Appendix A

EXTREMOPHILE NEMATODES IN AND AROUND MONO LAKE DEMONSTRATE ADAPTATION TO AN ARSENIC-RICH ENVIRONMENT

(This work was done in collaboration with Shih P.Y., Shinya R., Badroos J.M., Goetz E., and Sapir A.)

A.1 Abstract

Studying extremophile organisms have expanded our understanding of the limits and adaptability of life. Nevertheless, the dynamics of animal habitation of harsh environments and the mechanisms of resilience and plasticity underlying this habitation remain largely unknown. Here we describe the discovery of extremophile nematodes in and around Mono Lake, CA, a unique basic, arsenic-rich, and hypersaline environment. In contrast to the limited number of animal species previously reported to live in the lake, we have isolated at least eight species of nematodes, including five previously unidentified species. Finding live nematodes in the same niches of Mono Lake in two consecutive years show that the lake hosts a stable population of worms. Phylogenetic analyses show that the nematodes belong to diverse clades across the phylum Nematoda, supporting a model of multiple colonization events. Consistent with this model, different mouth morphologies of these nematodes suggest diverse feeding strategies including bacterial grazers and predatory nematodes. We were able to culture one species of Mono Lake worms, *Auanema tufa* n. sp., and found that it is resistant to arsenite (As(III)) and arsenate (As(V)) — the two primary arsenic species in the lake. Integration of niche environmental conditions with the prevalence of worms at each of these niches suggests that arsenic resistance preceded the adaptation to other environmental conditions in the lake. Our finding highlights the previously unappreciated complexity of the animal life in the unique ecosystem of Mono Lake and provides insights into the dynamics and type of adaptations of animals to extreme environments.

A.2 Introduction

Among the largest habitats on Earth are “extreme” environments where the physical and chemical conditions differ from the habitable zone of humans. These environments include, for example, the Deep Sea, sub-terrestrial niches, the high atmosphere, and specific terrestrial lakes. However, we know very little about the organisms that live in these habitats (extremophiles) and their strategies for adapting and thriving in such hostile environments, partly due to sampling challenges and limited access to these habitats. Moreover, the difficulties of growing and maintaining organisms from extreme habitats in the laboratory limit our understanding of the dynamics and the mechanisms underlying the adaptation of these organisms to their niches.

One phylum of organisms that seem to be particularly adapted to thrive in extreme environments are nematodes. These roundworms have been found in a variety of hostile environments, including deep subterranean niches (1, 2), extreme arid soil (3), frozen Antarctic water (4) and the Deep Sea (5, 6). Moreover, nematodes were found to dominate many of the habitats with environmental conditions so harsh as to almost not support animal life including the subterrane surface (7) and anoxic underwater sediments (8).

Nematodes have developed several protective strategies of modified life cycle to ensure the survival of the current or subsequent generations. For example, in response to unfavorable environmental conditions *Caenorhabditis elegans* enters an alternative developmental stage, the dauer, that allows its survival in harsh conditions (9, 10). Specific adaptive genetic programs facilitate the unique

physiology of the dauer state including the development of specialized morphology such as thickening of the cuticle, and an anaerobic metabolism. These adaptations result in an animal that is highly resistant to environmental insults and long-lived. The diverse lifestyle and feeding strategies of nematodes that range from free-living bacterial and fungal feeders, predator nematodes, to parasitic worms of plant and animal hosts often result in the cohabitation of worms in the same ecological niche. It is not clear, however, what specific adaptations enable nematodes to survive and thrive in extreme environments. Moreover, the sequences of events that underlie the habitation of nematodes in hostile environments remain largely unknown.

Mono Lake, a natural basin located in the Inyo National Forest of California, is an extreme environment that is high in pH, salt, and arsenic (11). It was formed as a closed basin since at least 50,000 years ago (12), but in 1941 some freshwater streams feeding the lake were diverted, making the drop of the lake level even more severe (13). The result of this level drop not only concentrated the salts (14, 15), but also facilitated arsenic to dissolve from sediments to its aqueous forms (16). Arsenic is a chemical element that is toxic to most organisms. At a biochemical level, inorganic arsenic in concentrations found in Mono Lake replace phosphate in several reactions and may react with critical thiols in proteins and inhibit their activity. Thus, arsenic has a negative pleiotropic effect on living organisms causing genotoxicity, altered DNA methylation and cell proliferation, oxidative stress, apoptosis, and mutagenesis (17). The level of arsenic in Mono Lake is approximately 0.2 mM, which is 1,500 times higher than the maximum limit

for drinking water (18). Consistent with the harshness of the environment, the number of living animals reported in the lake has been limited to two animal species, the alkali fly (*Ephydra hians*) and brine shrimp (*Artemia monica*) (19). The adaptation of these two species is polyphyletic, suggesting that an independent habitation of the lake took place in a process of strong purifying selection. Nevertheless, the sequence of events of this colonization process and the type of the specific adaptations that enable these animals to live in Mono Lake remain largely unknown.

Here we report eight species isolated in and around Mono Lake. These species were isolated from polyphyletic nematode clades, suggesting that Mono Lake has been inhabited by nematodes independently and multiple times. One of these species, *Auanema tufa* is culturable in laboratory conditions and exhibits resistance to arsenic, highlighting a probable hallmark of adaptation of animals to arsenic-rich environments.

A.3 Results

Nematodes were isolated from three sampling sites around Mono Lake

Mono Lake covers 13 miles east to west and 8 miles north to south, and the lake shores are characterized by variable levels of human intervention and environment conditions. To survey for animal life in the sediments of Mono Lake, we collected soil from three different sites around Mono Lake to sample across various levels of human activities, and chemical and physical conditions. The three sites located in the north-east (site A), south (site B), and west (site C) (**Figure A.1A** and **Figure A.2**). Not approachable by vehicles, site A (Pristine Beach) on the north-east side of the lake is a large, sandy open field with the least visitors and observable biological activity of the three sites. Site B (Navy Beach) on the south attracts the most tourists. It contains emerged tufa structures, which are the precipitation products of calcium-bearing springs and the lake's carbonated waters (19). Site C (Old Marina) on the west has a rocky shore with small tufa structures. In all the sites we found the brine shrimps *Artemia monica* in the lake water and upper surface of the sediment, larvae of the *Ephydra hians* alkali fly in the sediments, and adult alkali flies on the lake's shores.

At each sampling site, we collected soil samples from three zones with various distances relative to the shore: dry zone, tide zone and in-lake (**Figure A.1B**). Within each niche, we sampled different sub-niches, for example, "in-lake" sampling involved the sampling of sediments in an increasing distance from the shoreline. We isolated live nematodes from all three sampling sites. From site A, most samples were collected from the tide zone and in-lake, and nematodes were

isolated in samples across -1 to 100 m away from the shore in sediments under water columns of 0 to 110 cm deep (**Figure A.1B-C**). Nematodes were also found from site B dry and tide zones, and from dry, tide and in-like zones in site C. In contrast to the sediments, we did not find nematodes along the water columns. During the survey of the soil samples, we found that nematodes that were isolated in the wet tide zone and in-lake niches coexist with brine shrimp and the larvae of the alkali fly. These three taxa were the only animals isolated from the samples demonstrating the harshness of the environment that apparently can host a limited number of animal species that developed specific adaptations.

Mono Lake is not an isolated ecological system; it collects the waters of several streams from the nearby mountains, and it is amenable for different human interventions. To rule out the possibility that the isolated nematodes are the result of an environmental contamination, for example due to human activity, we sampled the isolated Pristine Beach site (**Figure A.1A**). Sampling this site in two consecutive years, 2016 and 2017, we found nematodes at Pristine Beach at both years indicating that the lake hosts an ecologically-stable community of nematodes (**Figure A.1C**). From the many morphologically different nematodes we found, we choose to characterize eight morphologically distinct species by DNA analysis (species a-h. **Figure A.1D** (species b), **Figure A.1E** (species e) and **Figure A.3**). One species was isolated from in-lake in site A (species g), six species were isolated in site B (species a-f), and one was from both site B and C (species h). Importantly, in 2017, we found two of the species (species e and f) again, from different locations (site B tide zone in 2016 and site C dry zone in 2017) (**Figure**

A.4E). This observation suggests that particular species of nematodes are ecologically stable and widespread in the lake.

Some of the nematodes in Mono Lake live in pH 10.

To understand the environmental conditions at the niches inhabitant by Mono Lake nematodes, we measured the pH and soil salinity of our samples (**Table A.1**). Consistent with previous reports, the average pH of the samples fell within the range of 9-10 across different zones and sites (minimum: 9.0 ± 0.7 from site B tide zone, maximum: 10.01 ± 0.1 from site C dry zone), except for one sample from site B dry zone (pH=7.815). In contrast, the salinity of the samples varied by site and zone (**Figure A.4A**). Overall, samples from site A were more saline (tide zone 15.0 ± 3.0 ppt and in-lake 11.5 ± 3.5 ppt), and samples from site B were less saline (dry zone 1.0 ppt, tide zone 0.9 ± 0.7 ppt and in-lake 2.7 ± 1.9 ppt). This chemical analysis is consistent with the geography of Mono Lake in which site A, the most secluded from human interventions and the entry points of freshwater streams, is the most chemically extreme among the three sites we sampled. Nevertheless, site A hosts a large population of nematodes in the tide zone and in the lake, suggesting that nematodes were adapted to thrive even at extreme niches of the lake.

Mono Lake's nematodes belong to different nematode clades and represent diverse lifestyles

We integrated morphological and phylogenetic tools to study the biodiversity of

the isolated nematodes and their lifestyle in the Mono Lake ecosystem. Within the eight species, we identified a variety of mouth structures (**Figure A.5**), including grinders (**Figure A.4B**, species a), teeth (**Figure A.4C**, species d) and long esophagus (**Figure A.4D**, species f). The mouth structure of nematodes is an indicator of its feeding style (28). Base on the mouth structure analysis, we predict that species a and species d are a bacterial feeder and a predator, respectively. Species d may develop its tooth to prey on nematodes that are bacterial feeders in cases of harsh environmental conditions similar to what was shown for the interaction between the predatory nematode, *Pristionchus pacificus,* and its prey, *C. elegans* (29). Species e belongs to the family Mermithidae (see below), whose members have been observed to parasitize arthropods, such as spiders and grasshopper (30). This structure raises the possibility that species e could be parasitic of the other animals living in the lake. Taken together, our data show that the ecosystem of Mono Lake is much more complex than previously thought encompassing bacterial grazers, predators of other animals, and probably parasitic nematodes.

Five nematodes isolated are likely new species

We used molecular signatures, including ribosome large subunit (LSU) 28rDNA and small subunit (SSU) 18rDNA, to identify the species in order to understand the course and dynamics of lake colonization by these nematodes. The sequence analysis suggested that three of the isolated nematodes are known species, and five of the isolated nematodes are likely new species (**Figure A.4E**, **Figure A.6**).

Moreover, the isolates are from across the phylogeny of Nematoda (Blaxter and Helder classification (31)(32)) (**Figure A.4E-F**). The known species include Clade V/9 nematodes *Mononchoides americanus* (species c, **Figure A.11-12**) and *Diplogaster rivalis* (species d, **Figure A.13-14**), and Clade II/1 nematode *Prismatolaimus dolichurus* (species f, **Figure A.17**). Two of the new species belong to Clade V/9, including *Auanema sp.* (species a, **Figure A.7-8**) and *Pellioditis sp.* (species b, **Figure A.9-10**). We assigned the other three new species in family instead of genus because of the lack of phylogenetically close species: species e is in *Mermithidae* family, which belongs to Clade I/2 (**Figure A.4F** and **Figure A.15**); species g and h are in *Diplolaimelloides* family, which is classified between Clade II and III/5 (**Figure A.4F** and **Figure A.18-19**). We concluded that species g and h are different because the sequence similarity between them is 96.77%, which is less than our criteria of 98% (**Figure A.20**). Taken together, the diverse distribution across the phylum Nematoda suggests that the colonization of Mono Lake by nematodes happened independently and multiple times.

Auanema tufa **is culturable in lab**

The difficulty in replicating the exact conditions of extreme environments in order to culture the organisms that live in these habitats is a major obstacle in the study of life in the extremes. Thus, employing different culturing methods and conditions, we sought to establish a stable culturing system of Mono Lake nematodes in the laboratory. Of the eight species identified, we were able to

culture in our laboratory, using *C. elegans* culturing methods, one species which we identified as belonging to the *Auanema* genus (species a). Because this *Auanema sp.* shares only 89% (LSU) and 96%(SSU) sequence identity with its closest related *A. rhodensis* (**Figure A.6-8**), we concluded that *Auanema sp.*is a new species. Based on the tufa-rich environment we isolated it from, we named the species *Auanema tufa.* Notably, while its close related nematode species have been found in diverse habitats, only *A. tufa* was isolated from extreme environment (**Figure A.21**). The reproductive lifespan of *A. tufa* at 22.5°C is around 2.5-3 days, which is comparable to *C. elegans*. *A. tufa* shares some similarities with *A. rhodensis* and *A. freiburgensis* but also show some unique characteristics of their reproduction traits (33). The adult of all three species has a vulva located at mid-body and a two-armed gonad (**Figure A.21B-C**). *A. rhodensis* and *A. freiburgensis* have three genders (hermaphrodite, male and female), wherease *A. tufa* might be hermaphroditic or parthenogenic. We have observed male in *A. tufa*, but it appears very rarely. Moreover, *A. tufa* live-birth hatched larvae from their vulva (ovoviviparity) (**Figure A.21B**) instead of laying embryos like other nematodes of the *Auanema* genus such as *A. rhodensis* and *A. freiburgensis*. Ovoviviparity has been considered an adaptation to thrive in extreme environments (34, 35), thus yet representing another conceivable adaptation of *A. tufa* to the conditions of the lake.

A. tufa is an arsenic-resistant nematode

Mono Lake water and sediments are unique environments of high pH, salinity,

and high concentrations of arsenic species, primarily As(III) and (V) (11). It is known that a high concentration of arsenic is toxic to most living organisms thereby limiting animal life in arsenic-rich environments. To understand how Mono Lake nematodes survive in this hostile environment, we exposed *A. tufa* and a control nematode, the culturable soil worm *C. elegans,* with increasing concentrations of As(III) and As(V) solutions and examined their ability to survive over time. After 2.5 hours of exposure, we observed increased survival of *A. tufa* in both 1.5 and 3 mM of As(III) solutions in comparison to *C. elegans* (**Figure A.22A-B**). Even more striking is the ten fold more resistance of *A. tufa* to As(V). Specifically, *A. tufa* could withstand a concentration of 30mM As(V) compared to *C. elegans* (**Figure A.22C-D**). As a control we incubated the two strains in water and we detected 100% survival of the two species within the time window of the assay (**Figure A.22E**). *A. tufa* was isolated from near the surface of the tide zone, where As(V) is reported to be the dominant arsenic species (36). The results strongly suggest that evolving of mechanisms of arsenic resistance is a critical step in the adaptation of nematodes, including *A. tufa,* to the conditions of Mono Lake.

An increasing body of evidenc show that in *C.elegans*, SKN-1 is a transcription factor dedicated to promote many protective stress responses. Specifically, an activated form of *skn-1* mediates arsenic resistance in *C. elegans* (37). Thus, it is possible that activation of SKN-1 is one of the mechanisms that collectively underlie the adaptation of Mono Lake nematodes to Arsenic. To test if *skn-1* gene activity could explain the observed arsenic resistance of *A. tufa*, we compared the survival rate of *A. tufa* with different strains of *C. elegans*. These strains include the

wild-type background as a control and an *skn-1* allele, *lax188,* in which the SKN-1 protein is activated constitutively. We choose to expose the worms to 10mM As(V) solution in which the survival rate of *A. tufa* is significantly higher than wild-type *C. elegans* worms (**Figure A.22C**). Consistent with previous reports, we found that the activation of SKN-1 leads to arsenic resistance. Importantly, *A. tufa* survive better than wild-type and *skn-1* gain of function *C. elegans* worms (**Figure A.22F**). Thus, activation of the *skn-1* pathway might play a critical role in the adaptation of *A. tufa* and other Mono Lake nematodes to the extreme environmental conditions in the lake.

A.4 Discussion

Because Mono Lake is an extreme natural environment it was thought to host limited animal biodiversity. Here we report that, in addition to what was previously known, nematodes live in Mono Lake. We found spatial and temporal stable populations of nematodes all across the lake (A, B, and C sites) and at various zones (dry zone, tide zone, and in-lake), indicating there are multiple niches within the ecosystem of Mono Lake where nematodes can thrive. Mono Lake nematodes have multiple lifestyles for survival, as suggested by their diverse morphologies. In total we identified, using molecular phylogeny, eight species that belong to diverse clades across the phylum Nematoda. This polyphyletic diversity suggests that multiple colonization events took place in Mono Lake. Moreover, we found that one of the nematodes, *Auanema tufa* is culturable in lab and is more resistant to arsenic than *C. elegans*.

Due to the high level of protection of Mono Lake, we believe that our sampling was far from being saturated. Indeed, when we isolated the same species (species in *Mermithidae* and *Tripylidae*) in subsequent years, we did not find them in the same site. Our unsaturated sampling may also explain why the nematodes we observed at low abundance in the first year (*A. tufa*) were not observed in the subsequent year.

We suspect that there are several ways for the nematodes to adapt to Mono Lake. First, it is possible that nematodes around Mono Lake develop pre-adaptations to arsenic, which may allow them to evolve and further adapt to the high pH and salinity conditions in-lake. That could explain the adaptation strategy

of the arsenic-resistant *A. tufa* found in site B, where the salinity is the lowest and the pH varies the most among the three sampling sites. Secondly, upregulation of arsenic resistance genes, such as *skn-1,* may be a critical aspect of this adaptation. Further investigation is required to test directly if *skn-1* or other stress-related genes are involved. Finally, entering the dauer stage, a stress-resistant and developmentally arrested period (38)(39), might help nematodes survive in Mono Lake and find relatively favorable places within the harsh environment via dauer-specific dispersal behaviors (40). Our sampling technique did not favor the isolation of dauers, but it is possible that dauer formation is one strategy of resistance that facilitated the habitation of the lake by dauer-forming nematodes.

The fact that nematodes have been found in several harsh environments, including Mono Lake, raises the question: what makes nematodes good extremophiles? Because nematode genomes can very quickly and dramatically through high rates of gene acquisition and loss (41), it is likely that nematodes can adapt to challenging conditions. Moreover, the small size of nematodes is probably beneficial, allowing the utilization of neuroendocrine signaling to engage and enact whole animal survival programs in response to stress. Lastly, as mentioned before, dauer animals have well-equipped physiology and behaviors to cope with stress.

We have investigated extremophile biology in nematodes and have identified yet another harsh environment where nematodes can survive. We identified eight species from across the diversity of Nematoda, suggesting that Mono Lake was invaded independently and multiple times. The arsenic resistance of *A. tufa* that lives in the relatively safe harbor of the B site suggests that preadaptation to

arsenic could lead to the genomic evolution necessary to survive the pH and salinity of inner Mono Lake.

A.5 Material and Method

Sites and sampling

Soil and water samples were collected from three sites around Mono Lake (**Figure A.1**) in August 2016, June 2017 and July 2017. Site A, which we named Pristine Beach, (38° 3′ 27.91″ N, 119° 1′ 50.66″ W), site B is at Navy Beach (37° 56′ 21.90″ N, 119° 1′ 25.93″ W), and site C is at Old Marina (37° 59′ 12.80″ N, 119° 8′ 18.70″ W).

At each site, soil samples were collected from inside the lake, tide zone, and dry zone, with each sample weight ranging from 15 to 375 g. Total numbers of samples collected from each site were: 25 from site A (9 in 2016 and 16 in 2017), 34 from site B (19 in 2016 and 15 in 2017), and 22 from site C (7 in 2016 and 15 in 2017). The sampling permits were issued to Amir Sapir by the California Fish and Wildlife Department (SCP-13436) and from the Californian State Parks Department. All of the sample information, including location, pH, salinity, and the presence of nematodes, is listed in Table S1.

Soil salinity and pH measurement

Each soil sample was mixed with Milli-Q water in a 1:2 ratio (weight:volume) for salinity and pH measurements (20). Soil salinity was estimated by measuring the conductivity with two meters: Orion conductivity meter model 126 (for 2016 samples) and TPS WP-81 conductivity meter (for 2017 samples). Soil pH was measured using VWR pH meter model 8015.

Nematode isolation and species identification

Nematodes were isolated directly from the soil samples either using a dissecting microscope on-site or in the laboratory by the Baermann funnel method for overnight extraction (21). The isolated nematodes were further identified by morphology and molecular signatures. For molecular analysis, individual worm lysate was prepared in worm lysis solution (100µl DirectPCR lysis reagent (Viagen Biotech), 10.5µl proteinase K (10 mg/ml) and 5µl 1M DTT). The gene fragments of ribosome large subunit (LSU) 28rDNA and small subunit (SSU) 18rDNA were amplified (22)(23) and sequenced. MEGA7 was used to build phylogenetic tree from the resulting sequences (24). The tree was estimated by using Maximum Likelihood (ML) analysis and 1,000 bootstrap replicates, and the species identification was done with General Time Reversible model (25). The isolated nematode is considered as a new species when it exhibits <98% sequence similarity compared with its nearest neighbor (26, 27).

Nematode culture

Maintenance

Both *C. elegans* wild-type strain N2 (Bristol) and *Auanema tufa* n. sp. were grown using standard *C. elegans* culturing protocol with *Escherichia coli* strain OP50 as a food source (19). *Auanema tufa* was maintained at 22.5°C.

Freezing

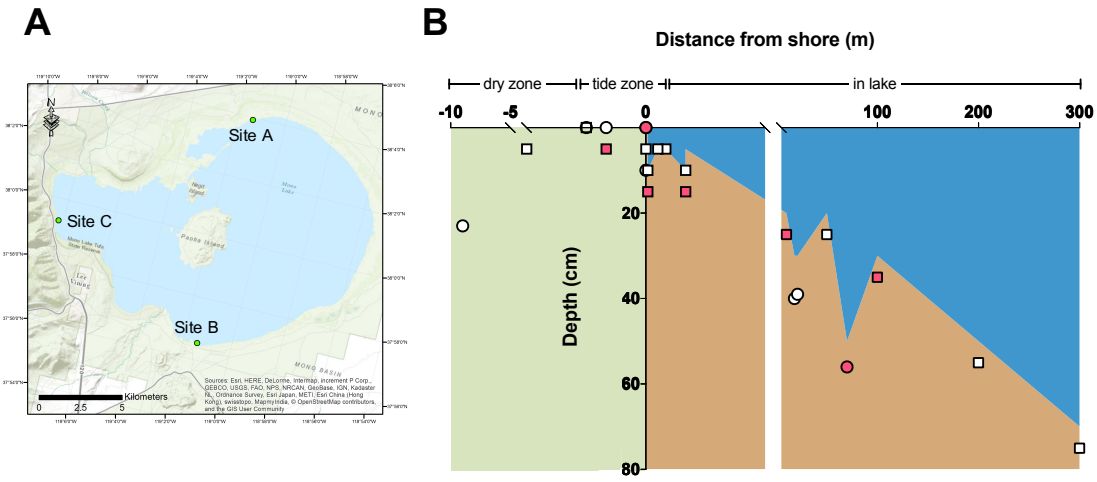
Auanema tufa was frozen using Trehalose-DMSO method (personal

communication with Dr. Kevin F. O'Connell). Briefly, *Auanema tufa* n. sp. from freshly starved plates was washed off with M9 buffer (3 g KH_2PO_4, 6 g Na_2HPO_4, 5 g NaCl and 1 ml 1 M $MgSO_4$ in 1L ddH_2O) and collected in a 15ml centrifuge tube. The worm pellet was washed once, re-suspended with Trehalose-DMSO freezing buffer (15.1 g Trehalose (Fisher BioReagents, PA, Cat# BP2687-25) and 17.7 ml DMSO in 500 ml M9 buffer), and transferred to cryogenic vials. The vials were stored in -80°C freezer after 30 minutes incubation at room temperature.

Survival assay

As(III) and As(V) solutions were prepared by dissolving sodium (meta)arsenite (Sigma-Aldrich, MO, Cat S7400) and sodium arsenate dibasic heptahydrate (Sigma-Aldrich, MO, Cat# S9663) in Milli-Q water, respectively. Adults of *C. elegans* wild-type N2, *skn-1(lax188)*, and *Auanema tufa* were washed with Milli-Q water for 4 times and transferred to 12-well tissue culture plates (Corning, NY) containing 0.9 ml of Milli-Q water and various concentration of As(III) or As(V) per well. Each well has on average 34 animals, ranging from 10 to 66. Final concentrations of 1.5 and 4.5 mM of As(III), and 10 and 30 mM of As(V) was used to treat animals. Animals were incubated at 22°C and the numbers of surviving animals, determined by their physiology and touch-provoked movement (in response to eyelash touch), were counted at different time points (1, 2.5, 5 and 7 hours).

A.6 Figures



C

Year	Site A			Site B			Site C		
	dry zone	tide zone	lake	dry zone	tide zone	lake	dry zone	tide zone	lake
2016	0/1	1/5	1/3	2/3	9/14	0/1	1/1	0/6	NA
2017	0/1	2/6	3/8	NA	1/3	0/9	6/14	3/4	4/11
Total	0% (0/2)	27% (3/11)	36% (4/11)	67% (2/3)	59% (10/17)	0% (0/10)	47% (7/15)	30% (3/10)	36% (4/11)

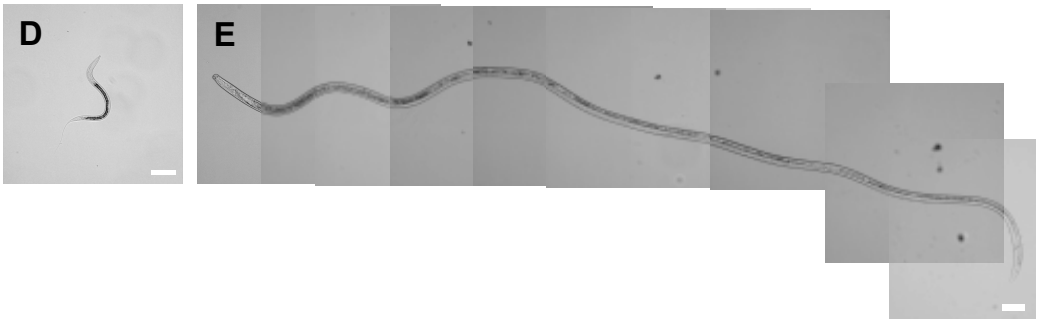


Figure A.1. Nematodes were isolated from in and around Mono Lake. (A) The locations of the three sampling sites A, B and C around Mono Lake. Samples were collected in August 2016 and June 2017. **(B)** Plot showing the locations at site A where samples were collected, relative to the shore (x-axis) and surface (y-axis). The boundaries of three different kinds of locations, dry zone, tide zone, and in-lake, were shown by the brackets. Blue indicates lake water and brown indicates

the soil. Circles and squares represent samples collected in 2016 and 2017, respectively. The samples in which nematodes were found were colored in pink. **(C)** Summary table of the origins of the samples. Samples were collected from dry zone, tide zone or lake from each site. The numbers in the cells indicate the number of samples with nematodes isolated versus the total number of samples collected. The locations that have nematodes found were highlighted in beige for 2016 samples and grey for 2017 ones. NA, non-applicable. **(D-E)** Representative images of two nematodes isolated. One was isolated from site B dry and tide zones in 2016 **(D)**, and the other one was isolated from site B tide zone in 2016 and site C dry zone in 2017 **(E)**.

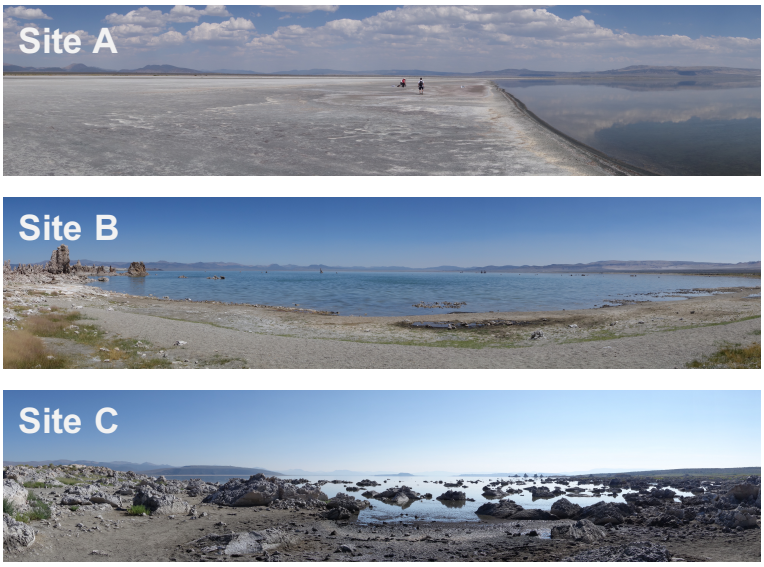
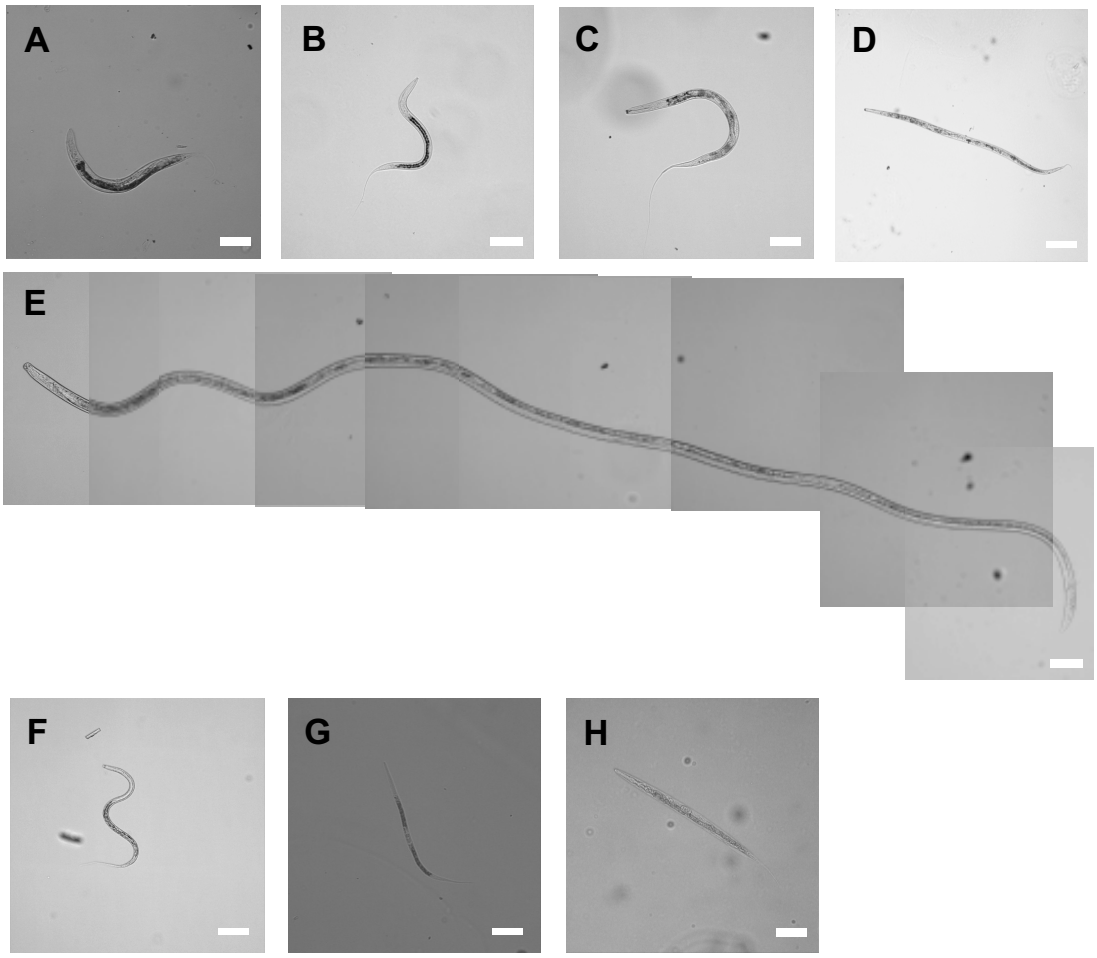


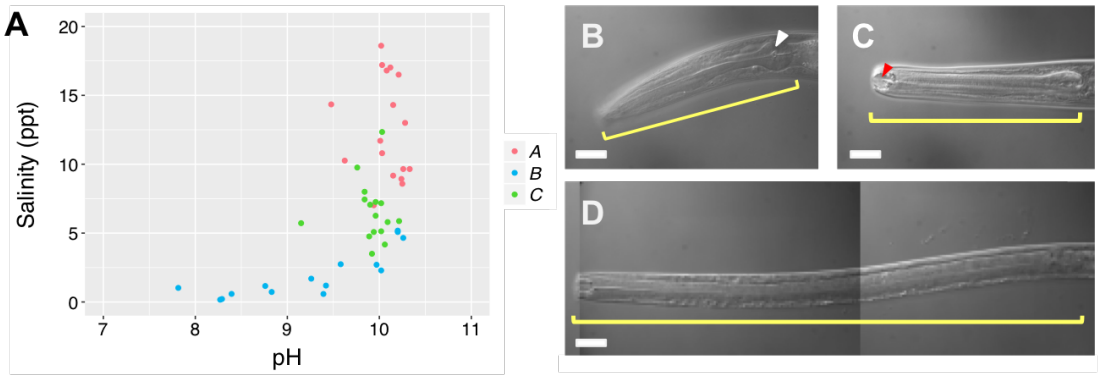
Figure A.2. Pictures of three sampling sites around Mono Lake. Pictures of site A (10 Mile Road), site B (Navy Beach), and site C (Old Marina).



I

Species	Clade		Site A		Site B		Site C		
	Blaxter[3]	Helder[4]	tide zone	lake	dry zone	tide zone	dry zone	tide zone	lake
a. *Auanema sp.*[1,2]	V	9				B8 (2016) B14 (2016)			
b. *Pellioditis sp.*[1,2]	V	9			B20 (2016)	B9 (2016)			
c. *Mononchoides americanus*[1,2]	V	9			B20 (2016)	B7 (2016) B9 (2016)			
d. *Diplogaster rivalis*[1,2]	V	9				B8 (2016)			
e. species in Mermithidae[1,2]	I	2				B9 (2016)	C131 (2017) C133 (2017)		
f. *Prismatolaimus dolichurus*[1]	II	1				B7 (2016)	C130 (2017)		
g. species in Monhysteridae[2]	(II, III)	5		A9 (2016)					
h. species in Monhystreidae[2]	(II, III)	5				B8 (2016)	C7 (2016)		

Figure A.3. Nematodes isolated from the three sites are diverse in morphology. (A-H) Morphology of species a-h under low magnification. **(I)** Identification and classification of the eight nematodes isolated. The species were identified by either 28S LSU rRNA (Footnote 1) or 18S SSU rRNA (Footnote 2). The classification system was based on the ones introduced by Blaxter (Clade I-V) or Heider (Clade 1-13). Some species fall between Clade II and III, which were indicated with parenthesis in the table. The sample number, location, and the year collected were indicated in the corresponding cell. Highlighted squares denote sites where the species was observed. Samples from 2016 are in beige, and those from 2017 are in grey. Scale bar: 100μm



E

Species	Clade		Site A		Site B		Site C		
	Blaxter[3]	Helder[4]	tide zone	lake	dry zone	tide zone	dry zone	tide zone	lake
a. *Auanema sp.*[1,2]	V	9				B8 (2016) B14 (2016)			
b. *Pellioditis sp.*[1,2]	V	9			B20 (2016)	B9 (2016)			
c. *Mononchoides americanus*[1,2]	V	9			B20 (2016)	B7 (2016) B9 (2016)			
d. *Diplogaster rivalis*[1,2]	V	9				B8 (2016)			
e. species in Mermithidae[1,2]	I	2				B9 (2016)	C131 (2017) C133 (2017)		
f. *Prismatolaimus dolichurus*[1]	II	1				B7 (2016)	C130 (2017)		
g. species in Monhysteridae[2]	(II, III)	5		A9 (2016)					
h. species in Monhystreidae[2]	(II, III)	5				B8 (2016)	C7 (2016)		

F

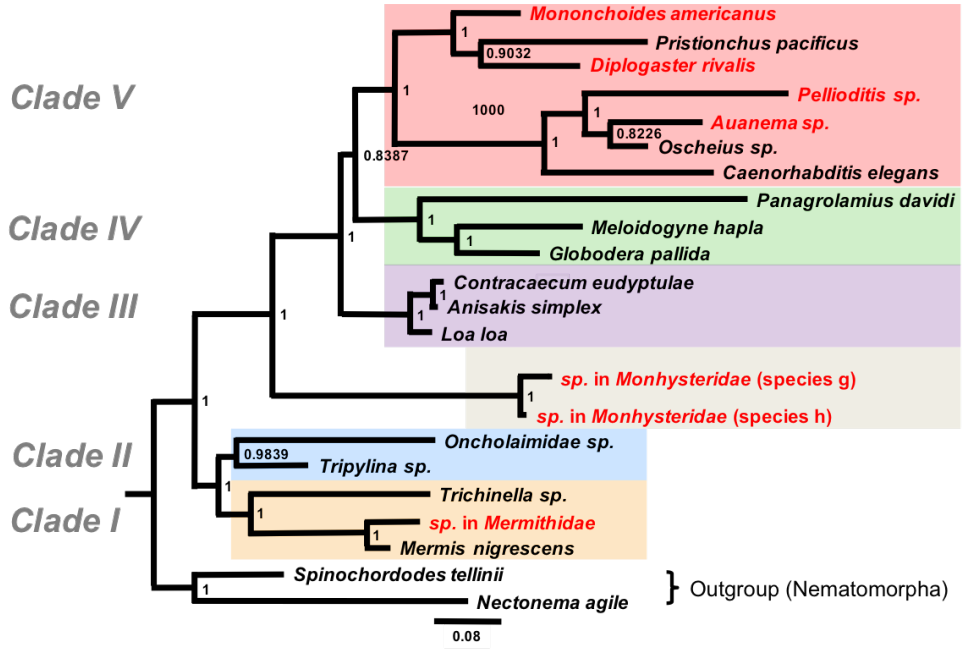
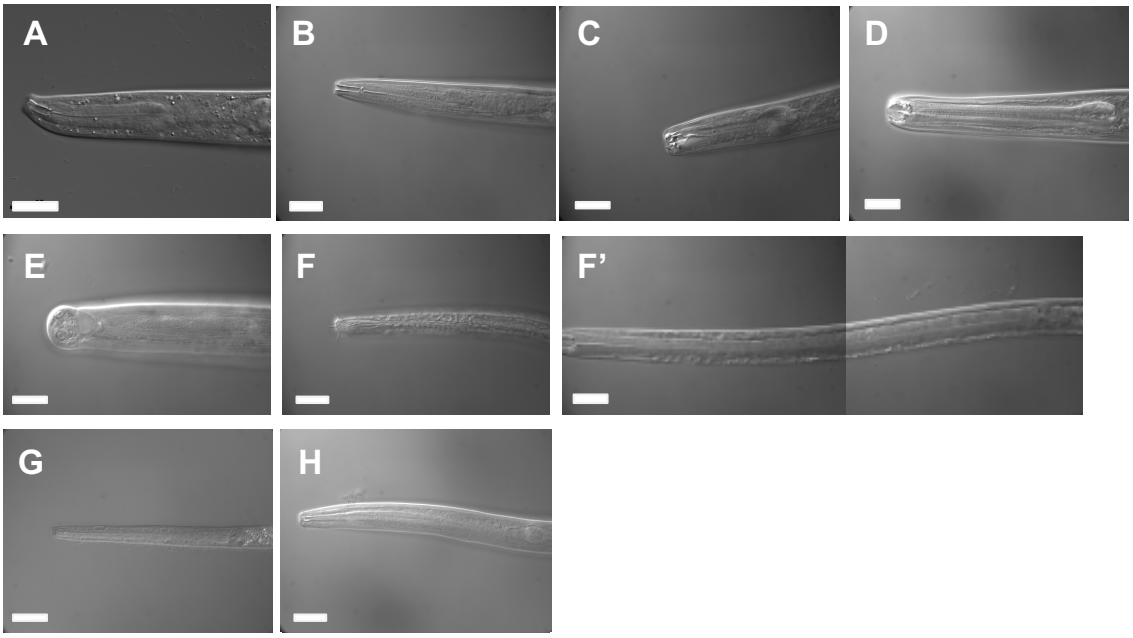


Figure A.4. The nematodes isolated are phylogenetically and morphologically diverse. (A) Plot showing the salinity and pH of all the samples collected. Each dot represents the measurements from one single sample, and the color corresponds to the site where the sample were collected from. **(B-D)** Mouth/head structures of three representative nematodes (Species a, d and f, respectively). The white and red arrowheads indicate the grinder and tooth, respectively. The yellow bracket shows the length of the esophagus. Scale bar: 20μm. **(E)** Identification and classification of the eight nematodes isolated. The species were identified by either 28S LSU rRNA or 18S SSU rRNA. The classification system was based on the ones introduced by Blaxter (Clade I-V) or Heider (Clade 1-13). Some species fall between Clade II and III, which were indicated with parenthesis in the table. The sample number, location, and the year collected were indicated in the corresponding cell. Highlighted squares denote sites where the species was observed. Samples from 2016 are in beige, and those from 2017 are in grey. **(F)** Phylogenetic tree of the eight of the nematodes based on SSU sequences. The nematodes we isolated were highlighted in red. The numbers show the bootstrap score out of 1000 runs. Footnotes: 1, confirmed by LSU rRNA sequence; 2, confirmed by SSU rRNA sequence; 3, reference (31) ; 4, reference (32)

Sample number	Location	From shore (cm)	Under ground (cm)	Water depth (cm)	pH	Salinity (ppt)	Presence of neamtdoes	Number of nematodes/species
A1	tide zone	0	0	0	ND	ND	NO	
A2	tide zone	-150	0	0	ND	ND	NO	
A3	tide zone	0	10	0	ND	ND	NO	
A4	tide zone	0	0	0	9.477	14.342	YES	1 / 1
A5	tide zone	-100	0	0	ND	ND	NO	
A6	dry zone	-900	23	0	ND	ND	NO	
A7	in lake	1800	10	30	ND	ND	NO	
A8	in lake	7000	6	50	9.624	10.26	YES	~5 / 1
A9	in lake	2100	9	30	ND	ND	NO	
A100	tide zone	5	10	0	10.21	16.5	NO	
A101	tide zone	5	5	0	10.08	16.8	YES	1 / 1
A102	dry zone	-300	5	0	10.12	17.02	NO	
A103	tide zone	50	0	5	10.15	14.3	NO	
A104	tide zone	0	5	0	10.02	18.6	NO	
A105	in lake	30	0	5	10.01	11.7	NO	
A106	tide zone	-150	0	0	10.03	17.2	NO	
A107	tide zone	-100	0	5	10.03	10.8	YES	4 / ND
A108	in lake	100	5	10	10.28	13	YES	33 / ND
A109	in lake	1000	5	20	10.33	9.65	YES	1 / 1
A110	in lake	5000	5	20	10.24	8.93	NO	
A111	in lake	10000	5	30	10.25	8.58	YES	4 / ND
A112	in lake	20000	5	50	9.94	7.02	NO	
A113	in lake	30000	5	70	10.15	9.17	NO	
A114	in lake	100	5	5	10.26	9.65	NO	
B1	tide zone	0	0	0	ND	ND	NO	
B2	tide zone	0	0	0	ND	ND	NO	
B3	in lake	300	0	10	ND	ND	NO	
B4	tide zone	0	8	0	ND	ND	NO	
B5	tide zone	20	10	0	ND	ND	YES	2 / 1
B6	tide zone	10	10	0	ND	ND	YES	1 / 1
B7	tide zone	0	5	0	9.3925	0.581	YES	~15 / 2
B8	tide zone	-100	5	0	ND	ND	YES	20 / ≥2
B9	tide zone	-100	5	0	5.967[a]	0.779	YES	~50 / 3
B10	tide zone	0	0	0	ND	ND	YES	1 / 1
B12	tide zone	0	0	0	ND	ND	YES	1 / 1
B13	tide zone	-75	8	0	ND	ND	NO	
B14	tide zone	-75	0	0	8.394	0.586	YES	~200 / ≥3
B15	tide zone	0	0	0	ND	ND	NO	
B16	dry zone	-300	6.5	0	ND	ND	YES	2 / ND
B19	dry zone	-300	5	0	ND	ND	NO	
B20	dry zone	0	0	0	7.815	1.022	YES	~20 / 2
B21	tide zone	-120	4	0	ND	ND	YES	1 / 1
B100	in lake	30	10	0	9.42	1.19	NO	
B101	tide zone	0	10	0	8.83	0.725	NO	
B102	in lake	300	10	0	8.27	0.166	NO	
B103	in lake	50	10	30	9.58	2.74	NO	
B104	in lake	10	10	10	9.97	2.7	NO	
B106	in lake	10	10	10	8.76	1.16	NO	
B107	in lake	30	10	10	9.26	1.692	NO	
B108	tide zone	-100	10	0	8.29	0.214	YES	40 / 1
B111	tide zone	0	10	0	10.02	2.29	NO	
B112	in lake	100	10	40	10.2	5.08	NO	
B113	in lake	300	10	50	10.26	4.65	NO	
B114	in lake	1000	10	100	10.2	5.18	NO	
C1	tide zone	0	5	0	ND	ND	NO	
C2	tide zone	0	0	0	ND	ND	NO	
C3	tide zone	-30	0	0	ND	ND	NO	
C4	tide zone	0	4	0	ND	ND	NO	
C6	tide zone	0	5	0	ND	ND	NO	
C7	dry zone	-200	5	0	10.2145	5.87	YES	3 / 2
C8	tide zone	0	3	0	9.175	22.361[a]	NO	
C100	in lake	300	0	0	10.06	4.17	YES	1 / 1
C101	tide zone	5	0	0	9.89	4.76	YES	2 / ND
C102	dry zone	-20	0	0	10.02	7.17	YES	8 / 1
C103	in lake	10	20	0	10.02	5.13	NO	
C104	dry zone	-1000	0	0	10.03	12.34	YES	52 / ND
C105	in lake	20	20	20	9.92	3.5	NO	
C106	tide zone	0	0	0	9.15	5.72	YES	1 / 1
C108	in lake	100	0	20	9.94	5.08	NO	
C109	in lake	100	10	0	9.96	7.26	NO	
C110	in lake	300	10	40	9.9	7.06	YES	2 / ND
C111	in lake	1000	0	50	10.09	5.8	YES	2 / ND
C112	in lake	3000	10	50	9.84	7.44	YES	1 / 1
C113	in lake	5000	0	50	9.96	6.26	NO	
C114	in lake	7000	10	70	9.84	8	NO	
C115	in lake	10000	10	100	9.76	9.76	NO	
C130	dry zone	-300	5	0	ND	ND	YES	~400 / 1
C131	dry zone	-500	5	0	ND	ND	YES	131 / 2
C132	tide zone	-30	5	0	ND	ND	NO	
C133	dry zone	-1000	5	0	ND	ND	YES	10 / 1
C134	dry zone	-1000	15	0	ND	ND	NO	
C135	dry zone	-1000	28	0	ND	ND	NO	
C136	dry zone	-500	15	0	ND	ND	YES	1 / 1
C137	dry zone	-500	48	0	ND	ND	NO	
C138	dry zone	-50	5	0	ND	ND	NO	
C139	dry zone	-50	48	0	ND	ND	NO	
C140	dry zone	-50	28	0	ND	ND	NO	
C141	dry zone	-50	15	0	ND	ND	NO	
C142	dry zone	-10000	2	0	ND	ND	NO	
C143	tide zone	-30	5	10	ND	ND	YES	1 / 1

Table A.1. Detailed information of the soil samples collected. Sample numbers include the information of both sampling site (A, B, or C) and sampling year (2016 samples start from 1, 2017 samples start from 100). The sign of the distance from the shore indicates the direction of the sampling site in respect to the lake: positive is into the lake, and negative is away from the lake. Footnote: a, outliers, excluded from further analysis. ND: not determined.



I

Species	Clade		Site A		Site B		Site C		
	Blaxter[3]	Helder[4]	tide zone	lake	dry zone	tide zone	dry zone	tide zone	lake
a. *Auanema sp.*[1,2]	V	9				B8 (2016) B14 (2016)			
b. *Pellioditis sp.*[1,2]	V	9			B20 (2016)	B9 (2016)			
c. *Mononchoides americanus*[1,2]	V	9			B20 (2016)	B7 (2016) B9 (2016)			
d. *Diplogaster rivalis*[1,2]	V	9				B8 (2016)			
e. species in Mermithidae[1,2]	I	2				B9 (2016)	C131 (2017) C133 (2017)		
f. *Prismatolaimus dolichurus*[1]	II	1				B7 (2016)	C130 (2017)		
g. species in Monhysteridae[2]	(II, III)	5		A9 (2016)					
h. species in Monhystreidae[2]	(II, III)	5				B8 (2016)	C7 (2016)		

Figure A.5. Nematodes isolated from the three sites are diverse in morphology. (A-H) Morphology of species a-i under high magnification. (F) and (F') were taken from the same animal but on different focal planes. **(I)** Identification and classification of the eight nematodes isolated. The sample number, location, and the year collected were indicated in the corresponding cell. Scale bar: 20μm

Species	Sequence identity （%）	
	LSU	SSU
a	89	96
b	88	95
c	90	98 (to Mononchoides americanus)
d	92	99 (to Diplogaster rivalis)
e	85	93
f	99 (to Prismatolaimus dolichurus)	NA
g	NA	92
h	NA	96

Figure A.6. Percent of sequence identity of each isolate compared to its closest related species. Based on LSU and SSU sequences. NA: not applicable

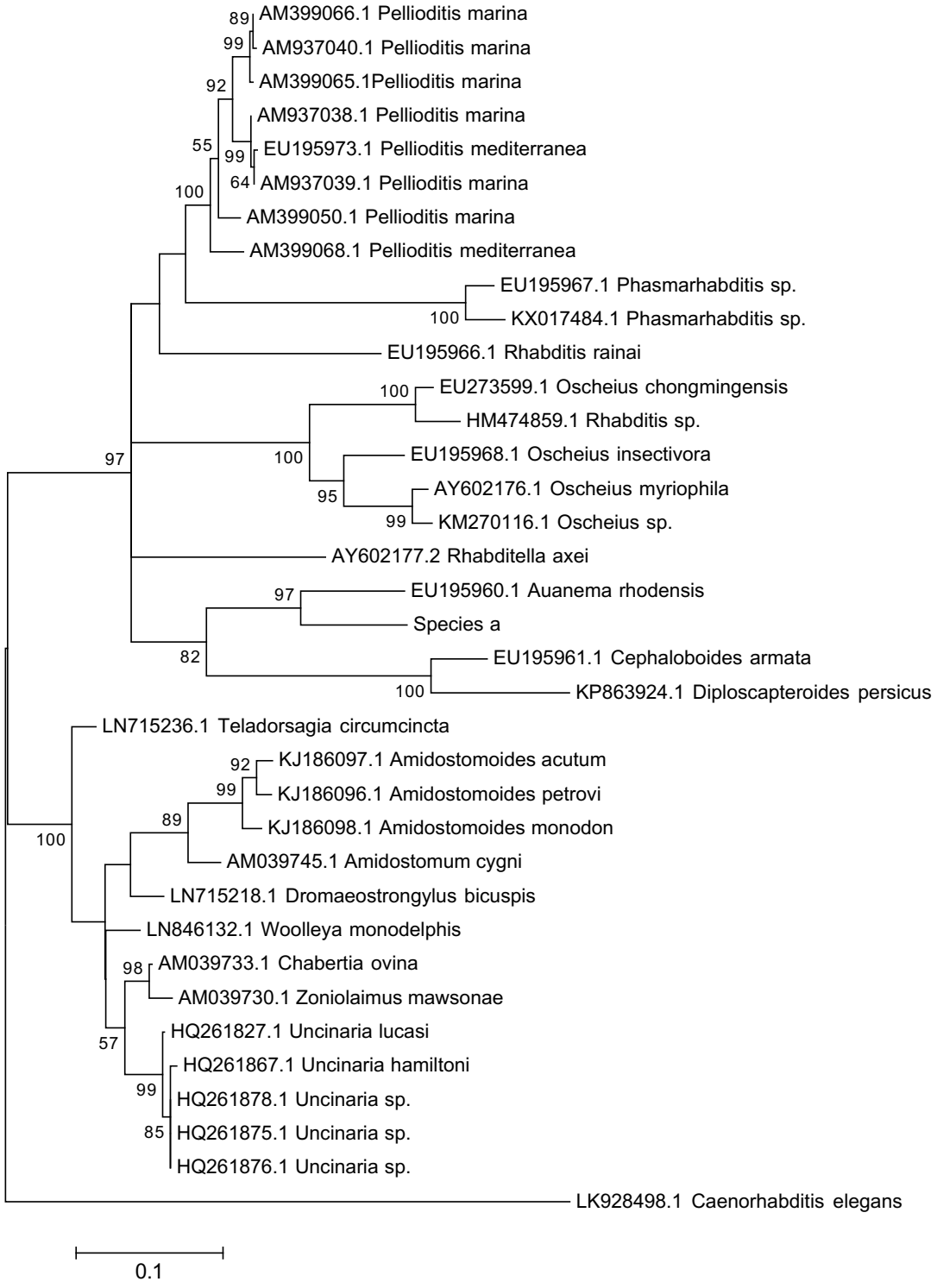


Figure A.7. Phylogenetic tree of species a (based on LSU sequence)

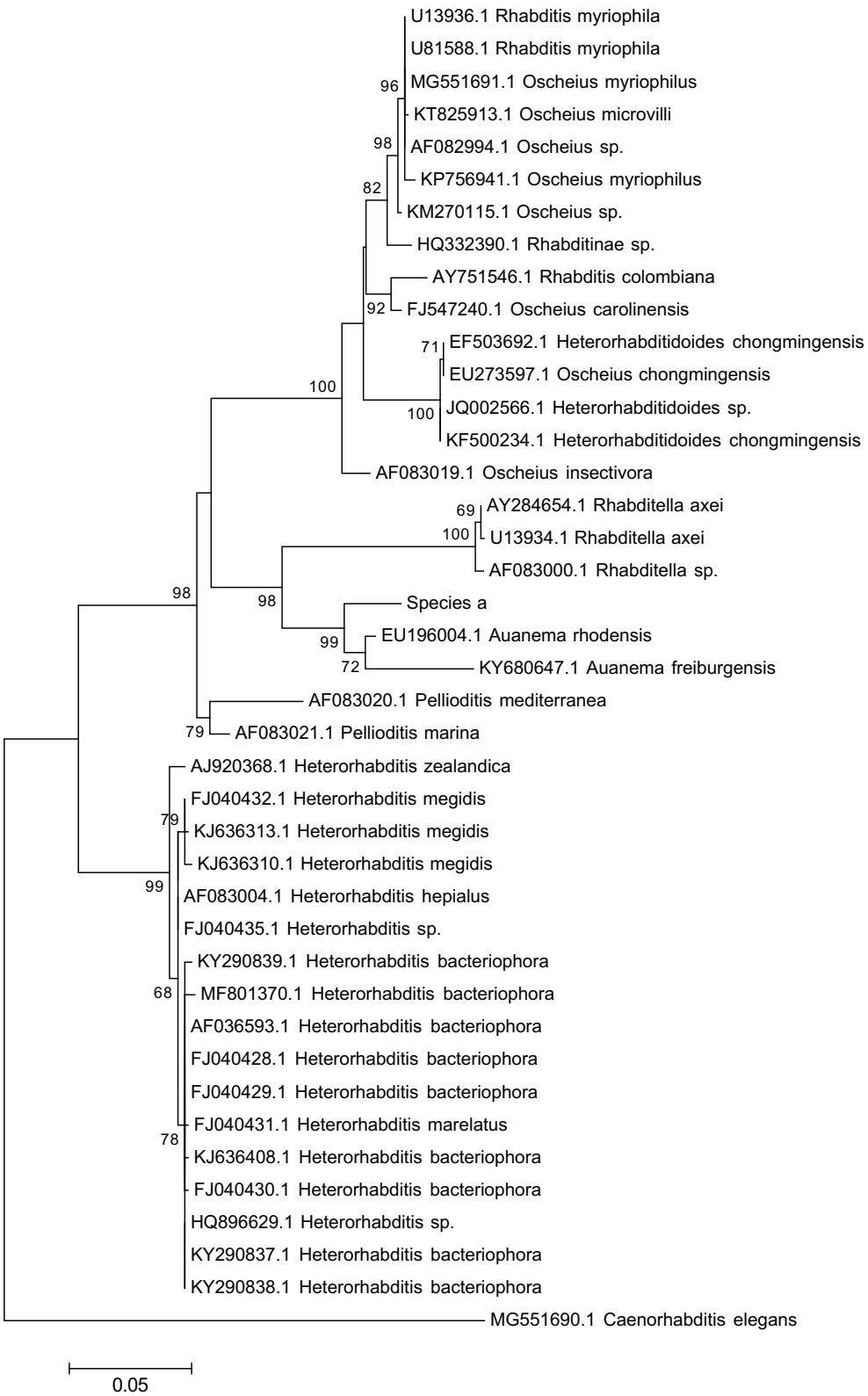


Figure A.8. Phylogenetic tree of species a (based on SSU sequence)

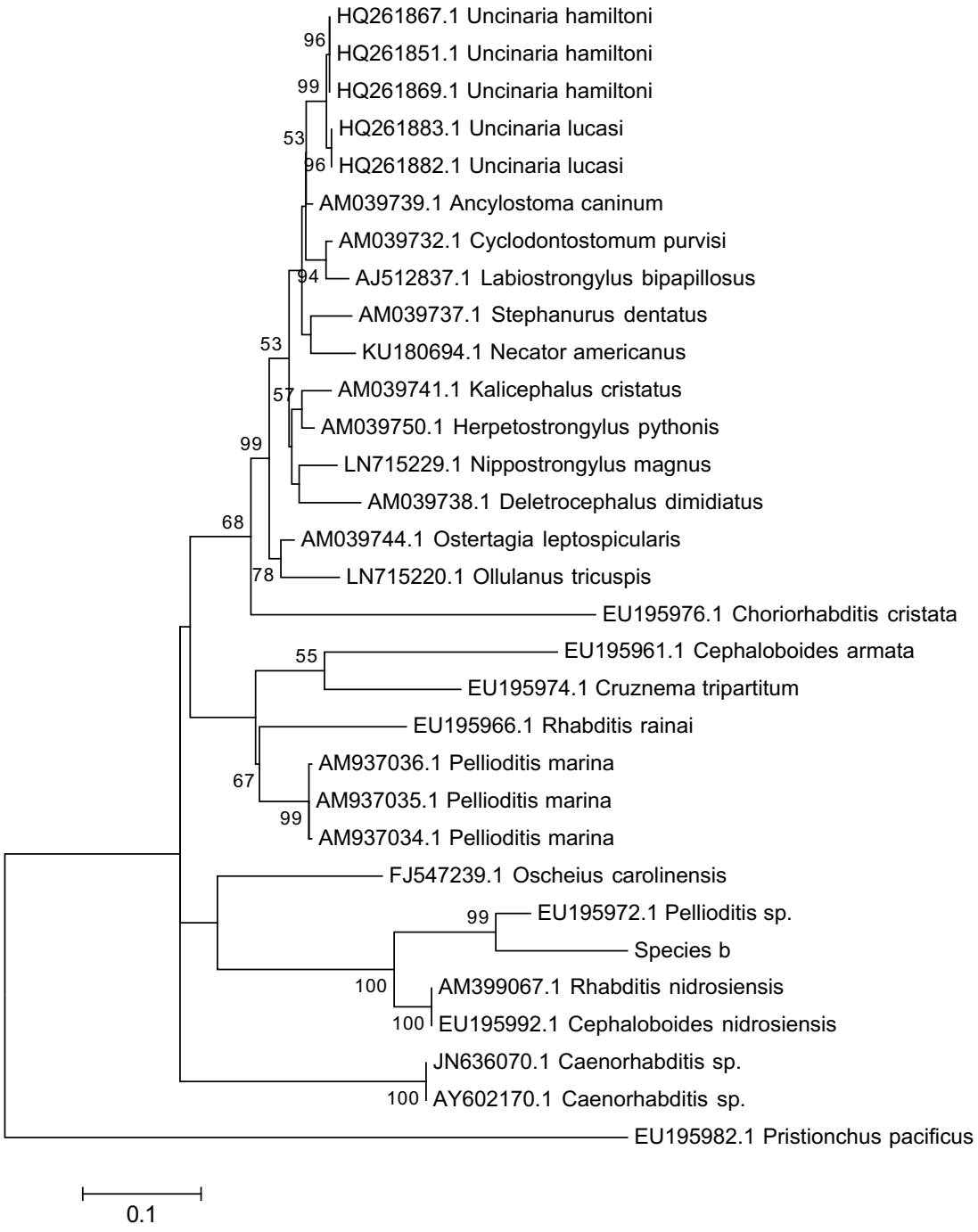


Figure A.9. Phylogenetic tree of species b (based on LSU sequence)

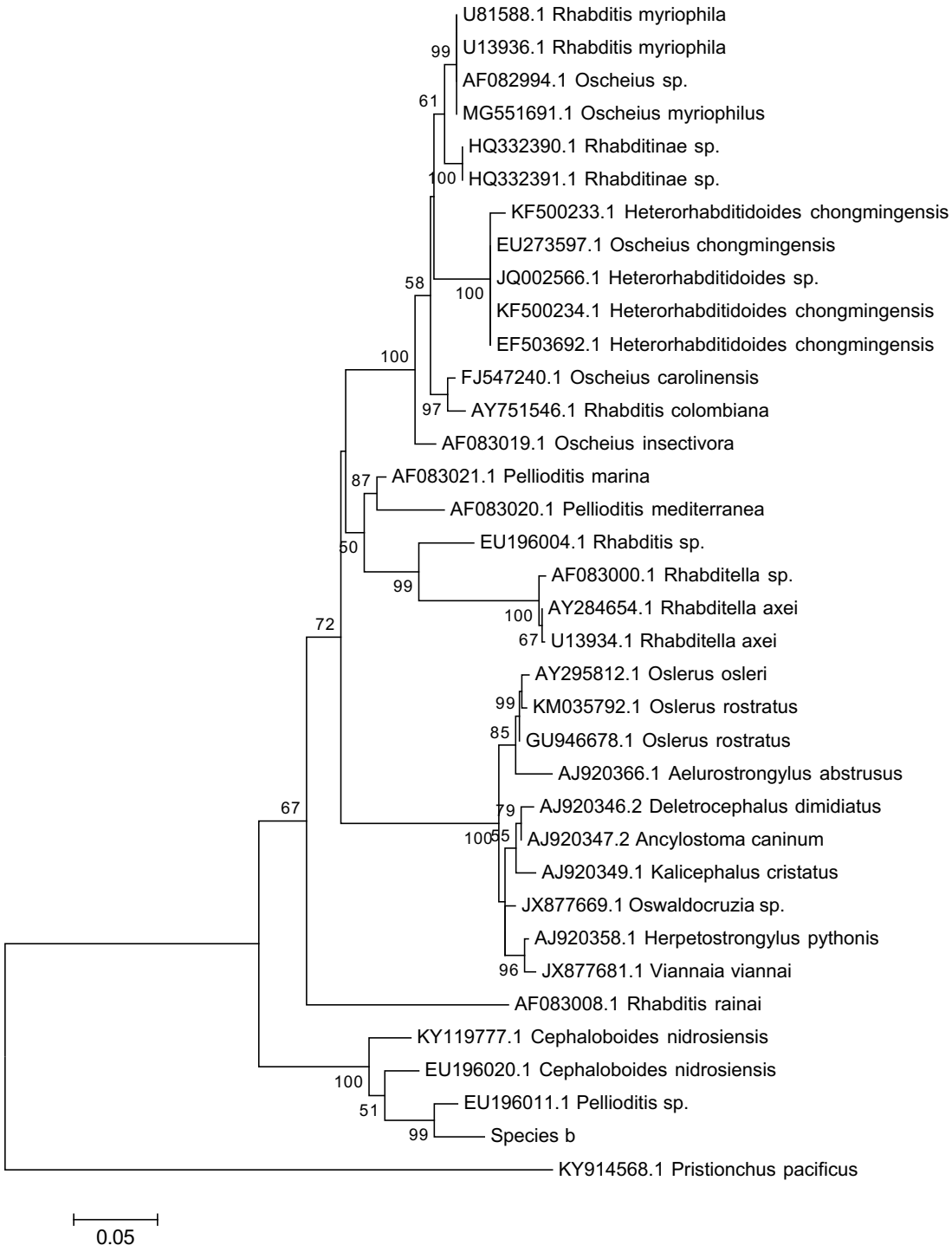


Figure A.10. Phylogenetic tree of species b (based on SSU sequence)

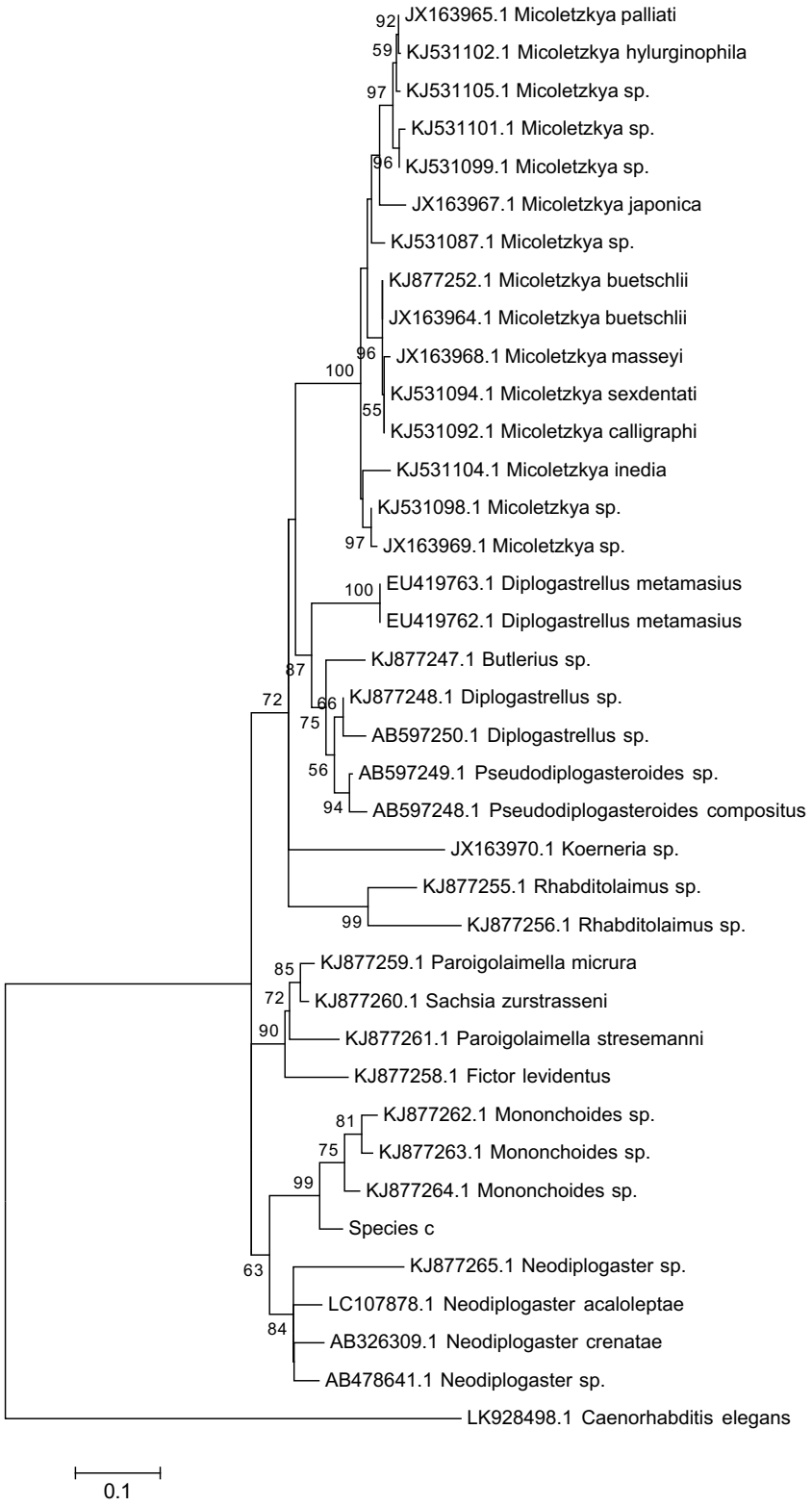


Figure A.11. Phylogenetic tree of species c (based on LSU sequence)

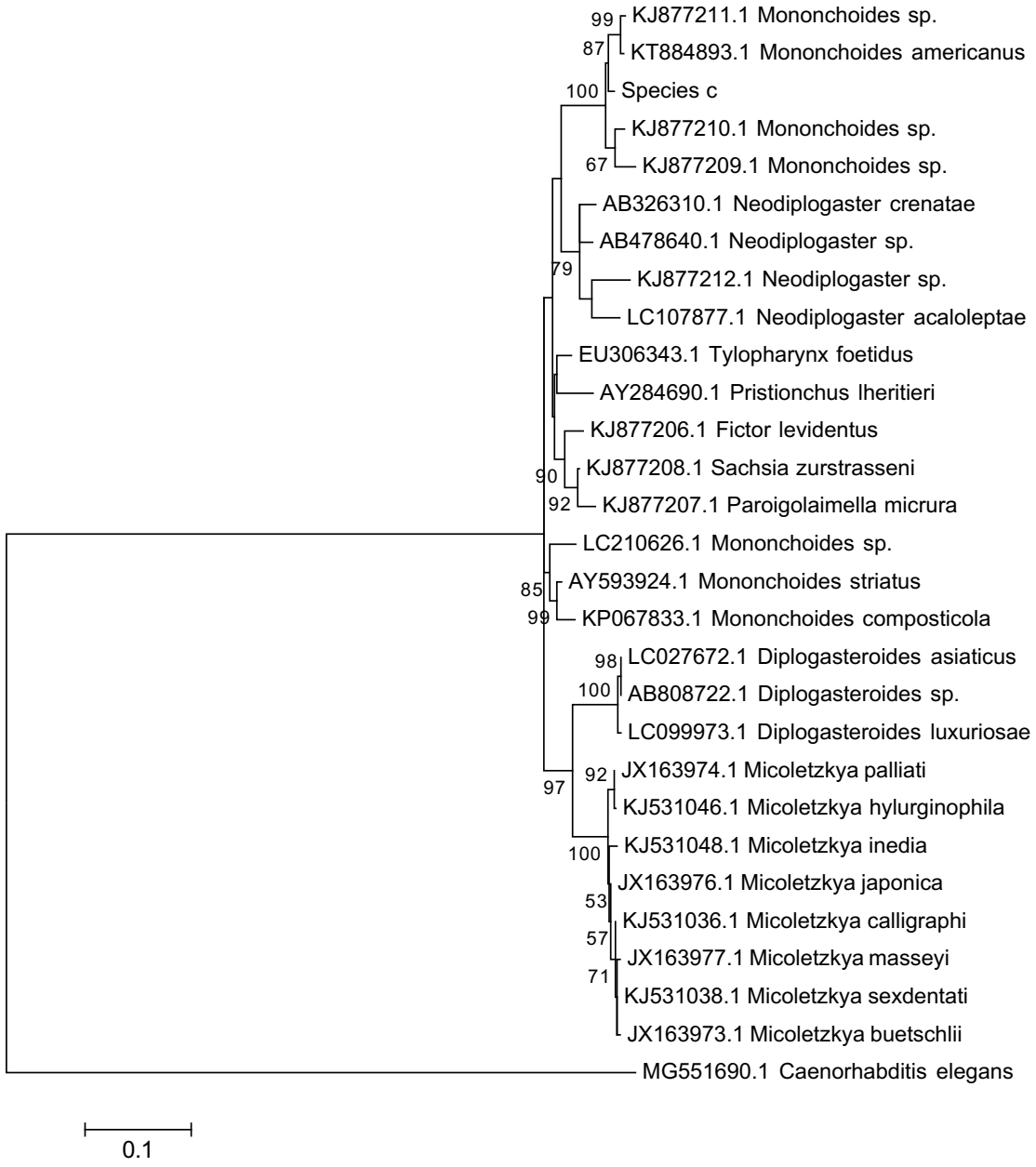


Figure A.12. Phylogenetic tree of species c (based on SSU sequence)

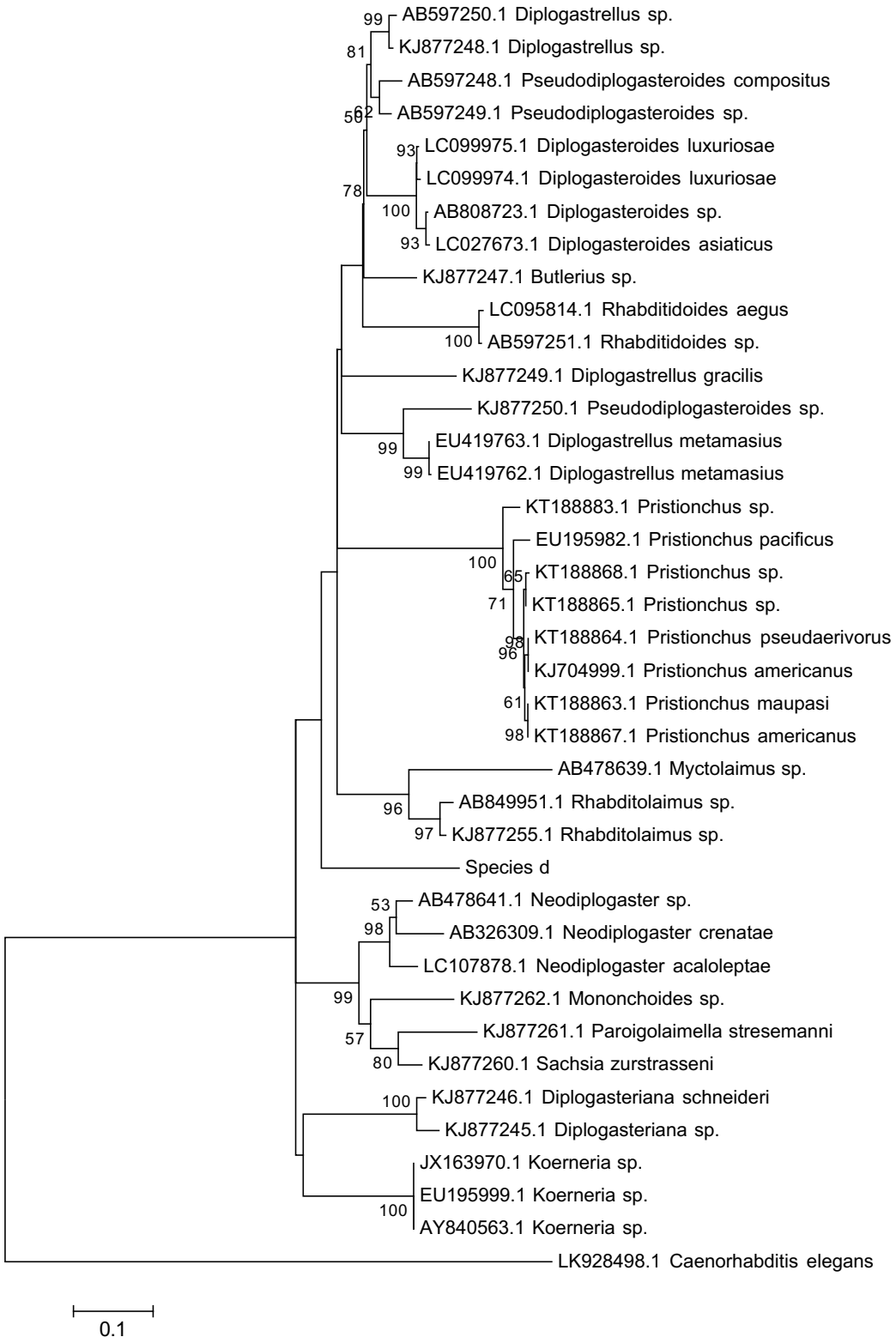


Figure A.13. Phylogenetic tree of species d (based on LSU sequence)

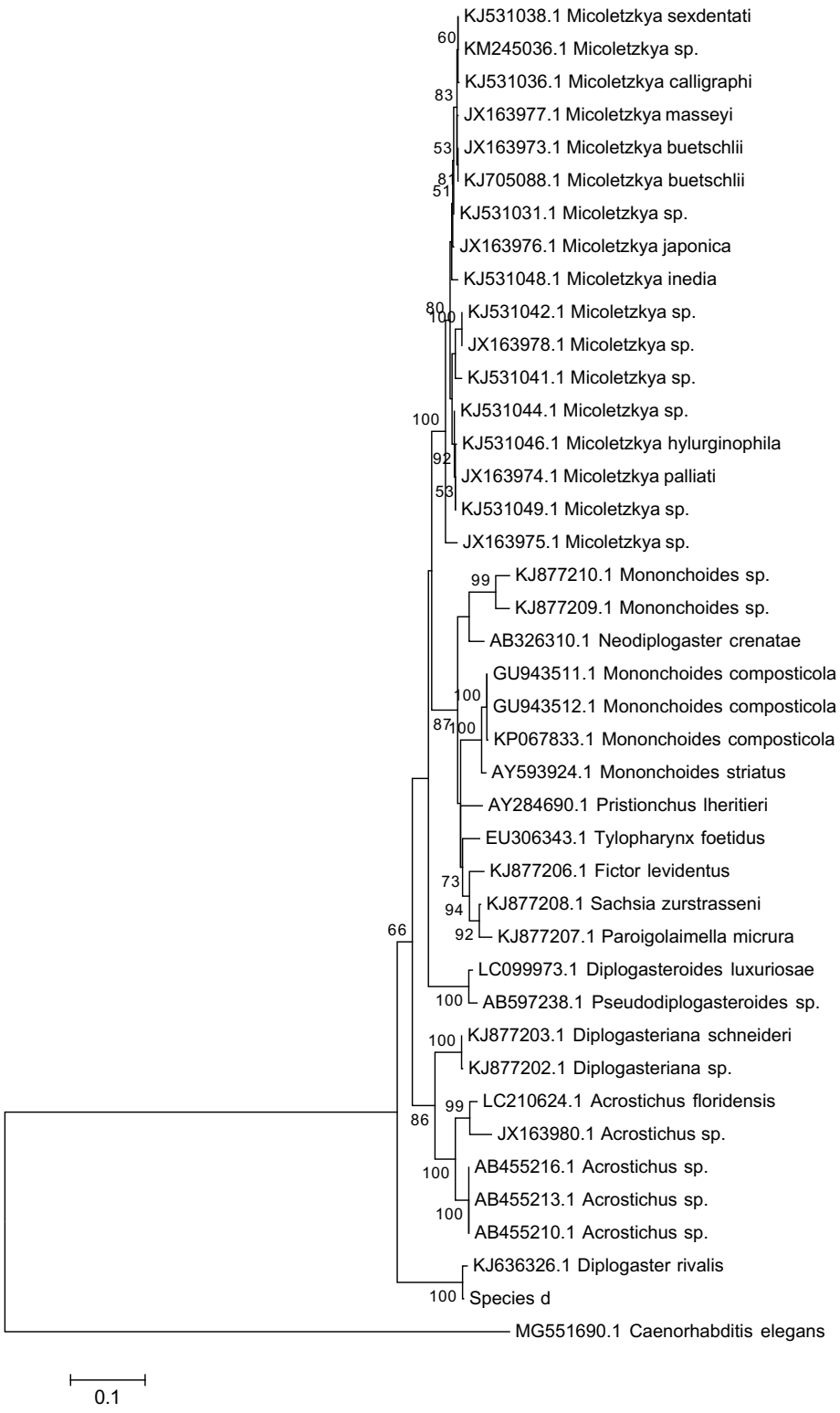


Figure A.14. Phylogenetic tree of species d (based on SSU sequence)

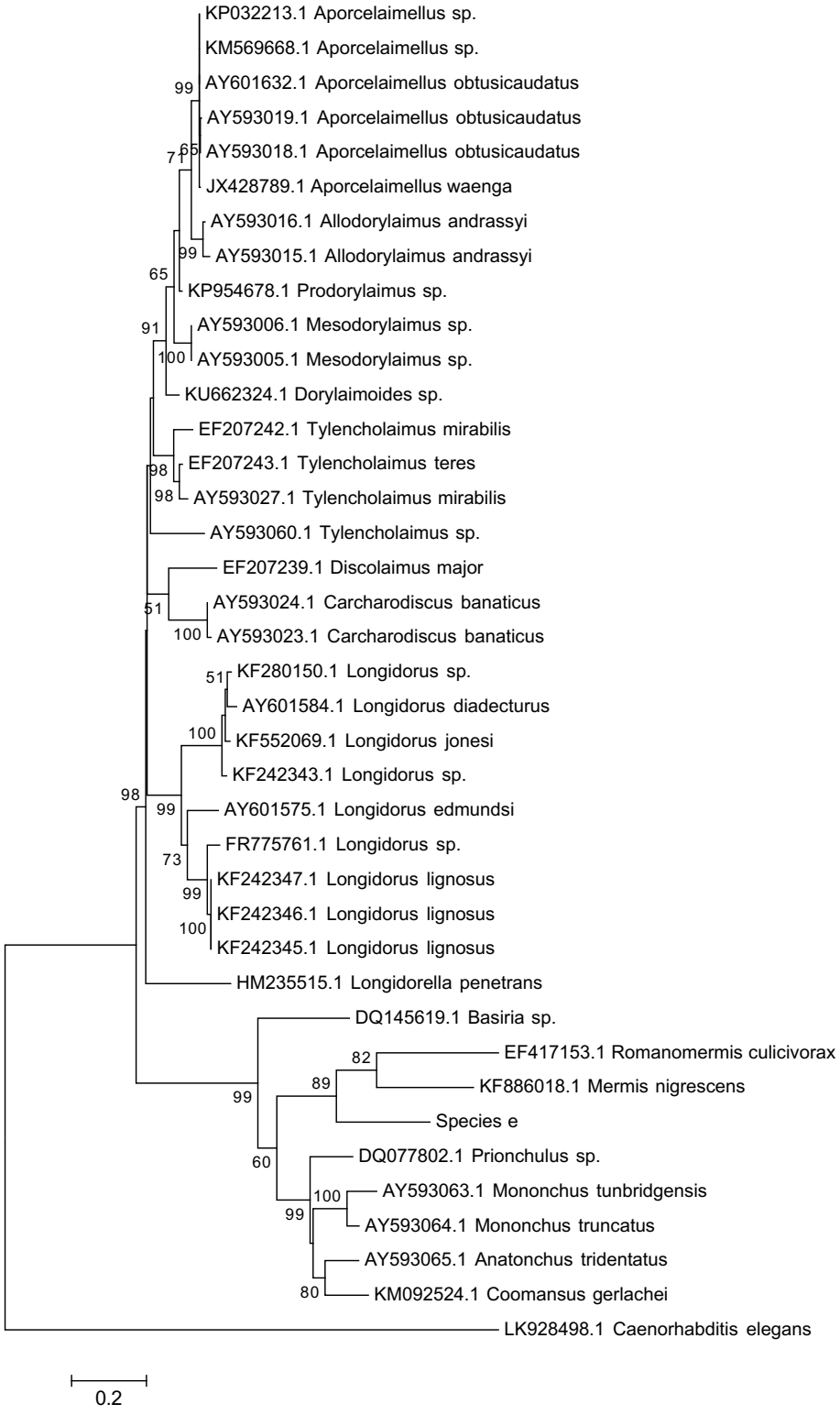


Figure A.15. Phylogenetic tree of species e (based on LSU sequence)

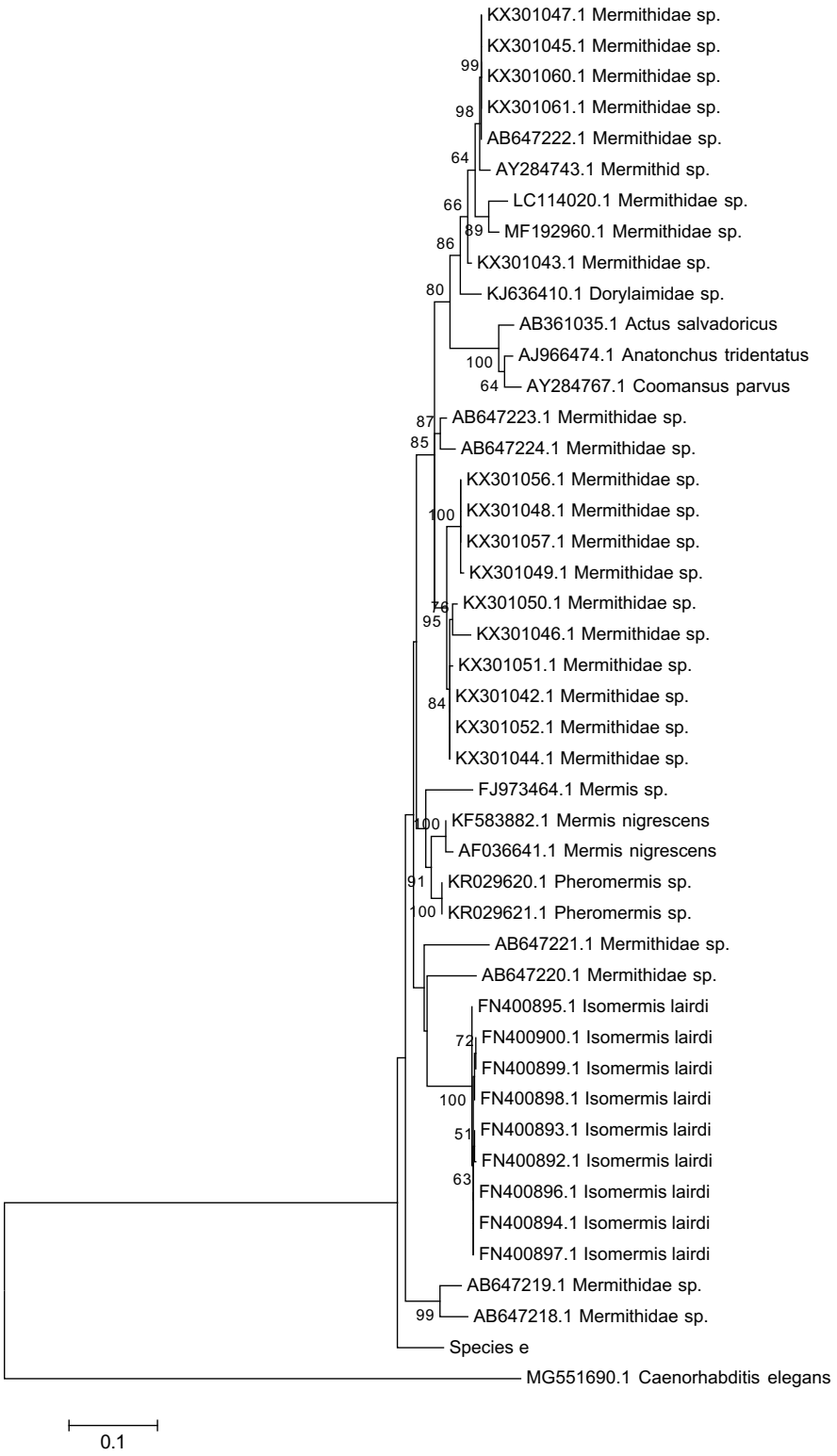


Figure A.16. Phylogenetic tree of species e (based on SSU sequence)

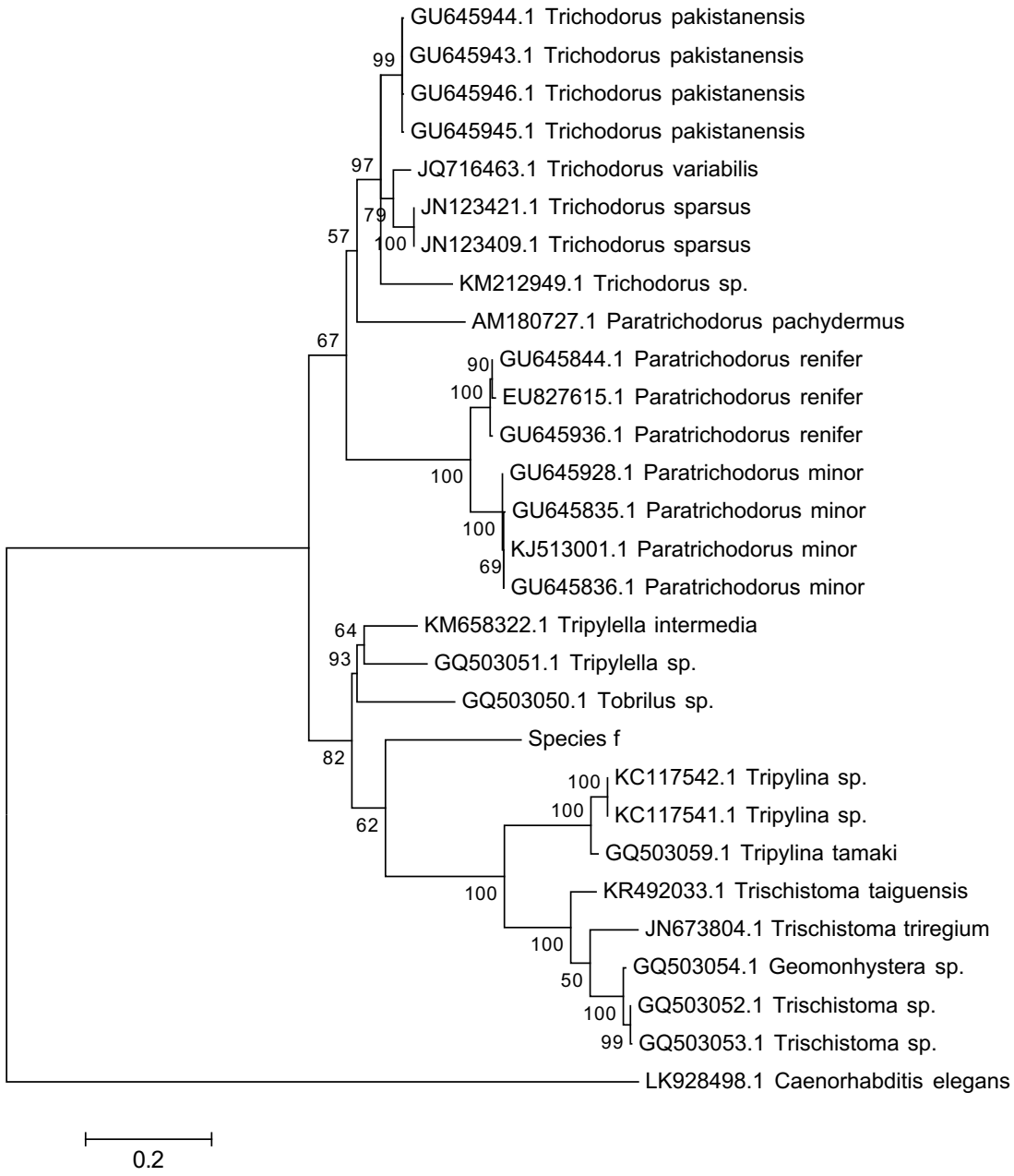


Figure A.17. Phylogenetic tree of species f (based on LSU sequence)

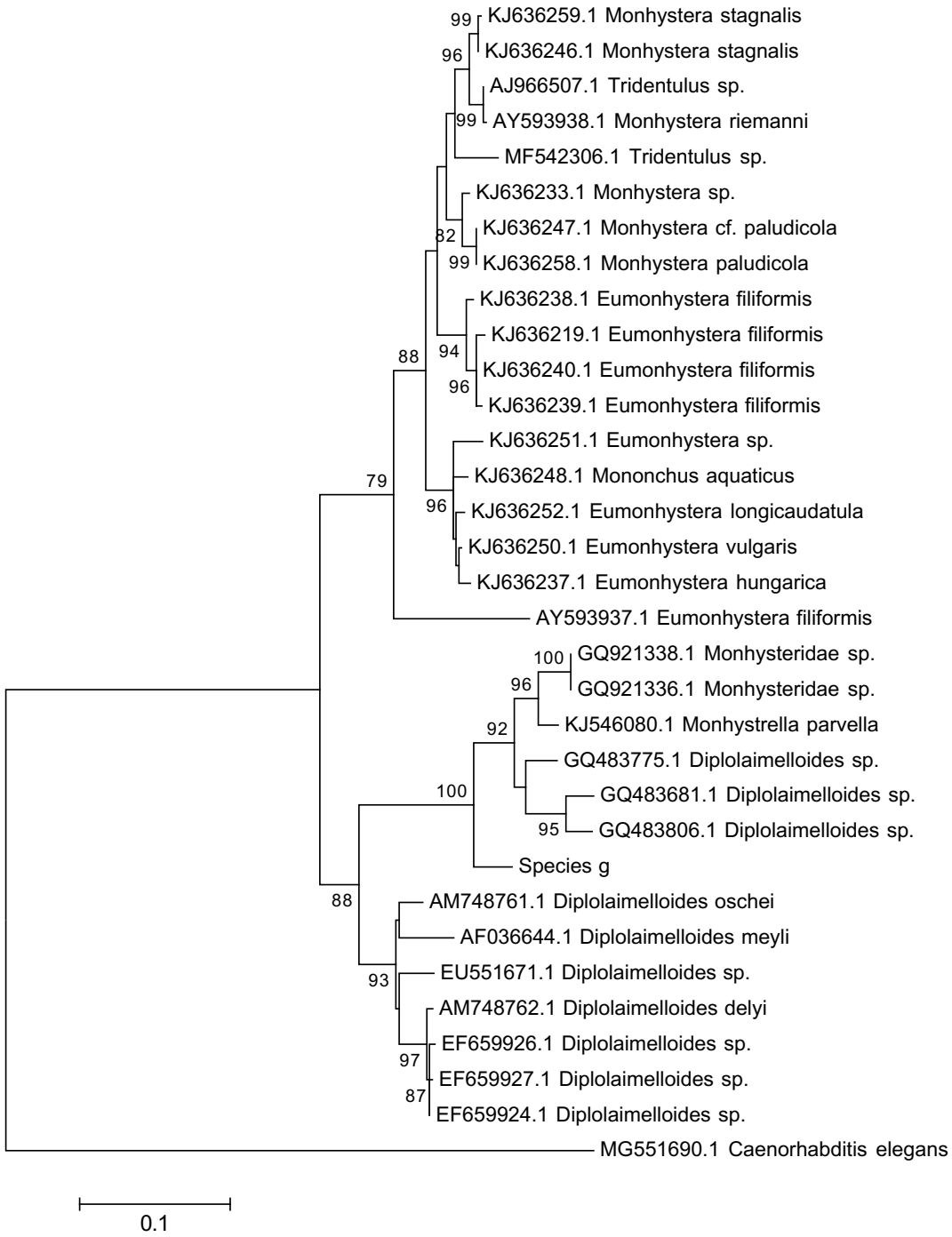


Figure A.18. Phylogenetic tree of species g (based on SSU sequence)

Figure A.19. Phylogenetic tree of species h (based on SSU sequence)

```
g       -------------------ATTACAACAGCCGTTGTTTCTTGGATCTCTTCTCTACTTGGA     42
h       ----AGCCGCGATAGCTCATTACAACAGCCGTTGTTTCTTGGATCTCTTCTCTACTTGGA     56
i       AGTGAGCCGCGATAGCTCATTACAACAGCCGTTGTTTCTTGGATCTCTTCTCTACTTGGA     60
                     ****************************************

g       TAACTGTGGTAATTCTAGAGCTAATACATGCAATCAAGCCCTGAACTTACGTGACGGGCG     102
h       TAACTGTGGTAATTCTAGAGCTAATACATGCAACCAAGCCCTGAACTTACGTGACGGGCG     116
i       TAACTGTGGTAATTCTAGAGCTAATACATGCAACCAAGCCCTGAACTTACGTGACGGGCG     120
        ******************************** ************************

g       CATTTATTAGAACAAAACCATCCGGCTTTGC--CGTGCTTTGGTGACTCTGAATAACTGA     160
h       CATTTATTAGAACAAAACCATCTGGCCTCGCGCCATCTAATGGTGACTCTGAATAACTGA     176
i       CATTTATTAGAACAAAACCATCTGGCCTCGCGCCATCTAATGGTGACTCTGAATAACTGA     180
        ********************** *** * **  * *    ********************

g       GCCGATCGCACGGGCTTGTCCCGGCGACATATCTTTCAAGTGTCTGCCTTATCAGGTTTC     220
h       GCCGATCGCACGGGCTCGTCCCGGCGACGTATCTTTCAAGTGTCTGCCTTATCAGGTTTC     236
i       GCCGATCGCACGGGCTTGTCCCGGCGACATATCTTTCAAGTGTCTGCCTTATCAGGTTTC     240
        **************** *********** ********************************

g       GTTGGCGGTTTATGTGACCGCCAAGCCTGTAACGGGTAACGGAGGATCAGGGTCTGACTC     280
h       GTTGGCGGTTTATGTGACCGCCAAGCCTGTAACGGGTAACGGAGGATCAGGGTCTGACTC     296
i       GTTGGCGGTTTATGTGACCGCCAAGCCTGTAACGGGTAACGGAGGATCAGGGTCTGACTC     300
        ************************************************************

g       CGGAGAGGGAGCCTGAGAAACGGCTACCACTTCTAAGGAAGGCAGCAGGCGCGCAAATTA     340
h       CGGAGAGGGAGCCTGAGAAACGGCTACCACTTCTAAGGAAGGCAGCAGGCGCGCAAATTA     356
i       CGGAGAGGGAGCCTGAGAAACGGCTACCACTTCTAAGGAAGGCAGCAGGCGCGCAAATTA     360
        ************************************************************

g       CCCACTCTTGGAGCAAGGAGGTAGTGACGAAAAATACCAAGGTCAGGCTCATTGAGCTTG     400
h       CCCACTCTTGGAGCAAGGAGGTAGTGACGAAAAATACCAAGGTCAGGCTCATCGAGCTTG     416
i       CCCACTCTTGGAGCAAGGAGGTAGTGACGAAAAATACCAAGGTCAGGCTCATCGAGCTTG     420
        ************************************************** *******

g       ACCATTGGAATGAGAACAATCTAAATCCTTTAACGAGGATCTAGTGGAGGGCAAGTCTGG     460
h       ACCATTGGAATGAGAACAATCTAAATCCTTTAACGAGGATCTAGTGGAGGGCAAGTCTGG     476
i       ACCATTGGAATGAGAACAATCTAAATCCTTTAACGAGGATCTAGTGGAGGGCAAGTCTGG     480
        ************************************************************

g       TGCCAGCAGCCGCGGTAATTCCAGCTCCGCAAGTGTATTCCTATACTGTTGCGTTTAAAT     520
h       TGCCAGCAGCCGCGGTAATTCCAGCTCCGCAAGTGTATTCCTATACTGTTGCGTTTAAAT     536
i       TGCCAGCAGCCGCGGTAATTCCAGCTCCGCAAGTGTATTCCTATACTGTTGCGTTTAAAT     540
        ************************************************************

g       AGCTCGTAGTTGGATCTGCGTGTTTAGCGCGTGGTGCTGCTTTTTGTGGTTACTGCGACG     580
h       AGCTCGTAGTTGGATCTGCGTGTTTAGCGCGCGGTGCTGCTTTTTGCAGTTACTGCGACG     596
i       AGCTCGTAGTTGGATCTGCGTGTTTAGCGCGCGGTGCTGCTTTTTGCAGTTACTGCGACG     600
        ******************************** **************  ************

g       CGACACAGTGTTGCTTTCTGCCACGATGCTCTCGCGAGTGTCGTGTCGATAAGCAGAGTT     640

h       CGACACAGTGTTGCTTGCTGCCACGATGCTCCCACAAGG----------------------     635
i       CGACACAGTGTTGCTTGCTGCCACGATGCTC------------------------------     631
        **************** **************

g       TACTTTGAACAAATCAGAGTGCTCAAAACGGGCGTTTCGCTCGAATGTTCTTGCATGGAA     700
h       ------------------------------------------------------------     635
i       ------------------------------------------------------------     631


g       TAATGGAATA       710
h       ----------       635
i       ----------       631
```

Figure A.20. Species g and h SSU sequence alignment

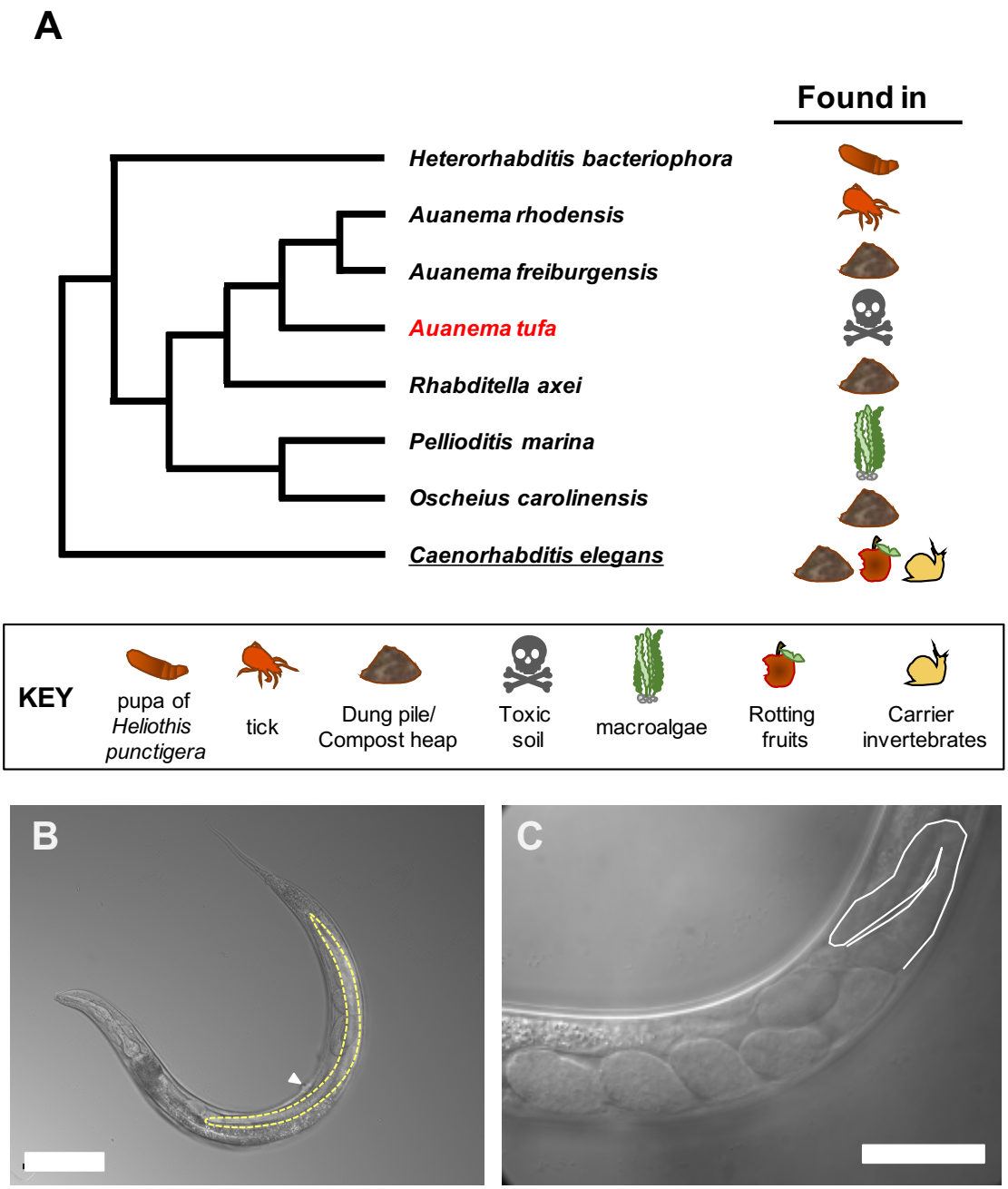


Figure A.21. Characteristics of *Auanema tufa*. (A) Simplified phylogenetic tree showing the phylogenetic relationships of *Auanema tufa* (highlighted in red) and selected Rhabditina based on SSU sequences. **(B)** One of the two arms of the *A. tufa* adult gonad. The gonad arm is outlined with white line. Scale bar: 20μm. **(C)**

The representative image of an adult *A. tufa.* The position of the vulva was indicated by the white arrow. Scale bar: 100μm

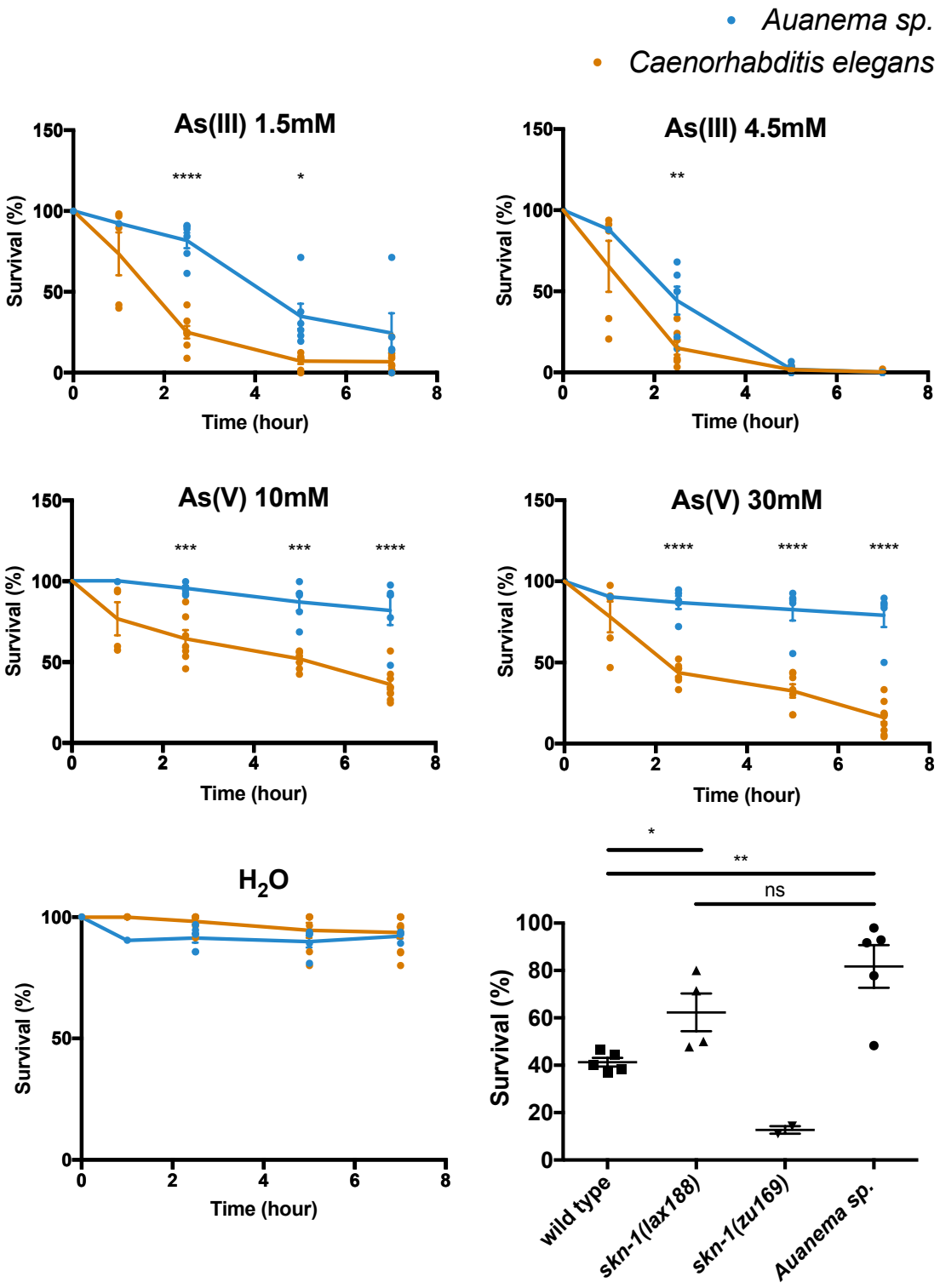

Auanema sp.
Caenorhabditis elegans
As(III) 1.5mM
As(III) 4.5mM
As(V) 10mM
As(V) 30mM
H2O
Survival (%)
Time (hour)
****
*
**
***
ns
wild type
skn-1(lax188)
skn-1(zu169)
Auanema sp.

Figure A.22. *A. tufa* is resistant to arsenic. (A-D) The survival curve of *A. tufa* (blue) and *C. elegans* (orange) in 1.5mM As(III) (A), 4.5mM As(III) (B),10mM As(V) (C), or 30 mM As(V) (D). **(E)** The survival of *Auanema sp.* (blue) and *C. elegans* (orange) in water over time. Statistics: two-way ANOVA with Bonferroni correction. "*"<0.05, "**"<0.01, "***"< 0.001, "****" <0.0001. **(F)** The survival percentage of *C. elegans,* wild-type animals, *skn-1* mutants (with gain-of-function *(lax188)* and *A. tufa* with 10mM As(V) treatment for seven hours. WT, wild-type; gf, gain-of-function. Statistics: One-way ANOVA with Tukey's post hoc test after the validation of normal distribution using the SPSS software "*" $p<0.05$. Error bars indicate the standard error of the mean.

A.7 References

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