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Spauligodon orobicus sp. nov. (Oxyurida: Pharyngodonidae) a parasite infecting the common wall lizard, *Podarcis muralis* (Laurenti, 1768) in northern Italy

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Abstract

Parasitic nematodes of the oxyurid family Pharyngodonidae are commonly found across reptile orders, with species of the genus *Spauligodon* Skrjabin, Schikhobalova & Lagodovskaja, 1960, being among the most common ones. However, scant information exists regarding the prevalence of *Spauligodon* nematodes in Italian reptile populations. Although two *Spauligodon* species have been reported from southern Italy, the presence of these worms in the rest of the country remains unknown. In this research, we describe *Spauligodon orobicus* sp. nov. from an Italian common wall lizard *Podarcis muralis* (Laurenti, 1768) of northern Italy. The new species is distinct from other known species both at the morphological and molecular level. The study increases the knowledge on the biodiversity of *Spauligodon* nematodes and in general on the biodiversity of Italy, and adds northern Italy to the geographical range of this nematode genus.

http://zoobank.org/urn:lsid:zoobank.org:act:29DB5E90-9CB5-46A3-8471-FEE0395FA29A

Keywords: *Nematoda*, *Pharyngodonidae*, Podarcis muralis, Spauligodon orobicus *sp. nov.*, *endoparasite*

Introduction

Reptiles are infected by a myriad of endoparasitic helminths, such as cestodes, trematodes, and nematodes (Jacobson 2007). Parasitic nematodes have been detected in individuals from all reptile orders. The order Oxyurida includes ~900 species, all parasites of the intestines of arthropod and vertebrate hosts. An important characteristic of these nematodes is haplodiploid reproduction (Adamson 1994). The Pharyngodonidae is a diversified oxyurid family comprising 24 genera, which are parasites of fish, amphibians, reptiles, and mammals (Pereira et al. 2018). This family is frequently found in reptiles, in particular species from the genera *Pharyngodon* Diesing, 1861, *Skrjabinodon* Inglis, 1968, *Thelandros* (Wedl, 1861), and *Spauligodon* Skrjabin, Schikhobalova & Lagodovskaja, 1960 (Pereira et al. 2018).

Nematodes of the genus *Spauligodon* are commonly found in many reptile families worldwide. The worms belonging to this genus are short in relation to their diameter, and show a conical tail, a concentrically lined cuticle, a short esophagus, an esophageal bulb with a non-sclerotized valve, and the ovary and uterus always posterior or at the level of the esophageal bulb (Álvarez et al. 2021).

Few records have been published on *Spauligodon* nematodes infecting wild reptiles in Italy (Casanova et al. 2003; Carbonara et al. 2023) and at the moment nothing is known about their prevalence in northern Italian populations of reptiles. In Italy, only two *Spauligodon* species have been recorded from the

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southern regions of the country: *Spauligodon extenuatus* (Rudolphi, 1819), reported in the 1940s (García-Calvente 1948), and *Spauligodon aloisei* Casanova et al. 2003 which was described as a new species in 2003 (Casanova et al. 2003) after being found in eight *Podarcis siculus* (Rafinesque, 1810) from Calabria. Since then, it was recently identified from three *P. siculus* (one from Lipari island, one from Basilicata, and one from Apulia), two *Podarcis filfolensis* (Bedriaga, 1876) from Linosa island and one *Chalcides ocellatus* (Forsskål, 1775) from Linosa island (Carbonara et al. 2023).

The herpetofauna of northern Italy is believed to include ~30 species, grouped in the orders of Squamata (snakes and lizards) and of Chelonia (turtles and tortoises). The most widespread reptile species in northern Italy is the common wall lizard *Podarcis muralis* (Laurenti, 1768). To the best of our knowledge, no studies on parasitic helminths of northern Italian reptiles have been published. Nevertheless, few studies on helminths that parasitize common wall lizards have been published regarding non-Italian specimens, and *Spauligodon* nematodes infecting *P. muralis* were reported from Spain, Bulgaria, and Turkey (García-Adell & Roca 1988; Kirin 2002; Yildirimhan & Sümer 2019).

In this study, we report the first documented case of a *Spauligodon* infection in northern Italy, occurring in an Italian common wall lizard, *P. muralis*. The assignment of the specimen to an already described species was impossible, considering both morphological and molecular data. This led us to the description of the new species *Spauligodon orobicus* sp. nov.

Materials and methods

A number of 82 lizards of the species *P. muralis* were collected in Salvano locality (municipality of Palazzago, Bergamo district, Italy) between July 2022 and October 2023 in the context of a parasitological survey of peri-urban reptiles (under the authorization of the Italian Ministry of Environment - Protocol number: 77772–21/06/2022).

On the 6th of October 2023, one oxyurid-like worm crawled outside of the cloaca of a male lizard after feces collection, and was stored in 96% Ethanol (Figure 1). The worm was collected at the exact coordinates of 45.737402, 9.556013.

The sample was taken to the EntoPar laboratory at the University of Milan and observed and photographed under a Keyence VHX-7000 digital stereomicroscope (Keyence Corporation, Osaka, Japan) and a Leica DM1000 optical microscope (Leica Microsystems, Germany). The diagnostic characters of the worm were measured using the ImageJ program (Schneider et al. 2012). Furthermore, DNA was extracted from the whole worm using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). A polymerase chain reaction (PCR) with the pan-metazoan primers LCO1490 and HCO2198 targeting the Cytochrome C oxidase subunit I gene (COI) (Folmer et al. 1994; Otim et al. 2021) was performed, and the PCR product was sequenced on both strands (Eurofins Genomics GmbH, Konstanz, Germany; GenBank, accession number OR864670.1). Utilizing the obtained sequence as a query, a nBLAST search was executed. The hits were downloaded and aligned in the Aliview software (Larsson 2014) using MUSCLE, version 3.8.425 (Edgar 2004). The alignment of the sequences was then subjected to a phylogenetic analysis using MEGA, version 11.0.10 (Tamura et al. 2021) with 1000 bootstrap replicates and GTR+G+I as the substitution model, selected based on BIC values after a model test analysis. Outgroups were included according to a global nematode phylogeny (Blaxter et al. 1998). The phylogenetic tree was visualized and edited

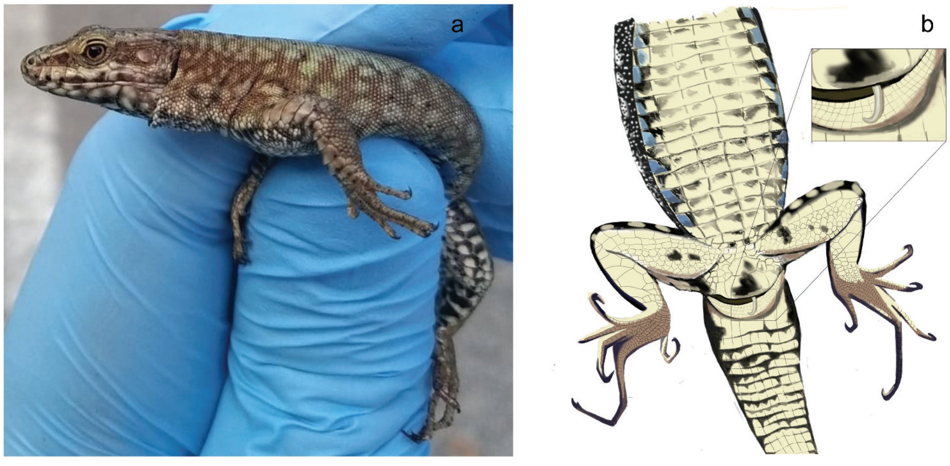

Figure 1. (a) The male *P. muralis* in which the *S. orobicus* sp. nov. specimen was found. (b) Drawing depicting the specimen crawling out of the lizard cloaca as noted in the field before the worm collection.

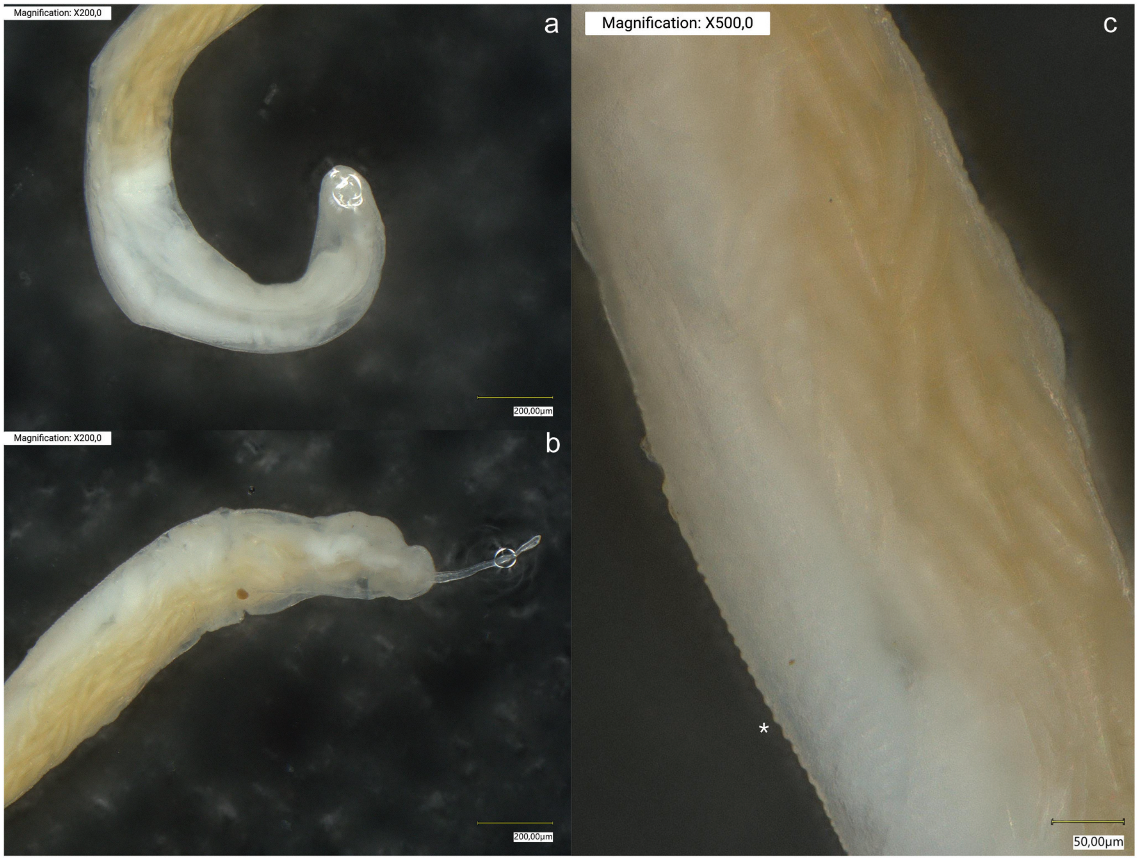

Figure 2. Photos of the *S. orobicus* sp. nov. specimen taken with a digital microscopy. (a) Cephalic region (200X magnification). (b) Detail of the caudal region (200X magnification). (c) Detail of the eggs and of the concentrically lined cuticle, indicated with the asterisk (500X magnification).

using the TreeViewer software (Bianchini & Sánchez-Baracaldo 2024).

Results

Taxonomic account

Family Oxyuridae

Genus *Spauligodon* Skrjabin, Schikhobalova & Lagodovskaja, 1960

Spauligodon orobicus sp. nov.

Holotype: One female; male unknown
Type locality: Salvano locality, Palazzago municipality, Bergamo district, Lombardy region, Italy (45.737402, 9.556013.) crawling out of the cloacal opening of a male *P. muralis* lizard on the 6th October of 2023, leg. Alessandro Alvaro and Lorenzo Sanchez-Ruiz.
Type host: *Podarcis muralis* (Laurenti, 1768) (Reptilia: Lacertidae)
Site of infection: presumably intestine; collected alongside feces and observed crawling outside of the cloaca of the host.
Etymology: The authors would like to name the species after the orobic portion of Alps, to which the type locality belongs.
Zoobank LSID: 29DB5E90-9CB5-46A3-8471-FEE0395FA29A

Description of the new species

Small nematode, white in coloration. Roughly cylindrical in body shape. Cephalic and caudal regions are narrower than the rest of the body (Figure 2). Length of the body 3.4 mm. Cuticle with fine transverse-ringed striation ~7–9 µm wide. Esophagus 182.83 µm long and 27.80 µm wide; bulb width 69.76 µm. Vagina localized caudally to the esophageal bulb (Figure 3). Spineless tail, 306.38 µm long and 26.97 µm wide (Figure 4a). Eggs ~110–117 µm long and ~24–27 µm wide, elliptical in shape and more convex on one side, with flattened extremities (Figure 4b–c). The morphological

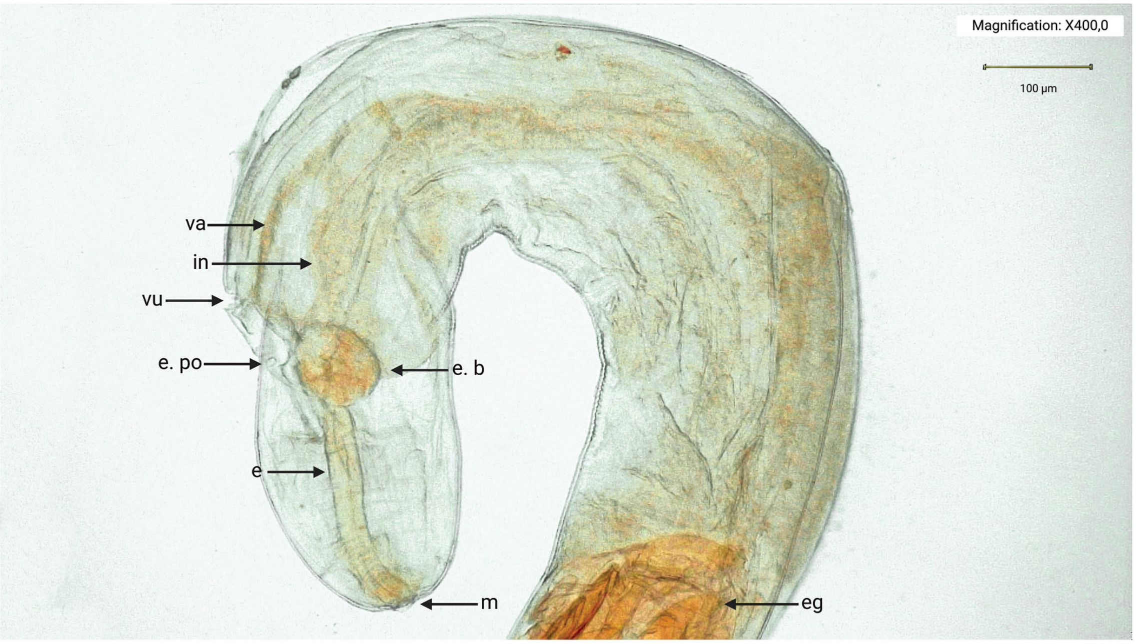

Figure 3. Cephalic region of the *S. orobicus* sp. nov. specimen photographed at the optical microscope (400X magnification). va = vagina; in = intestine; vu = vulva; e.po = excretory pore; e.b = esophageal bulb; e = esophagus; m = mouth; eg = eggs.

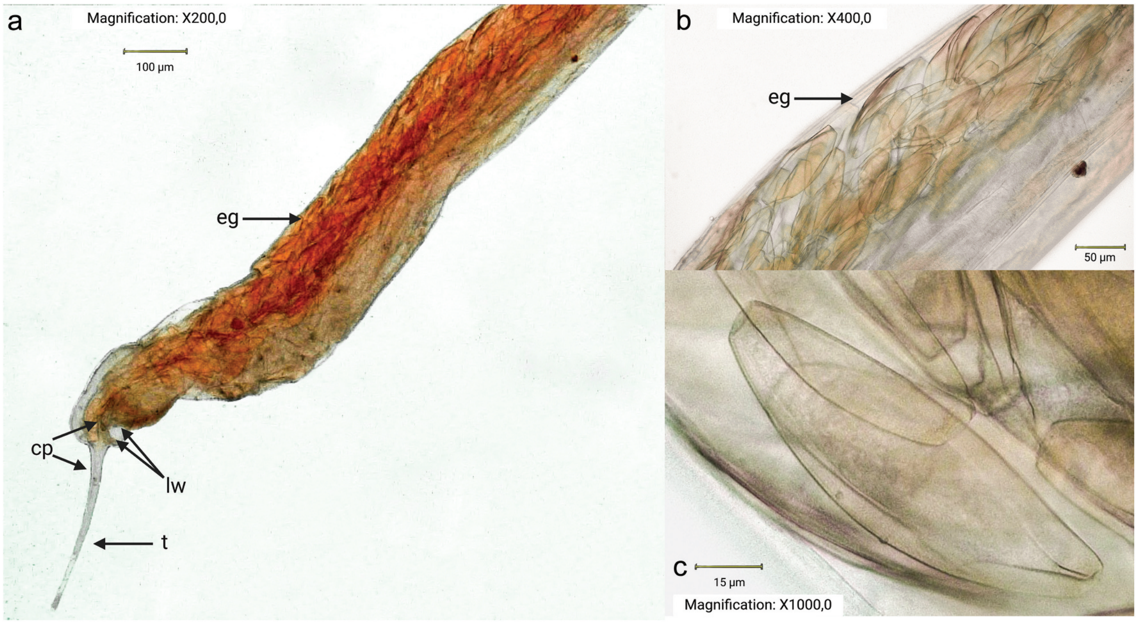

Figure 4. Caudal region of the *S. orobicus* sp. nov. photographed at the optical microscope. (a) Caudal region showing the tail (200X magnification). (b) Body showing the eggs (400X magnification). (c) Detail of egg shape (1000X magnification). eg = eggs, cp = caudal papillae, lw = lateral wings, t = tail.

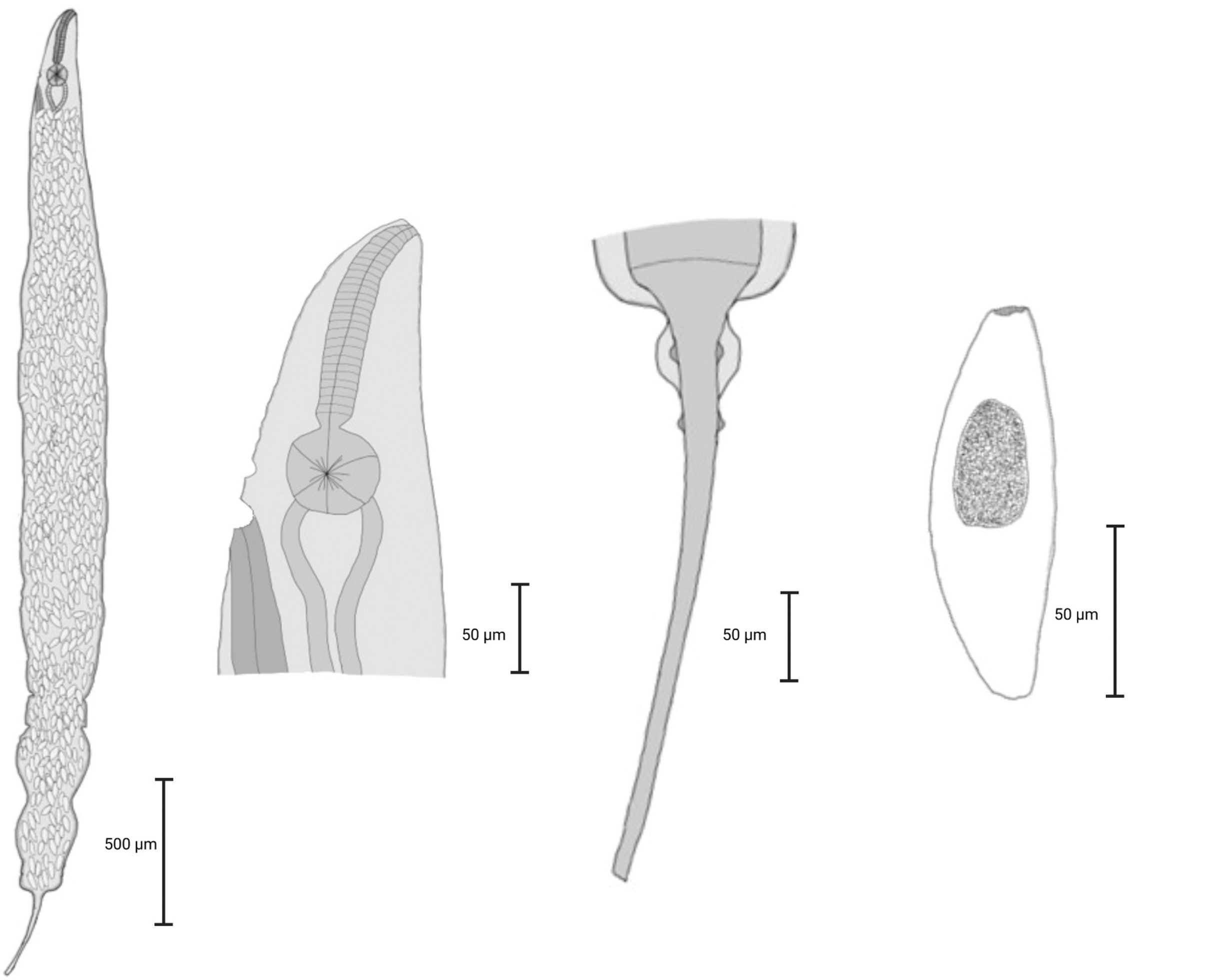

Figure 5. Drawings of the *S. orobicus* sp. nov. specimen. From left to right: specimen in ventral view, detail of the cephalic region, detail of the caudal region, egg.

features of the new species have been illustrated in drawings (Figure 5).

DNA sequence

COI: GenBank OR864670.1; 658 bp long.

Differential diagnosis

Morphological diagnosis

S. orobicus sp. nov. differs from *S. aloisei* at the morphological level for two main features. Firstly, both the genital pore and the vulva are located posteriorly to the esophageal bulb, while in *S. aloisei* these structures are localized anteriorly. Secondly, the tail lacks any sort of spines in the collected specimen, while in *S. aloisei* the female's tail features 4–9 epicuticular spines (Casanova et al. 2003).

Other than *S. aloisei*, only *S. extenuatus* has been recorded in Italy so far (García-Calvente 1948). However, the newly described species differs from *S. extenuatus* since it lacks spines on the tail. Other species from this genus, recorded from the Mediterranean area, are *Spauligodon cabrerae* Castano-Fernandez, Zapatero-Ramos & Solera Puertas, 1988, *Spauligodon paratectipenis* (Chabaud & Golvan, 1957), and *Spauligodon tectipenis* Gedoelst, 1919. The presented specimen differs from *S. cabrerae* because it lacks spines on the tail. The specimen is similar to *S. tectipenis* for the absence of spines on the tail and the position of the excretory pore and the vulva. However, *S. orobicus* sp. nov. differs in the shape of the eggs, for the lack of a constriction before the esophageal bulb, for the absence of teeth in the esophageal bulb and for the position of the excretory pore. Although *S. orobicus* sp. nov. shares the presence of lateral

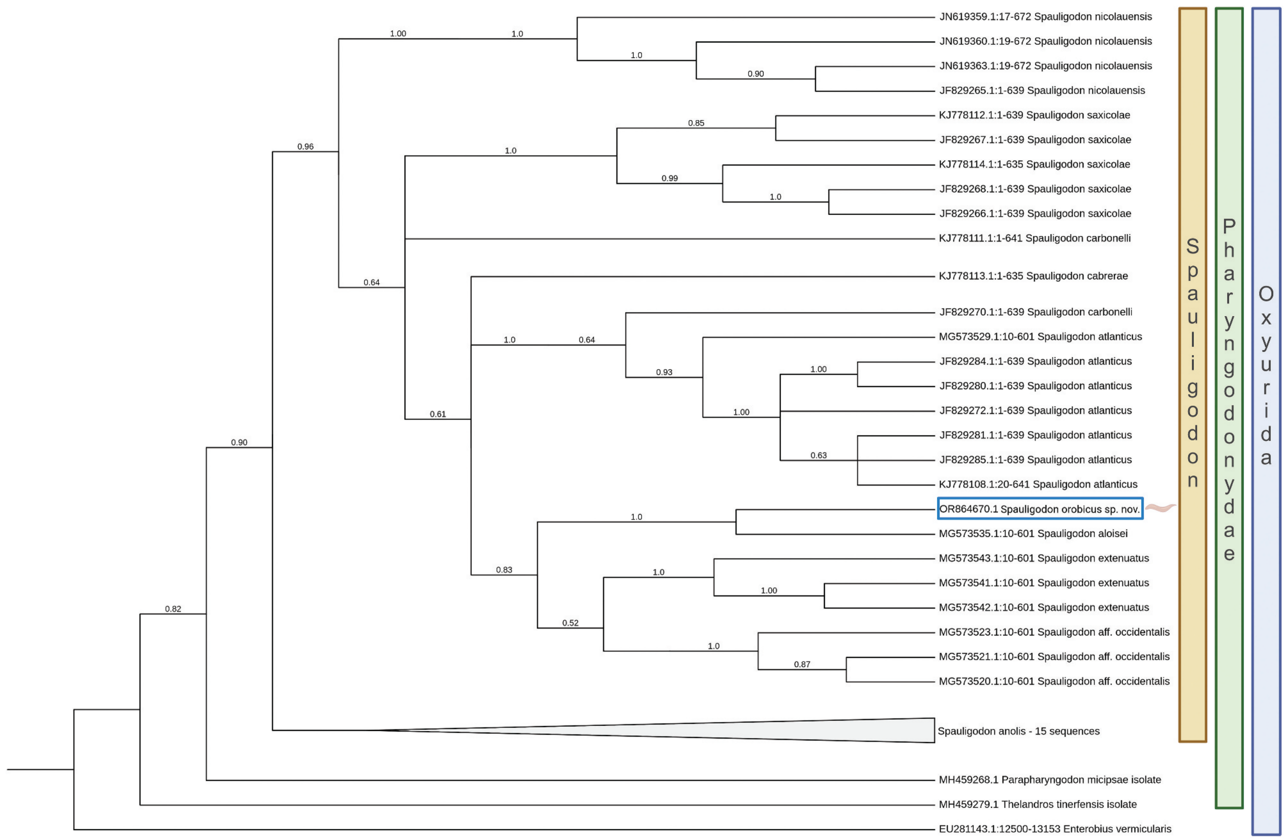

Figure 6. Maximum likelihood bootstrap consensus tree showing the position of the COI sequence of the described *S. orobicus* sp. nov. specimen (in the blue rectangle) in relation with sequences of other specimens of different *Spauligodon* species. Outgroups: *Parapharyngodon micipsae* (Nematoda: Oxyurida: Pharyngodonidae), *Thelandros tinerfensis* (Nematoda: Oxyurida: Pharyngodonidae), *Enterobius vermicularis* (Nematoda: Oxyurida: *Oxyuridae*). Phylogenetic analysis carried out using MEGA software, with GTR+G+I nucleotide substitution model and 1000 bootstrap replicates. Branch labels show bootstrap support values.

wings with *S. paratectipenis*, they are less developed than in *S. paratectipenis*, in which this character is well developed and begins near the cephalic region. Moreover, the eggs of *S. paratectipenis* are described as being barrel-shaped, while those of *S. orobicus* sp. nov. have a different morphology.

Molecular diagnosis

The sequence obtained from the specimen was 658 bp long, after excluding primer sequences. After running this sequence against the databases using nBLAST, the best hit was a sequence from *S. aloisei* (accession number: MG573535.1). Direct comparison of the common part of the two sequences (592 bp) showed 95.1% identity (29/592 bp differences). The alignment did not show any insertion or deletion.

In the phylogeny, these two sequences formed a monophyletic group (bootstrap support = 100%) (Figure 6). Other more distantly related sequences belong to the species *S. extenuatus*, *Spauligodon atlanticus* Astasio-Arbiza, Zapatero-Ramos, Ojeda-Rosas & Solera-Puertas, 1987, *S. cabrerae*, and *Spauligodon occidentalis* Jorge, Perera, Harris, J, Carretero, Roca, 2012, which show less than 90% sequence identity.

The DNA sequence comparison of the presented species with *S. extenuatus* shows a maximum of 88,35% identity of the common part of the sequences.

For the species *S. cabrerae*, *S. paratectipenis* and *S. tectipenis* no COI sequences are publicly available to the best of our knowledge.

Conclusion

The presence or absence of spines on the adult tails and egg morphology are the diagnostic characters used to distinguish the different *Spauligodon* between each other (Bursey et al. 2005). Geographical distribution is also taken into account in *Spauligodon* taxonomy, as it is considered by some authors to be the most important aspect in reptilian oxyurid speciation (Chabaud & Brygoo 1962).

Moreover, the threshold of COI gene identity used to discriminate species in nematodes is estimated to vary from 98% to 95% (Gonçalves et al. 2021).

The peculiar morphological characteristics, the location of the collection site, and the COI sequence percentage of identity of the described specimen indicate that it belongs to a species new to science, namely *S. orobicus* sp. nov. This species represents the first which has been reported from northern Italy, thus this area is now to be included in the distribution area of the *Spauligodon* genus.

Acknowledgments

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Disclosure statement

No potential conflict of interest was reported by the author(s).

Ethical standard

Reptile handling and manipulation were carried out under the authorization of the Italian Ministry of environment (Protocol number: 77772–21/06/2022).

Data availability statement

All the data that supports the conclusions of this study can be accessed in the paper itself and also in the GenBank database.

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