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Chronogaster troglodytes sp. n. (Nemata : Chronogasteridae) from Movile Cave, with a review of cavernicolous nematodes

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Summary – *Chronogaster troglodytes* sp. n. (Nemata : Chronogasteridae) is described as the first true cavernicolous nematode which was recovered from Movile Cave in Romania. This species is adapted for survival in floating fungal mats growing in hydrogen sulfide-rich thermomineral waters. Populations are composed of hermaphroditic females which feed on bacteria associated with the fungal mats. A review of cavernicolous nematodes, and their relationship to epigean freshwater and soil forms, is presented.

Résumé – Chronogaster troglodytes ***sp. n. (Nemata : Chronogasteridae) provenant de la caverne de Movile et liste des nématodes cavernicoles*** – *Chronogaster troglodytes* sp. n. (Nemata : Chronogasteridae) est décrit comme le premier véritable nématode cavernicole, récolté dans la caverne de Movile, en Roumanie. Cette espèce est adaptée à la survie dans le feutrage d'hyphes fongiques flottant à la surface d'eaux thermo-minérales riches en hydrogène sulfuré. La population est composée de femelles hermaphrodites se nourrissant à partir des bactéries associées aux champignons. Les nématodes cavernicoles sont passés en revue et leurs relations avec les formes vivant dans les eaux de surface et les sols sont présentées.

Key-words : *Chronogaster*, cavernicolous nematodes.

Cavernicolous nematodes have been little studied, thus it was of interest when, in the subterranean Movile Cave in Romania, samples of floating mats composed of stratified fungal mycelia and sulfide oxidizing bacteria revealed the presence of nematodes.

The Movile Cave, located in a limestone plateau in Southern Dobrogea, consists of a network of fissures and small cave passages partially flooded by hydrogen sulfide rich thermomineral waters. The chemoautotrophically based ecosystem appears to be the first subterranean community relying exclusively on autochthonous primary production (Sarbu & Popa, 1992).

Although representatives of three genera of nematodes were recovered from the floating mats in Movile Cave, the most abundant by far was a species of *Chronogaster* which was represented only by hermaphroditic females. This species is described below as a unique cave inhabiting nematode that has adapted to the specialized physiological conditions in Movile Cave. A review of cavernicolous nematodes, and their relationship to epigean soil and freshwater nematodes, is presented.

Samples of floating mats from the surface of the thermowaters of Movile Cave were fixed directly in 4 % formalin at 60 °C. Additional samples were taken at depths of 20 cm and 1.5 m, respectively. The nematodes were hand picked out of the samples, processed to glycerin and mounted on microscope slides. Microscopic examinations were conducted with a Nikon Optiphot microscope equipped with Differential Interference Contrast. Body inclusions tentatively called crystalloids were examined under polarized light.

Chronogaster troglodytes sp. n.

(Figs 1, 2)

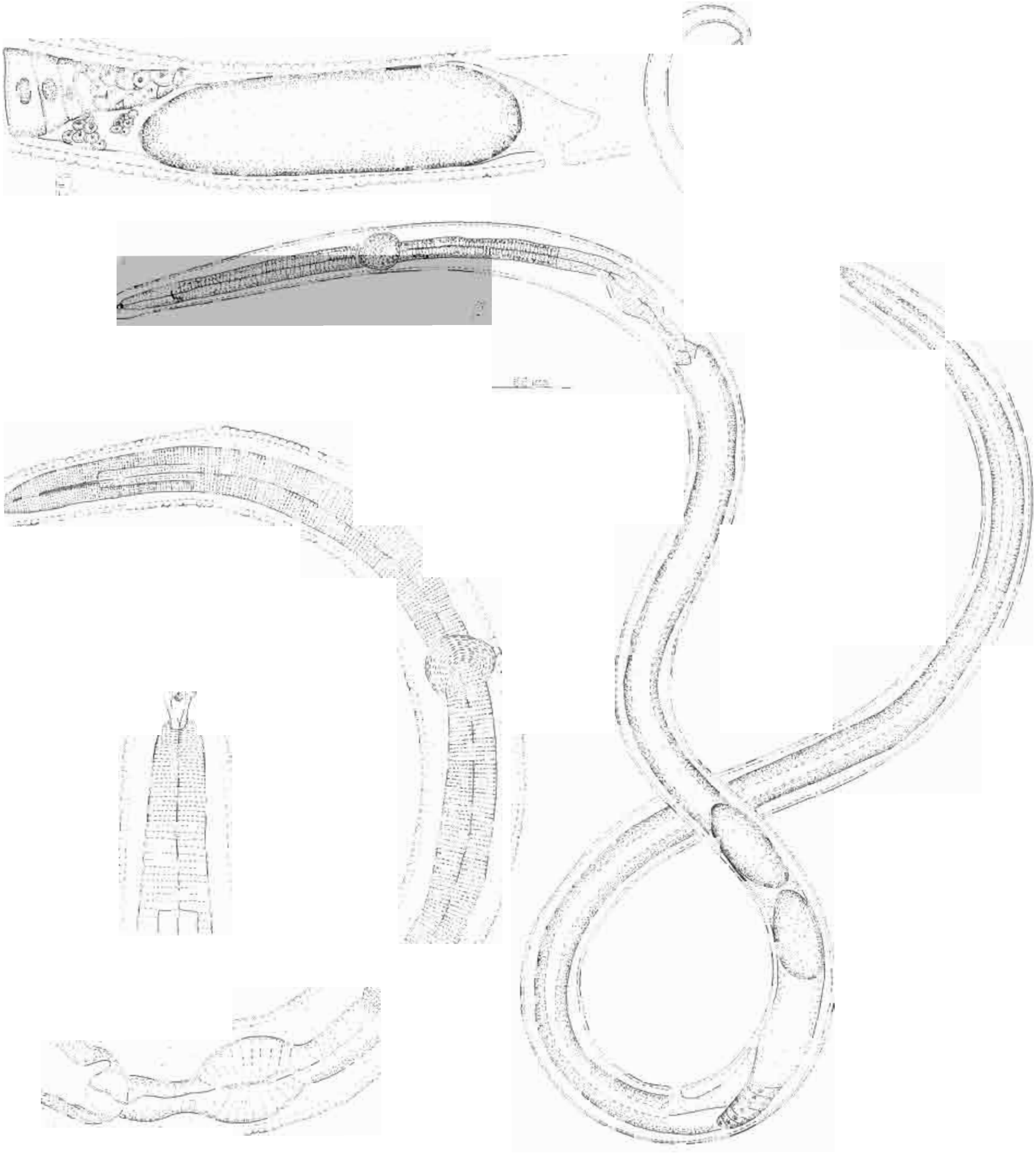
DIMENSIONS

Females (paratype, n = 10) : L = 1.29 (1.17-1.44) mm; a = 55 (47-65); b = 5.1 (4.3-5.9); c = 9.3 (8.0-10.8); V = 49 (48-50); tail = 141 (127-160) µm.

Holotype (female) : L = 1.26 mm; a = 57; b = 4.9; c = 9.8; V = 50; tail = 128 µm.

DESCRIPTION

Female : Body variable in shape when relaxed, but the tail strongly curved ventrally. Greatest width 24 (21-27) µm. Transverse cuticular striae distinct, the width varying from 0.8 to 1.4 µm behind the lip region, 1.3 to 1.9 µm at the base of the œsophagus, 1.5 to 2.4 µm at midbody and 0.6 to 1.9 µm opposite the anus. Lateral lines absent. Cephalic setae 4.9 (4.5 to 6.9) µm long. Unstriated lip region 1.7 (1.2 to 2.5) µm high. Mouth cavity funnel-shaped, the stoma measuring 7.0 (5.1-10.5) µm in length and 2.2 (1.8-2.6) µm in width. Distance between head and radial tubules 32 (26-42) µm. Amphids broadly stirrup-shaped with a circular opening located opposite the basal half of the stoma and measuring 3.1 (2.6-5.9) µm in greatest width and 2.9 (2.6-5.9) µm in greatest length. Nerve ring located 122 (109-161) µm posterior to mouth opening. Excretory pore



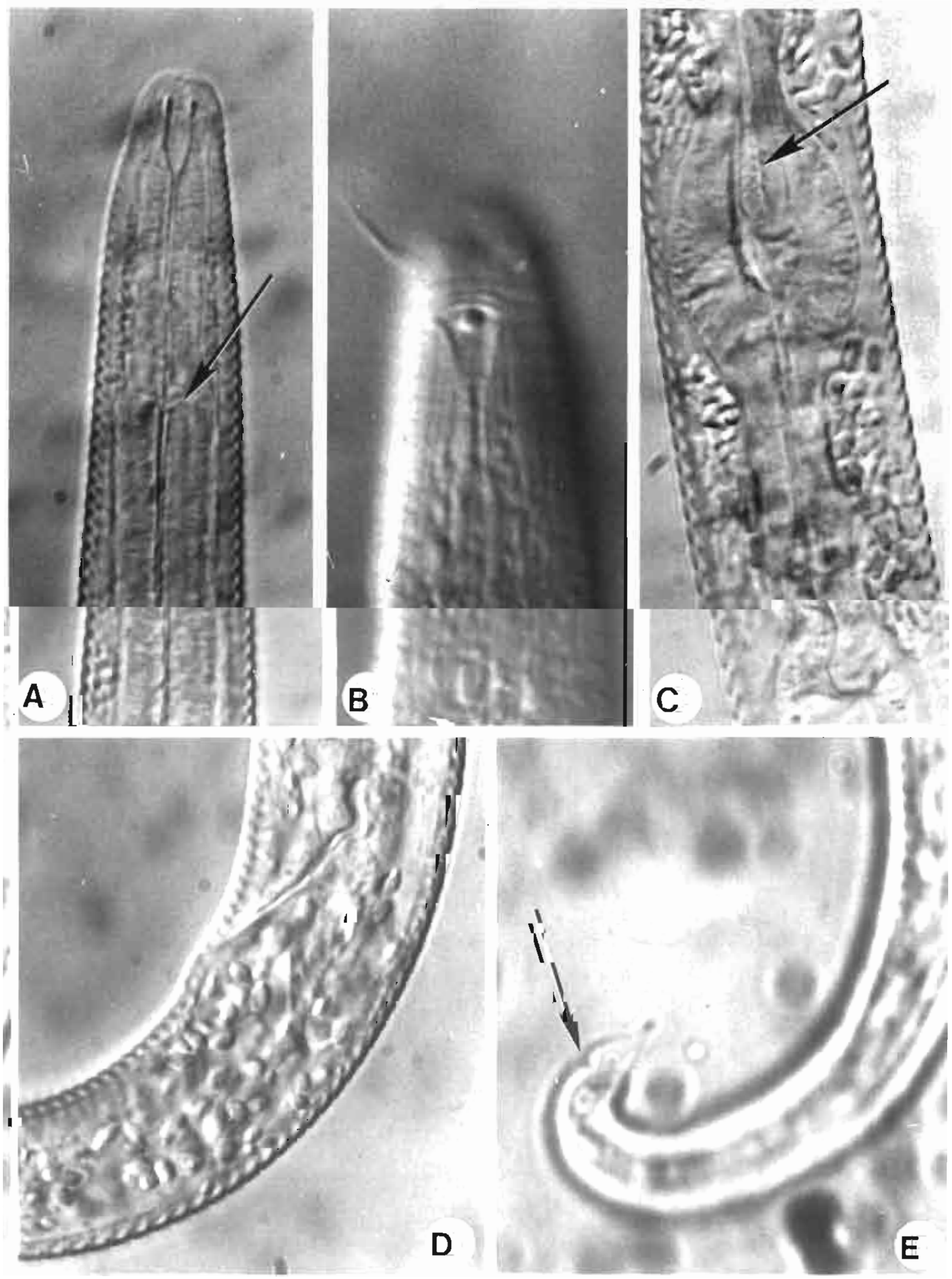


Fig. 2. Chronogaster troglodytes *n. sp. hermaphrodite. A : Anterior region showing position of radial tubules (arrow); B : Head area showing amphid with circular opening; C : Basal oesophageal region showing denticles (arrow) on valve in basal bulb and crystalloids surrounding the basal bulb; D : Anal area showing crystalloids posterior to the rectum; E : Tail showing notch on dorsal surface (arrow).*

and hemizonid absent. Total length of oesophagus 254 (222-272) µm. The oesophagus is divided into several sections, including the basal bulb 26 (22-32) µm in length with a dilated denticulate chamber in the anterior third, a prebulbar extension 14 (9-27) µm in length continuous with the basal bulb, a postbulbar extension, also continuous with the bulb, 15 (11-18) µm in length, and a cardia 7 (4-9) µm in length. Prebulbar extension not noted previously in members of this genus. Rectum 20 (17-22) µm in length and 1.3 (1.1-1.6) times the anal body diameter. Tail elongate conoid, terminating in a sharply pointed tip, with one or rarely two notches on the dorsal surface just before the tip. Anal body diameter 15 (13-17) µm. Caudal glands and caudal pore absent. Large lateral ovoid glands occasionally seen in some specimens but their position not constant (so it is not known if they correspond to the glandular bodies described by previous workers). Openings of these glands spherical, 4.5 µm in diameter. Hypodermal cords and occasionally the body cavity filled with rod or oval shaped bodies, 1.3-5.1 µm in length and 0.6-1.3 µm in width. Number of these bodies varying from individual to individual and probably corresponding to the crystalloids of previous authors. Vulva is a transverse opening leading to a short vagina, 5.9 (3.2-6.4) µm long. Posterior uterine sac extending 6.4 (1.9-16.0) µm in length. A single anterior reflexed gonad which extends 169 (120-216) µm from the vulva. Spherical bodies (2.3 to 2.8 µm in diameter) near the proximal portion of the gonad and often adjacent to the distal portion of the maturing eggs. These bodies are considered to be sperm, thus the females of *C. troglodytes* being actually hermaphrodites. Eggs in the proximal portion of the uterus in mature individuals 75 (36-102) µm in length to 20 (16-22) µm in width.

Male : Not found.

TYPE LOCALITY AND HABITAT

In fungal mats floating on thermomineral sulfur waters in the subterranean Movile Cave, Southern Dobrogea, Romania.

TYPE SPECIMEN

Holotype hermaphrodite deposited in the Nematology Collection at the Department of Nematology, University of California, Davis, CA. Paratype hermaphrodites deposited at the Laboratoire de Biologie Parasitaire, Protistologie, Helminthologie, Muséum National d'Histoire Naturelle, Paris.

DIAGNOSIS

Chronogaster troglodytes n. sp. is distinguished from all other species in the genus by the following combination of characters; stirrup-shaped amphids with a circular opening, cephalic setae not over 7.0 mm long, dorsal surface of tail with one or two small notches and tail tip pointed without obvious, set-off mucros or spines. The anterior extension of the oesophageal bulb has not been reported in other representatives of the genus but may have been overlooked. This is the only member of the genus that is thought to be hermaphroditic.

This description brings the total number of *Chronogaster* species to 31. The present species could not be identified in either the key of Heyns and Coomans (1983) including 25 species nor that of Raski and Maggenti (1984) which includes 29 previously known species, only omitting *C. zujarensis* Ocaña & Coomans, 1991.

BIOLOGICAL OBSERVATIONS

Entire populations (eggs, juveniles, hermaphrodites) of *C. troglodytes* have been recovered from the floating mats on the surface of the thermomineral waters (for the types and concentrations of ions found in water samples from Movile Cave, see Sarbu and Popa, 1992).

However, in sediment at 20 cm depth, eggs and juveniles were rarely found and in deep sediment at 1.5 meter depth, no nematode stages were recovered. This correlation is probably dependent on the amount of oxygen since while the surface water contained 1.0 to 1.5 mg of O_2/liter, the concentration dropped to 0.1 mg at a depth of 10 cm and to O at depths of 20 cm or greater. Thus *C. troglodytes* lives in the microbial-rich fungal mats which float on the surface of the water. Bacteria probably serve as the major source of food for these nematodes since bacterial cells were noted in the intestinal lumen of some individuals.

Hermaphroditism in *Chronogaster*

Males are known for only five of the 29 species of *Chronogaster* (Raski & Maggenti, 1984) and there has been little discussion concerning the mode of reproduction of these nematodes. Small spherical objects interpreted as spermatozoa occurred in the distal portion of the uterus of *C. troglodytes* and varied from 2.4 to 2.8 µm in diameter. Since males were absent fom all populations, it is concluded that hermaphroditism occurs in this species.

Although there is no previous mention of sperm production in any female *Chronogaster*, Heyns and Coomans (1980) remark that in *C. africana* and *C. multispinata,* both of which lack males, ovoid bodies could be seen near the flexure of the ovary. The authors interpreted these bodies as rejected and degenerating oocytes, however, they might have been related to sperm development. Clearly this issue should be further investigated.

Crystalloids

Crystalloids were first noted in *C. gracilis* Cobb, 1913. Heyns and Coomans (1980) discussed the presence of crystalloids in the body cavity of *Chronogaster* spp. and mentioned that they were uncommon. Later, Heyns and

Table 1. *Nematode genera reported from caves or subterranean waters.*

Nematode genera	Habitat	Location	Reference
Achromadora	–	Hungary	Andrássy, 1959
Actinolaimus	–	Poland	Andrássy, 1959
Alaimus	–	Belgium	Schuurmans-Stekhoven, 1943
"	mould	Mexico	Zullini, 1973
Amphidelus *	mud	Mexico	Zullini, 1977
Anaplectus	organic detritus	Belgium	Schuurmans-Stekhoven, 1943
Anatonchus	–	Belgium	Andrássy, 1959
Aphonolaimus	–	Jugoslavia	Andrássy, 1959
Aporcelaimellus	mud	Mexico	Zullini, 1977
Aporcelaimus	–	Belgium	Andrássy, 1959
Axonchium	mud	Mexico	Zullini, 1973
Cephalobus *	organic detritus	Belgium	Schuurmans-Stekhoven, 1943
Cervidellus	–	Hungary	Andrássy, 1959
Chiloplacus	–	Hungary	Andrássy, 1959
Chronogaster	floating fungal mats in thermomineral pools	Dobrogea, Romania	Present study
Clarkus	slime	Mexico	Zullini, 1973
Criconema	–	Jugoslavia	Andrássy, 1959
Criconemoides	–	Belgium, Jugoslavia	Andrássy, 1959
Cylindrolaimus	–	Hungary	Andrássy, 1959
Desmoscolex	–	Jugoslavia	Andrássy, 1959
Diplogaster	–	Hungary, Jugoslavia	Andrássy, 1959
Ditylenchus	–	Poland	Andrássy, 1959
Dorylaimus *	small pool rotting wood	Belgium	Schuurmans-Stekhoven, 1943
"	–	Yucatan	Chitwood, 1938
"	spring	Bihor, Romania	Schuurmans-Stekhoven, 1950
Enchodelus	–	Switzerland	Andrássy, 1959
Eucephalobus	–	Jugoslavia	Andrássy, 1959
Halalaimus	–	Jugoslavia	Andrássy, 1959
Helicotylenchus	–	Belgium	Andrássy, 1959
Hofmaenneria	–	Jugoslavia	Andrássy, 1959
Hoplolaimus	small pool	Belgium	Schuurmans-Stekhoven, 1943
Iotonchus	–	Belgium	Andrássy, 1959
Ironus *	water	Mexico	Zullini, 1973
Mermis	cave	Bihor, Romania	Schuurmans-Stekhoven, 1950
Mesodorylaimus	mud	Hidden Cave, New Mexico	Material submitted by James C. Cokendolpher 22 April 1992
"	mud	Mexico	Zullini, 1973, 1977
Mesorhabditis	–	Jugoslavia	Andrássy, 1959
Monhystera *	mud	Mexico	Zullini, 1973
Mononchus *	organic detritus	Belgium	Schuurmans-Stekhoven, 1943
"	mud	Mexico	Zullini, 1973, 1977
Mylonchulus *	mud	Mexico	Zullini, 1977
Panagrolaimus	floating fungal mats in thermomineral pools	Dobrogea, Romania	Present study
Plectus *	small pool organic detritus	Belgium	Schuurmans-Stekhoven, 1943
"	sediment	Mexico	Zullini, 1973
Prionchulus	–	Belgium Jugoslavia	Andrássy, 1959
Prismatolaimus	–	Belgium, Hungary	Andrássy, 1959
Prodesmodora	mud	Mexico	Zullini, 1973
Protorhabditis	floating fungal mats in thermomineral pools	Dobrogea, Romania	Present study
Rhabditis *	organic detritus	Belgium	Schuurmans-Stekhoven, 1943
Rhabditophanes	–	France	Andrássy, 1959
Rotylenchus	–	Jugoslavia	Andrássy, 1959
Stenonchulus	–	Jugoslavia	Andrássy, 1959
Teratocephalus	–	Poland	Andrássy, 1959
Tetylenchus	organic detritus	Belgium	Schuurmans-Stekhoven, 1943
Thalassoalaimus	–	Jugoslavia	Andrássy, 1959
Theristus	–	Jugoslavia	Andrássy, 1959
Trilobus *	small pool organic detritus	Belgium	Schuurmans-Stekhoven, 1943
Tripyla *	spring	Romania	Schuurmans-Stekhoven, 1943
"	sediment	Mexico	Zullini, 1973
Trischistoma	mud	Mexico	Zullini, 1973
"	mud	Capri	Meyl, 1954
Tylenchorhynchus	–	Belgium	Andrássy, 1959
Tylenchus *	organic detritus	Belgium	Schuurmans-Stekhoven, 1943
Xiphinema	mud	Mexico	Zullini, 1973

* Additional representatives of cavernicolous species in this genus are cited by Andrássy (1959).

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