Journal of Helminthology (2012) **86,** 215–221
doi:10.1017/S0022149X11000277

Diversity and distribution of nematodes associated with terrestrial slugs in the Western Cape Province of South Africa

J.L. Ross[1]*, E.S. Ivanova[2], W.F. Sirgel[3], A.P. Malan[4] and M.J. Wilson[1†]

[1]Institute of Biological and Environmental Sciences, University of Aberdeen, AB24 3UU, UK: [2]Centre of Parasitology, A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Science, Leninskii prospect 33, 119071 Moscow, Russia: [3]Department of Botany and Zoology, Faculty of Sciences, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa: [4]Department of Conservation Ecology and Entomology, Faculty of AgriSciences, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa

(Received 1 March 2011; Accepted 18 April 2011; First Published Online 1 June 2011)

Abstract

A survey of nematodes associated with native and introduced species of terrestrial slugs was conducted in the Western Cape Province of South Africa, in order to gather new data regarding diversity and distribution. A total of 521 terrestrial slugs were collected from 35 localities throughout the Western Cape. All slugs were dissected and examined for the presence of internal nematodes. Extracted nematodes were identified using a combination of molecular (18S rRNA gene sequencing) and morphological techniques. Nematodes were found parasitizing slugs at 14 of the 35 sites examined, amounting to 40% of sample sites. Of all slugs, 6% were infected with nematodes. A total of seven species of nematode were identified in the province, including *Agfa flexilis*, *Angiostoma* sp., *Phasmarhabditis* sp. SA1, *Phasmarhabditis* sp. SA2, *Caenorhabditis elegans*, *Panagrolaimus* sp. and *Rhabditis* sp. Of these species, four were thought to be parasitic to slugs (*A. flexilis*, *Angiostoma* sp., *Phasmarhabditis* sp. SA1 and *Phasmarhabditis* sp. SA2), as opposed to forming necromenic or phoretic associations. Three new species of slug-parasitic nematode were identified during this study (*Angiostoma* sp., *Phasmarhabditis* sp. SA1 and *Phasmarhabditis* sp. SA2).

Introduction

Nematodes associated with terrestrial slugs are understudied in comparison to entomopathogenic nematodes (Wilson & Grewal, 2005). Current understanding of the slug/nematode relationship is based on surveys of Europe (Mengert, 1953; Morand, 1988; Laznik *et al.*, 2009; Ross *et al.*, 2010a, b), North America (Gleich *et al.*, 1977; Ross *et al.*, 2010a, b), South-East Asia (Pham Van Luc *et al.*, 2005) and Australia (Charwat & Davies, 1999), along with numerous descriptions of individual parasites from around the world (for reviews see Grewal *et al.*, 2003; Morand *et al.*, 2004; Ross *et al.*, 2010a, b). These surveys reveal that there are a total of seven families of nematodes known to be associated with terrestrial slugs: Agfidae, Alloionematidae, Angiostomatidae, Cosmocercidae,

*E-mail: jenna.ross@abdn.ac.uk
†Current address: Agresearch, Ruakura Research Centre, East Street, Private Bag 3123, Hamilton 3240, New Zealand.

Diplogasteridae, Mermithidae and Rhabditidae. This low number of nematode taxa is considered to be an underestimation of the real taxonomic diversity of nematodes associated with terrestrial slugs. A greater understanding could be achieved by surveying countries such as South Africa, where there is a unique climate and habitat diversity (Coe & Skinner, 1993; Stattersfield *et al.*, 1998).

South Africa is located on the southern tip of Africa, between the Atlantic and Indian oceans. This location provides a range of climates, from subtropical in the east to semiarid in the west (Hatting *et al.*, 2009). In the Western Cape Province (WCP), the climatic range is favourable for European slugs, which has resulted in the widespread distribution of these invasive species (Herbert & Kilburn, 2004). The introduction of these slugs is believed to be due to the migration of European settlers during the eighteenth and early nineteenth century (Smith, 1992). The success of these pests is not completely understood, but the lack of associated nematodes has been implicated as an important factor in the invasion of European slugs into North America (Ross *et al.*, 2010a). Recent reports by the ARC-Plant Protection Research Institute, state that European slug species have established themselves as major pests in South African agriculture (G. Tribe, pers. comm.). Current methods for controlling slugs rely on chemical molluscicides, such as metaldehyde and carbamate compounds (Hata *et al.*, 1997). Both metaldehyde and methiocarb are poisonous to a wide range of vertebrates (Homeida & Cooke, 1982; Fletcher *et al.*, 1994) and studies have shown that methiocarb is toxic to a number of beneficial invertebrates, including earthworms and carabid beetles (Purves & Bannon, 1992). Therefore it is important that a method of biological control is identified. The most effective commercial method for the biological control of molluscs in Europe is the slug-parasitic nematode *Phasmarhabditis hermaphrodita* (Rae *et al.*, 2007). *Phasmarhabditis hermaphrodita* is currently mass produced by Becker Underwood UK Ltd (Littlehampton, UK) and sold under the trade name of Nemaslug®. To date, this product cannot be sold in South Africa due to current legislation (amendment of Act 18 of 1989 under the Agricultural Pest Act 36 of 1947) which prohibits the introduction of exotic animals. Therefore, only an indigenous nematode isolate may be developed into a new molluscicide product. However, before this can be done, a systematic survey of nematodes associated with terrestrial slugs must be conducted in South Africa. Therefore this paper presents new data on the diversity and distribution of nematodes associated with terrestrial slugs in the WCP of South Africa, where European slug species are known to be widespread and pestiferous.

Materials and methods

Sampling

Slugs were collected from 35 localities in the WCP of South Africa (fig. 1) between 15 May 2009 and 7 September 2009. Sample habitats included public/private gardens, agricultural land, vineyards and private/commercial nurseries. Slugs were identified on the basis of their anatomy (Sirgel, 1985; Herbert, 2010) and rinsed with 0.9% saline solution to remove surface-dwelling

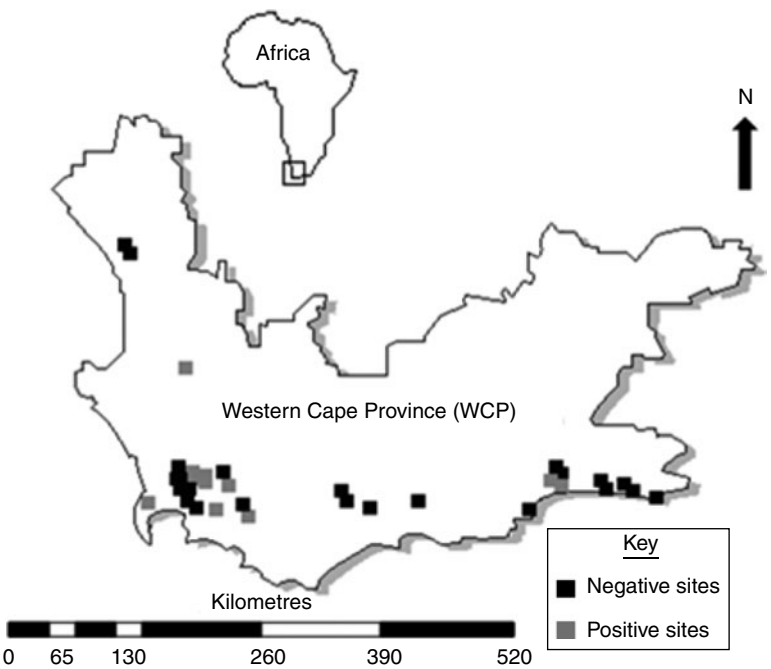

Fig. 1. Map showing the location of sample sites in the Western Cape Province (WCP) of South Africa.

https://doi.org/10.1017/S0022149X11000277 Published online by Cambridge University Press

nematodes. Slugs were then dissected and examined for the presence of internal nematodes.

Morphological and molecular identification

Adult nematodes were heat killed and fixed at 60°C in 5% formaldehyde, and were left in the fixative for a minimum of 2 h before being processed into anhydrous glycerol for mounting (Seinhorst, 1959). To aid identification, juvenile bacterial-feeding nematodes were cultured on modified kidney media plates (Wilson *et al.*, 1993), pre-inoculated with bacteria isolated from dead slugs (Wilson *et al.*, 1995). Light microscopic studies were done using a Zeiss Jenaval microscope (VEB Carl Zeiss, Jena, DDR).

For molecular identification, individual nematodes were picked directly into 70% ethanol. DNA extraction, polymerase chain reaction (PCR) amplification and sequencing of the 18s rRNA gene followed methods described by Ross *et al.* (2010b). Sequences of slug-parasitic nematodes were deposited in the National Center for Biotechnology Information (NCBI) GenBank and compared with the database (http://www.ncbi.nlm.nih.gov/) using the BLASTN search tool (Altschul *et al.*, 1990). The accession numbers, closest matches, identity and coverage of slug-parasitic nematodes were recorded.

Results

A total of 521 terrestrial slugs were collected from 35 sample sites (fig. 1), representing 12 different slug species from seven families (table 1). Of these, 33 slugs were native to South Africa and 488 slugs were European invasive species (table 1). Nematodes were found to parasitize six of the 12 slug species identified, leaving *Deroceras panormitanum*, *Chlamydephorus gibbonsi*, *Oopelta granulosa*, *O. polypunctata, Testacella maugei* and *Laevicaulis alte* free from nematode parasites in this survey (table 1).

Nematodes were found parasitizing slugs at 14 of the 35 sites examined (40%), (fig. 1, table 2). Of all slugs collected, 6% were infected with nematodes. These data are presented in tables 1 and 2, and show that of the 14 positive sites, two sites were found to have *Agfa flexilis*, one site had *Angiostoma* sp., two sites had *Phasmarhabditis* spp., eight sites had *Caenorhabditis elegans,* three sites had *Panagrolaimus* sp. and two sites had *Rhabditis* sp. These species represent four families: Agfidae, Angiostomatidae, Panagrolaimidae and Rhabditidae. Of these species, four are thought to have parasitic relationships with slugs, including *A. flexilis, Angiostoma* sp., *Phasmarhabditis* sp. SA1 and *Phasmarhabditis* sp. SA2 (Agfidae, Angiostomatidae and Rhabditidae) (table 3). The accession numbers, matches, identity and coverage of these slug-parasitic nematodes are recorded in table 3.

Discussion

This paper presents new data on the diversity and distribution of terrestrial slugs and their associated nematode parasites in the WCP of South Africa. A total of seven species of nematode were identified in the province, including *A. flexilis*, *Angiostoma* sp., *Phasmarhabditis* sp. SA1, *Phasmarhabditis* sp. SA2, *C. elegans,*

Table 1. Prevalence and abundance of nematodes associated with terrestrial slugs collected at various localities in the Western Cape Province (WCP) of South Africa.

Host species	Native or introduced	Number collected	Parasite species	Prevalence (%)
Agriolimacidae				
Deroceras reticulatum	Introduced	69	*Phasmarhabditis* sp. SA2*†	4.3
			Rhabditis sp.	4.3
Deroceras panormitanum	Introduced	26	–	–
Arionidae				
Ariopelta capensis	Native	1	*Rhabditis* sp.	100
Ariostralis nebulosa	Native	4	*Phasmarhabditis* sp. SA1*†	75
Oopelta granulosa	Native	14	–	
Oopelta polypunctata	Native	4	–	
Chlamydephoridae				
Chlamydephorus gibbonsi	Native	1	–	–
Limacidae				
Limax flavus	Introduced	32	*Agfa flexilis**	12.5
			Caenorhabditis elegans	3.0
			Panagrolaimus sp.	3.0
Limax valentianus	Introduced	313	*Agfa flexilis**	2.2
			Caenorhabditis elegans	0.6
			Panagrolaimus sp.	0.3
Milacidae				
Milax gagates	Introduced	44	*Angiostoma* sp.*†	6.8
			Panagrolaimus sp.	2.3
Testacellidae				
Testacella maugei	Introduced	4	–	–
Veronicellidae				
Laevicaulis alte	Native	9	–	–

†, New host association; *, thought to be true parasites rather than necromenic or phoretic association.

https://doi.org/10.1017/S0022149X11000277 Published online by Cambridge University Press

Table 2. Sites in the Western Cape Province (WCP) of South Africa with nematodes associated with terrestrial slugs.

Location	Co-ordinates	Habitat	Nematode infection	Family
Caledon	34.20.49S 19.15.87E	Agricultural	*Angiostoma* sp.†	Angiostomatidae
Cape Town	33.92.36S 18.37.97E	Garden	*Caenorhabditis elegans*	Rhabditidae
George	33.99.46S 22.52.15E	Nursery	*Phasmarhabditis* sp. SA2†	Rhabditidae
George	55.99.52S 22.53.67E	Nursery	*Caenorhabditis elegans*	Rhabditidae
Grabouw	34.09.03S 19.03.48E	Garden	*Caenorhabditis elegans*	Rhabditidae
Porterville	33.00.39S 18.59.44E	Garden	*Panagrolaimus* sp.	Panagrolaimidae
Stellenbosch	33.93.78S 18.86.74E	Botanical garden	*Panagrolaimus* sp.	Panagrolaimidae
Stellenbosch	33.55.46S 18.53.05E	Garden	*Agfa flexilis* † *Caenorhabditis elegans*	Agfidae Rhabditidae
Stellenbosch	33.55.45S 18.52.17E	Garden	*Agfa flexilis* † *Panagrolaimus* sp.	Agfidae Panagrolaimidae
Stellenbosch	33.93.93S 18.86.62E	Garden	*Caenorhabditis elegans*	Rhabditidae
Stellenbosch	33.93.14S 18.87.64E	Garden	*Caenorhabditis elegans*	Rhabditidae
Stellenbosch	33.55.45S 18.52.09E	Garden	*Caenorhabditis elegans*	Rhabditidae
Strand	43.07.24S 18.50.28E	Garden	*Caenorhabditis elegans*	Rhabditidae
Hottentots Holland Mountains	34.02.48S 18.59.11E	Mountain	*Phasmarhabditis* sp. SA1† *Rhabditis* sp.	Rhabditidae

†, First record in South Africa.

Panagrolaimus sp. and *Rhabditis* sp. (table 1). Of these seven species, four are thought to have parasitic relationships with slugs (*A. flexilis*, *Angiostoma* sp., *Phasmarhabditis* sp. SA1 and *Phasmarhabditis* sp. SA2), as opposed to forming necromenic or phoretic associations (tables 1 and 3). These nematodes represent three different families: Agfidae, Angiostomatidae and Rhabditidae.

The family Agfidae are obligate parasites of molluscs that associate with the salivary gland or genital tract (Morand & Hommay, 1990; Korol & Spiridonov, 1991; Ribas & Casanova, 2002). There are only three known species, all of which fall within the genus *Agfa* (Morand & Hommay, 1990; Korol & Spiridonov, 1991; Ribas & Casanova, 2002).

The Angiostomatidae family has two known genera, *Angiostoma* and *Aulacnema*. Molluscan angiostomatids are generally obligate parasites of the intestine (Campana-Rouget & Theodorides, 1956; Spiridonov, 1985; Morand & Spiridonov, 1989; Morand, 1992; Pham Van Luc *et al.*, 2005; Ivanova & Wilson, 2009); however, *Angiostoma glandicola* has been found to associate with the hepatopancreas (Ivanova & Spiridonov, 2010) and *Angiostoma aspersae* has been isolated from the pallial cavity of slugs (Morand, 1986). In addition to being mollusc parasites,

Table 3. Accession numbers, closest matches, identity and coverage of slug-parasitic nematodes isolated in the Western Cape Province (WCP) of South Africa.

Slug-parasitic nematode species	Accession number	Closest NCBI match	Identity (%)	Coverage (%)
Family: Agfidae *Agfa flexilis*	HQ115063	*Agfa flexilis* EU573704 (Ross *et al.*, 2010b)	100	100
Family: Angiostomatidae *Angiostoma* sp.	HQ115062	*Phasmarhabditis neopapillosa* FJ516754 (Ross *et al.*, 2010b)	98	98
Family: Rhabditidae *Phasmarhabditis* sp. SA1	HQ115060	*Phasmarhabditis* sp. EM434 EU196008 (Kiontke *et al.*, 2007)	96	99
Family: Rhabditidae *Phasmarhabditis* sp. SA2	HQ116061	*Angiostoma dentifera* FJ516752 (Ross *et al.*, 2010b)	98	100

https://doi.org/10.1017/S0022149X11000277 Published online by Cambridge University Press

angiostomatids have also been described from the intestine and bronchi of amphibian and reptile hosts (Chitwood, 1933; Bursey & Goldberg, 2000; Bursey & Manire, 2006; Falcon-Ordaz *et al.*, 2008).

There are several genera within the Rhabditidae family that associate with slugs, including *Rhabditis*, *Caenorhabditis* and *Phasmarhabditis*. However, *Phasmarhabditis* is the only genus that is considered to be truly parasitic to slugs (Morand *et al.*, 2004). *Phasmarhabditis* spp. parasitize the mantle cavity of slugs, in close association with the shell. However, unlike the obligate parasites *Agfa* and *Angiostoma, Phasmarhabditis* spp. are facultative parasites that are known to live on leaf litter and slug faeces (Tan & Grewal, 2001; MacMillan *et al.*, 2009).

Of the four slug-parasitic nematodes isolated in the WCP, three (*Angiostoma* sp., *Phasmarhabditis* sp. SA1 and *Phasmarhabditis* sp. SA2) have not been described previously. The identification of these undescribed nematodes implies that South Africa is rich in unidentified nematode species. Similar findings have been noted for entomopathogenic nematodes, where several newly described *Steinernema* and *Heterorhabditis* species have been identified (Nguyen *et al.*, 2006; Malan *et al.*, 2006, 2008; Hatting *et al.*, 2009). Therefore nematode surveys should be conducted throughout the African continent, especially areas with a moist, damp climate, where slugs are known to occur.

A total of 12 terrestrial slug species were identified in the WCP, representing seven families: Agriolimacidae, Arionidae, Chlamydephoridae, Limacidae, Milacidae, Testacellidae and Veronicellidae (table 1). These families have all been identified in previous surveys, but the species *C. gibbonsi* has not previously been isolated in the WCP (Herbert & Kilburn, 2004). *Chlamydephorus gibbonsi* is endemic to the Eastern Cape region, ranging from Zululand to East London, with occasional records in Transkei, Pietermaritzburg and the Colenso area (Herbert & Kilburn, 2004). This species occurs in a range of habitats, from indigenous forests to open thornveld (Herbert & Kilburn, 2004). It is possible that *C. gibbonsi* was introduced into the WCP though the importation of plant material, as a nursery was identified within 1 km of the isolation site.

A total of 521 terrestrial slugs were collected in the WCP, but only 33 slugs were native to South Africa and the remaining 488 slugs were exotic European species (table 1). There are numerous explanations for the success of these European invaders, but Ross *et al.* (2010a) demonstrated that parasite release played an important role in the invasion of European slugs into North America. They showed that parasite prevalence and species richness was higher in the home range (UK) compared to the invasive range (USA). The results of the present survey are very similar to those of the invasive range (USA) described by Ross *et al.* (2010a), where nematodes were present at 34% of USA study sites and 5.4% of all slugs examined were associated with nematodes. In South Africa, nematodes were present at 40% of study sites and 6% of slugs were infected. Species richness was also low in South Africa, with only seven species of nematode being identified, four of which were truly parasitic (tables 1 and 3). While the reduced prevalence of nematode parasites may have aided invasion by European slugs into South Africa, it is not possible from our data to be certain that parasite release is the only factor allowing invasion by these exotic slug species. Conversely, we cannot know whether invasion of European species may have been halted by nematode parasites indigenous to South Africa.

This paper presents the first systematic survey of nematodes associated with terrestrial slugs in the WCP of South Africa. This work will not only aid understanding of the slug/nematode association, but will also help with the identification of an indigenous isolate which could be developed as a biological molluscicide in South Africa. Future work should focus on the virulence and biocontrol potential of these nematodes, especially with regards to molluscs that have been identified as pestiferous in the region. However, before this can occur, tests should be conducted on the effects of these nematodes on native non-pestiferous slug species, as many molluscs are included in the Database of Threatened Invertebrates of South Africa (Herbert, 1997).

Acknowledgements

This work was carried out within the University of Aberdeen and the University of Stellenbosch, and was supported financially by Becker Underwood, the Biotechnology and Biological Sciences Research Council (BBSRC) and the British Society for Parasitology. We would like to thank Geoff Tribe, Annelize Lubbe, Scott Gilbert and staff in the Department of Conservation Ecology and Entomology at the University of Stellenbosch for their help with slug collections.

References

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.

Bursey, C.R. & Goldberg, S.R. (2000) *Angiostoma onychodactyla* sp. n. (Nematoda, Angiostomatidae) and other intestinal helminths of the Japanese clawed salamander, *Onychodactylus japonicus* (Caudata: Hynobiidae) from Japan. *Comparative Parasitology* **67**, 60–65.

Bursey, C.R. & Manire, C.A. (2006) *Angiostoma carettae* n. sp. (Nematoda: Angiostomatidae) from the loggerhead sea turtle *Caretta caretta* (Testudines: Cheloniidae), Florida, U.S.A. *Comparative Parasitology* **73**, 253–256.

Campana-Rouget, Y. & Theodorides, J. (1956) A propos de *Angiostoma limacis* parasite du tube digetif des limaces. *Annales de Parasitologie Humaine et Comparée* **30**, 23–28.

Charwat, S.M. & Davies, K.A. (1999) Laboratory screening of nematodes isolated from South Australia for potential as biocontrol agents of helicid snails. *Journal of Invertebrate Pathology* **74**, 55–61.

Chitwood, B.G. (1933) On some nematodes of the superfamily Rhabditoidea and their status as parasites of reptiles and amphibians. *Journal of the Washington Academy of Sciences* **23**, 508–520.

Coe, M.J. & Skinner, J.D. (1993) Connections, disjunctions and endemism in the eastern and southern

African mammal faunas. *Transactions of the Royal Society of South Africa* **48**, 233–255.

Falcon-Ordaz, J., Mendoza-Garfias, B., Windfield-Perez, J.C., Parra-Olea, G. & de Leon, G.P.P. (2008) *Angiostoma lamotheargumedoi* n. sp (Nematoda: Angiostomatidae) from the intestine of *Pseudoeurycea mixteca* (Caudata: Plethodontidae) in central Mexico. *Revista Mexicana de Biodiversidad* **79**, 107–112.

Fletcher, M.R., Hunter, K. & Barnet, E.A. (1994) *Pesticide poisoning of animals 1993*. 52 pp. London, MAFF Publications.

Gleich, J.G., Gilbert, F.F. & Kutscha, N.P. (1977) Nematodes in terrestrial gastropods from central Maine. *Journal of Wildlife Diseases* **13**, 43–46.

Grewal, P.S., Grewal, S.K., Tan, L. & Adams, B.J. (2003) Parasitism of molluscs by nematodes: types of associations and evolutionary trends. *Jounal of Nematology* **35**, 146–156.

Hata, T.Y., Hara, A.H. & Hu, B.K.S. (1997) Molluscicides and mechanical barriers against slugs *Vaginula plebeia* Fischer and *Veronicella cubensis* (Pfeiffer) (Stylommatophora: Veronicellidae). *Crop Protection* **16**, 501–506.

Hatting, J., Stock, P. & Hazir, S. (2009) Diversity and distribution of entomopathogenic nematodes (Steinernematidae, Heterorhabditidae) in South Africa. *Journal of Invertebrate Pathology* **102**, 120–128.

Herbert, D.G. (1997) The terrestrial slugs of KwaZulu-Natal: diversity, biogeography and conservation (Mollusca: Pulmonata). *Annals of the Natal Museum* **38**, 197–239.

Herbert, D.G. (2010) *The introduced terrestrial Mollusca of South Africa*. SANBI Biodiversity Series 15. Pretoria, South African National Biodiversity Institute.

Herbert, D. & Kilburn, D. (2004) *Field guide to the land snails and slugs of eastern South Africa*. 336 pp. Pietermaritzburg, Natal Museum.

Homeida, A.M. & Cooke, R.G. (1982) Pharmacological aspects of metaldehyde poisoning in mice. *Journal of Veterinary Pharmacology and Therapeutics* **5**, 77–82.

Ivanova, E.S. & Spiridonov, S.E. (2010) *Angiostoma glandicola* sp. n. (Nematoda: Angiostomatidae): a parasite in the land snail *Megaustenia* sp. from the Cat Tien Forest, Vietnam. *Jounal of Helminthology* **84**, 297–304.

Ivanova, E.S. & Wilson, M.J. (2009) Two new species of *Angiostoma* Dujardin, 1845 (Nematoda: Angiostomatidae) from British terrestrial molluscs. *Systematic Parasitology* **74**, 113–124.

Kiontke, K., Barriere, A., Kolotuev, I., Podbilewicz, B., Sommer, R., Fitch, D.H. & Felix, M.A. (2007) Trends, stasis, and drift in the evolution of nematode vulva development. *Current Biology* **17**, 1925–1937.

Korol, E.N. & Spiridonov, S.E. (1991) *Angiostoma kimmeriensis* sp. n. and *Agfa tauricus* sp. n. – parasitic Rhabditida (Nematoda) from Crimean terrestrial molluscs. *Helminthologia* **28**, 179–182.

Laznik, Z., Ross, J.L., Tóth, T., Lakatos, T., Vidrih, M. & Trdan, S. (2009) First record of the nematode *Alloionema appendiculatum* Schneider (Rhabditida: Alloionematidae) in Arionidae slugs in Slovenia. *Russian Journal of Nematology* **17**, 137–139.

MacMillan, K., Haukeland, S., Rae, R.G., Young, I.M., Crawford, J.W., Hapca, S. & Wilson, M.J. (2009) Dispersal patterns and behaviour of the nematode *Phasmarhabditis hermaphrodita* in mineral soils and organic media. *Soil Biology and Biochemistry* **41**, 1483–1490.

Malan, A.P., Nguyen, K.B. & Addison, M.F. (2006) Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) from the south western parts of South Africa. *African Plant Protection* **12**, 65–69.

Malan, A.P., Nguyen, K.B., De Waal, J.Y. & Tiedt, L. (2008) *Heterorhabditis safricana* n. sp. (Rhabditida: Heterorhabditidae), a new entomopathogenic nematode from South Africa. *Nematology* **10**, 381–396.

Mengert, H. (1953) Nematoden und Schneken. *Zeitschrift für Morphologie und Ökologie Tiere* **41**, 311–349.

Morand, S. (1986) *Angiostoma aspersae* sp. n. (Nematoda, Angiostomaidae) parasite de *Helix aspersa* Müller (Gastropoda, Helicidae). *Bulletin du Museé National d'Histoire Naturelle, Paris* **11**, 111–115.

Morand, S. (1988) Contribution a l'étude d'un système hôtes-parasites: nematodes associés à quelques mollusques terrestres. 265 pp. Doctoral thesis, Université de Rennes, France.

Morand, S. (1992) *Angiostoma spiridonovi* n. sp. (Nematoda, Angiostomatidae) parasite of *Limax flavus* (Gastropoda, Limacidae). *Proceedings of the Helminthological Society of Washington.* **59**, 212–217.

Morand, S. & Hommay, G. (1990) Redescription de *Agfa flexilis* (Nematoda: Agfidae) parasite de l'appareil genital de *Limax cinereoniger* (Gastropoda: Limacidae). *Systematic Parasitology* **15**, 127–132.

Morand, S. & Spiridonov, S.E. (1989) Redescription de trois espèces d'Angiostomatidae (Nematoda, Rhabditida), parasites de gastropodes pulmones Styllomatophores, et description de cycle évolutif de deux d'entre elles. *Bulletin du Musée National d'Histoire Naturelle, Paris, 4th series* **11**, 367–385.

Morand, S., Wilson, M.J. & Glen, D.M. (2004) Nematodes (Nematoda) parasitic in terrestrial gastropods. pp. 525–557 *in* Barker, G.M. (*Ed.*) *Natural enemies of terrestrial molluscs*. Wallingford, CABI Publishing.

Nguyen, K.B., Malan, A.P. & Gozel, U. (2006) *Steinernema khoisanae* n. sp. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from South Africa. *Nematology* **8**, 157–175.

Pham Van Luc, P., Spiridonov, S.E. & Wilson, M.J. (2005) *Aulacnema monodelphis* n.g.n. sp. and *Angiostoma coloaense* n. sp. (Nematoda: Rhabditida: Angiostomatidae) from terrestrial molluscs of Vietnam. *Systematic Parasitology* **60**, 91–97.

Purves, G. & Bannon, J.W. (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.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.

Ribas, A. & Casanova, J.C. (2002) *Agfa morandi* sp. n. (Rhabditida, Agfidae) a parasite of *Limax* sp. (Gastropoda, Limacidae). *Parasitology Research* **88**, 745–747.

Ross, J.L., Ivanova, E.S., Severns, P.M. & Wilson, M.J. (2010a) The role of parasite release in invasion of

the USA by European slugs. *Biological Invasions* **12**, 603–610.

Ross, J.L., Ivanova, E.S., Spiridonov, S.E., Waeyenberge, L., Moens, M., Nicol, G.W. & Wilson, M.J. (2010b) Molecular phylogeny of slug-parasitic nematodes inferred from 18S rRNA gene sequences. *Molecular Phylogenetics and Evolution* **55**, 738–743.

Seinhorst, J.W. (1959) A rapid method for the transfer of nematodes from fixative to anhydrous glycerine. *Nematologica* **4**, 67–69.

Sirgel, W.F. (1985) A new subfamily of Arionidae (Mollusca, Pulmonata). *Annals of the Natal Museum* **26**, 471–487.

Smith, V.R. (1992) Terrestrial slug recorded from sub-Antarctic Marion Island. *Journal of Molluscan Studies* **58**, 80–81.

Spiridonov, S.E. (1985) *Angiostoma asamati* sp. n. (Angiostomatidae: Rhabditida) – new species of nematodes from slugs (Mollusca). *Helminthologia* **22**, 253–261.

Stattersfield, A.J., Crosby, M.J., Long, A.J. & Wege, D.C. (1998) *Endemic bird areas of the world: Priorities for biodiversity conservation*. Cambridge, The Burlington Press.

Tan, L. & Grewal, P.S. (2001) Infection behaviour of the rhabditid nematode *Phasmarhabditis hermaphrodita* to the grey garden slug *Deroceras reticulatum*. *Journal for Parasitology* **87**, 1349–1354.

Wilson, M.J. & Grewal, P.S. (2005) Biology, production and formulation of slug-parasitic nematodes. pp. 421–429 *in* Grewal, P.S., Ehlers, R.-U. & Shapiro-Ilan, D.I. (*Eds*) *Nematodes as biocontrol agents*. Wallingford, CABI Publishing.

Wilson, M.J., Glen, D.M., George, S.K. & Butler, R.C. (1993) Mass cultivation and storage of the rhabditid nematode *Phasmarhabditis hermaphrodita*, a biocontrol agent of slugs. *Biocontrol Science and Technology* **3**, 513–521.

Wilson, M.J., Glen, D.M., George, S.K. & Pearce, J.D. (1995) Selection of a bacterium for the mass production of *Phasmarhabditis hermaphrodita* (Nematoda:Rhabditidae) as a biological control agent for slugs. *Fundamental and Applied Nematology* **18**, 419–425.