Journal of Helminthology

cambridge.org/jhl

Nematodes associated with terrestrial slugs in the Edmonton region of Alberta, Canada

T. Brophy[1], R.J. Mc Donnell[2], D.K. Howe[3], D.R. Denver[3], J.L. Ross[4,*] and L.T. Luong[1]

[1]Department of Biological Sciences, University of Alberta, Edmonton, AB, T6G 2E9, Canada; [2]Department of Crop and Soil Science, Oregon State University, Corvallis, OR 97331; [3]Department of Integrative Biology, Oregon State University, Corvallis, OR 97331 and [4]Department of Conservation Ecology and Entomology, Faculty of AgriSciences, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa

Research Paper

*Current address: Crop Health and Protection (CHAP), National Agri-Food Innovation Campus, Sand Hutton, York, YO411LZ, UK.

Cite this article: Brophy T, Mc Donnell RJ, Howe DK, Denver DR, Ross JL, Luong LT (2020). Nematodes associated with terrestrial slugs in the Edmonton region of Alberta, Canada. *Journal of Helminthology* **94**, e200, 1–5. https://doi.org/10.1017/S0022149X20000838

Received: 31 July 2020
Revised: 3 September 2020
Accepted: 3 September 2020

Key words:
Biological control; *Deroceras reticulatum*; gastropod; Mollusca; parasitism; *Phasmarhabditis*; slug pests

Author for correspondence:
L.T. Luong, E-mail: lluong@ualberta.ca

Abstract

A survey of nematodes associated with terrestrial slugs was conducted in residential gardens, nurseries, greenhouses and agricultural sites located in and around Edmonton, Alberta, Canada. A total of 2406 slugs were collected from 82 sites. Slugs were decapitated and cadavers were incubated for two weeks, with emerging nematodes removed and processed for identification. Nematodes were identified using molecular sequence data for the 18S ribosomal DNA. Nematodes were recovered from 20 of the 82 sites surveyed, with 24.4% of the slugs infected with nematodes. A total of seven nematodes were identified to species level, including *Caenorhabditis elegans*, *Panagrolaimus papillosus*, *Pellioditis typica*, *Pelodera pseudoteres*, *Rhabditella axei*, *Rhabditoides inermiformis* and *Phasmarhabditis californica*. An additional four specimens were identified to genus level, including *Oscheius* sp. (9), *Pristionchus* sp., *Rhabditis* sp. and *Rhabditophanes* sp. (1). The two most common nematode species were *C. elegans* and *P. pseudoteres*. The facultative parasite, *P. californica*, was recovered from a single *Arion rufus* specimen, collected from a seasonal nursery. To our knowledge, this study represents the first survey of slug-associated nematodes in Canada.

Introduction

Terrestrial gastropod molluscs (slugs and snails) (Mollusca: Gastropoda) are among the most damaging pests in residential gardens, nurseries, greenhouses and agriculture (Godan, 1983; DeAngelis, 1993; Barker, 2002). Current control measures rely on chemical molluscicides, primarily methiocarb, metaldehyde, iron phosphate and sodium ferric EDTA, either formulated into baited pellets or a liquid spray (Hata *et al*., 1997; Bailey, 2002; Henderson & Triebskorn, 2002). To date, the aforementioned methods have proven to be the most cost-effective approach for controlling molluscs; however, studies have demonstrated that methiocarb and metaldehyde are toxic to non-target organisms (Purves & Bannon, 1992; Fletcher *et al*., 1994; Bailey, 2002). Methiocarb has been banned in many countries but it still can be used in the US as an emergency molluscicidal treatment in ornamental nurseries. There has been increased pressure from regulatory bodies in regions like Europe to ban or limit the use of metaldehyde due to concerns relating to drinking water (Castle *et al*., 2017) and the impact on birds and small mammals (Ross, 2019). Metaldehyde was to be banned in Great Britain in spring 2020, but the ban was overturned by the High Court in 2019. Thus, a more effective and environmentally favourable method of mollusc control is needed. Nematodes offer a potential solution as they play an important role in the regulation of gastropod populations in nature (Morand *et al*., 2004), yet the association is not well studied relative to entomopathogenic nematodes (Wilson & Grewal, 2005).

Most surveys on nematodes associated with terrestrial slugs have, to date, focused on Australia, New Zealand, Europe, Africa and the USA (Ross *et al*., 2016; Wilson *et al*., 2016). Thus far, nematodes belonging to nine families have been found in association with slugs as phoretic or definitive hosts. These include: Agfidae, Alloionematidae, Angiostomatidae, Alaninematidae, Ascaridae, Cosmocercidae, Diplogastridae, Mermithidae and Rhabditidae (Pieterse *et al*., 2017). The nematode that has gained greatest interest due to its commercialization as a biological control agent is *Phasmarhabditis hermaphrodita* (Schneider) Andrássy. *Phasmarhabditis hermaphrodita* is a facultative parasite capable of infecting slug species from the families Agriolimacidae, Arionidae, Milacidae, Limacidae and Veronicellidae (Wilson *et al*., 1993; Grewal *et al*., 2003; Rae *et al*., 2007), as well as various snail species (Rae *et al*., 2007; Mc Donnell *et al*., 2018a, b). This slug-parasitic nematode is commercially available under the trade names Nemaslug® (BASF) and SlugTech® (Dudutech) in numerous European countries. However, these products are not legally available to date in North America as *P. hermaphrodita*, as well as other Phasmarhabditids

CAMBRIDGE UNIVERSITY PRESS

(*Phasmarhabditis californica* and *Phasmarhabditis papillosa*) have only recently been reported in California (Tandingan De Ley *et al.*, 2014; Tandingan De Ley *et al.*, 2016) and Oregon (Mc Donnell *et al.*, 2018a, b), and there is little information on the host range of these US strains.

European pestiferous slugs are a growing problem in Canada, yet there exist few comprehensive studies on the abundance and diversity of slugs in the region (Hawkins *et al.*, 1997; Moss & Hermanutz, 2010; L'Heureux & Angers, 2018). The widespread success of invasive slugs is likely due to a number of factors, including favourable climates and lack of associated natural enemies (Ross *et al.*, 2010). In a recent study, *P. californica* was isolated for the first time from a slug specimen in Edmonton, Alberta (Brophy *et al.*, 2020). This discovery was part of a broader survey conducted in and around Edmonton with the hope of isolating a potential biological control agent of invasive slugs in Canada. Here we present data from the survey of slugs and their associated nematodes collected from residential, agricultural and horticultural sites.

Materials and methods

Slug collection and identification

Slugs were collected from residential gardens, nurseries, greenhouses and agricultural sites in the Edmonton area of Alberta, Canada. The survey was conducted in 2019 between May and October. Slugs were collected by hand and transferred to plastic containers with preformatted lids to allow for air circulation. Once the slugs were returned to the laboratory, they were maintained in an incubator (18°C–12°C, 12 h light:12 h dark cycle) and fed *ad lib* carrot slices or lettuce until processing. Specimens from the same collection site were kept together to avoid cross-contamination. Slugs were initially identified through morphological examination (Forsyth, 2004; Mc Donnell *et al.*, 2009; Grimm *et al.*, 2009; Vlach, 2016; https://idtools.org/id/mollusc/index.php). A molecular diagnosis was only performed on specimens when examination of external morphology and internal genital morphology was inconclusive – for example, immature specimens. A piece of tail tissue was also taken from select slug specimens and placed in 95% ethanol for molecular analysis. DNA was extracted from a small piece (~2 mm × 2 mm) of this tissue using an Omega BioTek blood and tissue kit (Norcross, GA, USA). Partial fragments of the mitochondrial cytochrome *c* oxidase subunit I (*cox* 1) gene were sequenced from four of the specimens (table 1) following the methods of Reich *et al.* (2015) using the Folmer *et al.* (1994) *cox* 1 primer set LCO-1490 (5′-GGTCA ACAAATCATA AAGATATTGG-3′) and HCO-2198 (5′-TAA ACTTCAGGGTGACCAAAAAATCA-3′). After trimming and editing with Bioedit v 7.0.5.3 (Hall, 1999), the resulting sequences were 648–652 bp long. The closest match to specimens from the Barcode of Life (BOLD) database (Ratnasingham & Hebert, 2007) and GenBank (Benson *et al.*, 2013) database were then used to confirm our morphological identifications. The sequences from the current study have been deposited in GenBank under accession numbers (table 1).

Nematode identification

Slugs were decapitated and cadavers were incubated for two weeks in individual Petri dishes, which were covered and sealed with Parafilm (Wilson *et al.*, 2016). If nematodes were present on the cadaver at that time, three individuals were transferred with a sterile pin to 95% ethanol and later identified using molecular techniques. After removal of the storage ethanol, nematodes were transferred to a proteinase-K-based lysis buffer for DNA extraction as previously described (Williams *et al.*, 1992). The nematode species were identified using 18S ribosomal DNA amplification with the primer set 18A (5′-AAAGATTAAGCCATGCATG-3′) and 26R (5′-CATT CTTGGCAAATGCTTTCG-3′), resulting in an ~800 bp segment, which was directly end sequenced using primer 18A (Blaxter *et al.*, 1998). All 18S rRNA sequences were compared against GenBank's non-redundant (nr) database using blastn, NCBI Resource Coordinators. Database resources of the National Center for Biotechnology Information. Nucleic Acids Res.2018;46(D1):D8-D13. doi:10.1093/nar/gkx1095. Nematode BLAST matches with percent identity of 98–100 were used for species identification. One representative sequence for each nematode species identified in this study was submitted to GenBank (see table 2 for accession numbers).

Table 1. Number and prevalence of slugs associated with nematodes.

Slug species	Positive (total)	% Prevalence
Arionidae		
Arion fasciatus	2 (124)	1.6
Arion hortensis	0 (1)	–
Arion rufus	1 (9)	11.1
Prophysaon andersoni[a]	0 (11)	0
Agriolimacidae		
Deroceras invadens	0 (25)	0
Deroceras laeve[b]	1 (10)	10.0
Deroceras reticulatum	69 (2158)	3.2
Limacidae		
Ambigolimax valentianus[c]	16 (65)	24.6
Limax maximus	1 (2)	–
Total	90 (2405)	3.7

Species with a superscript letter indicate that morphological identifications were confirmed using partial COI gene sequences. This was achieved by finding the closest match on the Barcode of Life (BOLD) and GenBank databases.
[a]*Prophysaon andersoni* (GenBank accession number: MT680916): 97.82% match with a specimen from British Columbia, Canada, on GenBank and 100% match with a specimen from an unspecified location on BOLD.
[b]*Deroceras laeve*, two specimens: (1) (GenBank accession number: MT680915) 100% match with a specimen from British Columbia, Canada on GenBank and 100% match with a specimen from Manitoba, Canada, on BOLD; (2) (GenBank accession number: MT680918) 99.38% match with a specimen from an unspecified location on GenBank and 100% match with a specimen from West Gloucestershire, UK, on BOLD.
[c]*Ambigolimax valentianus* (GenBank accession number: MT680917): 99.07% match with a specimen from Aude, France, on GenBank and 99.84% match with a specimen from Antrim, Northern Ireland, on BOLD.

Results

A total of 2406 slugs were collected from Edmonton and surrounding areas. Specimens were collected from 72 residential gardens (harvested by homeowners and donated to the lab), four natural green spaces (parks, ravines), four seasonal nurseries and the University of Alberta Botanical Garden (exterior gardens and two year-round greenhouses). Slugs were also opportunistically obtained from two sites adjacent to agricultural fields, a strawberry patch in Thorsby and a ditch alongside a canola field in

https://doi.org/10.1017/S0022149X20000838 Published online by Cambridge University Press

Table 2. Nematode species, accession numbers and associated slug species.

Nematode species	Accession number[a]	Nematode family	Slug species
Caenorhabditis elegans	MT782338	Rhabditidae	*Deroceras reticulatum*
			Ambigolimax valentianus
Oscheius sp. (9)	MT782339	Rhabditidae	*D. reticulatum*
Panagrolaimus cf. *papillosus*	MT782340	Panagrolaimidae	*D. reticulatum*
Phasmarhabditis californica	MT135094	Rhabditidae	*Arion rufus*
Pellioditis typica	MT782341	Rhabditidae	*D. reticulatum*
Pelodera pseudoteres	MT782342	Rhabditidae	*A. fasciatus*
			D. reticulatum
Pristionchus sp.	MT782343	Diplogastridae	*D. reticulatum*
Rhabditella axei	MT782345	Rhabditidae	*D. reticulatum*
Rhabditis sp.	MT782346	Rhabditidae	*D. reticulatum*
Rhabditoides inermiformis	MT782344	Rhabditidae	*D. reticulatum*
Rhabditophanes sp. (1)	MT782347	Alloionematidae	*D. reticulatum*

[a]One representative sequence was submitted to GenBank for each nematode species identified in this study.

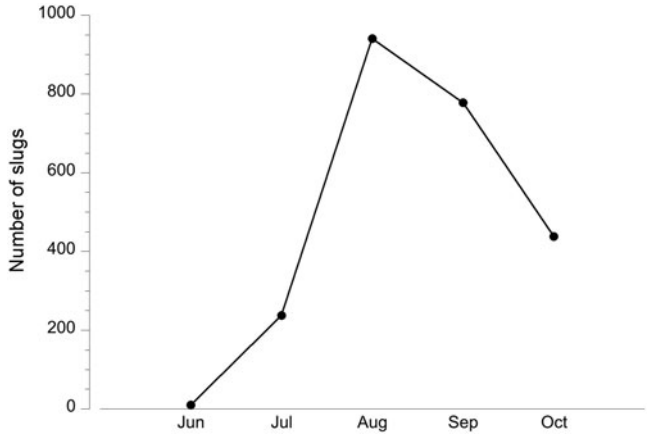

Fig. 1. Number of slugs collected in the 2019 survey between June and October.

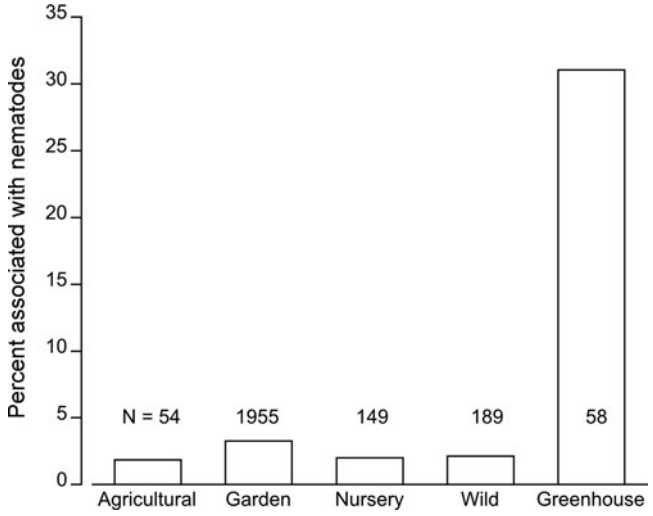

Fig. 2. Prevalence of nematodes in all slugs surveyed based on the type of collection site. Numbers represent sample sizes.

Falun. The vast majority of slugs were collected from residential gardens, due largely to the overwhelming cooperation of residential gardeners. In total, nine slug species were identified (table 1), representing three slug families (Arionidae, Agriolimacidae and Limacidae). Although we did not conduct a formal population estimate, the peak slug recovery (especially from home gardeners) occurred in August, suggestive of a seasonal peak in the population density of adult slugs (fig. 1).

Nematodes were recovered from 20 of the 82 sites surveyed (24.4%). Of all slugs examined, 3.74% were associated with nematodes. The highest prevalence occurred in year-round greenhouses, where 31.0% of the slugs harboured nematodes; only 3.27% of the nearly 2000 slugs from residential gardens harboured nematodes (fig. 2). *Deroceras reticulatum* was the most common slug, but these were rarely associated with nematodes (3.20%; table 1). By contrast, nematodes were recovered from 16 of 65 (24.6%) specimens of *Ambigolimax valentianus*, most of which were collected from the year-round greenhouses (table 1). A total of seven nematode species were identified, plus four specimens that could only be identified to genus level (table 2). The two most common nematodes were *Caenorhabditis elegans* and *Pelodera pseudoteres*. The facultative parasite *P. californica* was recovered from a single *Arion rufus* specimen, collected from a potted plant located on the exterior grounds of a seasonal nursery (Brophy *et al.*, 2020).

Discussion

This study is the first survey of nematodes associated with slugs in Canada, and specifically in the central Alberta region. A total of nine slug species and 11 nematode taxa (seven to species level; four to genus level) were identified. Overall, the nematodes represented four families: Alloionematidae, Diplogastridae, Panagrolaimidae and Rhabditidae. The most common family Rhabditidae is a large family with many genera associated with slugs, including *Rhabditis*, *Caenorhabditis* and *Phasmarhabditis*

https://doi.org/10.1017/S0022149X20000838 Published online by Cambridge University Press

(Grewal *et al.*, 2003; Pieterse *et al.*, 2017). Most of the nematodes we recovered were likely either free-living or phoretic on the slugs, with the exception of *P. californica*, which is a facultative parasite of slugs (Tandingan De Ley *et al.*, 2016). The reason for this may be due to the use of the decapitation technique (Wilson *et al.*, 2016), which can encourage the replication of free-living or phoretic nematodes. In the future, slug cadavers should be rinsed thoroughly prior to placing in Petri dishes to remove phoretic species. The two most common nematodes, *C. elegans* and *P. pseudoteres*, are considered free-living or phoretic on arthropods, slugs and earthworms (Schulte, 1989; Kiontke & Sudhaus, 2006). However, *C. elegans* has been shown to persist in the intestine of molluscs (Petersen *et al.*, 2015). Mollusc-associated nematodes have evolved independently four times (in the Nematoda) in two groups: Metastrongyloidea (Order Strongylida), most of which use molluscs as intermediate hosts, and the Rhabditoidea (Order Rhabditida), which primarily use molluscs as definitive hosts (Grewal *et al.*, 2003).

The invasive slug *D. reticulatum* was the most common slug in our survey, and is the most widespread and important pest in Canada (Grimm *et al.*, 2009). In spite of the over-representation of this species in our survey, the majority of which were collected from residential gardens, only 3.2% were found in association with nematodes. Previous studies have recovered nematodes from 3 to 6% of all slugs surveyed in the USA (Ross *et al.*, 2010), South Africa (Ross *et al.*, 2012) and Norway (Ross *et al.*, 2016). Nearly a quarter (24.6%) of the *Ambigolimax valentianus* slugs we collected harboured nematodes, most of which were collected from year-round greenhouses. Similarly, a study in Norway found that whilst the prevalence of infection was 18.7% among all slugs, 34.8% of *Arion vulgaris* examined were associated with nematodes (Ross *et al.*, 2016).

All slug species, except *Prophysaon andersoni* (native), in our survey are introduced and of European origin. It is also important to note that the Holarctic *Deroceras laeve* is thought to have both introduced and native populations in the US (Mc Donnell *et al.*, 2009) and a similar population structure likely exist in Canada. The economic and ecological impact of introduced molluscs on native species and habitats is not well understood in Canada. Introduced temperate species, such as *D. reticulatum* and *Arion* spp., are considered fairly intolerant of prolonged freezing temperatures (Storey *et al.*, 2007). Yet, *D. laeve* have been reported from Baffin Island in north-eastern Canada, known for bitterly cold winters and short summers (Storey *et al.*, 2007; Grimm *et al.*, 2009). Further research is needed to better understand how these temperate species survive winter in Alberta, particularly outside greenhouses.

Our discovery of the nematode *P. californica* in Canada is especially exciting because it is a facultative parasite and, therefore, should be tested for its potential to be a biological control agent. The congeneric *P. hermaphrodita* is commercially available in Europe as a bio-molluscicide; however, to date, this nematode cannot be sold in Canada as it has only recently been identified in the region (Brophy *et al.*, 2020). Notably, *P. californica* was first described in association with a slug in California (Tandingan De Ley *et al.*, 2016) and has recently been collected in New Zealand (Wilson *et al.*, 2016) and Ireland (Carnaghi *et al.*, 2017). Future studies are, therefore, needed to understand the virulence and biocontrol potential of *P. californica*, as well as the risk to non-target species.

In our study, we sampled opportunistically from a variety of sites, and, as such, did not achieve equal sampling from the various habitat types. Future surveys should increase sampling of molluscs from nurseries and greenhouses where high prevalence of nematodes were observed. In addition, horticultural sites, natural habitats and agricultural crops should be surveyed more intensively in future studies since the extent of the economic and ecological impact of invasive slugs is poorly understood. Moreover, a systematic survey of the abundance and diversity of nematodes associated with slugs in Canada is greatly needed. Lastly, if *P. californica* or other species of Phasmarhabditids are to be pursued as biological control agents in Canada, it is critical that their host range be determined, particularly their potential impacts on native gastropods.

Acknowledgements. Our thanks to all the residential gardeners, local growers, garden centres and nurseries that participated in the survey. We are also grateful to the undergraduate volunteers, K. Kent, L. MacLeod and G. Mulyk, for assisting with the collection and monitoring of slugs.

Financial support. This research was funded by Alberta Environment and Parks. J.L.R. was supported by the Nuffield Farming Scholarship Trust.

Conflicts of interest. None.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

References

Bailey SER (2002) Molluscicidal baits for control of terrestrial gastropods. pp. 33–54 *in* Barker GM (*Ed*) *Molluscs as crop pests*. Wallingford, CABI Publishing.

Barker GM (2002) *Molluscs as crop pests*. p. 558. Wallingford, CABI Publishing.

Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J and Sayers EW (2013) GenBank. *Nucleic Acids Research* **41**(database issue): D36–D42.

Blaxter ML, De Ley P, Garey JR, *et al.* (1998) A molecular evolutionary framework for the phylum Nematoda. *Nature* **392**(6671), 71–75.

Brophy T, Howe DK, Denver DR and Luong LT (2020) First report of a gastropod parasitic nematode *Phasmarhabditis californica* (Nematoda: Rhabditidae) in Alberta, Canada. *The Journal of Nematology* **52**, 1–3..

Carnaghi M, Rae R, Tandingan De Ley I, *et al.* (2017) Nematode associates and susceptibility of a protected slug *Geomalacus maculosus* to four biocontrol nematodes. *Biocontrol Science and Technology* **27**, 294–299.

Castle GD, Mills GA, Gravell A, Jones L, Townsend I, Cameron DG and Fones GR (2017) Review of the molluscicide metaldehyde in the environment. *Environmental Science: Water Research & Technology* **3**, 3415–3428.

DeAngelis JD (1993) *Slugs. Oregon State University Extension Bulletin FS 277*. Corvallis, Oregon, Oregon State University.

Fletcher MR, Hunter K and Barnet EA (1994) *Pesticide poisoning of animals*. p. 52. London, MAFF Publications.

Folmer O, Black M, Hoeh WR, Lutz R and Vrijenhoek RC (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit 1 from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3**, 294–299.

Forsyth RG (2004) *Land snails of British Columbia, Royal B.C. Museum handbook*. p. 188. Victoria, Royal British Columbia Museum.

Godan D (1983) *Pest slugs and snails*. Berlin, Springer-Verlag.

Grewal PS, Grewal SK, Tan L and Adams BJ (2003) Parasitism of molluscs by nematodes: types of associations and evolutionary trends. *Journal of Nematology* **35**, 146–156.

Grimm FW, Forsyth RG, Schueler FW and Karstad A (2009) *Identifying land snails and slugs in Canada*. 1st edn. p. 168. Ottawa, Canadian Food Inspection Agency.

https://doi.org/10.1017/S0022149X20000838 Published online by Cambridge University Press

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment [ed.], and analysis program for Windows 95/98/ NT. *Nucleic Acids Symposium Series* **41**, 95–98.

Hata T, Hara AH and Hu BKS (1997) Molluscicides and mechanical barriers against slugs, *Vaginula plebeia* Fischer and *Veronicella cubensis* Pfeiffer (Stylommatophora: Veronicellidae). *Crop Protection* **16**, 501–506.

Hawkins JW, Lankester MW, Lautenschlager RA and Bell FW (1997) Effects of alternative conifer release treatments on terrestrial gastropods in northwestern Ontario. *Forestry Chronicle* **73**, 91–98.

Henderson I and Triebskorn R (2002) Chemical control of terrestrial gastropods. pp. 1–32 *in* Barker GM (*Ed*) *Molluscs as crop pests*. Wallington, CABI Publishing.

Kiontke K and Sudhaus W (2006) Ecology of *Caenorhabditis* species. *In* WormBook: the online review of *C. elegans* biology [Internet]. Pasadena (CA): WormBook; 2005-2018. Available at https://www.ncbi.nlm.nih.gov/books/NBK19723/, accessed July 2020.

L'Heureux E and Angers B (2018) A discreet invasion: distribution, origins, and expansion of the European slug complex *Arion subfuscus* s.l. in Quebec. *Canadian Journal of Zoology* **96**, 325–331.

Mc Donnell RJ, Paine TD and Gormally MJ (2009) *Slugs: a guide to the invasive and native fauna of California*. Davis, University of California Agricultural and Natural Resources Publications.

Mc Donnell RJ, Tandingan De Ley I and Paine T (2018a) Susceptibility of neonate *Lissachatina fulica* (Achatinidae: Mollusca) to a US strain of the nematode *Phasmarhabditis hermaphrodita* (Rhabditidae: Nematoda). *Biocontrol Science and Technology* **28**, 1091–1095.

Mc Donnell RJ, Lutz MS, Howe DK and Denver DR (2018b) First report of the gastropod-killing nematode, *Phasmarhabditis hermaphrodita*, in Oregon, USA. *Journal of Nematology* **50**, 77–78.

Morand S, Wilson MJ and Glen DM (2004) Nematodes (Nematoda) parasitic in terrestrial gastropods. pp. 525–557 *in* Barker GM (*Ed*) *Natural enemies of terrestrial molluscs*. Wallington, CABI Publishing.

Moss M and Hermanutz L (2010) Monitoring the small and slimy – protected areas should be monitoring native and non-native slugs (Mollusca: Gastropoda). *Natural Areas Journal* **30**, 322–327.

Petersen C, Hermann RJ, Barg MC, Schalkowski R, Dirksen P, Barbosa C and Schulenburg H (2015) Travelling at a slug's pace: possible invertebrate vectors of *Caenorhabditis* nematodes. *BMC Ecology* **15**, 19.

Pieterse A, Malan AP and Ross JL (2017) Nematodes that associate with terrestrial molluscs as definitive hosts, including *Phasmarhabditis hermaphrodita* (Rhabditida: Rhabditidae) and its development as a biological molluscicide. *Journal of Helminthology* **91**, 517–527.

Purves G and Bannon JW (1992) Non-target effects of repeated methiocarb slug pellets application on carabid beetle (Coleoptera, Carbidae) activity in winter-sown cereals. *Annals of Applied Biology* **121**, 215–223.

Rae R, Verdun C, Grewal P, Robertson JF and Wilson MJ (2007) Biological control of terrestrial molluscs using *Phasmarhabditis hermaphrodita* – progress and prospects. *Pest Management Science* **63**, 1153–1164.

Ratnasingham S and Hebert PD (2007) BOLD: the Barcode of Life Data System (http://www.barcodinglife.org). *Molecular Ecology Notes* **7**, 355–364.

Reich I, Gormally M, Allcock AL, Mc Donnell R, Castillejo J, Iglesias J, Quinteiro J and Smith CJ (2015) Genetic study reveals close link between Irish and Northern Spanish specimens of the protected Lusitanian slug *Geomalacus maculosus*. *Biological Journal of the Linnean Society* **116**, 156–168.

Ross JL (2019) *Riding the slime wave: gathering global data on slug control*. Taunton, UK, Nuffield Farming Scholarships Trust Report.

Ross JL, Ivanova ES, Severns PM and Wilson MJ (2010) The role of parasite release in invasion of the USA by European slugs. *Biological Invasions* **12**, 603–610.

Ross JL, Ivanova ES, Sirgel WF, Malan AP and Wilson MJ (2012) Diversity and distribution of nematodes associated with terrestrial slugs in the Western Cape Province of South Africa. *Journal of Helminthology* **86**, 215–221.

Ross JL, Ivanova ES, Hatteland BA, Brurberg MB and Haukeland S (2016) Survey of nematodes associated with terrestrial slugs in Norway. *Journal of Helminthology* **90**, 583–587.

Schulte F (1989) Description of *Rhabditis (Pelodera) pseudoteresn.* sp. (Rhabditidae: Nematoda) with a redescription of its sibling *R.(P.) teres* (Schneider, 1866). *Revue de Nématologie* **12**, 387–394.

Storey KB, Storey JM and Churchill TA (2007) Freezing and anoxia tolerance of slugs: a metabolic perspective. *Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology* **177**, 833–840.

Tandingan De Ley IT, McDonnell RD, Lopez S, Paine TD and De Ley P (2014) *Phasmarhabditis hermaphrodita* (Nematoda: Rhabditidae), a potential biocontrol agent isolated for the first time from invasive slugs in North America. *Nematology* **16**, 1129–1138.

Tandingan De Ley I, Holovachov O, Mc Donnell RJ, Bert W, Paine TD and De Ley P (2016) Description of *Phasmarhabditis californica* n. sp and first report of *P. papillosa* (Nematoda: Rhabditidae) from invasive slugs in the USA. *Nematology* **18**, 175–193.

Vlach J (2016) *Slugs and snails in Oregon: a guide to common land molluscs and their relatives*. p. 39. Salem, Oregon Department of Agriculture.

Williams BD, Schrank B, Huynh C, Shownkeen R and Waterston RH (1992) A genetic mapping system in *Caenorhabditis elegans* based on polymorphic sequence tagged sites. *Genetics* **131**, 15.

Wilson MJ and Grewal PS (2005) Biology, production and formulation of slug-parasitic nematodes. pp. 421–429 *in* Grewal PS, Ehlers RU and Shapiro-Ilan DI (*Eds*) *Nematodes as biocontrol agents*. Wallingford, CABI Publishing.

Wilson MJ, Glen DM and George SK (1993) The rhabditid nematode *Phasmarhabditis hermaphrodita* as a potential biological control agent for slugs. *Biocontrol Science and Technology* **3**, 503–511.

Wilson MJ, Wilson DJ, Aalders LT and Tourna M (2016) Testing a new low-labour method for detecting the presence of *Phasmarhabditis* spp. in slugs in New Zealand. *Nematology* **18**, 925–931.

https://doi.org/10.1017/S0022149X20000838 Published online by Cambridge University Press