in a methane seep pockmark in the North Sea at 58°16.95'N 00°59.20'E.

Description

Holotype male 1	-- M 3244	3300 μ m, a=143, b=-, c=60, Sc=26 μ m
	9 - 19	19
Allotype female 1	-- V -	3810 μ m, a=106, b=-, c=-, V=60%
	9 - 29	-

Holotype male (range within males in parenthesis) (Figure 1)

Body 3.3 (3.03-3.3) mm long, slender, almost cylindrical except in head and tail region. Cuticle finely striated with 5-6 striations per 10 μ m in the post-amphidial region. Somatic sensilla absent except for sparsely and irregularly distributed 1- μ m long papillae in amphidial and post-amphidial region, and caudal papillae described below.

Table 1. *Measurements of Astomonema southwardorum* sp. nov. in μ m.

Character	Males			Females		Juveniles		
	1	2	3	1	2	1	2	3
Total body length	3300	3030	3270	3810	4160	1470	1010	1370
De Man's ratio a	143	112	156	106	115	70	53	60
De Man's ratio c	60	50	47	-	-	-	-	-
Head diameter	9	7	8	9	9	8	9	8
Labial sensilla	1	*	1	1	1	*	1	1
Cephalic setae	2	2	2	2	2	2	2	2
Amphid from anterior	26	24	22	26	26	20	15	19
Amphid diameter	7	7	7	6	6	5	4	5
Amphid c.b.d.	13	14	12	16	15	11	10	11
Maximum body diameter	23	27	27	36	36	21	19	23
Vulva from anterior	-	-	-	2280	2030	-	-	-
Vulva c.b.d.	-	-	-	29	25	-	-	-
Spicule length as chord	26	26	29	-	-	-	-	-
Gubernaculum length	5	6	11	-	-	-	-	-
Anal body diameter	19	16	21	-	-	-	-	-
Tail length	56	60	70	-	-	-	-	-

c.b.d., corresponding body diameter; *, character not seen

Head rounded with six short ($1\ \mu\text{m}$) and four long ($2\ \mu\text{m}$) square-ended papillae, termed labial and cephalic setae respectively in Table 1; two short papillae situated laterally $2\ \mu\text{m}$ from apex, then further back a circle of four short and four long papillae

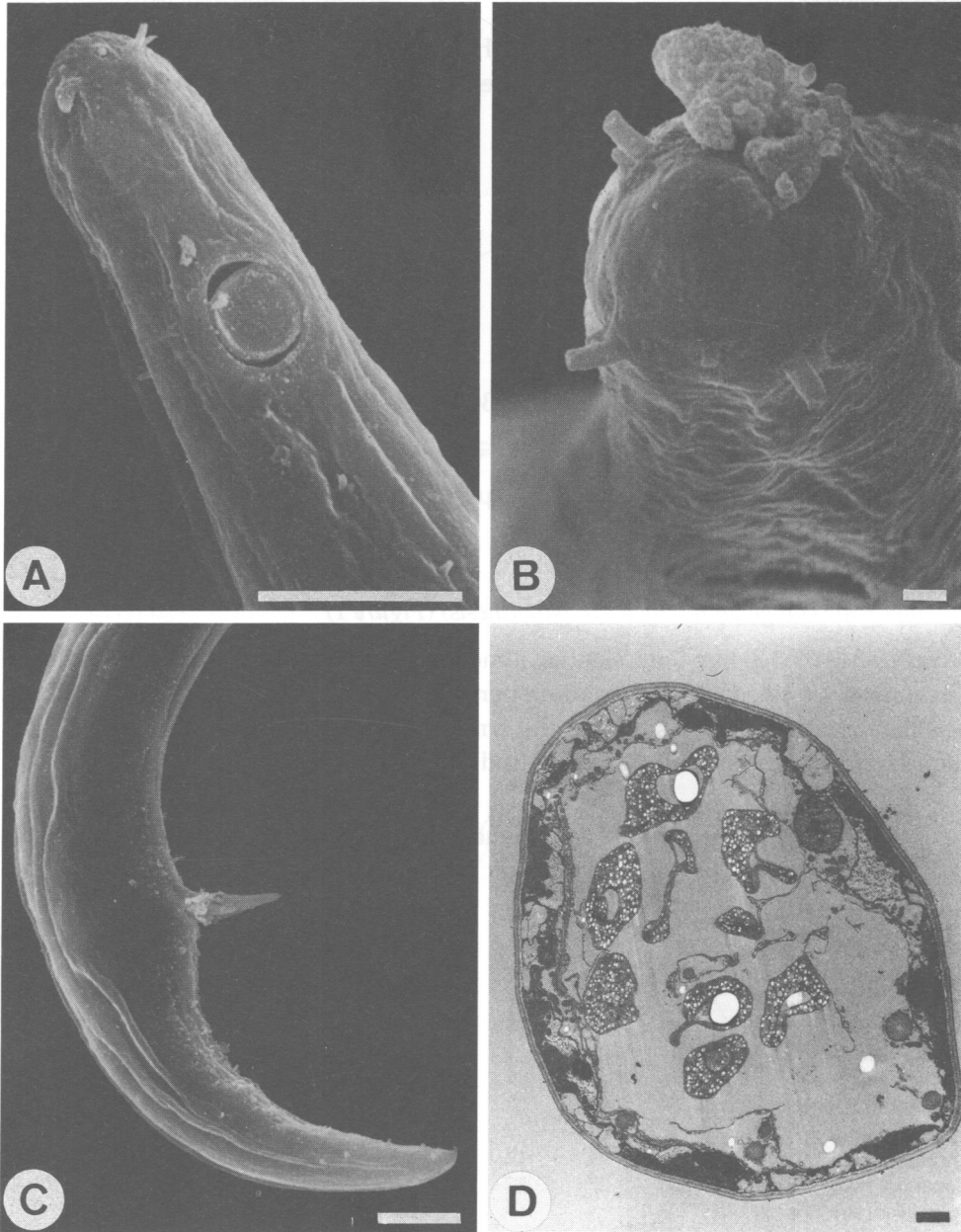


Figure 2. *Astomonema southwardorum* sp. nov. electron micrographs. Scanning electron micrographs of (A) head and neck region, (B) head showing 0+6+4 arrangement of head sensilla, and (C) tail of male. (D) Transmission electron micrograph of transverse section of mid-body region of juvenile showing vacuolated cells of micro-organisms. Some of the sculpturing in the scanning micrographs may be preparation artefacts, due to slow penetration of the fixative through the cuticle or due to the shrinkage which occurs during critical-point drying. Scale bars: (A) $10\ \mu\text{m}$; (B) $1\ \mu\text{m}$; (C) $10\ \mu\text{m}$; (D) $1\ \mu\text{m}$.

situated dorsally and ventrally (3 μm from apex); the homology of the head sensilla is uncertain. Amphids circular, diameter 7 μm or 54 (50-58)% of corresponding body diameter, situated 26 (22-26) μm or 2.9 (2.75-3.4) head diameters from anterior end and leading to a duct of the amphidial gland.

Alimentary canal apparently absent; mouth opening, buccal cavity and pharynx absent. Body cavity packed with small granules and unknown micro-organisms 5-9 μm long by 3-5 μm wide which have a vacuolated appearance. Testis single, outstretched, distal tip at 58 (58-62)% of body length from anterior. Spicules paired, equal, curved with a pointed distal tip, measuring 26 (26-29) μm or 1.4 (1.4-1.6) anal body diameters as chord. Gubernaculum with dorso-caudally directed apophyses 5 (5-11) μm long. One paired subventral pre-cloacal papilla; two paired subventral postcloacal papillae, one immediately posterior to the spicules, the other midway between the spicules and the tail tip. Tail conical, 56 (56-70) μm or 2.9 (2.9-3.7) anal body diameters long; four paired caudal papillae, 1 μm long, latero-ventrally positioned between the second post-cloacal supplement and the tail tip; four papillae 1 μm long on the tail tip.

Allotype female (paratype in parenthesis when differs from allotype) (Figure 1)

Similar to male in most features except tail which is rounded. No anus. Body 3.81 (4-16) mm long, slightly wider than male. Amphids 6 μm diameter, 37 (40)% of corresponding body diameter, 26 μm or 2.9 head diameters from anterior. Ovaries paired, outstretched; vulva cuticularized, 60 (49)% of body length from anterior.

Paratype juveniles (Figure 1)

Similar to adult in most features but tail is conical and characteristically curved at the tip (Figure 1F,G). Body 1.01-1.47 mm long. Amphids smaller than in adults, 4-5 μm , 40-45% of corresponding body diameter, 15-20 μm or 1.7-2.5 head diameters from the anterior. No cervical or caudal sensilla observed in any juveniles. Mouth opening, pharynx and vestigial buccal cavity not present.

Etymology

Astomonema southwardorum is named after Drs Alan and Eve Southward in recognition of their pioneering work on symbiotic associations in marine invertebrates. Alan Southward was present on the cruise of the MV 'Resolution' when this species was first collected and Eve Southward has kindly made the TEM photograph available to us and is presently working on the micro-organisms associated with this species.

Ecology

Astomonema southwardorum was the dominant nematode in two samples taken from the sides of a methane seep pockmark and was found in densities of 1168 and 2251 individuals per 10 cm^2 . It was also found in lower densities (5 and 33 individuals per 10 cm^2) in two samples taken from the bottom of the pockmark where the benthic faunal abundances were generally low. A preliminary study indicates that it has a maximum

density at a depth of 5-8 cm in the sediment, which coincides with a maximum in elemental sulphur (Dando *et al.*, 1991). In common with other *Astomonema* species its body appears to be densely packed with a large number of intracellular symbiotic organisms (Southward & Gire, personal communication). To our knowledge no similar specimens have been found in any other area of the North Sea, suggesting high specificity to the methane seep area biotope.

Differential diagnosis and taxonomic remarks

We have placed *Astomonema southwardorum* in this genus as it possesses the same head sensilla arrangement as *A. jenneri* Ott, Rieger, Rieger & Enderes, 1982 which is the type genus and which was described by Kito (1989) as a characteristic 0+6+4 arrangement for this genus. The head sensilla arrangement and the presence of only one testis distinguish it from a similar mouthless genus *Parastomonema* Kito, 1989. According to Kito (1989) another feature suggesting generic differences was the presence of a vestigial conoid stoma in juvenile *Parastomonema* whilst small *Astomonema* juveniles have been observed with vestigial tubular stoma. In our new species we have not observed any vestigial stoma.

The species within the genus *Astomonema* are all rather similar with the differential diagnosis being based on length and arrangement of somatic setae and length and width of the body.

Astomonema southwardorum can be distinguished from *A. otti* Vidakovic & Boucher, 1987, by its lack of a regular arrangement of cervical setae: *A. otti* has a 4+4+6+1 configuration of setae 0.5 µm long. These two species also differ in the arrangement of precloacal setae (1 seta in *A. otti*, 1 paired pre-cloacal papilla in *A. southwardorum*), postcloacal papillae (2-3 in *A. otti*, 2 in *A. southwardorum*), and caudal setae (sparse in *A. otti*, 4 paired, situated latero-ventrally in *A. southwardorum*). *Astomonema southwardorum* is mainly longer and thinner than *A. otti* (higher demanian a ratio). *Astomonema jenneri* Ott, Rieger, Rieger & Enderes, 1982, and *A. obscura* (Boucher & Helléouët, 1977) Vidakovic & Boucher, 1987, both have longer cephalic and cervical setae than *A. southwardorum* and *A. otti*.

Astomonema southwardorum is probably most like *A. brevicauda* (Vitiello, 1971) Vidakovic & Boucher (1987). However, this latter species has a rather confusing taxonomic history such that it is impossible to determine whether they are the same species. Consequently we have had to describe our *Astomonema* as a new species. *Astomonema brevicauda* (Vitiello, 1971) Vidakovic & Boucher (1987) was redescribed by Vitiello (1971) as *Rhabdocoma brevicauda* (Schuurmans Stekhoven, 1950) which he synonymized with *R. cylindricauda* (Schuurmans Stekhoven, 1950). Schuurmans Stekhoven's (1950) descriptions were of species with a buccal cavity, cylindrical oesophagus and an anal/cloacal opening towards the posterior part of the body. In Vitiello's (1971) redescription of *R. brevicauda* his specimens had no mouth, but his figure 17B shows a female with a distally expanded oesophagus, and he observed a minority of specimens with a cylindrical oesophagus extending from the level of the amphids down to that of the cardia. Without redescription or any explanation Vidakovic & Boucher (1987) transferred *Rhabdocoma*

brevicauda to the genus *Astomonema*. The drawings and descriptions of the setal arrangements and lengths of *A. brevicuda* are not clear, in separate drawings Vitiello (1971) shows both a 0+6+4 and a 0+0+4 head sensilla arrangement for his *Rhabdocoma brevicuda* but appears to describe only the 0+0+4 arrangement in his text. There does not appear to be any type material available for re-examination. Kito (1989) uses the arrangement of head sensilla as a major diagnostic feature separating the genera *Astomonema* (0+6+4) and *Parastomonema* (0+0+4). Because of the importance of the setal arrangement and length as diagnostic characteristics both within the genus *Astomonema* (Vidakovic & Boucher, 1987) and between the mouthless genera *Astomonema* and *Parastomonema* (Kito, 1989) we regard *Astomonema brevicuda* (Vitiello, 1971) Vidakovic & Boucher (1987) as a *species inquirenda*.

In all the *Astomonema* species so far described all females lack an anus and most authors describe a high incidence of tail breakage and wound healing anterior to the anus. We have been fortunate enough to observe hundreds of *A. southwardorum* specimens and have not yet observed one adult female with an anus. All females had cylindrical tails, only juveniles and males had conical tails and only adult males had visible cloacal openings. No adult males had rounded tails. We suggest that this is a sexually dimorphic characteristic for our species.

Since there is no formal description of the genus *Astomonema* we propose the following.

Family SIPHONOLAIMIDAE Filipjev, 1918

Astomonema Ott, Rieger, Rieger & Enderes, 1982

Siphonolaimidae. Body long, slender and cylindrical. Cuticle thin and finely annulated. Head rounded with 0+6+4 head sensilla. Amphids circular. Alimentary canal degenerate: mouth opening, buccal cavity, pharynx and anus absent in adults, cloacal opening present in male. Reproductive system with two outstretched and opposed ovaries in the female, and one testis in the male.

Type species: *Astomonema jenniferi* Ott, Rieger, Rieger & Enderes, 1982.

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