

 Open access • Journal Article • DOI:10.1038/NATURE09974

Nematoda from the terrestrial deep subsurface of South Africa — [Source link](#)

Gaetan Borgonie, Antonio García-Moyano, Antonio García-Moyano, Derek Litthauer ...+7 more authors

Institutions: Ghent University, University of Bergen, University of the Free State, Wageningen University and Research Centre ...+1 more institutions

Published on: 02 Jun 2011 - Nature (Nature Publishing Group)

Topics: Halicephalobus mephisto and Biosphere

Related papers:

- [Prokaryotes: The unseen majority](#)
- [Environmental genomics reveals a single-species ecosystem deep within Earth.](#)
- [Long-term sustainability of a high-energy, low-diversity crustal biome.](#)
- [Lithoautotrophic microbial ecosystems in deep basalt aquifers](#)
- [Desulfotomaculum and Methanobacterium spp. Dominate a 4- to 5-Kilometer-Deep Fault](#)

Share this paper:    

View more about this paper here: <https://typeset.io/papers/nematoda-from-the-terrestrial-deep-subsurface-of-south-34gryl6662>

Worms from hell: *Nematoda* from the terrestrial deep subsurface of South Africa

G. Borgonie¹, A. García-Moyano^{2†}, D. Litthauer², W. Bert^{1,3}, A. Bester², E. van Heerden², C. Möller², M. Erasmus², T.C. Onstott⁴

¹Department of Biology, Nematology Section, Ghent University, Ghent, Belgium.

²Department of Microbial, Biochemical and Food Biotechnology, University of the Free State, Bloemfontein, South Africa.

³Laboratory of Nematology,, Department of Plant Sciences, Wageningen University, Wageningen, The Netherlands.

⁴Department of Geosciences, Princeton University, Princeton, NJ.

†Present address: Department of Biology, University of Bergen, Bergen, Norway

Since its discovery over two decades ago, the deep subsurface biosphere has been considered to be the realm of single cell organisms, extending >3 km into the Earth's crust and comprising a significant fraction of the global biosphere^{1,2,3,4}. The constraints of temperature, energy, O₂ and space seemed to preclude the possibility of more complex multi-cellular organisms from surviving at these depths. Here we report species of the phylum *Nematoda* that have been detected in or recovered from 0.9-3.6 km deep fracture water encountered in the deep mines of South Africa, but have not been detected in the mining water. These subsurface nematodes, including a new species *Halicephalobus mephisto*, tolerate high temperature, reproduce asexually and preferentially feed upon subsurface bacteria. ¹⁴C data indicate that the fracture water in which the nematodes reside is 3-12 kyr old paleometeoric water. Our data suggest that nematodes should be found in other deep hypoxic settings where temperature permits and that they may control the microbial population density by grazing upon fracture surface biofilm patches. Our results expand the known *Metazoan* biosphere and demonstrate that deep ecosystems are more complex than previously accepted. The discovery of multi-cellular life in the deep subsurface of the Earth also has important implications for the search for subsurface life on other worlds in our solar system.

Halicephalobus mephisto sp. nov.; Phylum Nematoda Potts, 1932; Cephalobina, Panagrolaimidae. One holotype and nine paratypes deposited in the collection at the Museum voor Dierkunde (collection number UGMD 104182), Ghent University, Belgium. Six paratypes deposited at the University of the Free State, Bloemfontein, Republic of South Africa (collection number UFS GB0035). Type population collected from Beatrix Au mine at 1.3 km depth, shaft 3, level 26, corridor 28 approximately 1 km north of shaft 3 (28°14' 24.06"S 26°47'45.25"E). Nematodes were collected from fracture

¹Department of Biology, Nematology Section, Ghent University, Ledeganckstraat 35, B9000, Ghent, Belgium. ²Metagenomics Platform, Department of Microbial, Biochemical and Food Biotechnology, University of the Free State, P. O. Box 339, Bloemfontein 9300, South Africa. ³Laboratory of Nematology, Department of Plant Sciences, Wageningen University, Wageningen, The Netherlands. ⁴Department of Geosciences, Princeton University, Princeton, NJ 08544, USA. †Present address: Department of Biology, University of Bergen, Postbox 7803, N-5020 Bergen, Norway.

water expelling from a high-pressure valve. Etymology - Mephisto ("he who loves not the light") refers to the devil, Lord of the underworld, in medieval mythology from the Faust legend since the new species is found at -1.3 km into the Earth's crust. Differential diagnosis - Although *Halicephalobus* is a morphologically minimalistic genus, *H. mephisto* is a new typological morphospecies as it can be easily differentiated from all other species of *Halicephalobus* by the presence of a long tail (110-130 μm ; c': 9-10) with filiform terminus and the absence of reflexed ovary tip (Fig. 1). Phylogenetically, *H. mephisto* n. sp. has a maximally supported sister relationship with *H. gingivalis*-*Halicephalobus* spp., differing by 10% from *H. gingivalis* and by 8% from other *Halicephalobus* species. In comparison, the most closely related different genus, *Procephalobus*, differs by 17% from *H. mephisto* n. sp. Alternative alignment methods did not have a single effect on the tree topology outcome and resulted in a similar or increased branch support for the *H. gingivalis*-*Halicephalobus* spp. clade. The monophyly of *Halicephalobus* was always maximally supported, independently of the alignment method. Furthermore, 13 and 6 autapomorphic characters were present in the SSU rRNA sequences for the new species and its sister clade, respectively. Although based on limited available homologous sequences, multiple autapomorphic characters from two loci (18S and D2D3) and multiple autapomorphic characters in both sister lineages (18S) indicate lineage exclusivity for *H. mephisto* n. sp. with respect to other *Halicephalobus* species (Fig. 2). Hence, the species status of *H. mephisto* fulfills the requirements of an amalgamation of evolutionary and phylogenetic species concepts. Description - Body straight to slightly ventrally arcuate after fixation, 0.52 to 0.56 mm long with annulations. The tail is relatively long and the tail tip filiform, terminus straight to variably curved. The reproductive system was monodelphic, prodelphic, on right side of the intestine with posterior reflexed ovary extending 99 – 135 μm posterior vulva. Ovary tip is not reflexed back anteriorly. Temperature tolerant and parthenogenetic (See Supplementary Discussion for more detailed description).

Although eukaryotes, bacteria and archaea cohabit in almost all surface environments on the Earth, very few searches for eukaryotes in the subsurface have been published. Sinclair and Ghiorse⁵ discovered 0.1 - 10 eukaryotes g^{-1} comprised of algae, fungi, amoebae and flagellates at 200 m depth in South Carolina. Ekendahl et al.⁶ found 0.01 - 1 fungal cells mL^{-1} , $\sim 3 \mu\text{m}$ in size, in 200 - 450 m deep fractures in Sweden. In this study of the South African subsurface, we expanded the search for subsurface life to nematodes for the following reasons: 1) nematodes are one of the most successful metazoan phyla with respect to their abundances, distribution and physiological tolerance^{7,8}; 2) nematodes can enter a state of anabiosis for extended periods; 3) nematodes continue to metabolize aerobically in hypoxic environments where the pO_2 is only 0.4 kPa⁹.

The steps taken to determine whether the nematodes recovered were indigenous and not recent surface or mining contaminants were: 1) adaptation of filtration procedures that had been successfully used to collect planktonic microorganisms from thousands of liters of borehole water¹⁰ (Supplementary Methods); 2) soil samples around the boreholes and the mining water were tested for nematodes; 3) the chemical composition and the microbial community structure of the fracture water were determined; 4) the ^3H and ^{14}C

concentrations were measured. Twenty-two water samples were collected from 6 boreholes ranging in depth from 0.5 to 3.6 km located in 5 different South African mines (Table 1) Between 475 to 9,792 L of water were filtered for each sample. Eighteen soil samples were collected, 6 of which were from Beatrix Au mine. Seven mining water samples were collected, 3 of which were from Beatrix Au mine and for which 2 to 31,582 L of water were filtered.

Beatrix Au Mine borehole water yielded a new species, *Halicephalobus mephisto* sp. n. Borehole water from Driefontein Au Mine yielded 2 nematode species, *Plectus aquatilis* and a monhysterid specimen that survived but did not reproduce. Borehole water from the shallowest site, Star Diamonds Mine and from Northam Pt Mine did not yield any nematodes, but a fourth nematode, a monhysterid species, was detected in DNA extracted from borehole water from the deepest site at Tau Tona Au Mine. All three living nematode species preferentially fed on the borehole water bacteria versus *Escherichia coli* (Supplementary Methods), and the two that were able to reproduce did so by parthenogenesis (Table 2). None of these species were found in more than one borehole. Although these three boreholes were sampled on multiple dates, nematodes were only recovered from the first sample (Table 1). No other metazoans were detected. Only two of the soil samples yielded nematodes, but only when the soil was wet, and all were taxonomically distinct from the three borehole nematodes (Table 1). None of the soil samples from Beatrix Au Mine yielded nematodes. Nematodes were also not detected in any mining water samples.

The Beatrix and Driefontein Au mine borehole valves were closed for at least several months to a year prior to sampling and the high water pressure upon opening the valve and flushing the filtration apparatus precluded contamination from air. The possibility of nematodes being contaminants from borehole drilling is unlikely because of the mining water used for drilling is treated with Na hypochlorite and H₂O₂ to the point that its DNA is highly degraded and because the high fracture water pressure encountered during drilling flushes out drilling water (Supplementary Discussion). Nonetheless we tested for this possibility at Beatrix Au Mine, where a 6,480 L borehole water sample yielded *H. mephisto*, by filtering 31,582 L of the mining water used for drilling from a valve close to the borehole and no nematodes were detected. We cannot preclude the possibility that nematodes were present in the mining water at the time the borehole was drilled a year prior to sampling, but this seems unlikely given the disinfection procedures are standard operating protocols. Because the nematodes were parthenogenetic, crossing with surface species was impossible, and nematode morphology and genetics cannot be used as indicators of long-term isolation^{11,12}. Further evaluation of their indigeneity, therefore, relied upon environmental data.

The water sampled was hypoxic with dissolved O₂ concentrations ranging from 13 to 72 µM (Supplementary Tables). The Beatrix Au Mine borehole water yielded sulfate concentrations that were similar to that of the 3-5 myr old fracture water from 1.5 km depth¹⁴ and was 100 times less than that of the mining water¹³. The geochemistry of the Driefontein Au Mine borehole water was consistent with groundwater from the karstic, sulfidic, Transvaal dolomite aquifer¹³. The Tau Tona Au Mine borehole water was

consistent with other highly saline fracture water from >3 km depth and distinct from the mining water and acid mine drainage water in this mining district^{13,15}. The 16S rRNA gene clone libraries were comprised of sulfate reducing *Firmicutes* and δ *Proteobacteria*, heterotrophic *Proteobacteria* (fermenters and/or methanotrophs), *Nitrospira* and chemolithotrophic *Proteobacteria* that have been previously detected in the fracture water of these mines¹⁵ (Supplementary Tables and Discussion). With the exception of the Driefontein borehole water, the ³H concentrations were within 2 S.D. of the detection limit (Table 1). Compared to the 10-100 TR of regional precipitation for the late 80's¹⁶, these values indicate that <1% of the borehole water is comprised of post-1980's surface water and no more than ~3% of the Driefontein borehole water could be modern. The $\Delta^{14}\text{C}$ values for the dissolved inorganic carbon ranged from -932.8 to -619.6 and using these values along with regional recharge values and corrections for dead carbon (Supplementary Tables and Discussion), the estimated ¹⁴C ages ranged from ~2.9 to 12.1 kyr (Table 1).

The geochemical, isotopic and molecular data indicate that the nematode-bearing water represents paleometeoric, hypoxic water that contains a microbial assemblage comprised of both aerobic and anaerobic bacteria. The cultured nematodes preferentially fed upon these bacteria as opposed to *E. coli*. Nematodes were absent from the mining water, and when found twice in soils they were taxonomically distinct from those found in the paleometeoric water. No other metazoans were found in the paleometeoric water. The nematodes, therefore, do not appear to be contaminants from mining contamination or from modern water incursion, but appear to be indigenous to the paleometeoric water.

Can the subsurface microbial population density sustain nematodes for thousands of years? The ratio of planktonic microbial cells to nematodes was 10^8 to 10^{10} (Table 1) greatly exceeding the 10 to 100 microbial cells to protists ratios reported for other terrestrial subsurface environments⁶. More likely the nematodes are grazing on the $\sim 5 \times 10^4$ cells cm^{-2} patches of bacteria attached to the fracture surfaces¹⁷. This density corresponds to a sessile microbial concentration 100 times that of the planktonic cells making the ratio of microbial cells to nematodes $\sim 10^{10}$ to 10^{12} . Given the 2.6×10^{-8} g dry weight for *H. mephisto* (Supplementary Discussion), measured bacterivory rates of 6.6 to 15.2×10^5 bacterial cells $(\text{mm of nematode})^{-1} \text{ day}^{-1}$ and respiration/total C consumption ratios of 0.16 to 0.72¹⁸ for nematodes, $\sim 10^4$ bacterial cells could readily sustain *H. mephisto* for one day and 10^{11} for ~30 kyr. The association of nematodes with biofilms may explain why they were detected only when the boreholes were first opened and not in subsequent samples. The initial release of high-pressure water would have dislodged biofilms and nematodes from the fracture surfaces and some time would be required after the borehole was sealed again for the biofilm community to reform. This lack of reproducibility in eukaryote presence was also observed for subsurface fungi⁶.

Is O₂ limiting? Water samples yielded 13 to 72 μM O₂ concentrations (equivalent to 1.3 - 6.8 kPa of O₂ in Table 1), values that can sustain the maximum metabolic rate of 3×10^{-4} mols of O₂ $(\text{gm of nematode})^{-1} \text{ hr}^{-1}$ for *Caenorhabditis elegans*^{19,20}. If the paleometeoric water had an O₂ concentration of $\sim 350 \mu\text{M}$ upon recharge, then the ¹⁴C ages constrain the O₂ consumption rate to be $\sim 3\text{-}8 \times 10^{-8} \text{ M yr}^{-1}$, values which overlap those reported for the

Middendorf aquifer²¹. Given *H. mephisto*'s mass, its maximum metabolic rate would be $\sim 7 \times 10^{-8}$ mols of O₂ nematode⁻¹ yr⁻¹. The observed O₂ consumption rate could support ~ 1 nematode L⁻¹ at its maximum metabolic rate compared to the 1 nematode per 10⁴ liters detected. Even at 4 μM O₂ nematodes metabolize aerobically⁹, but at much slower rates¹⁹.

Temperature imposes a limit to the depth that nematodes could live. The temperature of Beatrix BH2 is higher than most terrestrial nematodes can tolerate, but *H. mephisto* exhibited a high temperature tolerance (Table 2) like that of the opportunistic pathogen *H. gingivalis*. The detection of the monhysterid species in the 48°C Tau Tona borehole is significantly higher than a reported occurrence of this order in a 43°C hot spring²², but comparable to the 51 to 61°C, hot spring nematode occurrences^{23,24,25}.

Our data suggest that the interactions between meiofaunal communities and chemolithotrophic biofilms found in hypoxic, sulfide, cave systems, e.g. Movile Cave in Romania²⁶, extend to greater depths and smaller confines and could control the size and turnover rate of subsurface microbial communities. Given their abundance on the sea floor and around hydrothermal vents nematodes should also be found beneath the seafloor in the sediments or in mid-ocean ridge basalt²⁷. Other meiofauna, such as the Loricifera inhabiting the deep anoxic hypersaline L'Atalante basin²⁸ could also be present. The ability of multi-cellular organisms to survive in the subsurface should be considered in the evolution of Eukaryotes and the search for life on Mars.

- 1 Pedersen, K. The deep subterranean biosphere. *Earth Sci. Rev.* **34**, 243-260 (1993).
- 2 Onstott, T. C. *et al.* in *Enigmatic Microorganisms and Life in Extreme Environments* (ed J. Seckbach) (Kluwer Publications, 1998).
- 3 Amend, J. P. & Teske, A. Expanding frontiers in deep subsurface microbiology *Palaeogeography, Palaeoclimatology, Palaeoecology* **219**, 131 – 155 (2005).
- 4 Whitman, W. B., Coleman, D. C. & Wiebe, W. J. Prokaryotes: the unseen majority. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 6578-6583, doi:10.1073/pnas.95.12.6578 (1998).
- 5 Sinclair, J. L. & Ghiorse, W. C. Distribution of aerobic bacteria, protozoa, algae and fungi in deep subsurface sediments. *Geomicrobiol. Jour.* **7**, 15-31 (1989).
- 6 Ekendahl, S., O'Neill, A., Thomsson, E. & Pedersen, K. Characterisation of yeasts isolated from deep igneous rock aquifers of the Fennoscandian shield. *Microb. Ecol.* **46**, 416-428 (2003).
- 7 Heip, C., Vincx, M. & Vranken, G. The ecology of marine nematodes. *Oceanogr. Mar. Biol. Annu. Rev.* **23**, 399-489 (1985).
- 8 Lambshead, P. in *Nematode morphology, physiology and ecology* Vol. 1 eds ZX Chen, SY Chen, & DW Dickson) 438-492 (Tsinghua University Press, 2004).
- 9 Föll, R. L. *et al.* Anaerobiosis in the nematode *Caenorhabditis elegans*. *Comp. Biochem. Physiol.* **124B**, 269–280 (1999).
- 10 Moser, D. P. *et al.* *Desulfotomaculum* spp. and *Methanobacterium* spp. Dominate 4-5 km Deep Fault. *Appl. Environ. Microbio.* **71**, 8773-8783, doi:DOI: 10.1128/AEM.71.12.8773-8783.2005 (2005).

- 11 Dorris, M., De Ley, P. & Blaxter, M. Molecular analysis of nematode diversity
and the evolution of parasitism. *Parasitol Today* **15**, 188-193 (1999).
- 12 Holterman, M. *et al.* Phylum-wide analysis of SSU rDNA reveals deep
phylogenetic relationships among nematodes and accelerated evolution toward
crown clades. *Mol. Biol. Evol.* **23**, 1792-1800 (2006).
- 13 Onstott, T. C. *et al.* The origin and age of biogeochemical trends in deep fracture
water of the Witwatersrand Basin, South Africa. *Geomicrobiol. J.* **23**, 369-414,
doi:doi: 10.1080/01490450600875688 (2006).
- 14 Lippmann, J. *et al.* Dating ultra-deep mine waters with noble gases and ³⁶Cl,
Witwatersrand Basin, South Africa. *Geochim. Cosmochim. Acta.* **67**, 4597-4619,
doi:doi: 10.1016/S0016-7037(03)00414-9 (2003).
- 15 Gihring, T. M. *et al.* The distribution of microbial taxa in the subsurface water of
the Kalahari Shield, South Africa. *Geomicrobiol. J.* **23**, 415-430,
doi:10.1080/01490450600875696 (2006).
- 16 Michel, R. L. in *Isotopes in the Water Cycle: Past, Present and Future of a
Developing Science* eds P.K. Aggarwal, J.R. Gat, & K.F.O. Froelich) Ch. 5, 53-
66 (Springer, 2005).
- 17 Wanger, G., Onstott, T. C. & Southam, G. Structural and Chemical
Characterization of a Natural Fracture Surface from 2.8 Kilometers Below Land
Surface: Biofilms in the Deep Subsurface. *Geomicrobiol. J.* **23**, 443-452,
doi:10.1080/01490450600875746 (2006).
- 18 Ferris, H., Venette, R. C. & Lau, S. S. Population energetics of bacterial-feeding
nematodes: carbon and nitrogen budgets. *Soil. Biol. Biochem* **29**, 1183-1194
(1997).
- 19 van Voorhies, W. & Ward, S. Broad oxygen tolerance in the nematode
Caenorhabditis elegans. *J. Exp. Biol.* **203**, 2467-2478 (2000).
- 20 van Voorhies, W. A. & Ward, S. Genetic and environmental conditions that
increase longevity in *Caenorhabditis elegans* decrease metabolic rate. *Proc. Natl.
Acad. Sci. U.S.A.* **96**, 11399–11403 (1999).
- 21 Phelps, T. J., Murphy, E. M., Pfiffner, S. M. & White, D. C. Comparison between
geochemical and biological estimates of subsurface microbial activities. *Microb.
Ecol.* **28**, 335-349, doi:10.1007/BF00662027 (1994).
- 22 Ocana, A. Relationship between nematode species and the physico-chemical
characteristics of spring waters. II Temperature. *Nematol. medit.* **19**, 25-28
(1991).
- 23 Neher, D. A. & Powers, T. O. in *Encyclopedia of Soils in the Environment* Vol. 3
eds D. Hillel *et al.*) 1-5 (Academic Press, 2004).
- 24 Hoeppli, R. & Chu, H. J. Free-living nematodes from hot springs in China and
Formosa. *Hong Kong Naturalist supplement* **1**, 15–29 (1932).
- 25 Jana, B. B. The thermal springs of Bakreswar, India: Physico-Chemical
Conditions, Flora and Fauna. *Hydrobiologia* **41**, 291-307 (1973).
- 26 Engel, A. S. Observations on the Biodiversity of Sulfidic Karst Habitats *Journal
of Cave and Karst Studies* **69**, 187-206 (2007).
- 27 Edwards, K. J., Bach, W. & McCollom, T. M. Geomicrobiology in oceanography:
microbe–mineral interactions at and below the seafloor. *Trends Microbiol.* **13**,
449-456 (2005).

- ²⁸ Danovaro, R. *et al.* The first metazoa living in permanently anoxic conditions. *BMC Biology* **8**, 30-40 (2010).
- ²⁹ Yeates, G. W., Bongers, T., Goede, R. G. M. d., Freckman, D. W. & Georgieva, S. S. Feeding habits in nematode families and genera — an outline for soil ecologists. *J. Nematology* **25**, 315-331 (1993).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgments GB is grateful to the University of Ghent for allowing a yearlong sabbatical and grants from the FWO, BOF and UFS. We wish to express our recognition of the support provided by the mines and the many people without whom this work would have been impossible. In particular we are grateful to F. Rheeder and H. Möller, Tau Tona Au Mine (AngloGold Ashanti Limited); T. Lineque, Driefontein Au Mine (Gold Fields Limited); A. Thwala, C. Rose and R. Fynn, Beatrix Au Mine (Gold Fields Limited); B. Visser, Star Diamonds Mine (Petra Diamonds); and O. Holovachev (UC Riverside, USA); T. Moens and A. Vierstraete (UGent, Belgium). TCO acknowledges support from a National Science Foundation Continental Dynamics Program grant, EAR 0409605. EvH acknowledges support from BioPAD/UFS Metagenomics Platform grant. The authors are grateful to the comments from three anonymous reviewers.

Author Contributions §AGM, DL and WB all contributed equally to this study. GB, AGM, DL, AB and ME collected the filtered samples, the control samples and performed field analyses. GB carried out the enrichments. AGM performed microbial DNA extraction and 16S rRNA amplification, sequencing and tree construction. WB contributed the nematode identification, their morphological description and their molecular analyses. TCO modeled the geochemical, ³H and ¹⁴C data. GB wrote the paper with input from WB, AGM, TCO and EvH. We are grateful to E. Botes and K. Albertyn of the Univ. of Free State for their contributions to the data analysis.

Author Information Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to G.B. (gborgonie@gmail.com) or T.C.O. (tullis@princeton.edu).

Table 1. Geochemical, isotopic and nematode results

	Star Diamonds	Driefontein	Beatrix BH1 BH2	Northam	Tau Tona	
Depth (km)	0.5	0.9	1.3	1.3	1.7	3.6
T°C	32	24	30	37	48	48
pH	8.3	7.5	7.7	7.9	8.3	7.7
pO ₂ (kPa) ¹	4.6	2.5	3.1	1.3-6.8	2.4	2.9
microbial counts (cells L ⁻¹)	NA ²	<10 ⁵	NA	3x10 ⁶	2x10 ⁵	3.4x10 ⁶
samples with nematodes/total samples	0/2	1/3 ³	0/6	1/6	0/1	1/4
Nematodes/L	NA	~7x10 ⁻⁵	NA	~3x10 ⁻⁵	<10 ⁻⁴	>~5x10 ⁻⁴
³ H (TR ⁴)	NA	0.270±0.026	NA	0.014±0.024	0.062±0.045	0.034±0.021
δ ¹³ C (‰ VPDB ⁵)	NA	-8	NA	-32	-17.4	-17.7
Δ ¹⁴ C	NA	-932.8±1.0	NA	-704.1±2.2	-645.9±2.4	-619.6±2.9
¹⁴ C age (yr)	NA	10,104- 12,084	NA	4,413-6,247	5,798	2,919-5,165
<i>H. mephisto</i>	-	-	-	+	-	-
<i>P. aquatilis</i>	-	+	-	-	-	-
monhysterid sp1	-	+	-	-	-	-
monhysterid sp2	-	-	-	-	-	+ ⁶
	Control Samples					
Soil ⁷	dorylaimids, mononchids and Annelids	<i>Diploscapter coronatus and Rhabditis regenfussi</i>	-	-	-	-
Mining water ⁸	-	-	-	-	-	-

¹ pO₂ (kPa) = 101.325 x [O₂] x 1.8x10⁴/K_H, where K_H is Henry's solubility constant (moles of O₂/moles of H₂O-atm) = EXP{[-286.942+15450.6/T°K+36.5593Ln(T°K)+ 0.0187662T°K]/1.987} and [O₂] is the dissolved O₂ concentration in μM.

² NA = not analyzed.

³ One sample yielded two nematodes of two separate species. In the case of Beatrix, only one nematode was found.

⁴ TR = Tritium units = 1 ³H per 10¹⁸ H atoms.

⁵ VPDB = Vienna Pee Dee *Belemnite* *americana*.

⁶ Samples did not yield a cultivable nematode, but the DNA from the fracture yielded an 18S rRNA gene sequence belonging to *Monhysteridae*.

⁷ Approximately 900 grams of soil were plated and in the cases where the soil was dry, an additional 900 grams was wetted and plated. The total number of nematodes was 17 for Star Diamonds and 18 for Driefontein.

⁸ Two to six liters of mining water were filtered for metazoans with the exception of Beatrix Au Mine where 31,582 liters of mining water was filtered (Supplementary Tables and Discussion).

Table 2. Nematode characteristics

Species	Maximum growth temperature (°C)	reproduction	feeding type
<i>H. mephisto</i>	41	parthenogenetic	bacteriophagous
<i>P. aquatilis</i>	31	parthenogenetic	bacteriophagous
monhysterid sp1	unknown	sexual?	bacteriophagous
monhysterid sp2	unknown	unknown	bacteriophagous ¹

¹ *Monhysteridae* are classified as bacterial feeding and/or substrate ingestion based upon their buccal morphology²⁹. Substrate ingestion is theoretically identical to bacterial feeding, predation and unicellular eukaryote feeding in many groups, because more than a pure food source is ingested.

Figure 1. General morphology of *Halicephalobus mephisto* n. sp. LM drawings of female holotype and SEM photograph of head. A: Entire body; B: Neck region; C: anterior region; D: SEM *en face* view; E: reproductive system; F: tail.

Figure 2. Bayesian interference 50% majority rule consensus phylogenies based on SSU rDNA data. *Halicephalobus mephisto* with GenBank sequences of closely related taxa. Branch support is indicated with Posterior Probability values. Scale bar indicates expected substitutions per site.