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First report of *Metathelazia capsulata* in red foxes (*Vulpes vulpes*) in Europe and new contributions to its identification

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ABSTRACT

Metathelazia capsulata is a lungworm that inhabit in the bronchi and bronchioles from mammal carnivore species, which life cycle is unknown. *M. capsulata*-like spirurid nematodes were isolated at necropsy from the respiratory tract of red foxes (*Vulpes vulpes*) from the Region of Murcia (SE Spain). The main objective of this study was to describe in detail the morphometric features of these nematodes, as well as to report some molecular markers. The principal morphometric difference compared to previous *M. capsulata* descriptions was the shorter total length for both males and females (6.6 mm and 7.4 mm, respectively). In addition, the mean values of buccal cavity depth and distance between the excretory pore and the anterior end of the nematode were also lower than those previously reported. On the other hand, sequence data of the mitochondrial (COI) and nuclear (rDNA) genes of *M. capsulata* were described, being the first time that molecular markers are reported for the genus *Metathelazia* and also for the entire family Pneumospiruridae. Based on data available from GenBank, these results indicate that *M. capsulata* sequences are closely related to the family Rhabdochonidae, which is assumed to belong to superfamily Thelazioidea, a superfamily including the family Pneumospiruridae, but also suggest the distant relations with the family Thelaziidae. This is the first time that *M. capsulata* is reported in red fox from Europe. This study provides valuable information for future phylogenetic studies on *Metathelazia* spp. nematodes and, in general, on species of the family Pneumospiruridae.

1. Introduction

The superfamily Thelazioidea comprises three families: Pneumospiruridae, Rhabdochonidae and Thelaziidae. This last family includes nematodes whose primarily host are birds and mammals, while those in Rabdochonidae parasitize marine and freshwater fishes (Chabaud and Bain, 1994). The Pneumospiruridae family includes nematodes parasitizing the respiratory system of carnivores, having little information about their transmission and life cycle (Anderson, 2000), although Gerichter (1948) suggested that an intermediate host could be involved, as occurs in other Thelazioidea nematodes.

Along with *Pneumospirura* and *Vogeloides*, *Metathelazia* is the main genus included in Pneumospiruridae (Chabaud, 1975), with a large number of species described worldwide (Chabaud and Bain, 1994) affecting lung parenchyma and bronchi of their host.

The identification of nematodes belonging to Pneumospiruridae family is frequently based on the morphological characteristics of the cephalic region. In this sense, along the years, different authors have taken into account this criterion to classify Pneumospiruridae nematodes (Dougherty, 1943; Wertheim and Chabaud, 1977); in particular, the genus *Vogeloides* presents six developed lips, the genus *Pneumospirura* is small-lipped, and in the genus *Metathelazia* lips are absent (Pence and Stone, 1977).

Metathelazia californica (Skinker, 1931), *M. bassarisci* (Pence and Stone, 1977) and *M. servalis* (Wertheim and Giladi, 1977) are some of the species that have been previously reported in carnivores such bobcat (*Lynx rufus*), cougar (*Puma concolor couguar*) or serval (*Leptailurus serval*). Moreover, the northern white-breasted hedgehog (*Erinaceus roumanicus sacer*) and the Egyptian mongoose (*Herpestes ichneumon*) have been described to host *M. multipapillata* and *M. oesophagea*, respectively (Gerichter, 1948).

Nile fox (*Vulpes vulpes nilotica*), European badger (*Meles meles*), American badger (*Taxidea taxus*) and marbled polecat (*Vormela peregusna*) are the three carnivore species in which *M. capsulata* has been

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Table 1
Mean, standard deviation (sd), range and coefficient of variation (CV) of the measures of male and female specimens, expressed in micrometers, except the total length of the nematode, which is expressed in millimeters.

	Male ($n = 14$)		Female ($n = 15$)	
	Mean ± sd (range)	CV (%)	Mean ± sd (range)	CV (%)
Length (mm)	6.6 ± 0.74 (5.2–8.4)	11.3	7.4 ± 0.75 (5.6–8.5)	10.0
Width	386 ± 0.04 (311–492)	11.0	477 ± 0.05 (318–592)	11.7
Oral cavity width	21 ± 4.6 (10.9–28.9)	21.7	20 ± 4.9 (10–30)	24.5
Oral cavity depth	11 ± 1.6 (7.2–14.2)	15.1	11 ± 2.3 (6.6–16.5)	21.0
Nerve ring – head	57 ± 8.2 (42.6–74.8)	14.4	60 ± 7.9 (43.4–74.1)	13.3
Excretory pore – head	99 ± 9.9 (87.6–125.7)	10.0	113 ± 9.3 (95.1–127.5)	8.2
Length esophagus	416 ± 32.9 (355.2–477.6)	7.9	439 ± 28.8 (381.7–493.7)	6.5
Width esophagus	64 ± 7.5 (50.8–78.5)	11.7	66 ± 8.7 (50.5–84.8)	13.2
Spicule right length	311 ± 20.7 (268.8–353.6)	6.6		
Spicule right width	17 ± 2.7 (10.8–22.8)	16.2		
Spicule left length	297 ± 19.9 (258.5–326.8)	6.7		
Spicule left width	16 ± 2.5 (11.3–20.7)	15.3		
Gubernaculum length	36 ± 4.9 (25.7–49.0)	13.5		
Gubernaculum width	10 ± 2.2 (6.4–14.5)	23.0		
Vulva – anus			122 (±17.6) 100.3–149.9	14.4
Anus – tail			67 (±10.2) 51.3–88.7	15.1
Eggs – length			45 (±6.9) 29.9–57.3	15.3
Eggs – width			34 (±6.5) 17.2–43.7	19.4

previously reported (Gerichter, 1948; Pence and Dowler, 1979). Additionally, *M. mexicana* is a new species that has been recently described from a Nine-banded armadillo (*Dasypus novemcinctus*) in Central Mexico (Jiménez et al., 2013).

To the author's knowledge, since Gerichter (1948) descriptions, no other species of the genus *Metathelazia* has been described until the moment in Europe. This lack of reports regarding nematodes of the genus *Metathelazia* contrasts with studies published in recent years concerning the genus *Vogeloides*. In particular, Blanco et al. (1993) isolated *V. oesophagea* in Egyptian mongooses from Spain, and a new species named *V. morowaliensis* has been recently described in Pallas's tube-nosed bats (*Nyctimene cephalotes*) from Indonesia (Purwaningsih et al., 2021).

In this study, a spirurid nematode detected in the bronchi of red foxes coming from the Region of Murcia (SE, Spain) was morphologically described. Also, six molecular markers were reported: one mitochondrial (cytochrome oxidase subunit I (COI) gene) and five nuclear markers (18S gene, 28S gene, 5.8S, and internal transcribed spacers (ITSs) ITS1 and ITS2) of the ribosomal DNA (rDNA). Therefore, the aim of this study was to enhance the knowledge of Pneumospiruridae family, providing morphological and molecular data of this spirurid found in red foxes.

2. Material and methods

Between 2015 and 2021, the thoracic viscera of 167 foxes from hunting programmes, wildlife recovery centres or road-killed in the Region of Murcia (SE, Spain) were obtained. Specifically, trachea, heart and lungs were extracted at necropsy and kept refrigerated in individual plastic bags until analysis. When it was not possible to complete the analysis within 24 h, these samples were frozen at −20 °C until further study. Each respiratory tract was longitudinally opened through the tracheobronchial tree and the large pulmonary vessels. Subsequently, the lung parenchyma was cut (3 × 3 cm pieces), immersed in water and manually squeezed. The content was filtered through a 63 μm of mesh sieve, and the remaining material was inspected under a Nikon SMZ645 stereomicroscope. Finally, lung tissue was artificially digested with a solution of pepsin and chlorhydric acid (Martínez-Rondán et al., 2019), in order to identify worms located in bronchioles of smaller diameter. All isolated helminths specimens were preserved in absolute ethanol until morphological identification or DNA extraction to molecular analysis. The prevalence (CI95%), median abundance (MA) and median intensity (MI) of the detected nematodes were determined according to Bush et al. (1997).

2.1. Morphometrical identification

A total of 29 nematodes (14 males and 15 females), were measured and cleared with lactophenol to carry out their description. The visualization of internal structures was performed using a Leica DM6000B microscope connected to a Leica DFC280 digital camera, while for length and width of the parasite Leica Z6 APO stereomicroscope connected to Leica DFC550 digital camera was used. Both system equipment presented Leica Application Suite V 2.5.0 software. All measurements were taking using ImageJ software and expressed in micrometers, with the exception of the total length of the nematodes, that was measured in millimeters.

2.2. Sample preparation and field emission scanning electron microscope imaging

For the study of lips details, field emission scanning electron microscopy (FE-SEM) was used. As previously mentioned, samples were fixed in absolute alcohol at laboratory temperature until processing. Subsequently, postfixation was performed with 1% osmium tetroxide during 2 h and the samples were washed in 0.1 M sodium cacodylate buffer with sucrose. Specimens were dehydrated with acetone series and dried at critical point on 0.2 μm Isopore filters (Merck Millipore Ltd). Lastly, the samples were subsequently submitted to platinum sputtering with a 5.0 nm thin film (Leica EM ACE 600) and were examined employing an FE-SEM, (ApreoS Lovac IML Thermofisher), with a selected voltage of 5 kV, 0.2 nA and working distance of 10 mm.

2.3. DNA extraction, PCR amplification, cloning and sequencing

The total DNA of six female nematodes was extracted from ethanol-fixed specimens with the standard phenol-chloroform procedures using the whole specimen for each DNA extraction. The different molecular markers (COI, 18S, 28S, 5.8S, ITS1 and ITS2) were amplified by PCR (Polymerase Chain Reaction) with specifics primer pairs (Supplementary material). Specific primer pairs were used to amplify COI, 18S, and 28S sequences (De Bellock et al., 2001; Casiraghi et al., 2001; Xiang et al., 2013), while for the ISTs and 5.8S three primer pairs combinations were used (Supplementary material). The combination of the primer pair ITS1-F and nema28_R68 allowed the amplification of the ITS1, 5.8S and ITS2 in a long fragment of about 2 kb (Itagaki et al., 2005; Xiang et al., 2013). As this fragment was difficult to amplify, to facilitate the amplification of these markers, two new primer ITS1-int-R and ITS2-int-F located on the 5.8S sequence were designed as internal primers to combine to the ITS1-F and nema28_R68 primers. Thus, the combination of ITS1-F and ITS1-int-R allowed the amplification of ITS1 and part of the 5.8S, and the combination of ITS2-int-F and nema28_R68 allowed the amplification of part of the 5.8S and the ITS2. PCRs were performed in 50 μl of reaction, containing 30–100 ng of template DNA. The PCR

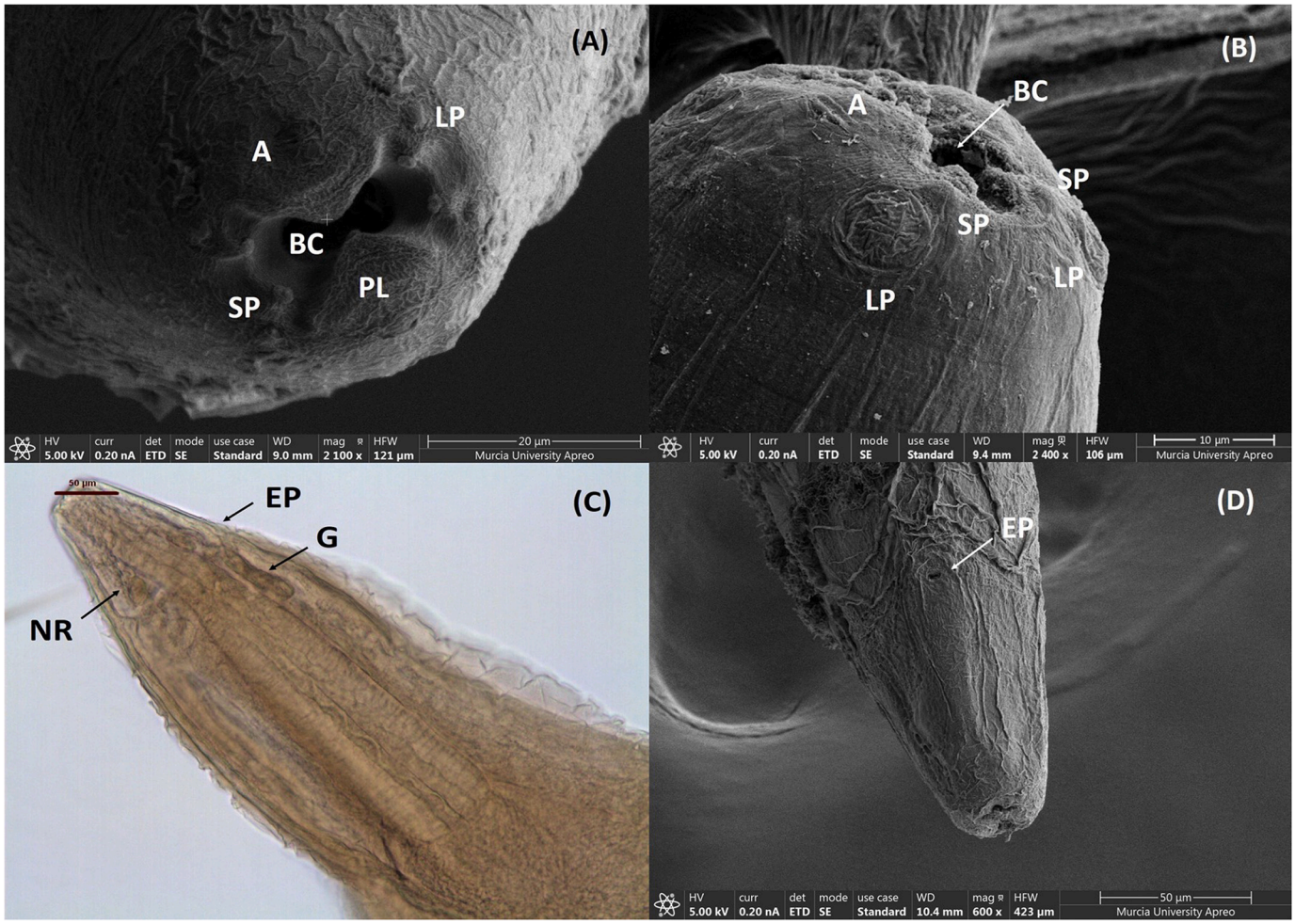

Fig. 1. Scanning electron micrograph with a frontal view (A) and lateral view (B) of the buccal cavity (BC) of a *Metathelazia capsulata* female. Four papillae pairs: two small pairs (SP) and two large pairs (LP) can be distinguished. The amphids (A) are at the bottom of the two pseudolabials (PL). (C) Excretory pore (EP) can be distinguished together with the gland (G), as well as the constriction at the level of the nerve ring (NR). (D) Scanning electron micrograph showing the opening of the excretory pore (EP).

conditions were as follows: initial denaturation at 95 °C for 4 min; 35 cycles with denaturation 30s at 95 °C, annealing temperature 90s and polymerase extension 120 s at 72 °C; and a final extension of 5 min at 72 °C. The annealing temperatures were 50 °C for COI, 18S, 28S and ITS1–5.8S-ITS2, 48 °C for ITS1, and 54 °C for ITS2. In order to get an accurate sequencing, the amplicons were resolved in 1% ethidium bromide-stained agarose gels, and they were isolated from the gel with a QIAquick gel extraction kit (Qiagen) and cloned in *Z*-Competent JM109 *E. coli* (Zymo Research) using the PGEMTeasy vector (Promega). Positive clones were Sanger sequenced with T7 and SP6 primers.

2.4. Sequence and phylogenetic analysis

The sequences were analysed and aligned using Bioedit program (version 7.0.9.0) (http://www.mbio.ncsu.edu/BioEdit/bioedit.html) and Clustal Omega (Sievers and Higgins, 2021; Madeira et al., 2022). The obtained sequences were aligned and compared with those belonging to other nematode species using the Blast tool (Altschul et al., 1990). The phylogenetic relationships were reconstructed with Maximum-Likelihood (ML) (Nei and Kumar, 2000) using MEGA X (Kumar et al., 2018). Node supports in the analysis were assessed with 2000 bootstrap replicates. For this analysis, the COI sequences from different nematode species available in GenBank considering a 100% of coverage and the highest percentages of identity with our sequences were used: *Chandlerella quiscali*, HM773029; *Cylicospirura subaequalis*, GQ342968; *Cylicospirura felineus*, GQ342967; *Dirofilaria* sp. *Hongkongensis*, KX265050; *Dirofilaria repens*, KF692102; *Gongylonema pulchrum*, AP017685; *Gongylonema nepalensis*, LC388892; *Onchocerca ochengi*, NC_031891; *Rhabdochona xiphophori*, MH778493; *Rhabdochona salgadoi*, MH778492; *Setaria labiatopapillosa*, NC_044071; *Spinitectus osorioi*, MN592671; *Spirocerca sp*, KJ605489; *Spirocerca lupi*, MT522373; *Thelazia rhodesi*, MT511659; *Thelazia callipaeda*, AM042556. The best-fit nucleotide substitution model with the lowest BIC (Bayesian Information Criterion) value was chosen using MEGA X, and for these sequences was GTR + G.

3. Results

Since the goal of the present study was to describe the morphometric characteristics and to determine some molecular markers of *M. capsulata*, only the results concerning this nematode species are presented. Specifically, the overall prevalence of foxes parasitized by *M. capsulata* was 32.9% (25.8–40.0), with a total of 407 nematodes recorded (MA = 0, range = 0–117; MI = 2, range = 1–117).

Measurements from the studied nematodes are shown in Table 1, as well as standard deviation, range and variation coefficient.

3.1. Species description

Nematodes have a tegumental sheath covering the body with thick

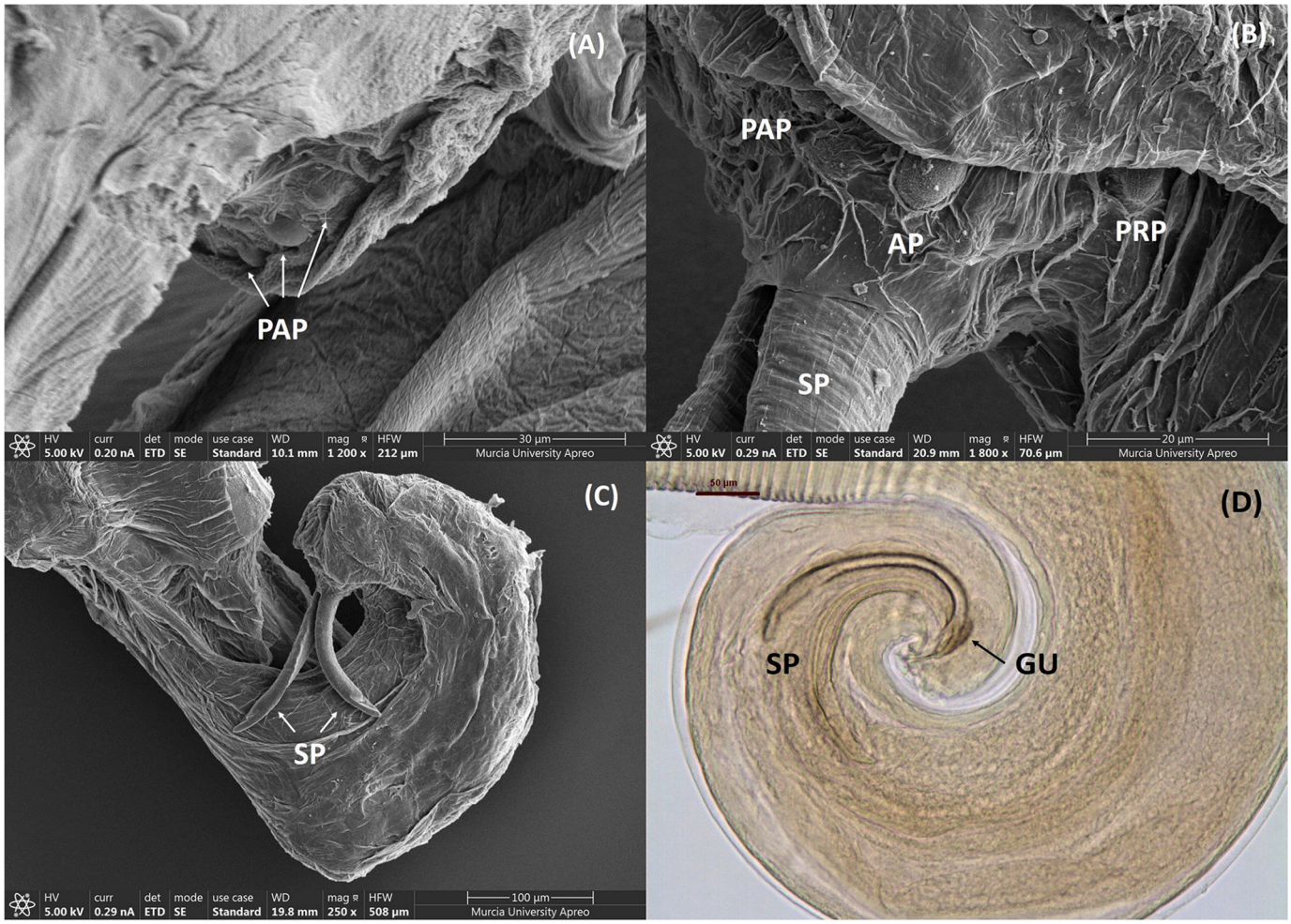

Fig. 2. Scanning electron micrograph of the caudal end of a male specimen of *M. capsulata*. (A) Postanal papillae (PAP). (B) Preanal papillae (PRP), adanal papillae (AP) and postanal papillae (PAP), and spicules (SP). (C) Falciform spicules (SP) with pointed end. (D) Optical microscope image in which the presence of double gubernaculum (GU) can be appreciated.

and smooth cuticle with a whitish appearance. The buccal cavity has two pseudolabia and four papillae pairs in two circles, a pair each located laterally at dorsal and ventral margins of buccal capsule. Also, there are two big lateral amphids (Fig. 1a,b). Excretory pore presents a small gland and the esophagus is club-shaped with a short muscular anterior part and a longer glandular posterior part, with a constriction coinciding with the nerve ring (Fig. 1c,d).

These parasites showed a clear sexual dimorphism. The width of males is uniform throughout the body. The tail is disposed in a spirally shaped, with conical shape ending and absent caudal alae. There are six pairs of lateral papillae with the following disposition: two preanal pairs, one adanal pair and three postanal pairs (Fig. 2a,b). The spicules are falciform and slightly different in length (Table 1) with pointed end and a double tear-shaped gubernaculum is present (Fig. 2c,d).

The body of females becomes gradually wider until the tail, which is rounded and presents an ending dorsal bending mucron. Vulva and anus are close to the posterior end, coinciding with a strong muscular system at the terminal portion of the vagina (Fig. 3a,b). Eggs are numerous, oval-shaped and thick-shelled. They are completely embryonated at the terminal part of the uterus (Fig. 3c,d).

3.2. Sequences analysis

Values of number of analysed sequences, length, A + T content, and the intraindividual and interindividual variation of each molecular markers are reported in Table 2.

COI sequences (ON995618-ON995623) were obtained from three nematodes and the alignment showed very little intraindividual and interindividual variation both due to several substitutions but not to deletion or insertion (Table 2). In fact, all of them could be translated to the corresponding protein fragment without stop codon. Nucleotide variation in COI sequences and the A + T content were similar to that of other nematode species (Otranto et al., 2005; Iorio et al., 2009; Bai et al., 2020). As no sequences for this genus or family are previously described we aligned the COI sequences with some that covered the same region and are available in GenBanK belonging from different nematode genera (*Chandlerella*, *Cylicospirura*, *Dirofilaria*, *Gongylonema*, *Onchocerca*, *Rhabdochona*, *Setaria*, *Spinitectus*, *Spirocerca* and *Thelazia*). The result of the alignment demonstrated that the highest identity percentage is observed with the COI sequence of the species *Rhabdochona salgadoi* (84.3%) (family Rhabdochonidae) and the lowest with the species *Thelazia callipaeda* (80.0%) (family Thelaziidae).

18S rDNA sequences (OP004062-OP004065) were obtained from two nematodes. As for COI sequences, we aligned the 18S sequences with sequences of the same region that are available in GenBanK belonging from different nematode genera (*Gongylonema*, *Ichtyobronema*, *Oxyspirura*, *Physaloptera, Rhabdochona*, *Spinitectus*, *Spirocerca*, *Streptopharagus* and *Thelazia*). The result of the alignment demonstrated that the highest identity percentage is observed with the 18S sequence of *Physaloptera turgida* (95.0%) (Physalopteridae) and the lowest with *Thelazia lacrymalis* (88.7%) (Thelaziidae).

28S rDNA sequences (OP021864-OP021869) were obtained from

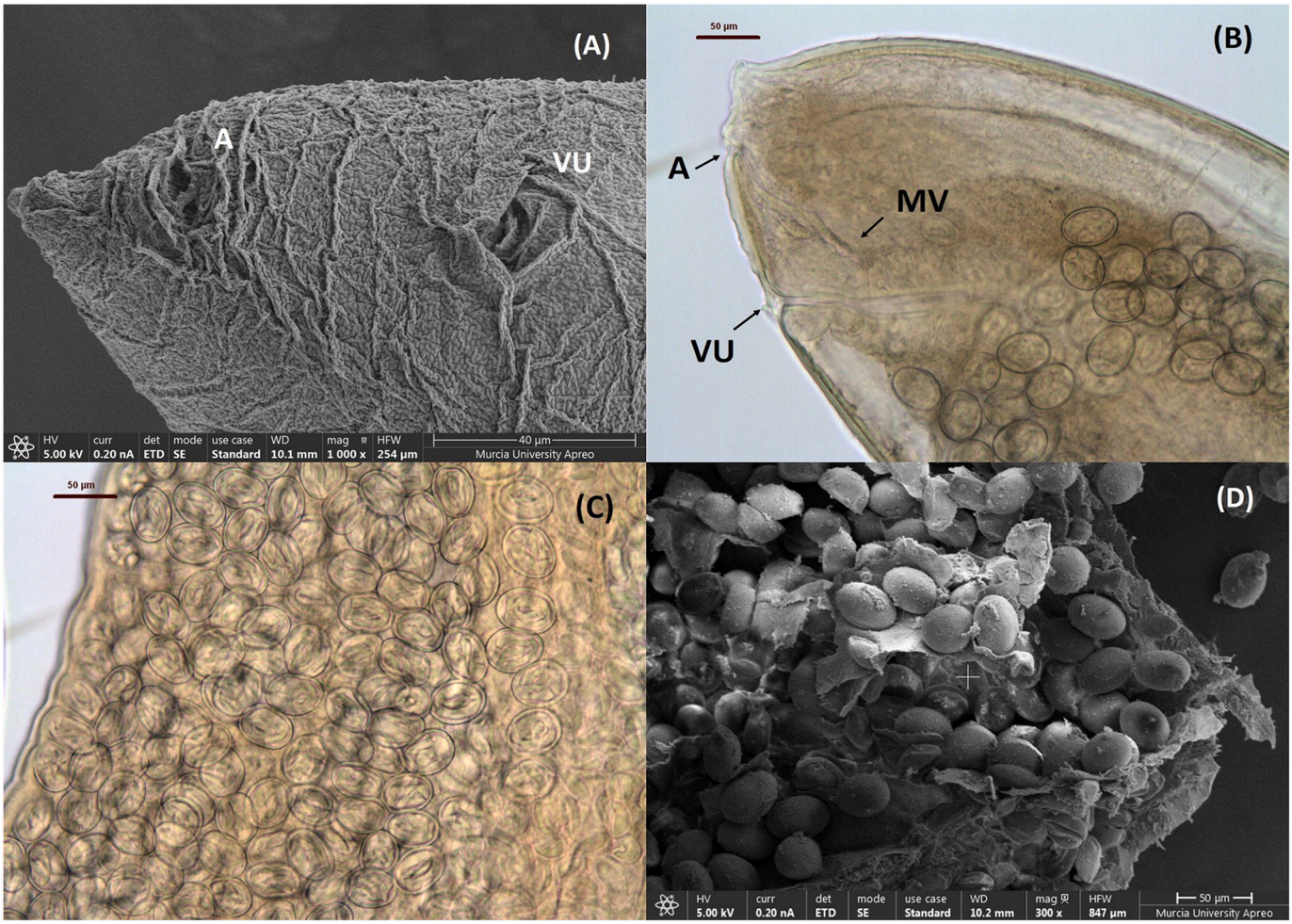

Fig. 3. (A) Scanning electron micrograph of a female specimen of *M. capsulata* showing the vulva (VU) and anus (A) close to the posterior end. (B) Optical microscope image showing the musculature of the vagina (MV) and the vulva (VU) and anus (A) position. Oval-shaped and thick-shelled eggs viewed through the optical (C) and the scanning electron micrograph (D).

Table 2
Sequence number, length, A + T content (%), intraindividual and interindividual variations (%) of each molecular marker.

Molecular Markers	N° of sequences	Length (bp)	A + T content (%)	Individual variation (%)	
				Intraindividual	Interindividual
COI	6	689	69.28–69.81	0.00–0.29	0.29–1.45
18S rDNA	4	1775	51.04–51.21	0.06–0.39	0.17–0.23
28S rDNA	6	972	54.32–54.53	0.0–0.41	0.0–0.31
5.8S rDNA	6	153	51.63	0.0–0.65	0.0–0.65
ITS1	6	734	57.90–58.17	0.54–0.69	0.54–0.69
ITS2	6	835–873	58.68–60.48	1.1–2.3	0.4–4.5

three nematodes. The Blast search performed with our sequences in GenBank reported sequences from other distant families of nematodes. However, the coverage was low (around 50%), and the highest identity (86.9%) for this coverage corresponded to the species *Gongylonema neoplasticum* belonging to the family Gongylonematidae.

With the different combinations of the ITSs primer pairs (Supplementary material), six ITS1, ITS2 and 5.8S sequences were obtained (OP059074-OP059083) from three nematodes. For ITS1, the intraindividual and interindividual are due to random distributed substitutions. For the ITS2, the length varied from 835 bp to 873 bp mainly due to variation in a microsatellite (TA)n, and the A + T content of these sequences of *Habronema microstoma* and *Habronema muscae* were similar being 70.5% and 64.2%, respectively (Traversa et al., 2004).

The interspecific variation of the ITSs sequences was very high even between closely related sequences (Blouin, 2002). As no sequences for closely related species were available in GenBank, the alignment of the ITSs sequences with those of other nematode species belonging from other families was very low. In fact, Blast search in GenBank resulted in coverages inferior to 12% of the length of the analysed sequences.

Of the 5.8S rDNA sequences analysed five were identical, while one presented a substitution. The Blast search in GenBank demonstrated that the sequences from species of the genera *Protospirura*, *Spirurina*, *Gongylonema* and *Mastophorus* have the highest identity percentages (96.7%, 96.1%, 96.0% and 95.4%, respectively).

3.3. Phylogenetic analysis

In this work, the partial sequence of the COI was used for the

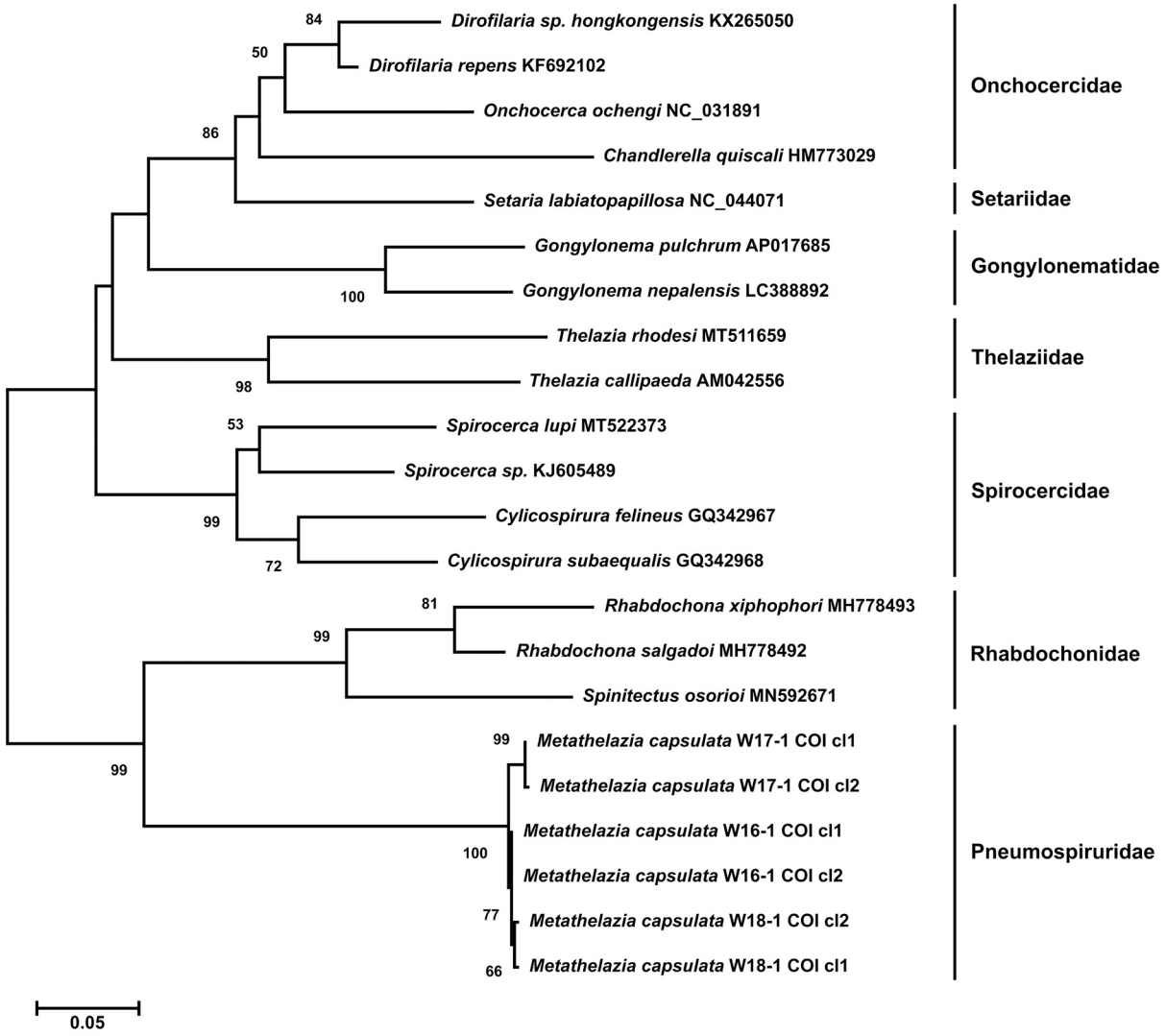

Fig. 4. Maximum likelihood tree using of COI sequences of *Metathelazia capsulata* and sixteen different nematode species (GTR + G model). Bootstrap supports of 2000 replicates greater than 50 are shown at each node. The scale bar represents the number of nucleotide substitutions per site.

phylogenetic analysis. Sixteen COI sequences from different nematode species were selected from GenBank considering a 100% coverture and the highest identity percentages with the analysed fragment (see Material and Methods section). The obtained Maximum-likelihood tree (Fig. 4) grouped all the sequences of *M. capsulata* showing a close relationship with the clade that included the species of the genera *Rhabdochona* and *Spinitectus* (family Rhabdochonidae) as expected for a species from the Pneumospiruridae family. However, the species of the family Thelaziidae (genera *Spirocerca* and *Thelazia*) are situated on clades more distant.

4. Discussion

To the authors' knowledge, this is the first record of the *Metathelazia* genus in red foxes in Europe, and, specifically, the first time that *M. capsulata* is found in the bronchial tree of this wild canid. This species was reported previously in other wild carnivore species in America and Palestine (Gerichter, 1948; and Pence and Dowler, 1979). The results indicate that this species is significantly prevalent in the study area. The most recent study about *M. capsulata* prevalence (Pence and Dowler, 1979) found a prevalence of 47% in American badgers. It is noteworthy, on the other hand, that one of the foxes in our study had a *M. capsulata* intensity (117 specimens) that is the highest value described to date in carnivores.

Most of the morphological characteristics found in the studied specimens were in accordance with those described by Skinker (1931): poorly developed lips and buccal capsule, similar male spicules and, in case of females, long, pointed and conical tail, and vulva distant from the anus in the posterior end.

As proposed by Wertheim and Chabaud (1977), the nematode species described in this study should be classified as *M. capsulata*, since the buccal capsule had two major lobes (pseudolabials) and four smaller submedian. Moreover, these characteristics of the cephalic region agrees with other species of the genus as *M. mexicana* (Jiménez et al., 2013).

As noted above, several species of the genus *Metathelazia* have been previously described (Table 3). Concerning *M. capsulata*, our results reveal some differences compared to previously descriptions. When comparing the measures with those described by Gerichter (1948) and Pence and Dowler (1979), one of the main differences is the individual size. Specifically, the mean length size of the nematodes found from red foxes in this study was 6.6 and 7.4 mm for males and females, respectively. However, previous authors reported higher values of the same measure (10 mm and 18 mm for males and females, respectively). The same happened to other measures as buccal cavity depth or distance between the excretory pore and the anterior end of the nematode; in both cases, our measures were lower than those previous described

Table 3
Measurements of some nematode species described from the family Pneumospiruridae, expressed in micrometers, except the total length of the nematode, which is expressed in millimeters.

	Pneumospirura bassarisci (Pence and Stone, 1977)		*Vogeloides felis* (Vogel, 1928) (Pence and Stone, 1977)		*Metathelazia californica* (Skinker, 1931) (Pence and Stone, 1977)		*Metathelazia multipapillata* (Gerichter, 1948)	
	Male *n* = 4	Female *n* = 9	Male *n* = 30	Female n = 30	Male n = 30	Female n = 30	Male	Female
Lenght	6.34 (5.30–6.42; 6.02)	10.69 (7.47–11.20; 9.49)	5.73–7.62 (6.63)	15.7–20.9 (18.9)	11.8–16.5 (13.4)	37.5–56.4 (43.1)	14–16	30–40
Width	227 (222–258; 235)	404 (216–408; 327)	162–346 (275)	442–589 (502)	104–125 (112)	184–236 (204)	130–140	190–260
Lenght esophagus							340–390	340–420
Width esophagus							40–50	70–80
Muscular esophagus	105 (110–117; 111)	117 (116–140; 125)	125–249 (167)	140–234 (177)	152–199 (171)	216–275 (236)		
Glandular esophagus	246 (237–257; 247)	242 (234–281; 250)	193–310 (251)	287–386 (332)	316–392 (358)	298–503 (404)		
Bucal deep	24 (22–25; 20)	26 (14–28; 19)	16–35 (22)	25–35 (28)	5–7 (6)	6–12 (10)		
Bucal lenght								
Nerve ring - head	80 (65–80; 75)	82 (76–88; 81)	61–113 (82)	64–99 (84)	99–135 (119)	117–140 (209)		
Excretory pore - head	82 (80–84; 82)	105 (90–108; 97)	70–146 (93)	59–94 (76)	140–216 (180)			
Spicule	181 (140–199; 173)		199–322 (244)		187–234 (210)		170–190	
Spicule width								
Gubernaculum	41 (41–47; 43)		32–53 (39)		29–37 (31)		27–30	
Gubernaculum width								
Cloacal opening - tail	59		47–70 (56)		40–70 (61)			
Vulva - anus		152 (106–164; 142)		118–220 (158)		74–182 (117)		100–160
Anus - tail		47 (47–59; 53)		37–88 (65)		258–353 (308)		60–70
Eggs - lenght		47 (47–53; 50)		41–49 (46)		40–48 (44)		47–49
Eggs - width		37 (36–40; 38)		26–36 (34)		28–34 (32)		33–35
Vagina								2400–3000

	Metathelazia oesophagea (Gerichter, 1948)		*Metathelazia capsulata* (Pence and Dowler, 1979)		*Metathelazia mexicana* (Jiménez et al., 2013)		*Metathelazia capsulata* (Gerichter, 1948)	
	Male	Female	Male	Female	Male n = 4	Female *n* = 11	Male	Female
Lenght	6.6–6.8 mm	7.8–8.2 mm	8.63–12.05 (10.07) mm	11.56–21.83 (17.13) mm	6.55–8.34 (7.23) mm	7.97–1.04 (1.04) mm	10–12 mm	18–20 mm
Width	260–280	340–370	131–346 (273)	294–603 (442)	238–273 (256)	261–461 (346)	290–360	440–470
Lenght esophagus	1000–1200	1230–1270			444–485 (474)	407–525 (466)	420–470	520–550
Width esophagus	190–210	230–270					70–90	70–100
Muscular esophagus			205–345 (269)	222–345 (293)				
Glandular esophagus			51–179 (117)	96–164 (137)				
Bucal deep			12–54 (30)	10–50 (27)	13–23 (17)	13–28 (21)		
Bucal lenght			12–44 (28)	12–47 (34)				
Nerve ring - head			49–140 (89)	82–132 (110)	59–79 (68)	58–81 (70)		
Excretory pore - head			72–130 (100)	62–204 (152)	69–109 (92)	90–121 (107)		
Spicule length	210–230		183–315 (260)		190–231 (214)		310–340	
Spicule width			12–15 (18)					
Gubernaculum length	38–39		25–53 (41)		40–79 (53)		45–54	
Gubernaculum width			8–18 (12)					
Cloacal opening - tail			36–76 (54)		74–83 (78)			
Vulva - anus		80–120		125–249 (187)		183–259 (204)		140–160
Anus - tail		100		35–94 (71)		66–87 (78)		100
Eggs - lenght		40–46		42–50 (44)		40–52 (44)		47
Eggs - width		30–35		29–37 (33)		24–39 (31)		38
Vagina		1000–1100						1900–2600

(Gerichter, 1948; Pence and Dowler, 1979). As for the remaining data, although the mean values were not similar, the ranges can be considered in agreement with the description of *M. capsulata*, as well as the general description of the parasite. On the other hand, the number and arrangement of the observed papillae coincides with those described by Pence and Dowler (1979).

Specimens from this study provided evidence of the wide metric variability that can occur in *M. capsulata*. In fact, Pence and Dowler (1979) discussed similar phenomenon when they compared their results with those of Gerichter (1948). In this sense, Jiménez et al. (2013) suggested that differences observed in nematodes recovered from badgers in America (New World), and those from badgers, foxes and polecats in Palestine (Old World), could in fact be different species. However, they did not dispose of the original samples to be able to carry out comparisons and verify his theory. As recommended these authors, based on the variability in the measures found in relation with previous *M. capsulata* descriptions, if the original specimens would be available, more detailed comparative studies could be carried out to determine

whether this is, in fact, a new species of *Metathelazia*.

On the other hand, this work is the first to present molecular markers for the genus *Metathelazia* and also for the entire family Pneumospiruridae. In fact, sequence data of the mitochondrial (COI) and nuclear (rDNA) genes of *M. capsulata* are described. The marker analysed presented the usual characteristic in relation to the sequence length, A + T content and variation described for the same marker in other nematode species (Pereira and Baldwin, 2016; Bai et al., 2020).

The analysed markers are commonly used for nematode species identification, diagnostic and phylogenetic analysis (Iorio et al., 2009; Pereira and Baldwin, 2016; Choudhury and Nadler, 2018; Bai et al., 2020). As no information has been reported about molecular markers of this genus or even of this family, we have not been able to compare our data with those from closely related species. In fact, for analysed sequences, the Blast searches in GenBank reported sequences from other nematode families.

Mitochondrial molecular markers are currently being used for species identification in several organism groups including nematode species (Blouin, 2002; Otranto et al., 2005, 2007). Especially the COI gene is a widely used gene as important barcoding for species identifications and for phylogenetic analysis. Our phylogenetic analysis results with this gene agree with the consideration that the genera *Rhabdochona* and *Spinitectus* are closely related (Choudhury and Nadler, 2018). Our results indicate that sequences of *M. capsulata* are closely related to the family Rhabdochonidae, which is assumed to belong to superfamily Thelazioidea (Černotíková et al., 2011), a superfamily that includes species of the family Pneumospiruridae, but also suggest the distant relations with the family Thelaziidae.

5. Conclusions

The morphological and molecular data provided by our study will be useful in future research for the identification of *M. capsulata*, offering valuable information that will serve as a basis for phylogenetic studies of nematodes belonging to the genus *Metathelazia* and, in general, of species of the family Pneumospiruridae.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rvsc.2022.12.016.

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