Journal of Nematology 25(3):500–502. 1993.

A Free-living *Panagrolaimus* sp. from Armenia Can Survive in Anhydrobiosis for 8.7 Years[1]

RAFFI V. AROIAN,[2] LYNN CARTA,[2] ISGOUHI KALOSHIAN,[3] AND PAUL W. STERNBERG[2]

Abstract: Although the ability of plant-parasitic nematodes to survive in a dehydrated or anhydrobiotic state for long periods of time has been well documented, the ability of free-living nematodes has not. Here we report on the survival of a free-living nematode, *Panagrolaimus* sp., from Armenia in the anhydrobiotic state for 8.7 years. This *Panagrolaimus* sp. can be cultured and maintained readily and may provide a good system for studying anhydrobiosis in nematodes.

Key words: anhydrobiosis, Armenia, free-living nematode, *Panagrolaimus*, survival.

Anhydrobiosis is a state of undetectable metabolic activity induced by dehydration that can allow nematodes and other organisms to survive for long periods without water (3,7). Important aspects of anhydrobiotic survival for some nematodes include (i) the production of carbohydrates such as glycerol and trehalose, which may prevent damage to membranes and macromolecules normally associated with desiccation and (ii) a slow rate of water loss in the nematode and the surrounding environment (14). Several examples exist of plant-parasitic nematodes surviving in anhydrobiosis for many years. Few such examples exist of free-living nematodes.

MATERIALS AND METHODS

The soil sample used in this study was taken 12 July 1983 from a residential driveway in Ashdarag district, Byurakan, a mountainous region of the Republic of Armenia (formerly Armenia, S.S.R.). It was stored in Pasadena, California, at room temperature in a glass jar with the lid tightly closed. The sample has remained dry the entire time. The present moisture content is 1.0 g H_2O per 25.0 g soil. The soil type is clay loam (33% sand, 32% silt, 35% clay).

To recover free-living nematodes, approximately 5–10 ml of this soil was placed on a 9-cm nematode growth medium (NGM) plate (2,12) containing a lawn of *Escherichia coli* strain OP50 (2). Soil was positioned away from the bacteria and hydrated by adding about 1 ml of M9 buffer (2,12). The plate was sealed with parafilm and kept at room temperature (ca. 22 C).

Specimens, heat-killed in M9 buffer, were mounted in 4% formaldehyde on 5% agar pads and measured with an ocular micrometer. The nematode was identified as *Panagrolaimus* sp. based on the morphology of the stoma and mouth parts (1). It was designated PS443 (*hy-1*) and stored at −70 C in glycerol using standard protocols for *Caenorhabditis elegans* (2,12).

RESULTS

Three attempts to recover living nematodes were made using the Armenian soil collected in July 1983 (Table 1). In the initial attempt (November 1987), one nematode migrated to the bacterial lawn 24 hours after plating, two more 29 hours after plating, and a final one 48 hours after plating. All four were juveniles, later identified as two males and two females. They were transferred to a fresh NGM plate with OP50 at 20 C. The females laid many

Received for publication 27 July 1992.
[1] Funded by an NSF-PYI award to P. W. S.
[2] Howard Hughes Medical Institute and Division of Biology, California Institute of Technology, Pasadena, CA 91125. Present address of first author: Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143–0448.
[3] Department of Nematology, University of California, Davis, CA 95616.
The authors thank Diana Freckman and John Crowe for discussions. Soil texture analysis was provided by the DANR Analytical Laboratory, University of California Cooperative Extension, Davis, CA. Live cultures of PS443 (*hy-1*) are available upon request.

TABLE 1. Summary of attempts to isolate free-living nematodes from an Armenian soil sample taken in July 1983.

Date of attempt	Years since sample taken	No. nematodes recovered	No. male/ female
2 November 1987	4.3	4	2/2
24 November 1991	8.4	1	?†
25 March 1992	8.7	3	0/3

† The nematode was never found. Its presence was inferred by tracks in the bacterial lawn.

eggs that hatched and developed into fertile adults. The isolate was identified as *Panagrolaimus* sp.

In the second attempt (November 1991), we saw the tracks of a nematode but were unable to recover it. In the third attempt (March 1992), three female nematodes migrated to the bacteria 33–56 hours after the soil was plated. Based on size, all were probably older than J1 stage, and one may have been as old as J4 stage. Two of the three were identified as *Panagrolaimus* sp. These two females successfully produced progeny upon mating with males derived from frozen stocks of the November 1987 isolation, indicating that they were the same species as isolated 4 years earlier. The third could not be identified definitively but was closely related, if not identical, to the other two. Several attempts to mate males with this third female were unsuccessful.

On both the November 1991 and March 1992 plates, the carcasses of several nematodes were also noted. One was coiled upon itself.

This *Panagrolaimus* sp. is readily cultured. The generation time from egg to adult at 20 C on NGM plates is about 8 days. Males are necessary for reproduction. Morphometric measurements of this nematode are as follows: *Female* (n = *10*): L = 1.05–1.45 mm (1.22 ± 0.15 mm); a = 15–21; b = 6.5–8; c = 14.5–18; v = 57–60%; *Male* (n = *10*): L = 0.80–1.05 mm (0.91 ± 0.08 mm); a = 18–21; b = 5–6; c = 18–25; spicules = 28 ± 3 μm. A comparison of these measurements with published data suggests this isolate represents a new species.

DISCUSSION

This Armenian *Panagrolaimus* isolate can survive dehydration at least 8.7 years. Based on the stage of the nematodes that migrated out of the soil and the size of carcasses seen, these Armenian nematodes probably become anhydrobiotic as juveniles. The coiled carcass seen is suggestive of the coiling associated with other anhydrobiotic nematodes (5).

Anhydrobiotic survival of *Panagrolaimus* has been reported before. *Panagrolaimus* was the second most common genus found in dry soil from Kinchega National Park in Australia (9). Experiments showed that this isolate could successfully enter the anhydrobiotic state and recover, provided the nematodes were slowly dehydrated.

There is only one other study on the long-term survival of anhydrobiotic free-living nematodes in soil. Specimens of *Acrobeloides nanus* were isolated from soil samples stored for 6.5 years in a plastic bag (10). In a study involving *Aphelenchus avenae* grown as a free-living nematode on fungi, live specimens were recovered from laboratory-prepared pellets stored in dry air for 18 months (4). The plant parasites *Helicotylenchus dihystera* and *Pratylenchus penetrans* survived anhydrobiotically in soil samples for at least 250 and 770 days, respectively (8,13). The longest records of nematode anhydrobiotic survival are for nematodes not stored in soil. *Anguina tritici* survived in seed galls for 28 years, and *Filenchus polyhypnus* survived in a herbarium specimen for almost 39 years (6,11).

Given its ability for anhydrobiotic survival and its relative ease of culture and maintenance, this *Panagrolaimus* sp. may be a useful system for studying anhydrobiosis in nematodes.

LITERATURE CITED

1. Andrássy, I. 1984. Klasse nematoda. Stuttgart: Gustav Fischer Verlag.

2. Brenner, S. 1974. The genetics of *Caenorhabditis elegans*. Genetics 77:71–94.

3. Crowe, J. H. 1971. Anhydrobiosis: An unsolved problem. American Naturalist 105:563–573.

4. Crowe, J. H., and A. C. Madin. 1975. Anhydrobiosis in nematodes: Evaporative water loss and survival. Journal of Experimental Zoology 193:323–334.

5. Demeure, Y., and D. W. Freckman. 1981. Recent advances in the study of anhydrobiotic nematodes. Pp. 205–226 *in* B. M. Zuckerman and R. A. Rohde, eds. Plant parasitic nematodes, vol. 3. New York: Academic Press.

6. Fielding, M. J. 1951. Observations on the length of dormancy in certain plant infecting nematodes. Proceedings of the Helminthological Society of Washington 18:110–112.

7. Freckman, D. W., and C. Womersley. 1983. Physiological adaptations of nematodes in Chihuahuan desert soils. Pp. 395–403 *in* P. Lebrun, H. M. Andre, A. De Medts, C. Gregoire-Wibo, and G. Wauthy, eds. New trends in soil biology: Proceedings of the VIII international colloquium of soil biology. Louvain-la-Neuve, Belgium: Dieu-Brichart.

8. McGlohon, N. E., J. N. Sasser, and R. T. Sherwood. 1961. Investigations of plant-parasitic nematodes associated with forage crops in North Carolina. Technical Bulletin 148, North Carolina Agricultural Experimental Station, North Carolina State College, Raleigh.

9. Nicholas, W. L., and A. C. Stewart. 1985. Cryptobiotic nematodes in dry soil from Kinchega National Park, NSW. Australian Nematologists' Newsletter 4:18–21.

10. Nicholas, W. L., and A. C. Stewart. 1989. Experiments on anhydrobiosis in *Acrobeloides nanus*. Nematologica 35:489–491.

11. Steiner, G., and F. E. Albin. 1946. Resuscitation of the nematode *Tylenchus polyhypnus*, n. sp., after almost 39 years' dormancy. Journal of the Washington Academy of Sciences 36:97–99.

12. Sulston, J., and J. Hodgkin. 1988. Methods. Pp. 587–606 *in* W. B. Wood, ed. The nematode *Caenorhabditis elegans*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

13. Townshend, J. L. 1984. Anhydrobiosis in *Pratylenchus penetrans*. Journal of Nematology 16:282–289.

14. Womersley, C. 1987. A reevaluation of strategies employed by nematode anhydrobiotes in relation to their natural environment. Pp. 165–173 *in* J. A. Veech and D. W. Dickson, eds. Vistas on nematology. Society of Nematologists.