First report of *Filaria martis* Gmelin, 1790 in the European mink, *Mustela lutreola* (Linnaeus, 1761)

Jordi Torres[1,2], Jordi Miquel[1,2], Christine Fournier-Chambrillon[3], Adrien André[4], Fermín Urra Maya[5], Gloria Giralda Carrera[6], Pascal Fournier[3]

[1] Departament de Biologia, Sanitat i Medi ambient, Universitat de Barcelona, Av. Joan XXIII, sn, 08028 Barcelona, Spain

[2] Institut de Recerca de la Biodiversitat (IRBio), Universitat de Barcelona, Av. Diagonal 645, 08028 Barcelona, Spain

[3] GREGE, Route de Préchac, 33730 Villandraut, France

[4] Laboratoire de Biologie Evolutive, Unité de Génetique de la Conservation, Université de Liège, Institut de Botanique B22, Quartier Vallée 1, Chemin de la Vallée 4, 4000 Liège, Belgium

[5] Gestión Ambiental de Navarra S.A., Padre Adoain, 219 Bajo, 31015 Pamplona, Spain

[6] Servicio de Conservación de la Biodiversidad del Gobierno de Navarra, C/ González Tablas 9, 31005 Pamplona, Spain

Corresponding author:

Jordi Torres

Departament de Biologia, Sanitat i Medi ambient, Facultat de Farmàcia, Universitat de Barcelona, Av. Joan XXIII, sn, 08028 Barcelona, Spain

Tel.: + (34) 93 402 45 00

Fax: + (34) 93 402 45 04

E-mail: jtorres@ub.edu

Abstract: The riparian European mink (*Mustela lutreola*), currently surviving in only three unconnected sites in Europe, is now listed as a critically endangered species according to the IUCN. Habitat loss and degradation, anthropic mortality, interaction with the feral American mink (*Neovison vison*), and infectious diseases are among the principal causes of its decline. Surveys of helminth parasites of this host that also include focus on subcutaneous potentially pathogenic helminths such as those belonging to the genus *Filaria* are very scarce. We report here the presence of specimens of *Filaria martis* in the subcutaneous connective tissues of three *M. lutreola* individuals from Spain. This is the first finding of a subcutaneous nematode in a representative of the genus *Mustela.* The report also enlarges the known range of the definitive hosts of this nematode. These worms were mainly located in the dorsal region of mink and more rarely in the knees, elbows and hips. Skin sloughing was only observed in one *M. lutreola* with both septicaemia and an associated high burden of *F. martis*. Therefore, more attention should be paid to potentially pathogenic helminths when designing conservation programs dedicated to *M. lutreola*.

Keywords: *Filaria martis*, *Mustela lutreola*, Spain, new host record

Introduction

The European mink, *Mustela lutreola* (L., 1761) is a small territorial riparian mustelid, which has suffered a dramatic distribution and population decline over the last century (Maran et al. 1998; Maizeret et al. 2002) being currently listed as critically endangered according to the IUCN (Maran et al. 2011). The European mink populations are currently distributed in three well-defined, unconnected areas (northern Spain and south-western France, Romania, and Belarus and Russia). These populations are still declining due to several factors including anthropic pressure (e.g. habitat loss and degradation, vehicle collisions, accidental trapping, dog predation, hunting), interspecific competition with the alien invasive American mink (*Neovison vison*, formerly *Mustela vison*), and several infectious diseases. In recent years numerous studies on habitat use, spatial behaviour, health and reproductive status, and conservation genetics have been conducted on the western population of *M. lutreola* to understand the causes of its decline and to propose conservation measures (Fournier-Chambrillon et al. 2004, 2010; Michaux et al. 2005;

Zabala et al. 2006; Fournier et al. 2007, 2008; Philippa et al. 2008; Moinet et al. 2010). The highest density of *M. lutreola* in its western population was detected in 2004 in Navarre (Spain), where a health survey was thus initiated by the government, including necropsies on animals found dead in the natural environment (Ceña et al. 2005; Fournier-Chambrillon and Fournier 2013). Earlier parasitological surveys have allowed a thorough characterization of the helminth fauna of *M. lutreola* in Europe (Shimalov et al. 1993; Sidorovich and Anisimova 1993; Torres et al. 2003, 2008) but most of the individuals surveyed were not examined for the presence of subcutaneous potentially pathogenic helminths such as those belonging to the genus *Filaria* Gmelin, 1790. Therefore, the results here presented provide novel information about the presence of these scantly known nematodes parasitizing mustelids in Europe.

Materials and methods

Carcasses of 91 *M. lutreola* specimens were collected between 1999 and 2013 in the Spanish Foral Community of Navarre (Fig. 1). They were kept frozen and then necropsied by trained veterinarians in order to determine the cause of death of each animal. A range of samples was taken at this time for genetic, virological, bacteriologic and histological studies. When it was possible the skin of several specimens was completely removed to enable examination for any subcutaneous lesions, but given the major traumas to which the animals were subjected by road collision (major cause of mortality) only 25 specimens could be completely examined. All the nematodes found in the subcutaneous tissues were removed and transferred to vials containing 70 % ethanol. Some individuals were later processed using conventional techniques for nematodes (cleared in Amman’s lactophenol) allowing their specific identification according to previous descriptions of *F. martis* given in Anderson (1960) and Chabaud and Mohammad (1989). Later this identification was molecularly corroborated.

Genomic DNA was isolated from an individual worm stored in ethanol using a QIAamp DNA Mini Kit and a Qiacube (Qiagen Inc, Hilden, Germany), according to manufacturer’s instructions with the following minor modifications: one single elution of 50 µl was performed with an incubation time of 3 min. A partial sequence of the mitochondrial cytochrome oxidase I (COI) gene was amplified using the primers CO1intF (5-TGATTGGTGG TTTTGGTAA-3) and CO1intR (5-ATAAGTACGAGTATCAATATC-3) designed by Casiraghi et al. (2001). PCR reactions were performed in a 25 µl final volume under the following conditions: 2.5 µl of each primer (10 µM), 10 µl of multiplex PCR Mastermix (Qiagen Inc., Hilden, Germany), 2 µl of DNA and 8 µl of HyPure Molecular Biology Grade Water (VWR, Radnor, USA). The thermal profile used was: an activation step at 94 °C for 5 min, followed by 40 cycles (denaturation at 94 °C for 45 sec, annealing at 52 °C for 45 sec, extension at 72 °C for 90 sec) and a final extension step at 72 °C for 5 min. All PCR reactions were performed with a Unocycler thermocycler (VWR, Radnor, USA). PCR products were visualized on a 1 % agarose gel. Sanger-sequencing was performed and chromatograms were inspected visually to resolve any ambiguities. Forward and Reverse sequences were aligned using Clustal-W inplanted in MEGA 6 (Tamura et al. 2013) and a resulting 663 bp long has been obtained. The sequence has been deposited in GenBank (accession number: KU761590) and compared to previously published GenBank sequences.

Results

The presence of several specimens of *F. martis* in the subcutaneous connective tissue was observed in three (one male and two females) adult European minks that represent a prevalence of 12.0 % (Fig. 1). The morphological and morphometric data of our *Filaria* specimens are consistent with those previously reported for this species in other hosts by Anderson (1960), Chabaud and Mohammad (1989) and Otranto et al. (2007). The molecular analysis (Fig. 2) presented a 99 % homology with the previously published GenBank 642 bp long *F. martis* sequence (AJ544880.1). Only two nematodes in the most infected *M. lutreola* specimen appeared trapped in subcutaneous membranous capsules whilst the remaining individuals occurred free in the subcutaneous tissue (Figs. 3 and 4). Nematodes were recovered mainly from the dorsal region of the hosts but also from the knees, elbows and hips. Furthermore, skin sloughing was observed in the most infected European mink, in which a septicaemia (*Enterococcus*, *Staphylococcus* and *Macrococcus*) was detected.

Discussion

Nematodes belonging to the genus *Filaria* Gmelin, 1790 have long caused controversy among parasitologists. The most common subcutaneous filarial findings in mustelid carnivores were attributed to *Filaria martis s.l.* having been reported repeatedly in Europe, North America and Japan. However, Chabaud and Mohammad (1989) studied specimens from various hosts and reported eleven species within the genus *Filaria*.

The type species *F. martis* Gmelin, 1790 was described for the first time parasitizing *Martes martes* (formerly *Mustela martes*) in Italy and later on, it was also reported in *Martes foina* in the same country (Otranto et al. 2007). This species seemed to have a cosmopolitan pattern of distribution infecting several mammals, but nowadays it is accepted that *F. martis* only affects Palaearctic Mustelidae species (Chabaud and Mohammad 1989). *Filaria martis* has been sporadically reported in *M. foina* specimens from Switzerland (Zimmerli 1981) and Spain (Sospedra 2000) but no consistent information is available about its prevalence in any host under natural conditions throughout its distribution range except for Italy (Otranto et al. 2007). The latter authors reported a prevalence of 52.3 % in road-killed *M. foina*, from 1997 to 2006 in the Italian province of Lecce, even though it was never found in *M. foina* specimens from the nearby Italian province of Basilicata. To our knowledge the present report of a filariid in *M. lutreola* constitutes the first finding of a subcutaneous nematode in this mustelid and also in any representative species of the genus *Mustela* Linnaeus, 1758. The relatively low number of specimens examined (n=25) does not allow any definitive conclusion about the true prevalence of *F. martis* in the current occidental population of the European mink.

Filarioids are all transmitted by haematophagous arthropods. The Filariidae elicit skin lesions and release eggs and/or larvae which attract arthropod vectors, mainly Muscidae. However, no data about the life cycle of *F. martis* are currently known but its transmission process should be similar to that of *F. taxideae*, the only representative of the genus *Filaria* for which reliable life cycle data are available (see Anderson 2000). The eggs of *F. taxideae*, a species that infects American badgers (*Taxidea taxus*) and striped skunks (*Mephitis mephitis*), produce skin lesions after being laid between the epidermis and the cutaneous basement membrane. The eggs and larvae become exposed at the surface of the skin, where they are available to intermediate hosts involved in transmission, that are attracted to open sores on mammals. The potential vectors, perhaps muscid dipterans attracted to lesions as pointed out by O'Toole et al. (1993), are not yet known with certainty. Otranto et al. (2007) found some *M. foina* infected by both *F. martis* and the tick *Haemaphysalis erinacei* but these authors could not draw any conclusions about the life cycle of *F. martis*. In all the surveys carried out on *M. lutreola* in Spain, so far no ticks and fleas have been detected, most likely because of the time interval between host death and subsequent discovery of the carcass.

Little information is currently available about the pathology that *F. martis* can produce in its hosts, but it is known that these infections usually do not evoke a significant inflammatory response (Anderson 1960). In our study, in the most infected specimen of *M. lutreola*, on which septicaemia was detected, two nematodes were apparently trapped in subcutaneous membranous capsules, and skin sloughing was also observed in this individual.

In a broader context it can be pointed out that nematodes of the genus *Filaria* cause a poorly known subcutaneous filariasis in mustelids and no robust data are available yet about the extent of their occurrence. However, the present report enlarges the known range of the definitive hosts of *F. martis* including now a representative of the genus *Mustela* (*M. lutreola*), which paradoxically is the more endangered and has the lowest population density of all representatives of the genus in Europe.

Concluding remarks

More attention should be paid in the future to the presence of this potentially pathogenic nematode (*F. martis*) when designing conservation programs dedicated to the critically endangered *M. lutreola*. To now the relatively low number of *M. lutreola* specimens examined worldwide does not allow taking any robust conclusion about the true prevalence and potential pathology of *F. martis* on the current populations of this very endangered carnivore.

Acknowledgments: The present study was partly supported by a Spanish grant from the “Agència de Gestió d’Ajuts Universitaris i de Recerca – AGAUR” (no. 2014 SGR 1241). The necropsies program was funded by the “Departamento de Desarrollo Rural, Medio Ambiente y Administración Local del Gobierno de Navarra” and the carcasses were collected by the “Sección de Hábitats y Sección de Guarderío de Gobierno de Navarra, Equipo de Biodiversidad de Gestión Ambiental de Navarra S.A.”

References

Anderson RC (1960) A study of *Filaria martis* Gmelin, 1790 from *Martes foina* and *Pedetes caffer*. Can J Zool 38:157–167. doi:10.1139/z60-018

Anderson RC (2000) Nematode parasites of vertebrates. Their development and transmission. CABI Publishing, Wallingford, 650 pp.

Casiraghi M, Anderson TJC, Bandi C, Bazzocchi C, Genchi C (2001) A phylogenetic analysis of filarial nematodes: comparison with the phylogeny of *Wolbachia* endosymbionts. Parasitology 122, 93–103. doi:10.1017/S0031182000007149

Ceña JC, Bidegain I, Itoiz U, Alfaro I, Berasategui G, Ceña A, Alvarez I, López de Luzuriaga J, Sánchez D, Cano MJ, Díez N, Hidalgo R, García-Marín F, Ferreras C, Carbajal A, Sánchez-Migallón D, Gómez-Moliner B, Cabría M, Urra Maya F, (2005) Estimación de la población de Visón Europeo (*Mustela lutreola*) en Navarra. 2004. Gestión Ambiental, Viveros y Repoblaciones de Navarra, S.A. & Gobierno de Navarra, 255 pp.

Chabaud AG, Mohammad MK (1989) Le genre *Filaria* Gmelin, 1790. Description de quatre espèces nouvelles. Bull Mus natn Hist nat, Paris 4ème sér, 11, sect A, 1:47–59.

Fournier P, Maizeret C, Jiménez D, Chusseau JP, Aulagnier S, Spitz F (2007) Habitat utilization by sympatric European mink *Mustela lutreola* and polecats *Mustela putorius* in south-western France. Acta Theriol 52:1–12. doi:10.1007/BF03194194

Fournier P, Maizeret C, Fournier-Chambrillon C, Ilbert N, Aulagnier S, Spitz F (2008) Spatial behaviour of European mink *Mustela lutreola* and polecat *Mustela putorius* in south-western France. Acta Theriol 53:343–354. doi:10.1007/BF03195195

Fournier-Chambrillon C, Aasted B, Perrot A, Pontier D, Sauvage F, Artois M, Cassiede JM, Chauby X, Dalmolin A, Simon C, Fournier P (2004) Antibodies to Aleutian mink disease parvovirus in free-ranging European mink (*Mustela lutreola*) and other small carnivores from southwestern France. J Wild Dis 40:394–402. doi:10.7589/0090-3558-40.3.394

Fournier-Chambrillon C, Bifolchi A, Mazzola-Rossi E, Sourice S, Albaret M, Bray Y, Ceña JC, Urra Maya F, Agraffel T, Fournier P (2010) Reliability of stained placental scar counts in farmed American mink and application to free-ranging Mustelids. J Mammal 91:818–826. doi:10.1644/09-MAMM-A-297.1

Fournier-Chambrillon C, Fournier P (2013) Seguimiento bio-sanitario de la población de visón europeo en Navarra, y particularmente de los tramos bajos del río Arga 2005-2013. In : Dossier de trabajo para el II taller de expertos para la conservación del visón europeo en Navarra. Life. Natura 2000. Territoriovison.eu. Gobierno de Navarra. Gestión ambiental de Navarra S.A., 1–191.

Maizeret C, Migot P, Rosoux R, Chusseau JP, Gatelier T, Maurin H, Fournier-Chambrillon C (2002) The distribution of the European mink (*Mustela lutreola*) in France: towards a short term extinction? Mammalia 66:525–532. doi:10.1515/mamm.2002.66.4.525

Maran T, Macdonald DW, Kruuk H, Sidorovich VE, Rozhnov VV (1998) The continuing decline of the European mink, *Mustela lutreola*: evidence for the intraguild aggression hypothesis. In: Dunstone N, Gorman ML (eds) Behaviour and ecology of riparian mammals. Cambridge University Press., Cambridge, pp 297–324.

Maran T, Skumatov D, Palazón S, Gómez A, Põdra M, Saveljev A, Kranz A, Libois R, Aulagnier S (2011) *Mustela lutreola*. In: IUCN Red List of Threatened Species. Version 2013.2. www.iucnredlist.org. Accessed December 11, 2013.

Michaux JR, Hardly OJ, Justy F, Fournier P, Kranz A, Cabria M, Davison A, Rosoux R, Libois R (2005) Conservation genetics and population history of the threatened European mink *Mustela lutreola*, with an emphasis on the west European population. Mol Ecol 14:2373–2388. doi:10.1111/j.1365-294X.2005.02597.x

Moinet M, Fournier-Chambrillon C, André-Fontaine G, Aulagnier S, Mesplède A, Blanchard B, Descarsin V, Dumas P, Dumas Y, Coïc C, Couzi L, Fournier P, (2010) Leptospirosis in free-ranging endangered European mink (*Mustela lutreola*) and other small carnivores (Mustelidae, Viverridae) from southwestern France. J Wild Dis 46:1141–1151. doi:10.7589/0090-3558-46.4.1141

Otranto D, Lia RP, Cantacessi C, Brianti E, Traversa D, Giannetto S (2007) *Filaria martis* Gmelin 1790 (Spirurida, Filariidae) affecting beech marten (*Martes foina*): morphological description and molecular characterisation of the cytochrome oxidase *c* subunit I. Parasitol Res 101:877–883. doi:10.1007/s00436-007-0554-3

O'Toole D, Wiliams ES, Welch V, Nunamaker CE, Lynn C (1993) Subepidermal vesiculobullous filarial dermatitis in free-ranging American badgers (*Taxidea taxus*). Vet Pathol 30:343–351.

Philippa JD, Fournier-Chambrillon C, Fournier P, Schaftenaar W, Van de Bildt MW, Van Herweijnen R, Kuiken T, Liabeuf M, Ditcharry S, Joubert L, Bégnier M, Osterhaus A (2008) Serologic survey for selected viral pathogens in endangered European mink (*Mustela lutreola*) and other free-ranging mustelids in south-western France. J Wild Dis 44:791–801. doi:10.7589/0090-3558-44.4.791

Shimalov VT, Sidorovich VY, Shimalov VV (1993) Helminths of mustelids inhabiting of ponds in Belarus. Vest Akad Navuk BSSR Ser Biyalagichnykh Navuk 4:96–101.

Sidorovich VE, Anisimova EI (1993) Helminth infestation in a declining population of European mink (*Mustela lutreola*) in Belarus. Small Carniv Conserv 9, 16–17.

Sospedra E (2000) Sobre las vermifaunas de las especies del género *Martes* Pinel, 1792 (Carnivora: Mustelidae) en España continental e insular. PhD Thesis, University of Barcelona, 353 pp.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol 30:2725–2729. doi:10.1093/molbev/mst197

Torres J, Mañas S, Palazón S, Ceña JC, Miquel J, Feliu C (2003) Helminths parasites of *Mustela lutreola* (Linnaeus, 1761) and *M. vison* Schreber, 1777 in Spain. Acta Parasitol 48:55–59.

Torres J, Miquel J, Fournier P, Fournier-Chambrillon C, Liberge M, Fons R, Feliu C (2008) Helminth communities of the autochthonous mustelids *Mustela lutreola* and *M. putorius* and the introduced *Mustela vison* in south-western France. J Helminthol 82:349–356. doi:10.1017/S0022149X08046920

Zabala J, Zuberogoitia I, Martínez-Climent JA (2006) Factors affecting occupancy by the European mink in south-western Europe. Mammalia 3–4:193–201. doi:10.1515/MAMM.2006.051

Zimmerli J (1981) Study of the parasites of *Martes foina* in the Vaud canton during 1980-1981. Schweiz arch Tierheilkd 124:419–422.

Legends to figures

Fig. 1 Geographic distribution of the 91 European mink (*Mustela lutreola*) that were found dead in Navarre including the three individuals infected by *Filaria martis*.

Fig. 2 *Filaria martis* partial mitochondrial cytochrome oxidase I (COI) gene.

Fig. 3 Nematode trapped in a dorsolumbar subcutaneous membranous capsule on the most infected European mink.

Fig. 4 Most nematodes occurred free in the subcutaneous tissue (here at the elbow).

Figure 1

Click here to download Figure Lutreola_Fig1.tif

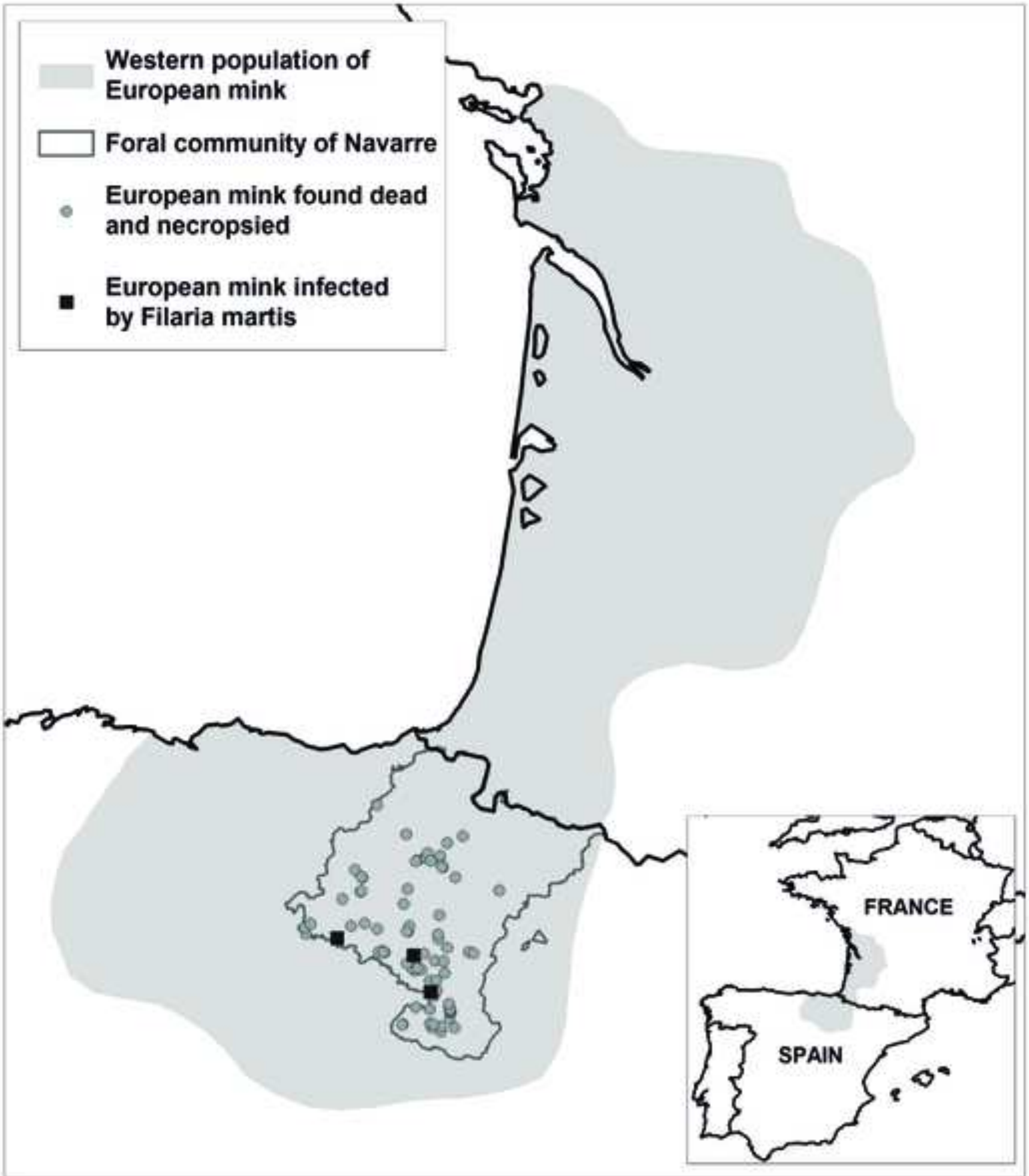

Figure 2

Click here to download Figure Filaria-genbank_Fig2.tif

Filaria martis mitochondrial partial COI gene for cytochrome oxidase subunit I

Sequence ID: emb|AJ544880.1| **Length:** 643 **Number of Matches:** 1

Range 1: 1 to 643 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1177 bits(637)	0.0	642/644(99%)	2/644(0%)	Plus/Plus

```
Query  13   GTTACCTGTTATGATTGGGTCTCCTGAGATAGCTTTTCCTCGTATTAATGCTTTATCTTT  72
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    GTTACCTGTTATGATTGGGTCTCCTGAGATAGCTTTTCCTCGTATTAATGCTTTATCTTT  60

Query  73   TTGGTTAACTTTAGTGGCTTTGTTTATGGTATATGGTTCtttttttATTGGTAATGGTCC  132
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   TTGGTTAACTTTAGTGGCTTTGTTTATGGTATATGGTTCTTTTTTTATTGGTAATGGTCC  120

Query  133  TGGAAGTAGTTGAACTTTATATCCTCCTTTAAGTGTCGATGGTCATCCGGATATGTCTTT  192
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  TGGAAGTAGTTGAACTTTATATCCTCCTTTAAGTGTCGATGGTCATCCGGATATGTCTTT  180

Query  193  AGATAGAATGATTTTAAGTTTGCATACTGTTGGGGCTGGTTCTTTATTAGGTGCTATTAA  252
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  AGATAGAATGATTTTAAGTTTGCATACTGTTGGGGCTGGTTCTTTATTAGGTGCTATTAA  240

Query  253  TTTTATGGTTACTGTTCAGAATATGCGTTCTACAGCAGTTACTTTAGATCAGTTGAGTAT  312
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  TTTTATGGTTACTGTTCAGAATATGCGTTCTACAGCAGTTACTTTAGATCAGTTGAGTAT  300

Query  313  GTTTGTTTGAACTACTTATTTAACTTCTGTGTTAATTTTGTTATCAGTACCTGTTTTAGC  372
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  GTTTGTTTGAACTACTTATTTAACTTCTGTGTTAATTTTGTTATCAGTACCTGTTTTAGC  360

Query  373  TGGTTCTTTGTTATTTTTGTTATTAGATCGTAATTTTGGTGGATCTTTTTATGATTCTAG  432
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  TGGTTCTTTGTTATTTTTGTTATTAGATCGTAATTTTGGTGGATCTTTTTATGATTCTAG  420

Query  433  TAGTGGTGGAAGTCCTTTACTTTATCAGCATTTGTTTTGATTTTTTGGTCATCCTGAGGT  492
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  TAGTGGTGGAAGTCCTTTACTTTATCAGCATTTGTTTTGATTTTTTGGTCATCCTGAGGT  480

Query  493  TTATATTGTTATTTTGCCTGCTTTTGGTATTATTAGTGAGAGTGTTTTGTTTTTAACTGA  552
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  481  TTATATTGTTATTTTGCCTGCTTTTGGTATTATTAGTGAGAGTGTTTTGTTTTTAACTGA  540

Query  553  TAAGGAGCGTTTATTTGGTCATGTAAGAATGATTTTTGCTTCTATTTGAATTTCTGTGTT  612
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  541  TAAGGAGCGTTTATTTGGTCATGTAAGAATGATTTTTGCTTCTATTTGAATTTCTGTGTT  600

Query  613  AGGAACTTCAGTGTGGGGTCAT-CATATGTATACTGCTGGTTTA  655
            | |||||||||||||||||||| |||||||||||||||||||||
Sbjct  601  A-GAACTTCAGTGTGGGGTCATCCATATGTATACTGCTGGTTTA  643
```

Figure 4

Click here to download Figure Lutreola_Fig4.tif