Short note

First record of a mermithid nematode in the leaf beetles *Galeruca laticollis* (Coleoptera: Chrysomelidae)

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Chrysomelidae are usually parasitised by nematodes of families Mermithidae, Allantonematidae, Steinernematidae and Heterorhabditidae (Poinar, 1998). The majority of the cases that report the presence of mermithids in Chrysomelidae refer to immature stages of nematodes (Poinar, 1998; Yaman *et al.*, 2011). To date, the only report of mermithids within the genus *Galeruca* Geoffroy, 1762 was of *Mermis nigrescens* Dujardin, 1842 in *G. tanaceti* (Linnaeus, 1758) (Siebold, 1842).

In the present work we report the first isolation of a nematode detected in an adult specimen of *G. laticollis* Sahlberg, 1837, the only known Chrysomelidae feeding on the toxic *Aconitum napellus* L. emend. Skalický.

Thirty-five specimens of *G. laticollis* were collected on Grigna N (Lecco – Italy; 45°57' N 9°23' E, 1300 m a.s.l.) and maintained in laboratory. During the dissection of a male specimen of *G. laticollis*, a live nematode was isolated from the abdomen (Fig. 1A). Morphological study was performed with a light microscope Zeiss Axioskop 2 combined with AxioCam ICc1 camera (Carl Zeiss Microscopy GmbH). Nematode DNA was extracted using DNeasy Blood and Tissue Kit (Qiagen). A fragment of 1142 base pairs of the 18S rRNA gene was amplified through specific PCRs and newly developed primers: 18SF2 (5'-AGGGAGCCTAGA AAYGGCTA-3'), 18SR1 (5'-AGCGACGGGCGG TGTGTAC-3') and 18SR2 (5'-GTCTCGYTCGTT ATCGGAAT-3'). Amplifications through PCR were performed as in Montagna *et al.* (2013), with an annealing temperature of 52°C. The obtained amplicons were sequenced by ABI technology and assembled in a consensus sequence (accession number LN623641). This sequence was subjected to BLAST analysis and compared with sequences available in GenBank. A first phylogenetic analysis was performed on a dataset that includes the homologous sequences of 58 nematodes (30 families) (Poinar *et al.*, 2007; Wang *et al.*, 2007; Yeates & Buckley, 2009). A second phylogenetic analysis was conducted on a reduced dataset with members of Mermithidae (14 taxa) and three out-groups (AF03663, AY284748, AF036596). The sequences included in each dataset were aligned with the G-INS-I algorithm implemented in MAFFT 5 (Katoh *et al.*, 2005). Bayesian analysis was performed in MrBayes 3.2 (Ronquist *et al.*, 2012) with GTR+G (Lanave *et al.*, 1984) as model of nucleotide substitution, selected according to AIC in jModelTest 2 (Darriba *et al.*, 2012). Number of runs, generations and sample frequency settled as in a previous work (Montagna *et al.*, 2012). Maximum likelihood (ML) analyses were performed with PhyML (Guindon *et al.*, 2010) implementing the same model of nucleotide evolution, the best of NNI and SPR, and 100 bootstrap replicates.

The isolated nematode was 76 mm long and 0.3 mm wide, with a tail appendage 59 µm long and a cuticle 11 µm thick (Figs 1B & C) forming a layer completely surrounding the nematode body. Even adopting electron-microscopy technique, the detection of structures useful to identify the specimen at genus or species level (*i.e.*, cephalic and labial papillae) was impossible due to the presence of the cuticle; moreover, no vulvar opening, vagina or spicules were detected. All these morphological features prompted the ascription of the isolated nematode to a juvenile of the family Mermithidae (Braun, 1883; Kaiser, 1991). The body-tail ratio is

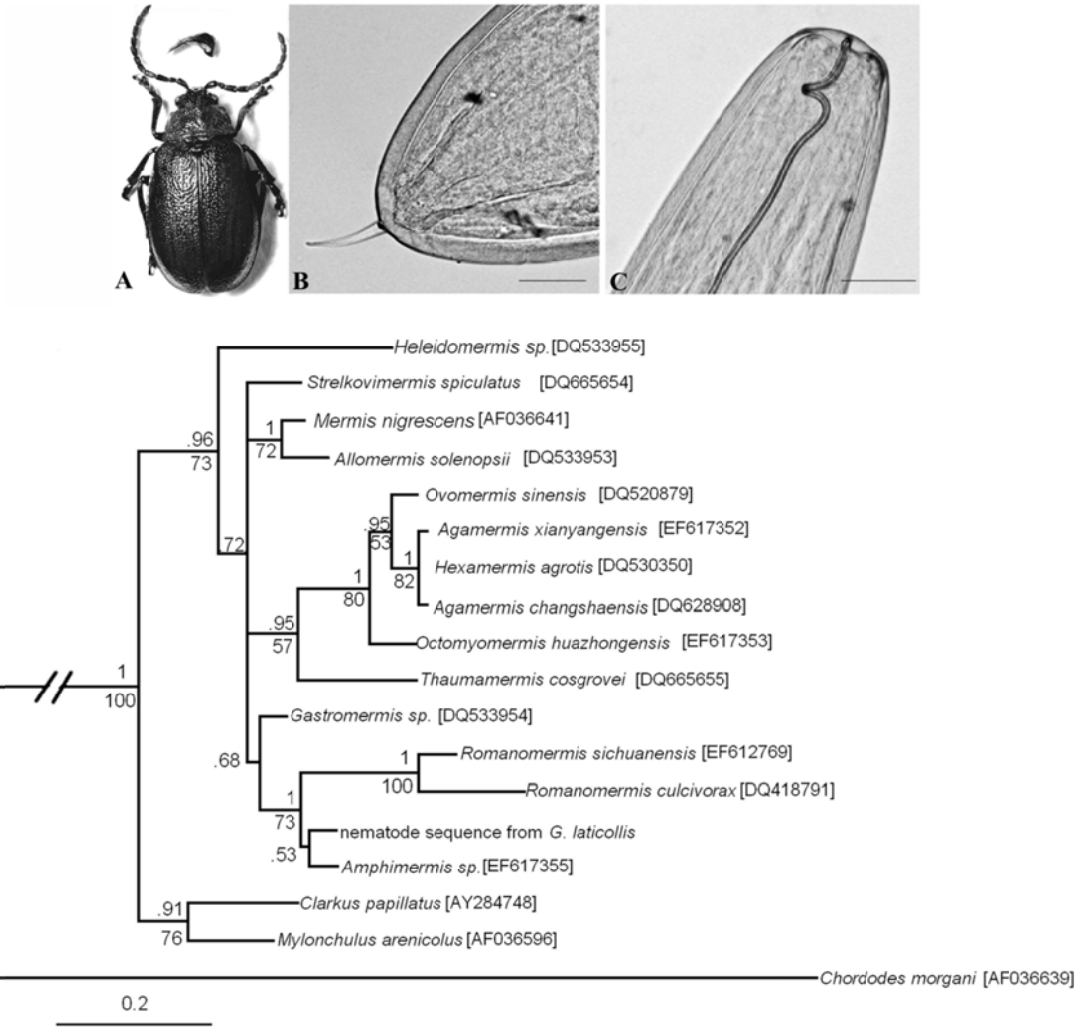

Fig. 1. A: *Galeruca laticollis* from which the nematode was extracted (obtained as described in Montagna, 2011). Above the specimens is present the median lobe of aedeagus; B: posterior part of the juvenile Mermithidae with the tail appendage, scale bar = 50 µm; C: anterior part of the nematode, scale bar = 50 µm; D: Majority rule consensus phylogram (threshold > 50%) based on the 18S rRNA gene sequences; support values > of 0.5 and of 50% are reported on the branches (BPP above and ML bootstraps below); the scale bar indicates the distance in substitutions per site.

very similar to that measured in specimens parasitising *Chrysolina fastuosa* (Scopoli, 1763) (Coleoptera: Chrysomelidae) (Yaman *et al.*, 2011). The obtained sequence shows the best match (identity 99%) with an unidentified nematode isolated from *Kosciuscola tristis* Sjöstedt, 1934 (Orthoptera: Acrididae) (JQ894731). The phylogenetic analysis performed on the whole 18S rRNA dataset confirms that the nematode isolated from *G. laticollis* belongs to the family Mermithidae (data not shown). The phylogram inferred by the reduced dataset is shown in Fig. 1D. The nematode from *G. laticollis* clusters with *Amphimermis* sp., resulting in a well-supported group together with two species of *Romanomermis* Coman, 1961. Nematodes of the genus *Amphimermis* Kaburaki & Imamura, 1932 are terrestrial, whereas those of *Romanomermis* are aquatic parasites (Kaiser, 1991; Kobylinski *et al.*, 2012). *Amphimermis* spp. has been previously reported to parasitise hosts from different insect orders (Baker & Poinar, 1994; Wang, 2007). Within coleopterans, members of *Amphimermis* have been usually isolated from Chrysomelidae (Poinar, 1998); for example, *A. elegans* (Hagmeier, 1912) and *A. volubilis* Rubzov & Koval, 1975 were reported from *Leptinotarsa decemlineata* (Say, 1824). Interestingly, only one specimen of *G. laticollis* out of 35 dissected (2.9%) was parasitised, indicating a low prevalence of the parasite. Since behavioural differences were not

observed between the infected and non-infected specimens, apparently the nematode did not heavily affect the host fitness. Based on the described life cycle of mermithids (Braun, 1883; Kaiser, 1991), we can hypothesise that *G. laticollis* and the nematode have a congruent life cycle. We hypothesise that in late winter and spring, juveniles hatch from eggs laid by adult mermithids and pre-infective juveniles invade the larval stages of *G. laticollis*. Once the host dies, in late autumn, juvenile mermithids leave the host and reach the soil to moult and mate.

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