Parasites & Vectors

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Squamata reptiles as a potential source of helminth infections when preyed on by companion animals

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Abstract

Background Squamate reptiles cohabiting with companion animals may represent a source of helminth infections, especially through predation by dogs and cats with an outdoor lifestyle.

Methods In order to assess the role of reptiles as intermediate/paratenic hosts of trophically transmitted helminths, synanthropic reptiles ($n = 245$) captured from different ecological settings (i.e., households, dog shelters, urban, peri-urban and rural areas or natural parks) of southern Italy were examined for endoparasites. Parasitic cysts (i.e., larval forms of acanthocephalans, cestodes and nematodes) and free helminths (i.e., adult nematodes and digeneans) were morphologically and molecularly identified, and statistical analysis was carried out to evaluate the correlations between reptiles, infections, and ecological settings.

Results Overall, 31% of reptiles were positive for at least one helminth, with *Podarcis siculus* (18.7%) and *Tarentola mauritanica* (8.1%) being the most frequently infected species. Among the parasites of medical interest, *Joyeuxiella echinorhyncoides* showed the highest prevalence (19.7%), followed by *Diplopylidium acanthotetra* (10.5%), *Joyeuxiella pasqualei*, *Mesocestoides lineatus* (5.6%) and *Physaloptera* sp. (3.9%). *Macracanthorhynchus hirudinaceus* was detected once. *Podarcis siculus* and *T. mauritanica* were associated with cestode infections.

Conclusions The wide range of helminths detected here in reptiles living in sympatry with pets and the fact that many of these helminth species are parasitic and may infect companion animals (e.g., *J. pasqualei*, *J. echinorhyncoides*, *D. acanthotetra*, *Physaloptera* sp.) and humans (i.e., *Macracanthorhynchus hirudinaceus*, *Mesocestoides lineatus*) indicate the potential health risk associated with pets preying on these small vertebrates. Our results indicate the need for complementary investigations of trophically transmitted parasites in dogs and cats living in sympatry with reptiles.

Keywords Synanthropic reptiles, Helminth fauna, Predation by pets

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Background

Squamate reptiles, especially geckos and lizards, are common synanthropic animals with a worldwide distribution [1–3]. Urbanization and habitat fragmentation have favored the encounters between these animals, pets and humans, as well as the transmission of diseases caused by pathogens that are shared between them [3, 4]. Interactions between companion animals and reptiles have also increased due to the popularity of these small vertebrates as pets [5], with snakes and lizards being the most common [6]. Although information on the care of reptiles is still limited, they represent 2.1% of the pet population in Italy [7, 8]. As a result of their widespread popularity as pets, the scientific community's interest in these animals has increased in recent decades, though little is known about their endoparasites [6]. Small reptiles preyed on by cats and dogs may represent a potential source of helminth infections, as some of these pathogens are trophically transmitted [9]. Indeed, many species of cestodes (i.e., *Joyeuxiella pasqualei*, *Joyeuxiella echinorhyncoides, Diplopylidium acanthotetra, Mesocestoides* spp.), and nematodes (i.e., *Aelurostrongylus abstrusus, Physaloptera rara*, *Spirocerca lupi, Toxocara canis, Troglostrongylus brevior*) have reptiles as intermediate or paratenic hosts and pets as definitive ones [10–19]. Therefore, lizards and geckos cohabiting with companion animals represent a potential risk for the introduction of helminths into households, especially if these reptiles are obtained directly from the wild [20]. To date, helminths associated with reptiles have been investigated mainly from an ecological perspective [21–24], and only marginally in relation with their role in the maintenance of parasitic diseases of dogs and cats [9]. The main helminth taxa associated with synanthropic reptiles include Digenea (e.g., *Paradistomum* spp., *Brachylaima* spp., *Renifer* spp.), Cestoda (e.g., *Diplopylidium* spp., *Joyeuxiella* spp. *Mesocestoides* spp.), Nematoda (e.g., oxyurids, ascarids, strongyles, *Rhabdias* spp., *Strongyloides* spp.) and Acanthocephala (e.g., *Sphaerirostris* spp., *Centrorhynchus* spp., *Oligacanthorhynchus* spp.) [24–29]. Cestodes and acanthocephalans are usually detected as larval forms, localized mostly in the coelomic cavity, liver and intestinal serosa. Digeneans and oxyurid nematodes, however, are generally detected as adults in the digestive tract [24, 26, 30, 31].

Although studies have been conducted on *Leishmania* spp., *Borrelia burgdorferi* and *Rickettsia* spp. associated with reptiles [32–34], there is still a lack of data on the endoparasites of these vertebrates. Thus, the aim of the present study was to evaluate the role of Squamata reptiles in different epidemiological settings (i.e., households, dog shelters, urban, peri-urban, and rural areas or natural parks) as sources of helminth infections when preyed on by companion animals.

Methods

Study area and reptile sampling

Squamata reptiles were collected between April 2020 and July 2021 from various locations in four southern Italian regions (i.e., Apulia, Basilicata, Calabria, Sicily), within the framework of a study on zoonotic parasites of reptiles [34]. Specifically, the study locations (i.e., households, dog shelters, urban, peri-urban, and rural areas or natural parks) of each region were chosen based on the presence of different reptile species living in sympatry with the feline, canine and human population (Fig. 1). Data on the reptiles, including geographical origin (i.e., region, city/town, study location), capture status (i.e., dead or alive) and biological status (i.e., young, young adult, adult) were recorded in individual files, along with information on the presence of companion animals at the same location, and antiparasitic treatments (i.e., insecticides/repellents, anthelmintics). Two hundred and thirty of the captured reptiles were humanely euthanized according to protocols [35]. Necropsies were performed on these and on the reptiles captured dead ($n=15$). The body wall was opened by longitudinal incision and the coelomic cavity, organ surfaces, mesenteries and organ lumens examined for the presence of helminths by optical observation using a stereomicroscope (Leica MS5; Leica, Germany). Protocols for the collection of reptiles were authorized by the Ministry for Environment, Land and Sea Protection of Italy (approval no. 0073267/2019).

Morphological identification of helminths

Parasitic cysts were separated from non-parasitic ones and examined under a light microscope (Leica DMLB2), as were free helminths (Fig. 2). A representative number of individuals of each parasite group (i.e., Acanthocephala, Nematoda, Cestoda, and Digenea) was fixed and cleared on a glass slide. For cestodes, digeneans and acanthocephalans, formalin/acetic acid/alcohol solution was used, while nematodes were cleared in lactophenol solution and examined as temporary preparations. All of the remaining cysts and/or free helminths collected were stored in individual vials containing 70% ethanol. After storage for a few hours (for nematodes) or a maximum of 2 days (for other helminths) at 25 °C, the slides were examined using light microscopy; dichotomous keys and original descriptions were used for morphological identification [16, 31, 36–56]

Molecular procedures

For the molecular identification of helminths, genomic DNA was extracted using a commercial kit (Qiagen

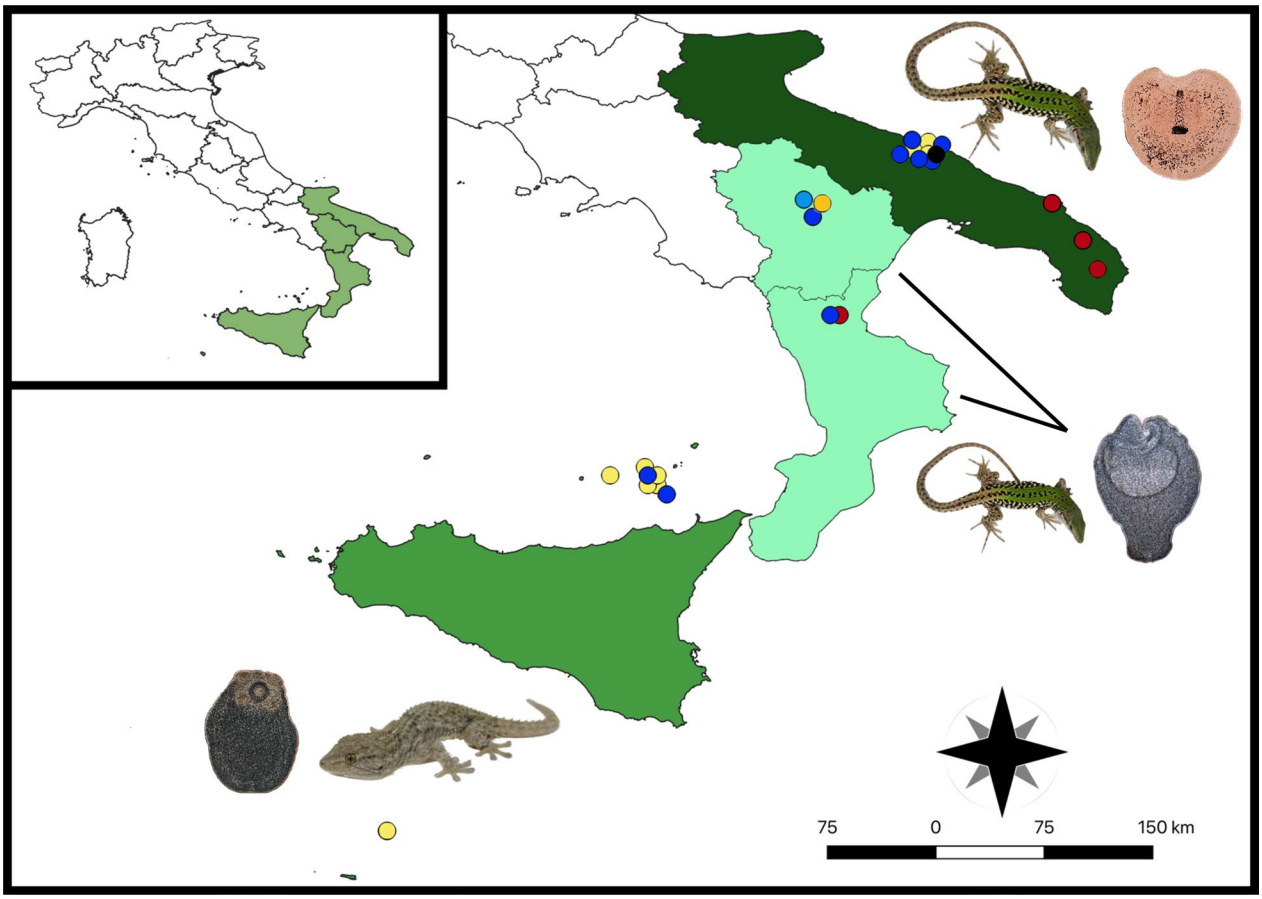

Fig. 1 Map showing the sampling locations and the main parasite species of medical/veterinary interest and host species for the four Italian regions included in the study. Study locations/areas are represented by colored circles as follows: dog shelters (red circles), households (black circles), natural park (orange circle), peri-urban (blue circles), rural (light blue circle), urban (yellow circles). Shades of green indicate the abundance of collected hosts per region (e.g., darker green indicates higher abundance in the Apulia region). The most abundant species of parasite and host are indicated (i.e., *Joyeuxiella echinorhyncoides* and *Podarcis siculus* in Apulia, *Mesocestoides lineatus* and *Podarcis siculus* in Basilicata and Calabria, *Diplopylidium acanthotetra* and *Tarentola mauritanica* in Sicily)

Micro Kit; Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions, for a representative number of specimens. At least one sample (or more than one in the case of different host species and/or regions of collection) for each parasite of veterinary and medical concern was processed, along with three acanthocephalan specimens, as these were the most common helminth group detected. The quantity of the DNA of eight samples was evaluated by Qubit 2.0 fluorometer (Applied Biosystems, USA). Conventional polymerase chain reaction (PCR) was performed for molecular identification of acanthocephalans as well as for cestodes and nematodes; details regarding sample processing, including the target genes and primers used, are reported in Table 1. Amplified PCR products were visualized by gel electrophoresis in 2% agarose gel containing GelRed nucleic acid gel stain (VWR International, Milan, Italy) and viewed on a GelLogic 100 gel documentation system (Kodak, New York). Negative (i.e., ultra-pure sterile water) and positive DNA controls collected in previous studies (i.e., *Sphaerirostris picae*, *J. pasqualei*, *T. brevior*) were included in all PCR runs. All the positive PCR products were purified and sequenced in both directions using the same forward and reverse primers by employing Big Dye Terminator v.3.1 chemistry (3130 Genetic Analyzer; Applied Biosystems, CA) in an automated sequencer (ABI-PRISM 377). Nucleotide sequences were edited, aligned and analyzed using Geneious version 9.0 (Biomatters, Auckland, New Zealand) [57], and compared with publicly available sequences in the GenBank database, using the Basic Local Alignment Search Tool (BLAST; http://blast.ncbi.nlm.nih.gov/Blast.cgi) for species identification.

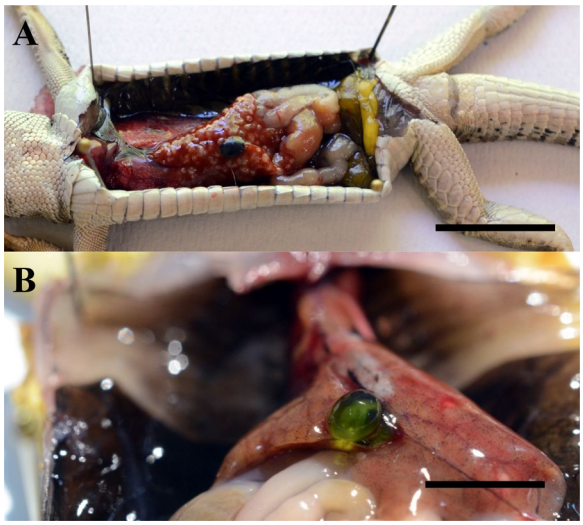

Fig. 2 Ventral view of dissected reptiles showing (**a**) liver cysts (scale bar = 4 cm), and (**b**) gall bladder with digenean (scale bar = 1 cm)

Statistical analysis

For reptile species from various ecological and environmental settings that were or were not infected by helminth parasites, a multiple correspondence analysis (MCA) was carried out to graphically represent the relationship structure of two or more qualitative variables through positioning maps [58]. Before conducting the MCA analyses, several responses were transformed into dichotomous variables (yes/no) [58]. The suitability of the MCA variables was assessed by chi-squared tests. Variables showing significant correlations with less than half of the total variables were discarded. Eigen values that were more than the value of the mean [59] and Cronbach's alpha [60] were used for the selection of an appropriate number of dimensions. The MCA analysis conducted here was undertaken to assess the potential relevance of selected ecological, epidemiological, and environmental variables with respect to the health risk of companion animals linked to potential reptile predation. Eleven variables were included in the analysis: reptile capture status (dead or alive); region (Apulia, Basilicata, Calabria, and Sicily); type of sampling location (urban, peri-urban, rural, regional park, household and dog shelter); presence of pets (yes/no); treatment of pets with insecticides/repellents (yes/no); treatment of pets with anthelmintics (yes/no); presence of cysts (yes/no); positivity of the examined reptiles with respect to Acanthocephala (yes/no), Nematoda (yes/no), Cestoda (yes/no), Digenea (yes/no). Exact binomial 95% confidence intervals (CIs) were established for proportions by using Epitools—Epidemiological Calculators software [61].

Results

A total of 245 squamate reptiles belonging to five families (i.e., Scincidae, Gekkonidae, Lacertidae, Pythonidae and Phyllodactylidae) and seven species (see Table 2), of which 75.1% were classified as adults and the remaining as young adults (15.9%) or young (8.9%), were subjected to necropsy. The species of reptiles captured are reported in Table 2 together with geographical location (i.e., sampling location, city/town, region) and capture status (dead or alive).

Of the 245 reptiles examined, 42% had at least one cyst (103/245, 95% CI 0.36–0.48) (Table 2). Of these, 62.1% (64/103, 95% CI 0.52–0.71) had cysts containing parasite

Table 1 Molecular tools employed for helminth identification

Helminth	Target gene	Primer	Sequence 5′-3′	Annealing temperature (°C)	Fragment length (base pairs)	References
Macracanthorhynchus hirudinaceus	*cox*1	JB3	TTTTTTGGGCATCCTGAGGTTAT	48	~400	[89]
Diplopylidium acanthotetra						
Joyeuxiella echinorhyncoides		JB4.5	TAAAGAAAGAACATAATGAAAATG			
Mesocestoides lineatus						
Sphaerirostris picae		Cyclo *cox*1Fa	CARCATATGTTTTGRTTTTTTGG	52	~420	[49, 90]
Diplopylidium acanthotetra						
Joyeuxiella echinorhyncoides		Cyclo *cox*1Rb	CCTAAYGACATAACATAATGRAAATG			
Physaloptera sp.	*cox*1	NTF	TGATTGGTGGTTTTGGTAA	54	~555	[91]
		NTR	ATAAGTACGAGTATCAATATC			
	18S	NC18SF	AAAGATTAAGCCATGCA	57	~1700	[92]
		NC5BR	GCAGGTTCACCTACAGAT			
		Worm AF	GCGAATGGCTCATTAAATCAG	54	~530	[93]
		1270R	CCGTCAATTCCTTTAAGTTT			

Table 2 Reptile species and number of individuals captured with respect to sampling location, capture status and presence of at least one cyst and/or one helminth

Species (no.)	Region (no.)	City/town (no.)	Sampling location (no.)	Capture status (no.)	Cysts (no.)	Helminths (no.)
Chalcides ocellatus (25)	Sicily (25)	Linosa (25)	Urban (25)	Alive (25)	4	6
Hemidactylus turcicus (2)	Calabria (2)	Cassano All'ionio (2)	Dog shelter (2)	Alive (2)	0	0
Hierophis carbonarius (3)	Sicily (1)	Salina (1)	Urban (1)	Alive (1)	1	1
	Apulia (2)	Torre a Mare (1)	Urban (1)	Dead (1)	1	1
		Noicattaro (1)	Urban (1)	Dead (1)	0	0
Podarcis filfolensis (22)	Sicily (22)	Linosa (22)	Urban (22)	Alive (22)	1	2
Podarcis siculus (125)	Apulia (81)	Brindisi (17)	Dog shelter (17)	Alive (17)	6	6
		Lecce (30)	Dog shelter (30)	Alive (30)	21	16
		Noicattaro (15)	Household (9)	Dead (1)	0	0
				Alive (8)	6	4
			Urban (4)	Dead (4)	3	0
			Peri-urban (2)	Dead (2)	0	0
		Torre a Mare (3)	Peri-urban (2)	Alive (2)	2	2
			Urban (1)	Dead (1)	0	0
		Valenzano (16)	Peri-urban (16)	Dead (1)	1	1
				Alive (15)	11	5
	Basilicata (10)	Parco regionale (10)	Natural park (10)	Alive (10)	6	4
	Calabria (17)	All'ionio (17)	Dog shelter (12)	Alive (12)	7	3
			Peri-urban (5)	Alive (5)	2	1
	Sicily (17)	Filicudi (1)	Urban (1)	Alive (1)	1	1
		Linosa (1)	Urban (1)	Alive (1)	0	0
		Lipari (9)	Urban (9)	Alive (9)	2	3
		Malfa (4)	Peri-urban (4)	Alive (4)	0	0
		Vulcano (2)	Peri-urban (2)	Alive (2)	0	0
Python molurus (1)	Apulia (1)	Valenzano (1)	Urban (1)	Dead (1)	0	0
Tarentola mauritanica (67)	Apulia (25)	Adelfia (1)	Peri-urban (1)	Dead (1)	1	0
		Bari (6)	Peri-urban (6)	Alive (6)	5	5
		Brindisi (1)	Dog shelter (1)	Alive (1)	0	0
		Lecce (3)	Dog shelter (3)	Alive (3)	1	1
		Noicattaro (6)	Household (3)	Alive (3)	2	2
			Urban (2)	Alive (2)	2	0
			Peri-urban (1)	Dead (1)	0	0
		Tricase (1)	Dog shelter (1)	Alive (1)	1	1
		Valenzano (7)	Peri-urban (7)	Alive (7)	6	4
	Basilicata (1)	Pietrapertosa (1)	Rural (1)	Alive (1)	0	0
	Calabria (5)	Cassano All'ionio (5)	Dog shelter (5)	Alive (5)	0	0
	Sicily (36)	Linosa (22)	Urban (22)	Alive (22)	3	2
		Lipari (4)	Urban (4)	Alive (3)	2	2
				Dead (1)	0	0
		Malfa (8)	Peri-urban (6)	Alive (6)	3	2
			Urban (2)	Alive (2)	2	1
		Pollara (2)	Urban (2)	Alive (2)	0	0
Total 245					103	76

larval forms. Specifically, 13% (32/245, 95% CI 0.09–0.18) of the animals were infected with acanthocephalans, 12.6% (31/245, 95% CI 0.09–0.17) with cestodes, and 4% (10/245, 95% CI 0.02–0.07) with nematodes. In addition, 9.7% of the reptiles examined (i.e., 24/245, 95% CI 0.07–0.14) had at least one free helminth, and 6.1% (15/245, 95% CI 0.04–0.10) were parasitized by adult nematodes and 3.6% (9/245, 95% CI 0.02–0.07) by digeneans, which

were localized in the intestinal lumen and gall bladder, respectively. Overall, 31% of the reptiles (76/245, 95% CI 0.26–0.37) were positive for at least one helminth (Table 2) and 22.3% (17/76, 95% CI 0.14–0.33) of them were co-infected with two or three parasite groups, with Acanthocephala-Nematoda the most common association recorded (Table 3). *Podarcis siculus* (18.7%, 46/245, 95% CI 0.14–0.24) and *T. mauritanica* (8.1%, 20/245, 95% CI 0.05–0.12) were the species most frequently found to be positive for parasites (Table 2). All of the infected reptiles lived in sympatry with pets, of which 43.4% had been previously treated with repellent/insecticide and 7.9% also with anthelmintics.

A total of 12 parasite taxa were morphologically identified, including larvae of two acanthocephalans (*S. picae*, *Macracanthorhynchus hirudinaceus*) and four cestodes (*D. acanthotetra*, *J. pasqualei*, *J. echinorhyncoides*, *Mesocestoides lineatus*), third-stage larvae of two nematodes (family Acuariidae, *Physaloptera* sp.), adults of three nematodes (*Parapharygodon micipsae*, *Spauligodon aloisei*, *Moaciria icosiensis*), and an adult of one digenean (*Paradistomum mutabile*). The main morphological features allowing macroscopic identification, along with morphometric measurements and photos, are provided in additional files (Additional file 1: Text S1, Figure S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, S12, Table S1).

In the BLAST analysis, the *cox*1 sequences of *S. picae* (Cyclo cox1Fa/Cyclo cox1Rb) and *M. lineatus* (JB3/JB4.5) shared 99.7% and 98.9% nucleotide identity with GenBank sequences MK471355 and JF268501, respectively. The 18S nucleotide sequence of *Physaloptera* sp. (worm AF/1270R) was 99.4% similar to sequence MN855524. For the other parasite taxa investigated by molecular methods (i.e., *D. acanthotetra*, *J. echinorhyncoides*, *M. hirudinaceus*) no amplification was recorded, or the sequences were of low quality; the sequence of *J. pasqualei* was previously reported [54].

Of the helminth parasites of medical interest identified in the reptiles ($n=76$), occurrence was highest for *J. echinorhyncoides* (19.7%, 15/76, 95% CI 0.12–0.30), followed by *D. acanthotetra* (10.5%, 8/76, 95% CI 0.05–0.19), *J. pasqualei* and *M. lineatus* (5.6%, 4/76, 95% CI 0.02–0.13), and *Physaloptera* sp. (3.9%, 3/76, 95% CI 0.01–0.11). *Macracanthorhynchus hirudinaceus* was detected in one *P. siculus* specimen, which was also infected with *S. picae*. Information on the identified parasites, including taxa, helminth stage, anatomical site, host species and sampling locations, is given in Table 4. Among the infected *P. siculus* specimens ($n=46$), *S. picae* (43.4%, 20/46, 95% CI 0.30–0.58) and *J. echinorhyncoides* (23.9%, 11/46, 95% CI 0.14–0.38) were the most frequent helminths detected, followed by nematodes of the family Acuariidae (13%, 6/46, 95% CI 0.06–0.26). The most frequently recorded association was that of *S. picae* with nematodes of the family Acuariidae (Tables 2, 3, 4). Of the 20 *T. mauritanica* positive on necropsy, occurrence was highest for *S. picae* (40%, 8/20, 95% CI 0.22–0.61), followed by *D. acanthotetra* (25%, 5/20, 95% CI 0.11–0.47) (Tables 2, 3, 4).

The MCA analysis conducted using the 11 variables identified two dimensions which explained 89% of the

Table 3 Reptile species infected by more than one parasite group [Acanthocephala (*A*), Cestoda (*C*), Nematoda (*N*), Digenea (*D*)], and location from which each individual was collected

Species	City/town	Region	Sampling location	Parasite group
Tarentola mauritanica	Noicattaro	Apulia	Household	C+D
Podarcis siculus	Lecce	Apulia	Dog shelter	A+N
Podarcis siculus	Lecce	Apulia	Dog shelter	A+N
Podarcis siculus	Lecce	Apulia	Dog shelter	A+N
Tarentola mauritanica	Noicattaro	Apulia	Household	N+C
Podarcis siculus	Valenzano	Apulia	Peri-urban	A+N+D
Tarentola mauritanica	Valenzano	Apulia	Peri-urban	A+N+C
Podarcis siculus	Valenzano	Apulia	Peri-urban	A+C
Podarcis siculus	Torre a mare	Apulia	Peri-urban	A+C+D
Podarcis siculus	Brindisi	Apulia	Dog shelter	A+D
Podarcis siculus	Lecce	Apulia	Dog shelter	N+C
Podarcis siculus	Lecce	Apulia	Dog shelter	A+N
Chalcides ocellatus	Linosa	Sicily	Urban	A+N+D
Podarcis filfolensis	Linosa	Sicily	Urban	A+C
Podarcis siculus	Valenzano	Apulia	Peri-urban	C+D
Podarcis siculus	Cassano All'Ionio	Calabria	Peri-urban	A+C
Tarentola mauritanica	Valenzano	Apulia	Peri-urban	A+C

Table 4 Taxa and stage of identified parasitic helminths, anatomical site [liver (*L*), intestinal serosa (*IS*), mesentery (*M*), peritoneum (*P*), intestinal lumen (*IL*), stomach (*S*), gall bladder (*G*)], host species and sampling location

Helminth taxa	Stage	Anatomical site	Host (no.)	Region (no.)	City/town (no.)	Sampling location
Acanthocephala						
Sphaerirostris picae	Larval	L, IS, M, P	*Chalcides ocellatus* (1)	Sicily (1)	Linosa (1)	Urban
			Podarcis filfolensis (1)	Sicily (1)	Linosa (1)	Urban
			Podarcis siculus (22)	Calabria (1)	Cassano All'ionio (1)	Peri-urban
				Apulia (20)	Brindisi (5)	Dog shelter
					Lecce (8)	Dog shelter
					Noicattaro (2)	Household
					Torre a mare (1)	Peri-urban
					Valenzano (4)	Peri-urban
				Sicily (1)	Filicudi (1)	Urban
			Tarentola mauritanica (8)	Apulia (8)	Bari (3)	Peri-urban
					Lecce (1)	Dog shelter
					Tricase (1)	Dog shelter
					Valenzano (3)	Peri-urban
Macracanthorhynchus hirudinaceus	Larval	L	*Podarcis siculus* (1)	Apulia (1)	Valenzano (1)	Peri-urban
Nematoda						
Acuariidae gen. sp.	Larval	IS, P	*Chalcides ocellatus* (1)	Sicily (1)	Linosa (1)	Urban
			Podarcis siculus (5)	Apulia (5)	Lecce (4)	Dog shelter
					Brindisi (1)	Dog shelter
			Tarentola mauritanica (1)	Apulia (1)	Valenzano (1)	Peri-urban
Physaloptera sp.	Larval	IS, S	*Hierophis carbonarius* (1)	Sicily (1)	Salina (1)	Urban
			Podarcis siculus (2)	Apulia (1)	Valenzano (1)	Peri-urban
				Calabria (1)	Cassano All'ionio (1)	Peri-urban
Parapharygodon micipsae	Adult	IL	*Tarentola mauritanica* (5)	Sicily (4)	Lipari (3)	Urban
					Linosa (1)	Urban
				Apulia (1)	Noicattaro (1)	Household
Spauligodon aloisei	Adult	IL	*Chalcides ocellatus* (1)	Sicily (1)	Linosa (1)	Urban
			Podarcis filfolensis (2)	Sicily (2)	Linosa (1)	Urban
			Podarcis siculus (3)	Sicily (1)	Lipari (1)	Urban
				Basilicata (1)	Regional park (1)	Natural park
				Apulia (1)	Lecce (1)	Dog shelter
Moaciria icosiensis	Adult	IL	*Chalcides ocellatus* (4)	Sicily (4)	Linosa (4)	Urban
Cestoda						
Diplopylidium acanthotetra	Larval	L, IS	*Podarcis siculus* (3)	Apulia (2)	Noicattaro (2)	Household
				Calabria (1)	Cassano All'ionio (1)	Dog shelter
			Tarentola mauritanica (6)	Apulia (3)	Noicattaro (2)	Household
					Valenzano (1)	Peri-urban
				Sicily (3)	Malfa (3)	Urban
						Peri-urban
Joyeuxiella pasqualei	Larval	L	*Podarcis siculus* (3)	Apulia (2)	Lecce (2)	Dog shelter
				Basilicata (1)	Regional park (1)	Natural park
			Tarentola mauritanica (1)	Apulia (1)	Noicattaro (1)	Household
Joyeuxiella echinorhyncoides	Larval	L, IS	*Hierophis carbonarius* (1)	Apulia (1)	Torre a Mare (1)	Urban
			Podarcis siculus (11)	Apulia (10)	Lecce (6)	Dog shelter
					Torre a Mare (1)	Peri-urban
					Valenzano (3)	Peri-urban
				Sicily (1)	Lipari (1)	Urban
			Tarentola mauritanica (3)	Apulia (3)	Bari (1)	Peri-urban
					Valenzano (2)	Peri-urban

Table 4 (continued)

Helminth taxa	Stage	Anatomical site	Host (no.)	Region (no.)	City/town (no.)	Sampling location
M. lineatus	Larval	L	*Podarcis siculus* (4)	Basilicata (2)	Regional park (2)	Natural park
				Calabria (2)	Cassano All'ionio (2)	Dog shelter
Digenea						
P. mutabile	Adult	G	*Chalcides ocellatus* (1)	Sicily (1)	Linosa (1)	Urban
			Podarcis siculus (6)	Apulia (5)	Brindisi (1)	Dog shelter
					Valenzano (2)	Peri-urban
					Torre a Mare (2)	Peri-urban
				Sicily (1)	Lipari	Urban
			Tarentola mauritanica (2)	Apulia (2)	Noicattaro (1)	Household
					Bari (1)	Peri-urban

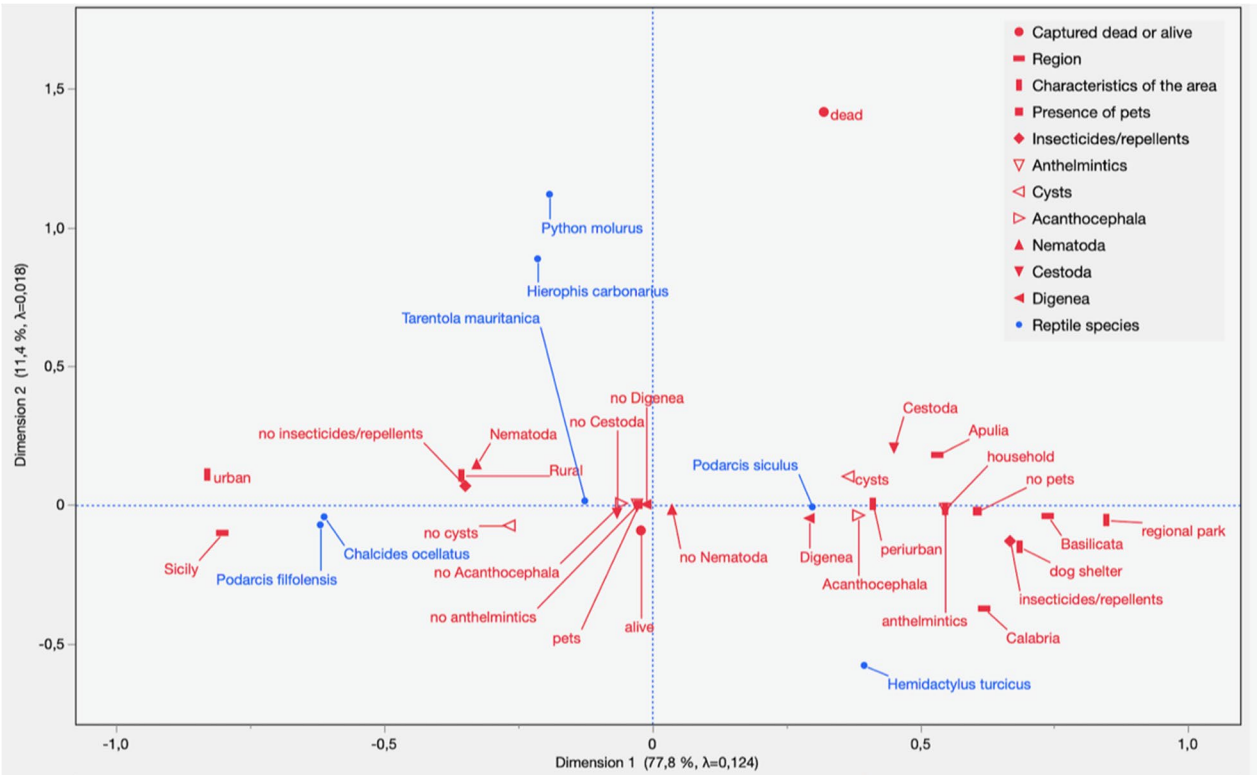

Fig. 3 Multiple correspondence analysis biplot for reptile species infected or not infected with helminth parasites in various ecological, epidemiological, and environmental settings

variability, with a Cronbach's α of 0.406 (Fig. 3). The latter is related to a weighted average of the correlations between the variables in the MCA, and is used to assess the overallreliability of the new measurement scale, created using the extracted dimensions, so as to ascertain if the obtained value that is retained is acceptable [58, 62]. Dimension 1 was associated with region (eigenvector > 0.6—Basilicata 0.738, Calabria 0.619, Sicily − 0.802), study location (dog shelter 0.686, regional park 0.847, urban − 0.830), pet presence (no pets 0.607), use of insecticides/repellents (yes 0.668), and explained 77.8% of the variability (Fig. 3) (Additional file 2: Table S2). Dimension 2 was associated with status when captured (dead 1.417) and explained 11.4% of the variability (Additional file 2: Table S2). Chi-square test results, inertia and the explained percentage variability for each singular value are given in Table 5. Figure 3 shows that *P. siculus* was associated with Apulia, the presence of cysts and Cestoda, while *T. mauritanica* was associated with urban environments, and to a

Table 5 Multiple correspondence analysis showing chi-square test results, inertia and percentage explained variability for each singular value

Singular value	Inertia	χ^2	% Explained variability	Cumulative %
0.35276	0.12444	335.37	77.79	77.79
0.13503	0.01823	49.14	11.40	89.18
0.10108	0.01022	27.53	6.39	95.57
0.06651	0.00442	11.92	2.77	98.34
0.04745	0.00225	6.07	1.41	99.74
0.02027	0.00041	1.11	0.26	100.00

minor extent, the presence of Nematoda and pets not treated with insecticides or repellents.

Discussion

The wide range of helminths detected in the present study in reptiles living in sympatry with pets and the fact that many of these are parasitic and can infect companion animals (e.g., *D. acanthotetra*, *J. pasqualei*, *J. echinorhyncoides, Physaloptera* sp.) and humans (i.e., *Macracanthorhynchus hirudinaceus*, *Mesocestoides lineatus*) indicate the potential importance of reptiles as intermediate/paratenic hosts of parasitic helminths. Indeed, published data indicate that predation by pets could represent a transmission route for endoparasites [9].

The percentage of reptiles found to be infected by helminths (28.9%) is lower than that previously reported (i.e., 67–98%) [26, 28, 63], probably because the majority of those studies were focused on a single host species [24, 26, 28] or used different diagnostic methods (i.e., coprological techniques) [6, 64]. In addition, the number of individuals of some reptile species (i.e., *Hemidactylus turcicus*, *Hierophis carbonarius*, *Python molurus*) analyzed here was low, which likely led to underestimation of their helminth fauna. The high infection rates recorded here for *P. siculus* and *T. mauritanica* may be related to the fact that these two species were the most abundant, and especially so in dog shelters and urban and periurban areas. The detection of *M. hirudinaceus* in *P. siculus* is unprecedented and may be explained by the high prevalence of this zoonotic helminth in wild boar and intermediate hosts from the same area [65, 66]. Although little is known about the role of reptiles as paratenic hosts of *M. hirudinaceus* [67], they have been implicated in the life cycle of other zoonotic species of *Macracanthorhynchus* (i.e., *Macracanthorhynchus ingens* and *Macracanthorhynchus cutulinus*) [4, 68, 69]. To date, human cases of accidental infection through the ingestion of intermediate/paratenic hosts parasitized by *M. hirudinaceus* have been described mostly from other countries (i.e., Morocco, Argentina, Iran, and Tunisia) [66].

The detection of *J. echinorhyncoides*, *J. pasqualei* and *D. acanthotetra* in reptiles living in sympatry with companion animals highlights a potential link between pets and infection with these parasites through ingestion of these small vertebrates. Indeed, these cestodes have been reported from domestic [54, 70, 71] and feral cats [72, 73] and, to a lesser extent, from dogs [54, 74]. *M. lineatus* is known to circulate in wild and domestic carnivores in Italy [75–77]. Its detection in the present study from dog shelters shows the importance of treating pets with anthelmintics. Indeed, *Mesocestoides* sp. infections in dogs can become severe, e.g. with the development of peritoneal larval cestodiasis, where larvae penetrate the host's intestinal wall and cause potentially life-threatening peritonitis [78]. Although no human cases of infection with *Mesocestoides* spp. have been recorded in Europe, these species may be of zoonotic relevance due to the incidental ingestion of their intermediate hosts [9, 79]. Among the nematodes found here, the only species of veterinary relevance was *Physaloptera* sp., a well-known agent causing vomiting and weight loss in cats and dogs [80–82]. Although its occurrence here is lower than that previously reported [25, 45], its detection confirms the circulation in southern Italy [83, 84], which should not be overlooked since *Physaloptera* spp. can cause severe clinical signs in cats, including gastric erosions and marked catarrhal gastritis [85, 86]. A massive infection by *Physaloptera* sp. was described in a young cat from a shelter in southern Louisiana [82], which indicates the importance of parasite control strategies for cats kept in these facilities.

Overall, the results discussed above suggest cats as more susceptible than dogs to helminth infections through the ingestion of reptiles, as they are more likely to show predatory behavior and can adapt to many types of environments [87]. In an Italian study, 21% of the prey of cats, which are considered excellent hunters, were reptiles [87, 88], and the preying of cats on these animals may support the trophic transmission of parasitic diseases [9]. The association between the lizards *P. siculus* and cestodes, along with *T. mauritanica* geckos and untreated pets, highlights the possible role of reptiles as sources of helminth infections and potentially zoonotic parasites.

Conclusions

Synanthropic geckos and lizards represent an interface between wildlife and domestic settings. Encounters between these small vertebrates, companion animals and humans may lead to health issues, such as

the transmission of parasites through predation of these small animals by dogs and cats. The results of the present study highlight the presence of helminth parasites in squamate reptiles that could be transmitted to companion animals (e.g., *J. pasqualei*, *J. echinorhyncoides, D. acanthotetra*; *Physaloptera* sp.) and humans (i.e., *Macracanthorhynchus hirudinaceus*, *Mesocestoides lineatus*) when they live in sympatry. In this context, reptiles may play a role in the maintenance of parasitic diseases of pets, which reinforce the importance of regular anthelmintic treatment of companion animals. Finally, whenever a gecko, a lizard or a snake is captured from the wild and brought into a domestic setting, it would be good practice to screen it for the presence of parasites to reduce the risk of pathogen introduction.

Abbreviations

DNA	Deoxyribonucleic acid
BLAST	Basic Local Alignment Search Tool
CI	Confidence interval
MCA	Multiple correspondence analysis
PCR	Polymerase chain reaction
µm	Micrometer
cm	Centimeter

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13071-023-05852-8.

Additional file 1: Text S1. Main morphological features used for the macroscopic diagnosis of each parasite taxon. **Figure S1** Larval stage of *Sphaerirostris picae*. **Figure S2** Larval stage of *Macracanthorhynchus hirudinaceus*. **Figure S3** Larval stage of *Diplopylidium acanthotetra*. **Figure S4** Larval stage of *Joyeuxiella echinorhyncoides*. **Figure S5** Larval stage of *Joyeuxiella pasqualei*. **Figure S6** Larval stage of *Mesocestoides lineatus*. **Figure S7** An adult of *Paradistomum mutabile*. **Figure S8** Larval stage of the nematode family Acuariidae. **Figure. S9** Larval stage of *Physaloptera* sp. **Figure S10** An adult of *Parapharygodon micipsae*. **Figure S11** An adult of *Moaciria icosiensis*. **Figure S12** An adult of *Spauligodon aloisei*. **Table S1** Morphometric measurements [length (*L*), width (*W*)] of helminths collected from reptiles (all measurements are given in micrometers)

Additional file 2: Table S2 Eigenvectors of variables associated with dimensions 1 and 2 of the multiple correspondence analysis

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Author contributions
MC: Conceptualization, data curation, formal analysis, investigation, methodology, writing—original draft, writing—review and editing. JAM-R: Conceptualization, data curation, formal analysis, investigation, methodology, supervision, writing—review and editing. RPL: Conceptualization, data curation, formal analysis, investigation, methodology, supervision. GA: Data curation, formal analysis, methodology. RI: Methodology, writing—review and editing. AV: Methodology, writing—review and editing. GC: Data curation, formal analysis, methodology, writing—review and editing. GB: Data curation, formal analysis, methodology, writing—review and editing. DO: Data curation, formal analysis, investigation, supervision, writing—review and editing. All authors contributed to the study and approved the final version of the manuscript.

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Availability of data and materials
The data that support the findings of this study are available from the corresponding author upon reasonable request. All sequences generated in this study have been deposited in the GenBank database (*S. picae* OQ451868, *M. lineatus* OQ451939, *Physaloptera* sp. OQ466450).

Declarations

Ethics approval and consent to participate
Protocols for collection of reptiles were authorized by the Ministry for Environment, Land and Sea Protection of Italy (approval no. 0073267/2019).

Consent for publication
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Competing interests
The authors declare that they have no competing interests.

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