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Original article

Presence of nematodes in giant African land snails kept as pets in Poland

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Abstract

The aim of this study was to assess the presence and identity of nematodes in pet giant African land snails (*Lissachatina fulica*) in Poland using microscopic and molecular techniques. *Lissachatina fulica*, *syn. Achatina fulica,* a giant African land snail is not only considered a free-living invasive species and an intermediate host of some parasites, but is also gaining importance as a pet animal living in close contact with humans. In this research, pooled fecal samples and mucus swabs were obtained from 49 pet giant land snails (11 private collections) living in different regions of Poland. The samples were examined using microscopic techniques (Lugol staining, Baermann larvoscopy) and PCR to investigate the presence of nematodes. The microscopic examinations of fecal samples revealed the presence of nematodes in 63.6% (7/11) of the snail groups. Rhabditid nematodes were found in 27.3% (3/11) of the examined groups. Sequencing of PCR products revealed the presence of gastropod nematodes *Phasmarhabditis* sp. (KEN1), *Poikilolaimus oxycercus* and *Caenorhabditis nigoni*. The genetic material of mammalian parasites, including *Crenosoma*, was not detected. Given the increasing popularity of *L. fulica* as pets, understanding their parasitological status is essential for both animal and public health. It also helps meet the expectations of owners who wish to provide proper care for their pet snails.

Keywords: *Caenorhabditis*, mollusks, *Phasmarhabditis*, *Poikilolaimus*, rhabditid

Introduction

The giant African land snail, *Lissachatina fulica,* is regarded as a free-living and invasive species in many regions, including southeast Asia, the islands of the Pacific Ocean and the Americas. In Africa, this species is widely investigated due to its status as a pest in agricultural settings. Additionally, it is an intermediate host for certain helminth parasites including *Crenosoma vulpis, Aelurostrongylus abstrusus, Angiostrongylus cantonensis, Angiostrongylus costaricensis*, or *Angiostrongylus malaysiensis* (Carvalho et al. 2003, Dumidae et al. 2019, Penagos-Tabares et al. 2019, Cardoso et al. 2020, Rodrigues et al. 2022, Lopes-Torres et al. 2024). Giant African land snails have been gaining popularity in many European countries (including Poland) as exotic pets, and they are becoming more frequent patients of veterinary clinics. Therefore, it is important to know the risk factors for infections with helminth parasites in humans and animals. There is a limited number of studies considering giant African land snails as pets, and their European population kept in households remains poorly understood. To date, there have been no studies from Poland addressing the presence of parasites in these snails. The aim of the study was to investigate: what is the prevalence and species composition of nematodes in pet giant African land snails (*Lissachatina fulica*) kept in private households in Poland, and are these nematodes potentially pathogenic or environmentally significant?

Materials and Methods

Sample collection

Fecal samples and mucus swabs were collected from March to July 2024 from 49 snails kept as pets in 11 private collections located in different regions of Poland. The number of individuals in the groups ranged from 1 to 17 and the age of the snails ranged from 3 months to over 2 years. Snails were kept on a substrate made of coconut fiber (10 out of 11 terrariums) or garden soil (1 out of 11 terrariums). The material was collected by veterinarians. None of the snails had ever been dewormed or received any treatment. All groups were provided with washed, plant-based food sourced from grocery stores or private gardens. From each snail group, a pooled fecal sample of at least 3 ml was collected into sterile containers. Mucus swabs were taken individually from the snails using sterile swabs. For collections of more than five snails, mucus samples were taken from five randomly selected individuals and pooled into a single sample. The material was transported to the laboratory at the Division of Parasitology and Invasive Diseases, Wroclaw University of Environmental and Life Sciences, within a maximum of 24 hours. Before examination, the samples were carefully purified from residue of the substrate. The procedures carried out for the purposes of this study did not require approval from an ethics committee. According to the currently applicable law, studies involving crustaceans do not require such approval. Moreover, the procedures performed were not painful and did not cause evident stress.

Microscopic examination

Microscopic examination of fecal smears stained with Lugol solution was performed from each pooled fecal sample to check the presence of nematodes (adults, larvae and eggs). Additionally, each pooled fecal sample was also examined using a modified Baermann technique to isolate the nematode larvae. Larvoscopy was conducted according to previously described procedures (Gelaye et al. 2021). The obtained sediment was examined using a stereo microscope (ZEISS Stemi 508). Morphological identification of nematodes was performed following the methods described previously (Scholze and Sudhaus 2011).

DNA extraction and amplification

DNA was extracted from the nematodes obtained from the feces by larvoscopy and from the mucus swabs, using the *EXTRACTME GENOMIC* DNA KIT (BLIRT S.A, Poland) as per the manufacturer's instruction. DNA extracted from the nematodes was used to identify the species of nematode belonging to Rhabditidae, while DNA obtained from the mucus swabs was used to detect the genetic material *Crenosoma* spp. The DNA samples were stored at 4°C and used shortly after extraction.

To identify Rhabditidae, a fragment of rDNA containing the internal transcribed spacer regions (ITS1, 5.8S, ITS2) as well as a fragment of rDNA containing the gene for 18S rRNA were amplified (Vrain et al. 1992, Ross et al. 2010, d'Ovidio et al. 2019). To identify the genetic material of *Crenosoma* spp. a part of a fragment of mitochondrial 12S rDNA was amplified using primers Fila 12SF and Fila 12SR (Latrofa et al. 2015). All PCR mixtures contained DreamTaq DNA polymerase and optimized DreamTaq buffer (Thermo Scientific, USA), dNTPs (A&A Biotechnology, Poland), appropriate primers (Genomed, Poland), DNA and PCR-grade water. Details are provided in Table 1.

All PCR reactions were conducted using a T100™ thermal cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA). PCR-generated products were detected

Table 1. PCR method – detailed information on conditions.

Identified nematode	Amplified fragments	Primers	PCR mixture (25 µl)	PCR conditions
Rhabditidae	ITS1, 5.8S, ITS2	Forward: 18S: 5′-TTG ATT ACG TCC CTG CCC TTT-3′, Reverse: 28S: 5′-TTT CAC TCG CCG TTA CTA AGG-3′	PCR buffer (2.5 µl); dNTPs (0.5 µl); primers (0.125 µl of both forward and reverse); polymerase (0.125 µl); DNA (0.5 µl); water	94°C for 2 min; 30 cycles at: 94°C for 30 s, 55°C for 60 s; 72°C for 60 s; and 72°C for 7 min
	18S rRNA	Forward: 22F: 5′- TCC AAG GAA GGC AGC AG GC-3′ Reverse: 1080JR: 5′- TCC TGG TGC CCT TCC GTC AAT TTC-3′		94°C for 2 min; 30 cycles at: 94°C for 30 s, 55°C for 60 s 72°C for 90 s; and 72°C for 7 min
Crenosoma spp.	12S rDNA	Forward: Fila 12SF (5′-CGG GAG TAA AGT TTT GTT TAA ACCG-3′) Reverse: Fila 12SR (5′-CAT TGA CGG ATG GTT TGT ACC AC-3′)	PCR buffer (2.5 µl); dNTPs (0.5 µl); primers (0.125 µl of both forward and reverse); polymerase (0.125 µl); DNA (1 µl); water	95°C for 3 min; 30 cycles at: 95°C for 30 s, 52°C for 60 s, 72°C for 60 s, and 72°C for 7 min

Fig.1. *Phasmarhabditis spp.* A – anterior end, B – perivulvar area, C – female tail, D – lateral view of male tail

on electrophoresis in 1.5% agarose gels stained with Midori Green DNA Stain (Nippon Genetics Europe GmbH, Düren, Germany) alongside a DNA Marker 1, 100-1000 bp marker ladder (A&A Biotechnology, Gdynia, Poland) using a PowerPacTM Basic (Bio-Rad Laboratories Inc., Hercules, CA, USA) at 95 V and 400 mA. The electrophoresis results were read using the GelDoc Go Imaging System with Image Lab v.6.1 software (Bio-Rad Laboratories Inc., Hercules, CA, USA). The PCR products were sequenced by Genomed S.A. (Warsaw, Poland) in both directions. The identity of the obtained sequences was checked by a blast search (https://blast.ncbi. nlm.nih.gov/Blast.cgi).

Results

The microscopic examination of fecal samples confirmed the presence of nematodes in 7 of 11 (63.6%) examined private snail collections. Adult rhabditid nematodes were observed in 3 of 11 (27.3%) snail groups (Fig. 1). In 4 of 11 collections (36.4%), only isolated nematode larvae were detected, with no accompanying eggs or adult forms. Molecular examination

Table 2. Detailed results of larvoscopy and PCR.

Sample number	Adult nematodes in fecal samples	Number of larvae[1]	Results of the first PCR[2]	Results of the second PCR[3]
1	Present	+++	+ *(Phasmarhabditis sp.* KEN1)	+ *(Phasmarhabditis sp.* KEN1)
2	Absent	+	-	-
3	Absent	-	-	-
4	Absent	-	-	-
5	Absent	-	-	-
6	Present	+++	+ Rhabditidae	+ *Poikilolaimus oxycercus*
7	Absent	+++	-	-
8	Absent	+	-	-
9	Absent	++	-	-
10	Absent	-	-	-
11	Present	+++	+ *Not sequenced*	+ *Caenorhabditis nigoni*

[1] Number of larvae detected by larvoscopy: "-" No larvae, "+" single larvae in a sample (not visible in every field of view), "++" 1-5 larvae in every field of view, "+++" > 6 larvae in every field of view; [2] PCR using primers 18S and 28S; [3] PCR using primers 22F and 1080JR

detected Rhabditidae genetic material in three samples (no 1, no 6 and no 11), which were the same in which adult nematodes were visible. Sequencing of PCR products revealed over 98% identity to *Phasmarhabditis* sp. (KEN1), *Poikilolaimus oxycercus* and *Caenorhabditis nigoni,* which confirmed the result of the microscopic observation. Genetic material of *Crenosoma* was not detected in any mucus sample. Detailed information is presented in Table 2.

Discussion

In Europe, giant African land snails are continually gaining popularity as household pets, even finding their place in primary schools as classroom animals. Interestingly, children often interact with these snails, frequently touching them with bare hands (Hirschenhauser and Brodesser 2023). This close contact underscores the importance of understanding the potential risks associated with keeping such pets. *L. fulica* may act as an intermediate host for exotic human parasites, such as *Angiostrongylus cantonensis* (a major causative agent of human neuroangiostrongyliasis), as well as for certain helminths that are dangerous to animals (e.g., *Crenosoma vulpis, Aelurostrongylus abstrusus*) (Penagos-Tabares et al. 2019, Rodrigues et al. 2022). Thus, it is crucial to investigate the health implications and any zoonotic concerns that may arise from handling these snails. To date, no data have been published regarding the presence of any parasites in giant African land snails kept as household pets in Poland. This lack of information has led us to initiate an investigation in this area.

Breeding conditions and nutrition may affect the health status of snails. As many as 90.9% (10/11) of snail groups were kept on a substrate made of coconut fiber. The substrate was sterilized before placing it in a terrarium in only one case. However, this collection was one of the three in which the presence of parasites was identified using both microscopic and molecular methods. In the one of the snail groups garden soil was used as the substrate, although it was commercially purchased. All groups of snails in which the parasite species were identified by PCR received products from private gardens. All these data suggest that the parasitic infections may have occurred from external sources through ingestion of food containing parasite eggs. Nevertheless, we cannot rule out the possibility that hidden infections become active with animal age.

Grewal et al. distinguished a few types of associations between mollusks and nematodes. First, the mollusks are paratenic or intermediate hosts of some species belonging to Nematoda (especially *Metastrongyloidae*), which can be dangerous for many vertebrates, including humans. Moreover, mollusks can be definitive hosts of many roundworms that can be pathogenic or apathogenic to them. Some of the nematode species most commonly using mollusks as the definitive host belong to Rhabditidae (Grewal et al. 2003). Rhabditidae include many species of nematodes, usually free-living, but they are also found in some invertebrates (e. g. snails). So far, species such as *Rhabditella axei, Rhabditis terricola, Cruznema sp*.,

and *Pristionchus entomophagus* have been found in giant land snails (Odaibo et al. 2000, d'Ovidio et al. 2019). In this study, three species of Rhabditidae were found in fecal samples of *L. fulica*: *Phasmarhabditis* sp. (KEN1), *Poikilolaimus oxycercus* and *Caenorhabditis nigoni.*

Species belonging to the genus *Caenorhabditis* have many morphological similarities but they differ in terms of ecology. *Caenorhabditis nigoni* has not been identified in snails so far, and this is the first record of this species in *L. fulica. C. nigoni* belongs to the Elegans group, and is morphologically and genetically similar to *Caenorhabditis briggsae,* which has been found in naturally infected giant African land snails from South America (Felix et al. 2014, Guerrero et al. 2018). Another species belonging to the same group, *Caenorhabditis brenneri* was identified in *L. fulica* collected from a banana plantation in the Philippines (Diano et al. 2022). Importantly, in this study, the snails affected by *C. nigoni* were kept on sterilized subsoil, which minimizes the risk of contamination. Moreover, propagating individuals of *C. nigoni* predominantly do not occur in the soil, where only dauer stages are found. Previous investigations suggest that some *Caenorhabditis spp.* can develop in rooting plants (especially fruit), which suggests that the nematode could be eaten by snails and only pass through their digestive system. Nevertheless, the snails were fed with fresh vegetables and the terrarium was regularly cleaned. The above facts indicate that the parasite inhabited the snail intestine (Kiontke et al. 2011).

Poikilolaimus oxycercus was included into the *Rhabditis* subgenus in 1930 by Fuchs. Roundworms belonging to the *Poikilolaimus spp.* are bacteriophagous and oviparous. They were found in sewage, slurry and litter from drains and ditches (Tahseen et al. 2009).

P. oxycercus was also observed in the rectum of snails, for the first time by Örley in 1886 (Örley 1886, Sudhaus 2018). Some research suggests that *P. oxycercus* can only pass through the snail guts where it survives for 1-3 days (Sudhaus 2018). Taking into account the above information, *P. oxycercus* does not seem to be pathogenic for snails or vertebrates, and probably has only environmental importance.

Phasmarhabditis spp. are relatively well-known and widely described, as they are used in the biological control of slug populations (Sudhaus 2018). According to data deposited in the NCBI Taxonomy Browser, 10 species and over 20 unclassified strains have been distinguished within the genus of *Phasmarhabditis*. All species tested for their activity against snails seem to have a potential as biological control agents. The most well-known member of the genus is *P. hermaphrodita,* which is lethal for many gastropod mollusks, and is even a component of commercially available products against snails (Rae et al. 2007, Schurkman et al. 2022). It was previously believed that *Phasmarhabditis spp*. are not parasitic and occur inside the snail body only as a dauer stage. In 1987 this theory was disproved, and this nematode is known to actively reproduce in snails (Rae et al. 2007). In our study, sequencing of the genetic material allowed for detection of *Phasmarhabditis sp*. (KEN1). This is a relatively new nematode species, isolated from *Polytoxon robustum* snails in Kenya. Females of the species have a conoid tail shape, and their vulva is located in the middle of the body. Males have nine bilateral pairs of genital bursal papillae and a pair of cephalate spicules. The larva can penetrate the dorsal integumental pouch of snails to reach the shell cavity and develop into adults. The nematode can cause swelling of the mantle and death of the susceptible mollusks within 4-21 days after exposure (Wilson et al. 1993, Tan and Grewal 2001). Studies conducted by Williams and Rae suggest that *L. fulica* is highly resistant to *P. hermaphrodita*. Investigation showed no influence on animal weight or loss of appetite over 70 days of exposure, even though encapsulated and dead nematodes were found in the shell cavity (Williams and Rae 2015). Nevertheless, we found numerous larvae and adult parasites on microscopic examination of fecal samples. It is possible that, despite being resistant to it, *L. fullica* participates in the environmental spread of *Phasmarhabditis spp*.

Molecular detection of *Crenosoma vulpis* was conducted via amplification of the mitochondrial 12S rDNA fragment. Although *L. fullica* is known as the intermediate host for these nematodes, their genetic material was not detected in the examined samples. This may be caused by limited contact of the pet snails with the external environment; however, the type of examined sample (mucus swab) may also play a role. The L3 larvae of certain parasites can be excreted into snail slime (Barcante et al. 2003, Ferdushy and Hasan 2010, Rollins et al. 2023). Nevertheless, they are released by snails mainly exposed to certain factors, such as stress, and examination of mollusk tissue provides the most accurate results.

In a portion of the samples (4 out of 11), only larvae were detected, without eggs or adult nematodes. The lack of distinguishing features made it impossible to assign them to specific species. Amplification of ITS1, 5.8S, ITS2 and 18S rDNA also did not allow for the identification of these nematode larvae. Nevertheless, the presence of larvae alone suggests that they originated from the environment rather than from the snails.

Conclusion

This study provides the first documented evidence of nematode presence in pet giant African land snails (*Lissachatina fulica*) kept in private households in Poland. Adult nematodes or their developmental stages were found on microscopy in 7 out of 11 examined groups, a prevalence of 63.6%. Molecular techniques allowed us to detect three species of Rhabditidae: *Phasmarhabditis* sp., *P. oxycercus* and *C. nigoni.* Notably, this is the first report of *C. nigoni* in *L. fulica*, expanding the known ecological range of this species and raising questions about its transmission dynamics in captive environments. Although the detected nematodes are not known to be zoonotic, their presence underscores the importance of monitoring parasitic fauna in exotic pets, particularly those in close contact with humans. It is also necessary to mention and discuss noticeable limitations of this study. The first one is the small number of examined collections (11). Nevertheless, to the best of our knowledge, this is the study encompassing the largest number of collections of pet snails in Europe. While a study on snails from Italy included a larger number of individual animals, they originated from only three snail groups (d'Ovidio et al 2019). Due to the limited number of collections, it was impossible to perform reliable statistical tests to demonstrate any correlations between the breeding conditions and the presence of nematodes. The absence of mammalian parasitic DNA (e.g., Crenosoma spp.) suggests a low zoonotic risk under controlled domestic conditions; however, the use of mucus swabs alone may have limited detection sensitivity. Future studies incorporating tissue samples and broader geographic sampling are warranted to refine these findings. Despite the limited number of collections examined, this study represents the most extensive parasitological screening of pet *L. fulica* in Europe to date. These findings contribute to a growing body of knowledge on the microbiological and parasitological status of exotic invertebrate pets and highlight the need for standardized health surveillance protocols in non-traditional companion animals.

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Author declarations

Ethics approval

The study did not require approval from an ethics committee (according to the currently applicable law, studies involving crustaceans do not require such approval).

Use of generative artificial intelligence

No artificial intelligence tools were used during manuscript preparation.

Conflict of interest

The authors declare that they have no conflict of interest.

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