Parasitology Research (2024) 123:344
https://doi.org/10.1007/s00436-024-08364-1

RESEARCH

Co-introduction into a delicate island ecosystem: metastrongyloid nematodes (superfamily Metastrongyloidea) of veterinary and medical importance circulating in aquatic and terrestrial environments of Tenerife (Canary Islands)

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Received: 27 November 2023 / Accepted: 30 September 2024

Abstract

Metastrongyloid nematodes typically reside as adults in the cardiopulmonary systems of their mammalian definitive hosts, potentially causing severe diseases. Of particular concern are *Angiostrongylus cantonensis* and *A. costaricensis*, which can cause eosinophilic meningitis and abdominal angiostrongyliasis, respectively, in their accidental human hosts. Several metastrongyloid species of medical and veterinary importance have been documented in the Canary Islands. However, the gastropod species acting as intermediate hosts for some of these nematodes in the archipelago remained unknown. This study aimed to investigate the occurrence of metastrongyloid nematodes in terrestrial and aquatic gastropods, including both endemic and non-native species, on Tenerife. Foot samples from terrestrial and aquatic gastropods were analyzed using a multiplex PCR targeting the Internal Transcribed Spacer 1 (ITS1), allowing the specific detection of *A. cantonensis*, *A. vasorum*, *Aelurostrongylus abstrusus*, *Crenosoma striatum*, *Troglostrongylus brevior*, and *Crenosoma vulpis*. Five metastrongyloid species, namely *C. striatum*, *A. cantonensis*, *Ae. abstrusus*, *A. vasorum*, and an unidentified metastrongyloid, were identified within both non-native and endemic terrestrial gastropods. In the aquatic snail *Physella acuta*, only *A. cantonensis* and *C. striatum* were detected. This study confirms the introduction of various metastrongyloids associated with non-native mammalian fauna and provides new data on the occurrence of these nematodes in non-native and endemic gastropod species, including their presence in aquatic environments on the Canary Islands.

Keywords *Angiostrongylus cantonensis* · *Angiostrongylus vasorum* · *Aelurostrongylus abstrusus* · *Crenosoma striatum* · Metastrongyloids · Canary Islands

Handling Editor: Julia Walochnik.

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Published online: 09 October 2024

Introduction

The nematode superfamily Metastrongyloidea includes helminths most commonly residing in the cardiopulmonary system of mammals (Anderson 2000). Some species, such as *Angiostrongylus cantonensis* (Alto 2001) and *A. costaricensis* (Romero-Alegría et al. 2014), are capable of infecting humans as aberrant hosts, while other infect domestic animals including dogs (*A. vasorum*, *Crenosoma vulpis*) (Morgan et al. 2005; Maksimov et al. 2017), cats (*Aelurostrongylus abstrusus*, *Troglostrongylus* spp., *Oslerus rostratus*) (Hamilton 1963; Brianti et al. 2012), and wildlife (Cowie 2019; Anderson 2000).

The life cycle of metastrongyloid nematodes commonly involves gastropods as intermediate hosts, in which the larvae develop until their third stage. Definitive hosts acquire the metastrongyloids by accidental or deliberate ingestion of infected gastropods, though carnivorous species may also become infected by consumption of paratenic hosts such as reptiles, rodents, amphibians (Anderson 2000).

The Canary Islands is an oceanic archipelago of volcanic origin in the northern Atlantic, included in the Macaronesia region, together with Madeira, Selvagens, and Cape Verde archipelagos (Carracedo and Pérez-Torrado 2013). As the Canarian archipelago is spatially isolated from the African and European continents, it has a highly endemic and simplified fauna of terrestrial vertebrates (Arechavaleta et al. 2010). However, at least 14 mammal species have been intentionally or unintentionally introduced by humans and have established uncontrolled populations across the archipelago. Six of these species are present in the main seven islands including the feral cat *Felis catus*, the black rat and brown rat *Rattus rattus* and *R. norvegicus*, the house mouse *Mus musculus domesticus*, the goat *Capra hircus*, and the European rabbit *Oryctolagus cuniculus* (Arechavaleta et al. 2010). In Tenerife, the Algerian hedgehog *Atelerix algirus*, the pygmy white-toothed shrew *Suncus etruscus*¸ the European mouflon *Ovis orientalis*, and the ferret *Mustela putorius furo* are also present (Nogales et al. 2006; Barone Tosco 2018).

The introduction of mammals into island ecosystems often has significant ecological consequences, and the Canary Islands are no exception. Rats, cats, and ferrets have been documented to feed on endemic vertebrate species of the archipelago, causing in some cases the reduction of their populations to the point close to extinction; the impact on endemic vegetation is of similar magnitude, caused mainly by seed consumption (Medina and Nogales 2009; Nogales et al. 2006; Kennerley 2019; Pino et al. 2021).

In the Canary Islands, metastrongyloid nematodes *Crenosoma striatum*, *A. cantonensis*, *Ae. abstrusus*, *Troglostrongylus brevior*, *O. rostratus*, and *Angiostrongylus chabaudi* have been reported in their respective definitive hosts (Foronda et al. 2010; Sánchez Vicente 2013; Rodríguez-Ponce et al. 2016; García Livia et al. 2023). Also, accidental infections of *A. cantonensis* in feral cats, presumably following ingestion of infected paratenic hosts, have been documented in the archipelago (Martin-Carrillo et al. 2023). Recent studies on the endemic lizard *Gallotia galloti* (Lacertidae) have shown the presence of a variety metastrongyloid nematodes that utilize these lizards as paratenic hosts (Anettová et al. 2022; Izquierdo-Rodriguez et al. 2023).

Numerous gastropod species are known to act as intermediate hosts of *A. cantonensis* in the archipelago, including *Insulivitrina* (= *Plutonia*) *lamarckii*, *Cornu aspersum*, *Theba pisana*, *Limacus flavus*, *Milax gagates*, *I. emmersoni*, and *I. oromii*. Additionally, *A. vasorum* and *Ae. abstrusus* larvae have been found in *Rumina decollata*, *C. aspersum*, and *I. lamarckii*, although without DNA-based identification (Martin-Alonso et al. 2015; Segeritz et al. 2021; Martin-Carrillo et al. 2023).

The high prevalence of metastrongyloid larvae in lizards of Tenerife (Anettová et al. 2022; Izquierdo-Rodriguez et al. 2023), the new data on metastrongyloids present in the archipelago (García Livia et al. 2023), and the scarcity of data regarding their lifecycle in the archipelago prompted this study to investigate the occurrence of metastrongyloid nematodes in terrestrial and aquatic gastropods. In this study, several endemic and non-native terrestrial gastropod species were tested by multiplex-nested PCR to confirm their involvement in the epidemiology of mammalian lungworm infections. In addition, specimens of the non-native aquatic snail *Physella acuta*, collected from multiple freshwater sources on the island, were examined to assess their potential as sentinel hosts for metastrongyloid infections.

Materials and methods

Samples collection

For the terrestrial gastropod study, two model localities with known presence of *A. cantonensis* in definitive hosts were selected to compare metastrongyloid prevalence and diversity in urban and native forest environment of Tenerife (Fig. 1). The endemic semislug *I. lamarckii* (Vitrinidae) and snail *H. bidentalis* (Helicidae) were collected in the Rural Park of Anaga (28.536640544837674,16.3020098686852 4). The non-native species *R. decollata* (Subulinidae), *C. aspersum* (Helicidae), and *Ambigolimax* sp. (Limacidae) were collected in urban Tegueste (28.525043, -16.336855), in a park close to where positive lizards were examined previously (Table 1 and Fig. 2). Aquatic snail specimens of *P.*

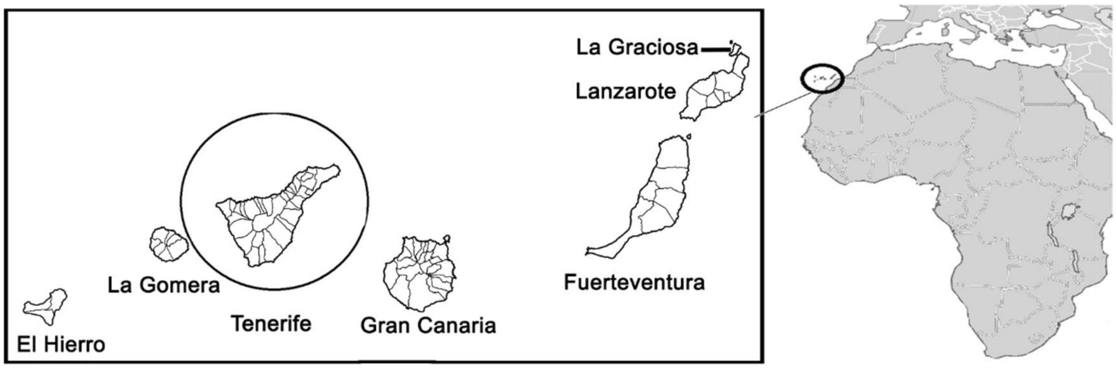

Fig. 1 Geographical location of the Canary Islands, highlighting Tenerife, where the study was conducted. The original image used for the map was extracted from https://es.wikipedia.org/wiki/Anexo:Municipios_de_Canarias#/media/Archivo:Mapa_Canarias_municipios.svg, where permission for its use and edit is granted. The image was edited using Photoshop CS6

Table 1 Collection sites and numbers of specimens examined. *I.l.*, *Insulivitrina lamarckii*; *H.b.*, *Hemicycla bidentalis*; *C.a.*, *Cornu aspersum*; *R.d.*, *Rumina decollata*; *A.sp.*, *Ambigolimax* sp.; *P.a.*, *Physella acuta*

Municipalities	Locality	No	Species					
			I.l	H.b	C.a	R.d	A.sp.	P.a
Tegueste	28.5193494, – 16.325842	21	-	-	-	-	-	21
	28.5248800, – 16.334178	10	-	-	-	-	-	10
	28.525043, – 16.336855	84	-	-	45	32	7	-
La Laguna	28.5459158, – 16.368691	4	-	-	-	-	-	4
Santa Cruz	28.5732811, – 16.188972	4	-	-	-	-	-	4
	28.562615, – 16.252017	3	-	-	-	-	-	3
	28.5059247, – 16.232769	10	-	-	-	-	-	10
	28.5262431, – 16.204700	10	-	-	-	-	-	10
	28.5366405 – 16.302009	96	65	31	-	-	-	-
Garachico	28.3687050, – 16.758663	10	-	-	-	-	-	10
Santiago del Teide	28.2726731, – 16.839540	4	-	-	-	-	-	4
	28.2640014, – 16.822964	10	-	-	-	-	-	10
Buenavista del Norte	28.2965544, – 16.849539	10	-	-	-	-	-	10

acuta (Physidae) were surveyed in 11 freshwater localities across the island of Tenerife, from the North-East (Tegueste, La Laguna, Santa Cruz) and North-West of Tenerife (Garachico, Santiago del Teide, Buenavista del Norte) (Table 1 and Fig. 2). Gastropods were identified following MolluscaBase (2023).

Captured gastropods were placed individually into vials and taken to Instituto Universitario de Enfermedades Tropicales y Salud Pública de Canarias (IUETSPC) where they were euthanized by immersion in 5% ethanol followed by beheading. Foot samples were preserved in absolute ethanol until further analysis.

Sample preparation and molecular analysis

Foot samples for each individual were cut and then DNA isolation was performed using DNEasy Blood&Tissue Qiagen with the following modifications: increasing the proteinase K to 25 µl and extending the lyse phase overnight, resulting in the total digestion of the foot tissue. A multiplex-nested PCR was set up for the detection of *A. cantonensis*, *A. vasorum*, *A. abstrusus*, *C. striatum*, *C. vulpis*, and *T. brevior*, following previously published methods by Izquierdo-Rodriguez et al. (2023). The first round amplifies the entire ITS1 region of all targeted species; second round uses species-specific primers to produce different product sizes for gel-based discrimination. Both rounds were performed using Qiagen Multiplex PCR plus kit (100) in a total volume of 25 µl. The PCR products were visualized in a 2% agarose gel, and the bands were purified using Gel/PCR DNA Fragments Kit and sequenced in Macrogen Europe (Netherlands) using the amplification primers. For sequencing, representative bands were randomly selected according to their intensity and suspected identity in compliance with their molecular weights (Fig. 3).

The obtained sequences were assembled and edited using Geneious Prime® 2019.2.1 software (Kearse et al. 2012) and their identity was confirmed by BLAST

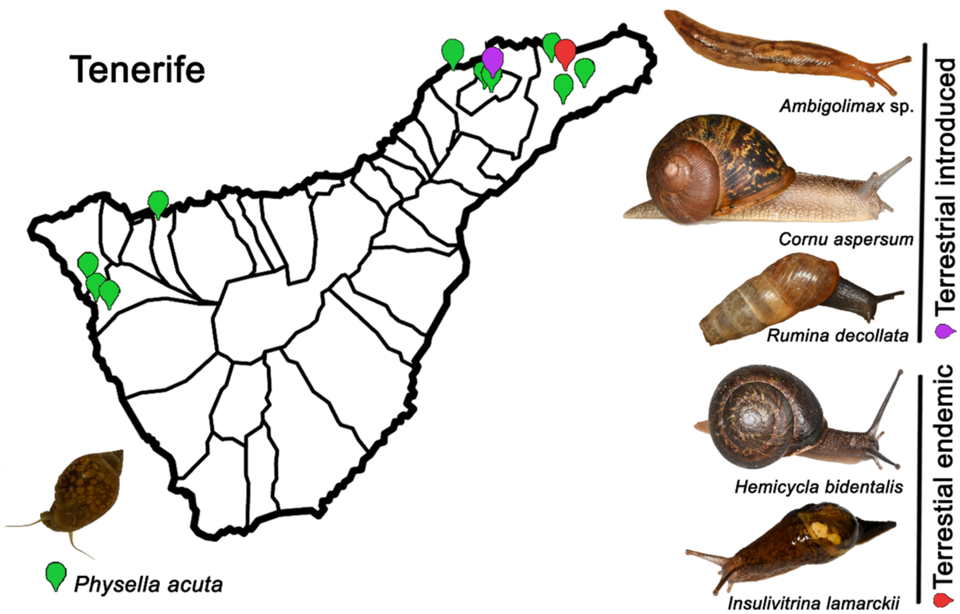

Fig. 2 Collection localities and gastropod species examined, regarding the terrestrial gastropods, the endemic species were collected in Anaga rainforest while the non-native in Tegueste; the aquatic specimens were collected in various locations of the island; *C. aspersum*, *H. bidentalis*, and *I. lamarckii* were photographed by David Modrý; *Ambigolimax* sp. and *R. decollata* specimens were photographed by Radovan Coufal. The *P. acuta* picture was downloaded from: https://www.inaturalist.org/observations/150989860 where permission for sharing and adaptation is granted (CC BY-NC 4.0). The original image used for the map was extracted from https://es.m.wikipedia.org/wiki/Archivo:Tenerife_municipios.svg, where permission for its use and edit is granted. The image was edited using Photoshop CS6

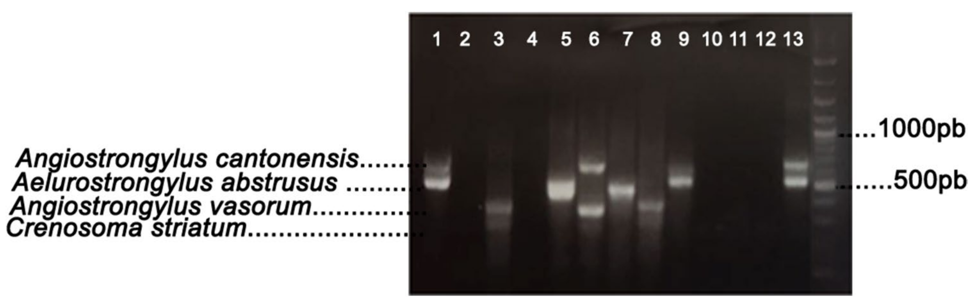

Fig. 3 Representative result of the multiplex-nested PCR in semislug *I. lamarckii* showing the identifying/differentiation power of the assay in a 2% agarose gel with 1-kb Plus DNA ladder molecular weight marker (New England Biolabs, Ipswich, MA, USA). Bands represent *Angiostrongylus cantonensis* (642 bp), *Aelurostrongylus abstrusus* (537 bp), *Angiostrongylus vasorum* (492 bp), *Crenosoma striatum* (377 bp), and an unidentified metastrongyloid (an additional band around 300 bp). *Troglostrongylus brevior* (579 bp) and *Crenosoma vulpis* (299 bp) bands are omitted as none of the samples was positive

analysis of the NCBI GenBank database. All sequences were obtained from both DNA strands, and 11 high-quality representative sequences were uploaded to GenBank under the accession numbers OR565227, OR565228, OR565230, and OR565232-OR565239 (Table 2). The results were statistically analyzed using the chi-square test, with the significance level set at 0.5.

Results

A total of 57 terrestrial gastropods (31.67%) were positive for DNA of tested metastrongyloid nematodes. Divided between endemic and non-native, 34 (35.42%) of the terrestrial endemic gastropods were positive, while in the

Table 2 Results from the BLAST analysis with the NCBI GenBank database of the sequences obtained in this study (*A. cantonensis*, *Angiostrongylus cantonensis*; *A. vasorum*, *Angiostrongylus vasorum*; *Ae. abstrusus*, *Aelurostrongylus abstrusus*; *C. striatum*, *Crenosoma striatum*; *C. vulpis*, *Crenosoma vulpis*)

Accession numbers	Sequence length	Closest matches	Species assigned
OR565227	583 bp	99.7% *A. cantonensis* (OR119900)	*A. cantonensis*
OR565228	158 bp	97.3% *Crenosoma* sp. slug (MG878893); 93.1% *C. vulpis* (KF836608); 88.8% *C. striatum* (KT257662)	Unknown metastrongylid
OR565230	145 bp	97.3% *Crenosoma* sp. slug (MG878893); 93.1% *C. vulpis* (KF836608); 88.8% *C. striatum* (KT257662)	Unknown metastrongylid
OR565232	450 bp	99.8% *Ae. abstrusus* (KX518353)	*Ae. abstrusus*
OR565233	422 bp	98.3% *A. vasorum* (MT345058)	*A. vasorum*
OR565234	594 bp	99.8% *A. cantonensis* (OR119900)	*A. cantonensis*
OR565235	591 bp	100% *A. cantonensis* (OR119900)	*A. cantonensis*
OR565236	204 bp	100% *C. striatum* (KR868716)	*C. striatum*
OR565237	170 bp	99.3% *Crenosoma* sp. slug (MG878894); 96.1% *C. striatum* (OP210307); 93.9%, *C. vulpis* (KF836608)	Unknown metastrongylid
OR565238	533 bp	99.6% *A. cantonensis* (OR119901)	*A. cantonensis*
OR565239	171 bp	99.3% *Crenosoma* sp. slug (MG878894); 96.1% *C. striatum* (OP210307); 93.8% *C. vulpis* (KF836608)	Unknown metastrongylid

case of non-native gastropods, 23 individuals (27.38%) harbour DNA from any of the nematodes. Among terrestrial gastropods, *A. cantonensis* was the most prevalent species (11.67%), followed by *C. striatum* (11.11%), *Ae. abstrusus* (9.44%), the band of approx. 300 bp, visually different from the *C. vulpis* positive control, later confirmed by sequencing as the unknown metastrongyloid (5%), and lastly *A. vasorum* (4.44%). The identity of amplicons based on their length was confirmed by sequencing for all of 20 randomly chosen bands with sequence identity > 99.5% (Table 2). Neither *T. brevior* nor *C. vulpis* were detected (Tables 2 and 3). All tested nematode species were found in both endemic and non-native terrestrial species, with no significant difference in prevalence between those groups ($p < 0.05$).

Regarding *P. acuta*, only *A. cantonensis* and *C. striatum* were detected. The presence of the rat lungworm in this aquatic gastropod was restricted to the North-East of the island, to the municipalities of Tegueste and Santa Cruz. A total of six individuals were positive, accounting for 6.25% prevalence. On the other hand, *C. striatum* was found in 16 *P. acuta* individuals (16.67%) throughout the North of Tenerife (Tegueste, La Laguna, Santa Cruz, Buenavista del Norte).

Coinfections were present among the endemic species of gastropods studied. Among the nine positive *H. bidentalis* individuals, two were coinfected with *A. cantonensis* and the unidentified metastrongyloid. In *I. lamarckii*, ten out of 25 positive gastropods were coinfected, *A. cantonensis* and *Ae. abstrusus* being the most common combination, occurring in seven individuals, three of which were also combined with *A. vasorum*. The most prevalent parasite in the single-infected *I. lamarckii* was *A. cantonensis*, found in 12% of positive samples.

Table 3 Results from the multiplex PCR analysis in endemic and non-native gastropods of Tenerife; * Total prevalence = prevalence of positive samples for any of the metastrongyloid studied

Gastropod	*n*	*A. cantonensis* (%)	*A. vasorum* (%)	*Ae. abstrusus* (%)	*C. striatum* (%)	Unknown metastrongyloid (%)	Total prevalence (%)*
I. lamarckii	65	15.38	10.77	15.38	7.69	6.15	38.46
H. bidentalis	31	12.90	-	-	12.90	9.68	29.03
C. aspersum	45	2.2	-	-	11.11	2.2	15.56
R. decollata	32	15.63	-	21.88	12.50	-	34.38
Ambigolimax sp.	7	14.29	14.29	-	28.57	14.29	71.43
Total terrestrial	**180**	**11.67**	**4.44**	**9.44**	**11.11**	**5**	**31.67**
P. acuta	96	6.25	-	-	16.67	-	21.88
Total	**276**	**9.78**	**2.90**	**6.16**	**13.04**	**3.26**	**28.26**

In urban environments, five of the 11 positive *R. decollata* specimens were coinfected, being *A. cantonensis* + *Ae. abstrusus* present in four individuals. Among the single-infected individuals of this species, *C. striatum* was the most prevalent. Only one *P. acuta* individual from Tegueste was coinfected with *A. cantonensis* and *C. striatum*. Neither *C. aspersum* nor *Ambigolimax* sp. positive individuals, none was coinfected, and for both species *C. striatum* was the most commonly detected parasite.

The comparison of the obtained ITS1 sequences with those available in the GenBank confirmed their identity with the following GenBank entries: *A. cantonensis* (100%, OR119900), *A. vasorum* (98.3%, MT345058), *Ae. abstrusus* (99.8%, KX518353), *C. striatum* (100%, KR868716). Additionally, nine samples showed double bands of approximately 300 bp; these sequences showed 97.28–99.33% similarity with two nematode sequences obtained from slugs of Germany tagged as *Crenosoma* sp. (MG878893, MG878894). These unidentified metastrongyloids were previously reported in lizards from Tenerife (OR753433) (Izquierdo-Rodriguez et al. 2023).

Discussion and conclusions

The primary challenges in biodiversity conservation often revolve around habitat degradation or destruction and the introduction of non-native or exotic species into ecosystems (Van Dyke 2008). In island ecosystems, where species have evolved in relative isolation, the introduction of invasive species represents a significant threat to the survival of endemic fauna (Courchamp et al. 2003).

The results of this study, together with the previous findings of a range of metastrongyloid nematode parasites in endemic lizards of Tenerife (Izquierdo-Rodiguez et al. 2023), demonstrate that mammalian invasion into a pristine island ecosystem has also led to the establishment of life cycles of metastrongyloid nematodes that were introduced along with their respective mammalian hosts.

Metastrongyloid nematodes are generalists concerning their gastropod intermediate hosts (Hobmaier and Hobmaier 1935; Baruš and Blažek 1971; Kim et al. 2014). The data from this study confirm the involvement of non-native as well as endemic gastropods in the circulation of introduced metastrongyloids, including presence of *A. cantonensis* in aquatic environments of Tenerife. However, despite initial expectations, the aquatic snails *P. acuta* do not appear to play a significant role in the life cycle of metastrongylid gastropods. Therefore, it is not suitable sentinel hosts for monitoring the distribution of zoonotic *A. cantonensis* across the island. Individuals of *P. acuta* positive for *A. cantonensis* DNA were found in Tegueste and Santa Cruz, areas linked to water sources in Anaga, a region known for its high prevalence of this zoonotic nematode in both rats and gastropods (Martin-Alonso et al. 2015; Martin-Carrillo et al. 2021). Notably, the brown garden snail *C. aspersum* showed the lowest prevalence of metastrongyloid larvae among all the studied gastropods, corroborating previous studies where few damaged L3 larvae of *A. cantonensis* were collected from this snail species, unable to infect rats (Alicata 1965).

The spectrum of detected nematodes perfectly matches the spectrum of mammalian definitive hosts introduced to the island. The PCR data confirm the findings of Segeritz et al. (2021), who had morphologically identified larvae of *A. vasorum* and *Ae. abstrusus* in gastropods of Tenerife and El Hierro. Interestingly, the presence of *A. vasorum* DNA in gastropods and lizards suggests circulation of this nematode species among carnivores. Although no clinical nor asymptomatic infections in local dogs have been reported, the fact that synanthropic gastropods collected in public parks were found harbouring this nematode implies a risk of transmission to pets.

Previous research on lizards (Izquierdo-Rodriguez et al. 2023) revealed sequences of an unidentified metastrongyloid close to *Crenosoma* spp., with high similarity to nematode sequences retrieved from slugs from Germany (MG878893, MG878894) labelled as *Crenosoma* sp. (Lange et al. 2018). Remarkably, the resulting sequences found in all tested terrestrial gastropod species except *R. decollata* were highly similar (99.3%) to those detected in lizards, confirming circulation of this yet unidentified metastrongyloid among Canarian mammals. The detailed characterization of this metastrongyloid exceeds the scope of this manuscript and based on preliminary data from several genes, it will require extensive work aiming to identify also the paratenic/definitive host and the analysis of the adult worms. The low diversity of Tenerife mammals makes it possible to hypothesize about the definitive host of this enigmatic species. Domestic ferrets *Mustela putorius furo* were introduced to Canary Islands from Europe probably during the sixteenth century for rabbit hunting and nowadays have established feral populations in several of the islands in the archipelago (Medina and Martín 2010; Arechavaleta et al. 2010). Wild European populations of *Mustela putorius* host *C. melesi* and *C. schachmatovae*, one of which could hypothetically be associated with the findings of this study (Kretschmar 2016; Deak et al. 2023). The presence of highly similar metastrongyloid DNA sequences in gastropods in Germany, where the polecat (a wild form of a domestic ferret) occurs, also suggests mustelid hosts. Future studies focused on the helminth fauna of ferrets in the Canary Islands and comparative molecular analyses with European mustelids could provide insights into the identity of this enigmatic worm. Although its closest matches when comparing these unidentified metastrongyloids sequences to those available on GenBank are tagged as *Crenosoma* sp. (MG878893, MG878894), the brevity of the sequences obtained in this study, together with the scarcity of ITS1 *Crenosoma* spp. sequences available in the GenBank, prevents the confirmation of their identity, hence these sequences being referred as unknown or identified metastrongyloids along the text.

The frequency of coinfections with several metastrongyloid nematodes reported in intermediate and paratenic hosts emphasizes the usefulness of multiplex-nested PCR in epidemiological studies. Specifically, in cases when relative abundance matters, the DNA metabarcoding approaches can be applied in future research, similar to increasing usage in gastrointestinal nemabiome research (Pafčo et al. 2018; Francis and Šlapeta 2022; Halvarsson et al. 2022).

The presence of metastrongyloids nematodes of public and veterinary health importance in gastropods of Tenerife implies a risk of transmission not only to humans, but also to domestic and free ranging animals. Veterinary and healthcare professionals of Tenerife should consider metastrongyloid infection as a possible cause of meningitis in humans and dogs (in case of *A. cantonensis*) or respiratory distress in domestic animals.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00436-024-08364-1.

Acknowledgements We would like to thank the Government of the Canary Islands and Tenerife for granting the necessary permits for the fulfilment of this study (No Ref. 2022/14555; 2022/11052 and 2022-00920). We would like to thank MRIR, JCRB, MIS, and PGL for their help during the development of this article and LMA for the identification of some of the terrestrial gastropods.

Author contributions DM and PF designed and supervised the study. EIR, LA, AŠ, and RC conducted field sampling. EIR performed the DNA isolation. KH and EIR designed the primers and conditions of the multiplex PCR. EIR performed the laboratory assays. KH supervised the laboratory work and performed sequencing results analyses.

Funding EIR is granted a scholarship by the Spanish Ministry of Science, Innovation and Universities and Universidad de La Laguna (Becas M-ULL, convocatoria 2019), This study was funded by "Consejería de Economía, Industria, Comercio y Conocimiento, Gobierno de Canarias" (ProID2021010013) and FEDER-FSE Canarias 2014–; and "Consejería de Transición Ecológica, Lucha contra el Cambio Climático y Planificación Territorial, Gobierno de Canarias" (Orden Nº248/2020, 4th December, 2020/4727). DM, LA, AŠ, and RC were supported by the Czech Science Foundation (22-26136S). Fieldwork of LA and RC was funded by Masaryk University's PhD Mobility scholarship and MUNI/IGA/1182/2021. KH was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103)—Funded by the European Union—Next Generation EU.

Data availability No datasets were generated or analyzed during the current study.

Declarations

Ethical approval Not applicable.

Competing interests The authors declare no competing interests.

Animal welfare Not applicable.

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