# Report of *Neomisticius rhizomorphoides* (Rühm, 1955) Siddiqi, 1986 (Tylenchida: Anguinidae) from a cherry tree in California, USA

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**Summary.** *Neomisticius rhizomorphoides* was reported from a cherry tree in California, USA. Free-living adult and juvenile nematodes were found in tunnels made by bark beetles *Xeleborus dispar* and *Xyleborinus saxeseni*. The tunnels were infested by two species of ascomycete fungi from the genus *Ophiostoma*. Morphological and molecular characterisations of free-living adult stages of *N. rhizomorphoides* are given. Phylogenetic analysis of partial 28S rRNA gene sequences revealed that *N. rhizomorphoides* belongs to the family Anguinidae.

**Key words:** *Ophiostoma*, Paurodontidae, phylogeny, *Xeleborus dispar*, *Xyleborinus saxeseni*.

In July 2018, several branches of a cherry tree collected in Trinity County, California, USA and infected by bark beetles were received by the Plant Pest Diagnostic Centre of California Department of Food and Agriculture, Sacramento. Analysis of this wood sample revealed the presence of numerous nematodes inhabiting bark tunnels. Based on morphology this nematode was identified as *Neomisticius rhizomorphoides* (Rühm, 1955) Siddiqi, 1986. The genus *Neomisticius* belongs to the subfamily Paurodontinae Thorne, 1941, family Paurodontidae Thorne, 1941, superfamily Sphaerularioidea Lubbock, 1861 (Poinar, 1975), suborder Hexatylina Siddiqi, 1980 of the order Tylenchida Thorne, 1949 according to Siddiqi (2000). The genus *Neomisticius* contains only one species. The present study provides a short morphological and molecular characterisation of free-living adult stages of *N. rhizomorphoides* and a position of this species within the order Tylenchida based on the partial 28S rRNA gene sequences.

## MATERIAL AND METHODS

**Nematode sample.** Wood samples were collected from a cherry tree (*Prunus* sp.) growing in an orchard in Junction City, Trinity County, California, USA. Nematodes were extracted by placing small pieces of tree branches into a Petri dish with distilled water. Measurements and light micrographs were taken of live specimens with an automatic Infinity 2 camera attached to a compound Olympus BX51 microscope equipped with Nomarski interference contrast.

**DNA extraction, PCR, sequencing and phylogenetic analysis.** DNA was extracted from several specimens using the proteinase K protocol. DNA extraction, PCR and cloning protocols were used as described by Tanha Maafi *et al.* (2003). The following primer set was used for PCR: the forward D2A (5'-ACA AGT ACC GTG AGG GAA AGT TG-3') and the reverse D3B (5'-TCG GAA GGA ACC AGC TAC TA-3') primers (Subbotin *et al.*, 2006) for amplification of the D2-D3 expansion segments of 28S rRNA gene and the forward TW81 (5'-GTT TCC GTA GGT GAA CCT GC-3') and the reverse AB28 (5'-ATA TGC TTA AGT TCA GCG GGT-3') primer (Tanha Maafi *et al.*, 2003) for amplification of the ITS1-5.8-ITS2 rRNA gene. The D2-D3 of 28S rRNA PCR product was purified using QIAquick PCR purification kit (Qiagen, USA) and submitted for direct sequencing, whereas the ITS rRNA PCR product was purified using QIAquick Gel extraction kit (Qiagen), cloned into the pGEM-T vector and transformed into JM109 High Efficiency Competent Cells (Promega, USA).

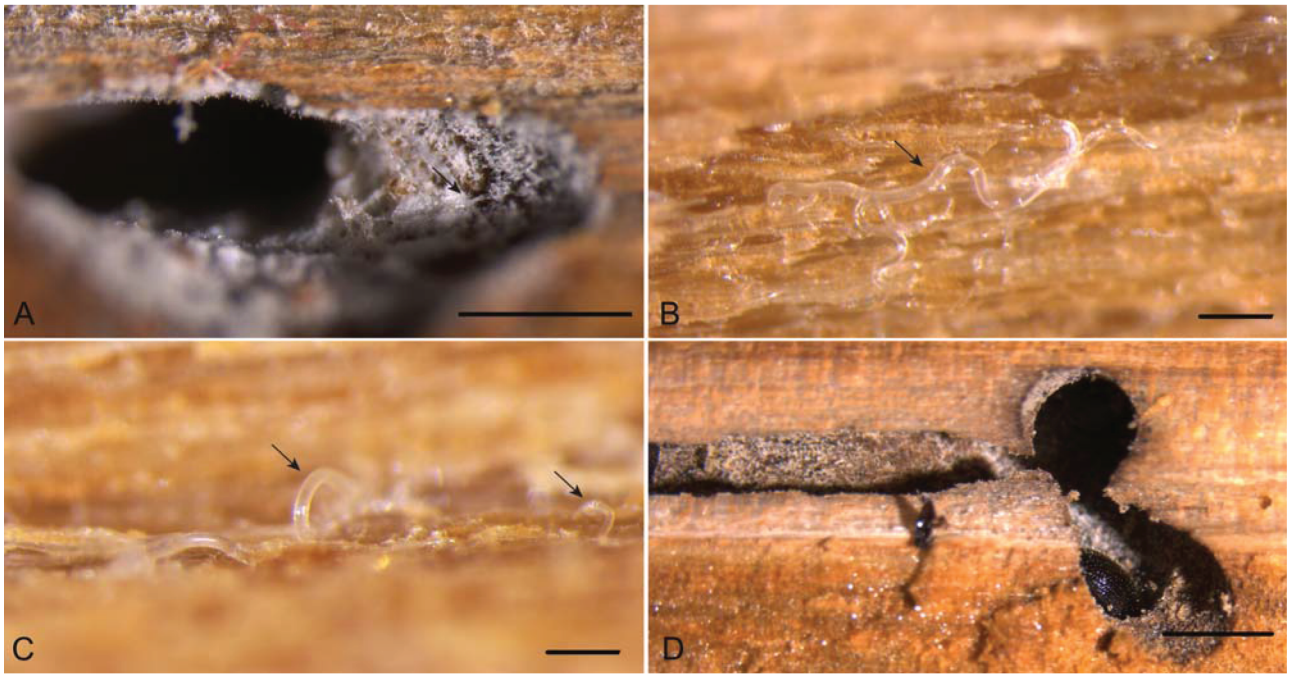


**Fig. 1.** A & D: Bark tunnels in cherry wood; B & C: Nematodes on wood surface. Arrows indicate nematode specimens. Scale bars: A & D – 1 mm; B & C – 100 µm.

The ITS clone was submitted to PCR with same primers and then sequenced. Sequencing was conducted at Quintara Biosciences (CA, USA). The newly obtained sequences were submitted to the GenBank database under accession numbers: MK929283 (D2-D3 expansion fragments of 28S rRNA) and MK929281 (ITS rRNA).

The new sequence of the D2-D3 of 28S rRNA gene was aligned using ClustalX 1.83 (Thompson *et al.*, 1997) (multiple alignment parameters: gap opening – 5 and gap extension – 3) with published gene sequences of related genera (Chizhov *et al.*, 2012; Yaghoubi *et al.*, 2014, 2018; Esmaeili *et al.*, 2017, 2018; Mobasseri *et al.*, 2017). Outgroup taxa for each dataset were chosen based on previously published data (Subbotin *et al.*, 2006). Gblock version 0.91b was used to eliminate poorly aligned positions and divergent regions of an alignment (Talavera & Castresana, 2007). Sequence alignment was analysed with Bayesian inference (BI) using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) under the GTR + G + I model. BI analysis for each gene was initiated with a random starting tree and was run with four chains for $1.0 \times 10^6$ generations. Two runs were performed for each analysis. The Markov chains were sampled at intervals of 100 generations. After discarding burn-in samples (10%), a 50% majority rule consensus tree was generated. Posterior probabilities (PP) in percentage are given on appropriate clades.

## RESULTS AND DISCUSSION

### *Neomisticius rhizomorphoides* (Rühm, 1955) Siddiqi, 1986 (Figs 1-3)

**Free-living females** (n = 4): L = 1431.8 ± 231.8 (1137.5-1670) µm; maximum body width = 25.6 ± 2.4 (22.5-27.5) µm; a = 56.2 ± 10.6 (45.5-68.9); b = 7.9 ± 4.6 (4.9-10.3); c = 50.7 ± 0.9 (49.8-51.6); V = 90.8 ± 1.2 (89.4-91.9)%; stylet = 11.7 ± 0.9 (10.6-12.5) µm; tail = 26.6 ± 3.8 (22.5-30) µm.

**Free-living males** (n = 6): L = 976.3 ± 135.5 (770-1137.5) µm; maximum body width = 24.4 ± 1.0 (23.8-26.3) µm; a = 40.0 ± 5.1 (32.4-47.9); b = 8.1 ± 1.0 (6.7-9.3); c = 32.4 ± 5.7 (28-43.3); stylet = 12.5 ± 0.4 (11.9-13.1) µm; tail = 30.4 ± 3.5 (26.3-35) µm; spicules = 21.3 ± 1.6 (18.8-22.5) µm; gubernaculum = 7.6 ± 0.3 (7.5-8.1) µm.

**Free-living female.** Body long. Cuticle finely annulated. Lip region low and smooth. Stylet with small knobs. Orifice of dorsal pharyngeal gland very close to stylet knobs. Procorpus cylindroid, metacorpus weakly developed, isthmus narrow and basal bulb large. No basal bulb extension projecting into lumen of intestine observed. Excretory pore near base of stylet. Postvulval uterine sac large. Female tail subcylindrical, sometimes with a short terminal process.

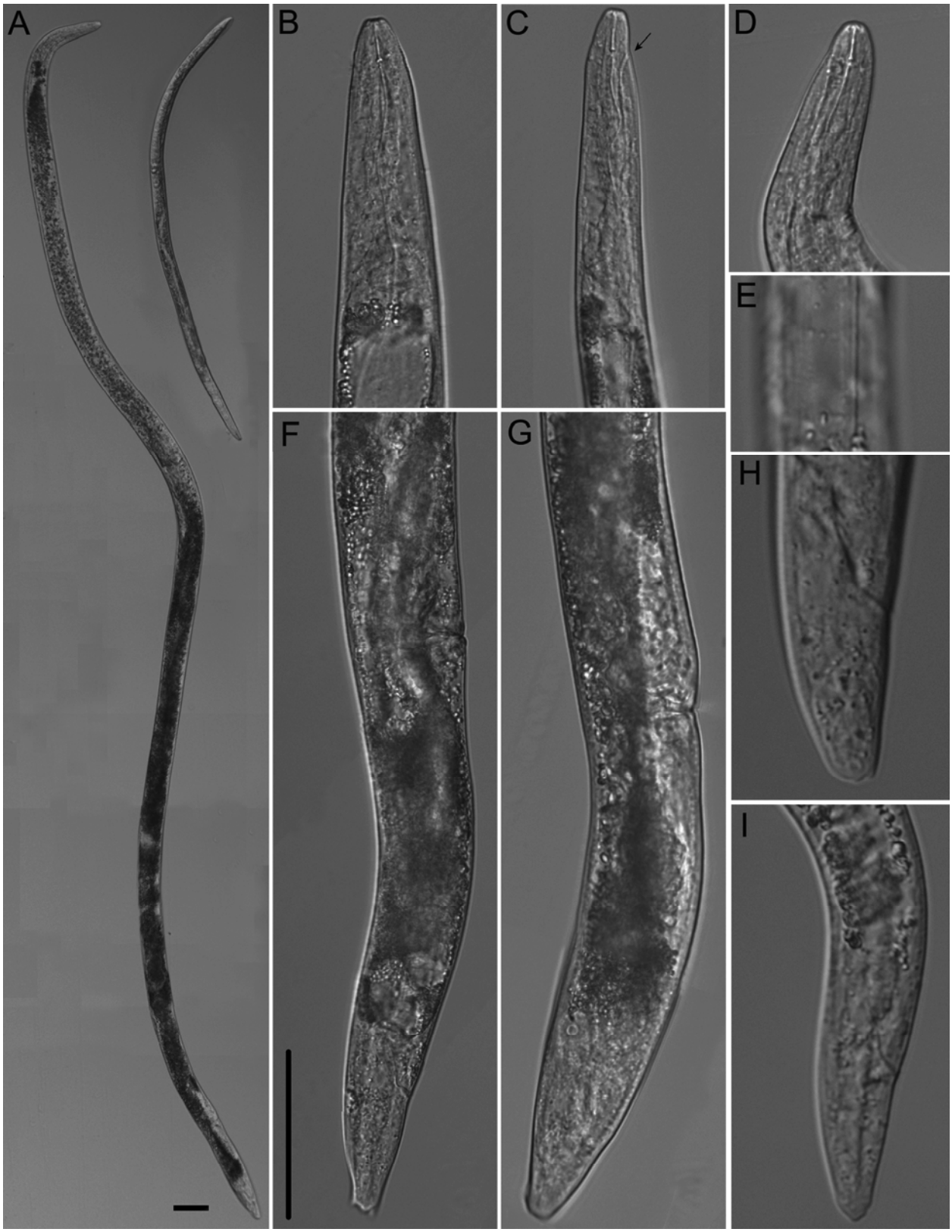


**Fig. 2.** *Neomisticius rhizomorphoides*. Adults. A: Female and male, whole bodies; B-D: Anterior region of females; F, G, H, I: Posterior region of females, E: Lateral field of female. Arrow indicates excretory pore. Scale bars: 20 µm.

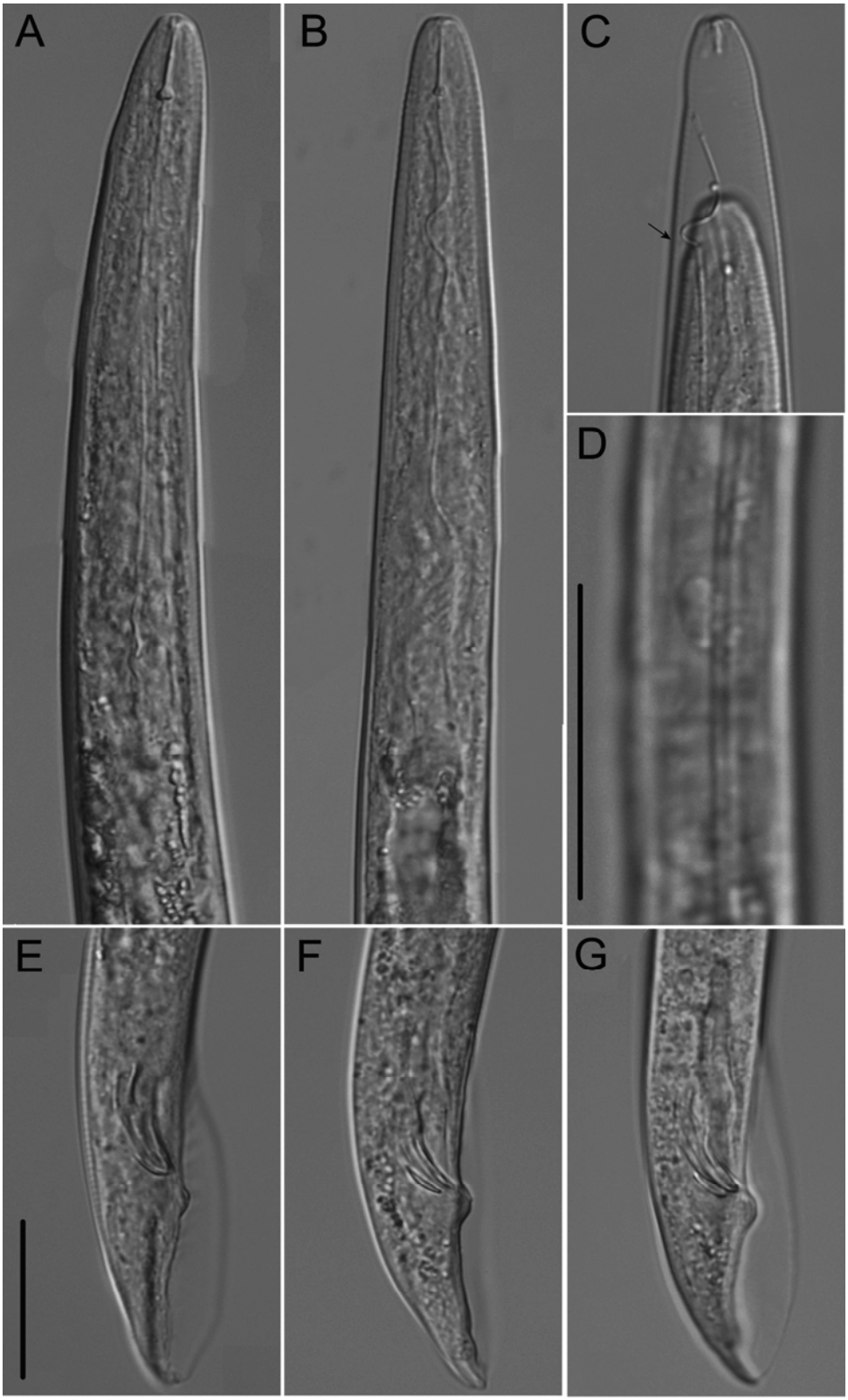


**Fig. 3.** *Neomisticius rhizomorphoides*. Male and male juvenile. A & B: Anterior region of males; C: Anterior region of male juvenile; D: Lateral field of male; E-G: Posterior region of males. Arrow indicates excretory pore. Scale bars: 20 μm.

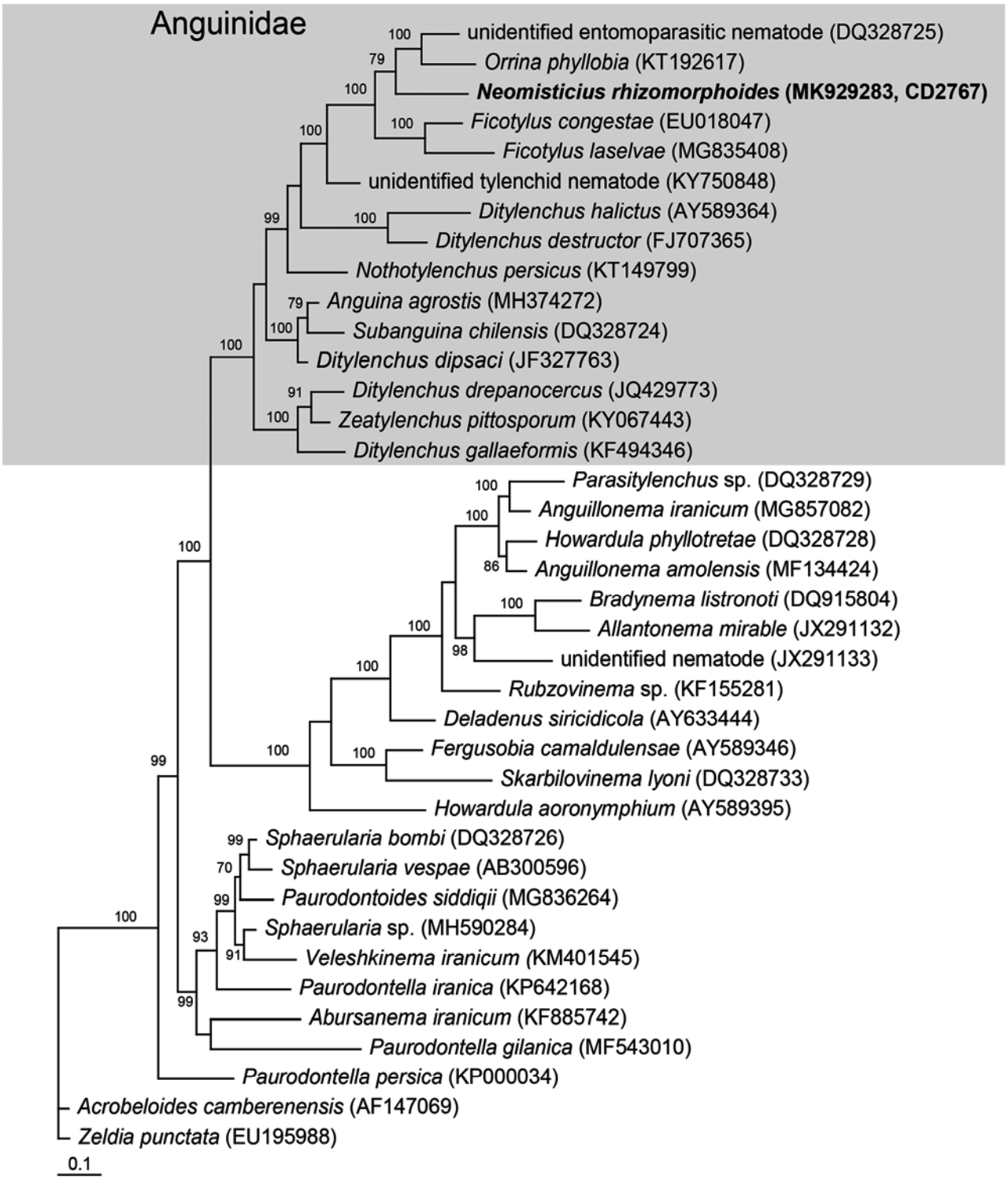


**Fig. 4.** Phylogenetic relationships within Anguinidae and Hexatylina. Bayesian 50% majority rule consensus tree from two runs as inferred from analysis of the D2-D3 of 28S rRNA gene sequence alignment under the GTR + I + G model. Posterior probabilities equal or more than 70% are given for appropriate clades. Original sequence of *Neomisticius rhizomorphoides* is indicated by bold font.

**Free-living males.** Body shorter than in female. General morphology similar to female. Excretory pore near base of stylet. Lateral field with two lines. Testis single, anteriorly outstretched. Spicules paired, ventrally arcuate. Gubernaculum simple, arcuate. Bursa large and covering the entire tail.

**Location and habit.** Nematode adults and juveniles were found in bark tunnels (Fig. 3) of cherry tree collected in Trinity County, California, USA. The tunnels made by bark beetles *Xeleborus dispar* (Fabricius 1792) and *Xyleborinus saxeseni* (Ratzeburg 1837) (family Curculionidae) (Dr Alexey K. Tishechkin, pers. comm.) were infested by two species of ascomycete fungi from the genus *Ophiostoma* (Dr Suzanne Rooney Latham, pers. comm.).

**Molecular characterisation and phylogenetic position.** Phylogenetic position of *N. rhizomorphoides* within the Tylenchida obtained from the analysis of the D2-D3 of 28S rRNA gene sequence using the BI is given in Fig. 4. In the partial 28S rRNA gene phylogenetic tree, *N. rhizomorphoides* was nested within Anguinidae and it was a sister taxon to *Orrina phyllobia* and an unidentified entomoparasitic nematode, although the statistical support for such relationships was low. BLAST search of the ITS rRNA gene sequence of *N. rhizomorphoides* revealed highest similarity with those of *Orrina phyllobia* (similarity – 79.2%, coverage – 42%) and *Ditylenchus persicus* (79.9% and 39%).

*Neomisticius rhizomorphoides* was described by Rühm (1955) in Germany as a representative of the genus *Anguillonema*, *A. rhizomorphoides*. Different stages of this nematode were discovered from the body cavity and genitalia of the beetle *Xyleborus dryographus* (Ratzeburg, 1837) (family Curculionidae) and from bark tunnels, which were infested with fungal mycelium. Nematode eggs and other stages have been found in large numbers in layers of fungal mycelium (Sumenkova, 1975). The genus *Anguillonema* was poorly described by Fuchs (1938) and never correctly re-described in spite of the works of Rühm (1956) and Massey (1974). Golden (1971), Sumenkova (1975) and Andrássy (1976) placed this genus in Misticiinae (Paurodontidae) (Fortuner & Raski, 1987). Siddiqi (1986) erected a new genus *Neomisticius* for *A. rhizomorphoides*. He described the genus with stem-like basal extension. The base of the pharynx of *A. rhizomorphoides* was not described in the original publication by Rühm (1955) (Fortuner & Raski, 1987). *Neomisticius* differed from *Misticius* in having different tail shape and bursa enveloping entire tail. Fortuner and Raski (1987) noticed that *N. rhizonzorphoides* was very similar to *Sychnotylenchus* (Anguinidae) by anterior position of excretory pore, cylindroid female tail with rounded end, caudal alae enveloping male tail, and to *Stictylus* (synonym of *Prothallonema*, Sphaerulariidae *sensu* Siddiqi, 2000) by the corpus of the pharynx but without a stem at the posterior end of the glands. These authors considered *N. rhizomorphoides* as *species inquirendum*.

In the family Paurodontidae Siddiqi (2000) included seven genera: *Paurodontus* Thorne, 1941, *Bealius* Massey & Hinds, 1970, *Luella* Massey, 1974, *Misticius* Massey, 1967, *Neomisticius*, *Paurodontella* Husain & Khan, 1968 and *Paurodontoides* Jairajpuri & Siddiqi, 1969. Siddiqi (2000) questioned the validity of Paurodontidae and believed that most probably this family was junior synonym of Sphaerulariidae, since the type genus and other genera included in it were morphologically similar and were suspected of having similar life cycles to members of the latter group. Andrássy (2007) agreed and placed *Neomisticius* and other genera in the family Sphaerulariidae Lubbock, 1861 and thesubfamily Paurodontinae Thorne, 1941. Recent phylogenetic analysis studies using 18S and partial 28S rRNA gene sequences confirmed that some species of *Paurodontella* (Yaghoubi *et al.*, 2018) clustered with representatives of Sphaerulariidae; however, the positions of other species are still uncertain (Esmaeili *et al.*, 2017). Thus, the revision of some genera of this family is still needed. It should be also noticed that the name of Paurodontidae was already used by Marsh (1887) for a family of Late Jurassic to Early Cretaceous mammals in the order Dryolestida.

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**С.А. Субботин и В.Н. Чижов.** Сообщение о *Neomisticius rhizomorphoides* (Rühm, 1955) Siddiqi, 1986 (Tylenchida: Anguinidae) из вишневого дерева в Калифорнии, США.

**Резюме.** *Neomisticius rhizomorphoides* был выделен из вишневого дерева в Калифорнии, США. Свободноживущие взрослые стадии и личинки нематоды были обнаружены в туннелях, сделанных короедами: *Xeleborus dispar* и *Xyleborinus saxeseni*. Туннели были заражены двумя видами грибов аскомицетов из рода *Ophiostoma*. Даны морфологические и молекулярные характеристики свободноживущих взрослых стадий этой нематоды. Филогенетический анализ последовательностей гена 28S рРНК показал, что *N. rhizomorphoides* принадлежит к семейству Anguinidae.