

Preliminary data on the parasite survey of terrestrial gastropods of Sicily

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Summary. A short survey on the parasite fauna of terrestrial molluscs in south-east of Sicily was performed. In total, 432 specimens of 12 different snail species from the families Cochlostomatidae, Clausiliidae, Subulinidae, and Helicidae and five slug species from the families Agriolimacidae, Limacidae and Milacidae were collected and examined. Seven out of 17 examined gastropod species in the area were susceptible to nematode infection. Representatives of three nematode taxa, *Angiostoma margaretae* Ross, Malan & Ivanova, 2011, *Phasmarhabditis* spp. and *Caenorhabditis elegans* (Maupas, 1900), were isolated and molecularly characterised. New host, *Papillifera papillaris affinis* (Philippi, 1836), was recorded for *A. margaretae*, originally described from South Africa. The discovery of two different strains or even putative species of *Phasmarhabditis* at close localities is indicative of its possible diversity in the area. Trematodes from four families were found at four sites in *Rumina decollata* (Linnaeus, 1758), *Cornu aspersum* (O.F. Müller, 1774), *Ambigolimax valentianus* (Férussac, 1822), *Tandonia sowerbyi* (A. Férussac, 1823) and *Milax nigricans* (Philippi, 1836). Mixed trematode/nematode infection was observed in *R. decollata* at the University of Catania grounds in the Catania city centre, *M. nigricans* and *T. sowerbyi* at Villasmundo in Syracuse Province. No significant difference in parasite diversity and parasite prevalence between urban and wild habitats was found.

Key words: *Angiostoma margaretae*, *Caenorhabditis elegans*, morphology, morphometrics, *Phasmarhabditis* sp., rDNA.

Terrestrial gastropods are an important component of natural and agricultural ecosystems of the world, especially the Mediterranean region. In Sicily, particularly, geological, paleo-climatic, evolutionary and biogeographic events have contributed to a high species diversity of gastropod molluscs (Giusti & Manganelli, 1984; Manganelli *et al.*, 1995; Bank, 2017; Bank & Neubert, 2017) with high levels of endemism (see, for example, Liberto *et al.*, 2016; Viviano *et al.*, 2019). Many gastropod taxa (Cochlostomatidae, Clausiliidae, Helicidae) are adapted for life in limestone areas (Colomba *et al.*, 2011) where they can form populations of different sizes consisting of representatives of the genera *Rupestrella* Monterosato, 1894, *Siciliaria* Vest, 1867, *Marmorana* W. Hartmann, 1844 *etc.* There is also a number of invasive species in Sicily known to affect the native species diversity (McNeely, 2001; Douglas *et al.*, 2013; Early *et al.*, 2016; Sparacio *et*

al., 2017, 2018). Economically, some native as well as alien gastropod species have impact as pests in agriculture, horticulture and gardens (Manganelli *et al.*, 1995), while others are traditionally used as food and therefore regarded as beneficial despite occasionally being a nuisance in agriculture.

It is also known that one of the edible gastropods of Mediterranean origin, *Cornu aspersum* (O.F. Müller, 1774) (syn. *Helix aspersa*), may act as an intermediate host for a number of metastrongyloid nematodes (Seneviratna, 1959; Anderson, 2000; Gianelli *et al.*, 2015). Such gastropod-borne parasites pose risks to health and well-being of humans and animals. Aquatic gastropods take a greater part than terrestrial ones in the distribution of trematodes, such as *Schistosoma*, that are dangerous for humans. However, several species of *Brachylaima* Dujardin, 1843, typical trematode parasite of birds, can be dangerous to humans and are transmitted by species of *Monacha*

Fitzinger, 1833 (Hygromiidae) and other helioids native in the Mediterranean region (Rashed, 2008; Ismail & Güreli, 2018). Another important nematode parasite, *Angyostrongylus* Kamensky, 1905, causative agent of ailments with different symptoms of domestic animals and even humans is transmitted mainly through terrestrial gastropods. Although the majority of cases of eosinophilic meningitis caused by the rat lungworm *A. cantonensis* (Chen, 1935) were registered outside Europe, global warming and spread of tropical gastropods around the earth by a human should be another concern in the control of major helminthiases. The wide range of gastropod hosts used by *A. cantonensis* shows the great adaptation potential of this parasite and points at the need for the control of its presence in gastropods. Another big group of gastropod-borne parasites is represented by nematodes (Rhabditida, Mermithida and Ascaridida) associated with gastropods as sole hosts, be it truly parasitic relationships or necromeny. *Phasmarhabditis hermaphrodita* (A. Schneider, 1859), a facultative (opportunistic) parasite of slugs, has been developed as the biocontrol agent sold in many countries in Europe (Wilson *et al.*, 1993; Rae *et al.*, 2007). The search for such natural enemies of pestilent gastropod species was recently started in Italy, and *Phasmarhabditis apuliae* has been described from milacid slugs (Nermet' *et al.*, 2016). We did not find any other reports on any parasites associated with land gastropods in Italy and, to our knowledge, no studies on parasite fauna of gastropods in Sicily have been carried out. A short systematic survey on helminths of terrestrial gastropods was undertaken by the authors in Sicily in February 2019. Survey results are presented below with the focus on nematodes completing their life cycle in gastropods. Finally, some additional data on gastropod distribution in the south-east of Sicily are provided.

MATERIAL AND METHODS

Nematode material. Gastropods were collected in Sicily and transported to the Centre of Parasitology, Moscow where dissections were made. Gastropods were identified based on their morphology. Taxonomical references were based on the checklist of land and freshwater Gastropoda of Europe (Bank & Neubert, 2017). Parasites extracted were preserved for morphological and molecular studies using preservation in a hot 4-5% formalin for nematodes, 96% ethanol for trematodes for morphology and freezing for molecular work. Except for the preserved specimens, the remaining rhabditid juveniles were placed in nutrient medium (soaked in water oatmeal or preliminary frozen gastropod meat)

to obtain adult stages. Prior to molecular characterisation, the nematodes collected were identified to the generic level using light microscopy. The samples used were designated as Si5 for *Angiostoma* sp. from *Tandonia sowerbyi* (A. Férussac, 1823) collected at Villasmundo, *Phasmarhabditis* sp. Si14 from *Monacha* sp. (Villasmundo) and Si15 from *Eobania vermiculata* (O.F. Müller, 1774) (Librino) and Si9 for an unidentified rhabditid from *Ambigolimax valentianus* (Férussac, 1822) (University of Catania grounds).

Molecular characterisation and phylogenetic analysis. The nematodes discovered in the course of mollusc dissection were transferred individually into 25 µl of sterile water in 0.7 ml Eppendorf tubes. DNA was extracted from worms in the worm-lysis solution (950 µl of a mixture of 2 ml of 1M NaCl, 2 ml of 1M Tris-HCl, pH 8 plus 5.5 ml of deionised water plus 10 µl of mercaptoethanol and 40 µl (proteinase K, 20 mg ml⁻¹), which was prepared immediately before DNA extraction (Holterman *et al.*, 2006). The worm-lysis solution was incubated at 65°C for 90 min, with the following deactivation of proteinase K at 99°C during 5 min.

Primers D2A (5'-ACA AGT ACC GTG AGG GAA AGT TG-3') and D3B (5'-TCG GAA GGA ACC AGC TAC TA-3') were used to amplify and sequence the D2-D3 LSU rDNA expansion segments (Nunn, 1992). The PCR cycling parameters for these two primers included a primary denaturation step at 94°C for 5 min followed by 34 cycles of 94°C for 1 min, 50°C for 1 min and 72°C for 1 min, followed by a postamplification extension step at 72°C for 6 min.

A pair of primers TW81 (5'-GTT TCC GTA GGT GAA CCT GC-3') and AB28 (5'-ATA TGC TTA AGT TCA GCG GGT-3') was used to amplify approx. 800 bp long sequence of ITS region of ribosomal DNA (Curran & Driver, 1994). PCR cycling parameters included primary denaturation at 95°C for 5 min followed by 35 cycles of 94°C for 45 s, 56°C for 60 s and 72°C for 70 s. Obtained sequences were deposited in NCBI Genbank (*Phasmarhabditis*, Si14, ITS – MN366391 and the same species D2D3 LSU rDNA – MN366385; *Phasmarhabditis*, Si15, D2-D3 LSU rDNA – MN366384). For comparative purposes and phylogeny construction, some sequences from GenBank were also used. Sequence alignments were generated using Clustal_X (Thompson *et al.*, 1997) under default values for gap opening and gap extension penalties. All alignments were analysed using PAUP* 4.0b10 (Swofford, 1998) for maximum likelihood, maximum parsimony and neighbour joining methods.

Table 1. List of gastropod species collected in south-east Sicily in February 2019.

Species	Location Catania Uni. garden & Villa Bellini	Villasmundo	Librino	Pantalica	Mineo	Oasi del Simeto	Caltagirone
Architaenioglossa							
Megalostomatidae							
<i>Cochlostoma (Auritus) westerlundi dionysi</i> (Paulucci, 1879)	0	0	0	47	0	0	0
Littorinimorpha							
Bithynidae							
<i>Chondrula (Mastus) pupa</i> (Linnaeus, 1758)	10	0	0	0	0	0	0
Stylomatophora							
Achatinidae							
<i>Rumina decollata</i> (Linnaeus, 1758)	32	7	45	0	1	8	0
Clausiliidae							
<i>Muticaria</i> cfr. <i>neuteboomi</i> Beckmann, 1990	0	0	0	0	9	0	0
<i>Papillifera papillaris affinis</i> (Philippi, 1836)	3	35	0	34	0	7	3
Milacidae							
<i>Milax nigricans</i> (Philippi, 1836)	3	1	1	0	0	0	0
<i>Tandonia sowerbyi</i> (A. Férussac, 1823)	1	1	0	0	0	0	0
Limacidae							
<i>Ambigolimax valentianus</i> (Férussac, 1822)	132	0	0	0	0	11	0
<i>Limacus flavus</i> (Linnaeus, 1758)	4	0	0	0	0	0	0
Agriolimacidae							
<i>Deroceras</i> cfr. <i>panormitanum</i> (Lessona & Pollonera, 1882)	0	0	2	0	0	2	1
Trossexodontidae							
<i>Caracollina lenticula</i> (Férussac, 1821)	0	2	0	0	0	0	0
Hygromiidae							
<i>Monacha</i> sp.	1	1	1	0	0	0	0
Geomitridae							
<i>Cernuella (Cernuella) virgata</i> (Da Costa, 1778)	0	0	0	0	3	0	0
Helicidae							
<i>Theba pisana</i> (Müller, 1774)	1	0	0	2	4	6	0
<i>Cornu aspersum</i> (O.F. Müller, 1774)	4	1	2	0	0	0	0
<i>Eobania vermiculata</i> (O.F. Müller, 1774)	0	1	2	0	0	0	0
Examined	190	49	53	84	17	35	4
Infected (%)	3.3	20.8	17.0	0	0	5.9	0

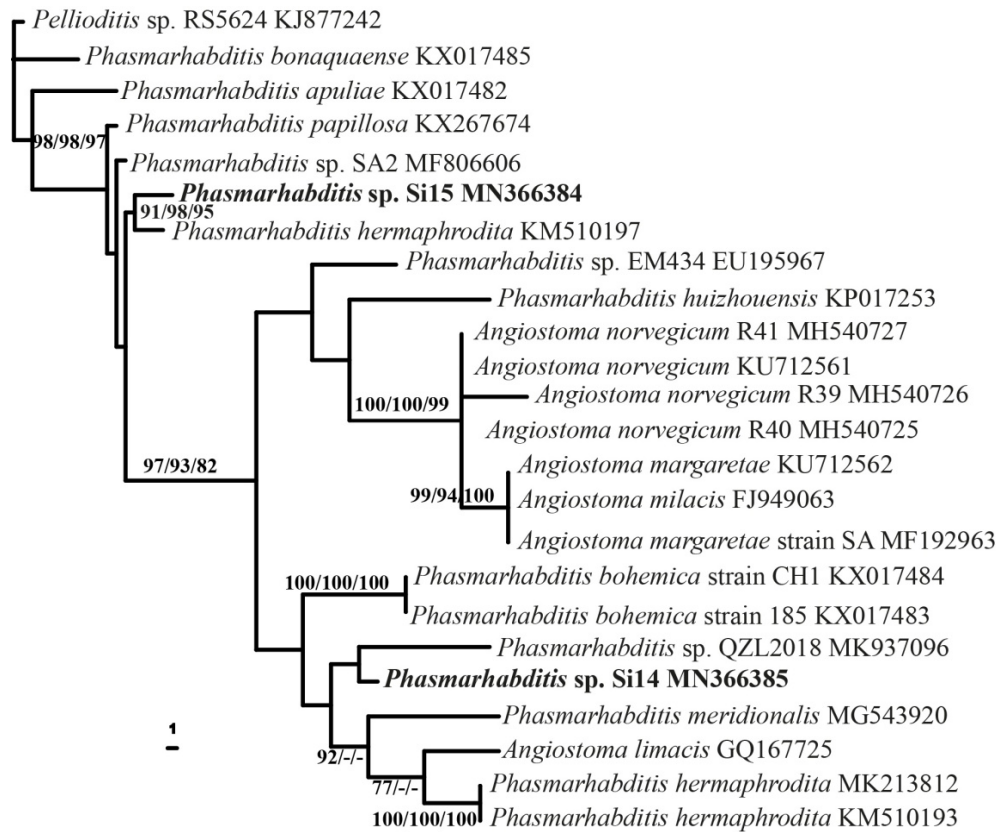


Fig. 1. Phylogenetic relationships of Sicilian *Phasmarhabditis* strains within other nematodes based on D2-D3 LSU rDNA. Bootstrap support values are presented near nodes as MP/NJ/ML. ML analysis (500 bootstrap replications), K2 + G model. For MP and NJ – 1000 bootstrap replications.

RESULTS

A total of 432 terrestrial snails and slugs were collected from seven sample sites around Sicily in 25-28 February 2019 (Table. 1). A total of 11 different snail species and five slug species were identified representing four snail families, Cochlostomatidae, Clausiliidae, Helicidae and Subulinidae, and three slug families, Agriolimacidae, Limacidae and Milacidae. Trematodes were found at four sites and were represented by four families. Mollusc species infected by trematodes were *Rumina decollata* (Linnaeus, 1758), *Cornu aspersum*, *Ambigolimax valentianus*, *Tandonia sowerbyi* and *Milax nigricans* (Philippi, 1836). Mixed trematode/nematode infection was observed in *R. decollata* collected at University of Catania grounds in the centre of Catania, *M. nigricans* and *T. sowerbyi* at Villasmundo Natural Reserve in Syracuse Province.

Molecular characterisation. The sequences of D2-D3 expansion segment were obtained for four

samples (Si5, Si9, Si14 and Si15) of nematodes from Sicilian molluscs. The approx. 500 bp long D2-D3 LSU rDNA sequence for the sample Si5 *Angiostoma* sp. was found to be identical with South African *Angiostoma margaretae* Ross, Malan & Ivanova, 2011 (deposited sequences KU712562 and MF192963 of specimens isolated from *Milax gagates* Draparnaud, 1801 and *Deroceras panormitanum* (Lesson & Pollonera, 1882), respectively). A BLAST-search demonstrated that the deposited ITS sequence MF192968 of *A. margaretae* from South African slug *Deroceras panormitanum* was the closest to the 730 bp long ITS rDNA sequence of our sample.

The 590 bp long sequence for sample Si9 was found to be 100% identical to the LSU sequence of the nematode *Caenorhabditis elegans* isolated in South Africa from the cosmopolitan slug *Deroceras panormitanum* (MF192964) and *C. elegans* from Asian horned frogs *Megophrys montana* (FJ589010). The 940 bp long ITS rDNA sequence was obtained for this sample. This ITS sequence of Si9

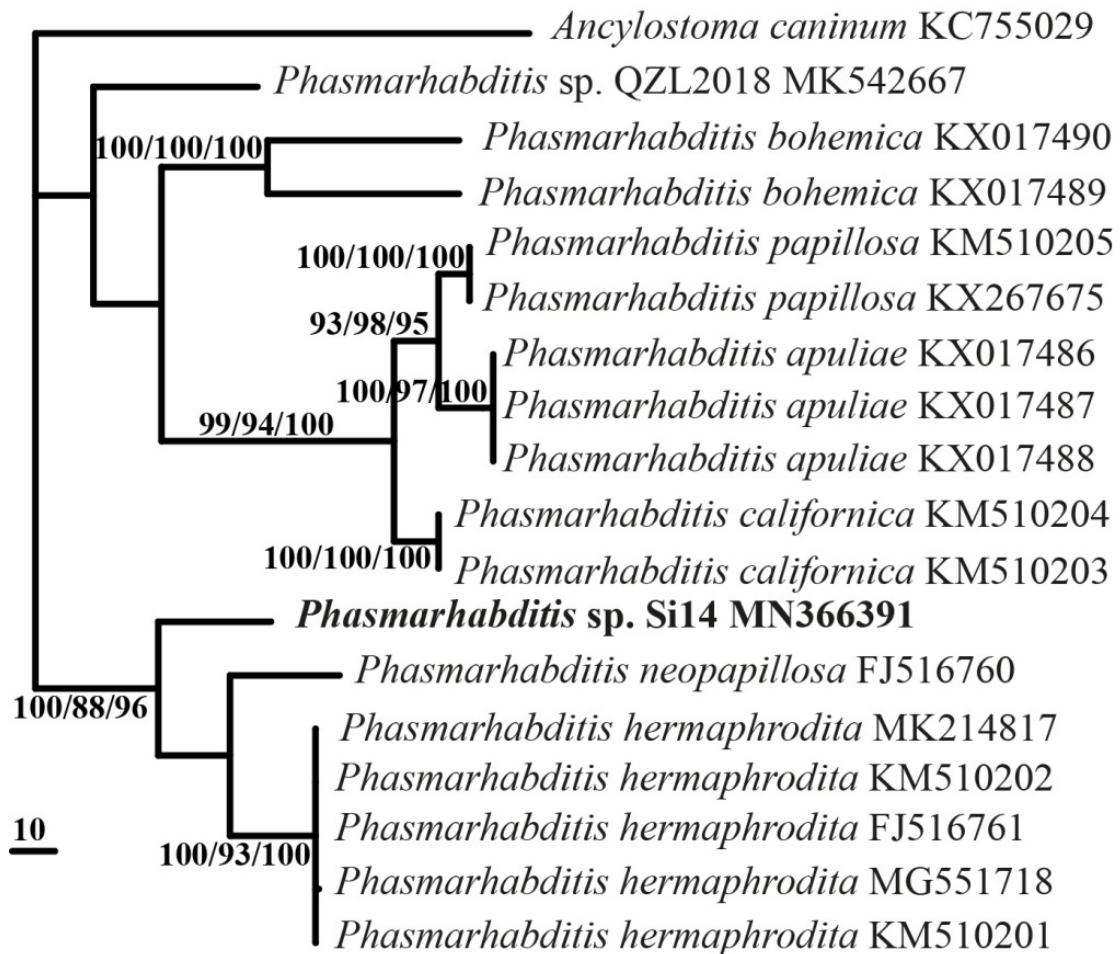


Fig. 2. Phylogenetic relationships of Sicilian *Phasmarhabditis* strains within other nematodes based on ITS rDNA. Bootstrap support values are presented near nodes as MP/NJ/ML. ML analysis (500 bootstrap replications), T92 + G model. For MP and NJ – 1000 bootstrap replications.

demonstrated complete identity in 820 corresponding positions of ITS sequence for *C. elegans* from an unidentified European slug (MG551716).

The D2-D3 LSU rDNA sequences obtained for samples Si14 and Si15 as expected corresponded to members of *Phasmarhabditis*. The 540 bp long sequence of Si14 clustered in the phylogenetic tree (Fig. 1) with a group of *Phasmarhabditis* species, which included *P. bohemica* Nermut, Půža, Mekete & Mráček, 2017, *P. hermaphrodita* (Schneider, 1859) Andrásy, 1983, *P. meridionalis* Ivanova & Spiridonov, 2017, and an unidentified *Phasmarhabditis* sp. QZL2018 from China (MK937096), and also *Angiostoma limacis* Dujardin, 1845 GQ167725. The 649 bp long ITS rDNA sequence of Si14 demonstrated another phyletic links. It clustered with *P. hermaphrodita* and *P. neopapillosa* (Mengert in Osche, 1952)

Andrásy, 1983 ITS sequences under strong bootstrap support (Fig. 2).

The 496 bp long D2-D3 LSU rDNA sequence of Si15 was quite different from sample Si14 clustering with *P. hermaphrodita* (KM510197) from *Deroceras reticulatum* slug collected in the USA.

Characterisation of collection sites, their gastropod population and associated parasites. I. Catania, the University garden and Villa Bellini city park. At Villa Bellini, we were able to find just a few gastropod specimens (*Milax nigricans*, *Tandonia sowerbyi* and *Cernuella virgata*), all free of the helminth's infection. At the same time, the University garden, a small area around the Section of Animal Biology, was inhabited by large numbers of gastropods, which sought shelter under lids of technical appliances (hatches and manhole covers). There, the gastropod population was represented by two slug species (*Limacus flavus* and *Ambigolimax*

valentianus) and five snail species (*Rumina decollata*, *Chondrula (Mastus) pupa*, *Monacha* sp., *Cornu aspersum*, *Cerņuella virgata* and *Papillifera papillaris affinis*). Predominant species were *A. valentianus* and *R. decollata*, which accounted to 72.9% and 17.7% of sampled gastropods, respectively. Total prevalence of helminth infection at the University grounds was 6% (*A. valentianus*, *R. decollata* and *C. aspersum*). Two species of nematodes and a species of a trematode were recorded: unidentified *Phasmarhabditis* sp. (*C. aspersum* and *R. decollata*); *Caenorhabditis elegans* (*A. valentianus*), and Dicrocoeliidae gen. et sp. (*R. decollata*). The infected specimen of *R. decollata* has served as a host for two parasites at the same time: a nematode *Phasmarhabditis* sp. and trematode sporocysts of Dicrocoeliidae, a parasite of mammals and birds with a complicated life cycle. To the best of our knowledge, this gastropod species was not reported previously as the intermediate host for Dicrocoeliidae.

While the juveniles of *Phasmarhabditis* sp. inhabited the mantle cavities of *C. aspersum* and *R. decollatum* and accounted from 3 to 10 specimens, hundreds of juveniles of *C. elegans* were found in the intestine of one out of 132 collected *A. valentianus*. The *C. elegans* dauer juveniles were tightly packed in distal parts of the intestine and were alive, while obviously getting ready for evacuation. What was the source of infection for slugs and why only one slug specimen was infected remained unclear. In the lab, the nematodes were able to multiply on oatmeal medium and on the frozen *Helix* meat with better results on the latter.

II. Librino. Librino is the suburb district of Catania, located in the south-west part of the town. The gastropods were collected in an uncultivated green area, on the edge of the town (slugs *Milax nigricans*, *Deroceras* cfr. *panormitanum* and snails *R. decollata*, *C. aspersum*, *E. vermiculata* and *Monacha* sp.). Here, *R. decollata* were predominant and 17.8% were found infected with *Phasmarhabditis* sp. Si15. The same strain was recovered from the only specimen of *E. vermiculata* collected nearby. One of two specimens of *C. aspersum* hosted metacercariae of Brachylaimidae. Total prevalence was 16.9%.

III. Highway 417 by Mineo. Examination of the small collection of snails (*Cerņuella virgata*, *Papillifera papillaris affinis*, *Muticaria* cfr. *neuteboomi*, *Theba pisana*, *R. decollata*), collected at limy slopes by Mineo did not reveal parasites.

IV. The Natural Reserve “Oasi del Simeto”. Oasi del Simeto is a wetland with the pioneer vegetation of dunes at the mouth of Simeto river, south to Catania, providing a home for numerous

birds. The site hosted a small population of gastropods, represented by both snails and slugs (*Deroceras* cfr. *panormitanum*, *A. valentianus*, *R. decollata*, *C. virgata* and *Theba pisana*). Total prevalence was 5.9%: a specimen of *A. valentianus* was infected by metacercariae of Brachylaimidae and *R. decollata* by *Phasmarhabditis* sp. (no molecular characteristics yet).

V. The Natural Reserve “Speleological Complex Villasmundo – S. Alfio”. The sampling site in the Villasmundo Reserve in Syracuse Province was located on the mountain slope with the steppe vegetation with rare carob and olive trees and hawthorns. Here, representatives of eight gastropod species were collected (*Milax nigricans*, *Tandonia sowerbyi*, *Papillifera papillaris affinis*, *R. decollata*, *E. vermiculata*, *C. aspersum*, *Caracollina lenticular* and *Monacha* sp.). Their examination has shown a surprisingly high diversity of its helminths (two species of nematodes and two species of trematodes) together with the highest prevalence in the survey (20.8%). Gastropod species infected at this site were *M. nigricans*, *T. sowerbyi*, *P. papillaris affinis*, *E. vermiculata* and *Monacha* sp. *Phasmarhabditis* sp. Si14 was found in the representatives of each gastropod species. The intestinal parasite *Angiostoma margaretae* (Si5) was found in *T. sowerbyi* and *P. papillaris affinis*. This nematode (1 to 6 specimens) was recovered from the oesophagus of its gastropod host and was represented by adult forms as well as late juveniles.

Both slug species examined (one specimen of each species was collected) showed cases of co-infection: in *M. nigricans*, it was *Phasmarhabditis* sp. + sporocysts of trematodes and in *T. sowerbyi*, metacercariae of Brachylaimidae + *Phasmarhabditis* sp. and *Angiostoma margaretae*.

VI. The Pantalica Nature Reserve. The sampling site at Pantalica necropolis was located close to Sortino (Syracuse Province) at 328 m a.s.l. and characterised mostly by vegetation of Mediterranean scrub. The following gastropod species were found there: *Cochlostoma (Auritus) westerlundii dionysi* (predominant), *P. papillaris affinis* and *T. pisana*. None was infected.

VII. Caltagirone. Several specimens of *P. papillaris affinis* and *Deroceras* cfr. *panormitanum* were collected in the city park and the latter was infected by an unidentified *Phasmarhabditis* sp.

DISCUSSION

Our survey covered just a minor portion of terrestrial gastropod diversity of the south-east part of Sicily. In general, the gastropods collected were

represented by species with a wide distribution range and the majority of gastropods examined during our study were snails. No significant difference in the parasite prevalence between them was found and both slugs and snails were found associated with parasites in our study (three species of slugs and four of snails). Slugs are considered to be more often affected by nematodes due to their better exposure to the soil (Rae, 2018). There is probability that in Sicily and similar habitats snail species may play the greater role as nematode hosts in the area due to their predominance over slugs.

Examination of more than four hundred land gastropods in Sicily showed quite low infestation rate typical for gastropod populations in Europe (Ivanova *et al.*, 2013; Ross *et al.*, 2015; Singh *et al.*, 2019) and USA (Ross *et al.*, 2010), while considering that previous surveys were focused only on slugs and their nematode parasites. No significant difference in parasite diversity and parasite prevalence between urban and wild habitats was found (highest values 17% at Librino and 20.8% at Villasmundo and lowest 3.3% at Catania University grounds and 0% Pantalica). Moreover, in the two well represented sites, the urban (Librino) and the wild (Villasmundo), parasite prevalence values were similar despite different gastropod species being present. However, more collections should be made and analysed to obtain a large body of data to allow further conclusions.

In our study, some of collected gastropod species were found in small quantities, thus preventing speculations on its susceptibility to parasites. Conversely, the only non-pulmonate snail species, *Cochlostoma (Auritus) westerlundi dionysi*, was found in quite large numbers and all snails were free of infection. To determine if the species is resistant to nematodes, examination from another localities should be made.

However, even the small scale of the survey demonstrated that many gastropod species (at least seven out of 17 examined) in the area were susceptible to nematode infection. Representatives of three taxa (*Angiostoma margaretae*, *Phasmarhabditis* spp. and *C. elegans*) were molecularly characterised, while yet another rhabditid species recovered and possible *Alloionema* sp. are in need of further collection and examination. All nematodes mentioned were recovered from living gastropod specimens. The discovery of two different strains or even putative species of *Phasmarhabditis* at close localities points at its possible variety in the area. A new host (*Papillifera papillaris affinis*) was recorded for *A. margaretae*. The nematode was found in Villasmundo in two non-related gastropod hosts: *Papillifera papillaris affinis*

(Clausiliidae) and *Tandonia sowerbyi* (Milacidae). It was originally described from a cosmopolitan milacid slug *Milax gagates* in South Africa (Ross *et al.*, 2011). Later, it was found in several slug species native for South Africa (own data, unpubl.). How many gastropod species inhabiting the same site were utilised by this parasite has yet to be investigated.

Petersen *et al.* (2015) reported that *C. elegans* was found in slugs quite often (9.9%) in the course of their survey on invertebrate vectors of *C. elegans* in northern Germany and slugs' intestines were the common habitat. In that study, the gastropod host range of *C. elegans* included mostly slugs of the Arionidae family, whereas no arionid slugs were collected during our survey. These authors tested the ability of *C. elegans* from an intestine to proliferate and proved it possible. They suggested that slug's intestine provides the suitable (humid and microbe-rich) environment for *C. elegans* and discussed the nature of relationships between *C. elegans* and gastropods (phoretic, commensal or even parasitic?). In our study, the behaviour of *C. elegans* rather supports the possible phoretic nature of the relationship between the nematode and the gastropod considering the huge amount of *C. elegans* juveniles (hundreds) carried in *A. valentianus* intestines.

In our study, we did not use the specific (histopathology) methods for the discovery of tissue-dwelling nematode juveniles living in a gastropod as an intermediate host. However, it is known that juveniles of Metastrongyloidea are being shed from the foot where they accumulate in the environment after reaching the invasive stage (Gianelli *et al.*, 2015). In our study, the examination of the mucus of all gastropods collected did not reveal the presence of metastrongylid juveniles.

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E.A. Ivanova, M. Clausi, I. Sparacio and S.E. Spiridonov. Предварительные результаты обследования паразитов наземных гастропод Сицилии.

Резюме. Проведено обследование паразитофауны наземных гастропод юго-востока Сицилии. Было собрано и обследовано 432 моллюска, принадлежащих к 12 видам улиток из семейств Cochlostomatidae, Clausiliidae, Subulinidae и Helicidae и 5 видам слизней из семейств Agriolimacidae, Limacidae и Milacidae. Инвазия нематод была обнаружена у 7 из 17 обследованных видов гастропод. Представители трех таксонов нематод (*Angiostoma margaretae* Ross, Malan & Ivanova, 2011, *Phasmarhabditis* spp. и *Caenorhabditis elegans* (Maupas, 1900) были выделены и охарактеризованы молекулярными методами. Для *A. margaretae*, первоначально описанной из Южной Африки, был зарегистрирован новый хозяин – *Papillifera papillaris affinis* (Philippi, 1836). Обнаружены два штамма (или, возможно, вида) *Phasmarhabditis*, что указывает на высокую вероятность их разнообразия в регионе. Трематоды были представлены 4 семействами и обнаружены в 4 из 7 мест сбора у *Rumina decollata* (Linnaeus, 1758), *Cornu aspersum* (O.F. Müller, 1774), *Ambigolimax valentianus* (Férussac, 1822), *Tandonia sowerbyi* (A. Férussac, 1823) и *Milax nigricans* (Philippi, 1836). Смешанная нематодно-трематодная инвазия была обнаружена у *R. decollata* на территории Университета Катании, а также у *M. nigricans* и *T. sowerbyi* в Вилласмундо, провинция Сиракузы. Не обнаружено существенной разницы в индексах разнообразия и распространенности паразитов между городскими и дикими популяциями гастропод.