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Two nematodes (Nematoda: Diplogastridae, Rhabditidae) from the invasive millipede *Chamberlinius hualienensis* Wang, 1956 (Diplopoda, Paradoxosomatidae) on Hachijojima Island in Japan

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Abstract

Millipedes may cause unexpected damage when they are introduced to new locations, becoming invaders that leave behind their old parasites and predators. Therefore, it was interesting to find numerous rhabditid nematodes within the gut of the invasive phytophagous millipede *Chamberlinius hualienensis* Wang, 1956 (Diplopoda, Paradoxosomatidae) from Hachijojima (Japan) in November, 2014. This millipede originated in Taiwan but was discovered in Japan in 1986. The nematodes were identified as juvenile *Oscheius rugaoensis* (Zhang et al., 2012) Darsouei et al., 2014 (Rhabditidae), and juvenile and adult *Mononchoides* sp. (Diplogastridae) based on images, morphometrics, and sequences of 18S and 28S rDNA. A novel short 28S sequence of a separate population of *Oscheius necromenus* SB218 from Australian millipedes was also included in a phylogenetic comparison of what can now be characterized as a species complex of millipede-associated *Oscheius*. The only other nematode associates of millipedes belong to Rhigonematomorpha and Oxyuridomorpha, two strictly parasitic superorders of nematodes. These nematode identifications represent new geographic and host associations.

Key words

Invertebrate phoresy, Nematode ecology, Ribosomal DNA, Phylogeny, Systematics, Taxonomy.

The invasive phytophagous millipede *Chamberlinius hualienensis* Wang, 1956 was originally described from Taiwan, where it is extremely common (Chen et al., 2011). It was discovered in Okinawa, Japan in 1986 (Higa and Kishimoto, 1986) and outlying islands of the Ryukyu Archipelago (Nakamura and Korsós, 2010). Fourteen years ago it was detected on Hachijo Island, Japan (Fujiyama et al., 2012). Remarkably these millipedes are not known to swarm in Taiwan (Chen et al., 2011), but in Japan mass occurrences of the species have even led to disruptions of railway traffic (Niijima and Arimura, 2002). While most millipedes in the world are harmless to humans, they can vector plant and animal diseases caused by bacteria such as *Citrobacter*, *Enterobacter*, *Salmonella*, and *Raoultella* species (Kania and Klapeć, 2012). Millipedes may cause unexpected damage when they are introduced to new locations, becoming invaders that leave behind their old parasites and predators. A taxonomic inventory of their invertebrate associates may shed light on their biology and ecology. Therefore, it was interesting that nematodes of the Rhabditida were recently discovered within the gut of the invasive species *C. hualienensis* (Meyer-Rochow, 2015). Their morphological and molecular characterization, phylogenetic relationships and ecological associations are detailed in this report.

Materials and methods

Nematodes were discovered to be associated with the phytophagous millipede *C. hualienensis*, specimens of which were collected in November on Hachijojima (33°05'N; 139°47'E) from mass aggregations on the concrete walls of a freeway (Meyer-Rochow, 2015). Nematodes were rinsed under tap water strictly from the internal cavity (hemocoel and intestine were not specified) of millipedes after dissection and placed in 65% ethanol before sending to Beltsville in December, 2014 and January 2015 to process for slides and PCR. Culture was not possible due to inadequate laboratory facilities in Japan.

Microscopy

Nematodes were imaged at ×40–60 on an Olympus BX51 microscope with a DP71 camera (Olympus America Inc., Center Valley, PA) equipped with polarization optics. Measurements in micrometers were made with an ocular micrometer on a Zeiss Ultraphot II compound microscope with Nomarski optics on alcohol-distorted specimens before formalin fixation, and images were also directly measured with CellSens ver 1.6 imaging software integrated with the camera (Olympus America LLC, Center Valley, PA). Fixed specimens of Rhabditidae juveniles were processed for permanent slides according to the formalin-glycerine method (Golden, 1990). Imaged specimens of Diplogastridae were subsequently processed for PCR so no vouchers are available. For scanning electron microscopy (SEM) *Oscheius* nematodes were fixed according to a method recently described by Takaku et al. (2013). Specimens were observed in a JEM7100F JEOL high-pressure environmental scanning electron microscope at 1 kV.

DNA analysis

Specimens of each nematode were mechanically disrupted in 20 µl of extraction buffer (Baldwin et al., 1997) then stored in PCR tubes at –80°C until needed. Extracts were prepared from thawed pools by incubating the tubes at 60°C for 60 min, followed by 95°C for 15 min. to deactivate proteinase K. Two microliters of the extract was used for each 25 µl PCR reaction.

The ribosomal LSU D2-D3 expansion segment was amplified with primers D2A 5′-ACAAGTACCG TGAGGGAAAGTTG-3′ and D3B 5′TCGGAAGGAA CCAGCTACTA-3′ (Nunn et al., 1996) using previously published amplification procedures (Baldwin et al., 1997 for *O. necromenus*; Ye et al., 2007 for others).

The 18S sequences reaction components included, per 25 µL reaction: 17.55 µL H_2O, 2.5 µL 10X PCR buffer, 0.5 µL dNTP mix (10 mM each dNTP), 0.75 µL $MgCl_2$, 50 mM, 0.75 µL 18S-G18S4 primer, 10 µM, 0.75 µL 18S-18P primer, 10 µM, 0.2 µL Taq (Invitrogen platinum, 1 unit), 23 µL of the above mix + 2 µL template DNA; cycling conditions were 94 C – 2 min, 94 C – 30 sec, 50 C – 30 sec, 68 C – 2 min, repeat 40 times: steps 2 through 4, 68 C – 10 min, 4 C – Hold, with primers of Thomas et al. (1997) used for PCR and sequencing.

PCR products were visualized and purified within the Lonza FlashGel™ DNA system (VWR International, Radnor, PA), and sequence was generated with an ABI BigDye Terminator v3.1 kit with sample sequence data analyzed on an ABI 3130XLAutomated DNA sequencer (Applied Biosystems, Foster City, CA, USA). The 28S sequence was determined on both strands using D2A and D3B primers.

The 28S rDNA sequences related to *Oscheius* (Table 1) and 18S rDNA sequences of *Mononchoides* Rahm, 1928 and relatives (Table 2) were aligned with MAFFT ver 7.017 (Katoh, et al., 2005). Bayesian likelihood trees were made with the MrBayes (Huelsenbeck and Ronquist, 2001) plugin within Geneious 7.1.7 (Biomatters, Auckland, New Zealand) using ModelTest ver. 3.7 (Posada and Crandall, 1998) AIC parameters generated within PAUP* (Sinauer Associates, Sunderland, MA).

Results

Systematics

Oscheius rugaoensis Zhang et al., 2012.
(Fig. 1)

Description

Measurement

Oscheius rugaoensis juvenile dauer stage N = 1: body length = 754 µm, body width = 35 µm, V = 56.6%, a = 21.7, c = 10.4, c′ = 5, stoma length = 20.6 µm. The stoma opening (Fig. 1A) was occluded. The pharynx especially (Fig. 1B) was shrunken and not well defined due to initial preservation in alcohol, so the 'b' ratio was not reliable. The lateral field was composed of five ridges (Fig. 1C).

Differential diagnosis

This juvenile of *Oscheius rugaoensis* has no clear counterpart for comparison in the descriptive

Table 1. Nematode 28S rDNA sequences for selected taxa in Figure 3.

Species	Isolate	Locality	Accession
Cephaloboides nidrosiensis	DF5075	The UK	EU195992
Metarhabditis blumi	DF5010	Spain	EU195965
Metarhabditis rainai	DF5091	Fiji	EU195966
Oscheius carolinensis		USA	FJ547239
Oscheius chongmingensis		China	EF503691
Oscheius chongmingensis	Tumian154	China	EU273599
Oscheius insectivorus	SB169	Germany	EU195968
Oscheius myriophilus	DF5020	The USA	AY602176
Oscheius necromenus	SB218	Australia	This paper
Oscheius rugaoensis	YNb59	China	AY177182
Oscheius rugaoensis		Japan	This paper
Rhabditella axei	DF5006	France	AY602177

Table 2. Nematode 18S rDNA sequences for selected taxa in Figure 4.

Species	Isolate	Locality	Accession
Caenorhabditis elegans	N2	The UK	AY268117
Heterorhabditis bacteriophora	1206	–	FJ040430
Fictor stercorarius	RS9003	Germany	KJ877235
Fictor sp. 2	RS9002	The USA	KJ877234
Koerneria luziae	Luc1	Japan	AB597232
Leptojacobus dorci	EJR2014	Japan	KF924399
Mononchoides cf. *americanus*	100D10	Japan	This paper
Mononchoides composticola	wb31	Belgium	GU943512
Mononchoides striatus	MonEStr	–	AY593924
Mononchoides sp.	FDL-2015 M63_39	Italy	LN827618
Mononchoides sp 1	VS-2014 RS5441	France	KJ877210
Mononchoides sp 2	VS-2014 RS9007	Mexico	KJ877209
Mononchoides sp 3	VS-2014 RS9008	New Caledonia	KJ877211
Neodiplogaster crenatae	NK126	Japan	AB326310
Paroigolaimella micrura	VS-TU-2014-2	Germany	KJ877207
Sachsia zurstrasseni	VS-TU-2014-3	Germany	KJ877208
Sudhausia aristotokia	RS9011	Ghana	KJ877231
Sudhausia crassa	RS9012	South Africa	KJ877232
Tylopharynx foetidus	wb3	Belgium	EU306343

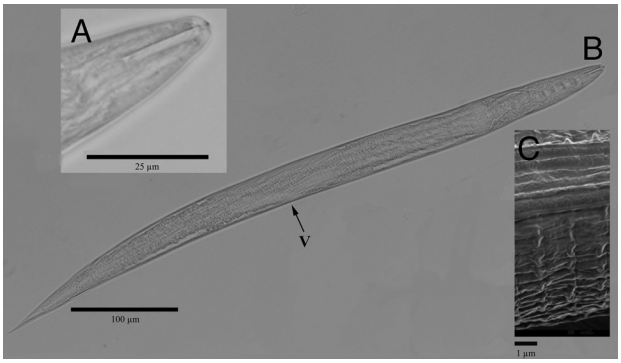

Figure 1: *Oscheius rugaoensis* Female juvenile (A) Stoma, (B) Body, ventral view, with vulva primordium (V), C. SEM lateral field cuticle.

morphological literature, being intermediate in length between the limits for dauer juveniles (666 µm) and adults (921 µm) (Zhang et al., 2012), yet having rudimentary gonad arms, similar stoma length (Darsouei et al., 2014) and five ridges (Zhang et al., 2012) as in adult females.

Molecular sequences and phylogeny

The small distance tree based on a 175 base pair (bp) MAFFT alignment (Fig. 3, Table 1) demonstrated that *O. rugaoensis* from Japan had a nearly identical sequence to that of a population from China (1/144 nucleotide difference or 0.7%), which had been described as *Heterorhabditidoides rugaoensis* by Zhang et al. (2012), and formally synonymized four years later (Darsouei et al., 2014). The longer sequence of *Oscheius rugaoensis* from Japan (vs that of China) had 5/288 nucleotide differences (98.3% similarity), compared to the closest species, *Oscheius necromenus* SB218. In contrast, two conspecific populations of *Oscheius chongmingensis* (Zhang et al., 2008; Ye et al., 2010 had only 4 out of 488 nucleotide differences between them (0.8%). The three millipede-associated nematode species resided in a clade with 100% support in a Bayesian likelihood tree (Fig. 3).

Oscheius necromenus Sudhaus and Schulte, 1989

Description

Measurements

N = 10 heat-killed hermaphrodite specimens, body length = 1,661 ± 220 (1,340–2,120) µm, body width = 130 ± 24 (100–180) µm, a = 13.0 ± 1.8 (8.7–15.1), b = 7.5 ± 0.7 (6.1–8.8), c = 14.3 ± 1.6 (12–17.4), c' = 2.6 ± 0.2 (2.3–2.9), V = 50 ± 2 (47–54)% , stoma length = 23 ± 1 (21–25) µm, stoma width = 5.7 ± 0.5 (5–6) µm, stoma length/stoma width = 4.5 ± 0.5 (3.3–4.9).

The original description (Sudhaus and Schulte, 1989) is supplemented by these measurements of adult females made from specimens cultured on *E. coli* OP50 in 1996. Some measurements of this cultured population interface with measures of the original population, extending the range compared to *O. necromenus* types for body length (vs 830–1,500 µm), body width (vs 54–90 µm), and stoma length (vs 18–20 µm). These measurements overlap with *O. necromenus* stoma width, 'c' and 'c'' ratios better than with *O. myriophilus* (vs 4–6 µm, 9–13, 3–5, respectively).

Locality and host

Oscheius necromenus Sudhaus and Schulte (1989) population SB169 from the diplopod *Oncocladosoma castaneum*, Adelaide Hills, South Australia (Sudhaus and Schulte, 1989), kept in culture since 1988 by the Sudhaus laboratory in Germany and later the Fitch laboratory at New York University (NYU), from where we obtained it in 1996. Although a LSU 28S rDNA D3 sequence was incorporated into a larger *Oscheius* tree

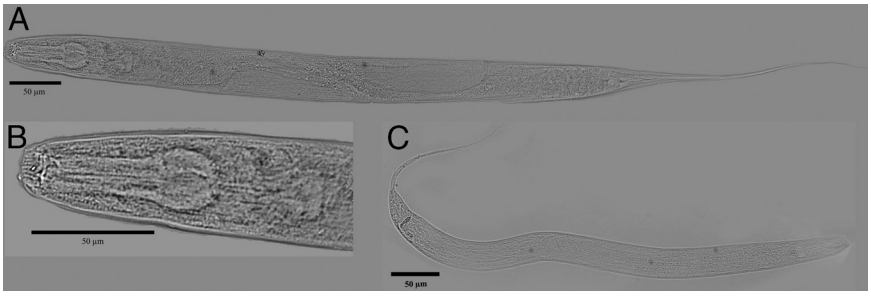

Figure 2: *Mononchoides* sp. cf. *americanus* (A) Female body, ventral view, (B) Female pharynx, (C) Male body, lateral view.

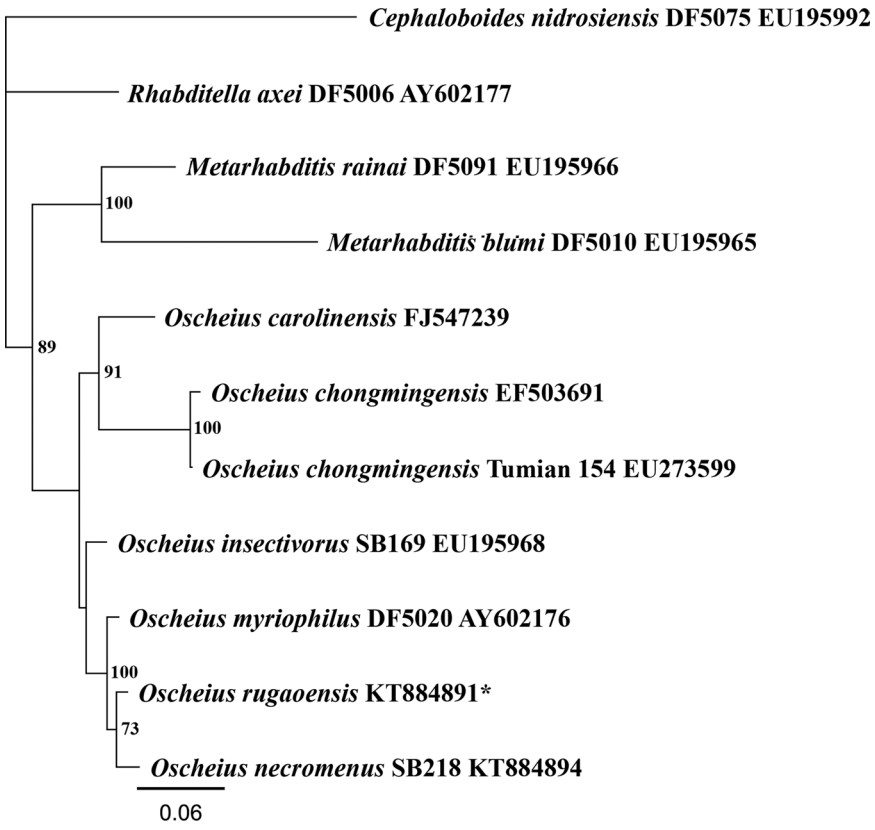

Figure 3: *Oscheius rugaoensis* 28S rDNA MrBayes phylogenetic tree of MAFFT aligned 175 base pair (bp) sequences of *Oscheius* with *Metarhabditis, Rhabditella, Cephaloboides* outgroups as implemented in Geneious ver. 7.1.7. ModelTest AIC GTR+G+I model with gamma rates, alpha shape parameter = 0.77. Chain Length 1,100,000, Burnin 110,000, Average LnL for two runs = –11529.5. Bayesian likelihood support values indicated above branches.

(Carta et al., 2001), this culture is no longer available, and molecular marker sequences other than the one generated in this work are not available in GenBank.

Mononchoides sp.

(Fig. 2)

Description

Measurements

Female body N = 1 (Fig. 2A) length = 890 µm, body width = 44.2 µm, stoma length = 14.9 µm, pharynx (Fig. 2B) length = 139 µm, anterior pharynx/posterior pharynx ratio = 1.7, vulva-anus distance = 278.8 µm, tail length = 254.0 µm, V = 40%, a = 20.1, b = 6.4, c = 3.5, c′ = 11, Paired gonads = 24.4% body length, anterior gonad = 116, posterior gonad = 101.

Male body n = 1 (Fig. 2C) length = 642 µm, body width = 29.2 µm, stoma length = 13.6 µm, tail length = 192 µm, spicule length = 26.2 µm, a = 22, b = 6.6, c = 3.4, c′ = 7.5.

Stoma of male and female with eight prominent cheilostom striations visible laterally defining seven plates, anterior esophagus about 1.7 × the posterior esophagus. Vulva-anus distance = 1.2 × tail length, long female tail ending in a thread. Pharynx, intestine, gonad, and genital papillae of male poorly defined.

Differential diagnosis

The specimens from the millipede come closest to *Mononchoides americanus* Steiner (1930) (=*Glauxinema americanus*; Steiner, 1930; Tsalolikhin, 2009) in major morphometrics except a shorter male spicule (26 vs 30–45 µm) and smaller value for female ‘c′’ (7.5 vs 8.7–12).

Molecular sequences and phylogeny

The DNA sequences were submitted to GenBank: for *Oscheius necromenus* SB 169 28S KT884894, *Oscheius rugaoensis* 28S KT884891, *Mononchoides*

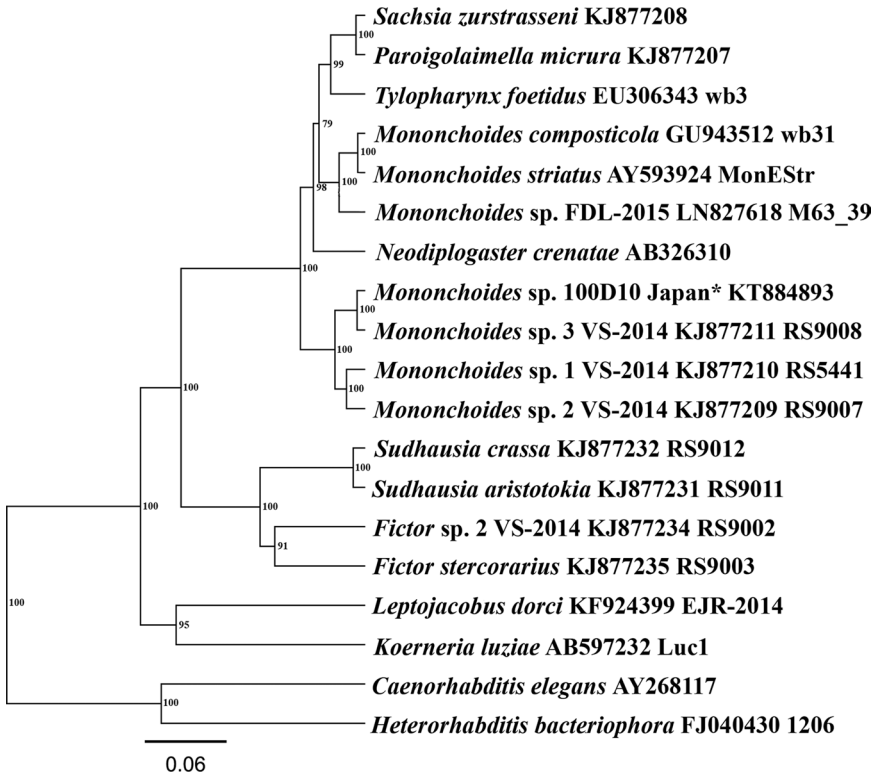

Figure 4: *Mononchoides* and diplogastrid relatives MrBayes phylogenetic tree of 18S rDNA based on a 3,410bp MAFFT alignment as implemented in Geneious ver. 7.1.7. ModelTest AIC GTR+G+I model with gamma rates, alpha shape parameter = 0.60. Chain Length 1,100,000, Burnin 110,000, Average LnL for two runs = –9630.4. Support values indicated above branches for Bayesian likelihood.

sp. cf. *americanus* 28S KT884892, 18S KT884893. The closest taxon for both 18S and 28S rDNA markers was *Mononchoides* sp. 3 from New Caledonia (in Susoy et al., 2015). For the 18S marker, a 1,088bp alignment showed 99% similarity between those isolates, and a 28S 779bp alignment showed a 96.8% similarity. A MrBayes phylogenetic tree for 18S rDNA (Fig. 4, Table 2) demonstrated 100% support for two independent clades of three and four isolates of putative *Mononchoides*, separated by *Neodiplogaster*. The second clade with four isolates included this millipede isolate of *Mononchoides* cf. *americanus*.

Discussion

Three primary groups of nematodes have been reported as associates of millipedes: Rhigonematomorph millipede gut parasite species (Hunt, 1996; Malysheva and Spiridonov, 2013), Oxyuridomorph, Thelastomatoidea and Coronostomatoidea (Adamson and van Waerebeke, 1982; Jex et al., 2005; Phillips et al., 2016), and Rhabditida: *Oscheius myriophilus* (Poinar, 1986) and *O. necromenus* (Sudhaus and Schulte, 1989). The first two nematode groups are true parasites, but the rhabditids may have a spectrum of parasitic to phoretic associations (Sudhaus, 2008).

The *Oscheius rugaoensis* population described in this work had the same 28S rDNA sequence as a nematode population incorrectly identified as an entomopathogenic *Heterorhabditis* sp. in 2003 (AY177182), later published under the name *Heterorhabditidis rugaoensis* (Zhang et al., 2012), and recently synonymized as *Oscheius* (Darsouei et al., 2014). *Oscheius necromenus* from Australia and *O. rugaoensis* are closely related (Fig. 3). Since *Oscheius myriophilus* (Poinar, 1986) and *O. necromenus* (Sudhaus and Schulte, 1989) were previously reported from millipedes, *O. rugaoensis* becomes now the third species from a millipede.

The nematode family Diplogastridae is one of the most phenotypically diverse within Rhabditida (Susoy and Herrmann, 2012). It is especially difficult to diagnose genera such as *Mononchoides, Koerneria* Meyl,

1960, and *Fictor* Paramonov, 1952 with superficially similar and highly plastic mouthparts (Huang et al., 2010). These three genera are only distantly related to one another in a recent, comprehensive phylogenetic tree of diplogastrids (Susoy et al., 2015). That tree did not include *Mononchoides composticola* and *M. striatus* seen here in the Figure 4 three-taxon clade. It demonstrates a lack of monophyly among the few GenBank sequences of *Mononchoides* out of a possible 43 nominal morphospecies (Sudhaus and Fürst von Lieven, 2003). This *Mononchoides* sp. represents an exceptional ecological association with millipedes. Other species in the genus *Mononchoides* were associated with rotting plant material and beetles (Sudhaus and Fürst von Lieven, 2003). The millipede association is probably secondary rather than primary (Sudhaus, 2008) for these *Mononchoides* due to the small numbers of adults that were recovered.

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References

Adamson, M. L. and van Waerebeke, D. 1982. Revision of the Thelastomatoidea, Oxyurida of invertebrate hosts I. Thelastomatidae. Systematic Parasitology 21:21–63.

Baldwin, J. G., Frisse, L. M., Vida, J. T., Eddleman, C. D., and Thomas, W. K. 1997. An evolutionary framework for the study of developmental evolution in a set of nematodes related to *Caenorhabditis elegans*. Molecular Phylogenetics and Evolution 8:249–59.

Carta, L. K., Morris, K., and Thomas, W. K. 2001. A new species of *Oscheius* (Nematoda: Rhabditida) from Formosan termites (*Coptotermes formosanus*) and a 28S rDNA molecular phylogeny with related taxa. Journal of Nematology 33:252–3.

Chen, C.-C., Golovatch, S.I., Chang, H.-W., and Chen, S.-H. 2011. Revision of the Taiwanese millipede genus *Chamberlinius* Wang, 1956, with descriptions of two new species and a reclassification of the tribe Chamberlinini (Diplopoda, Polydesmida, Paradoxosomatidae, Paradoxosomatinae). ZooKeys 98:1–27.

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.

Fujiyama, S., Ishida, T., and Shah, S. K. 2012. Ecology of immigrated diplopoda *Chamberlinius hualienensis* with special reference to that of *Parafontaria laminata armigera*. Annals of Environmental Science of Shinshu University 34:110–6, (in Japanese).

Golden, A. M. 1990. Preparation and mounting nematodes for microscopic observation, Zuckerman, B. M., Mai, W. F., and Krusberg, L. R. (Eds), Plant nematology laboratory manual, University of Massachusetts Agricultural Experiment Station, Amherst, MA: 197–205.

Higa, Y., and Kishimoto, T. 1986. Unusual outbreak and control of millipedes, *Chamberlinius haulienensis* Wang in Okinawa. Annual Report of Okinawa Prefectural Institute of Public Health 20:62–72, (In Japanese).

Huang, R.-E., Liang, J. U. N., Quan, L. U., and Zhang, X. -Y. 2010. *Micoletzkya chinaae* n. sp. (Nematoda: Diplogastridae), a potential predacious nematode and *Ektaphelenchus macrobulbosus* (Nematoda: Ektaphelenchinae) isolated from Simao pine in South-western China. Journal of Nematology 42:298–306.

Huelsenbeck, J. P., and Ronquist, F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics. 17:754–5.

Hunt, D. J. 1996. A synopsis of the Rhigonematidae (Nematoda), with an outline classification of the Rhigonematida. Afro-Asian. Journal of Nematology 6:137–50.

Jex, A. R., Schneider, M. A., Rose, H. A., and Cribb, T. H. 2005. The Thelastomatoidea (Nematoda: Oxyurida) of two sympatric Panesthiinae (Insecta: Blattodea) from southeastern Queensland, Australia: taxonomy, species richness and host specificity. Nematology 7:543–75.

Kania, G., and Klapeć, T. 2012. Seasonal activity of millipedes (Diplopoda) – their economic and medical significance. Annals of Agricultural and Environmental Medicine 19:649–50.

Katoh, K., Kuma, K.-I., Toh, H., and Miyata, T. 2005. MAFFT version 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Research 33:511–8.

Malysheva, S. V., and Spiridonov, S. E. 2013. *Ichthyocephaloides sumbatus* n. sp. (Nematoda: Rhigonematoidea) from Indonesia and additional data on *Xystrognathus phrissus* Hunt, Pham Van Luc and Spiridonov, 2002. Nematology 15:575–88.

Meyer-Rochow, V. B. 2015. New observations – with older ones reviewed – on mass migrations in millipedes based on a recent outbreak on Hachijojima (Izu Islands) of the polydesmid diplopod (*Chamberlinius hualienensis*, Wang 1956): nothing appears to make much sense. Zoological Research 36:119–32.

Nakamura, Y., and Korsós, Z. 2010. Distribution and diversity of millipedes of the Ryukyu Archipelago,

with the Senkaku and Daito island groups: a literature review (Arthropoda: Diplopoda). Acta Arachnologica 59:73–86.

Niijima, K., and Arimura, T. 2002. Obstruction of trains by the outbreaks of a millipede *Chamberlinius hualienensis* Wang (Diplopoda: Polydesmida). Edaphologia 69:47–9, (In Japanese).

Nunn, G. B., Theisen, B. F., Christensen, B., and Arctander, P. 1996. Simplicity-correlated size growth of the nuclear 28S ribosomal RNA D3 expansion segment in the crustacean order Isopoda. Journal of Molecular Evolution 42:211–23.

Phillips, G., Bernard, E. C., Pivar, R. J., Moulton, J. K., and Shelley, R. M. 2016. *Coronostoma claireae* n. sp. (Nematoda: Rhabditida: Oxyuridomorpha: Coronostomatidae) from the indigenous milliped *Narceus gordanus* (Chamberlain, 1943) (Diplopoda: Spirobolida) in Ocala National Forest, Florida. Journal of Nematology 48:159–69.

Poinar, G. O. Jr. 1986. *Rhabditis myriophilus* sp.n. (Rhabditidae: Rhabditida), associated with the millipede, *Oxidis gracilis* (Polydesmida: Diplopoda). Proceedings of the Helminthological Society of Washington 53:232–6.

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

Steiner, G. 1930. The nemic fauna of the slime flux of the Carolina poplar. Journal of Agricultural Research, USDA 41:427–34.

Sudhaus, W. 2008. Evolution of insect parasitism in rhabditid and diplogastrid nematodes, in Makarov, S. E., and Dimitrijevic, R. N. (Eds), Advances in arachnology and developmental biology, Institute of Zoology, Belgrade: 143–61.

Sudhaus, W., and Fürst von Lieven, A. 2003. A phylogenetic classification and catalogue of the Diplogastridae (Secernentea, Nematoda. Journal of Nematode Morphology and Systematics 6:43–90.

Sudhaus, W., and Schulte, F. 1989. *Rhabditis (Rhabditis) necromenus* sp. n. (Nematoda: Rhabditidae) from south Australian Diplopoda with notes on its siblings *R. myriophilus* Poinar, 1986 and *R. caulleryi* Maupas, 1919. Nematologica 35:15–24.

Susoy, V., and Herrmann, M. 2012. Validation of *Rhabditolaimus* Fuchs, 1914 (Nematoda: Diplogastridae) supported by integrative taxonomic evidence. Nematology 14:595–604.

Susoy, V., Ragsdale, E. J., Kanzaki, N., and Sommer, R. J. 2015. Rapid diversification associated with a macroevolutionary pulse of developmental plasticity. eLife 2015:1–39.

Takaku, Y., Suzuki, H., Ohta, I., Ishii, D., Muranaka, Y., Shimomura, M., and Hariyama, T. 2013. A thin polymer membrane nano-suit, enhancing survival across the continuum between air and high vacuum. Proceedings of the National Academy of Sciences 110:7631–5.

Thomas, W. K., Vida, J. T., Frisse, L. M., Mundo, M., and Baldwin, J. G. 1997. DNA sequences from formalin-fixed nematodes: integrating molecular and morphological approaches to taxonomy. Journal of Nematology 29:250–4.

Tsalolikhin, S. Y. 2009. A new species of free-living nematode *Glauxinema przhiboroi* sp. n. (Nematoda: Rhabditida) from Tuva. Zoosystematics Rossica 18:3–4.

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

Ye, W., Giblin-Davis, R. M., Davies, K. A., Purcell, M. F., Scheffer, S. J., Taylor, G. S., Center, T. D., Morris, K., and Thomas, W. K. 2007. Molecular phylogenetics and the evolution of host plant associations in the nematode genus *Fergusobia* (Tylenchida: Fergusobiinae). Molecular Phylogenetics and Evolution 45:123–41.

Zhang, C., Liu, J., Xu, M., Sun, J., Yang, S., An, X., Gao, G., Lin, M., Lai, R., He, Z., Wu, Y., and Zhang, K. 2008. *Heterorhabditidoides chongmingensis* 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.