



Data Article

Dataset supporting the use of nematodes as bioindicators of polluted sediments



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ABSTRACT

We provide the dataset supporting the research article “Nematodes as bioindicators of polluted sediments using metabarcoding and microscopic taxonomy” [1]. Nematodes are frequently used as bioindicators and the NemaSPEAR[%] is an validated index that is originally based on morphological data. The index was compared to molecular sequence data for the 28S rDNA, 18S rDNA and COI gene for 7 locations. This dataset includes chemical analyses of the sediments for 33 different substances. The sequence data for OTU-based analyses for the 28S rDNA, 18S rDNA and COI gene is given, together with the read distribution during bioinformatics processing. We furthermore include alternative ASV data, based on a cluster-independent approach. The morphological data is presented, including the biomass for each species, as well as an overview about whether the species is represented in the NCBI database. Furthermore, rarefaction analysis is given for the morphological data, and furthermore NMDS plots for the species and genus level based on morphological and molecular data. The correlation between the mean PEC-Q and the NemaSPEAR[%] values is

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given in order to compare the efficiency of the index, based on morphological and molecular data.

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Specifications table

Subject	Pollution
Specific subject area	An nematode-based index (NemaSPEAR[%]), which is based on morphological data, is validated with molecular data, amplifying fragments of the 28S rDNA, 28S rDNA and COI gene.
Type of data	Table Graph Figure
How data were acquired	Data was acquired using a Zeiss microscope and Illumina MiSeq sequencing (V3 chemistry; 2 × 300 bp; 2.5 M read pairs). Software used for further processing: PRIMER_v6, R Studio, mothur, cutadapt, SigmaPlot 11
Data format	NGS data is deposited under PRJNA513975 and PRJNA608650. Raw morphological data is provided here. Analyzed Filtered
Parameters for data collection	Sediment samples were collected at seven locations across Germany, whose pollution status was analysed before.
Description of data collection	Sediment samples were taken in 2017 and 2018 using a corer (2.6 cm diameter), using five subsamples per replicate.
Data source location	Institution: Bielefeld University City: Bielefeld Country: Germany Sample collection: Furlbach: N51°53.724 E008°42.931 Veerse: N53°08.483 E009°30.060 Oertze: N53°00.944 E010°04.974 Saale-Rischmühle: N51°21.038 E012°00.213 Elbe-Hitzacker: N53°09.643 E011°02.787 Elbe-Cumlosen: N53°02.432 E011°38.592 Luppe: N51°23.116 E012°00.526
Data accessibility	With the article and under PRJNA513975 and PRJNA608650 https://www.ncbi.nlm.nih.gov/bioproject/PRJNA513975 https://www.ncbi.nlm.nih.gov/bioproject/PRJNA608650
Related research article	Janina Schenk, Sebastian Höss, Marvin Brinke, Nils Kleinbölting, Henrike Brüchner-Hüttemann, Walter Traunspurger Title: Nematodes as bioindicators of polluted sediments using metabarcoding and microscopic taxonomy Journal: Environment International DOI: https://doi.org/10.1016/j.envint.2020.105922

Value of the data

- This dataset supports a pilot study evaluating suitability of molecular-based data for bioindication purposes with nematodes.
- This dataset provides benefit for molecular-based studies with biomonitoring aims, especially in the field of meiofauna, which is often difficult to identify morphologically.
- This data can be used to further verify this approach, e.g. with the use of bulk samples instead of isolated nematode communities.

- This dataset will give further insight into nematode-based bioindication using the NemaS-PEAR[%] index.

1. Data description

This dataset contains the chemical analyses for the seven locations for 33 substances traceable in the sediment samples (Table 1). The number of analysed nematodes for each sample and replicate is given, together with the information if samples were combined due to low specimen numbers (Table 2). For the molecular approach, using Operational Taxonomic Units (OTU) clustering, the list of the final OTU inventory is given for three genetic markers (Tables 4, 5, 6), together with the read distribution during the bioinformatic pipeline using mothur (Table 7). Amplicon Sequence Variants (ASVs) for an alternative cluster-independent approach are given for the three genetic markers in Table 3. For the COI gene, OTUs and ASVs phylogenetic trees show the relationship between the sequence variants, as the majority of OTUs and ASVs in the analyses was classified as “unknown” (Fig. 4 and 5). The morphological approach includes a list of all species found by microscopic analysis, including the biomass, based on measured spec-

Table 1

Chemical analysis of sediment samples. Given is the measured value for each of the 33 substances for each of the seven locations investigated; PEC = consensus-based probable effect concentrations as defined by De Deckere et al. [2] as “Consensus 2; dw = dry weight; values with < are limits of quantification (LOQ); for calculation of PEC quotients LOQ values were divided by two.

Substance	Unit	PEC	RM ¹	LU ¹	CUM ¹	HI ¹	VE ¹	ÖR ¹	FB ¹
As	mg/kg dw	50	14	64	25	34	1	<0.5	<2.5
Pb	mg/kg dw	118	48	333	124	107	<2.5	<2.5	1.6
Cd	mg/kg dw	7.8	0.5	45	3.1	4.9	<0.05	<0.05	<0.1
Cr	mg/kg dw	68	77	1174	124	97	2	4	2.8
Cu	mg/kg dw	60	48	571	97	101	1	3	<0.5
Ni	mg/kg dw	32	52	308	53	60	1	3	1.5
Hg	mg/kg dw	1.2	0.46	2.75	3.43	1.67	<0.025	<0.025	0.05
Zn	mg/kg dw	800	169	3507	772	950	<5	<5	6
Naphthalene	mg/kg dw	6.6	0.2	6.6	0.088	0.22	<0.005	<0.005	<0.005
Acenaphthylene	mg/kg dw	5.2	0.035	0.55	0.013	0.031	<0.005	<0.005	<0.005
Acenaphthene	mg/kg dw	3.3	0.025	2	0.02	0.047	<0.005	<0.005	<0.005
Fluorene	mg/kg dw	0.26	0.053	2.6	0.025	0.066	<0.005	<0.005	<0.005
Phenanthrene	mg/kg dw	0.89	0.41	4.4	0.18	0.4	0.012	0.01	0.015
Anthracene	mg/kg dw	0.17	0.13	1.7	0.048	0.12	<0.005	<0.005	<0.005
Fluoranthene	mg/kg dw	1.2	0.81	3.9	0.31	0.75	0.026	0.018	0.022
Pyrene	mg/kg dw	0.94	0.61	4.5	0.26	0.61	0.015	0.01	0.012
Benzo(a)anthracene	mg/kg dw	0.6	0.3	0.71	0.14	0.32	0.01	<0.005	0.011
Chrysene	mg/kg dw	0.83	0.27	0.44	0.18	0.35	<0.005	<0.005	0.01
Benz(b)fluoranthene	mg/kg dw	0.66	0.23	0.47	0.18	0.32	<0.005	<0.005	<0.005
Benzo(k)fluoranthene	mg/kg dw	0.32	0.083	0.17	0.076	0.13	<0.005	<0.005	0.005
Benzo(a)pyrene	mg/kg dw	0.6	0.21	0.25	0.11	0.26	<0.005	<0.005	0.011
Dibenz(a,h)anthracene	mg/kg dw	0.12	0.022	0.044	0.02	0.044	<0.005	<0.005	<0.005
Indeno(1,2,3-cd)pyrene	mg/kg dw	0.48	0.13	0.07	0.1	0.2	<0.005	<0.005	<0.005
Benzo(ghi)perylene	mg/kg dw	0.45	0.17	0.23	0.13	0.25	<0.005	<0.005	<0.005
PCB 28	µg/kg dw	2	0.13	26	1.2	2.9	<0.025	<0.025	<0.005
PCB 52	µg/kg dw	4.6	0.24	71	1.4	2.7	<0.025	<0.025	<0.005
PCB 101	µg/kg dw	6.7	0.41	88	3.1	4.4	<0.025	<0.025	<0.005
PCB 118	µg/kg dw	6.9	0.24	110	1.8	2.7	<0.025	<0.025	<0.005
PCB 138	µg/kg dw	7.5	0.51	100	9.7	11	<0.025	<0.025	<0.005
PCB 153	µg/kg dw	9.7	0.58	78	10	13	<0.025	<0.025	<0.005
PCB 180	µg/kg dw	5.5	0.17	28	7.9	11	<0.025	<0.025	<0.005
p,p'-DDD	µg/kg dw	3.2	0.9	210	11	44	<0.025	<0.025	<0.005
p,p'-DDE	µg/kg dw	6.8	0.91	87	5.1	13	<0.025	<0.025	<0.005

¹ Abbreviation for locations: Rischmühle (RM), Luppe (LU), Cumlosen (CUM), Hitzacker (HI), Veerse (VE), Örtze (ÖR) and Furlbach (FB).

Table 2

Overview about the number of nematodes for each replicate for the seven locations and the morphological and molecular approach. A black box indicates that these replicates were combined into a new replicate due to low individual number. For the location VE more than five replicates were available for morphological analysis, no further samples were available for molecular analysis, though.

Replicate	FB	VE	ÖR	RM	CUM	HI	LU
<i>Morph.</i>							
I	87	26	95	98	99	102	99
II	100	98	101	98	100	102	100
III	98	9	101	104	110	100	92
IV	100	71	102	99	96	99	100
V	100	10	100	99	98	101	100
VI		99					
VII		100					
VIII		73					
IX		33					
SUM	485	516	499	498	503	504	491
<i>Mol.</i>							
I	500	80	200	200	200	200	50
II		26	200	200	200	200	50
III		15	100	200	200	200	50
IV		9		100	200	200	40
V					200	200	60
SUM	500	130	500	700	800	1000	250

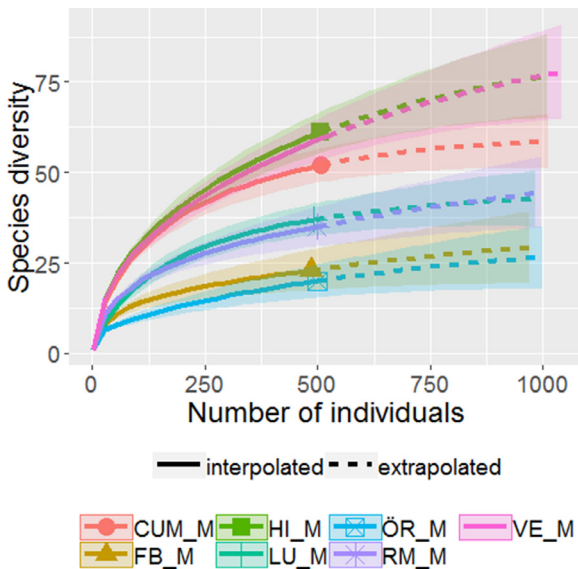


Fig. 1. Rarefaction analyses based on morphological data for the 7 locations (CUM, HI, ÖR, VE, FB and RM). Given is the species diversity that was observed, as well as extrapolated estimates for up to 1000 individuals inspected (dashed lines).

imens from this study, for each species (separated for female and male) and the information whether a reference sequence can be found at National Center for Biotechnology Information (NCBI) (Table 8). A rarefaction analysis based the morphological data provides insights into the (extrapolated) number of species in order to compensate that different numbers of individuals were analysed for the locations, due to samples with rare individual numbers (Fig. 1). For each

Table 3

Results of the cluster-independent bioinformatic approach. Amplicon Sequence Variants (ASVs) are given for the three genetic markers (COI = mICOInt/dgHCO, 28S rDNA = 1274/706, and 18S rDNA = 3Ndf/C_1132f). Numbers are given for all samples and replicates combined, as this is only an alternate approach, which was not regarded further.

ASV (mICOInt/dgHCO)	overall	ASV (1274/706)	overall	ASV (3Ndf/C_1132f)	overall
unknown_4	19,900	Nematoda environmental sample	67,051	Diplogaster rivalis	2717
unknown_5	13,129	Aporcelaimellus obtusicaudatus	25,065	Homalozoon vermiculare	553
unknown_6	11,719	Tobrilus gracilis;Tobrilus sp. BI-LSU-312	23,119	Oscheius tipulae	399
unknown_7	10,593	Eucephalobus oxyuroides	19,260	Mesorhabditis belari	290
unknown_9	8837	Tobrilus gracilis	15,565	invertebrate environmental sample	274
unknown_12	6880	Tobrilus pellucidus	11,906	Semitobrilus pellucidus	75
unknown_2	5113	unknown_1	7706	56c	24
unknown_18	3899	Merlinius sp. MerISp1	5177	unknown_9	24
unknown_19	3809	unknown_2	4139	Punctodora ratzeburgensis	21
unknown_22	3355	Rhabditis sp. DF5059	2857	unknown_11	21
unknown_21	2992	Rhynchocoelax simplex	2798	Bacillidium sp.	19
unknown_23	2929	unknown_3	2733	unknown_12	19
unknown_20	2851	Acrobelloides tricornis	2548	unknown_3	16
unknown_24	2802	unknown_4	1931	Microsporidium sp. BPAR7	16
unknown_25	2693	Mononchus truncatus	1052	Aphelenchoides bicaudatus	14
Polypedilum pullum	2657	unknown_5	969	unknown_14	13
unknown_26	2636	uncultured Paractinolaimus sp.	895	Daptonema sp. 1255	12
unknown_11	2325	Amplimerlinius paraglobigerus	813	uncultured fungus	11
unknown_27	2022	unknown_6	735	Mesorhabditis sp. WB-2009	10
unknown_28	1787	Ethmolaimus pratensis	648	Tobrilus cf. zakopanensis 1 JH-2014	9
unknown_29	1729	unknown_7	615	unknown_19	9
unknown_32	1654	Mononchus aquaticus	610	Frontonia leucas	7
Coccinella trifasciata perplexa	1648	Cephalobus cubaensis	596	Oscheius sp. AW-2011	6
unknown_33	1585	Mesodorylaimus bastiani	578	unknown_13	6
unknown_35	1447	Zeldia punctata	512	unknown_26	6
unknown_37	1315	Eumonhystera filiformis	418	Chromadorina bioculata	6
Limnodrilus hoffmeisteri	1145	unknown_8	412	unknown_27	5
unknown_38	1131	unknown_9	405	unknown_29	3
unknown_39	1017	unknown_10	398	Rhabditis sp. DF5059	3
unknown_40	1004	Eucephalobus striatus	337	Basiria duplexa	3
unknown_42	941	Monhystera paludicola;Monhystera stagnalis	267	uncultured eukaryote	2
Tubifex tubifex	927	unknown_11	262	Acrobelloides_nanus-7-3Ndf	2
Limnodrilus claparedianus	867	Homalozoon vermiculare	259	Ironus sp. 199_89c	2
unknown_45	821	Tripyla glomerans	249	Aporcelaimellus sp. SSU_3	2
unknown_46	801	unknown_12	224		
unknown_47	769	Anaplectus granulatus	220		
unknown_48	687	Pratylenchus convallariae	204		
unknown_49	681	unknown_13	197		
unknown_50	666	unknown_14	184		
unknown_51	619	Monhystera paludicola/Monhystera sp.	178		
Nais communis	594	Prodesmodora circulata	166		
unknown_53	585	unknown_15	166		
Phasmarhabditis papillosa	582	uncultured Tobrilus	164		

(continued on next page)

Table 3 (continued)

ASV (mCOLint/dgHCO)	overall	ASV (1274/706)	overall	ASV (3Ndf/C_1132f)	overall
unknown_54	496	<i>Oscheius tipulae</i>	163		
unknown_55	486	<i>Mesorhabditis</i> sp. 'Bursilla' PS1179	155		
unknown_56	483	unknown_16	148		
unknown_43	482	unknown_17	142		
unknown_57	464	unknown_18	121		
<i>Fejervarya granosa</i>	418	unknown_19	118		
unknown_60	410	unknown_20	117		
unknown_61	379	unknown_21	117		
unknown_62	376	unknown_22	116		
unknown_63	360	unknown_23	109		
unknown_65	325	unknown_24	107		
unknown_66	312	unknown_25	104		
unknown_67	300	unknown_26	104		
unknown_70	265	<i>Aphelenchus</i> sp. OH-2016	102		
unknown_69	261	unknown_27	100		
<i>Bos taurus</i>	247	unknown_28	100		
<i>Limnodrilus</i>	227	unknown_29	89		
hoffmeisteri complex lineage VIII					
<i>Meloidogyne naasi</i>	223	unknown_30	82		
unknown_72	223	<i>Cephaloboides</i> cf. <i>armata</i> SB363	81		
unknown_73	220	<i>Dorylaimus stagnalis</i>	80		
unknown_76	209	unknown_31	79		
unknown_71	204	unknown_32	79		
<i>Blastomussa loyae</i>	203	unknown_33	75		
<i>Naididae</i> sp.	201	unknown_34	75		
JCOCH052-10					
unknown_78	188	unknown_35	71		
unknown_79	186	unknown_36	71		
unknown_80	186	<i>Boleodorus</i> sp. 2 TJP-2012	64		
unknown_81	185	unknown_37	60		
unknown_86	156	unknown_38	54		
unknown_88	152	<i>Rhabditis brassicae</i>	53		
<i>Gammarus pulex</i>	145	unknown_39	52		
unknown_89	140	unknown_40	51		
unknown_90	139	unknown_41	50		
unknown_92	128	unknown_42	50		
unknown_93	128	unknown_43	49		
unknown_84	127	unknown_44	49		
unknown_94	127	unknown_45	46		
unknown_95	123	unknown_46	43		
unknown_97	115	unknown_47	43		
unknown_98	106	unknown_48	42		
unknown_99	106	unknown_49	39		
unknown_100	105	<i>Plectus exinocaudatus</i>	38		
<i>Amphichaeta raptisae</i>	104	unknown_50	38		
<i>Rhabditida</i> sp. 3028ed	103	unknown_51	37		
unknown_101	102	<i>Trichodorus similis</i>	36		
unknown_103	101	unknown_52	36		
unknown_104	101	unknown_53	34		
<i>Tubificinae</i> sp. 1 RV-2016	100	unknown_54	34		
unknown_105	100	unknown_55	33		
<i>Enochrus ater</i>	88	unknown_56	28		
unknown_106	88	<i>Coslenchus costatus</i>	27		
unknown_107	87	<i>Plectus velox</i>	26		
unknown_109	86	unknown_57	25		
<i>Rhabditida</i> sp. 3003ed	84	<i>Chiloplectus andrassyi</i>	22		
unknown_111	84	<i>Plectus opisthocirculus</i>	21		

(continued on next page)

Table 3 (continued)

ASV (micolint/dgHCO)	overall	ASV (1274/706)	overall	ASV (3Ndf/C_1132f)	overall
unknown_112	84	unknown_58	20		
unknown_113	81	unknown_59	19		
unknown_114	81	Trichodorus arasbaranensis	18		
unknown_115	80	unknown_60	18		
unknown_116	80	unknown_61	18		
unknown_117	79	unknown_62	18		
unknown_119	76	unknown_63	17		
unknown_120	74	unknown_64	17		
unknown_122	73	unknown_65	17		
unknown_124	66	Trischistoma sp. 2 ZQZ-2010a	16		
unknown_126	65	unknown_66	16		
unknown_127	65	unknown_67	16		
unknown_128	65	unknown_68	16		
Corynoneura carriana	64	unknown_69	16		
Limnodrilus	64	Plectus aquatilis/Plectus acuminatus	15		
hoffmeisteri complex lineage X					
unknown_129	64	unknown_70	15		
unknown_130	61	unknown_71	15		
unknown_118	59	unknown_72	15		
unknown_132	58	unknown_73	15		
unknown_133	58	Pellioiditis sp. VS-2014	14		
unknown_135	56	unknown_74	14		
unknown_137	55	unknown_75	14		
unknown_138	53	unknown_76	14		
unknown_136	52	unknown_77	14		
unknown_15	51	unknown_78	13		
unknown_140	48	unknown_79	13		
unknown_141	48	Acrobeloides sp. ES-2017	12		
unknown_142	48	Meloidogyne graminicola	12		
Limnodrilus	47	unknown_80	12		
hoffmeisteri complex lineage IX					
unknown_143	47	unknown_81	12		
unknown_144	46	unknown_82	12		
unknown_139	45	unknown_83	12		
unknown_145	45	unknown_84	12		
Chironomus melanescens	42	unknown_85	11		
unknown_125	39	unknown_86	11		
unknown_149	39	unknown_87	11		
unknown_150	38	unknown_88	11		
unknown_151	38	unknown_89	11		
unknown_152	38	Prismatolaimus dolichurus	10		
unknown_148	37	unknown_90	10		
Cricotopus sylvestris	36	unknown_91	10		
unknown_17	36	unknown_92	10		
unknown_153	35	unknown_93	10		
unknown_154	34	unknown_94	10		
unknown_155	34	unknown_95	10		
unknown_156	34	unknown_96	10		
unknown_158	33	unknown_97	10		
unknown_159	32	unknown_98	10		
unknown_160	31	unknown_99	10		
unknown_36	31	unknown_100	9		
unknown_161	30	unknown_101	9		
unknown_162	29	unknown_102	9		
unknown_163	29	unknown_103	9		
unknown_164	29	unknown_104	9		
unknown_165	28	unknown_105	8		

(continued on next page)

Table 3 (continued)

ASV (mCOLint/dgHCO)	overall	ASV (1274/706)	overall	ASV (3Ndf/C_1132f)	overall
unknown_166	28	unknown_106	8		
unknown_167	28	unknown_107	8		
unknown_168	28	unknown_108	8		
Prodiamesa olivacea	27	unknown_109	8		
unknown_169	27	Frontonia sp. 4 WS-2014	7		
unknown_171	27	unknown_110	7		
Propappus volki	25	unknown_111	7		
unknown_172	25	unknown_112	7		
unknown_34	25	unknown_113	7		
unknown_173	24	unknown_114	7		
unknown_175	24	unknown_115	6		
unknown_176	24	unknown_116	6		
unknown_178	24	unknown_117	6		
unknown_180	23	unknown_118	6		
unknown_58	23	unknown_119	6		
unknown_184	22	unknown_120	6		
unknown_31	22	unknown_121	6		
unknown_189	21	unknown_122	6		
unknown_170	20	unknown_123	6		
unknown_190	20	unknown_124	6		
unknown_191	20	unknown_125	6		
unknown_192	20	unknown_126	6		
unknown_193	20	unknown_127	6		
unknown_194	20	unknown_128	6		
unknown_195	20	Arabidopsis thaliana	5		
unknown_197	19	uncultured Asellus sp.	5		
Potamothenix moldaviensis	18	unknown_129	5		
unknown_198	18	unknown_130	5		
unknown_199	18	unknown_131	5		
unknown_200	18	unknown_132	5		
unknown_201	18	unknown_133	5		
Tanytarsus pallidicornis	17	unknown_134	5		
unknown_202	17	unknown_135	5		
unknown_203	17	unknown_136	4		
unknown_204	17	unknown_137	4		
unknown_205	17	unknown_138	4		
uncultured fungus	16	unknown_139	4		
unknown_206	16	unknown_140	4		
unknown_207	16	unknown_141	4		
unknown_209	16	unknown_142	4		
unknown_210	16	Daubaylia potomaca	3		
Arthropoda	15	Tobrilus medius	3		
environmental sample					
Ophidonais serpentina	15	unknown_143	3		
unknown_212	15	unknown_144	3		
unknown_213	15	unknown_145	3		
unknown_214	15	unknown_146	3		
unknown_215	15	unknown_147	3		
Asellus aquaticus	14	unknown_148	3		
Penicillium sclerotiorum	14	Bos indicus x Bos taurus	2		
unknown_216	14	Mesocriconema xenoplax	2		
unknown_217	14	unknown_149	2		
unknown_218	14	unknown_150	2		
unknown_219	14	unknown_151	2		
unknown_220	13	unknown_152	2		
unknown_223	13	unknown_153	2		
unknown_224	13	unknown_154	2		
unknown_225	13	unknown_155	2		

(continued on next page)

Table 3 (continued)

ASV (miCOlint/dgHCO)	overall	ASV (1274/706)	overall	ASV (3NDf/C_1132f)	overall
unknown_226	13	unknown_156	2		
Homo sapiens	12	unknown_157	2		
unknown_227	12	unknown_158	2		
unknown_229	12	unknown_159	2		
unknown_230	12	unknown_160	2		
Nais sp. N12 RV-2017	11	unknown_161	2		
unknown_231	11	unknown_162	2		
unknown_232	11	unknown_163	2		
unknown_234	11				
unknown_235	11				
unknown_238	11				
unknown_182	10				
unknown_239	10				
unknown_240	10				
Parasitidae sp.	9				
BOLD:AAF9233					
unknown_157	9				
unknown_221	9				
unknown_241	9				
unknown_242	9				
unknown_244	9				
unknown_245	9				
unknown_247	9				
unknown_44	9				
Eucyclops cf.	8				
serrulatus ZISP					
11SNM-549					
unknown_249	8				
unknown_250	8				
Chironomus	7				
pallidivittatus					
Micospina	7				
auribohorum					
unknown_181	7				
unknown_208	7				
unknown_251	7				
unknown_252	7				
unknown_254	7				
unknown_255	7				
unknown_258	7				
Acanthocyclops	6				
vernalis					
Anomala ruficapilla	6				
Paragordius	6				
tricuspidatus					
unknown_260	6				
unknown_261	6				
unknown_262	6				
unknown_263	6				
unknown_264	6				
unknown_266	6				
Potamothenix heuscheri	5				
unknown_196	5				
unknown_271	5				
unknown_272	5				
unknown_273	5				
unknown_274	5				
unknown_275	5				
unknown_276	5				

(continued on next page)

Table 3 (continued)

ASV (mCOLint/dgHCO)	overall	ASV (1274/706)	overall	ASV (3Ndf/C_1132f)	overall
unknown_277	5				
unknown_278	5				
unknown_279	5				
unknown_280	5				
unknown_3	5				
Eucyclops cf. serrulatus ZISP 11SNM-547	4				
unknown_281	4				
unknown_282	4				
unknown_284	4				
unknown_285	4				
unknown_286	4				
unknown_287	4				
unknown_291	4				
unknown_292	4				
unknown_293	4				
unknown_294	4				
unknown_295	4				
unknown_68	4				
Chydoridae sp. SHDT150804	3				
Habrotrocha constricta	3				
unknown_296	3				
unknown_297	3				
unknown_298	3				
unknown_299	3				
unknown_300	3				
unknown_301	3				
unknown_302	3				
unknown_303	3				
unknown_304	3				
unknown_305	3				
unknown_306	3				
unknown_307	3				
unknown_308	3				
unknown_309	3				
unknown_310	3				
unknown_311	3				
unknown_312	3				
unknown_314	3				
unknown_1	2				
unknown_315	2				
unknown_316	2				
unknown_318	2				
unknown_319	2				
unknown_320	2				
unknown_321	2				
unknown_322	2				

of the Nematode Species at Risk (NemaSPEAR) a NCBI inspection was performed to validate if reference sequences are available for the genus and the species level (Table 9). For molecular-based and morphology-based data, NMDS plots were calculated comparing the nematodes communities for each replicate at the species and genus level (Fig. 2). The NemaSPEAR[%] values for morphological and molecular data were plotted against mean probable-effect-concentration quotients (mean PEC-Q), in order to give insights about the coherences between the indices and the toxic potential of the sediment (Fig. 3).

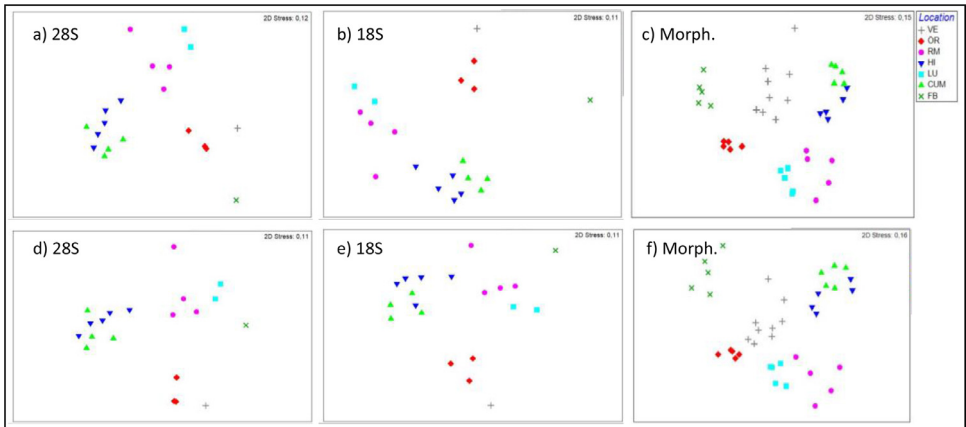


Fig. 2. -nMDS plots based on not-transformed relative abundances of species (a, b, c) and genera (d, e, f) found in sediments sampled from the seven locations based on taxonomic data for the 28S marker (a, d), the 18S marker (b, e) and the morphological analysis (c, f); Bray-Curtis similarity was applied; each location is encoded by a different color and symbol.

2. Experimental design, materials, and methods

Seven locations were sampled, with five replicates for morphological and molecular analysis each. Each replicate was comprised from five subsamples. The detailed process is described in ref [1]. Sediment samples were processed as described by Higgins and Thiel [4]. From each of

Table 4

List of OTUs determined for the 28S rDNA gene fragment, using the primer pairs 1274/706 for the seven locations (CUM, HI, LU, ÖR, RM, VE and FB). Clustering was performed at 99%.

OTU 1274/706	CUM	HI	LU	ÖR	RM	VE	FB
Acrobeloides sexlineatus	5.39	2.47	0.00	0.16	0.06	0.00	0.00
Anaplectus granulatus	0.37	0.62	0.00	0.00	0.01	0.00	10.22
Aphanolaimus aquaticus	0.00	0.00	0.00	0.00	0.00	0.00	0.04
Aphelenchus spec	0.14	0.17	0.00	0.00	0.01	0.00	0.00
Aporcelaimellus obtusicaudatus	32.23	42.80	0.07	0.82	2.29	0.00	0.00
Basiria sp. SAN-2005	0.04	0.00	0.00	0.00	0.00	0.00	0.00
Boleodorus sp. 2 TJP-2012	0.03	0.00	0.00	0.00	0.00	0.00	0.00
Bursilla sp. PS1179	0.15	1.05	0.00	0.01	0.05	0.00	0.00
Cephaloboides cf. armata SB363	0.00	0.00	0.00	0.00	0.31	0.00	0.00
Cephalobus cubaensis	1.41	0.80	0.00	0.03	0.01	0.00	0.00
Chromadorita leuckarti	0.00	0.20	0.45	0.16	30.98	0.08	6.92
Coslenchus costatus	12.63	1.03	0.00	0.54	0.09	0.00	0.00
Diplogastrellus sp. NK-2010	0.00	0.87	5.50	0.00	3.68	0.00	0.00
Dorylaimus stagnalis	0.00	0.06	0.00	0.16	0.50	0.00	0.00
Epitobrilus medius	0.02	0.02	0.00	0.01	0.00	0.00	0.00
Ethmolaimus pratensis	0.22	0.20	0.00	1.17	0.00	0.27	7.11
Eucephalobus cf oxyuroides	37.53	26.20	0.00	1.13	0.99	0.00	0.00
Eucephalobus striatus	0.45	1.66	0.02	0.01	0.04	0.00	0.00
Eudorylaimus sp. 2 KH-2018	0.26	0.00	0.00	0.00	0.00	0.00	0.00
Eumonhystera filiformis	0.00	0.16	0.80	0.00	0.26	0.25	0.00
Ironus longicaudatus	0.00	0.00	0.00	0.00	0.00	0.00	0.12
Ironus tenuicaudatus	0.00	0.00	0.00	0.06	0.00	0.00	0.00
Meloidogyne arenaria	0.03	0.00	0.00	0.00	0.00	0.00	0.00
Mesocriconema curvatum	0.02	0.83	0.44	0.00	0.06	0.00	0.00
Mesodorylaimus bastiani	0.13	0.62	0.00	0.00	0.00	0.00	0.00
Monhystera paludicola	0.00	0.00	0.00	0.08	0.36	0.05	0.04

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Table 4 (continued)

OTU 1274/706	CUM	HI	LU	ÖR	RM	VE	FB
Monhystera spec	0.00	0.00	0.00	0.04	0.00	0.00	0.00
Mononchoides sp. 1 VS-2014	0.05	0.00	0.00	0.00	0.00	0.00	0.00
Mononchus aquaticus	1.33	0.58	0.00	0.00	0.02	0.00	0.00
Mononchus truncatus	0.02	0.10	0.00	0.05	0.55	0.48	0.00
Neotobrilus longus	0.63	0.02	0.00	0.52	0.01	1.71	29.59
Panagrolaimus detritophagus	0.00	0.05	0.00	0.00	0.00	0.00	0.00
Panagrolaimus sp. PS1159	0.00	0.02	0.00	0.00	0.00	0.00	0.00
Panagrolaimus	0.03	0.00	0.00	0.00	0.00	0.00	0.00
Paractinolaimus sp.	0.07	0.01	0.00	0.01	0.00	0.00	0.00
Paratylenchus microdorus	0.00	0.03	0.00	0.00	0.00	0.00	0.00
Pellioiditis sp. VS-2014	0.00	0.04	0.00	0.00	0.00	0.00	0.00
Plectus aquatilis	0.02	0.00	0.00	0.00	0.00	0.00	2.75
Plectus exinocaudatus	0.09	0.05	0.22	0.00	0.05	0.08	0.16
Plectus opisthocirculus	0.00	0.05	0.00	0.00	0.00	0.00	0.08
Plectus velox	0.01	0.10	0.00	0.00	0.01	0.00	0.00
Pratylenchoides ritteri	0.02	0.07	0.00	0.00	0.00	0.00	0.00
Pratylenchoides sp. 1 MP-2013	1.60	15.80	0.00	0.04	0.40	0.00	0.00
Pratylenchus convallariae	0.06	0.00	0.00	0.00	0.00	0.00	0.00
Prionchulus punctatus	0.01	0.04	0.00	0.00	0.00	0.00	0.00
Prismatolaimus dolichurus	0.34	0.27	0.00	0.01	0.01	0.00	0.08
Prismatolaimus intermedius	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Prodesmodora circulata	0.00	0.00	0.00	0.00	0.00	1.11	0.00
Protolitonotus magnus	0.00	0.00	0.00	0.01	0.00	0.00	0.00
Rhabditoides sp. NK-2010	0.00	0.00	0.00	0.00	0.02	0.00	0.00
Rhabditis sp. DF5059	0.04	0.02	0.01	0.00	0.00	0.00	0.00
Rhabditoines regina	0.00	0.00	0.00	0.00	0.00	0.00	0.08
Rotylenchus robustus	0.81	0.09	0.00	0.02	0.00	0.00	0.00
Semitobrilus pellucidus	0.19	0.03	2.28	89.37	0.98	88.46	0.00
Theristus agilis	0.00	0.05	0.00	4.77	1.07	6.86	38.35
Tobrilus cf. helveticus KR-2013	0.04	0.00	0.00	0.00	0.00	0.00	0.00
Tobrilus gracilis	1.59	1.89	90.21	0.72	56.58	0.51	0.00
Tobrilus sp. BI-LSU-318	0.00	0.00	0.00	0.00	0.00	0.04	0.00
Trichodorus arasbaranensis	0.17	0.00	0.00	0.00	0.00	0.11	0.00
Tripyla glomerans	0.00	0.14	0.00	0.04	0.57	0.00	0.00
Tripyla setifera	0.00	0.07	0.00	0.03	0.00	0.00	4.48
unknown_1	0.03	0.00	0.00	0.00	0.00	0.00	0.00
unknown_10	0.07	0.00	0.00	0.00	0.00	0.00	0.00
unknown_11	0.02	0.00	0.00	0.00	0.00	0.00	0.00
unknown_12	0.03	0.00	0.00	0.00	0.00	0.00	0.00
unknown_13	0.14	0.00	0.00	0.00	0.00	0.00	0.00
unknown_2	0.00	0.04	0.00	0.00	0.00	0.00	0.00
unknown_3	0.02	0.00	0.00	0.00	0.00	0.00	0.00
unknown_4	0.08	0.10	0.00	0.00	0.00	0.00	0.00
unknown_5	0.07	0.00	0.00	0.00	0.00	0.00	0.00
unknown_6	0.05	0.00	0.00	0.00	0.00	0.00	0.00
unknown_7	0.00	0.04	0.00	0.00	0.00	0.00	0.00
unknown_8	0.02	0.00	0.00	0.00	0.00	0.00	0.00
unknown_9	0.05	0.00	0.00	0.00	0.00	0.00	0.00
Zeldia punctata	1.19	0.52	0.00	0.03	0.02	0.00	0.00

the five replicates 100 nematodes were isolated for morphological analyses, if possible. For the location VE more replicates were analysed, due to low nematode densities (Table 2). For molecular analyses 200 nematodes per replicate were isolated, as this gave more DNA yield in previous studies [5]. For several replicates with low nematodes densities, two or more replicates were combined as shown in Table 2.

At each of the seven locations, for analysis of chemical contamination 5–10 subsamples of sediment were collected with a stainless-steel grab sampler and then pooled in a stainless-steel tub to avoid the effects of local variability. After the removal of large debris, the sediment ma-

Table 5

List of OTUs determined for the 18S rDNA gene fragment, using the primer pairs 3NDf/C_1132f for the six locations (CUM, HI, LU, ÖR, RM and VE). Clustering was performed at 99%.

OTU_3NDf/C_1132f	CUM	HI	LU	ÖR	RM	VE
Cephalobus sp./Acrobeloides nanus	46.64	24.14	0.00	5.19	1.85	0.00
Amplimerlinius macrurus	0.50	0.46	0.00	0.00	0.00	0.00
Anaplectus sp. PDL-2005	0.05	0.23	0.00	0.00	0.00	0.00
Aphelenchoides bicaudatus	0.00	0.00	0.00	0.00	0.15	0.00
Aphelenchoides sp. Asp6753	0.00	0.07	0.00	0.00	0.00	0.00
Aphelenchoides sp. BE1	0.00	0.00	0.00	0.00	0.00	0.00
Aphelenchus sp. JH-2004	0.72	0.48	0.00	0.00	0.00	0.00
Aporcelainellus sp. AMS-2013	0.29	0.00	0.00	0.00	0.00	0.00
Basiria duplexa	10.72	0.49	0.00	0.41	0.06	0.00
Boleodorus thylactus	0.07	0.00	0.00	0.00	0.00	0.00
Cephaloboides cf. armata SB363	0.00	0.00	0.00	0.00	11.43	0.00
Cephalobus persegnis	0.00	2.72	0.00	0.00	0.08	0.00
Chromadorina bioculata	0.00	0.00	0.00	0.00	0.71	0.00
Coslenchus turkeyensis	0.05	0.31	0.00	0.00	0.00	0.00
Daptonema hirsutum	0.00	0.00	0.00	0.00	1.13	0.00
Daptonema sp. 1255	0.00	0.12	0.00	0.00	7.85	0.00
Diphterophora communis	7.09	0.00	0.00	0.42	0.00	0.00
Diplogaster rivalis	0.14	5.35	26.11	0.01	13.91	0.00
Ditylenchus sp. 10 JH-2014	1.20	2.22	0.00	0.10	0.07	0.00
Ditylenchus sp. 6 JH-2014	0.00	0.14	0.00	0.00	0.00	0.00
Ditylenchus sp. 8 JH-2014	0.00	0.11	0.00	0.00	0.00	0.00
Ethmolaimus pratensis	0.00	0.00	0.00	0.13	0.00	0.00
Eumonhystera sp. 1 JH-2014	0.00	0.00	0.00	8.53	0.00	7.60
Filenchus vulgaris	0.50	0.00	0.00	0.00	0.00	0.00
Geocenamys quadrifer	0.00	6.70	0.00	0.00	0.27	0.00
Hemileius microclava	0.00	0.00	0.00	0.00	0.03	0.00
Heterocephalobus elongatus	1.03	0.86	0.08	0.02	0.00	0.00
Ironus sp.	0.00	0.00	0.00	0.00	0.02	0.00
Merlinius brevidens	0.97	1.15	0.00	0.00	0.03	0.00
Merlinius joctus	0.00	0.44	0.00	0.00	0.00	0.00
Mesorhabditis sp. FI-Type-10	1.07	4.24	0.00	0.00	0.88	0.00
Monhystera cf. paludicola JH-2014	0.00	0.00	0.00	0.50	0.16	0.00
Monhystera sp. 1 JH-2014	0.00	0.00	0.00	0.00	1.84	0.00
Mononchus aquaticus_100c	0.00	0.00	0.01	12.55	0.01	17.73
Neotobrilus longus	0.00	0.00	0.00	2.20	0.00	2.33
Neotobrilus longus	2.27	1.00	8.88	3.06	1.48	3.70
Oscheius tipulae	0.63	4.43	0.00	0.00	1.27	0.00
Panagrolaimus cf. rigidus AF40	0.00	0.91	0.00	0.00	0.00	0.00
Panagrolaimus sp. AS01	0.00	0.50	0.00	0.00	0.00	0.00
Paratylenchus dianthus	0.00	0.15	0.00	0.00	0.00	0.00
Phasmarhabditis sp. SA2	0.63	0.00	0.00	0.00	0.00	0.00
Phasmarhabditis sp. SA4	1.12	0.62	0.00	0.00	0.25	0.00
Plectus opisthocirculus	0.00	0.07	0.00	0.00	0.00	0.00
Plectus sp. 3 JS-2016	0.00	0.06	0.00	0.00	0.03	0.00
Prismatolaimus cf. dolichurus JH-2004	0.41	0.00	0.00	0.02	0.00	0.00
Prismatolaimus dolichurus	0.14	0.73	0.00	0.00	0.01	0.00
Pristionchus lheritieri	0.98	0.00	0.00	0.13	0.00	0.00
Punctodora ratzeburgensis	0.00	0.00	0.00	0.00	5.28	0.00
Rhabditis sp. DF5059	12.40	23.22	1.62	1.05	2.36	0.00
Rhabditis sp. RA5	8.14	14.50	0.00	0.00	2.13	0.00
Rhynchocolex simplex	0.00	0.00	0.00	0.31	0.00	0.00
Semitobrilus pellucidus	0.00	0.00	0.00	0.00	0.00	1.67
Semitobrilus pellucidus /Tobriidae sp. 1 JH-2014	0.14	0.00	0.00	63.54	0.00	62.82
Tobrilus cf. zakopanensis 1 JH-2014	0.00	0.00	0.16	0.00	0.03	0.00
Tobrilus gracilis/Tobrilus sp. 1 JH-2014	0.34	2.38	62.80	1.49	45.39	0.00
Trichodoros similis	1.09	0.00	0.00	0.00	0.00	0.00
Tridentulus sp. PDL-2005	0.00	0.01	0.34	0.00	0.00	4.15
Tripyla glomerans	0.00	0.49	0.00	0.06	0.80	0.00
Tripyla sp. JH-2004	0.00	0.17	0.00	0.00	0.00	0.00

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Table 5 (continued)

OTU_3Ndf/C_1132f	CUM	HI	LU	ÖR	RM	VE
unknown_13	0.00	0.00	0.00	0.00	0.51	0.00
unknown_16	0.00	0.17	0.00	0.00	0.00	0.00
unknown_17	0.00	0.09	0.00	0.00	0.00	0.00
unknown_19	0.31	0.01	0.00	0.00	0.00	0.00
unknown_21	0.00	0.00	0.00	0.27	0.00	0.00
unknown_22	0.12	0.00	0.00	0.00	0.00	0.00
unknown_4	0.00	0.00	0.00	0.00	0.00	0.00
unknown_6	0.00	0.27	0.00	0.00	0.00	0.00
unknown_9	0.21	0.00	0.00	0.00	0.00	0.00

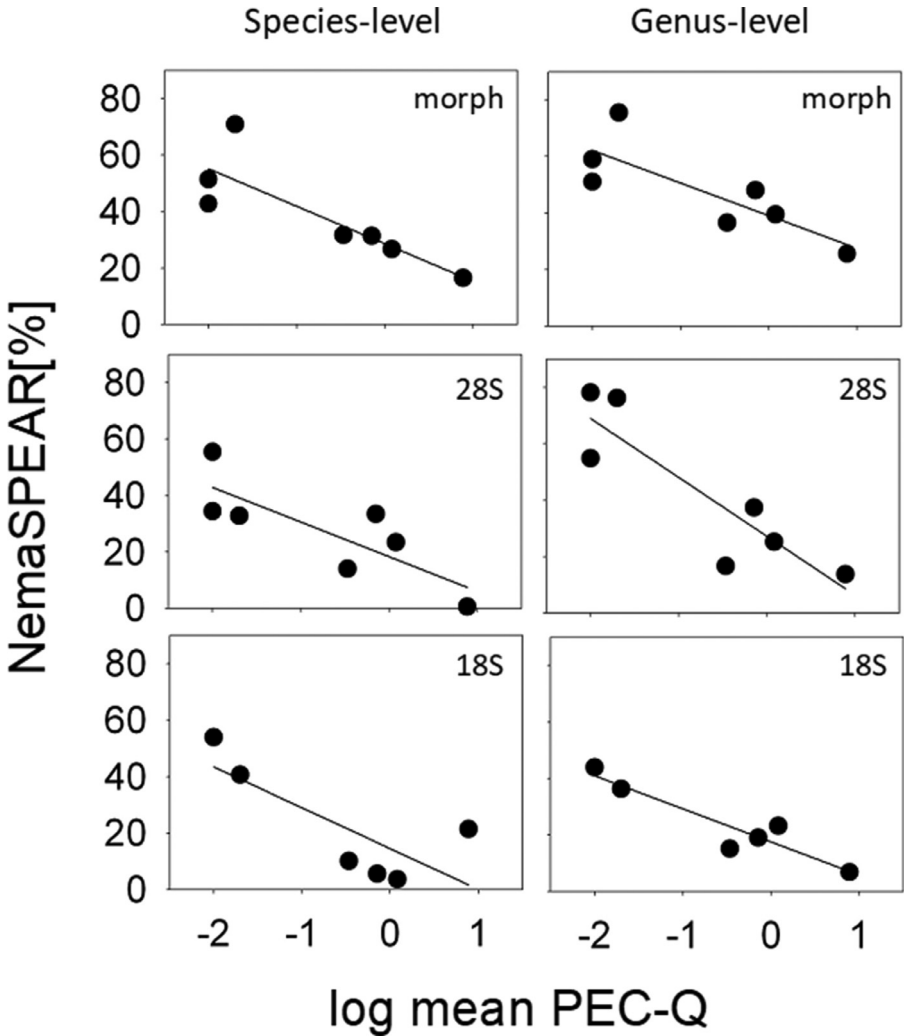


Fig. 3. NemaSPEAR[%] (a, c, e) and NemaSPEAR[%]_{genus} (b, d, f) calculated based on morphological (morph; a, b) and molecular (28S-rDNA: c, d, 18S-rDNA: e, f) taxonomic nematode species and genus data of river sediment sampled at 7 sites (6 sites for 18S-rDNA) plotted against the mean PEC-Q values calculated for the respective sediments; a: $r^2 = 0.71$; $p = 0.017$; b: $r^2 = 0.66$; $p = 0.027$; c: $r^2 = 0.65$; $p = 0.029$; d: $r^2 = 0.78$; $p = 0.008$; e: $r^2 = 0.60$; $p = 0.070$; f: $r^2 = 0.88$; $p = 0.006$.

Table 6

List of OTUs determined for the COI gene, using the primer pairs mICoInt/dgHCO for the six locations (CUM, HI, LU, ÖR, RM, and VE). Clustering was performed at 97%.

COI_OTU	CUM	HI	LU	ÖR	RM	VE
Acanthocyclops vernalis	0.00	0.00	0.00	0.01	0.01	0.00
Amphichaeta raptisae	0.02	0.00	0.00	0.49	0.00	0.00
Arthropoda environmental sample	0.00	0.00	0.00	0.06	0.00	0.00
Asellus aquaticus	0.06	2.29	0.00	0.00	0.00	0.00
Blastomussa loyae	0.00	0.00	0.00	0.00	0.00	6.77
Bos taurus	0.64	11.20	0.12	0.08	0.18	0.59
Candona candida	0.00	0.00	0.00	0.00	0.01	0.00
Chironomus melanescens	0.00	0.00	0.00	0.20	0.00	0.00
Chironomus pallidivittatus	0.00	0.00	0.11	0.01	0.01	0.00
Chydoridae sp. SHDT150804	0.00	0.00	0.00	0.01	0.00	0.00
Coccinella trifasciata perplexa	0.00	5.80	0.00	0.00	0.56	0.00
Corynoneura kadalinka	0.00	0.00	0.00	0.00	0.35	0.00
Cricotopus sylvestris	0.05	0.00	0.00	0.02	0.01	0.00
Endochironomus albipennis	0.00	0.00	0.00	0.00	0.01	0.00
Eucyclops cf. serrulatus ZISP 11SNM-547	0.00	0.00	0.00	0.00	0.03	0.00
Eucyclops cf. serrulatus ZISP 11SNM-549	0.00	0.00	0.00	0.07	0.02	0.00
Fejervarya granosa	0.00	1.30	0.00	0.00	0.20	0.00
Homo sapiens	0.15	0.00	0.00	0.00	0.00	0.00
Limnodrilus claparedianus	0.00	0.00	0.00	0.81	0.58	0.00
Limnodrilus hoffmeisteri complex lineage IX	0.00	0.00	0.00	0.18	0.00	0.00
Limnodrilus hoffmeisteri complex lineage X	0.00	0.00	0.00	0.00	0.20	0.00
Naididae sp. JCOCH052-10	0.00	0.00	0.00	0.87	0.00	0.00
Nais communis	0.00	0.00	0.00	0.02	0.00	0.00
Nais sp. N12 RV-2017	0.00	0.01	0.00	0.00	0.04	0.00
Ophidonais serpentina	0.00	0.00	0.00	0.00	0.04	0.00
Parasitidae sp. BOLD:AAF9233	0.00	0.00	0.00	0.03	0.00	0.00
Penicillium sclerotiorum	0.01	3.33	0.00	0.02	0.02	0.00
Polypedilum albinodus	0.00	0.00	0.00	0.01	0.00	0.00
Polypedilum pullum	0.22	1.44	0.85	0.06	1.24	20.46
Potamothenix heuscheri	0.00	0.00	0.00	0.01	0.01	0.00
Potamothenix moldaviensis	0.00	0.00	0.00	0.13	0.00	0.00
Prodiamesa olivacea	0.01	0.00	0.00	0.15	0.00	0.00
Propappus volki	0.00	0.00	0.00	0.00	0.05	0.00
Pseudo-nitzschia subfraudulenta	0.00	0.00	0.00	0.00	0.00	0.40
Tanytarsus pallidicornis	0.00	0.00	0.00	0.04	0.00	0.00
Tubifex tubifex	0.00	0.00	3.39	1.63	0.37	0.00
Tubificinae sp. 1 RV-2016	0.00	0.00	0.00	0.14	0.00	0.00
Tubificinae sp. T2_336	0.00	0.00	0.00	0.01	0.00	0.00
uncultured fungus	0.15	0.00	0.00	0.01	0.00	0.00
unknown_10	0.00	0.00	0.00	28.46	0.00	0.00
unknown_102	0.00	4.91	0.00	0.00	0.09	0.00
unknown_104	0.00	0.00	1.06	0.00	0.00	0.00
unknown_105	0.00	0.00	0.00	0.02	0.01	0.00
unknown_107	0.62	0.00	0.00	0.02	0.00	0.00
unknown_109	0.00	0.00	0.00	0.32	0.00	0.00
unknown_11	0.00	0.00	0.00	0.00	1.37	0.00
unknown_110	0.00	0.00	0.00	0.00	0.29	0.12
unknown_112	0.00	0.00	0.00	1.18	0.00	0.00
unknown_113	0.00	0.00	0.00	0.00	0.14	0.00
unknown_114	0.00	0.00	0.03	0.00	0.00	0.00
unknown_115	0.11	0.00	0.00	0.00	0.00	0.00
unknown_116	0.58	0.59	0.00	0.00	0.10	0.00
unknown_117	0.00	0.00	0.00	0.00	0.01	1.73
unknown_118	0.00	0.19	0.00	0.00	0.00	0.00
unknown_12	1.18	0.00	0.00	0.05	0.00	0.00
unknown_120	0.00	0.00	0.00	0.00	0.02	0.00
unknown_121	0.00	0.00	0.00	0.00	10.68	0.00
unknown_122	0.01	0.00	0.00	0.02	0.00	0.03

(continued on next page)

Table 6 (continued)

COI_OTU	CUM	HI	LU	ÖR	RM	VE
unknown_123	0.00	0.00	4.55	0.00	0.00	0.00
unknown_124	0.05	0.00	0.00	0.00	0.00	0.00
unknown_125	0.00	0.03	0.00	0.00	0.00	0.00
unknown_127	0.01	0.00	0.00	0.03	0.00	0.00
unknown_128	0.00	0.00	0.00	0.02	0.00	0.00
unknown_129	0.01	0.00	0.01	0.00	0.00	0.00
unknown_13	2.76	0.00	0.00	0.06	0.00	0.00
unknown_131	0.00	0.00	0.00	0.00	0.19	1.11
unknown_132	0.00	0.01	0.08	0.00	0.35	0.00
unknown_133	0.00	0.00	0.24	0.00	0.11	0.00
unknown_134	14.74	0.00	0.91	0.74	0.00	0.00
unknown_15	0.02	0.00	0.00	52.83	0.08	67.54
unknown_16	0.00	0.00	0.00	0.00	0.03	0.03
unknown_17	0.00	0.00	0.00	0.01	0.00	0.00
unknown_18	0.00	0.62	0.00	0.00	0.02	0.00
unknown_2	0.00	0.00	0.00	0.00	0.17	0.00
unknown_21	0.00	0.07	0.00	0.00	0.00	0.00
unknown_22	0.00	0.00	0.00	0.00	0.20	0.00
unknown_23	0.00	0.00	0.00	0.04	0.01	0.09
unknown_24	0.13	0.01	0.00	0.00	0.01	0.00
unknown_25	0.00	0.00	0.00	0.02	0.03	0.00
unknown_26	0.00	0.00	0.03	0.00	0.00	0.00
unknown_27	2.43	0.00	0.00	0.04	0.00	0.00
unknown_28	36.22	0.31	0.00	0.31	0.04	0.00
unknown_3	10.11	0.00	0.00	0.22	0.00	0.00
unknown_30	1.25	0.96	0.00	0.06	0.01	0.00
unknown_31	0.00	0.00	0.00	0.15	0.00	0.00
unknown_32	0.13	0.00	0.00	0.00	0.00	0.00
unknown_34	0.00	0.11	0.00	0.00	0.02	0.00
unknown_35	0.00	0.00	0.00	0.00	0.01	1.08
unknown_36	0.00	0.00	0.02	0.00	0.00	0.00
unknown_37	1.04	0.00	0.00	0.08	0.00	0.00
unknown_38	0.00	0.39	0.00	0.00	0.01	0.00
unknown_39	0.00	0.00	0.10	0.00	0.00	0.00
unknown_4	10.11	0.00	0.00	0.24	0.00	0.00
unknown_41	0.00	0.39	0.00	0.00	0.01	0.00
unknown_43	0.00	1.10	0.00	0.00	0.02	0.00
unknown_44	0.43	0.00	0.00	0.00	0.00	0.00
unknown_45	0.30	1.64	0.00	0.00	0.00	0.00
unknown_46	0.00	0.00	0.02	0.00	0.00	0.00
unknown_47	0.28	0.00	0.00	0.00	0.00	0.00
unknown_48	0.15	0.00	20.89	0.00	0.00	0.00
unknown_49	0.00	0.00	0.00	0.24	0.00	0.00
unknown_50	0.02	3.97	4.67	0.00	5.68	0.00
unknown_51	0.00	0.00	0.00	0.08	0.00	0.00
unknown_52	0.00	0.00	0.00	0.00	0.02	0.00
unknown_53	0.00	0.43	0.00	0.00	0.02	0.00
unknown_55	0.00	0.00	16.83	0.00	0.01	0.03
unknown_56	0.00	0.00	0.00	0.00	0.01	0.00
unknown_57	0.00	0.05	0.00	0.00	0.00	0.00
unknown_59	2.63	5.54	0.00	0.11	0.72	0.00
unknown_6	3.85	3.25	0.00	0.13	0.00	0.00
unknown_60	0.00	0.00	0.00	0.00	0.48	0.00
unknown_61	0.00	1.18	0.00	0.00	0.03	0.00
unknown_62	0.00	11.55	0.00	0.00	0.26	0.00
unknown_63	0.00	0.00	0.10	0.00	0.00	0.00
unknown_64	0.00	5.57	0.00	0.00	0.09	0.00
unknown_66	0.00	0.93	0.00	0.00	0.03	0.00
unknown_68	0.32	0.00	0.00	0.00	0.00	0.00
unknown_70	0.00	1.29	0.00	0.00	0.02	0.00

(continued on next page)

Table 6 (continued)

COI_OTU	CUM	HI	LU	ÖR	RM	VE
unknown_71	0.82	0.01	0.00	0.00	0.00	0.00
unknown_72	0.00	0.01	16.30	0.00	0.01	0.00
unknown_75	4.02	0.00	0.00	0.13	0.00	0.00
unknown_76	0.00	0.11	0.00	0.00	0.00	0.00
unknown_77	0.07	0.00	0.00	0.00	0.00	0.00
unknown_79	0.24	0.00	0.00	0.01	0.00	0.00
unknown_8	0.00	0.00	0.00	0.00	0.01	0.00
unknown_81	0.00	0.00	0.00	0.02	0.00	0.00
unknown_83	0.00	0.00	0.00	0.07	0.00	0.00
unknown_84	0.00	0.00	0.00	8.28	0.00	0.00
unknown_85	0.02	0.37	0.00	0.00	0.00	0.00
unknown_86	0.12	0.00	21.31	0.75	25.03	0.00
unknown_87	0.00	0.00	0.00	0.00	0.00	0.00
unknown_89	0.12	0.00	0.00	0.00	21.52	0.00
unknown_9	0.00	0.02	8.29	0.00	0.64	0.00
unknown_90	0.00	0.00	0.00	0.00	0.74	0.00
unknown_91	0.00	0.17	0.00	0.00	1.21	0.00
unknown_92	0.00	0.00	0.00	0.00	0.13	0.00
unknown_94	0.00	0.00	0.07	0.00	0.00	0.00
unknown_95	0.08	0.06	0.00	0.00	0.00	0.00
unknown_96	0.56	0.00	0.00	0.01	0.00	0.00
unknown_97	0.00	0.06	0.00	0.00	0.10	0.00
unknown_98	0.03	25.79	0.01	0.01	25.00	0.00
unknown_99	3.16	2.96	0.00	0.12	0.29	0.00

terial was homogenized, and 1 kg was transferred to glass containers. For each pooled sample, chemical analyses were conducted for 33 substances and according to the following methods: arsenic (As; ISO 17,294–2-E29:2005–02), 7 metals (Cd, Cu, Pb, Cr, Ni, Zn; ISO 17,294–2-E29:2005–02 and Hg; ISO 16,772:2005–06), 16 polycyclic aromatic hydrocarbons (PAHs according to the US EPA; ISO 18,287), 7 polychlorinated biphenols (PCB 28, 52, 101, 118, 138, 153, 180; EN 15,308), *p,p'*-DDD and *p,p'*-DDE (both ISO 10,382); Table 1).

2.1. Morphological data

Nematodes were assigned to the lowest taxonomic level as described in ref [1]. All specimens were measured and for each species the biomass contribution was calculated following Andrassyi [6], differentiating between male, female and juvenile stages 1 and 4, as also described in ref [1]. A rarefaction curve was calculated with R, using the package “iNEXT” [7] and the functions “iNEXT” and “ggiNEXT”. It was furthermore checked, whether a NCBI sequence was available for the morphological identified species, by using the species as search term and verifying if a 28S rDNA, 18S rDNA or COI sequence was found (Table 8).

2.2. Molecular data

Molecular data was analysed with mothur [8], following the standard protocol with alterations for the BLAST identification [9]. For the 18S rDNA and 28S rDNA gene fragment a cluster threshold of 99% was chosen, while for the COI gene 97% were chosen, due to the accelerated mutation rate. The distribution of reads in the process of bioinformatics was summed up after every filtering step, including merging of paired-end reads, filtering of long/short and homopolymer reads, and filtering of reads not fitting the alignment. The final OTU inventory for the three markers is listed (Table 4, 5, 6), based on BLAST with a cut-off of 95% identity. The results of an alternative approach, using no clustering, is given in Table 3. Data was generated with DADA2

Table 7
Distribution of reads within the bioinformatic pipeline using mothur. Given is the number of reads at each current step of the analysis for each sample sequenced for the three genetic markers (1274/706 = 28S rDNA, mlCOLint/dgHCO=COI, 3Ndf/C_1132f = 18S rDNA).

1274/706	total	Cum1-N	Cum4-N	Cum8-N	Cum9-N	Hi1-N	Hi2-N	Hi3-N	Hi4-N	Hi5-N
Reads	939,932	33,297	40,542	41,783	39,667	20,260	20,468	32,322	43,424	40,865
After Merging	664,456	23,157	27,574	29,279	25,755	11,774	13,932	23,267	30,755	27,636
After filtering of long or short reads/homopolymers	664,456	23,157	27,574	29,279	25,755	11,774	13,932	23,267	30,755	27,636
After filtering of not fitting reads	598,184	21,414	26,353	27,435	17,702	11,076	10,800	17,782	24,452	25,978
After uchime	505,353	15,849	20,226	20,712	13,709	8860	6768	12,015	19,517	21,399
After removal of rares	461,690	13,595	18,177	18,249	11,596	8073	5491	10,223	17,474	19,660
1274/706	LU1-N	LU2-N	OE1-N	OE10-N	OE2-N	RM10-N	RM2-N	RM3-N	RM4-N	VE1-N
Reads	83,965	60,945	67,117	60,600	83,511	67,836	53,470	39,092	40,649	70,119
After Merging	60,262	43,294	46,395	44,289	63,039	47,965	37,983	28,172	28,203	51,725
After filtering of long or short reads/homopolymers	60,262	43,294	46,395	44,289	63,039	47,965	37,983	28,172	28,203	51,725
After filtering of not fitting reads	59,015	42,881	45,941	43,803	62,745	44,758	28,141	13,683	23,067	51,158
After uchime	52,309	37,938	42,381	37,418	57,892	39,016	25,006	9457	17,141	47,740
After removal of rares	48,053	35,766	40,195	33,875	54,537	36,025	23,152	7870	15,095	44,584
mlCOLintF/dgHCO	total	Cum1-N	Cum4-N	Cum8-N	Cum9-N	Hi1-N	Hi2-N	Hi3-N	Hi4-N	Hi5-N
Reads	219,693	4732	6793	4174	19,136	2675	13,087	11,210	4270	3889
After Merging	156,481	2721	2879	1374	13,759	78	5548	7199	2033	1397
After filtering of long or short reads/homopolymers	156,481	2721	2879	1374	13,759	78	5548	7199	2033	1397
After filtering of not fitting reads	156,481	2721	2879	1374	13,759	78	5548	7199	2033	1397
After uchime	155,930	2714	2879	1369	13,749	78	5542	7192	2033	1397
After removal of rares	155,473	2691	2829	1350	13,706	73	5522	7178	2012	1369

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Table 7 (continued)

mlCOLintF/dgHCO	LU1-N	LU2-N	OE1-N	OE10-N	OE2-N	RM10-N	RM2-N	RM3-N	RM4-N	VE1-N
Reads	41,914	6133	16,755	9593	8252	11,629	30,348	8156	11,088	5859
After Merging	38,149	4024	13,830	7100	5804	9172	25,265	4899	7993	3257
After filtering of long or short reads/homopolymers	38,149	4024	13,830	7100	5804	9172	25,265	4899	7993	3257
After filtering of not fitting reads	38,149	4024	13,830	7100	5804	9172	25,265	4899	7993	3257
After uchime	37,690	4010	13,827	7094	5804	9158	25,262	4899	7976	3257
After removal of rares	37,657	4007	13,801	7074	5766	9131	25,233	4881	7958	3235
3Ndf/C_1132f	total	Cum1_N	Cum4_N	Cum8_N	Cum9_N	Hi1_N	Hi2_N	Hi3_N	Hi4_N	Hi5_N
Reads	526,519	22,607	25,268	24,394	33,179	22,067	22,673	23,612	23,519	24,289
After Merging	112,014	5666	4285	6265	4356	5879	6479	7263	6142	3330
After filtering of long or short reads/homopolymers	112,014	5666	4285	6265	4356	5879	6479	7263	6142	3330
After filtering of not fitting reads	110,098	5587	4214	6196	4224	5799	6372	7187	6066	3269
After uchime	105,997	5312	3999	5818	3969	5653	5957	6737	5889	3058
After removal of rares	56,835	2906	1913	3233	1955	3177	3185	3960	3236	1403
3Ndf/C_1132f	LU1_N	LU2_N	OE10_N	OE1_N	OE2_N	RM10_N	RM2_N	RM3_N	RM4_N	VE1_N
Reads	39,345	29,816	31,399	25,150	36,830	31,745	29,051	25,816	31,383	24,376
After Merging	9663	8764	6809	3420	6902	8243	2453	3408	8666	4021
After filtering of long or short reads/homopolymers	9663	8764	6809	3420	6902	8243	2453	3408	8666	4021
After filtering of not fitting reads	9525	8680	6669	3351	6731	8142	2383	3266	8554	3883
After uchime	9397	8575	6463	3285	6595	7984	2216	3084	8182	3824
After removal of rares	5297	5419	2903	1564	3226	4446	888	1250	4898	1976

Table 8

Inspection of NCBI reference database for the Nematode SPEcies At Risk (NemaSPEAR[%]) as defined by Höss et al. [3] at the species and at the genus level. An "x" indicates whether the species or genera is found at NCBI for the 18S rDNA or 28 rDNA gene, while an "n" indicates that no reference sequence was found. The number of deposited sequences is given, the numbers in parentheses indicate reference sequences that were generated in our lab but are not yet published.

NemaSPEAR	18S	n	28S	n	NemaSPEAR _{genus}	18S	n	28S	n
<i>Achromadora ruricola</i>	x	1	n		<i>Achromadora</i>	x	17	n	
<i>Achromadora terricola</i>	x	1	n		<i>Aglenchus</i>	x	30	x	6
<i>Aglenchus agricola</i>	x	25	x	2	<i>Alaimus</i>	x	18	x	3
<i>Alaimus meylli</i>	n		n		<i>Amphidelus</i>	x	1	n	
<i>Alaimus parvus</i>	x	2	n		<i>Aphanolaimus</i>	x	3	x	5
<i>Alaimus primitivus</i>	n		n		<i>Aphelenchus</i>	x	>50	x	>50
<i>Amphidelus cf elegans</i>	n		n		<i>Aporcelaimellus</i>	x	>50	x	38
<i>Aphanolaimus aquaticus</i>	x	3	x	3	<i>Bastiania</i>	x	4	n	
<i>Aporcelaimellus obtusicaudatus</i>	x	24	x	17	<i>Cephalenchus</i>	x	>50	x	>50
<i>Coslenchus costatus</i>	x	6	x	3	<i>Coslenchus</i>	x	30	x	20
<i>Cuticularia oxyerca</i>	n		n		<i>Crassolabium</i>	n		x	3
<i>Cylindrolaimus communis</i>	x	1	n		<i>Criconema</i>	n	51	x	15
<i>Epidorylaimus agilis</i>	n		n		<i>Cylindrolaimus</i>	x	6	x	12
<i>Epitobrilus medius</i>	x	1	x	17	<i>Diplogasteritus</i>	x	6	x	12
<i>Epitobrilus steineri</i>	n		n		<i>Epidorylaimus</i>	x	9	x	3
<i>Ethmolaimus pratensis</i>	x	3	x	7(+7)	<i>Ethmolaimus</i>	x	3	x	7
<i>Eucephalobus oxyuroides</i>	x	12	x	5	<i>Eudorylaimus</i>	x	>50	x	18
<i>Eudorylaimus acuticauda</i>	n		n		<i>Factor</i>	x	13	x	25
<i>Eudorylaimus carteri</i>	x	5	x	4	<i>Hemicyclophora</i>	x	30	x	>50
<i>Eumonhystera andrassy</i>	n		n		<i>Hirschmanniella</i>	x	28	x	32
<i>Eumonhystera barbata</i>	n		n		<i>Hofmaenneria</i>	n		n	
<i>Eumonhystera longicaudatula</i>	x	1	n		<i>Ironus</i>	x	15	x	7
<i>Eumonhystera simplex</i>	x	1	n		<i>Monhystrella</i>	x	2	n	
<i>Eumonhystera vulgaris</i>	x	1	n		<i>Paramphidelus</i>	x	4	n	
<i>Helicotylenchus pseudorobustus</i>	x	61	x	38	<i>Paraphanolaimus</i>	x	4	n	
<i>Hemicyclophora typica</i>	x	4	x	2	<i>Paraplectonema</i>	x	1	x	2
<i>Hirschmanniella gracilis</i>	n		n		<i>Plectus</i>	x	>50	x	>50
<i>Hofmaenneria brachystoma</i>	n		n		<i>Pratylenchus</i>	x	>50	x	>50
<i>Ironus ignavus</i>	n		n		<i>Prismatolaimus</i>	x	>50	x	8
<i>Ironus longicaudatus</i>	x	2	x	2	<i>Prodesmodora</i>	x	7	x	3
<i>Ironus tenuicaudatus</i>	n		x	3	<i>Prodorylaimus</i>	x	6	x	4
<i>Mesodoryaimus conurus</i>	n		n		<i>Punctodora</i>	x	5	n	
<i>Mesodoryaimus subtiliformis</i>	n		n		<i>Rhabditis</i>	x	>50	x	>50
<i>Monhystera lemani</i>	n		n		<i>Rhabdolaimus</i>	x	>50	n	
<i>Monhystrella paramacrura</i>	n		n		<i>Semitobrilus</i>	x	4	x	1
<i>Mononchus truncatus</i>	x	5	x	3	<i>Theristus</i>	x	33 (+>50)	x	3 (+>50)
<i>Neotobrilus longus</i>	x	3	x	16	<i>Thornia</i>	x	1	n	
<i>Panagrolaimus cf thienemanni</i>	n		n		<i>Trischistoma</i>	x	19	x	8
<i>Paramphidelus dolichurus</i>	n		n						
<i>Paraphanolaimus anisitsi</i>	n		n						
<i>Paraplectonema pedunculatum</i>	x	1	n						
<i>Plectus aquatilis</i>	x	7	x	5					
<i>Plectus cirratus</i>	x	1	x	2					
<i>Plectus opisthocirculus</i>	x	1	x	1					
<i>Plectus rhizophilus</i>	x	2	x						
<i>Prismatolaimus dolichurus</i>	x	3	x	7					
<i>Prismatolaimus intermedius</i>	x	3	n						
<i>Prismatolaimus tenuicaudatus</i>	n		n						
<i>Prodesmodora circulata</i>	x	4	x	3					
<i>Punctodora dudichi</i>	n		n						
<i>Punctodora ratzeburgensis</i>	x	5	n						
<i>Rhabditis gracilicauda</i>	n		n						
<i>Rhabdolaimus terrestris</i>	x	1	n						
<i>Semitobrilus pellucidus</i>	x	2	x	16					
<i>Theristus agilis</i>	x	3	(x)	(>150)					
<i>Theristus vesentinae</i>	n		n						
<i>Thornia propinqua</i>	n		n						
<i>Trischistoma monhystera</i>	n		n						

Table 9

Species inventory for the seven locations (VE, ÖR, RM, HI, LU, CUM, FB). Given are the species identified by morphological inspection, together with the biomass for a male (m biomass) and female (fm biomass) based on measured specimen in the dataset. It is indicated if a NCBI sequence for the 28S rDNA or 18S rDNA gene is found, an "n" showing that this species is missing.

Species	NCBI	fm biomass	m biomass	VE	ÖR	RM	HI	LU	CUM	FB
<i>Achromadora cf. terricola</i>	18S	0.93		0.00	0.60	0.00	0.00	0.00	0.59	1.24
<i>Achromadora longicauda</i>	n	0.05		0.19	0.00	0.00	0.00	0.00	0.00	0.00
<i>Achromadora ruricola</i>	18S	0.19	0.14	4.27	0.00	0.00	0.00	1.02	0.00	0.00
<i>Achromadora sp.</i>	18S	0.13		0.00	0.00	0.00	0.99	0.20	0.00	0.00
<i>Achromadora micoletzky</i>	n	0.17	0.08	11.06	0.00	0.60	4.96	0.41	14.31	0.00
<i>Aglenchus sp.</i>	18S,28S	0.11	0.10	0.00	0.00	0.40	0.00	0.00	0.00	0.00
<i>Alaimus parvus</i>	18S	0.13		0.00	0.00	0.00	0.00	0.00	0.40	0.00
<i>Alaimus primitivus</i>	n	0.34	0.12	0.59	0.00	0.00	0.40	0.00	0.79	0.00
<i>Amphidelus dudichi</i>	n	1.34		0.19	0.00	0.00	0.00	0.00	0.00	0.00
<i>Amphidelus elegans</i>	n	0.45		0.19	0.00	0.00	0.00	0.00	0.40	0.00
<i>Anaplectus grandepapillatus</i>	18S	2.34	1.72	0.00	0.00	0.00	0.00	0.00	0.00	18.56
<i>Anaplectus granulatus</i>	28S	0.92	0.69	0.00	0.00	0.00	2.38	0.00	1.00	0.00
<i>Aphanolaimus aquaticus</i>	18S,28S	0.82	0.43	0.00	0.00	0.80	0.00	0.00	0.00	0.00
<i>Aphelenchoides bicaudatus</i>	18S	0.07	0.06	0.19	0.00	0.00	0.20	0.00	0.00	0.00
<i>Aphelenchoides parietinus</i>	28S	0.23	0.20	0.00	0.00	0.00	0.99	0.00	0.79	0.00
<i>Aphelenchus cf. avenae</i>	18S,28S	0.21	0.25	0.00	0.00	0.00	0.20	0.00	0.40	0.21
<i>Aporcelaimellus obtusicaudatus</i>	18S,28S	9.99		0.97	0.00	0.00	3.37	0.00	2.98	0.00
<i>Bastiania gracilis</i>	18S	0.14	0.11	0.19	0.00	0.00	0.00	0.00	0.00	0.00
<i>Bitylenchus dubius</i>	18S	0.29	0.14	0.00	0.00	0.20	9.91	0.00	3.18	0.00
<i>Bursilla monhystera</i>	n	0.63	0.13	0.00	0.00	0.20	0.40	0.00	1.20	0.00
<i>Cephalenchus hexalineatus</i>	18S,28S	0.12	0.10	0.00	0.00	0.00	0.20	0.00	0.00	0.00
<i>Cephalobus persegnis</i>	18S,28S	0.43	0.23	0.19	0.00	0.60	20.22	0.61	11.52	0.00
<i>Chromadorina bioculata</i>	18S,28S	0.27	0.22	0.00	0.00	2.01	0.20	0.61	0.00	0.00
<i>Chromadorita leuckarti</i>	18S,28S	1.48	0.80	2.14	0.40	0.00	0.20	2.03	0.00	10.10
<i>Chromadorina viridis</i>	28S	0.79	0.54	0.00	0.00	1.81	0.00	0.00	0.00	0.00
<i>Chrysonema holsaticum</i>	n	1.90	0.55	0.00	0.00	0.00	0.00	0.00	0.20	0.00
<i>Clarkus papillatus</i>	18S,28S	1.46	1.95	0.00	0.00	0.00	0.40	0.00	0.00	0.00
<i>Coslenchus costatus</i>	18S,28S	0.11	0.07	0.00	0.00	0.00	2.18	0.61	1.59	0.00
<i>Crassolabium ettersbergensis</i>	n	0.30		0.00	0.00	0.00	0.00	0.00	0.59	0.00
<i>Crocodylaimus flavomaculatus</i>	n	1.27	1.23	0.00	0.00	0.00	0.00	0.00	0.40	0.00
<i>Cryptonchus tristis</i>	18S	1.57	1.71	0.38	0.00	0.00	0.00	0.00	0.00	0.00
<i>Cylindrolaimus melancholicus</i>	n	1.36	0.99	0.00	0.00	0.00	0.00	0.00	0.00	0.82
<i>Daptonema dubium</i>	n	5.06	2.20	0.00	0.00	21.90	3.37	0.00	0.00	0.00
<i>Diplogaster rivalis</i>	18S,28S	1.92	0.65	0.00	0.00	0.00	0.40	1.02	0.00	0.00
<i>Ditylenchus sp.</i>	18S,28S	0.92	0.46	0.00	0.00	0.00	0.99	0.00	0.00	0.00
<i>Dorylaimidae sp.</i>	18S,28S	3.41	2.50	0.00	0.00	0.20	0.00	0.20	0.00	0.00
<i>Dorylaimoides limnophilus</i>	18S,28S	1.22		0.00	0.00	0.20	0.20	0.00	0.00	0.00
<i>Dorylaimus stagnalis</i>	18S,28S	47.64	42.07	0.00	0.00	0.00	0.40	0.61	0.40	0.00
<i>Epidorylaimus agilis</i>	n	2.35		0.38	0.00	0.20	0.00	0.00	0.00	0.21
<i>Epitobrilus stefanskii</i>	18S	3.27	2.51	0.19	0.40	0.20	0.00	1.63	0.00	0.00
<i>Ethmolaimus pratensis</i>	18S,28S	0.68	0.44	0.19	9.02	0.00	0.00	0.00	0.00	1.03
<i>Eucephalobus oxyuroides</i>	18S,28S	0.26	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Eudorylaimus acuticauda</i>	n	3.94	2.69	0.00	0.00	0.00	0.99	0.00	0.79	0.00
<i>Eudorylaimus carteri</i>	18S,28S	4.65	3.27	0.00	0.00	0.00	0.00	0.00	0.00	0.21
<i>Eudorylaimus centrocercus</i>	28S	3.71	1.72	0.00	0.00	0.00	0.00	0.00	0.40	0.00
<i>Eumonhystera barbata</i>	n	0.12		0.19	0.00	0.20	0.00	0.00	0.00	0.21
<i>Eumonhystera dispar</i>	n	0.43		1.16	0.20	0.20	0.40	1.22	0.00	0.82
<i>Eumonhystera filiformis</i>	18S,28S	0.27	0.22	5.43	6.01	6.22	6.74	26.88	0.40	0.00
<i>Eumonhystera longicaudatula</i>	18S	0.22		1.94	17.43	3.41	0.99	0.00	0.00	1.86
<i>Eumonhystera pseudobulbosa</i>	n	0.04		1.75	0.80	1.41	0.59	2.03	0.40	0.21
<i>Eumonhystera simplex</i>	18S	0.03		2.71	0.00	1.00	1.19	0.00	3.38	0.00
<i>Eumonhystera vulgaris</i>	18S	0.19		6.41	19.24	0.00	0.59	1.42	0.00	2.27
<i>Eumonhystera sp.</i>	18S	0.14		18.62	0.00	0.00	0.00	0.00	0.00	0.00
<i>Euteratocephalus palustris</i>	18S	0.24		0.00	0.00	0.00	0.20	0.00	0.20	0.00
<i>Eutobrilus grandepapillatus</i>	n	10.80	5.89	0.00	0.00	0.00	0.20	0.00	0.00	0.00
<i>Fictor fictor</i>	n	1.58	0.59	0.19	0.00	0.00	0.20	0.00	0.00	0.00
<i>Filenchus sp.</i>	18S,28S	0.09		0.00	0.00	0.00	0.00	0.00	0.00	0.21

(continued on next page)

Table 9 (continued)

Species	NCBI	fm biomass	m biomass	VE	ÖR	RM	HI	LU	CUM	FB
<i>Filenchus vulgaris</i>	18S,28S	0.10	0.09	5.43	0.00	4.62	8.33	0.61	16.69	0.00
<i>Helicotylenchus pseudorobustus</i>	18S,28S	0.35		0.38	0.00	0.00	0.20	0.00	1.00	0.00
<i>Hemicycliophora typica</i>	18S,28S	0.52		4.27	0.00	0.00	0.40	0.41	0.20	0.00
<i>Heterocephalobus elongatus</i>	18S	0.41	0.32	0.00	0.00	0.00	0.59	0.00	0.20	0.00
<i>Hirschmanniella gracilis</i>	n	1.36	1.02	0.19	0.00	0.00	0.00	0.00	0.00	0.00
<i>Hofmaenneria niddensis</i>	n	0.58	0.41	0.00	0.00	0.00	0.00	0.00	0.00	1.65
<i>Ironus longicaudatus</i>	18S,28S	1.00		0.19	0.00	0.00	0.00	0.00	0.00	0.00
<i>Laimaphelenchus penardi</i>	18S	0.16	0.13	0.19	0.00	0.00	0.00	0.00	0.00	0.00
<i>Malenchus bryophilus</i>	18S,28S	0.06	0.03	0.00	0.00	0.00	0.40	0.41	0.20	0.00
Mermithidae				0.19	0.00	0.40	0.00	0.00	0.00	0.41
<i>Mesodorylaimus bastiani</i>	18S,28S	1.45	1.04	0.19	0.00	0.00	0.00	0.00	1.79	0.00
<i>Mesodorylaimus paetzoldi</i>	n	2.81		0.00	0.00	0.00	0.00	0.20	0.00	0.00
<i>Mesodorylaimus</i> sp.	18S,28S	1.94		0.00	0.20	0.00	0.00	0.20	0.00	0.00
<i>Monhystera paludicola</i>	18S,28S	0.86	0.56	5.04	2.00	1.81	0.00	2.44	0.00	0.41
<i>Monhystera stagnalis</i>	18S,28S	1.24	0.68	0.00	0.00	0.80	0.00	0.20	0.00	0.21
<i>Monhystera</i> sp.	18S,28S	0.43		0.00	0.00	3.01	0.00	0.00	0.00	0.00
<i>Monhystrella macrura</i>	n	0.16	0.08	0.00	0.00	0.20	0.00	0.00	0.00	0.00
<i>Monhystrella paramacrura</i>	n	0.04	0.06	0.00	0.00	5.82	0.20	0.20	0.79	0.00
<i>Mononchus aquaticus</i>	18S,28S	4.21	4.33	0.19	0.40	0.00	0.20	0.20	0.00	0.00
<i>Mononchus truncatus</i>	18S,28S	4.94	3.21	0.00	0.00	0.20	0.40	0.00	0.00	0.00
<i>Mylonchulus brachyuris</i>	18S	1.38	1.06	0.19	0.00	0.00	1.19	0.00	0.00	0.00
<i>Mylonchulus sigmaturus</i>	18S	1.86	1.07	0.00	0.00	0.00	1.78	0.00	0.20	0.00
<i>Neotobrilus diversipapillatus</i>	n	5.33	1.54	0.00	0.00	0.00	0.20	0.00	0.00	0.00
<i>Panagrolaimus rigidus</i>	18S,28S	1.07	0.54	0.59	0.00	0.00	2.57	0.41	1.59	0.00
<i>Paractinolaimus macrolaimus</i>	18S,28S	10.82	7.87	0.00	0.00	0.00	0.00	0.41	0.00	0.00
<i>Paraplectonema pedunculatum</i>	18S	0.68		0.00	0.00	0.00	0.20	0.00	0.00	0.00
<i>Pellioiditis pellioides</i>	n	7.97	3.11	0.38	0.00	0.00	0.00	0.00	0.00	0.00
<i>Plectus aquatilis</i>	18S,28S	1.30		0.19	0.00	0.00	0.00	0.20	0.20	0.00
<i>Plectus opisthocirculus</i>	18S,28S	0.13		0.59	0.20	0.80	0.99	2.24	0.00	0.00
<i>Plectus parvus</i>	18S,28S	0.17		0.59	0.20	0.00	0.00	0.20	1.00	0.00
<i>Plectus rhizophilus</i>	18S	0.76		0.00	0.00	0.00	0.00	0.81	0.00	0.00
<i>Plectus tenuis</i>	18S	0.64		0.00	0.00	0.00	0.00	0.61	0.00	0.00
<i>Pratylenchus</i> sp.	18S,28S	0.09		0.00	0.00	0.00	0.40	0.00	0.00	0.00
<i>Prismatolaimus dolichurus</i>	18S,28S	0.47	0.33	0.00	0.00	0.00	0.20	0.00	0.40	0.00
<i>Prismatolaimus intermedius</i>	18S	0.10	0.14	1.55	0.20	0.80	6.15	0.00	9.34	0.21
<i>Prismatolaimus</i> sp.	18S	0.37		0.00	0.00	0.00	0.59	0.00	0.00	0.00
<i>Prodesmodora arctica</i>	n	0.07		0.59	0.00	0.00	0.00	0.00	0.00	0.00
<i>Prodesmodora circulata</i>	18S,28S	0.33		0.38	0.00	0.00	0.20	0.00	0.20	0.00
<i>Prodorylaimus fliiarium</i>	n	2.31	2.12	0.38	0.00	0.00	0.00	0.00	0.00	0.00
<i>Prodorylaimus</i> sp.	18S,28S	3.52		0.00	0.20	0.00	0.00	1.02	0.00	0.00
<i>Punctodora ratzeburgensis</i>	18S	0.53	0.34	0.00	0.00	19.29	0.20	0.00	0.00	0.00
Rhabditidae	18S,28S	1.00		0.97	0.00	0.00	0.00	0.00	1.39	0.00
<i>Rhabditidae</i> sp.	18S	0.42		0.00	0.00	0.00	1.98	0.61	0.20	0.00
<i>Rhabditis gracilicauda</i>	n	0.51	0.66	0.19	0.00	0.00	0.20	0.00	0.20	0.00
<i>Rhabdolaimus aquaticus</i>	18S	0.08		0.00	0.00	0.20	0.00	0.00	0.00	0.00
<i>Rhabdolaimus terrestris</i>	18S	0.11		2.33	0.00	0.00	0.00	0.00	0.00	0.00
<i>Semitobrilus pellucidus</i>	18S,28S	4.86	5.08	10.47	41.89	0.20	0.00	0.00	0.00	9.07
Species 1		0.51	0.42	0.19	0.00	0.00	0.00	0.00	0.00	0.00
Species 2 (Cephalobidae)		0.46	0.37	0.00	0.00	0.00	0.00	0.00	0.59	0.00
<i>Teratocephalus tenuis</i>	n	0.10	0.05	0.00	0.00	0.00	0.20	0.00	0.20	0.00
<i>Teratocephalus terrestris</i>	18S	0.08		0.19	0.00	0.00	0.20	0.00	1.20	0.00
<i>Theristus agilis</i>	18S	1.19	0.68	0.19	0.00	0.00	0.20	0.00	0.00	37.31
<i>Theristus vesentinae</i>	n	0.21	0.15	0.00	0.00	0.00	0.00	0.00	0.79	3.30
<i>Tobrilus gracilis</i>	18S,28S	6.32	4.19	0.38	0.20	19.09	0.00	47.04	0.00	0.00
<i>Trichodoros sparsus</i>	18S,28S	0.94	0.60	1.36	0.00	0.00	0.00	0.00	0.00	0.00
<i>Tripyla glomerans</i>	18S,28S	17.79	12.80	0.00	0.00	0.60	0.20	0.61	0.00	0.00
<i>Tripyla setifera</i>	28S	1.61	1.43	0.38	0.20	0.00	0.00	0.00	0.00	9.48
<i>Trischistoma monohystera</i>	n	1.19	0.74	0.97	0.00	0.00	0.20	0.00	0.40	0.00
Tylenchidae	18S,28S	0.43	0.33	0.00	0.00	0.00	0.00	0.00	0.59	0.00

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Table 9 (continued)

Species	NCBI	fm biomass	m biomass	VE	ÖR	RM	HI	LU	CUM	FB
<i>Tylencholaimellus affinis</i>	18S	1.80	1.33	0.19	0.00	0.00	0.00	0.00	0.00	0.00
<i>Tylencholaimus teres</i>	18S	0.48	0.44	0.19	0.00	0.00	0.00	0.00	2.58	0.00
<i>Tylenchus davanei</i>	n	0.69	0.54	0.00	0.20	0.40	6.35	0.41	9.93	0.00
<i>Tylenchus</i> sp. 1	18S,28S	0.69	0.51	0.19	0.00	0.00	0.00	0.00	0.20	0.00
<i>Tylenchus</i> sp. 2		0.26		0.38	0.00	0.00	0.40	0.00	0.00	0.00
<i>Tylenchus</i> sp. 3		0.19		0.00	0.00	0.00	0.00	0.00	1.39	0.00
<i>Wilsonema otophorum</i>	18S,28S	0.06	0.03	0.00	0.00	0.00	0.00	0.00	0.20	0.00

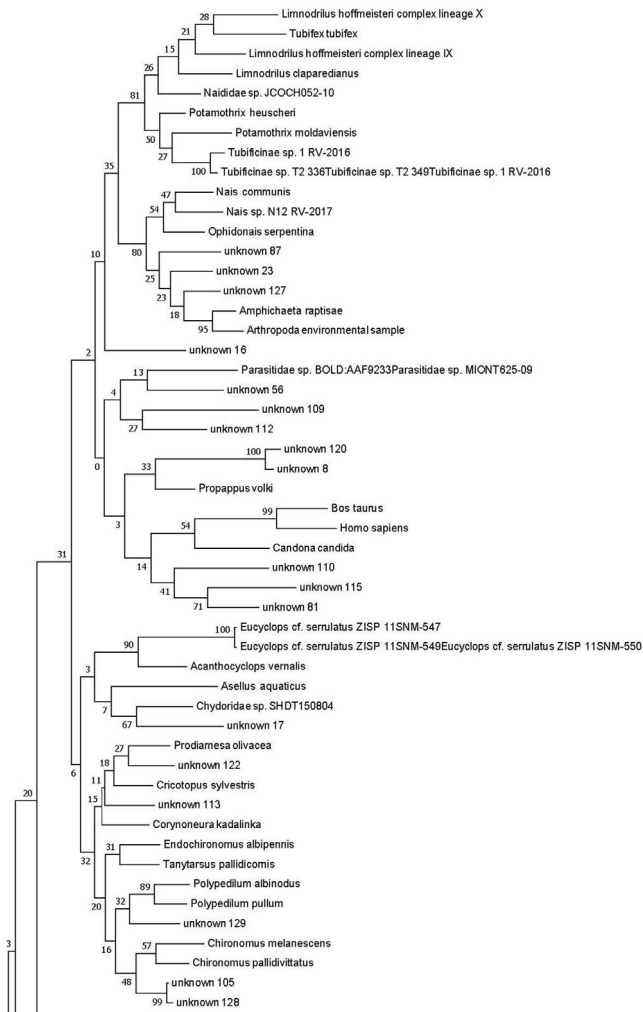


Fig. 4. Maximum likelihood tree based on the OTU inventory for the COI gene. The numbers at the branches represent the support values for the nodes.

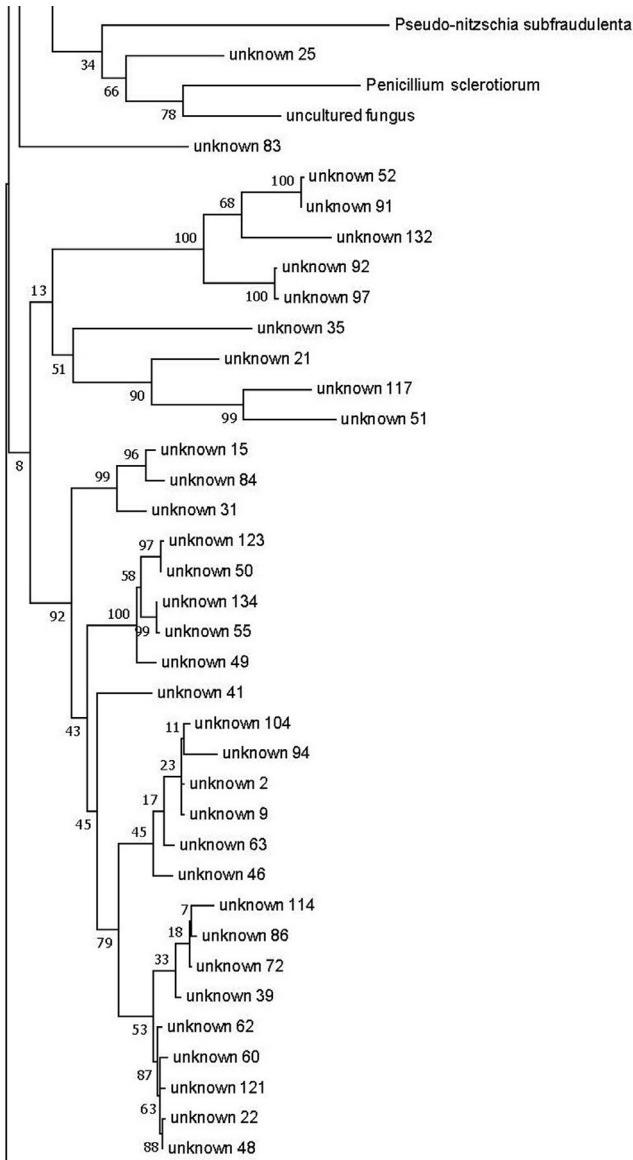


Fig. 4. Continued

[10], using default parameters with slight adaptations (length cutoff (280,250), maxEE = c (2,2), truncQ = 2). For the 18S gene fragment no length cut-off was chosen, as the overlap was very low due to the long amplicon length (around 540bp). The BLAST comparison was run with an identity cut-off of 97%. For the COI phylogenetic trees were built in MEGA7 [11] for the ASV and OUT inventory, using maximum likelihood with 500 bootstrap replicates and the Jukes-Cantor model.

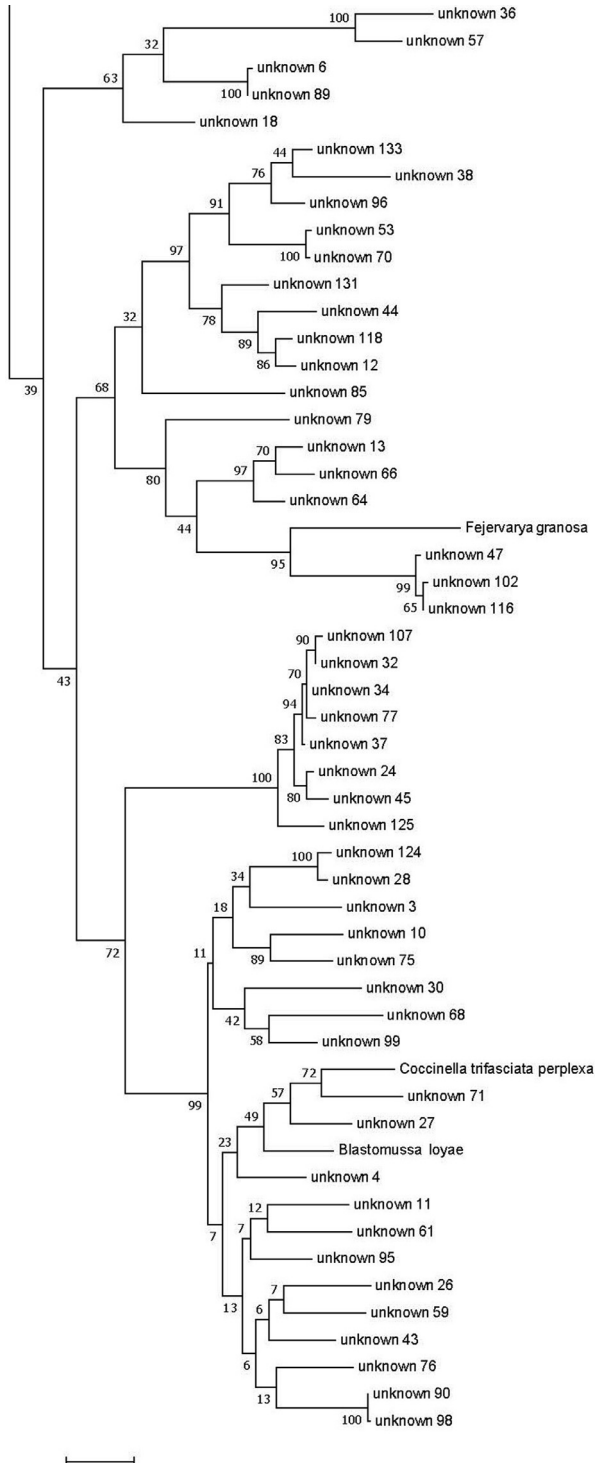


Fig. 4. Continued

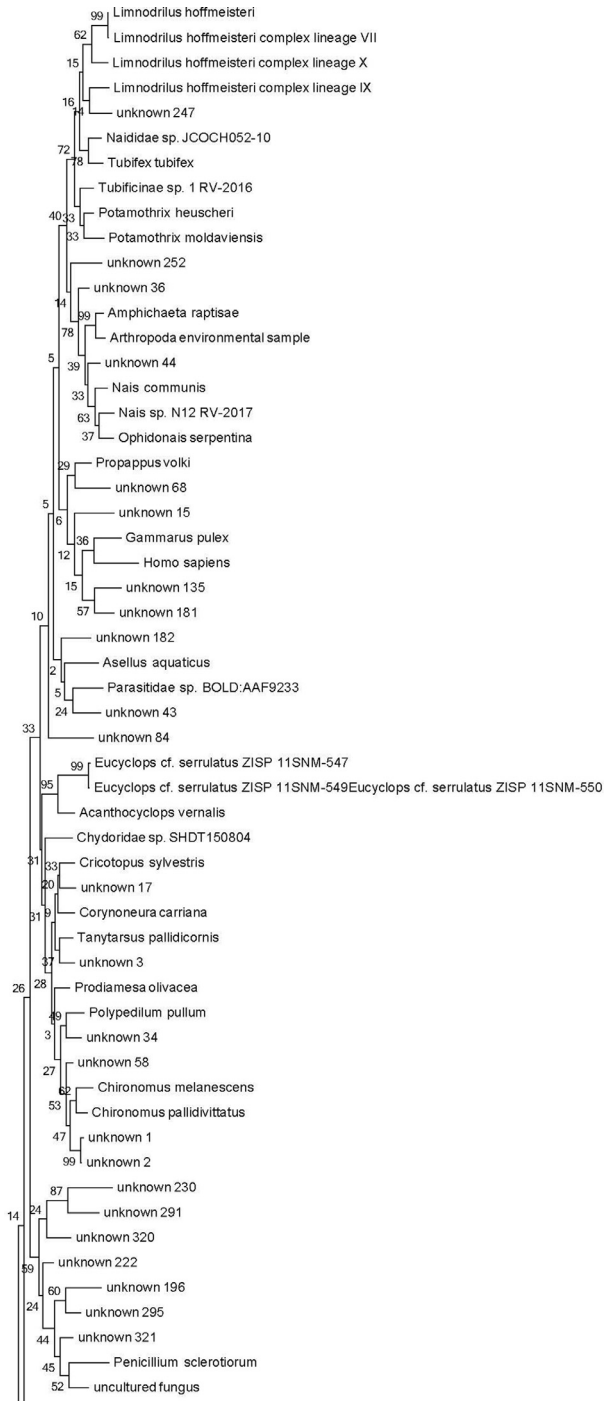


Fig. 5. Maximum likelihood tree based on the ASV inventory for the COI gene. The numbers at the branches represent the support values for the nodes.

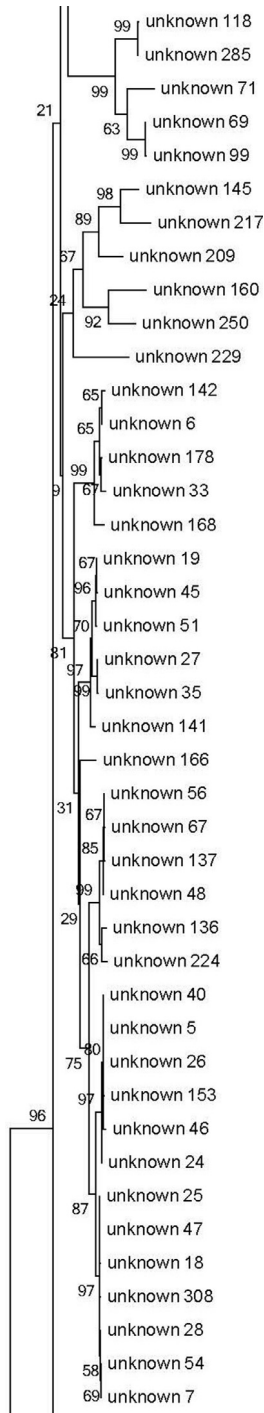


Fig. 5. Continued

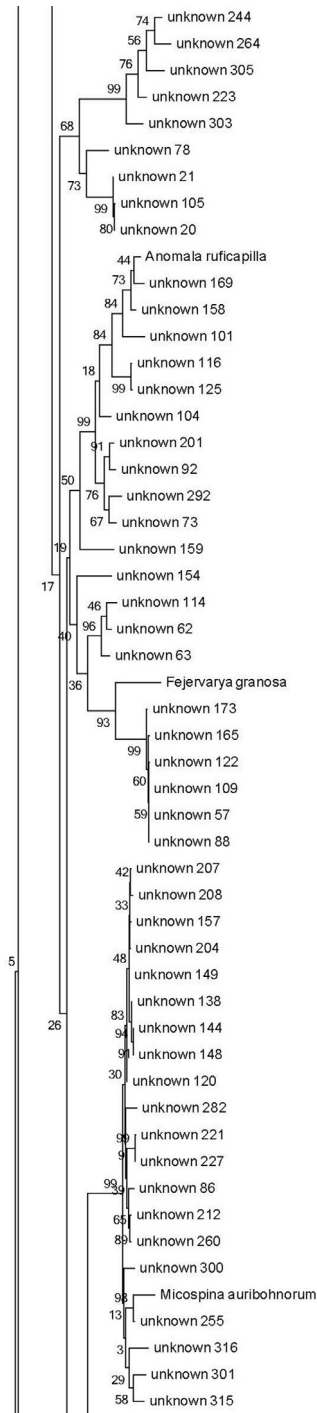


Fig. 5. Continued

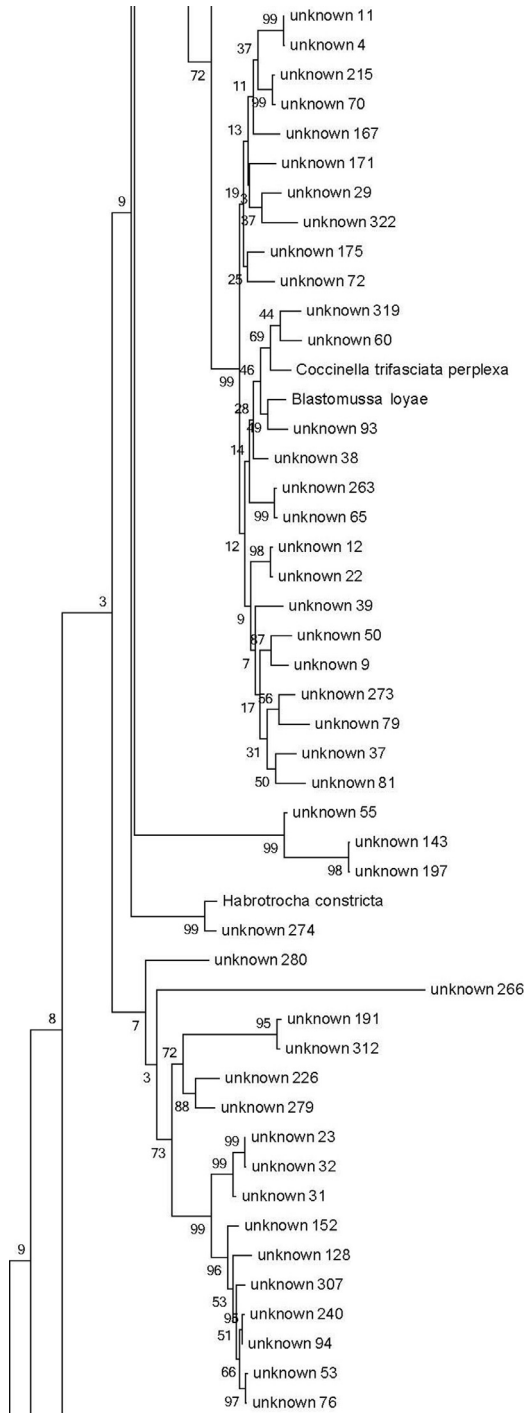


Fig. 5. Continued

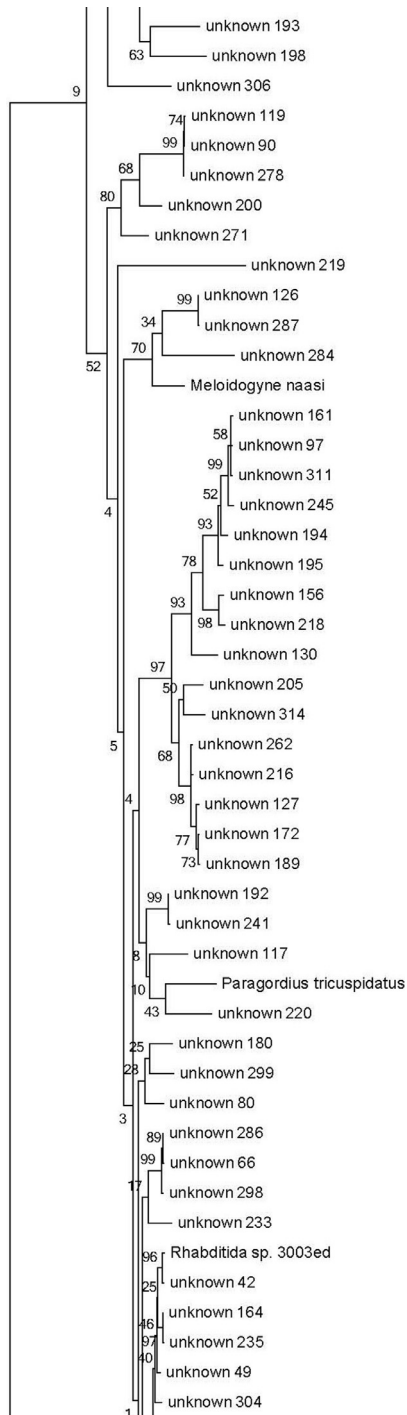


Fig. 5. Continued

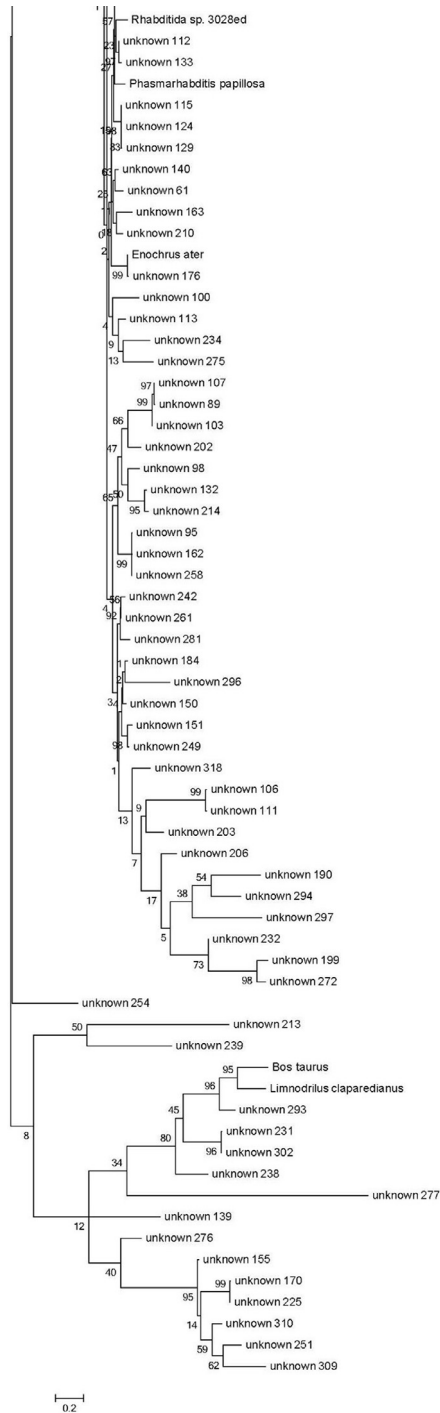


Fig. 5. Continued

2.3. Statistical data

NMDS plots were generated with PRIMER_v6 [12], using all replicates for the molecular and morphological data for the seven locations. The data was not transformed and Bray-Curtis similarity was applied. NMDS plot was generated with default parameters in PRIMER. Correlations between the NemaSPEAR[%] values for the morphological approach, the 28S rDNA gene fragment and the 18S rDNA gene fragment for the species and genus level against the logarithmized mean PEC-Q values were plotted using SigmaPlot11 (Fig. 3).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships which have, or could be perceived to have, influenced the work reported in this article.

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References

- [1] J. Schenk, S. Höss, M. Brinke, N. Kleinbölting, H. Brüchner-Hüttemann, W. Traunspurger, Nematodes as bioindicators of polluted sediments using metabarcoding and microscopic taxonomy, *Environ. Int.* 143 (2020) 105922, doi:10.1016/j.envint.2020.105922.
- [2] E. de Deckere, W. de Cooman, V. Leloup, P. Meire, C. Schmitt, P.C. von der Ohe, Development of sediment quality guidelines for freshwater ecosystems, *J. Soils Sediments* 11 (2011) 504–517, doi:10.1007/s11368-010-0328-x.
- [3] S. Höss, E. Claus, Von der Ohe, P. C. M. Brinke, H. Güde, P. Heininger, W. Traunspurger, Nematode species at risk—a metric to assess pollution in soft sediments of freshwaters, *Environ. Int.* 37 (2011) 940–949, doi:10.1016/j.scitotenv.2011.03.041.
- [4] H. Thiel, R.P. Higgins, *Introduction to the Study of Meiofauna*, Smithsonian Institution Press, Washington, 1988.
- [5] J. Schenk, S. Geisen, N. Kleinbölting, W. Traunspurger, Metabarcoding data allow for reliable biomass estimates in the most abundant animals on earth, *Metabarcoding Metagenomics* 3 (2019) e46704, doi:10.3897/mbmg.3.46704.
- [6] I. Andrassy, *Die Rauminhalts- und Gewichtsbestimmung der Fadenwürmer (Nematoden)*, *Acta Zool Hungarica* 2 (1956) 1–5.
- [7] T.C. Hsieh, K.H. Ma, A. Chao, iNEXT: an R package for rarefaction and extrapolation of species diversity (Hill numbers), *Methods Ecol. Evol.* 7 (2016) 1451–1456, doi:10.1111/2041-210X.12613.
- [8] P.D. Schloss, S.L. Westcott, T. Ryabin, J.R. Hall, M. Hartmann, E.B. Hollister, R.A. Lesniewski, B.B. Oakley, D.H. Parks, C.J. Robinson, Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities, *Appl. Environ. Microbiol.* 75 (2009) 7537–7541, doi:10.1128/AEM.01541-09.
- [9] J. Schenk, N. Kleinbölting, W. Traunspurger, Comparison of morphological, DNA barcoding, and metabarcoding characterizations of freshwater nematode communities, *Ecol. Evol.* (2020) 1–15, doi:10.1002/ece3.6104.
- [10] B.J. Callahan, P.J. McMurdie, M.J. Rosen, A.W. Han, A.J.A. Johnson, S.P. Holmes, DADA2: high-resolution sample inference from Illumina amplicon data, *Nat Methods* 13 (2016) 581–583, doi:10.1038/nmeth.3869.
- [11] K. Tamura, G. Stecher, D. Peterson, A. Filipski, S. Kumar, MEGA6: molecular Evolutionary Genetics Analysis Version 6.0, *Mol. Biol. Evol.* 30 (2013) 2725–2729.
- [12] K.R. Clarke, R.N. Gorley, *PRIMER v6 User Manual and Program*, PRIMER-E Ltd: Plymouth, UK, 2006.