Severe parasitism by *Versteria mustelae* (Gmelin, 1790) in the critically endangered European mink *Mustela lutreola* (Linnaeus, 1761) in Spain

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Abstract

The riparian European mink (*Mustela lutreola*), currently surviving in only three unconnected sites in Europe, is now listed as a critically endangered species in the IUCN Red List of Threatened species. Habitat loss and degradation, anthropogenic mortality, interaction with the feral American mink (*Neovison vison*), and infectious diseases are among the main causes of its decline. In the Spanish Foral Community of Navarra, where the highest density of *M. lutreola* in its western population has been detected, different studies and conservation measures are ongoing, including health studies on European mink, and invasive American mink control. We report here a case of severe parasitism with progressive physiological exhaustion in an aged free-ranging European mink female, which was accidentally captured and subsequently died in a live-trap targeting American mink. Checking of the small intestine revealed the presence of seventeen entangled *Versteria mustelae* worms. To our knowledge, this is the first description of hyperinfestation by tapeworms in this species.

Key words: critically endangered, severe parasitism, *Mustela lutreola*, physiological exhaustion, Spain, *Versteria mustelae*.

Introduction

The European mink, *Mustela lutreola* (Linnaeus, 1761), a small semiaquatic mustelid, has suffered a dramatic decline over the last 150 years and is now listed as critically endangered (Maran et al. 2016). Remaining populations are currently distributed in well-separated nuclei, in Northern Spain and Southwestern France, in Romania, in Ukraine and in 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*), and infectious diseases. In recent years numerous studies on ecology, health status, and conservation genetics have been conducted on the western population of European mink to better understand the causes of its decline and to propose conservation measures (Michaux et al. 2005; Fournier et al. 2008; Philippa et al. 2008; Fournier-Chambrillon et al. in press). Several parasitological surveys have allowed a thorough characterization of the helminth fauna of the species, which showed that cestodes rarely infected this riparian mustelid, and if at all, only in low numbers (Shimalov et al. 1993; Sidorovich and Bychkova 1993; Torres et al. 2003, 2008). The case reported here provides novel information about the potentially fatal impact of tapeworms on European mink, specifically of severe infestation by *Versteria mustelae* (Gmelin, 1790). The genus *Versteria* was recently proposed by Nakao et al. (2013), being *V. mustelae* the type species, which has several synonyms: *Taenia mustelae*, *Fimbriotaenia mustelae* and *Taenia tenuicollis*.

Materials and methods

The highest density of *M. lutreola* in its western population was detected in 2004 in the Spanish Foral Community of Navarra, where a health survey was initiated by the government (Ceña et al. 2005; Fournier-Chambrillon et al. in press). Recent data of American mink in Navarra led the government to control its invasion using mink rafts (Reynolds et al. 2004). One free-ranging European mink female accidentally captured on November 2016 on the river Ega, was found dead in a live-trap targeting the invasive species. It was kept frozen until necropsy performed shortly thereafter. Biometric and biological parameters were recorded, age class estimated through dental wear and calculus, and reproductive status determined by external examination and uteri analysis (Fournier-Chambrillon et al. 2010). A range of samples was taken for genetic, virological, bacteriological and histological studies, following the usual protocol established for necropsies.

This female revealed numerous macroscopically visible intestinal tapeworms and nasolacrimal sinuses parasitism by metastrongylid nematodes. These parasites were removed and transferred to vials containing 70% ethanol. The stomach, intestine and lungs were additionally checked under a stereoscopic microscope. All helminths found were processed according to classical helminthological methods, identified on the basis of previous descriptions, and counted. Some little hooks compatible with those of *V. mustelae* were observed on the tapeworms, although intact scolices could not be found. Therefore, a molecular identification was performed following the protocol described in Torres *et al.* (2016) with these modifications: the primers used were the ones designed by Bowles *et al.* (1992) (Forward 5'-TTTTTTGGGCATCCTGAGGTTTAT-3'; Reverse: 5'-TAAAGAAAGAACATAATGAAAATG -3'), with an annealing temperature of 50°C. The resulting sequence has been deposited in GenBank under the accession number MH431789; and compared to previously published GenBank sequences.

Results and Discussion

Necropsy revealed a cachectic relatively old female of 402 g in state of physiological exhaustion and without any traumatic lesion. No sign of recent lactation was observed, and absence of placental scars could not be interpreted because of rapid disappearance at this time of year due to tissue regeneration. Internal exam revealed congestion of all abdominal organs, and a pale almost translucent hepatic parenchyma. The other major macroscopic lesion was a mass of entangled tapeworms in the small intestine (Fig. 1). After incision, thickening of the gastrointestinal wall was observed, with presence of greenish to black mucus in the stomach, black rectal content suggesting digested blood, and diffuse congestion of the gastrointestinal mucosa. Opening of the skull revealed mild brain congestion and the presence of numerous metastrongylid nematodes in the nasolacrimal sinuses with ventral osseous deformation and right dorsal cranial perforation. Helminthological study concretely revealed the presence of 17 tapeworms (*V. mustelae*) and 10 *Molineus patens* in the small intestine as well as 18 *Skrjabingylus nasicola* in the nasolacrimal sinuses. Mitochondrial COI gene sequence obtained from the tapeworms presented a 99 % homology with the previously published GenBank *V. mustelae* sequence (AJ544880.1). Only one out of the 381 bases differed between the two sequences (Fig. 2). In contrast, our sequence matched the *Taenia martis* sequence with a lower identity score of 85%. In addition, both species are easily distinguished by the size of their hooks, which in *T. martis* are longer (around 150-200 µm) than those of *V. mustelae* (around 20-25 µm). Histological analysis was limited by autolysis and freezing artifacts. Nevertheless, marked hepatocellular atrophy, moderate multifocal verminous bronchopneumonia, moderate chronic interstitial nephritis, and lymph node and splenic lymphoid hyperplasia were observed. No histological lesions could be identified on the heart, bladder, adrenals, brain, stomach and intestines, but autolysis was severe on the latter. The bacteriological cultures were sterile after 48 h of incubation.

Live-trapping is a common method for monitoring European mink (Maizeret et al. 2002), and with adequate precautions and conditions, mortalities are extremely rare, affecting generally only strongly debilitated animals. Our results suggest a severe parasitism by *V. mustelae* responsible for the exhaustion of the female. The capture related stress of this old individual in poor body condition likely constituted the proximate cause of death. Hypothetically, the presence of numerous tapeworms caused mucosal irritation and bleeding, coupled with intestinal dilatation by the mass of entangled worms. The physiological exhaustion was confirmed by hepatic atrophy, indicative of excessive catabolism. Sinus parasitism with osseous lesions were probably aggravating medical conditions and the multicenter lymphoid hyperplasia was likely a consequence of multicentric parasitism.

Idiopathic chronic interstitial nephritis is frequent with age. No indication of other infectious process was detected through ancillary tests.

Among riparian mustelids living in France and Spain, the autochthonous European mink harbors the highest helminth richness and some species also affect this mustelid with high prevalence and intensities of parasitation (Torres et al. 2003, 2008). However, to our knowledge, cestodes have not been observed in *M. lutreola* in the currently contiguous French and Spanish populations. Nevertheless *V. mustelae* and even *T. martis* have been selectively found in the introduced *N. vison* (Torres et al. 2003) and *V. mustelae* infect more regularly European polecat *Mustela putorius* (Torres et al. 1996, 2008). This scenario can only be understood through the diet of these mustelids. The life cycle of *V. mustelae* involves one intermediate host that is a small mammal. In the Iberian Peninsula, *Myodes glareolus,* and several species of the genus *Microtus* have been identified as intermediate hosts of *V. mustelae* (Feliu et al. 1991). While the percentage of these terrestrial species remains particularly low in the European mink diet, in the American mink is a little higher, and can reach a quarter of the prey items in the polecat (Sidorovich 1992). Indeed, the European mink diet is highly variable between individuals, seasons and regions, but in a general way is composed of similar parts of fish, amphibians, birds and small mammals mainly related to the aquatic environment.

The high worm burden found for *V. mustelae* obviously resulted in the intestinal dilatation evidenced in the present case report. Previously, when *V. mustelae* has been found in the other above mentioned riparian mustelids, the infestation was always very low with no more than two specimens in the intestine, which strongly contrasts with the present case. Furthermore, the very low level of genetic diversity characterising Western European populations of *M. lutreola,* observed on neutral genetic markers (Cabria et al. 2015; Michaux et al. 2005) but also on markers associated to the immune system, like the Major Histocompatibility Complex (MHC) genes, could contribute to this high level of infestation.

To our knowledge, the present case is the first description of severe parasitism by tapeworms in the critically endangered European mink.

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Figure legends

Fig. 1. Heap of tapeworms (visible by transparency left hand of the photography) in the small intestine of an European mink female of Navarra, Spain.

Fig. 2. Match between our new sequence and the deposited *Versteria mustelae* partial mitochondrial cytochrome oxidase I (COI) gene sequence.

Figure 1

Click here to download Figure Fournier-Chambrillon_V_mustelae_E_Mink_MS_FIG_1.jpg

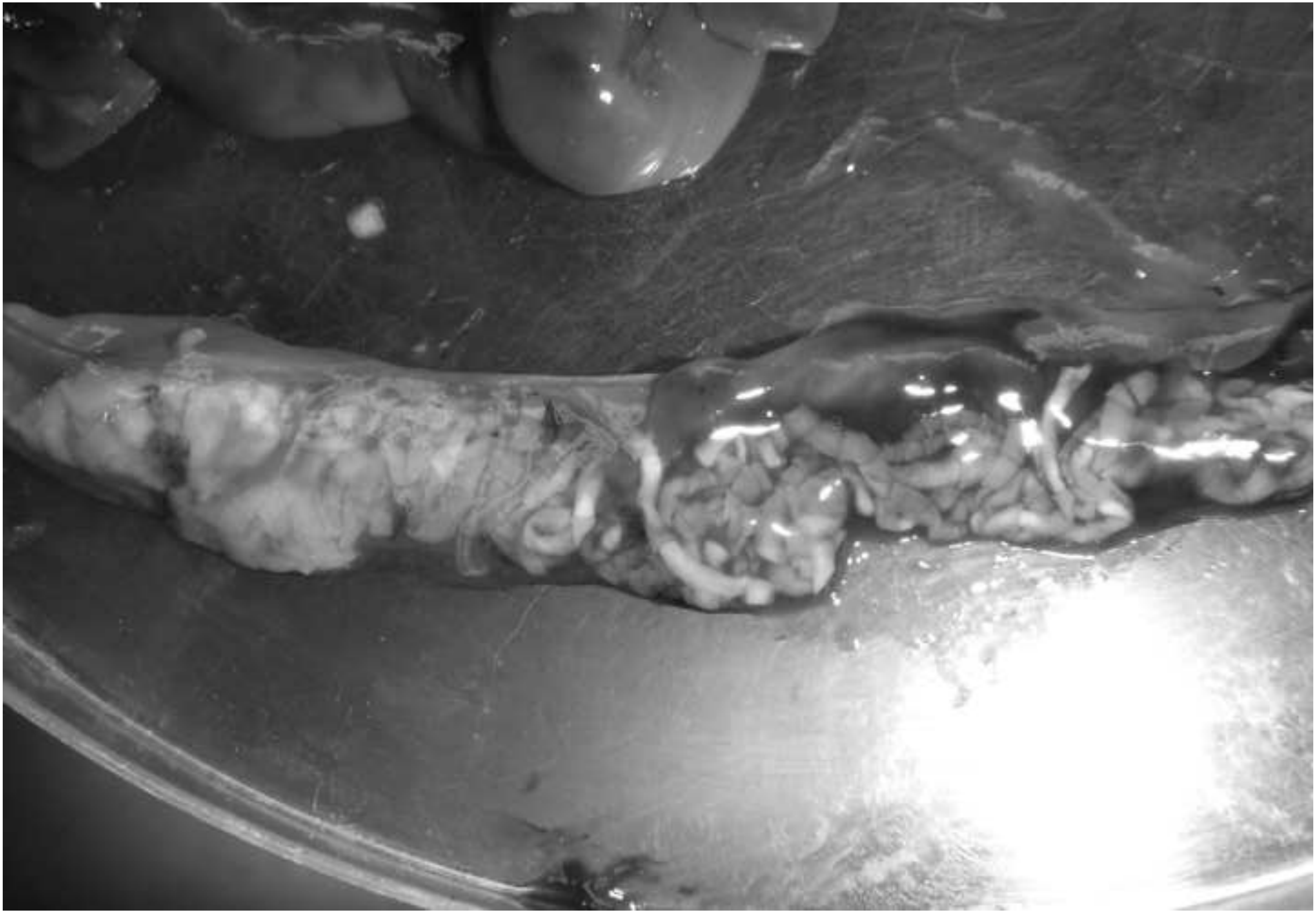

Figure 2

Click here to download Figure Fournier-Chambrillon_V_mustelae_E_Mink_MS_FIG_2.jpg

Versteria mustelae mitochondrial DNA, complete genome, isolate: TmuFi9

Sequence ID: AB732957.1 **Length:** 13582 **Number of Matches:** 1

Range 1: 9800 to 10180 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
699 bits(378)	0.0	380/381(99%)	0/381(0%)	Plus/Plus

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Query  1      ATTGGTCATATATGTTTGAGTATAAGGATGTGTTCTGATGCTTTTGGGTTTTATGGATTG  60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  9800   ATTGGTCATATATGTTTGAGTATAAGGATGTGTTCTGATGCTTTTGGGTTTTATGGATTG  9859

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Query  121    ACTGTTGGTTTAGATGTTAAGACTGCTGttttttttAGTTCTGTTACTATGATTATAGGA  180
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Query  181    GTTCCTACTGGTATAAAGGTGTTTACTTGGTTGTATATGTTACTGAATTCTAGTGTTAAT  240
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Sbjct  9980   GTTCCTACTGGTATAAAGGTGTTTACTTGGTTGTATATGTTACTGAATTCTAGTGTTAAC  10039

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Query  301    GTTACTGGTATAGTTTTGTCTGCTTGTGTATTAGATAATGTTTTGCATGATACTTGATTT  360
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