

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

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Accepted for publication 26 July 1993.

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 epigeal 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 epigeal 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) μ m.

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

DESCRIPTION

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

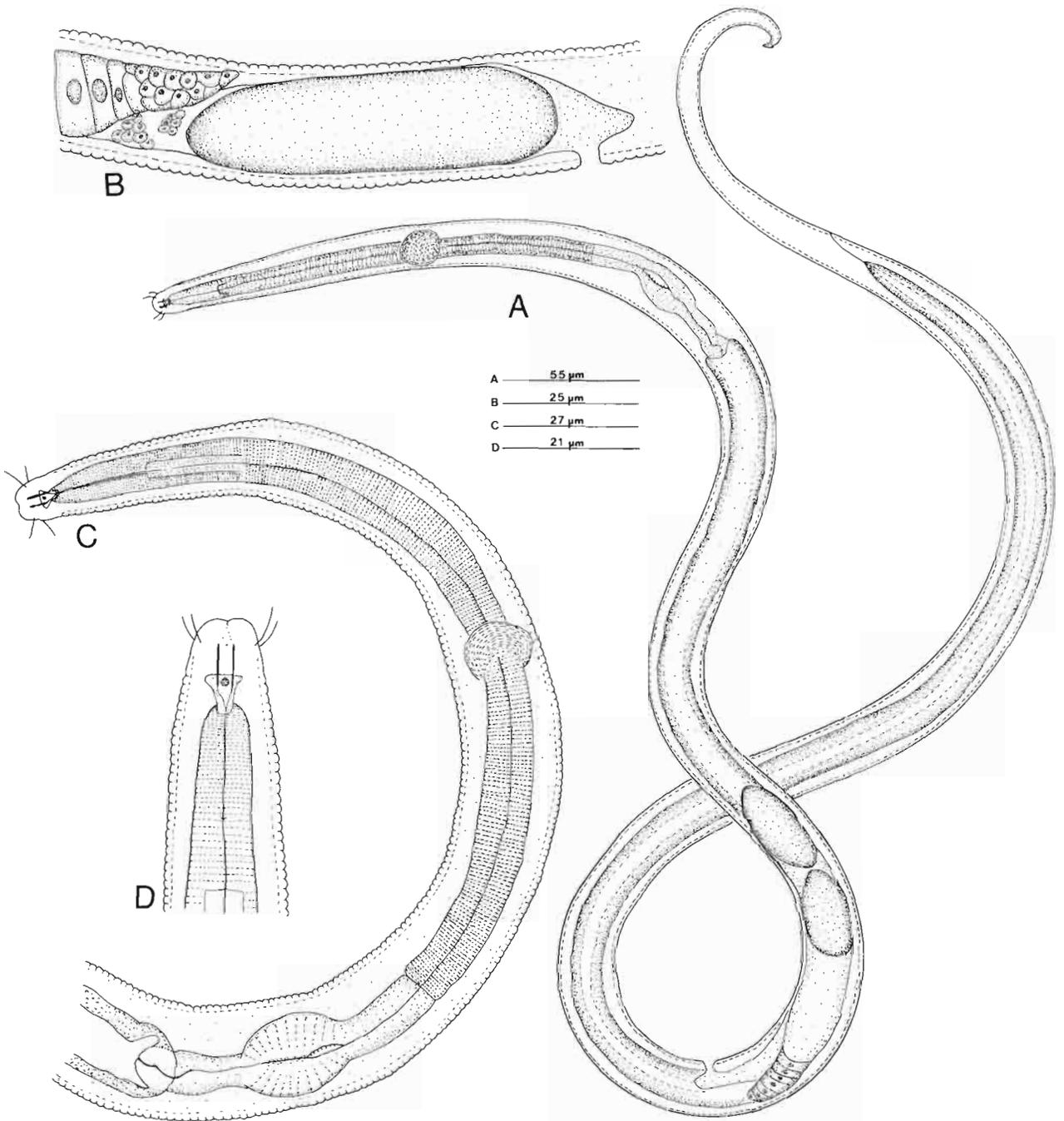


Fig. 1. *Chronogaster troglodytes* n. sp. hermaphrodite. A: General view; B: Vulvar region; C: Anterior region. D: Head.

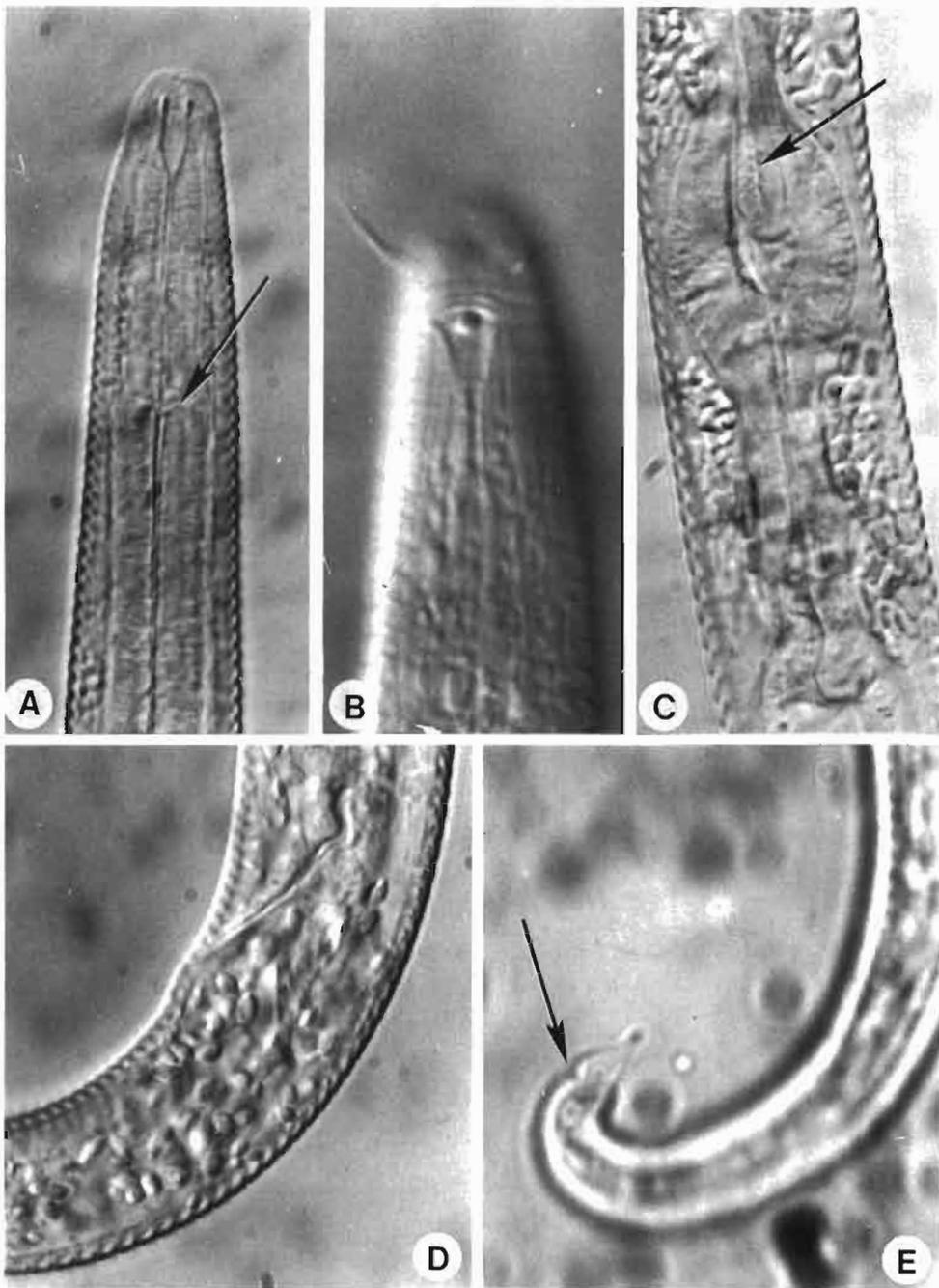


Fig. 2. *Chronogaster troglodytes n. sp. hermaphrodite*. A : Anterior region showing position of radial tubules (arrow); B : Head area showing amphid with circular opening; C : Basal oesophageal region showing denticles (arrow) on valve in basal bulb and crystalloids surrounding the basal bulb; D : Anal 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 0 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 from 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

Coomans (1983) illustrated crystalloids in the body wall of *C. rotundicauda*.

Although the exact nature of these crystalloids is unknown, even as to whether they represent concentrated waste material or some type of biological entity, their presence in only certain species of *Chronogaster* is intriguing. In the present study, these "crystalloids" were variable in shape (from rod- to oval-shaped), size (from 1.3 to 5.1 μm in length and from 0.6 to 1.5 μm in width), location (hypodermal cords, body cavity) and from nematode to nematode (some had few, some many, some had mostly rod-shaped bodies, others oval-shaped and others mixed shapes and sizes).

The fact that less than half of the known species of *Chronogaster* possess these crystalloids suggests that ecological conditions may play a role in determining their presence. The high mineral content of the thermal waters in which *C. troglodytes* lives could explain the presence of these bodies if they were mineral deposits, however they did not exhibit birefringence under polarized light, therefore it is doubtful that they are inorganic crystals. Until they can be examined ultrastructurally, a possible microbial nature cannot be ruled out.

Lateral glands

These structures represent another enigma found in representatives of *Chronogaster*. Heyns and Coomans (1980) describe the lateral glands in *C. glandifera* as large ovoid glandular bodies located in the body cavity, variable in number per individual and more prevalent on the right side of the body. Unless these are some type of free-floating coelomocytes, their variable nature is difficult to comprehend. The above authors also mentioned that some of these glands possessed indistinct tubes leading to the body surface. In *C. troglodytes*, similar lateral glands were only seen in three out of ten specimens and there were never more than two per individual. They were roughly spherical in outline (8 to 16 μm in diameter) and varied in position (in the tail region, adjacent to the vulva, mid-body region). In only one was there an indication of an opening leading to the body surface. The nature and function of these glands is unknown, and again, a microbial nature cannot be ruled out.

Cavernicolous nematodes

Are there nematodes which have become adapted or specialized for survival only in caves? Arthropods that have become adapted to cave habitats generally have physical modifications which differ from their epigeal counterparts. Such modifications are the reduction or loss of pigmentation, visual organs and an increase in size or number of tactile organs (Culver, 1982). Since most nematodes lack pigmentation and visual organs,

and by living in soil or in plant and animal tissues, their development already occurs in reduced light or darkness, morphological modifications of cave nematodes would not be expected.

In examining genera of nematodes that have been reported from caves or subterranean waters throughout the world (Table 1), all have species living in epigeal terrestrial or freshwater habitats. Many nematodes are probably carried by water into caves where they subsist for some time. This is why Andr assy (1965) noted that most cave nematodes are in a quiescent state and that cave nematodes normally represent a diverse assemblage. Of twelve species of cave nematodes collected by Zullini (1973) in Mexico, seven were freshwater forms, four were microbiotrophs, three were stylet bearing plant or invertebrate predators and five were non-stylet bearing predators.

It would appear that there are no true cavernicolous genera of nematodes. What about species? Speciation could occur when specialized developmental habitats occurred in caves that did not exist anywhere else. One such habitat could be bat guano and although nematodes have been found associated with this deposit (Zullini, 1973), they did not appear to be specialized for survival in this medium.

If specialization did occur in the cave habitat, it is more likely that adaptation would occur in response to special physiological, rather than physical conditions. Floating fungal mats on thermomineral waters constitute a specialized physiological habitat that is quite uncommon. In fact, such a habitat might have existed for millions of years since a Miocene date for the origin of Movile Cave has been postulated (Lascau, 1989). Conditions which lead to speciation are isolation and selection for survival in a unique environment. It appears that *C. troglodytes* sp. n. is a cavernicolous nematode species adapted for survival under the specialized conditions found in Movile Cave.

Although *Chronogaster* species are usually cited as aquatic nematodes occurring in freshwater streams, lakes, ditches, ponds and marshy areas, some species have been collected from a terrestrial habitat: sandy soil, sandy loam soil, heavy clay soil, sorghum field, pasture soil, forest soil (Heyns & Coomans, 1980, 1983; Raski & Maggenti, 1984). In fact, *C. africana* is cited as occurring in multiple aquatic and terrestrial habitats (Heyns & Coomans, 1980). Thus, it appears that a certain amount of genetic plasticity occurs in *Chronogaster* regarding habitat selection and adaptation.

It is speculated that several million years ago, during the formation of Movile Cave (Sarbu & Popa, 1992), an epigeal *Chronogaster* sp. was washed into the cave. This species survived on bacteria and slowly adapted to the changing trophic environment, finally now being restricted to the specialized fungal mat habitat in this unique cave.

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

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

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

Acknowledgements

Field work was supported by the National Geographic Society (4639-91), the Cave Research Foundation, the Explorer Club, the Department of Biological Sciences of the University of Cincinnati, and the « Emil Racovitza » Speleological Institute in Bucharest.

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