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Expanding Host Range: First Record of *Eustrongylides excisus* in *Padogobius bonelli* (Gobiidae) from the Po River (Northwest Italy)

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Abstract: Fish-borne parasites encompass diverse taxonomic groups, including nematodes of the genus *Eustrongylides* (family Dioctophymatidae), which infect fish, fish-eating birds, and, occasionally, humans. *Eustrongylides* spp. are globally distributed, with *E. ignotus* and *E. tubifex* prevalent in North America, while *E. excisus* is more frequent in Europe and the Middle East. This study investigates the presence of *E. excisus* in a fish community of the Po River (Turin, northwest Italy). Among 170 fish sampled from three sites along the Po River, only two *Padogobius bonelli* individuals from the Murazzi site were found to be infected, each hosting a single larva within the abdominal cavity. Molecular analysis (ITS rDNA sequencing) confirmed the parasite as *E. excisus*, showing 100% identity with known sequences. Although prevalence in this study was low (1.18%), the findings significantly expand the known host range of *E. excisus*, previously reported only in other gobiid species in Eastern Europe. The parasite is widely distributed in Italy, especially in commercial fish from large lakes, and its spread is facilitated by oligochaete intermediate hosts and piscivorous birds like cormorants. Given the benthic habits and diet of *P. bonelli*, increased monitoring is needed to better understand the parasite's transmission dynamics and the potential risks to native biodiversity and human health.

Keywords: fish-borne parasites; freshwater fish; nematodes; zoonosis

Key Contribution: This study reports the first detection of *Eustrongylides excisus* in *Padogobius bonelli* from the Po River, expanding the known host range of this zoonotic parasite. Molecular confirmation and ecological context highlight the need for enhanced monitoring to assess transmission dynamics and potential risks to biodiversity and public health.

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Academic Editor: Pierre Sasal

Received: 5 May 2025
Revised: 23 May 2025
Accepted: 24 May 2025
Published: 29 May 2025

Citation: Maganza, A.; Mossotto, C.; Gabetti, A.; Gozlan, R.E.; Combe, M.; Esposito, G.; Bondavalli, F.; Bertoli, M.; Pizzul, E.; Pastorino, P.; et al. Expanding Host Range: First Record of *Eustrongylides excisus* in *Padogobius bonelli* (Gobiidae) from the Po River (Northwest Italy). *Fishes* **2025**, *10*, 254. https://doi.org/10.3390/fishes10060254

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1. Introduction

Fish-borne parasites include a variety of organisms from different taxonomic groups, such as nematodes (family Anisakidae), trematodes (families Opisthorchiidae and Het-

erophyidae), and cestodes (family Dibothriocephalidae) [1]. The genus *Eustrongylides* Jägerskiöld, 1909 (family Dioctophymatidae) consists of nematodes that parasitize numerous fish species and fish-eating birds in freshwater ecosystems [2]. These nematodes have a heteroxenous life cycle with five developmental stages, requiring two intermediate hosts and one definitive host. Most infections are associated with freshwater environments [3]. In their adult stage, *Eustrongylides* nematodes inhabit the mucosal tissues of the esophagus, proventriculus, and/or intestines of piscivorous birds, particularly those of the orders Ciconiformes and Phalacrocoracidae. The eggs are excreted in the feces of these definitive hosts and develop into second and third larval stages when consumed by aquatic oligochaetes (e.g., *Tubifex* spp., *Lumbricus* spp. and *Limnodrilus* spp.). Eutrophication of aquatic environments, by increasing nutrient levels, favors the proliferation of oligochaete populations and can significantly increase the prevalence of *Eustrongylides* infections [4].

Several fish species serve as secondary intermediate hosts for *Eustrongylides* spp. [2–5]. Predatory fish, such as perch and largemouth bass, are at greater risk of severe infection due to the consumption of infected prey, which amplifies parasite loads [5]. Furthermore, large predatory fish, amphibians (family Bufonidae), and reptiles (e.g., *Natrix tessellata*) can act as paratenic hosts, facilitating transmission of the parasite to definitive hosts [6,7], as shown in Figure 1.

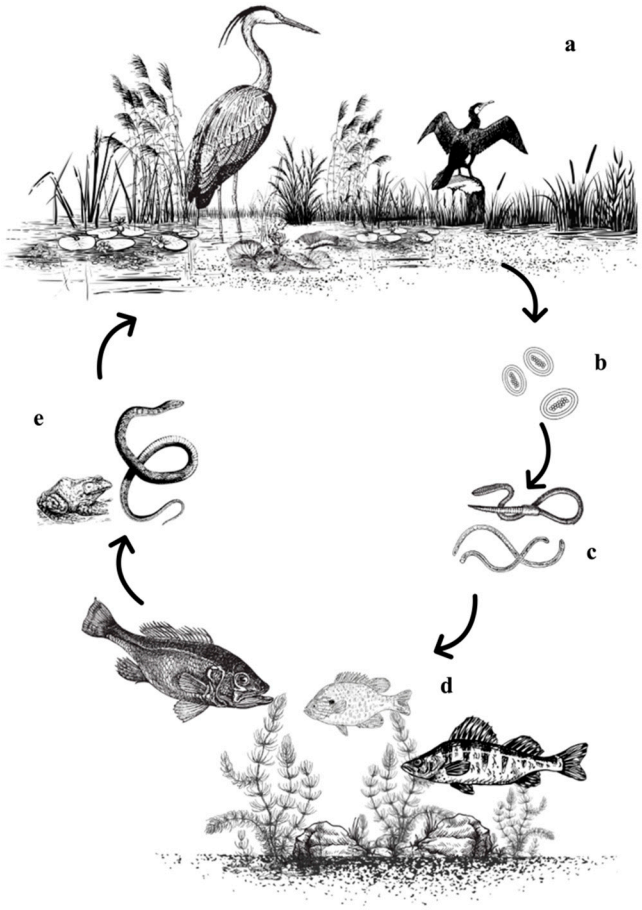

Figure 1. Life cycle of *Eustrongylides* nematodes. (**a**) Definitive hosts; (**b**) eggs; (**c**) aquatic oligochaetes as first intermediate hosts; (**d**) fishes as second intermediate hosts; (**e**) paratenic hosts. Image created by Alice Gabetti, co-author.

Eustrongylides parasites are known to infect birds, fish, and humans [8–10]. In avian hosts, the parasites localize primarily in the proventriculus, ventriculus, and intestines, where they create tunnels, eliciting an inflammatory response [11]. In fish, infections are

associated with severe pathological effects and pronounced inflammation, with larvae commonly found within the musculature, internal organs, or body cavity [8,12,13]. Parasitized fish generally do not exhibit high mortality rates but may experience chronic inflammation, a reduction in erythrocyte counts (or anemia in more severe cases), and an increase in neutrophils. These changes lead to greater vulnerability to infections by opportunistic pathogens [7,14,15].

Eustrongylidiasis is a zoonotic parasitic disease in humans transmitted through the ingestion of raw or undercooked fish infected with the larval stages of the parasite [16]. The number of officially registered cases was eight, all originating from North America and Sudan [14]; however, unregistered cases may also exist. The clinical manifestations of the disease predominantly include gastrointestinal symptoms, such as gastritis or enteritis [17–19], with progression to intestinal perforation reported in severe cases [9]. The symptoms were diagnosed as appendicitis, but it was only during the surgical procedure that the presence of parasites in the abdominal cavity was discovered [16,19]. A rare cutaneous presentation was documented in two cases from South Sudan. Even in this case, the definitive treatment is surgical excision of the larvae [20]. Due to its prevalence in fish and its potential danger to humans if ingested, scientific interest about the prevalence and geographic distribution of this parasite has increased over the last years, particularly concerning fish intended for human consumption [2].

Nematodes belonging to the genus *Eustrongylides* are now globally distributed, with reports documented on all continents [21]. The prevalence of dominant species varies geographically; *E. ignotus* and *E. tubifex* are predominantly identified in North America, while *E. excisus* is more frequently reported in Europe and the Middle East [22]. A bibliographic review indicates that *E. excisus* was not recorded in European regions prior to 2009 [23], with subsequent reports emerging only in 2015 [8]. From that point forward, interest in this parasite has increased, leading to a surge in related studies, as shown in Figure 2. An analysis of the scientific literature indexed in the Scopus database (https://www.scopus.com/home.uri?zone=header&origin=sbrowse; accessed on 23 May 2025) using the keyword "*Eustrongylides*" reveals a clear and consistent increase in research attention from 1980 to 2024. While the number of publications remained relatively low and stable through the 1980s and 1990s, a notable rise began in the early 2000s, with a sharp acceleration observed after 2015 (Figure 2A). The peak in publication output occurred in 2023, with over 60 documents, indicating heightened global interest in the parasite, likely driven by its ecological and zoonotic relevance. Geographically, the United States has contributed the highest number of publications, followed by Brazil and Canada, with significant input also coming from Germany, Italy, India, and other countries (Figure 2B). This trend underscores the growing recognition of *Eustrongylides* as a topic of international concern, particularly in relation to aquatic health and food safety.

Recent research has documented an expanding distribution of *Eustrongylides* nematodes in Italy, potentially driven by the rising population of cormorants around lakes and various biotic and abiotic factors [5,24]. However, existing knowledge has predominantly been derived from studies in commercially significant fishing areas, particularly lakes, such as Lake Trasimeno and Lake Garda [5,25,26]. Unfortunately, limited information is available regarding the presence and the prevalence of this parasite in natural fish populations and its broader ecological implications, a knowledge gap particularly evident in lotic ecosystems. Therefore, this study aims to investigate the occurrence of *Eustrongylides* nematodes in the Po River (northwestern Italy). To achieve this, comprehensive fish community monitoring was conducted at three sampling sites along the metropolitan stretch of the river.

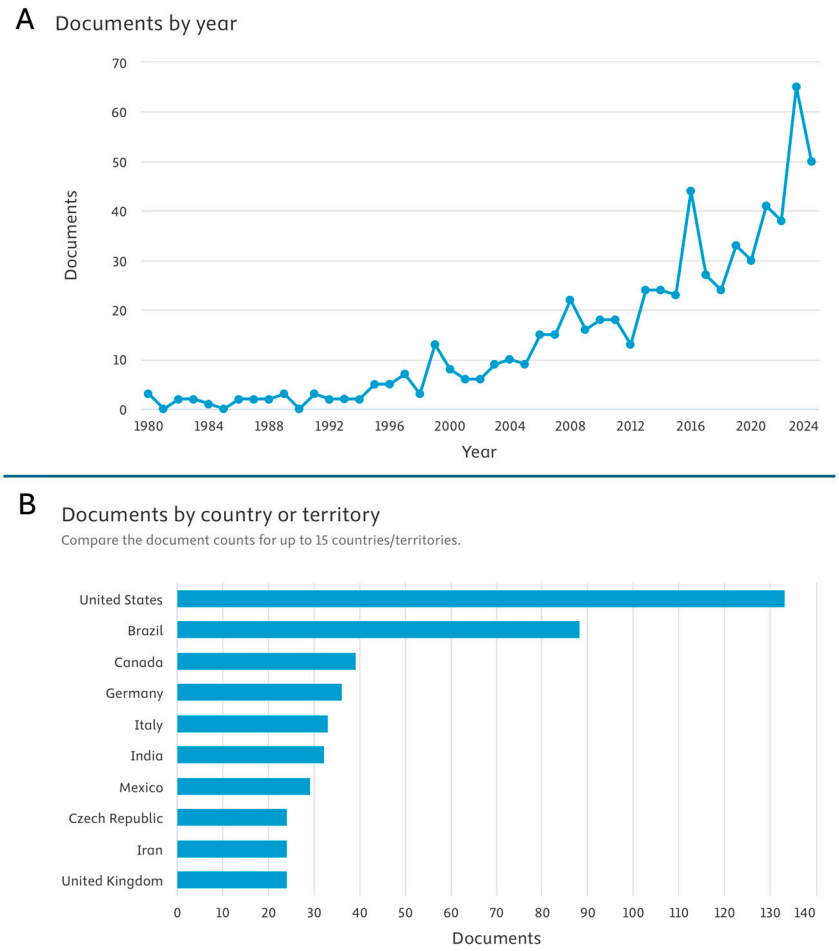

Figure 2. (**A**) Number of scientific articles related to "*Eustrongylides*" published per year (1980–2024); (**B**) geographical distribution of publications by country (Scopus database; https://www.scopus.com/home.uri?zone=header&origin=sbrowse (accessed on 23 May 2025)).

2. Materials and Methods

2.1. Study Area

The Po River originates from Mount Monviso (2020 m a.s.l.) in the Piedmont region (NW Italy) and extends approximately 650 km, fed by 141 tributaries. The Po River basin encompasses regions characterized by diverse hydrological and geomorphological features, as evidenced by the highly variable drainage system of the river and its tributaries. The ichthyofauna consists of 35 freshwater species and 10 euryhaline species, amounting to at least 45 native fish species, including 16 Italian endemics or sub-endemics [27].

For this study, three sampling sites along the urban stretch of the Po River in the Turin metropolitan area were chosen (Figure 3): one in Moncalieri (260 m a.s.l.) near the confluence with Sangone Creek, another in central Turin called Murazzi (239 m a.s.l.), and a third downstream of the San Mauro dam (210 m a.s.l.). All sites are subject to significant anthropogenic pressure, with wastewater from homes, hospitals, and industry particularly affecting the area between Moncalieri and the Murazzi station [27].

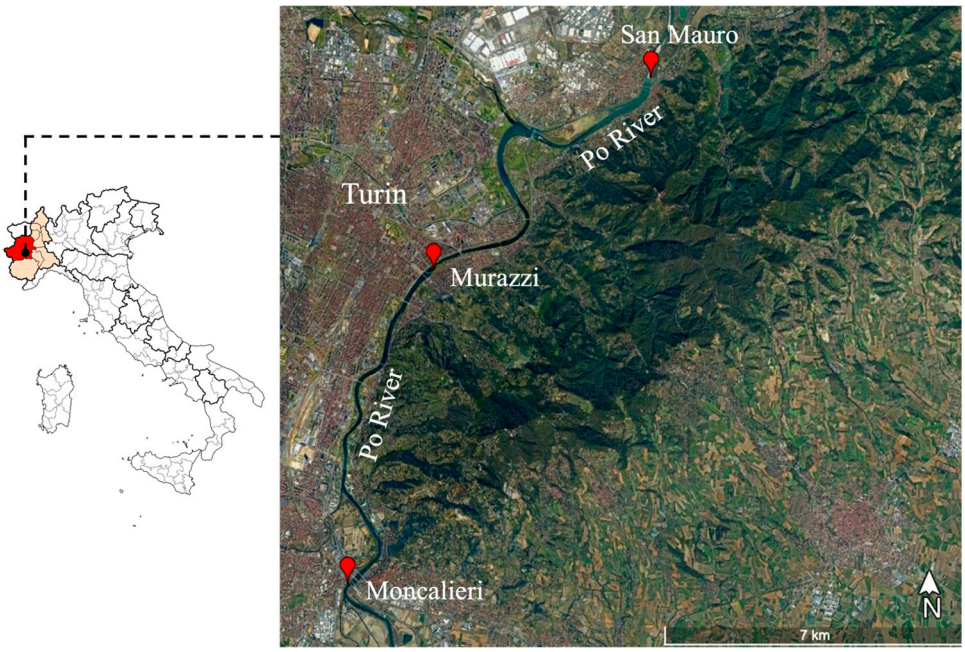

Figure 3. Location of the three sampling sites along the Po River: Moncalieri (44°59′56.5″ N 7°40′29.5″ E), Murazzi (45°03′52.8″ N 7°42′00.8″ E), and San Mauro (45°06′18.9″ N 7°45′53.4″ E). The map was generated using QGIS software, version 3.40.2-Bratislava.

2.2. Fish Sampling

A single fish sampling session was conducted on 20 August 2024, employing electrofishing in accordance with the guidelines detailed in the "Protocol for Sampling and Analysis of the Fish Fauna in Lotic Ecosystems" [28]. For this study, a standardized quali-quantitative fish sampling protocol was employed, consistent with national guidelines for lotic systems and aligned with CEN EN 14011:2003 standards [28]. Each sampling station was delineated using a 50 m transect as the unit of monitoring, positioned to represent the range of mesohabitats present (e.g., pools, riffles, runs). This length was selected to ensure comparability across sites while capturing a representative sample of the local fish assemblage. Fishing was conducted in an upstream direction to avoid disturbing fish ahead of the sampling area. Electrofishing was conducted using a battery-powered backpack electrofisher (Model IG200-2, Hans-Grassl GmbH, Schönau am Königssee, Germany) with the following operating parameters: pulsed direct current with a voltage range of 150–200 V and a current intensity between 15 and 25 A, adjusted in the field according to water conductivity and site-specific conditions to ensure effective fish capture and minimize stress. Electrofishing operations were conducted with the authorization and participation of the specialized Wildlife and Flora Protection function of the Metropolitan City of Turin, Italy (resolution of the provincial council No. 844-22328 of 5 June 2009, and No. 922-49228 of 9 December 2013). The fish were then euthanized using an overdose (150 mg/L) of tricaine methanesulfonate (MS-222) and subsequently transported to the laboratory.

2.3. Fish Identification and Parasite Investigation

Specimens were identified using the Italian freshwater fish checklist [29], and measurements of their body weight and total length were recorded. Muscles and organs were inspected for zoonotic parasites. Skeletal muscles were sectioned into slices measuring 2–3 mm and examined under a transilluminator (UVP White Light Transilluminators, TW-43, Analytik Jena, Jena, Germany) to detect encysted parasites.

Nematodes were isolated using pointed light metal forceps and a fine needle, rinsed in deionized water, and fixed in 70% ethanol. After fixation, the head and tail of each

larva were removed for morphological examination under light microscopy at up to 40× magnification, while the central body segment, which lacks taxonomically relevant features, was used for molecular analysis [30,31]. Specimens preserved in ethanol were classified to the genus level based on morphological keys provided by Moravec [12].

2.4. Molecular Analysis

Molecular analyses were performed following the method described by Mazzone et al. [31]. Species identification was achieved by sequencing the internal transcribed spacer (ITS), a conserved genomic region [32,33]. Both DNA strands were sequenced, and the resulting sequences were compared to similar entries in the GenBank database using BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 23 May 2025). A minimum sequence identity of 98% was applied as the threshold for species assignment.

3. Results

A total of 170 fish were collected across the three sampling sites, with 42 individuals obtained from Moncalieri, 57 from Murazzi, and 71 from San Mauro. Table 1 provides an overview of the identified species, along with their mean total lengths and weights. At the Moncalieri site, the fish community was predominantly composed of *Barbus barbus* (61.90%), followed by *Padogobius bonelli* (19.05%). The Murazzi site exhibited greater diversity, with *Leuciscus* sp. (38.60%), *Barbus barbus* (15.79%), *Rhodeus amarus* (14.04%), *Cobitis bilineata* (10.53%), and *Padogobius bonelli* (10.53%). Furthermore, at Moncalieri, *Barbus barbus* (39.44%) remained dominant, followed by *Squalius squalus* (29.58%) and *Cobitis bilineata* (11.27%). The San Mauro site exhibited a lower proportion of non-native species (49.30%) compared to Moncalieri (73.81%) and Murazzi (77.19%).

Of the 170 fish sampled, only two specimens of *Padogobius bonelli* (1.18%) from the Murazzi site were found to harbor a single nematode larva each in their abdominal cavity, as shown in Figure 4. The parasites measured 43 mm and 39 mm in length, respectively.

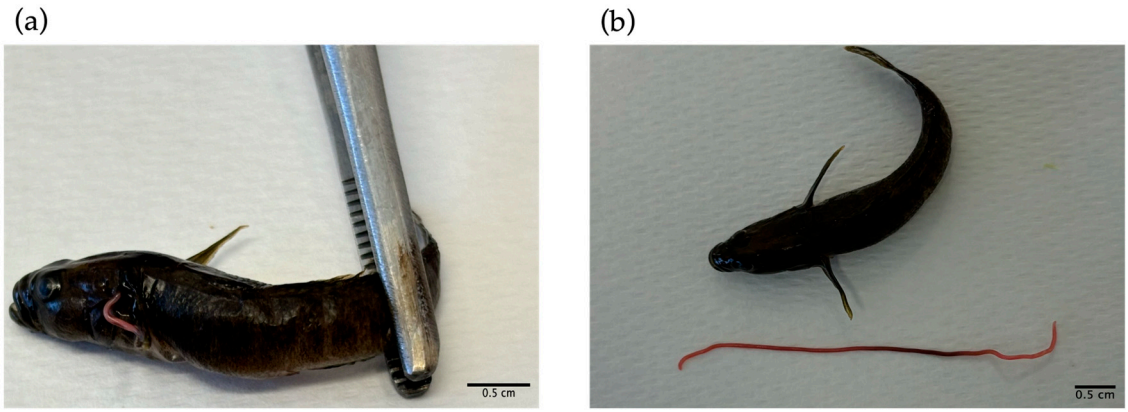

Figure 4. *Padogobius bonelli* parasitized by *Eustrongylides excisus:* (**a**) the parasite protruding from the fish abdominal cavity, and (**b**) the parasite removed and placed next to the host for size comparison.

As illustrated in Figure 5, the parasite's body is widest at the center and becomes thinner toward both ends. The anterior end is not swollen, the cuticle lacks spines, and the transverse shape becomes more distinct near the extremities [14].

Table 1. Species, origin (native, N/non-native, NN), and number (No) of specimens sampled per site, with mean and standard deviation of total weight and total length.

Sampling Sites	Species	Origin	Mean Weight (g)	Mean Length (cm)	No.
Moncalieri	*Barbus barbus*	NN	26.67 ± 28.32	12.58 ± 4.38	26
	Telestes muticellus	N	11.16	10	1
	Squalius squalus	N	1.03 ± 0.27	4.5 ± 0.71	2
	Rhodeus amarus	NN	2.61 ± 0.56	6.75 ± 0.96	4
	Padogobius bonelli	N	2.49 ± 1.16	6.31 ± 1.44	8
	Salmo trutta	NN	90.39	21	1
Murazzi	*Leuciscus* sp.	NN	1.74 ± 2.39	5.05 ± 1.86	22
	Barbus barbus	NN	9.16 ± 5.97	9.56 ± 3.11	9
	Romanogobio benacensis	N	8.95	11	1
	Padogobius bonelli	N	1.90 ± 0.93	4.92 ± 1.02	6
	Carassius carassius	NN	2.14 ± 1.64	5 ± 1.41	2
	Lepomis gibbosus	NN	7.48	8.5	1
	Pseudorasbora parva	NN	2.31 ± 0.25	7.25 ± 0.35	2
	Cobitis bilineata	N	1.69 ± 0.86	6.75 ± 2.07	6
	Rhodeus amarus	NN	2.51 ± 0.38	5.69 ± 0.37	8
San Mauro	*Padogobius bonelli*	N	3.43 ± 3.44	6.13 ± 2.29	4
	Silurus glanis	NN	56.52	21	1
	Rhodeus amarus	NN	1.46	5	1
	Cobitis bilineata	N	1.99 ± 0.88	7.13 ± 1.03	8
	Telestes muticellus	N	7.21 ± 2.25	8.17 ± 0.76	3
	Perca fluviatilis	NN	63.44	18.5	1
	Pseudorasbora parva	NN	2.68 ± 0.34	6.58 ± 0.10	4
	Barbus barbus	NN	12.05 ± 5.43	9.73 ± 1.45	28
	Squalius squalus	N	0.64 ± 0.21	4.02 ± 0.80	21

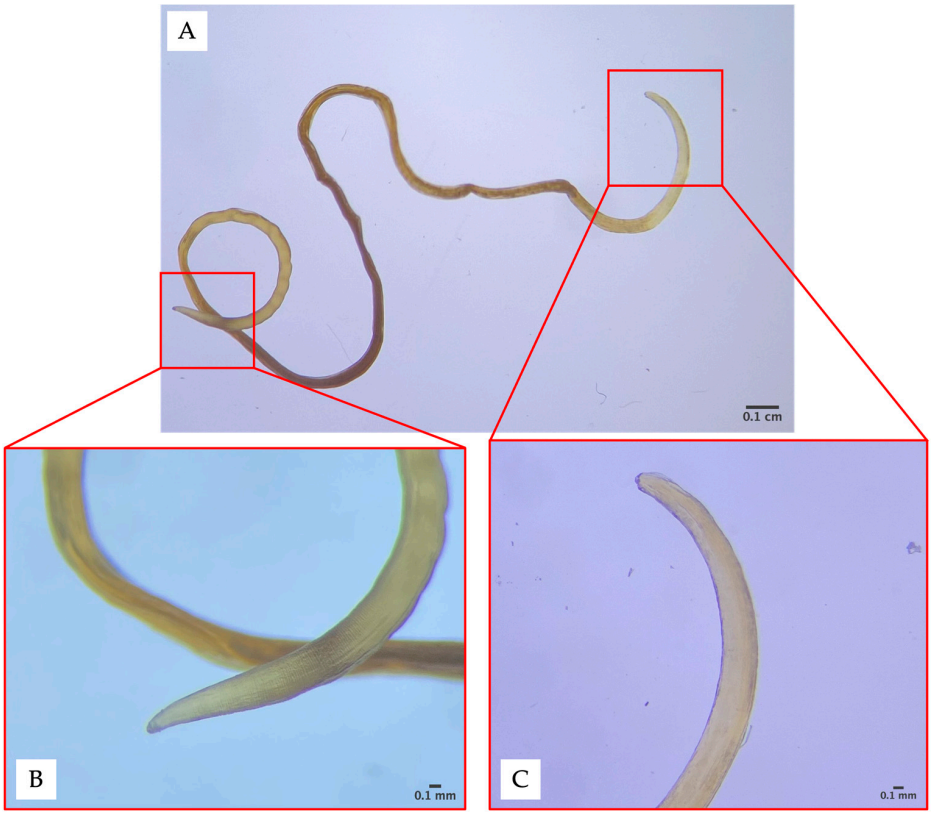

Figure 5. Microscopic view of *Eustrongylides excisus* larva (**A**); details of anterior part (**B**) and tail (**C**).

Amplification of the ITS rDNA sequences of the nematodes morphologically ascribable to the genus *Eustrongylides* spp. showed they were identical to each other. BLAST analysis gave 100% identity with the sequence of *E. excisus* deposited by Mazzone et al. [31].

4. Discussion

The fish community found at the three sites analyzed is similar to that found in previous work carried out in the same area [27]. The presence of *Eustrongylides excisus* was detected in only two individuals of *Padogbius bonelli* from Murazzi site, which is located in the centre of Turin.

Padogobius bonelli (Bonaparte, 1846), a species within the family Gobiidae, is an endemic fish native to the northern Adriatic region. Its distribution spans northern Italy, including the Po and Adige River basins, as well as parts of Dalmatia [34].

The presence of this parasite within the Gobiidae family has never been documented in *P. bonelli* and only in the species *Babka gymnotrachelus* and *Neogobius fluviatilis* in the Bulgarian section of the Danube [35]. In that study, the prevalence ranged from 9.09% to 25.81% in *N. fluviatilis* and from 25% to 40% in *B. gymnotrachelus*. Similarly, in the Po River, the prevalence of *E. excisus* in *P. Bonelli* ranged from 0 to 33.3%. However, *E. excisus* is commonly found in edible and commercially important fish species in Italy, including European perch (*Perca fluviatilis*), largemouth bass (*Micropterus salmoides*), and sand smelt (*Atherina boyeri*) [5,8,24,25,30,36–38]. Among these, the highest prevalence was recorded in perch from Lake Trasimeno (67.99%) [36] and in largemouth bass from Lake Garda (30%) [5]. Additionally, a notable prevalence has been reported in the non-native species *Lepomis gibbosus* in Lake San Michele (18.3%), Lake Candia (92.04%), and Lake Viverone (40.83%), all located in Piedmont (northwest Italy) [30,38]. The parasite's range also appears to be expanding. In fact, while no presence was detected in the Lakes of Avigliana (northwest Italy) during studies conducted between 2019 and 2022 [38], in 2023, it was reported in *L. gibbosus* and *Ameiurus melas* [39].

The most recent reports from Italy, with a particular focus on the Piedmont region, are presented in Table 2.

Table 2. Reports of *Eustrongylides excisus* in Italy. Sampling location, fish host, and the parasitological indexes are indicated.

Region	Water Basin	Year of Sampling	Fish Species	Parasitological Index *			References
				P%	MI	MA	
Umbria	Lake Trasimeno	2015	*P. fluviatilis*	6.84	1	NA	[25]
			M. salmoides	1.89	1	NA	
			A. boyeri	0.13	1	NA	
	Lake Trasimeno	2021	*P. fluviatilis*	67.99	8.28	6	[36]
			M. salmoides	0.26	1	0.003	
			T. tinca	0.13	1	0.0013	
			A. melas	2.72	1	0.0273	
			C. carpio	0.02	1	0.0002	
Tuscany	Lake Massacciuccoli	2019	*A. boyeri*	2.3	1	0.2	[40]
	Lake Massacciuccoli	2022	*A. melas*	28.6	1.7	0.49	[37]
			S. glanis	75	5.7	4.3	
			C. ramada	NA	NA	0.07	
			L. gibbosus	4.3	1	0.04	
			M. salmoides	25	1	0.25	
			A. boyeri	5.1	1	0.05	

Table 2. *Cont.*

Region	Water Basin	Year of Sampling	Fish Species	Parasitological Index * P%	MI	MA	References
Lombardy–Veneto	Lake Garda	2020	*P. fluviatilis* *M. salmoides* *L. gibbosus*	14.41 30 3.10	1 1.33 1	0.014 0.4 0.023	[5]
Piedmont–Lombardy–Swiss	Lake Maggiore	2019–2022	*P. fluviatilis*	8.20–20	1.54	0.15	[38]
Piedmont	Lake Viverone	2019–2022	*P. fluviatilis* *L. gibbosus*	25.33 40.83	1.11 1.01	0.28 0.44	
	Lake Candia	2019–2022	*L. gibbosus* *M. salmoides*	92.04 83.82	2.86 1	2.63 0.84	
	Lake Sirio	2019–2022	*L. gibbosus*	2.86	1	0.03	
	Lake Pistono	2019–2022	*L. gibbosus*	2.67	1.58	0.04	
	Lake Nero	2019–2022	*P. fluviatilis* *L. gibbosus*	5.41 10.32	1.25 1.23	0.07 0.13	
	Lake San Michele	2019–2022	*P. fluviatilis* *L. gibbosus* *M. salmoides*	10 18.29 17.86	1.67 1.53 1	0.17 0.28 0.179	
	Lake Alice Superiore	2019–2022	*P. fluviatilis* *L. gibbosus*	4.29 2.57	1 1.12	0.04 0.03	
	Great Lake of Avigliana	2023	*L. gibbosus* *A. melas*	NA NA	NA NA	NA NA	[39]

* P%: prevalence; MI: mean intensity; MA: mean abundance; NA: not available.

Several environmental factors may contribute to the expansion of both the range and the host diversity of *E. excisus*. Once released into the environment, the eggs of *E. excisus* can hatch at any time of the year and retain infective capacity for up to two years. Furthermore, the larvae have been observed to survive within the intermediate host for a period exceeding one year [13]. Piscivorous birds serve as definitive hosts for various parasite species, playing a key role in their distribution [30,41]. In particular, cormorants, once rare as wintering birds until 1980, have significantly increased in number in the Piedmont region, now inhabiting both large and small water bodies [24,42]. Additionally, climate change is known to influence bird distribution [43,44] and may alter parasite dynamics by shifting spatial ranges, host–parasite relationships, life cycle timing, and ecosystem interactions [45]. A recent review by Menconi et al. [2] also reported *Eustrongylides* nematodes in new fish hosts, including *Anguilla anguilla* (eel), *Ameiurus melas* (black bullhead catfish), *Tinca tinca* (tench), *Cyprinus carpio* (carp), *Silurus glanis* (wels catfish), and *Chelon ramada* (small thin-lip gray mullet).

The Po River basin is one of the most agriculturally productive and densely populated areas in Europe, but it is also among the most affected by nutrient pollution [46]. In such environments, low-oxygen conditions favor the proliferation of oligochaetes, which thrive by feeding on organic material [47]. Their abundance in eutrophic waters may create favorable conditions for the life cycle of *E. excisus*, as these worms play a crucial role in the parasite's transmission to fish hosts [5]. *Padogobius bonelli* is primarily a benthic predator, feeding on stream-dwelling invertebrates and fish eggs [48]. Given its feeding habits, it is reasonable to hypothesize that its diet may also include aquatic oligochaetes, which serve as the first intermediate hosts in the life cycle of *E. excisus*. Despite the absence of

additional evidence in our study, previous research has documented that other fish can consume infected prey and acquire multiple parasite infections [4,30]. Therefore, extending monitoring to larger fish is essential to accurately assess the prevalence of the parasite in predatory species.

In Italy, most reports of *E. excisus* originate from large lakes (e.g., Lake Trasimeno and Lake Garda) with intensive fishing activity and a commercial market for fish products intended for human consumption [5,25,36]. In contrast, the parasitological study of river fish not intended for consumption remains of limited scientific and economic interest.

5. Conclusions

This study reports for the first time the presence of *Eustrongylides excisus* in *Padogobius bonelli*, highlighting its expanding geographical range and host diversity. Parasites play a crucial yet often overlooked role in ecosystem and biodiversity research [49]. To improve our understanding of the parasite's various hosts, continued monitoring of Po River fish fauna and, potentially, its macroinvertebrates communities, particularly oligochaetes, is essential. Furthermore, helminthological studies on predatory fish that consume gobies and are part of the human diet are recommended to assess potential health risks.

Author Contributions: Conceptualization, A.M., A.G., R.E.G., M.C., G.E., M.B., E.P., P.P. and M.P.; data curation, A.M.; investigation, A.M., C.M., G.E., F.B., P.P. and M.P.; methodology, A.M., C.M., A.G., F.B., E.P., P.P. and M.P.; project administration, M.P.; supervision, P.P. and M.P.; writing—original draft, A.M. and P.P.; writing—review and editing, C.M., A.G., R.E.G., M.C., G.E., M.B., E.P., P.P. and M.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Fondazione CRT, grant number 23D04.

Institutional Review Board Statement: The sampling was carried out as part of a monitoring campaign led by the Fish Diseases Laboratory of the Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta (IZSPLV, Turin, Italy), aimed at assessing the health status of wild fish populations. This activity was conducted within the framework of a research project funded by the Fondazione CRT (grant number 23D04). Electrofishing operations were authorized and performed in collaboration with the specialized Wildlife and Flora Protection Unit of the Metropolitan City of Turin, in accordance with the resolutions of the Provincial Council No. 844-22328 (5 June 2009) and No. 922-49228 (9 December 2013).

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

Acknowledgments: The authors would like to thank Paolo Lo Conte (Città Metropolitana di Torino) for the fish sampling and for his technical support.

Conflicts of Interest: The authors declare no conflicts of interest.

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