[Research Note]

**Insect parasitic nematodes,
Contortylenchus sp. (Tylenchida:
Allantonematidae) from the spruce
bark beetle *Ips typographus* and the
large larch bark beetle *I. cembrae*
(Coleoptera: Scolytidae) in Japan**

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Parasitic nematodes were found in the two bark beetle species *Ips typographus* and *I. cembrae* in Japan. Based on their ecological and morphological characteristics, these nematodes are identified as members of the genus *Contortylenchus*. They show some differences in not only parasitic manners but also molecular profiles. Ribosomal DNA sequences for small subunit (SSU), and D2-D3 expansion segments of the large subunit (D2-D3 LSU) of the nematodes in the two different beetle species are almost identical, but they show one base-pair substitution in the D2-D3 LSU. These results suggest that the nematodes from *I. typographus* and *I. cembrae* are closely related but separate species. Interestingly, the D2-D3 LSU of the nematode in *I. typographus* was identical to those of both *Contortylenchus* sp. from the Moscow region, the Russian Federation, and *C. diplogaster* in *I. cembrae* from the Czech Republic. This result indicates that the nematode in *I. typographus* is closely related to or could be conspecific to the European *Contortylenchus* species, including especially *C. diplogaster* or a related species, *C. pseudodiplogaster*. Nematol. Res. 49(2), 45–47. (2019)

Key words: *Contortylenchus* spp., Czech Republic, D2-D3 LSU, Russian Federation, SSU

Bark beetles of the genus *Ips* usually propagate as secondary pests on declined trees. Outbreaks of the bark beetles, however, render them the status of primary pests that can cause tree mortality. In Japan, the spruce bark beetle *I. typographus* is a pest of *Picea jezoensis* and *P. glehnii*; and the large larch beetle *I. cembrae* is a pest of *Larix leptolepis*. During exploratory research for natural enemies of these beetles, we discovered parasitic nematodes in the hemocoel of the adult *I. typographus* (Fig. 1A). Mature parasitic females and their progenies (eggs and juveniles) were found in

the beetle, but no males were detected. The female body curved dorsally with a cleft vulva (Fig. 1B). Juveniles had a stylet without a metacarpus. Based on these ecological and morphological characteristics, we identified the nematode as a member of the genus *Contortylenchus* according to Nickle (1963) and Siddiqi (2000). Parasitic nematodes of the genus *Contortylenchus* were also found in the adult *I. cembrae* (Fig. 1C). The many female nematodes in *I. typographus* were encapsulated alive (Fig. 1D), whereas the nematodes from *I. cembrae* were free in the hemocoel. The female nematodes in *I. typographus* appeared more obese than the females in *I. cembrae* (Fig. 1B, C). The prevalence of *Contortylenchus* sp. in the adult *I. typographus* population was close to 50%, but that of the *I. cembrae* population was only several percent in samples obtained from both Hokkaido and Nagano Prefecture (Table 1). The reason for such a marked difference in nematode prevalence between *I. typographus* and *I. cembrae* is not known and remains to be investigated.

So far, only one *Contortylenchus* species, *C. genitalicola*, has been described in Japan (Kosaka and Ogura, 1993). However, *C. genitalicola* is a parasite of the Japanese pine sawyer *Monochamus alternatus* (Ogura and Kosaka, 1991). Parasitic females of *C. genitalicola* occur in the reproductive organ of the adult host insect, and are much bigger than *Contortylenchus* sp. in this study. Thus, our present *Contortylenchus* specimens are clearly different from *C. genitalicola*.

A single female nematode was obtained from an *I. typographus* adult collected in Tomakomai, Hokkaido (42°44'N, 141°29'E), and another single female from an *I. cembrae* adult collected in Karuizawa (36°20'N, 138°31'E) close to Komoro, Nagano Prefecture in Table 1. A near-full-length of the small subunit (SSU) and D2-D3 expansion segments of the large subunit (D2-D3 LSU) of ribosomal RNA genes were sequenced by using the methods of Kanzaki and Futai (2002) and Ye *et al.* (2007). The determined sequences were deposited in the GenBank database with accession numbers LC219517-LC219520. When compared with each other, the molecular sequences of the two Japanese nematode populations (1726 bps in SSU and 761 bps in D2-D3 LSU) proved to be almost identical, but showed one base-pair substitution in D2-D3 LSU.

A comparison by using the GenBank database shows that the SSU of *Contortylenchus* sp. is closest

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to that of *Delatylyus andersoni* (KY907662, Yu *et al.*, 2018), with only 89% similarity (1729 bps). This comparatively low SSU similarity was probably derived from the shortage of tylenchid (allantonematid) SSU sequences deposited in the GenBank database, *i.e.*, phylogenetically close relatives of *Contortylenchus* have not been sequenced for deposition in the database. Interestingly, the D2-D3 LSU of the nematode in *I. typographus* is identical to that of *Contortylenchus* sp. from the Moscow region, the Russian Federation (DQ328731, 680 bps, Subbotin *et al.*, 2006) and to *C. diplogaster* in *I. cembrae* from the Czech Republic (KM245035, 590 bps, Grucmanová *et al.*, 2016).

The nematodes from *I. typographus* and *I. cembrae* show some differences in their parasitic manners. They are also different in their molecular profiling with one base pair substituted in the D2-D3 LSU, although they are identical to each other in the SSU. These results suggest that the nematodes from *I. typographus* and *I. cembrae* are closely related but separate species. Interestingly, however, the D2-D3 LSU of the nematode in *I. typographus* was identical to that of *Contortylenchus* species from Europe. This result indicates that the nematode in *I. typographus* is closely related or could be conspecific to European *Contortylenchus* species, especially to *C. diplogaster*.

Contortylenchus diplogaster has been reported in *I. typographus*, *I. cembrae*, and *I. sexdentatus* (Rühm, 1956; Grucmanová and Holuša, 2013). *C. pseudodiplogaster*, a species closely related to *C. diplogaster*, has also been described from *I. sexdentatus* (Slankis, 1969). Morphologically, these two species differ in the shape of the tail in young females in the host beetle, a difference that becomes less prominent in older females (Slankis, 1969). *Contortylenchus* species in this study may be related to *C. pseudodiplogaster*, although we cannot make a definitive comparison of the tail shapes because we observed only old (egg-laying) females.

To identify species among allantonematid nematodes including *Contortylenchus*, morphological characteristics of males and infective females are generally considered better criterion because those in host insects usually show a degree of variety (Nickle, 1963). Because we could not observe the males and infective females of nematodes, nor young females in the host insects, we could not identify *Contortylenchus* sp. down to the species level by using morphological characteristics.

Several species of *Contortylenchus* nematodes have been described with neither morphological information of males and/or infective females nor

Fig. 1. *Contortylenchus* sp. found in adult *Ips typographus* and *I. cembrae*. A: Mature parasitic females of nematode (arrows) and juvenile nematodes found in *I. typographus*. B: Mature parasitic female with cleft vulva (arrow). C: A mature parasitic female and juvenile of nematode found in *I. cembrae*. D: Encapsulated mature parasitic female in *I. typographus*.

Table 1. Prevalence of *Contortylenchus* sp. in adult *Ips typographus* and *I. cembrae*^a populations.

Host species	Locality	Year	<i>n</i>	Prevalence (%)
<i>I. typographus</i>	Tomakomai ^b (42°44'N, 141°29'E)	2006	40	47.5
<i>I. cembrae</i>	Sapporo ^b (42°59'N, 141°23'E)	2005	66	6.1
	Komoro ^c (36°21'N, 138°28'E)	2007	29	3.4

^a Adult *I. typographus* and *I. cembrae* for propagation (overwintered generation) were collected from dead *Picea jezoensis* and *Larix leptolepis* trees, respectively.

^b Hokkaido, Japan.

^c Nagano Prefecture (Honshu), Japan.

little molecular information. Recently, *C. diplogaster* has been reported from *I. duplicatus* (Grucmanová *et al.*, 2014) and *I. cembrae* (Grucmanová *et al.*, 2016). We think that the integration of existing knowledge on *Contortylenchus* nematodes in *Ips* bark beetles is necessary for the species identification.

Acknowledgements

We thank Dr. Sergei Spiridonov, at the Center of Parasitology of A. N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, for translating Russian literature into English.

Literature cited

- Grucmanová, Š. and Holuša, J. (2013) Nematode associated with bark beetles, with focus on the genus *Ips* (Coleoptera: Scolytinae) in central Europe. *Acta Zoologica Bulgarica* 65, 547–556.
- Grucmanová, Š., Holuša, J. and Nermuť, J. (2014) Nematodes associated with the double-spined bark beetle *Ips duplicatus* (Coleoptera: Curculionidae) in central Europe. *Journal of Applied Entomology* 138, 723–732.
- Grucmanová, Š., Holuša, J., Čermák and Nermuť, J. (2016) Nematodes associated with *Ips cembrae* (Coleoptera: Curculionidae): comparison of generations, sexes and sampling methods. *Journal of Applied Entomology* 140, 395–403.
- 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.
- Kosaka, H. and Ogura, N. (1993) *Contortylenchus genitalicola* n. sp. (Tylenchida: Allantonematidae) from the Japanese pine sawyer, *Monochamus alternatus* (Coleoptera: Cerambycidae). *Applied Entomology and Zoology* 28, 423–432.
- Nickle, W. R. (1963) Notes on the genus *Contortylenchus* Rühm, 1956, with observations on the biology and life history of *C. elongatus* (Massey, 1960) n. comb., a parasite of a bark beetle. *Proceedings of the Helminthological Society of Washington* 30, 218–223.
- Ogura, N. and Kosaka, H. (1991) Biology of a tylenchid nematode parasitic on the Japanese pine sawyer, *Monochamus alternatus*. *Nematologica* 37, 455–469.
- Rühm, W. (1956) Die Nematoden der Ipiden. *Parasitologische Schriftenreihe* 6, 1–437.
- Siddiqi, M. R. (2000) Tylenchida. Parasite of Plants and Insects, 2nd Edition. CABI Publishing, Oxon, UK, xvii + 833 pp.
- Slankis, A. (1969) A new species of endoparasitic nematode, *Contortylenchus pseudodiplogaster* n. sp. from the bark beetle, *Ips sexdentatus*. *Materialy Nauchnoi Konferencii Vsesoyuznogo Obshchestva Gel'mintologov* 2, 302–305. (in Russian)
- Subbotin, S. A., Sturhan, D., Chizhov, V. N., Vovlas, N. and Baldwin, J. G. (2006) Phylogenetic analysis of Tylenchida Thorne, 1949 as inferred from D2 and D3 expansion fragments of the 28S rRNA gene sequences. *Nematology* 8, 455–474.
- 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, 1185–1197.
- Yu, Q., Munawar, M., Gu, J. and Ye, W. (2018) *Delatylenchus andersoni* n. gen., n. sp. (Nematoda: Neotylenchidae) isolated from white pine (*Pinus monticola*) Lumber from USA and intercepted in Ningbo, China. *Journal of Nematology* 50, 69–76.

Received: May 22, 2019