



Identification and phylogenetic analysis of *Taenia* spp. parasites found in wildlife in the Emilia-Romagna region, northern Italy (2017–2022)

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ABSTRACT

The recent expansion of the habitat of several wildlife species, comprising anthropized areas, is a relevant risk factor for many zoonotic diseases and should be considered in national and regional sanitary monitoring systems. We evaluated adult intestinal *Taenia* spp. parasites isolated from wild carnivores and cystic larval forms isolated from wild mammals analysed at the Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia-Romagna (IZSLER) as part of the regional wildlife sanitary surveillance plan. Then, we assessed parasite species through molecular analysis (multiplex PCR followed by ribosomal 12S subunit gene sequencing) in order to update the epidemiological situation on Taeniids infection in the Emilia-Romagna wildlife, reporting the prevalence of each isolated species. The most commonly isolated species was *Taenia serialis*, which we detected in both wolves and foxes as definitive hosts and in roe deer as intermediate host. More attention on the distribution of Taeniids in wildlife should be paid, considering their potential zoonotic role: several *Taenia* spp. (*Taenia solium*, *Taenia multiceps*, *Taenia serialis*, *Taenia brauni*, *Taenia glomerulatus*) are known for causing coenurosis in humans, with possible severe or fatal outcomes.

1. Introduction

Taeniidae are a family of cestodes whose life cycle requires two hosts: the adult form of the parasite resides in the intestinal tract of a definitive host (which can be either a strict mammalian carnivore or a human), while larvae are fluid-filled cysts which develop in the peritoneal cavity, visceral organs, skeletal muscle or central nervous system of an intermediate host (Taylor et al., 2015). Infection is trophic-related in both sylvatic and domestic cycles: in the first case, transmission occurs from preys to predators and vice versa, while in the second case humans and domestic animals are involved (Fig. 1 is a schematic representation of *Taenia* spp. life cycle). For the species that involve humans as definitive hosts (*Taenia solium*, *Taenia saginata* and *Taenia asiatica*) the intermediate hosts are specific: for *T. solium* and *T. asiatica* it is the domestic pig (Dixon et al., 2021; Galán-Puchades and Fuentes, 2013), while for *T. saginata* is cattle (Braae et al., 2018). The definitive host species in European wildlife include the red fox (*Vulpes vulpes*), the wolf (*Canis lupus*), the golden jackal (*Canis aureus*), the European lynx (*Lynx lynx*), the raccoon (*Procyon lotor*), the beech marten (*Martes foina*) and

the bear (*Ursus arctos*). A wider range of mammal species, including ruminants (Filip et al., 2019), wild boars (Sgroi et al., 2020), rodents (Bajer et al., 2020) and lagomorphs (Remesar et al., 2021) can act as intermediate hosts.

Many of the potential definitive hosts of *Taenia* spp. have been increasing in number in the last decades. In particular, there has been a relevant increase in wolf populations, with a direct impact on parasitic richness and diversity, as demonstrated by Lesniak et al. (2017): this could lead to an increase, at the same time, of the prevalence and abundance of different parasitic species, including Taeniidae. A similar upward trend for wolves happened in Italy, after a period of scarce number of packs in the country, as observed in the last census carried out by the Italian Institute for Protection and Research on Environment -ISPRA- (La Morgia et al., 2022); according to the last report available, Italian wolves are estimated to be between 2945 and 3608 (95% confidence interval), occupying 41600 km² in the Alps and 108534 km² in the Apennines. Another noteworthy element is the ability to occupy anthropized areas, as recently described by Macchioni et al. (2021) and Coppola et al. (2022).

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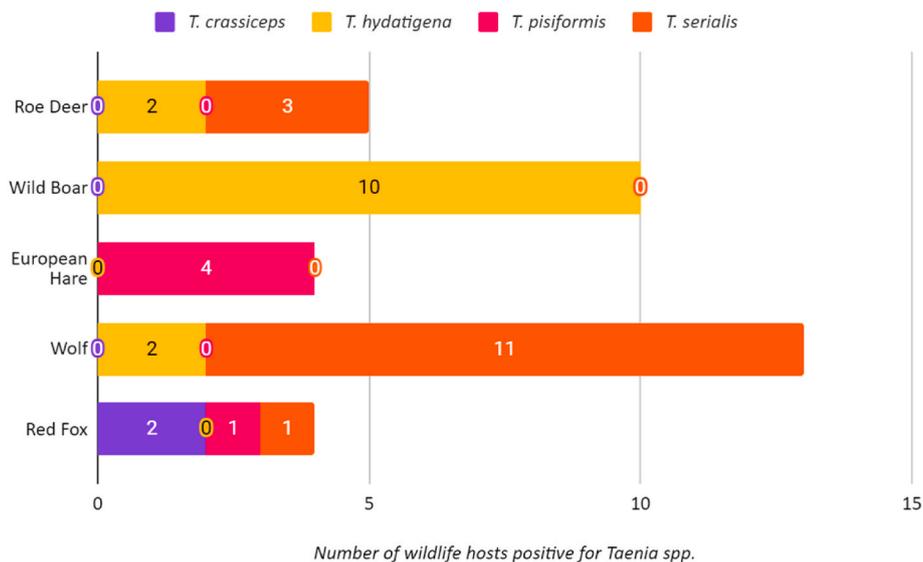


Fig. 2. – Distribution of different *Taenia* species in each host species considered.

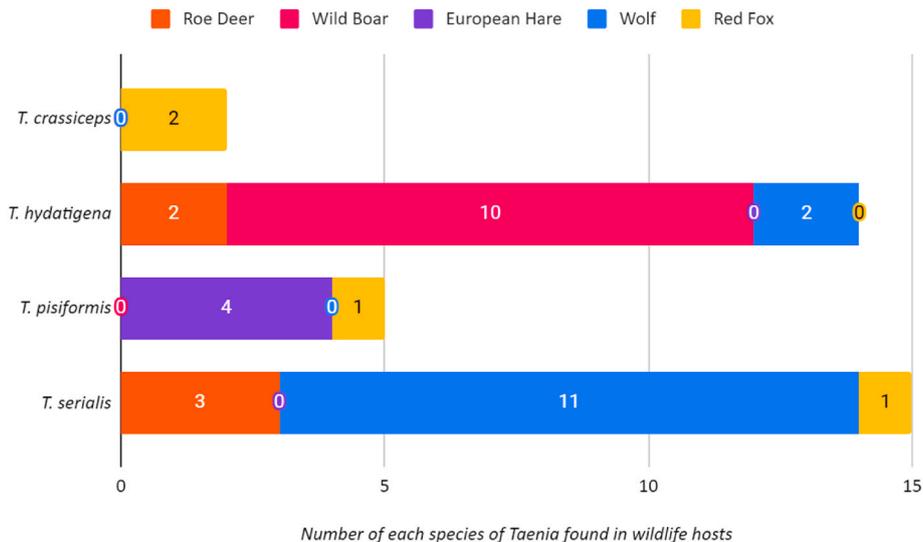


Fig. 3. Distribution of wildlife species in which each *Taenia* spp. were detected.

2. Materials and methods

2.1. Anatomopathological evaluation and sample collection

This study has considered the data obtained from 2738 carcasses of wild animals found in the Emilia-Romagna region in the period from January 2017 to December 2022.

Wild mammals analysed in this study were collected as part of the regional surveillance plan in force since 2017 (Deliberation of Emilia-Romagna Regional Council n. 1763, November 29, 2017). This plan comprises a targeted search for various infectious agents in wild animals, such as parasites (*Trichinella* spp., *Leishmania infantum*, *Toxoplasma gondii*), bacteria (*Brucella* spp., *Mycobacterium* spp., *Francisella tularensis*) and viruses (Aujeszky Disease Virus, West Nile and Usutu Virus, Influenza Virus, Newcastle Disease Virus, Classical and African Swine Fever, Blue Tongue Virus, Rabies lyssavirus), with analyses carried out on animals found dead in the natural environment, deceased at wildlife rescue centres, or culled according to control plans of certain species. The examinations include anatomopathological evaluation and laboratory analysis.

Carcasses or samples were accompanied by a form reporting relevant information such as geolocalization data, sex and age of animals. Forms are reported in the supplementary materials section (Figs. 1–3, in Italian).

Necropsies were performed by veterinarians working at the IZSLER (Istituto Zooprofilattico Sperimentale della Lombardia e dell’Emilia-Romagna) following a common protocol, derived from the method proposed by King et al. (2014). When cestodes were found at necropsy, the adult stages from definitive hosts or larval forms from intermediate hosts were carefully collected with forceps, paying attention not to disrupt them, and then transferred in plastic containers stored at –20 °C for PCR identification.

2.2. Molecular analysis

For PCR analysis, 59 samples were diluted in sterile PBS at a 10% weight/volume ratio and then homogenised. DNA was extracted from samples (either adult cestodes or cystic forms) through a commercial kit (DNeasy Blood & Tissue Kit, QIAGEN) and amplified through a multiplex polymerase chain reaction, following the protocol described by

Table 1
Detailed description of primers used for multiple PCR.

Primer	Target species	Sequence (5' → 3')	Forward (F)/ Reverse (R)	Size (bp)	Reference
Cest1	<i>E. multilocularis</i>	TGC TGA TTT GTT AAA GTT AGT GAT	F	25	Trachsel et al. (2007)
Cest2	<i>E. multilocularis</i>	CAT AAA TCA ATG GAA ACA ACA ACA AG	R	26	Trachsel et al. (2007)
Cest3	<i>Taenia</i> spp.	YGA YTC TTT TTA GGG GAA GGT GTG	F	24	Trachsel et al. (2007)
Cest4	<i>E. granulosus</i>	GTT TTT GTG TGT TAC ATT AAT AAG GGT G	F	28	Trachsel et al. (2007)
Cest5	<i>Taenia</i> spp.	GCG GTG TGT ACM TGA GCT AAA C	R	22	Trachsel et al. (2007)

Table 2
- GenBank sequences used for molecular analysis.

Species	GenBank submission code	Host	Geographical origin of samples
<i>Taenia serialis</i>	MF495483	Golden Jackal (<i>Canis aureus</i>)	Croatia
	MF495484	Golden Jackal (<i>Canis aureus</i>)	Croatia
	MF495485	Golden Jackal (<i>Canis aureus</i>)	Croatia
	DQ104238	Coyote (<i>Canis latrans</i>)	USA (California)
	EU219546	Dog (<i>Canis lupus familiaris</i>)	Germany
<i>Taenia pisiformis</i>	KP965913	Arctic fox (<i>Vulpes lagopus</i>)	Greenland
	AB031353	n/d	Japan
	AB329716	Japanese hare (<i>Lepus brachyurus angustidens</i>)	Japan
	AB704403	Dog (<i>Canis lupus familiaris</i>)	Japan
	DQ104227	European rabbit (<i>Oryctolagus cuniculus</i>)	USA (California)
	DQ104229	Coyote (<i>Canis latrans</i>)	USA (California)
	DQ104233	Coyote (<i>Canis latrans</i>)	USA (California)
	GU569096	Dog (<i>Canis lupus familiaris</i>)	China
	NC_013844	Dog (<i>Canis lupus familiaris</i>)	China
	<i>Taenia crassiceps</i>	AB031358	n/d
AF216699		n/d	USA
KP965908		Arctic fox (<i>Vulpes lagopus</i>)	Greenland
KP965910		Arctic fox (<i>Vulpes lagopus</i>)	Greenland
<i>Taenia hydatigena</i>	KP965911	Arctic fox (<i>Vulpes lagopus</i>)	Greenland
	MN505206	Red Fox (<i>Vulpes Vulpes</i>)	Poland
	NC_002547	n/d	USA
	KX094340	Sheep (<i>Ovis aries</i>)	Iran
	LC672187	Goat (<i>Capra aegagrus hircus</i>)	Bangladesh
	LC672189	Goat (<i>Capra aegagrus hircus</i>)	Bangladesh
	LC749827	Sheep (<i>Ovis aries</i>)	Iraq
	MK858250	Sheep (<i>Ovis aries</i>)	Iraq
	MT784876	Sheep (<i>Ovis aries</i>)	China

Trachsel et al. (2007): for *E. multilocularis* identification, primers Cest 1 and Cest 2 were used to amplify a 395 bp sequence of the mitochondrial gene Nad1 (NADH dehydrogenase subunit 1); for *E. granulosus* and *Taenia* spp., a common reverse primer (Cest 5) and two specific reverse

Table 3

Positivity for *Taenia* spp. in different species. Animals tested for *Taenia* spp. by PCR were the animals in which either adult cestodes or cystic larval forms were found at necropsy.

Species tested	Number of carcasses analysed	Animals tested for <i>Taenia</i> spp. by PCR	Animals positive for <i>Taenia</i> spp. (PCR)	% of animals positive for <i>Taenia</i> spp.
Roe Deer (<i>Capreolus capreolus</i>)	1227	8	5	0.41% (CI 95%: 0.05–0.76%)
Red Fox (<i>Vulpes vulpes</i>)	575	5	4	0.70% (CI 95%: 0.02–1.38%)
Brown hare (<i>Lepus europaeus</i>)	446	7	4	0.90% (CI 95%: 0.02–1.77%)
Wild boar (<i>Sus scrofa</i>)	238	11	10	4.20% (CI 95%: 1.65–6.75%)
European hedgehog (<i>Erinaceus europaeus</i>)	153	1	0	0%
Wolf (<i>Canis lupus lupus</i>)	90	23	13	14.44% (CI 95%: 7.18–21.71%)
Beech marten (<i>Martes foina</i>)	6	1	0	0%
Golden jackal (<i>Canis aureus</i>)	2	2	0	0%
European dormouse (<i>Glis glis</i>)	1	1	0	0%
TOTAL	2738	59	36	1.28% (CI 95%: 0.89–1.74%)

primers (Cest 3 and Cest 4, for *E. granulosus* and *Taenia* sp., respectively) were used to amplify a 267 bp sequence of 12S ribosomal RNA gene (rrnS) in *Taenia* sp. and a 117 bp sequence in *E. granulosus*.

For 100 µL of primer mix, 20 µL of Cest5 10 µL of Cest4, 10 µL of Cest3, 10 µL of Cest1 and 10 µL of Cest2 were added to 40 µL of DNase-free water. A detail on each primer sequence can be found in Table 1.

For each primer, a working concentration of 100 pmol/µl was obtained, while the concentration of the DNA templates was 2.5 mM for each dNTP.

The thermal profile of PCR is the following:

- 1 cycle of denaturation (5 min at 95 °C)
- 40 cycles of denaturation (30 s at 95 °C) + annealing (30 s at 52 °C) + extension (45 s at 72 °C)
- 1 cycle of final extension (8 min at 72 °C).

Samples testing positive for *Taenia* spp. were then further analysed to identify the species, by automatic Sanger sequencing of the amplified fragments of PCR through the Applied Biosystems platform (Genetic Analyzer 3500xl, Life Technologies). For sequencing, the Big dye terminator ready reaction v1.1 kit (Life Technologies) was used, while purification of sequencing products was obtained through the BigDye Xterminator Purification kit (Life Technologies).

The software BEAST2.6 (Bouckaert et al., 2019), which perform Bayesian phylogenetic analysis of molecular sequences, was used to infer the phylogenetic tree. The HKJ + G substitution model was chosen according to the model selection function of MEGAX software (Tamura et al., 2021): the number of iterations was selected to ensure an ESS Effective Sampling Size >200, as observed in Tracer. With the coalescent constant population as prior, the chain length was established at 10 million of iterations. The phylogenetic tree was then created using FigTree software v. 1.4.4 (available at <http://tree.bio.ed.ac.uk/software>

Distribution of different *Taenia* spp. parasites found in wildlife in the Emilia-Romagna region (2017-2022)

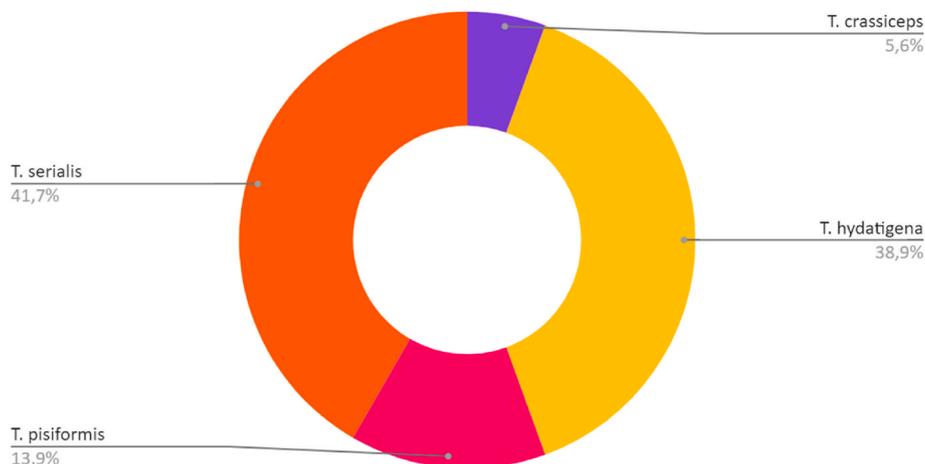


Fig. 4. Percentage of isolation of different *Taenia* species.

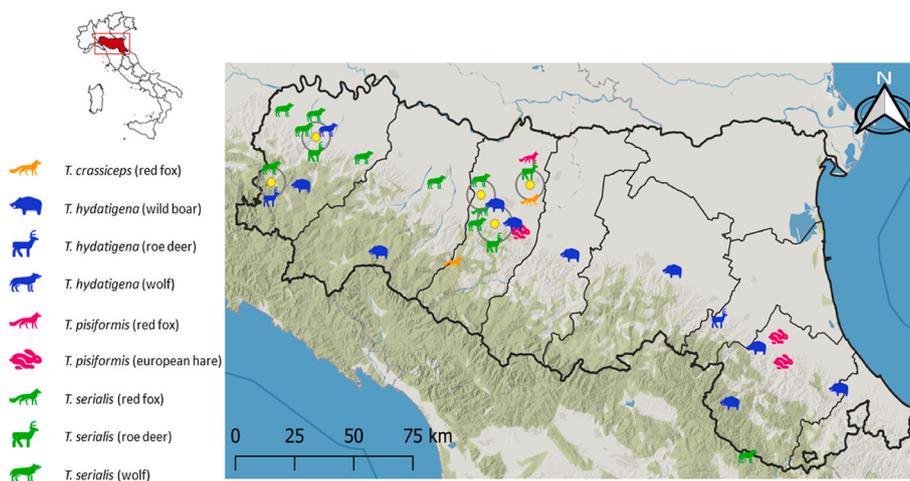


Fig. 5. Geographical location of animals resulted positive for *Taenia* spp. Icons placed around circles are located in the same position, represented by the yellow point. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

/figtree/). Our sequences were compared to other publicly available on GenBank, the details of which are reported in Table 2.

2.3. Map creation

We obtained the coordinates of the centroids of each municipality in which wild animals were found from the Geonames database. Such coordinates were then inserted in QGIS software v. 3.30.1: the basemap was obtained from ©MapTiler service.

3. Results

3.1. Prevalence of *Taenia* spp. findings

Among all the carcasses of wildlife animals we evaluated in the period of time considered, we found tapeworms macroscopically compatible with Taeniids in 31 carnivores, and 16 of them (12 wolves and four red foxes) were confirmed as *Taenia* spp. by PCR. 3 distinct adult cestodes were extracted from 1 wolf, so we analysed a total of 33 adult Taeniids from 31 animals.

Conversely, cystic formations macroscopically compatible with Taeniids larvae were found in 26 mammals, and PCR revealed positivity

for *Taenia* spp. in 19 samples (10 wild boars, five roe deer, four hares).

Taenia spp. was confirmed in 35 animals (1.28% of animals examined at the Institute under the wildlife sanitary surveillance plan, as detailed in Table 3).

According to our data, the most frequently isolated species in the Emilia-Romagna region in the period considered was *T. serialis*, found in 11 wolves, one fox and three roe deer, followed by *T. hydatigena*, which was identified in two wolves, 10 wild boars and two roe deer. Other less frequently isolated species were *T. pisiformis* (isolated from four hares and one fox) and *T. crassiceps* (isolated from two foxes). In this calculation is included one wolf, which resulted infested by both *T. serialis* and *T. hydatigena*.

The percentage of isolation of each *Taenia* species is represented in the graph reported in Fig. 4.

The locations of the carcasses resulted positive for *Taenia* spp. are reported in the map in Fig. 5.

3.2. Phylogenetic analysis

According to the results of phylogenetic analysis, different *Taenia* species form homogeneous clades, with well-supported nodes as can be seen in the phylogenetic tree in Fig. 6. In particular, the sequences

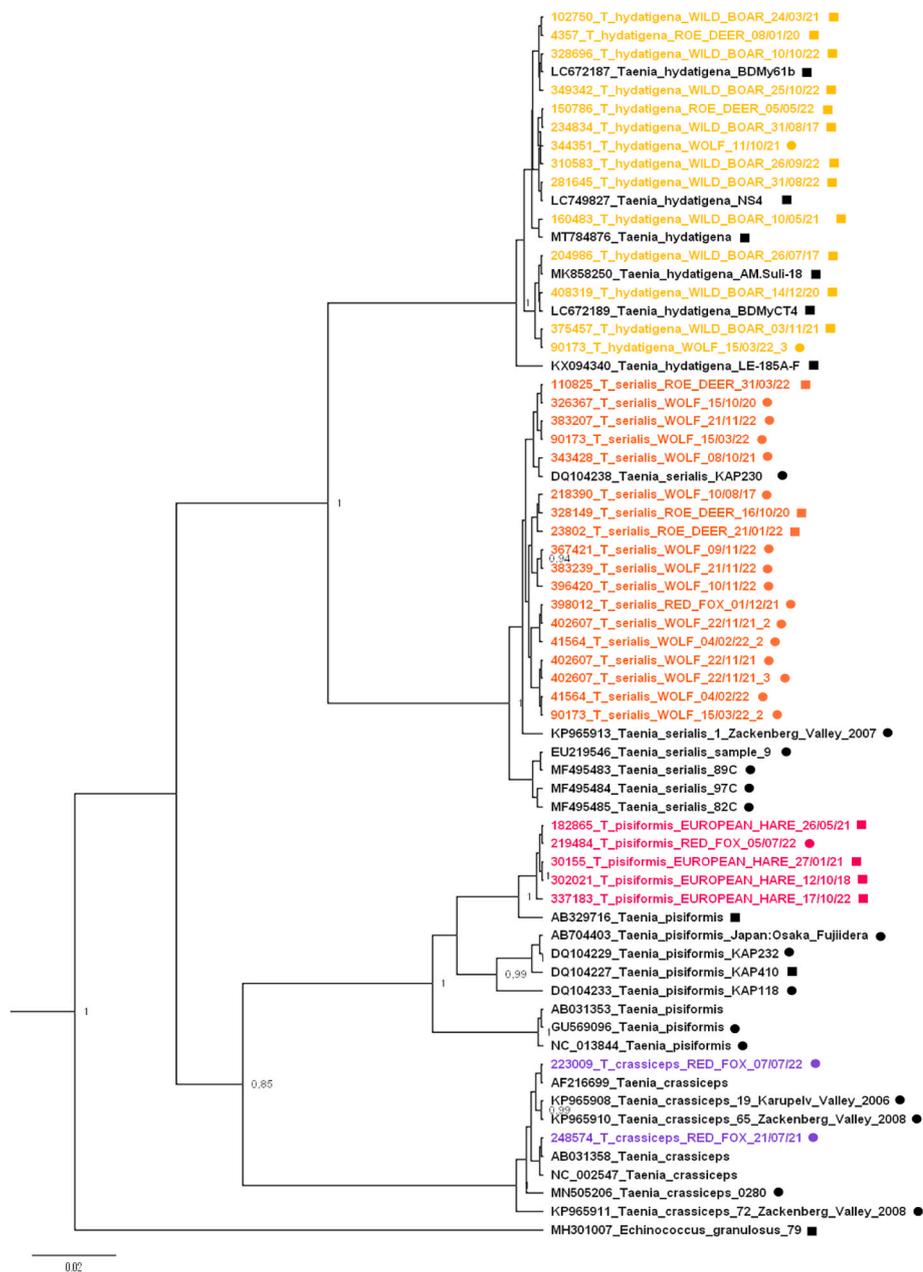


Fig. 6. Phylogenetic tree with the sequences of small ribosomal RNA subunit (*rrnS*) of *T. hydatigena* (yellow), *T. serialis* (orange), *T. pisiformis* (magenta), *T. crassiceps* (purple) sequenced at the IZSLER, compared with public sequences deposited in GenBank (black). The tree was obtained using the HKJ + G substitution model. Chain length = 10 million iterations. Node labels are posterior probabilities ≥ 0.9 . Squares = sequence from intermediate host; circles = sequence from definitive host. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

highlighted with different colours are the ones we analysed at the IZSLER (one colour per species), while the sequences highlighted in black text are the publicly deposited sequences we extracted from GenBank. A detailed list of the GenBank accession codes for the sequences analysed at IZSLER is reported in Tables 1–4 in the supplementary material section.

4. Discussion

According to our results, different species of cestodes belonging to the genus *Taenia* circulate in the wildlife of northern Italy. In particular, our data suggest that the main cycle of *T. serialis* (the most frequently isolated species) in the Emilia-Romagna region mainly involves wolves as definitive hosts and roe deer as intermediate hosts. Such a sylvatic cycle has already been hypothesized in the article presented by Morandi et al. (2021), who studied a case of neurocenurosis in a roe deer from central Italy, in an area bordering the Emilia-Romagna region, with similar geographical characteristics. The trophic transmission from roe

deer to wolves is supported by studies that question the common feeding behaviour of wolves in Europe. In particular, Sin et al. (2019) and Guimarães et al. (2022) agreed that wild ungulates are the main components of their diet. In addition to wolves, we also identified such parasite species in a red fox, which can act as a definitive host as previously reported in northeastern Italy by Citterio et al. (2021).

To our knowledge, the only other reported case of *T. serialis* infection in Italian wolves was reported by Crotti et al. (2023), while the prevalence of the same parasite in foxes was estimated to be relatively low (0.03% according to the study published by Citterio et al. in 2021). In Europe, *T. serialis* has been reported in wolves from Portugal (Pereira et al., 2023) with a prevalence of 5.9% and from Serbia (Ćirović et al., 2015) with a prevalence of 1.0%. Our data on the prevalence of *T. serialis* ($12.23\% \pm 0.07$ 95% CI), although lower than the prevalence reported by Crotti et al. (2023), 21.6%, is still the most common *Taenia* spp. isolated from wolves in our study. These data differ from previous literature on *T. serialis* in wildlife, as the most commonly reported intermediate hosts were lagomorphs, such as hares and rabbits

(Pffaffenberger and Valencia, 1988; Zhang et al., 2018).

We identified adult forms of *T. hydatigena* mainly in wolves and larval stages of the same parasite species in the wild boar, a common prey of wolf packs in Italy. This parasite species has already been reported in wolves from Italy (Gori et al., 2015; Poglajen et al., 2017) and in wild boar from Estonia (Järvis et al., 2007), Slovakia (Jarošová et al., 2022), Poland (Filip et al., 2019), Germany (Barutzki et al., 1991) and Spain (De La Muela et al., 2001). *T. hydatigena* has been frequently reported in Italian wild boars from central Italy by Di Nicola et al. (2015) and by Paoletti et al. (2019), and in southern Italy by Sgroi et al. (2020). According to our data, *T. hydatigena* was detected in 4.2% of the analysed boars, and the prevalence is within the range reported in the Italian literature (3.4%–15%).

T. hydatigena was found in almost all provinces of Emilia-Romagna, probably due to the wide spatial distribution of wild boar populations in the region. Conversely, *T. serialis* was mostly restricted to the contiguous provinces of Piacenza, Parma and Reggio Emilia, as in the hilly and mountainous areas of these provinces more wolves are reported (La Morgia et al., 2022).

Another wild carnivore, the red fox, is the main definitive host for *T. pisiformis*, another parasite species we have detected. In Europe, this species has already been reported in Slovenia (Vergles Rataj et al., 2013), Germany (Loos-Frank and Zeyhle, 1982), France (Petavy and Debloek, 1980) and Spain (Sanchis-Monsonís et al., 2020). We also identified larval stages of the same parasite species in a common prey of red foxes, the European hare, as investigated in the review conducted by Soe et al. (2017). In particular, predation on lagomorphs is more common in areas with a higher human footprint index.

One of the main limitations of our study is that the finding of adult cestodes in the intestine of definitive hosts, as well as larval stages in the muscle or visceral organs of intermediate hosts, was only incidental. This is because the regional wildlife surveillance plan does not require the opening of the intestines of carnivores, and low levels of infection in intermediate hosts may be challenging to detect, particularly with muscular localisation of larval stages. Since we only sampled cestodes from obvious cases of infection, the calculated prevalence of *Taenia* spp. is likely to be underestimated. We have, however, proved that different *Taenia* spp. are circulating in wildlife from the geographical area we investigated, even though additional research is needed to evaluate the exact prevalence of each species.

Another constraint of the study is dependent on molecular techniques: we adopted the PCR protocol that is normally used in diagnostics to determine the presence of *Taenia* spp. in samples, but such analysis can have limited efficiency if multiple targets are amplified. Furthermore, despite the sequences we used for species recognition are highly conserved, they are short in size and provide less information compared to whole genome sequencing.

5. Conclusion

Implementing a parasitological examination of the animals in the plan for sanitary assessment of wildlife species is crucial to gather reliable epidemiological data on the prevalence of parasitic infections in a geographical area. At present, wildlife surveillance in Italy is carried out and organized at regional level, so that the prioritized diseases searched in animals are not the same across different regions. Identification of *Taenia* spp. is not a requested analysis, so it represents an accidental finding most of the times.

We demonstrated that the life cycle of *T. serialis* can be maintained in this area of Italy, as the parasite has been found in both intermediate and definitive hosts. Wolves are capable of preying on roe deer, so transmission through a prey-predator cycle is likely. The recent increase in density of both species (La Morgia et al., 2022) sets a favourable condition for *T. serialis* and Taeniids diffusion (Lesniak et al., 2017). Moreover, the impact of urbanization on increasing the probability of spillover from sylvatic to domestic cycles of many pathogens should be

considered (Hassell et al., 2017).

This finding is particularly important for parasitic species, such as *T. serialis* itself, which could be transmitted to humans by ingestion of material contaminated by faeces of definitive hosts. The recent occupation by different wildlife carnivores of anthropized areas is a relevant risk factor for transmission to humans. Another factor to consider is the possibility of infection by domestic dogs, which living in strict contact with humans noticeably increase the risk of transmission (Mutwiri et al., 2023). The zoonotic potential of *T. serialis* has been only recently demonstrated (Yamazawa et al., 2020), but previous clinical reports in non-human primates had already been published in past years (Schneider-Crease et al., 2017; Deplazes et al., 2019). Therefore, a systematic and more detailed search for parasites of wildlife with relevance for public health is required and desirable in the future planning for sanitary monitoring of wildlife in Italy.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijppaw.2023.08.001>.

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