Russian Journal of Nematology, 2009, 17 (2), 145 - 147

Short note

First record of the nematode *Alloionema appendiculatum* Schneider (Rhabditida: Alloionematidae) in Arionidae slugs in Slovenia

Žiga Laznik*, Jenna L. Ross, Tímea Tóth***, Tamás Lakatos***, Matej Vidrih* and Stanislav Trdan***

***University of Ljubljana, Biotechnical Faculty, Dept. of Agronomy, Chair of Phytomedicine, Agricultural Engineering, Crop Production, Grassland and Pasture Management, Jamnikarjeva 101, SI-1111 Ljubljana, Slovenia, e-mail: ziga.laznik@bf.uni-lj.si**
****University of Aberdeen, Institute of Biological and Environmental Sciences, AB24 3UU, UK,**
*****Research and Extension Centre for Fruit Growing, Vadastag 2, H-4244 Újfehértó, Hungary.**

Accepted for publication 25 October 2009

Parasitic and pathogenic nematodes associated with terrestrial gastropod molluscs (slugs and snails) have received little attention compared with entomopathogenic nematodes (Wilson & Grewal, 2005). The most intensively studied species of slug-parasitic nematode is *Phasmarhabditis hermaphrodita* (Schneider) (Rhabditidae), a lethal parasite to many terrestrial molluscs (slugs and snails), particularly slug species from the families Agriolimacidae, Arionidae, Limacidae, Milacidae and Vagnulidae (Wilson *et al.*, 1993ab; Speiser *et al.*, 2001; Iglesias & Speiser, 2001; Grewal *et al.*, 2003; Rae *et al,* 2007). As a result of its high virulence and broad host range, *P. hermaphrodita* has been commercialised as a biological control agent for slugs and is sold throughout Europe (Rae *et al.*, 2007). Another species of nematode known to be associated with terrestrial gastropod molluscs is *Alloionema appendiculatum* (Morand *et al.*, 2004) This species has a broad geographical distribution and has been found in areas including Europe, Australia and North America (Cabaret *et al.*, 1988; Ross *et al.*, 2009). In 1969, *A. appendiculatum* was identified in Slovenia, following a survey of the nematological fauna. The nematode was found free-living in soils of a vineyard in the Primorska region (Hržič, 1969; Urek *et al.*, 2003).

In September/October 2008, 500 slugs, belonging to the Arionidae family, were collected from the Ljubljana and Prekmurje areas of Slovenia. All slugs were rinsed to remove surface-dwelling nematodes then dissected and examined for the presence of internal nematodes. The pathogenicity of isolated nematodes was then investigated by infecting five *Arion lusitanicus* Mabille with 1 ml of a 300 infective juveniles (IJ) ml^{-1} suspension on 9 cm diameter Petri dishes. After 4 days the slugs died and cadavers were kept at room temperature for a total of 10 days. White traps (Bedding & Akhurst, 1975) were then used to separate the nematodes from the dead slug tissue. The nematodes were washed in 5% sodium hypochlorite solution and DNA was extracted using a QIAmp DNA Mini Kit. The 18S gene was amplified using a combination of universal primer pairs: G18S4, 26R, 24F, and 18P (Blaxter *et al.* 1998). An additional primer pair, 22F, and a newly designed primer, 1080R (TCC-TGG-TGG-TGC-CCT-TCC-GTC-AAT-TTC), were also used to ensure complete coverage of the 18S region (J. Ross, unpubl.). PCR cycling parameters involved primary denaturation at 94°C for 5 minutes, followed by 35 cycles of 94°C for 60 s, 55°C for 90 s, and 72°C for 2 min. Post-amplification extension occurred at 72°C for 10 min (J. Ross, unpubl.). The PCR products were visualized on a 1% agarose gel and cleaned using a Qiagen QIAquick® PCR Purification Kit. Sequencing was carried out using an ABI3730 sequencer. The sequences were assembled using Sequencer 4.1 (Genes Codes Corp. Ann Arbor, Michigan, USA). The sequence was then deposited in the NCBI GenBank, under FJ665982. The results were then compared with the GenBank Database, (http://www.ncbi.nlm.nih.gov/), using the BLASTn search tool (Altschul *et al.*,

1990). The closest matches to this sequence were *Alloionema appendiculatum* (FJ516751.1) (Ross & Wilson, unpubl.) with 100% identity over 100% coverage, followed by *Alloionema appendiculatum* (EU573707.1) (Spiridonov *et al.*, unpubl.) with 99% identity over 100% coverage. Genetic studies of this partial 18S gene sequence (1425 bp) reveal that this nematode species is *Alloionema appendiculatum* Schneider (1859).

The nematode *Alloionema appendiculatum* is known to have a free-living and parasitic life cycle (Cabaret & Morand, 1990). During the parasitic cycle, third-stage juveniles (J3) enter the slug's body through its foot, where the nematodes moult to the fourth-stage juvenile (J4) which become encapsulated in the pedal musculature. These juveniles then exit the slug and moult into free-living immature adults (Morand & Daguzan, 1986). *Alloionema appendiculatum* has been reported from several species of slugs including *Deroceras* sp. (Schneider, 1859), *Arion ater* (Schuuramns – Stekhoven, 1950), *A. circumscriptus* (Mengert, 1953), *A. intermedius* (Cabaret *et al.*, 1988), *A. silvaticus* (Morand & Bonnet, 1989), *A. subfuscus* (Cabaret & Morand, 1990), *Cantareus aspersus* (Charwat & Davies, 1999) and *Prietocella barbara* (Morand, 2004). Morand and Daguzan (1986) noticed that *A. appendiculatum* was rarely reported from natural populations of slugs, when its presence in cultivated species of slugs is more common (Cabaret *et al.*, 1988).

ACKNOWLEDGEMENT

This work was carried out within Horticulture No P4-0013-0481, a programme funded by the Slovenian Research Agency, and within the L4-1013 project funded by the Slovenian Research Agency and Ministry of Agriculture, Food, and Forestry of the Republic of Slovenia and the company Unichem d.o.o. Part of the research was funded within Professional Tasks from the Field of Plant Protection, a programme funded by the Ministry of Agriculture, Forestry, and Food of Phytosanitary Administration of the Republic of Slovenia. This study was also supported by Becker Underwood and BBSRC.

REFERENCES

FRANKLIN, M.T. AND SIDDIQI, M.R. 1972. *Aphelenchoides besseyi. C.I.H.Description of Plant Parasitic Nematodes.* Set 1, No. 4.

ALTSCHUL, S.F., GISH, W., MILLER, W., MYERS, E.X. & LIPMAN, D.J. 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215: 403-410.

BEDDING, R. A. & AKHURST, R. J. 1975. Simple technique for the detection of insect parasitic rhabditid nematodes in soil. *Nematologica* 21: 109-110.

CABARET, J., MORAND, S., AUBERT, C. & YVORÉ, P. 1988. Snail farming: a survey of breeding management, hygiene and parasitism of the garden snail, *Helix aspersa* Müller. *Journal of Molluscan Studies* 54: 209-214.

CABARET, J. & MORAND, S. 1990. Single and dual infection of the land snail *Helix aspersa* with *Muellerius capillaris* and *Alloionema appendiculatum* (Nematoda). *Journal of Parasitology* 76: 579-580.

CHARWAT, S.M. & DAVIES, K.A. 1998. A nematode isolate for biocontrol of pest snails in south Australia. *Nematologica* 44: 469-470.

GREWAL, S.K., GREWAL, P.S. & HAMMOND, R.B. 2003. Susceptibility of North American native and non-native slugs (mollusca: Gastropoda) to *Phasmrahabditis hermaphrodita* (Nematoda: Rhabditidae). *Biocontrol Science and Technology* 13:119-125.

HRŽIČ, A. 1969. *Investigation of nematodes as direct plant pests and vectors of plant diseases*. Agricultural Institute of Slovenia, Ljubljana, Technical Report, 26 pp.

IGLESIAS, J. & SPEISER, B. 2001. Consumption rate and susceptibility to parasitic nematodes and chemical molluscicides of the pest slugs *Arion hertensis* s. s. and *A. distinctus*. *Journal of Pest Science* 74:159-166

MENGERT, H. 1953. Nematoden und Schnecken. *Zeitschrift für Morphologie und Ökoligie der Tiere* 4: 311-349.

MORAND, S. & BONNET, J.C. 1989. Importance des nématodes en héliciculture et méthode de prophylaxie. *Haliotis* 19: 69-75.

MORAND, S. & DAGUZAN, J. 1986. Contribution à l'escargot petit-gris (*Helix aspersa* Müller): Premiers résultats concernants l' acarien *Riccardoella limacum* (Schrank) et le nématode *Alloionema appendiculatum* (Schneider). *Haliotis* 15: 31-39.

MORAND, S., WILSON, M.J. & GLEN, D.M. 2004. *Nematodes (Nematoda) Parasitic in Terrestrial Gastropods*. In Barker, G.M. (ed.) Natural Enemies of Terrestrial Molluscs. CABI Publishing, Wallingford, UK, 644 pp.

RAE, R.G., VERDUN, C., GREWAL, P.S., ROBERTSON, J.F. & WILSON, M.J. 2007. Biological control of terrestrial molluscs using *Phasmarhabditis hermaphrodita* – progress and prospects. *Pest Management Science* 63: 1153-1164.

ROSS, J.L., IVANOVA, E.S., SEVERNS, P.M. & WILSON, M.J. 2009. The role of parasite release in invasion of the USA by European slugs. Biological Invasions. (in press).

SCHUURMANS STEKHOVEN, L.H. 1950. *Alloionema appendiculatum*. *Acta Zoologica Lilloana* 9: 481-485.

SPEISER, B., ZALLER, J.G. & NEWDECKER, A. 2001. Size-specific susceptibility of the pest slugs *Deroceras reticulatum* and *Arion lusitanicus* to the nematode biocontrol agent *Phasmarhabditis hermaphrodita*. *Biocontrol* 46:311-320

UREK, G., ŠIRCA, S. & KARSSEN, G. 2003. A review of plant-parasitic and soil nematodes in Slovenia. *Nematology* 5: 391-403.

WILSON, M.J. & GREWAL, P.S. 2005. Biology, production and formulation of slug-parasitic nematodes. In: Grewal, P.S., Ehlers, R.-U. & Shapiro-Ilan, D.I. (eds.) *Nematodes as biocontrol agents*. CABI Publishing, Wallingford, UK, 505 pp.

WILSON, M.J., GLEN, D.M. & GEORGE, S.K. 1993A. The rhabditid nematode *Phasmarhabditis hermaphrodita* as a potential biological control agent for slugs. *Biocontrol Science and Technology* 3:503-511

WILSON, M.J., GLEN, D.M., GEORGE, S.K. & BUTLER, R.C. 1993B. Mass cultivation and storage of the rhabditid nematode *Phasmarhabditis hermaphrodita*, a biocontrol agent for slugs. *Biocontrol Science and Technology* 3: 513-521.