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Incidence of *Oscheius onirici* (Nematoda: Rhabditidae), a potentially entomopathogenic nematode from the marshlands of Wisconsin, USA

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Abstract

In a search for an entomopathogenic nematode to control cranberry insect pests, three *Oscheius* populations (Rhabditidae) were recovered through the *Galleria*-bait method from one sample taken in a wild cranberry marsh in Jackson County, Wisconsin, USA. Morphological studies with light microscopy and scanning electron microscopy, as well as molecular analyses of the near-full-length small subunit rDNA gene, D2/D3 expansion segments of the large subunit rDNA gene, internal transcribed spacer, and mitochondrial cytochrome oxidase subunit 1 (Coxl) genes revealed this as *Oscheius onirici,* a species recently described from a karst cave soil of central Italy. The species belongs to the *dolichura*-group and is characterized by its DNA sequences; hermaphroditic reproduction; and males not found. A *Bacillus*-like bacterium appears to be associated with this nematode based on our microscopic and SEM observations; however its identity and persistent association with the nematode has not been confirmed. Nonetheless, this nematode is capable of infecting and killing the sparganothis fruitworm *Sparganothis sulfureana* Clemens (Lepidoptera: Tortricidae), the brown-banded cockroach *Supella longipalpa* Fabricius (Blattodea: Ectobiidae), and the cranberry fruitworm *Acrobasis vaccinii* Riley (Lepidoptera: Pyralidae), under laboratory conditions, and each in less than 72 hr. The mealworm *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae) and the greater wax moth *Galleria mellonella* Linnaeus (Lepidoptera: Pyralidae), are also susceptible, but take 3.5 and 5.2 days to die, respectively. This species is a new potential bio-control agent on insects.

Key words

Cranberry, characterization, DNA sequencing, entomopathogenic nematode, *Dolichura*-group, molecular phylogeny, morphology, rDNA.

Oscheius Andrassy, 1976 belongs to the family Rhabditidae. These bacteria-feeding nematodes live in close association with insects (Stock et al., 2005; Ye et al., 2010; Campos-Herrera et al., 2015; Torrini et al., 2015). *Oscheius* was divided into two groups: *Insectivora* and *Dolichura* (Sudhaus and Hooper, 1994). Species of the *Insectivora* group have crochet-like needle-shaped spicules, leptoderan bursa and common rectum *versus* normal spicules, peloderan bursa and expansile rectum in *Dolichura* group (Sudhaus and Hooper, 1994). So far, 43 species were described in *Oscheius* with 29 in *Insectivora* and 14 in *Dolichura* (Tabassum et al., 2016).

Nematodes can be considered as entomopathogenic if they fulfill the following criteria for entomopathogenicity: bearing a pathogenic bacterium within a dauer (also known as infective) juvenile nematode, releasing the bacterium within the host, active host-seeking and -penetration by dauer juveniles, rapid insect death, nematode and bacterial reproduction, reassociation of the pathogenic bacteria with new generations of dauer juveniles; and emergence of IJs from the cadaver so that the cycle can be repeated (Dillman et al., 2012). Only seven species in *Insectivora* group including *O. amsactae* (Ali et al., 2011), *O. carolinensis* (Ye et al., 2010), *O. chongminensis* (Zhang et al., 2008), *O. microvilli* (Zhou et al., 2017), *O. niazii* (Tabassum and Shahina, 2010), *O. rugaoensis* (Zhang et al., 2012), *O. siddiqii* (Tabassum and Shahina, 2010) and one species in *Dolichura* group namely *O. onirici* (Torrini et al., 2015) were recognized as entomopathogenic. Phylogenetic analysis based on ribosomal DNA sequences revealed these two groups are monophyletic (Ye et al., 2010, Torrini et al., 2015). *Heterorhabditidoides* was proposed as a new genus (Zhang *et al*., 2008), but it was considered as a junior synonym of *Oscheius* (Ye et al., 2010) and it is in general not accepted as a valid genus (Liu et al., 2012; Campos-Herrera et al., 2015; Torrini et al., 2015; Tabassum et al., 2016; Valizadeh et al., 2017). Another species, *Heterorhabditidoides rugaoensis,* was also transferred to *Oscheius* (Darsouei et al., 2014; Tabassum et al., 2016).

Nematodes of the genera *Steinernema* and *Heterorhabditis*, are often entomopathogenic, and as such, they have been used as bio-control agents to suppress pest populations (Kaya and Gaugler, 1993). These nematodes have been found on every continent except for Antarctica, and are lethal to a wide variety of insect pests (Poinar and Thomas, 1984). For bio-control programs, nematodes represent particularly promising agents because they can be readily applied to crops using standard spray equipment. In the cranberry marshlands of Washington, for example, entomopathogenic nematodes (EPNs) have been used to control the black vine weevil *Otiorhynchus sulcatus* Fabricius (Coleoptera: Curculionidae) (Booth et al., 2002). In the bogs of Massachusetts, populations of the strawberry root weevil have been suppressed via applications of *Heterorhabditis bacteriophora* Poinar, 1976 (Simser and Roberts 1994). Typically, commercially available EPN are found in the genera *Heterorhabditis* or *Steinernema*; however, recent discoveries of nematodes in North Carolina, USA (Ye et al., 2010), Switzerland (Campos-Herrera et al., 2015, Jaffuel et al., 2016) and Italy (Torrini et al., 2015) have shown that nematodes in the genus *Oscheius* can sometimes be entomopathogenic.

A survey of the EPN fauna was initiated in Wisconsin during the summer of 2015, then repeated in 2016. This survey was conducted using a previously employed *Galleria*-bait method (Orozco et al., 2014). A total of three cadavers were recovered ($n=540$ wax worms used during each of the seasons), all from 2015. The EPN that emerged from them were maintained in culture. They appeared as hermaphroditic without males. DNA sequencing was performed to identify this population and it is identical to two hermaphroditic populations of PS2068 from Oregon, USA and JU179 from France (Felix et al., 2001) and it has minor differences from a recently described species *O. onirici* from a karst cave soil of central Italy. This paper presented the characterization of this nematode through combined approaches by morphological and molecular analysis to determine its identity and tested its entomopathogenicity.

Materials and methods

Isolation of EPN from cranberry marshes

Counties with commercial cranberry operations were chosen for sampling efforts. Sites were further refined by selecting locations that also had accessible wild cranberry populations. Based on these criteria, two sites were chosen in Jackson County and one was chosen in Warren County in Wisconsin.

Once sites were established, a modified method described in Orozco et al. (2014) was used to sample them. Briefly, a four square-meter grid was established around the first wild cranberry plant encountered at each site. The grid was randomly sampled three times by taking an approximately 200 cm^3 volume of substrate from the surface of the soil to a depth of about 15 cm. This sample was placed in a plastic bag. The process was repeated twice, within each site, on each sampling date. Sites were sampled during the first week of May, June, July, and August 2015.

Although sites were located in different geographic locations, there were some similar and noteworthy conditions present in each sampling grid. The presence of *Sphagnum* moss (Sphagnales: Sphagnaceae) was unmistakable in all sampling grids, with cranberry plants growing out of hummocks formed by this plant. Peat could be found approximately 30 centimeters below the moss. Plants like leatherleaf, *Chamaedaphne* sp. (Ericales: Ericaceae), sedges (Poales: Cyperaceae), and bog birch, *Betula pumila*

(Fagales: Betulaceae) were typically located at the sampling sites. These plants are typical of the peatland habitats that support cranberry plants (Rydin and Jeglend, 2006).

Upon returning to the laboratory, EPN were extracted with the insect baiting technique as described in Orozco et al. (2014). Each soil sample was baited by placing six fifth instar wax worms *Galleria mellonella* Linnaeus (Lepidoptera: Pyralidae) into the plastic bags containing the sampled soil, which were stored at room temperature in the dark for a week. Afterwards, bags were checked daily until wax worms began showing signs of infection, at which point, the insects were removed from the bags, rinsed with water, and stored in modified White traps (Orozco et al., 2014) until EPN emerged. After emergence, EPN from each cadaver were stored separately in water, within tissue flasks, in drawers.

Nematodes were maintained by inoculating sparganothis fruitworms, which were reared in the laboratory on Stonefly Heliothis Diet®, made available by Ward's Science. Over ten inoculations in sparganothis larvae took place between the time that the nematodes were collected and the time that they were submitted for sequencing. Furthermore, the nematodes that were sent for morphological observation had been collected from sparganothis larvae before they were shipped to the Nematode Assay Section of the Agronomic Division, at the North Carolina Department of Agriculture & Consumer Services for further study.

Morphological observation

Nematodes were heat killed and fixed in 4% formalin. The nematodes were processed to glycerin by a modification of a glycerin-ethanol series of Seinhorst's rapid method (Seinhorst, 1959) and permanently mounted on 25 × 75 mm^2 microscope slides. Specimens were examined with a Leica DM2500 and Zeiss Imager.A2 compound microscope with interference contrast at up to 1,000× magnification. Stoma terminology by De Ley et al. (1995) and Sudhaus and Fitch (2001) was employed for stoma description. Drawings and measurements were made using a drawing tube. Live nematodes were heat killed in temporary water mounts for all measurements and microphotographs to assure quality and accuracy. Morphometric data were processed using Excel software (Ye, 1996).

Scanning electron microscopy

Nematodes in distilled water were transferred by pipette to modified BEEM capsules whose ends were cut open and fitted with 20-µm nylon mesh to hold the specimens through the entire preparation procedure (Bozzola and Russell, 1999). The capped capsules were then transferred to a jar containing 3% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, at 4°C. Samples were washed in three changes of the same buffer; post-fixed in 2% osmium tetroxide in 0.1 M sodium cacodylate buffer; pH 7.2, for 16 hr at 4°C in the dark in a refrigerator; washed in three more changes of the same buffer and then dehydrated through a graded ethanol series to 100%, 30 min per change, all on ice. Samples were allowed to come to room temperature and given two changes of 100% ethanol. Capsules containing the samples were then critical-point-dried in liquid CO_2 (Samdri-795, Tousimis Research Corp., Rockville, MD), mounted on aluminum stubs with double-stick tape, sputter-coated with 50 Angstroms of gold-palladium (Hummer 6.2, Anatech USA, Hayward, CA) and viewed using a JEOL JSM-5900LV (JEOL USA, Peabody, MA) at 10KV at the Center for Electron Microscopy, North Carolina State University.

Molecular analyses

Three populations of nematodes collected from different insect larvae were used for molecular study. Extraction, PCR and sequencing of DNA were conducted separately for each population to account for DNA polymorphism among nematodes originating from different insects.

There were initial suspicions that the three populations represented different species of nematodes, because the original cadavers that were collected from the soil were red, grey and tan. Ten nematodes from each population were picked into distilled water and their morphological identity was confirmed with light microscopy before being placed to 10 µl AE buffer (10 mM Tris-Cl, 0.5 mM EDTA; pH9.0) on a glass microscope slide, then macerated with a pipette tip and collected in final volume of 50 µl AE buffer. DNA samples were stored at –20°C until used as a PCR template. Different sets of primers were used in the PCR reactions. Primers for small subunit amplification were forward primer 18S-G18S4 (5′ GCTTGTCTCAAAGATTAAGCC 3′) and reverse primer 18S-18P (5′ TGATCCWKCYGCAGGTTCAC 3′) (De Ley et al., 2002; Dorris et al., 2002), forward primer SSUF07 (5′ AAAGATTAAGCCATGCATG 3′) and reverse primer SSUR26 (5′ CATTCTTGGCAAATGCTTTCG 3′) (Floyd et al., 2002), forward primer 18s965 (5′ GGCGATCAGATACCGCCCTAGTT 3′) and reverse primer 18s1573R (5′ TACAAAGGGCAGGGACGTAAT 3′) (Mullin et al.,

2005), and forward primer 18SnF (5′-TGGATAACTGTGGTAATTCTAGAGC-3′) and reverse primer 18SnR (5′-TTACGACTTTTGCCCGGTTC-3′) (Kanzaki and Futai, 2002). Primers for large subunit D2/D3 amplification were forward primer D2a (5′ ACAAGTACCGTGAGGGAAAGT 3′) and reverse primer D3b (5′TGCGAAGGAACCAGCTACTA3′) (Nunn, 1992). Primers for ITS1 amplification were forward primer rDNA2 (5′ TTGATTACGTTCCCTGCCCTTT 3′) (Vrain et al., 1992) and reverse primer rDNA1.58S (5′ ACGAGCCGAGTGATCCACCG 3′) (Cherry et al., 1997), Primers for CoxI amplification were forward primer COI-F1 (5′ CCTACTATGATTGGTGGTTTTGGTAATTG 3′) and reverse primer COI-R2 (5′ GTAGCAGCAGTAAAATAAGCACG 3′) (Kanzaki and Futai, 2002).

The 25 µl PCR was performed using Apex *Taq* Red Master Mix DNA polymerase (Genesee Scientific Corporation, San Diego, CA) according to the manufacturer's protocol. The thermal cycler program for PCR was as follows: denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 45 s, and extension at 72°C for 2 min. A final extension was performed at 72°C for 10 min (Ye et al., 2007). PCR products were cleaned using ExoSap-IT (Affymetrix, Inc., Santa Clara, CA) according to the manufacturer's protocol and were sequenced by Genomic Sciences Laborator in North Carolina State University using an Applied Biosystems 3730 XL DNA Analyzer (Life Technologies, Carlsbad, CA).

The sequences were deposited into the GenBank database. DNA sequences were aligned by KAlign (Lassmann and Sonnhammer, 2005. www.ebi.ac.uk/Tools/msa/kalign/) using default settings. The DNA sequences were compared with those of the other nematode species available at the GenBank sequence database using the BLAST homology search program.

The model of base substitution was evaluated using MODELTEST (Posada and Crandall, 1998; Huelsenbeck and Ronquist, 2001). The Akaike-supported model, the base frequencies, the proportion of invariable sites and the gamma distribution shape parameters and substitution rates were used in phylogenetic analyses. Bayesian analysis was performed to confirm the tree topology for each gene separately using MrBayes 3.1.0 (Huelsenbeck and Ronquist, 2001) running the chain for 1×10^6 generations and setting the "burnin" at 1,000. We used the Markov Chain Monte Carlo (MCMC) method within a Bayesian framework to estimate the posterior probabilities of the phylogenetic trees (Larget and Simon, 1999) using 50% majority rule.

Other nematode samples

Cultures of PS2068 from Oregon, USA and JU179 from France reported in Felix et al. (2001) were obtained to do comparative studies due to their high similarity with the study populations. The nematodes arrived on Nematode Growth Medium (NGM) (Stiernagle, 2006), with no bacteria in the dishes. They were immediately transferred to new NGM plates, which had been plated with *E. coli* OP50 as a food source for the nematodes. After the new cultures were established in the laboratory, they were stored at room temperature and in the dark. Nematodes were rinsed off the plates using distilled water, and diluted down to 100 dauer stage nematodes per milliliter so that they could be used in entomopathogenicity trials, alongside the Wisconsin populations.

Nematode entomopathogenicity

The nematode entomopathogenicity towards cranberry pests and other insects was evaluated by using late instar larvae of the sparganothis and cranberry fruitworms, 6th instar larvae of the mealworm, *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae), nymphs of the brown banded cockroach *Supella longipalpa* Fabricius (Blattodea: Blatellidae), and 5th instar larvae of the greater wax moth. Wax moths were purchased from Rainbow Mealworms Incorporated. The cockroaches were captured in the break room on the fifth floor of Russell Laboratories, and a population was established by rearing them in Tupperware containers with peanut butter and oatmeal. All tests lasted for a total duration of one week.

For the sparganothis fruitworms, greater wax moths, brown banded cockroaches, and mealworms, testing arenas were constructed by collecting 90 mm wide by 15 mm tall style Falcon® Petri Dishes. Two pieces of 9 cm diameter qualitative P5 Fisher brand filter paper were placed inside each dish. Due to the fact that the three populations produced slightly different cadaver phenotypes, we decided to determine whether or not the three populations differed in terms of their abilities to kill insects. Therefore, five insects from the tested species were placed in a Petri dish, along with 200 infective juveniles of a single nematode population, suspended in two milliliters of water. The control treatments received two milliliters of distilled water.

Three replicates were created for each test species and nematode combination, and the experiment was repeated three times. Since three populations were recovered, the process was repeated for each population. A two-way ANOVA was conducted to

examine the influences of nematode population and insect type on insect mortality, and a Holm-Sidak multiple comparisons test was then implemented to make pairwise comparisons, using SigmaPlot version 12.3.

Petri dishes with insects were inspected daily to monitor insects. To verify that the nematodes had killed the insects, the cadavers were removed from the Petri dishes immediately after mortality was induced. They were kept in White traps until nematodes emerged. The purpose of this step was to limit the possibility of nematodes colonizing dead insects. Once nematodes had been recovered, the cadaver was considered to be killed by the nematode. Since the dates of insect deaths were recorded, it was possible to determine the amount of time, in days, that it took for half of the insects to die, in order to evaluate the nematodes using the Dillman's criteria (Dillman et al., 2012).

Results

The species is identified as *Oscheius onirici* (Measurements in Table 1, Figs. 1–3) based on morphological data and molecular analysis.

Description

Females

Body straight, slightly bent ventrally when heat-killed. Cuticle smooth, finely annulated, annules about 1 µm wide at midbody. Lateral fields with three longitudinal ridges (four lines); lateral fields about one-fifteenth the body width, extending from corpus to the level of tail, but inconspicuous under light microscope. Six lips separated, continuous with the body contour; six bristle-like labial sensilla, one on each lip; four bristle-like cephalic sensilla, one on each sublateral lip. Amphideal apertures elliptical in lateral lips; located just behind anterior lip next to labial sensilla. Stoma opening trigonal shaped, each side with two lips. Stoma rhabditoid, about four times as long as wide. Cheilostom not sclerotized (*ca.* 1/5 stoma length), gymnostom sclerotized cylinder (*ca.* 1/3 stoma length), stegostom about half of the total stoma length, pro/mesostegostom sclerotized cylinder (*ca.* 1/3 stoma length); glottoid apparatus well developed, isomorphic; metastegostomatal flaps difficult to observe under light microscope, but appear to hinge on refractile dots that separate metastegostom and telostegostom which appears as a stomatal floor. Pharyngeal collar surrounding about 50% of buccal capsule. Procorpus cylindrical; metacorpus not clearly differentiated. Basal bulb global, with duplex haustrulum posterior from valve plates. Nerve ring usually surrounding mid-part of isthmus. Excretory pore conspicuous, usually ventrally located at the level of basal bulb. Hemizonid not clearly observed. Reproductive system didelphic, amphidelphic. Ovaries reflexed anteriorly. Up to 12 embryos within uteri [7.2 ± 3.0 (3-12)]. Vulva a transverse slit with side vulva flaps (Fig. 3C), opening at midbody. Rectum long, about 3 times of anal body width [3.1 ± 0.3 (2.5-3.4)]. Tail elongate, slender, tapering gradually; about 3.5 times anal body width. Phasmids conspicuous under SEM, pore like, protuberant (Fig. 3H); located 1/3 of tail region posterior to anus.

Males

Not found.

Juveniles

Body straight when heat-killed. Stoma and esophagus morphology similar to female. Tail elongate, conical.

Table 1 presented the measurements to compare the Wisconsin population with PS2068 from Oregon, USA, Jul179 from France and DFL2014 from Italy. No significant difference was observed among these populations.

Molecular phylogenetic relationships

No DNA polymorphism on the nearly-full-length 18S rDNA, the internal transcribed spacer (ITS), the D2/D3 expansion segment of 28S rDNA, and mitochondrial DNA CoxI gene was found from three nematode populations in red, tan and grey color in the appearance in original insect cadavers. The DNA sequences of *O. onirici* are available in the GenBank database under the accession numbers MF196094 (18S and ITS), MF196097 (28S) and MF196100 (CoxI). These sequences are also identical to those from PS2068 and JU179 (Felix et al., 2001). It has 5 bp differences, 1 gap in 18S, 5 bp differences, 2 gaps in ITS, 0 to 1 bp difference in 28S and 1 bp difference in CoxI with sequences of *O. onirici* from Italy, but the same in encoded protein sequence in CoxI. The first 20 bp of LN613268 and LN613269 of CoxI have 7 bp differences with our sequences, but encoded a stop codon and appeared to be sequence errors, thus it's not included in this comparison. The DNA sequences from *O. onirici* from Wisconsin are most close to other

Table 1. Morphometrics of female *Oscheius onirici*.

Measurement or ratio	16-34833 Wisconsin	PS2068 Oregon	Jul179 France	DFL2014[a] Italy
N	20	13	14	20
L	615–830 (733.3 ± 63.8)	610–830 (670.4 ± 54.0)	565–835 (645.4 ± 69.3)	584–801 (671 ± 65.5)
a	13.5–36 (16.3 ± 4.6)	4.8–23.3 (18.1 ± 4.3)	14.1–21.4 (16.2 ± 1.8)	15.8–22.5 (19 ± 2)
b	4.8–6.5 (5.6 ± 0.4)	3.3–6.4 (5.1 ± 0.7)	3.9–5.4 (4.3 ± 0.4)	4.4–6.0 (4.9 ± 0.5)
c	8.1–15.4 (10.6 ± 2.1)	3.6–9.4 (7.6 ± 1.5)	6.1–9.9 (7.4 ± 1.1)	8.6–11.8 (9.7 ± 0.9)
c’	2.2–4.8 (3.5 ± 0.7)	3.7–5.7 (4.6 ± 0.7)	3.5–5.8 (4.8 ± 0.7)	3.5–5.0 (4.3 ± 0.4)
V	47.3–53.2 (50.6 ± 1.3)	47.2–51.5 (49.7 ± 1.2)	37.7–53.9 (49.3 ± 3.9)	47.3–52.6 (51.0 ± 1.3)
Pharynx	118–154 (132.0 ± 11.3)	104–190 (134.7 ± 18.3)	135–163 (148.4 ± 6.7)	126–146 (137 ± 6.2)
Midbody width	20–55 (46.8 ± 7.4)	30–131 (42.8 ± 25.8)	34–46 (40.0 ± 3.4)	30–51 (36.0 ± 5.3)
Tail	40–95 (72.1 ± 26.3)	74–176 (93.3 ± 34.9)	72–102 (87.6 ± 8.3)	63–81 (69 ± 5.5)
ABW	16–38 (21 ± 7.6)	15–44 (20.8 ± 9.0)	16–29 (18.5 ± 3.4)	13–23 (16 ± 2.2)
Stoma length	14–22 (17.6 ± 1.9)	16–19 (17.7 ± 1.1)	18–29 (20.9 ± 2.4)	12–16 (14 ± 1.1)
Stoma diameter	3–5 (4.1 ± 0.5)	3–5 (4.1 ± 0.5)	4–5 (4.2 ± 0.4)	3–4 (4 ± 0.4)
Basal bulb length	24–45 (30.5 ± 5.4)	25–30 (27.6 ± 1.8)	20–31 (26.6 ± 3.4)	21–25 (23 ± 1.3)
Basal bulb width	19–28 (22.9 ± 2.6)	16–26 (19.7 ± 3.1)	18–27 (23.3 ± 3.1)	17–21 (19 ± 1.2)
EP (Anterior end to excretory pore)	88–130 (113.1 ± 28.4)	95–122 (109.3 ± 8.6)	100–133 (112.7 ± 8.2)	92–146 (108 ± 13.2)
D (%)	72.4–100.8 (87.6 ± 7.1)	70.9–92.5 (83.19 ± 6.3)	64.5–88.9 (77.4 ± 7.1)	63.4–101.7 (78.9 ± 9.6)
Stoma length/ stoma diameter	3.6–5.5 (4.3 ± 0.5)	1.9–6 (4.2 ± 0.9)	1.12–5.50 (4.6 ± 1.1)	

[a]Data from Torrini et al. (2015).
Note: All measurements in µm and in the format: Range (mean ± S.D.).

Oscheius species in GenBank, and these sequences were selected for phylogenetic analysis. Unfortunately, no other *Oscheius* CoxI sequence is available in GenBank, thus phylogenetic analysis was not performed.

Figure 4 presents a phylogenetic tree based on the nearly-full-length 18S rDNA. Using *Heterorhabditis* as an outgroup taxon, all species of *Oscheius* are in a clade with 100% support, clearly divided all *Oscheius* species into *Insectivora*-group and *Dolichura*-group each with 100% support. The *Dolichura*-group comprises of *O. onirici*, *O. dolichurus, O. dolichuroides, O. guentheri* and *O. tipulae.* This is consistent with the morphological classification by Sudhaus and Hooper (1994) and molecular phylogeny (Sudhaus and Fitch, 2001; Kiontke and Fitch, 2005; Kiontke et al., 2007). Based on 18S tree, seven populations including 16-34833 from Wisconsin, PS2068 from Oregon, JU179 from France, MG67 P20 and MG69 P20 from Switzerland, FVV2 from Kansas, USA and FDL2014 from Italy are the same species as *O. onirici. Oscheius onirici* appeared to be a sister species to *O. tipulae.*

Figure 5 presents a phylogenetic tree based on rDNA 28S D2/D3 sequences. Rooted by *Pellioditis marina* Bastian, 1865, the genus *Oscheius* was in a monophyletic clade with 100% support. The *Insectivora*-group and *Dolichura*-group were inferred as monophyletic clades with 100% support, corroborating the 18S tree. Five populations of *O. onirici* are nearly identical and are sister to *O. tipulae.*

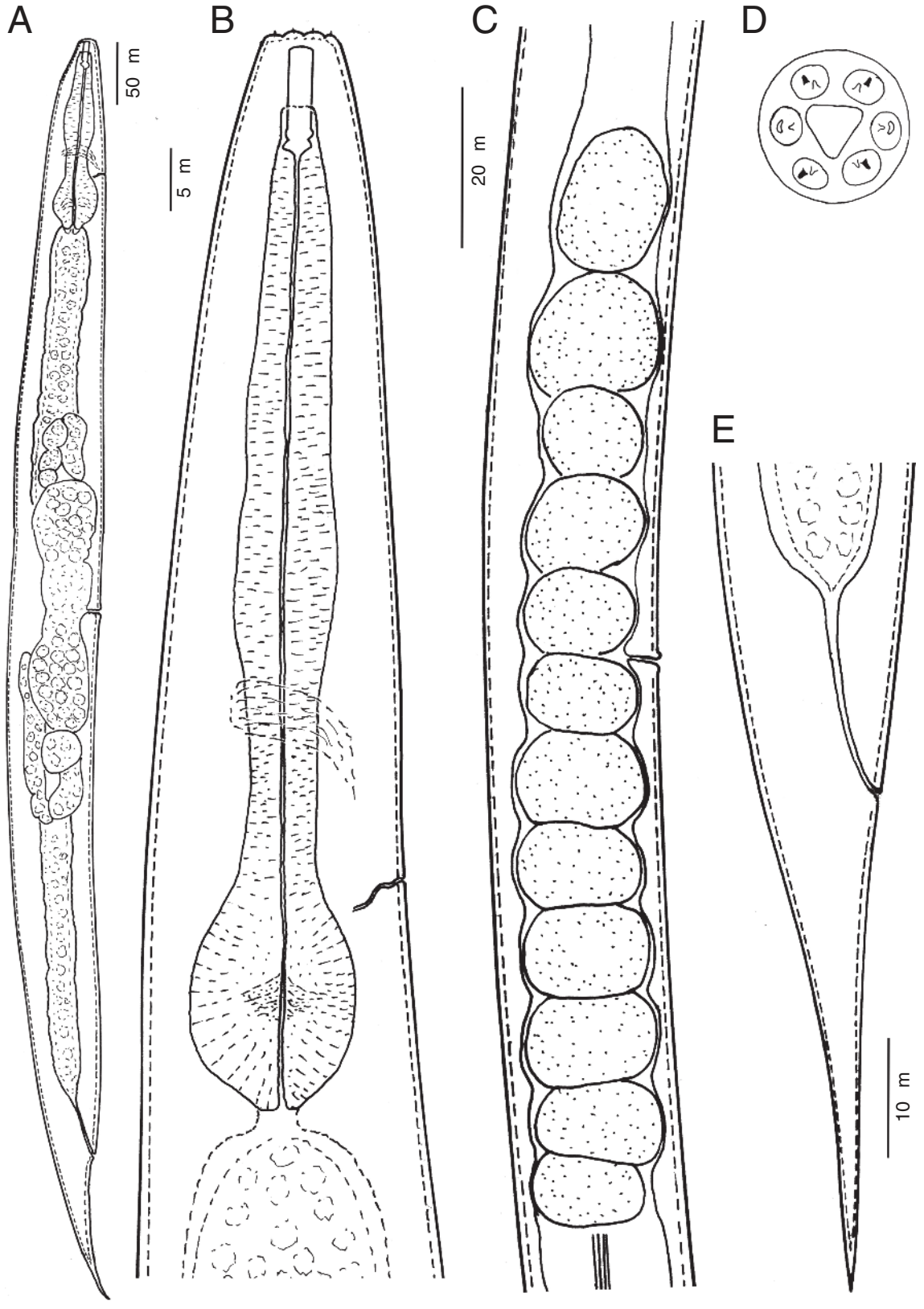

Figure 1: Oscheius onirici female. (A) Entire body. (B) Pharyngeal region. (C) Lateral view of vulva region and lateral field. (D) Schematic representation of En-face view of lip region. (E) Lateral view of tail region.

Figure 2: Photographs of Oscheius onirici female. (A) Entire body (arrow showing vulva). (B) Pharyngeal region (arrow showing excretory pore). (C) Head region. (D, E) Lateral view of vulva region (arrow showing vulva). (F) Lateral view of tail region (arrow showing anus). (G) Basal bulb (arrow showing excretory pore).

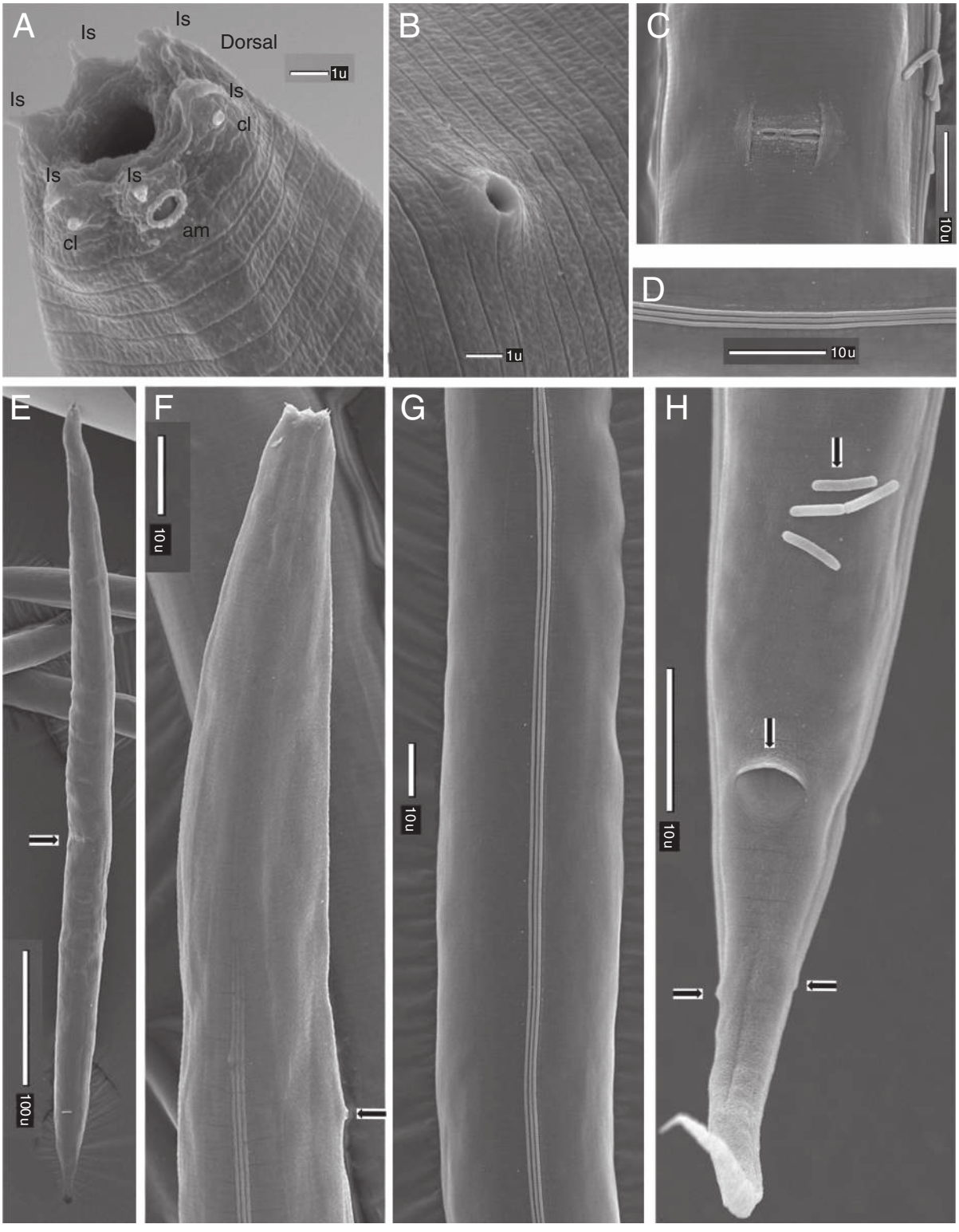

Figure 3: Scanning electron microscope photographs of Oscheius onirici female. (A) Lip region en-face view showing one amphideal aperture (am), six labial sensilla (ls) and two cephalic sensilla (cs). (B) Excrotory pore. (C) Ventral view of vulva. (D) Lateral lines. (E) Entire body lateral view (arrow showing vulva). (F) Esophageal region (arrow showing excretory pore). (G) Lateral field showing lateral lines. (H) Tail region ventral view (Top arrow showing four bacteria, middle arrow showing anus, two bottom arrows showing phasmids).

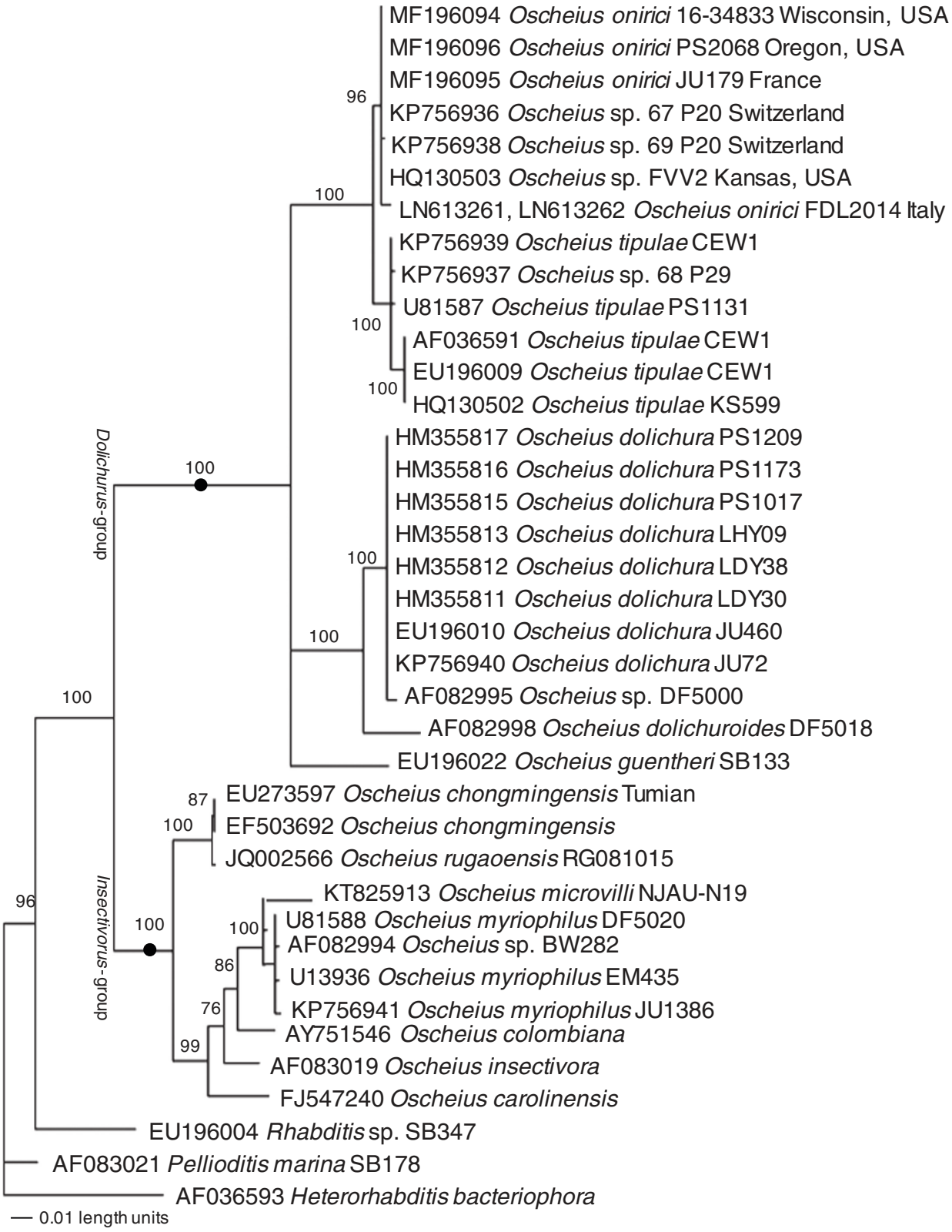

Figure 4: Bayesian consensus tree inferred from 18S under GTR+I+G model (–ln *L* = 5789.8442; AIC = 11599.6885; freqA = 0.2657; freqC = 0.202; freqG = 0.258; freqT = 0.2743; R(a) = 0.9275; R(b) = 3.6026; R(c) = 2.6802; R(d) = 0.6926; R(e) = 6.3237; R(f) = 1; Pinva = 0.4134; Shape = 0.6825). Posterior probability values exceeding 50% are given on appropriate clades.

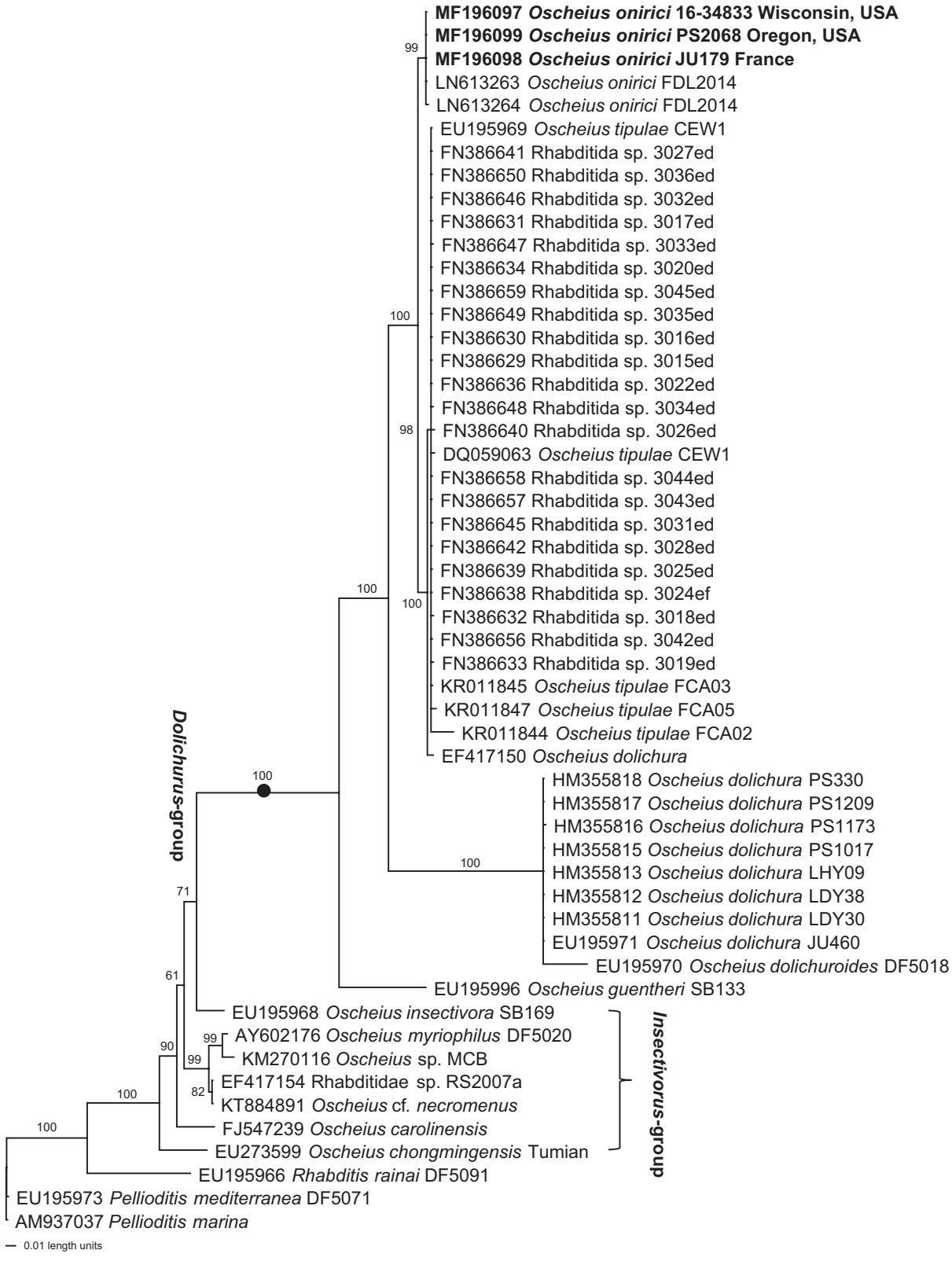

Figure 5: Bayesian consensus tree inferred from 28S D2/D3 under GTR+I+G model (–ln *L* = 4799.2056; AIC = 9618.4111; freqA = 0.2557; freqC = 0.182; freqG = 0.2892; freqT = 0.2732; R(a) = 0.6259; R(b) = 2.263; R(c) = 1.6102; R(d) = 0.5689; R(e) = 5.2205; R(f) = 1; Pinva = 0.2558; Shape = 0.6827). Posterior probability values exceeding 50% are given on appropriate clades.

Figure 6 presents a phylogenetic tree based on rDNA ITS sequences. Rooted by *Pellioditis marina*, the genus *Oscheius* was in a monophyletic clade with 100% support. The *Insectivora*-group and *Dolichura*-group were inferred as monophyletic clades with 98% and 100% support respectively, corroborating the 18S and 28S trees. Five unidentified populations of *Oscheius* are nearly identical to *O. onirici* from Wisconsin, Oregon, California, France, Switzerland and Italy and they should be considered as *O. onirici*. *Oscheius onirici* is sister also to *O. tipulae* as inferred in 18S and 28S trees.

Host and locality

Oscheius onirici was recovered by baiting soil samples collected in Jackson County, Wisconsin, USA, in 2015, using *Galleria mellonella* larvae. These nematodes are easily kept in culture by using the larvae of the native moth *Sparganothis sulfureana* Clemens (Lepidoptera: Tortricidae) as a host in subsequent *in vivo* propagations. Approximately 25 dauer stage nematodes are applied per host, and the incubation period is set for three days. Initial attempts to perform the experiment included a small amount of stonefly Heliothis diet®, but this step was removed because mold would grow on the provision. Therefore, insects were not given food during the trials. Applications took place in Petri dishes, as in the entomopathogenicity tests. Native moths like *S. sulfureana* are likely to be natural hosts.

Nematode entomopathogenicity

All three populations of *Oscheius onirici* were capable of inducing mortality in members of the Blattodea, Coleoptera, and two families of Lepidoptera, suggesting that the nematode is an EPN and has an extensive host range (Fig. 7A). The second population was significantly more effective at inducing mortality in the sparganothis fruitworm, compared to the other populations (p values < 0.0001 for all comparisons). Compared to the control treatments, all *Oscheius* populations induced significantly higher levels of mortality in the juvenile insects (p values < 0.0001 for all comparisons, Fig. 7A). For population 3, the mealworm and the sparganothis fruitworm did not have significantly different mortality rates (p = 0.138), but otherwise the sparganothis fruitworm mortality rate was significantly higher than the other insect mortality rates (p values < 0.0001 for all comparisons, Fig. 7A).

The sparganothis fruitworms and brown banded cockroaches died within 72 hr, whereas the *Galleria* wax moths took on average five days or more to die (Fig. 7B). The mealworms died in approximately three to four days (Fig. 7B), although no mealworms died in the control group during the experiment. The greater wax moth took significantly longer to die than any other insect, regardless of nematode treatment type (p values < 0.0001, Fig. 7B).

Experiments are underway to determine whether or not the nematodes are capable of inducing mortality in field conditions. Furthermore, efforts are being made to understand the bacteria associated with these three *Oscheius* populations. Bacteria have been seen on the outside of this nematode, and attempts to isolate this species are being planned. Furthermore, efforts to isolate bacterial cells from within the nematode are being considered.

Discussion

Nematode identification is challenging especially when a critical life stage is missing. In the genus *Oscheius* or many other Rhabditids, the male is the diagnostic stage because of the unique characters in the number and arrangement of genital papillae, the size and shape of spicules and the position and shape of bursa. Felix et al. (2001) characterized three biological species closely related to *Oscheius pseudodolichura* Korner in Osche, 1952 based on ribosomal DNA ITS sequences and a mating study from rare spontaneous males. All these populations are self-fertilizing hermaphrodites with very rare facultative males. From their study, three biological species were defined as sp. 1, sp. 2 and sp. 3, but no species names were proposed and no morphology study was attempted due to the nature of lack of male.

Numerous attempts using high/low temperature, changing diet or slowly drying the media were conducted in our experiments to induce males in culture but were not successful. It is noteworthy that males have been found from populations collected close to the equator in Italy (Torrini et al., 2015), but not from nematodes collected in Wisconsin, suggesting that the nematode may exhibit geographic parthenogenesis. The wasp *Pelecinus polyturator* Drury (Hymenoptera: Pelecinidae) produces both males and females in temperate climates, but does not produce males in the latitudes farther from the equator (Young, 1990). Perhaps *O. onirici* also uses this reproductive strategy, considering that he cold climate may provide barriers to reproduction. The nematodes might not be active in their search for mates during the cold winter months, because the ground is frozen. One could therefore speculate that if males are not needed, then the females can directly begin reproducing during the warm months, and could overcome the

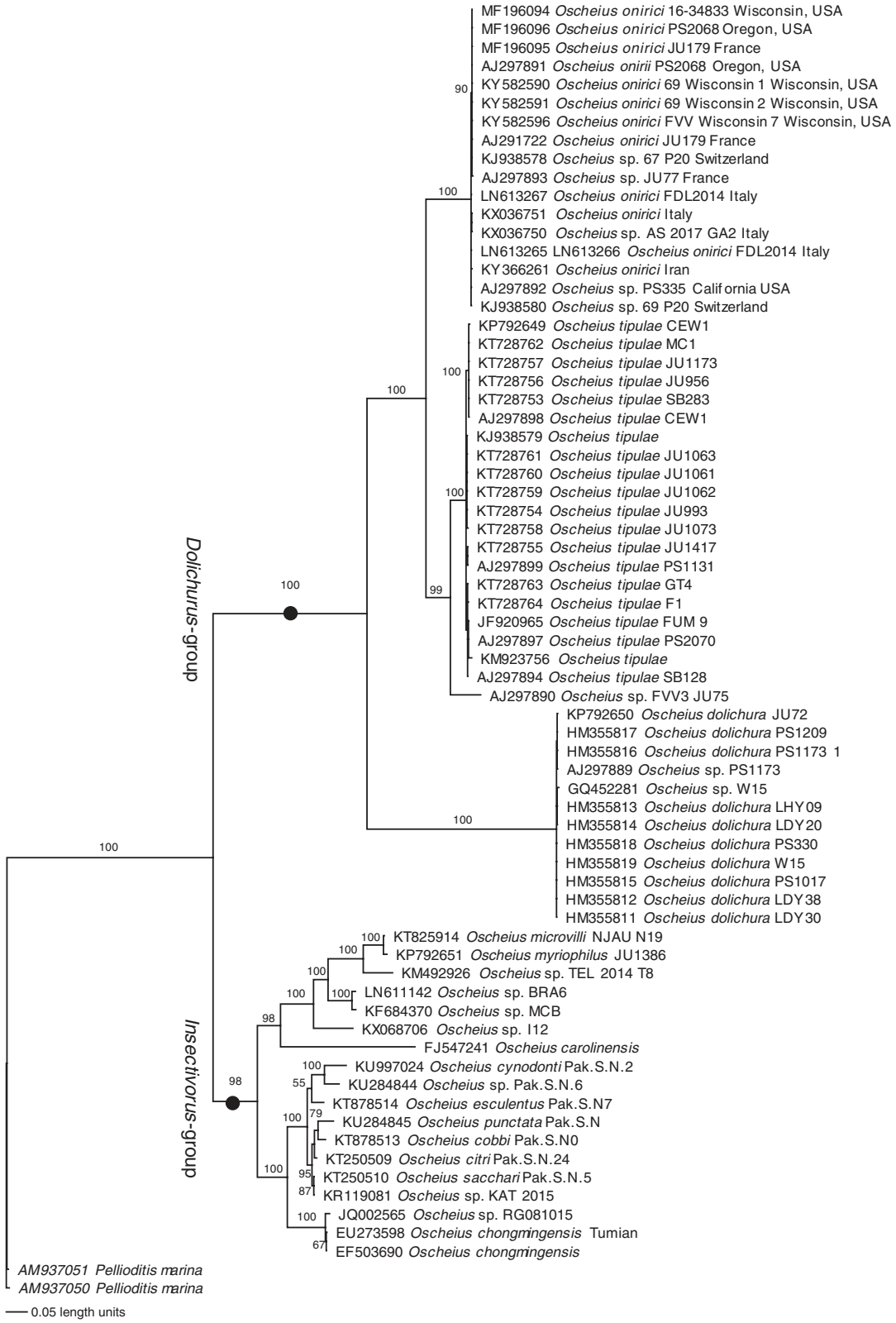

Figure 6: Bayesian consensus tree inferred from ITS under TVM+I model (–ln *L* = 9235.373; AIC = 18488.7461; freqA = 0.2255; freqC = 0.2115; freqG = 0.2393; freqT = 0.3237; R(a) = 1.3757; R(b) = 3.2802; R(c) = 1.847; R(d) = 1.2014; R(e) = 3.2802; R(f) = 1; Pinva = 0.1375; Shape = 1.5896). Posterior probability values exceeding 50% are given on appropriate clades.

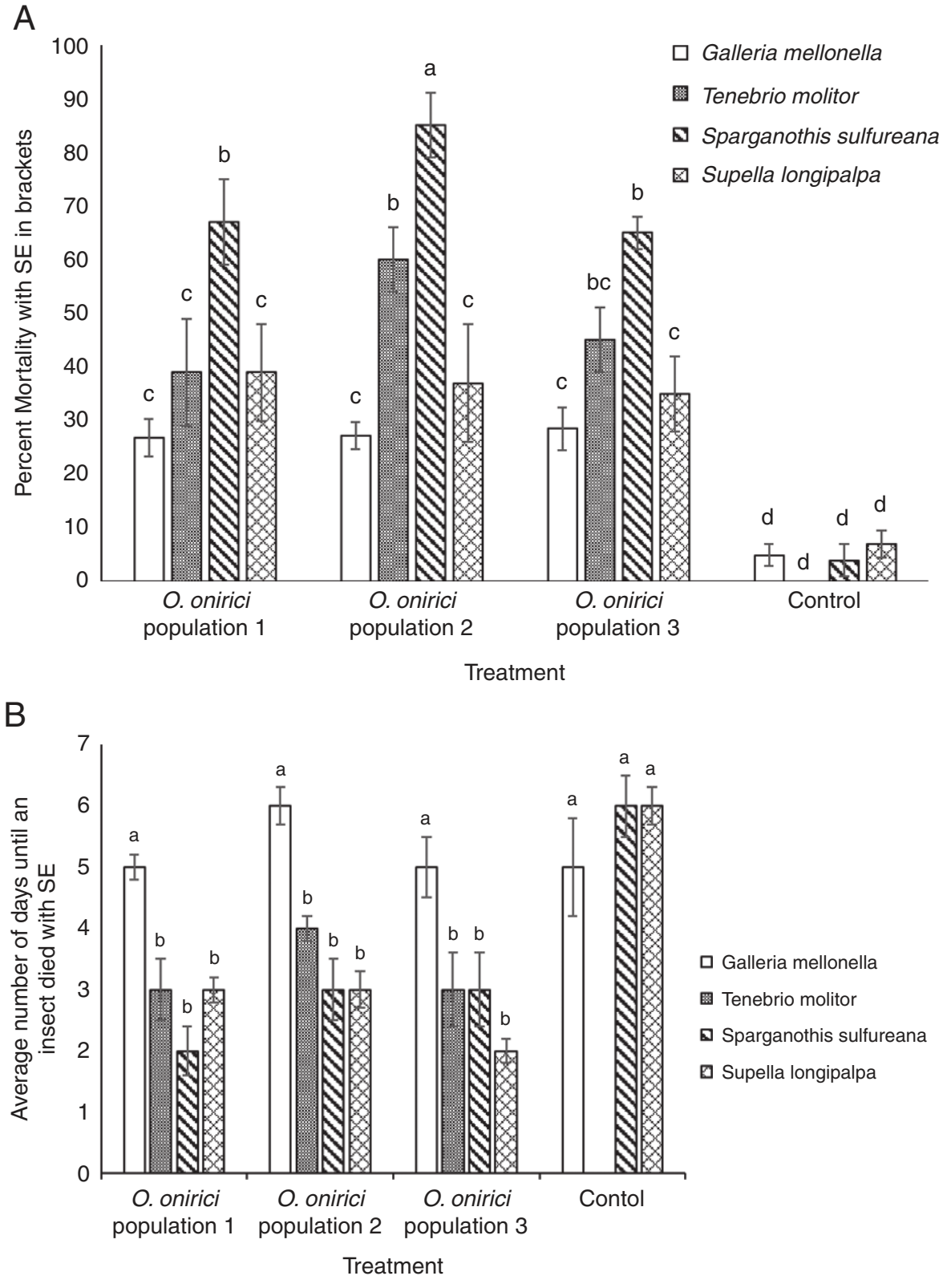

Figure 7: The mortality rates for different insect types treated with either an Oscheius onirici population, or a control treatment of two milliliters of distilled water. (B) The average number of days that it took for a larva to die after exposure to the treatment. Insects were treated with either an Oscheius onirici population, or a control treatment of two milliliters of distilled water.

climate-induced disadvantages that come from having less time to mate.

In a search for an EPN to control cranberry insect pests, an *Oscheius* nematode was recovered through the *Galleria*-bait method from a wild cranberry marsh in Jackson County, Wisconsin, and its sequence is identical to sp. 2 (PS2068 from Oregon and JU179 from France). *Oscheius onirici,* a species recently described from a karst cave soil of central Italian, is a hermaphroditic species, but fortunately, 12 rare males were discovered and thus the new species was proposed. This species is in *Dolichura* group, the study population from Wisconsin has minor sequence differences in ribosomal DNA and mitochrondrial DNA, but is identical for the encoded protein sequence in CoxI. Based on molecular phylogenetic analysis, these minor differences are not significant enough to separate them in different clade as different species. In addition to population 16-34833 from Wisconsin, PS2068 from Oregon, FVV2 from Kansas, JU179 from France, MG67 P20 and MG69 P20 from Switzerland are very close to each other on 18S and should be considered as *O. onirici* described from Italy. Recently, *O. onirici* was also reported from Iran (Valizadeh et al., 2017), indicating a wider distribution of this species. Further research is being conducted on the mass-propagation of this nematode for application as biological control agents in the cranberry marshlands of Wisconsin.

Less than one percent of insects used during the sampling process led to the recovery of a nematode isolate. This sampling effort recovered no *Oscheius* nematodes in 2016. The fact that the virulence assay shows that the *Oscheius* nematode infects other insects at higher rates may suggest that in the wild, these nematodes may attack other types of insects instead.

It is surprising that three *Oscheius* populations from this study belong to the same species, due to the fact that they have different levels of virulence. Perhaps the populations have different bacterial strains. Further research on the microbes associated with these populations may be helpful in evaluating that hypothesis.

According to a recent paper, a nematode must kill 50% of the hosts to which it is exposed within five days in order to be considered an entomopathogen (Dillman et al., 2012). By this standard, our nematode would not be considered as a true entomopathogen, when only the greater wax moth is used as a host, because it does not kill enough hosts in five days. When the sparganothis fruitworm is instead used as a potential host, the nematode is able to exceed the 50% mortality in five-day benchmark. Therefore, our study suggests that the greater wax moth may not be the best standard host for evaluating entomopathogenicity.

Kleptoparasitism or scavenging behavior (Campos-Herrera et al., 2015) raises the possibility that there may have been multiple nematode species that were collected in our initial bioassay. Studies have shown that *Oscheius onirici* can infect cadavers that are targeted by *Heterorhabditis megidis*, and can reproduce inside the cadaver, thus weakening the other EPN by way of taking their cadaver resources (Campos-Herrera et al. 2015). The fact that we maintained the nematodes in the laboratory through serial inoculations using sparganothis larvae suggests that there was most likely only one species left in any given line by the time we submitted our samples for analysis. This outcome is probable because theoretically one nematode species would have outcompeted the other over periods of successive inoculations.

If an insect died of starvation during the entomopathogenicity trials, a nematode could invade the cadaver as a survival strategy in the absence of live food (Sans-Blas and Gowen, 2008). Insects in control groups did not starve during the trial, so it is unlikely that starvation accounts for mortality in these entomopathogenicity screens. Since only one nematode species was used in the entomopathogenicity test, it is impossible to tell if the *O. onirici* nematodes collected from Wisconsin would prefer to kill an insect or kleptoparasitize one already infected by a different nematode species (Campos-Herrera et al. 2015), so a future study on this topic might be advisable in Wisconsin.

Studies have found that native EPN communities can experience strong competition from introduced nematodes (Jaffuel et al. 2016). Therefore, it may be important to check for EPN already in cranberry marshes when conducting these tests, to not only evaluate the potential for negative impacts on native EPN, but also to ensure only the introduced nematode is capable of having an impact on pests during trials.

In addition to *O. onirici*, other nematodes in the *Oscheius* genus are also at least facultative EPNs. *Oscheius microvilli* is capable of killing wax worms (Zhou et al., 2017), making it at least a facultative EPN. Other species, including *Steinernema kraussei* and *Steinernema affine*, are capable of facultative entomopathogenicity (Půza and Mrácek, 2010; Blanco-Perez et al., 2017). Given that *Steinernema* nematodes are also capable of facultative entomopathogenic, it is worth suggesting that there may be some evolutionary convergence between these groups.

In summary, a survey of nematodes in Wisconsin recovered three populations of *O. onirici* from a wild

marsh in Jackson County, Wisconsin. This identification was confirmed using extensive molecular and morphological screening procedures. The nematode can kill various insect pests, although it remains unclear as to how effective it may be in the commercial marshes.

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References

Ali, S.S., Pervez, R., Andrabi, R., Sharma, R., and Verma, V. 2011. *Oscheius amsactae* n. sp. (Nematoda: Rhabditida), a necromenic associate of red-hairy caterpillar, *Amsacta moori* (Lepidoptera: Arctiidae) from Kanpur district, India. *Archives of Phytopathology and Plant Protection* 44: 871–81.

Blanco-Perez, R., Bueno-Pallero, F.A., Neto, L., and Campos-Herrera, R. 2017. Reproductive efficiency of entomopathogenic nematodes as scavengers. Are they able to fight for insect's cadavers? *Journal of Invertebrate Pathology* 148: 1–9, available at: http://dx.doi.org/10.1016/j.jip.2017.05.003.

Booth, S.R., Tanigoshi, L.K., and Shanks, C.H. Jr. 2002. Evaluation of entomopathogenic nematodes to manage root weevil larvae in Washington state cranberry, strawberry, and red raspberry. *Environmental Entomology* 31: 895–902.

Bozzola, J.J., and Russell, L.D. 1999. *Electron Microscopy*, 2nd ed., Jones and Bartlett Publishers, Inc, 670pp.

Campos-Herrera, R., Půža, V., Jaffuel, G., Blanco-Perez, R., Čepulytė-Rakauskienė, R., and Turlings, T.C. 2015. Unraveling the intraguild competition between *Oscheius* spp. nematodes and entomopathogenic nematodes: implications for their natural distribution in Swiss agricultural soils. *Journal of Invertebrate Pathology* 132: 216–27.

Cherry, T., Szalanski, A.L., Todd, T.C., and Powers, T.O. 1997. The internal transcribed spacer region of *Belonolaimus* (Nemata: Belonolaimidae). *Journal of Nematology* 29: 23–9.

Darsouei, R., Karimi, J., and Shokoohi, E. 2014. *Oscheius rugaoensis* and *Pristionchus maupasi*, two new records of entomophilic nematodes from Iran. *Russian Journal of Nematology* 22: 141–55.

De Ley, I.T., De Ley, P., Vierstraete, A., Karssen, G., Moens, M., and Vanfleteren, J. 2002. Phylogenetic analysis of *Meloidogyne* small subunit rDNA. *Journal of Nematology* 34: 319–27.

De Ley, P., Van De Velde, M.C., Mounport, D., Baujard, P., and Coomans, A. 1995. Ultrastructure of the stoma in Cephalobidae, Panagrolaimidae and Rhabditidae, with a proposal for a revised stoma terminology in Rhabditida (Nematoda). *Nematologica* 41: 153–82.

Dillman, A.R., Chaston, J.M., Adams, B.J., Ciche, T.A., Goodrich-Blair, H., Stock, S.P., and Sternberg, P.W. 2012. An entomopathogenic nematode by any other name. *PLoS Pathogen* 8(3): e1002527, available at: https://doi.org/10.1371/journal.ppat.1002527.

Dorris, M., Viney, M.E., and Blaxter, M.L. 2002. Molecular phylogenetic analysis of the genus *Strongyloides* and related nematodes. *International Journal of Parasitology* 32: 1507–17.

Felix, M.A., Vierstraete, A., and Vaneleteren, J. 2001. Three biological species closely related to *Rhabditis* (*Oscheius*) *pseudodolichura* Korner in Osche, 1952. *Journal of Nematology* 33(2–3): 104–9.

Floyd, R., Abeb, E., Papert, A., and Blaxter, M. 2002. Molecular barcodes for soil nematode identification. *Molecular Ecology* 11: 839–50.

Huelsenbeck, J.P., and Ronquist, F. 2001. MR BAYES: Bayesian inference of phylogenetic tress. *Bioinformatics* 17: 1754–5.

Jaffuel, G., Mäder, P., Blanco-Perez, R., Chiriboga, X., Fliessbach, A., Turlingsa, T.C.J., and Campos-Herrera, R. 2016. Prevalence and activity of entomopathogenic nematodes and their antagonists in soils that are subject to different agricultural practices. *Agriculture, Ecosystems and Environment* 230: 329–40, available at: https://doi.org/10.1016/j.agee.2016.06.009.

Kanzaki, N., and Futai, K. 2002. A PCR primer set for determination of phylogenetic relationships of

Bursaphelenchus species within the *xylophilus* group. *Nematology* 4: 35–41.

Kaya, H.K., and Gaugler, R. 1993. Entomopathogenic nematodes. *Annual Review of Entomology* 38: 181–206.

Kiontke, K., and Fitch, D.H.A. 2005. The phylogenetic relationships of *Caenorhabditis* and other Rhabditids. The *C. elegans* Research Community, ed. WormBook, doi/10.1895/wormbook.1.11.1, available at: www.wormbook.org.

Kiontke, K., Barriere, A., Kolotuev, I., Podbilewicz, B., Sommer, R., Fitch, D.H.A., and Felix, M.A. 2007. Trends, stasis, and drift in the evolution of nematode vulva development. *Current Biology* 17: 1925–37.

Larget, B., and Simon, D.L. 1999. Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. *Molecular Biology and Evolution* 16: 750–9.

Lassmann, T., and Sonnhammer, E.L.L. 2005. Kalign – an accurate and fast multiple sequence alignment algorithm. *BMC Bioinformatics* 6: 298, available at: https://doi.org/10.1186/1471-2105-6-298.

Liu, Q.Z., Mráček, Z., Zhang, L.J., Půža, V., and Dong, L.M. 2012. Re-description of *Oscheius chongmingensis* (Zhang *et al*., 2008) (Nematoda: Rhabditidae) and its entomopathogenicity. *Nematology* 14: 139–49.

Mullin, P.G., Harris, T.S., and Powers, T.O. 2005. Phylogenetic relationships of Nygolaimina and Dorylaimina (Nematoda: Dorylaimida) inferred from small subunit ribosomal DNA sequences. *Nematology* 7: 59–79.

Nunn, G.B. 1992. Nematode molecular evolution. Ph.D. dissertation. University of Nottingham, UK.

Orozco, R.A., Lee, M.M., and Stock, S.P. 2014. Soil sampling and isolation of entomopathogenic nematodes (Steinernematidae, Heterorhabditidae). *Journal of Visualized Experiments Jul* 11;(89), doi: 10.3791/52083.

Poinar, G.O. Jr, and Thomas, G.M. 1984. *Laboratory Guide to Insect Pathogens and Parasites*, Plenum Press, New York, p. 392.

Posada, D., and Crandall, K.A. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817–8.

Půza, V., and Mrácek, Z. 2010. Does scavenging extend the host range of entomopathogenic nematodes (Nematoda: Steinernematidae)? *Journal of Invertebrate Pathology May* 104(1): 1–3.

Rydin, H., and Jegland, J. 2006. *Biology of peatlands*, Oxford University Press, Oxford, pp. 65–72.

Sans-Blas, E., and Gowen, S. 2008. Facultative scavenging as a survival strategy of entomopathogenic nematodes. *International Journal for Parasitology* 38: 85–91.

Seinhorst, J.W. 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica* 4: 67–9.

Simser, R., and Roberts, S. 1994. Suppression of strawberry root weevil, *Otiorhynchus ovatus*, in cranberries by entomopathogenic nematodes (Nematoda: Steinernematidae and Heterorhabitidae). *Nematologica* 40: 456–62.

Stiernagle, T. 2006. Maintenance of *C. elegans*. WormBook, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.101.1. available at: http://www.wormbook.org.

Stock, S.P., Caicedo, A.M., and Calatayud, P.A. 2005. *Rhabditis* (*Oscheius*) *colombiana* n. sp. (Nematoda: Rhabditida), a necromenic associate of the subterranean burrower bug *Cyrtomenus bergi* (Hemiptera: Cydnidae) from the Cauca Valley, Colombia. *Nematology* 7: 363–73.

Sudhaus, W., and Fitch, D. 2001. Comparative studies on the phylogeny and systematics of the Rhabditidae (Nematoda). *Journal of Nematology* 33: 1–70.

Sudhaus, W., and Hooper, D.J. 1994. *Rhabditis* (*Oscheius*) *guentheri* sp. n., an unusual species with reduced posterior ovary, with observations on the *Dolichura* and *Insectivora* groups (Nematoda: Rhabditidae). *Nematologica* 40: 508–33.

Tabassum, K.A., and Shahina, F. 2010. *Oscheius siddiqii* and *O. niazii*, two new entomopathogenic nematode species from Pakistan, with observations on *O. shamimi*. International. *Journal of Nematology* 20: 75–84.

Tabassum, K.A., Shahina, F., Nasira, K., and Erum, Y.I. 2016. Description of six new species of *Oscheius* Andrassy, 1976 (Nematoda: Rhabditida) from Pakistan with a key and diagnostic compendium to species of the genus. Pakistan. *Journal of Nematology* 34: 109–61.

Torrini, G., Mazza, G., Carletti, B., Benvenuti, C., Roversi, P.F., Fanelli, E., De Luca, F., Troccoli, A., and Tarasco, E. 2015. *Oscheius onirici* sp. n. (Nematoda: Rhabditidae): a new entomopathogenic nematode from an Italian cave. *Zootaxa* 3937: 533–48.

Valizadeh, A., Goldasteh, S., Rafiei-Karahroodi, Z., and Pedram, M. 2017. The occurrence of three species of the genus *Oscheius* Andrássy, 1976 (Nematoda: Rhabditida) in Iran. *Journal of Plant Protection Research* 57: 248–55.

Vrain, T.C., Wakarchuk, D.A., Levesque, A.C., and Hamilton, R.I. 1992. Intraspecific rDNA restriction fragment length polymorphism in the *Xiphinema americanum* group. *Journal of Nematology* 29: 250–4.

Ye, W. 1996. Applying Microsoft Works spreadsheet in statistics for morphometric data of nematode identification. *Afro-Asian Journal of Nematology* 6: 203–11.

Ye, W., Giblin-Davis, R.M., Braasch, H., Morris, K., and Thomas, W.K. 2007. Phylogenetic relationships among *Bursaphelenchus* species (Nematoda: Parasitaphelenchidae) inferred from nuclear ribosomal and mitochondrial DNA sequence data. *Molecular Phylogenetics and Evolution* 43: 1185–97.

Ye, W., Torres-Barragan, A., and Cardoza, Y.J. 2010. *Oscheius carolinensis* n. sp. (Nematoda: Rhabditidae), a potential entomopathogenic nematode from vermicompost. *Nematology* 12: 121–35.

Young, D.K. 1990. Distribution of *Pelecinus polyturator* in Wisconsin (Hymenoptera: Pelecinidae) with speculations regarding geographical parthenogenesis. *Great Lakes Entomologist* 23(1): 1–4.

Zhang, C.X., Liu, J.R., Xu, M.X., Sun, J., Yang, S.Y., An, X.H., Gao, G.F., Lin, M.S., Lai, R., He, Z.Y., Wu, Y.D., and Zhang, K.Y. 2008. *Heterorhabditidoides chongminensis* gen. nov., sp. nov. (Rhabditida: Rhabditidae), a novel member of the entomopathogenic nematodes. *Journal of Invertebrate Pathology* 98: 153–68.

Zhang, K.Y., Liu, X.H., Tan, J., Wang, Y., Qiao, L., Yedid, G., Dai, C.S., Qiu, R.L., Yan, X.W., Tan, H.W., Su, Z.Y., Lai, R., and Gao, G.F. 2012. *Heterorhabditidoides rugaoensis* n. sp. (Rhabditida: Rhabditidae), a novel highly pathogenic entomopathogenic nematode member of Rhabditidae. *Journal of Nematology* 44: 348–60.

Zhou, G., Yang, H., Wang, F., Bao, H., Wang, G., Hou, X., Lin, J., Yedid, G., and Zhang, K. 2017. *Oscheius microvilli* n. sp. (Nematoda: Rhabditidae): A facultatively pathogenic nematode from Chongming Island, China. *Journal of Nematology* 49: 33–41.