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Beavers are not alone: parasitic assessment of released Eurasian beavers in Central Italy

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Abstract

The Eurasian beaver *Castor fiber* L., 1758, absent in Italy for over 500 years, has established reproductive populations in Central Italy since 2019, most likely following unauthorized releases. Besides positive effects on local biodiversity, beavers may host a plethora of infective agents, including parasites. Therefore, an assessment of parasitic load of released beavers is pivotal to predict potential risks for other species and human health, following beaver releases. Microscopical and molecular analyses on collected beaver feces confirmed the presence of *Giardia duodenalis*, *Trichuris* spp., and *Travassosius rufus*, a species of nematode recorded in Italy for the first time. The necropsy carried out on a road-killed adult female beaver provided us the first records for Italy of the beaver beetle *Platypsyllus castoris*, and of the ectoparasite "fur mites," belonging to the genus *Schizocarpus*. The bacterium *Bordetella bronchiseptica* was isolated for the first time from the bronchi of *C. fiber*, and it may represent a threat to wild and domestic animals and human health. The dipteran species *Chrysomya albiceps* was also recorded for the first time on a beaver; this blowfly can cause myiasis in livestock and humans. Although the acquired data represent a first assessment for Italy, the high number of pathogens found in these few samples should be evaluated in terms of disease risk analysis.

Keywords *Castor fiber* · Parasites · *Platypsyllus castoris* · *Schizocarpus* spp. · *Travassosius rufus* · Reintroduction · Disease

Introduction

The Eurasian beaver *Castor fiber* L., 1758 (Rodentia: Castoridae) is the largest Eurasian rodent species (Rosell et al. 2005). Absent in Italy, for more than 500 years, *C. fiber* is now settled in Central Italy since 2018–2019, most likely following unauthorized releases from Central Europe (Salari et al. 2020; Pucci et al. 2021; Mori et al. 2021). Reproductive events have been confirmed for the first time since 2020, when beaver cubs were camera-trapped in two areas (Mori et al. 2022a). Two populations, in the Merse-Ombrone and Tiber river basins, occur in Central Italy, and other ones

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are present in southern regions (Mori et al. 2022a; Capobianco et al. 2023). On one side, beaver reintroduction implies an increase in local biodiversity and an improvement of the conservation status of endangered and native species and habitats (Law et al. 2016, 2019; Viviano et al. 2022). Furthermore, their presence is usually highly appreciated by humans, thus increasing local tourism (cf. Viviano et al. 2023). On the other hand, beavers could be potential hosts for a wide range of zoonotic endo- and ectoparasites species and may be included under the animal health legislation (Girling et al. 2019). In authorized reintroduction programs, beavers are subjected to pre-release health screens and post-release monitoring protocols (Goodman et al. 2012). Unmonitored populations can harbor pathogens which may be dangerous for the population itself, like *Leptospira* spp. (Marreros et al. 2018), or may introduce pathogens to disease-free environment as *Echinococcus multilocularis* and *Francisella tularensis* (the causative agent of tularaemia). Thus, introductions would be of concern: these pathogens, both of them being significant zoonoses, are notifiable under the EU law (Mörner 1992; Barlow et al. 2011). Other significant diseases and parasites associated with beaver reintroduction are *Mycobacterium bovis* and *Salmonella* spp. (Girling et al. 2019). The parasitic load of both the species of beavers, *C. canadensis* Kuhl, 1820 and *C. fiber*, has been studied in most of their distribution areas (e.g., Erickson 1944; Girling et al. 2019; Åhlen et al. 2021). Endoparasites such as the protozoa *Cryptosporidium* spp. and *Giardia* spp. are commonly found in the Eurasian beaver (Bystrianska et al. 2021). Currently, 33 helminth species have been detected in *C. fiber* in Eastern Europe, Denmark, and Sweden (Romashov 1969; 2012; Romashov and Romashova 2018). Among those, *Stichorchis subtriquetrus* is highly prevalent in both Eurasian and North American beavers. Snails represent intermediate hosts for the life cycle of this trematode (Romashov and Romashova 2018). Among other helminth species, *Psilotrema castoris*, *Echinococcus multilocularis*, *Fasciola hepatica*, and *Taenia martis* are reported (Erickson 1944; Girling et al. 2019). Another typical parasite, especially of *C. fiber* in Europe, is the nematode *Travassosius rufus*, easily identifiable for its reddish color. This species is a gastric monoxenous nematode, identifiable post-mortem in beaver stomachs, or by the presence of its eggs in beaver feces (Åhlén 2001). *Stichorchis subtriquetrus* and *T. rufus* are beaver-specific parasites (Romashov 2012). Furthermore, *Calodium hepaticum*, *Trichostrongylus capricola*, and two species of *Trichinella*: *T. spiralis* and *T. britovi* have been detected (Szekeres et al. 2022, for a review).

As to ectoparasites, several arthropods reported as generic parasites of birds and mammals may attack humans, acting as vectors for viral, bacterial, protozoan, or metazoan disease agents. Typical beaver ectoparasites are the follicle mite *Demodex castoris* and the specialized "fur mite" *Schizocarpus* spp., with 39 species described for the Eurasian beaver and 18 for the North American species (see Bochkov and Mironov 2008; Bochkov et al. 2012). These mites are highly specialized ectoparasites of beavers that live their entire life on their hosts, most likely representing an example of "high-speed evolution" (Bochkov and Mironov 2008; Bochkov et al. 2012). Up to ten mite species, inhabiting different fur zones, can simultaneously parasitize the same individual beaver (Bochkov et al. 2012).

Beavers may also host an extremely specialized beetle, the beaver beetle *Platypsyllus castoris*, characterized by a striking dorsoventral flattening in its adult form (Peck 2006). The larva is even more specialized than the adult, exhibiting hooked tarsi and mandibles. These adaptations are closely related, in the case of *P. castoris*, to the beaver high fur density and intense grooming behavior (Bailey 1923; Yavorskaya et al. 2023). The first certain record of the species on *C. fiber* was in the Camargue region of the Petit Rhone River near the Mediterranean coast of southern France, in the year 1884 (Peck 2006). Since then, European collection records of the beetle are from France, Germany, Norway, Russia, and Sweden (Pushkin 2014; Sazhnev and Budaev 2020; Åhlen et al. 2021). Further occurrences have been reported for Belgium, Belarus, Czech Republic, Netherlands, Poland, Slovakia, Switzerland, and Hungary (Besuchet 1978; Libois 2000; Prokin and Kirejtshuk 2007; Pushkin 2014; Bystrianska et al. 2021; Szekeres et al. 2022). Thus, the distribution of *P. castoris* covers most of the *C. fiber* current distribution in Europe (Halley et al. 2021).

Several examples of host-specific insect ectoparasites have been co-reintroduced along with their hosts throughout the world, enhancing the risk of parasite-mediated competition (Jørgensen 2015). The recent, unofficial reintroductions of Eurasian beavers in Central Italy did not consider health status of translocated animals. Therefore, an assessment of parasitic load of released beavers would be pivotal to predict potential risks for other species and human health.

The "Rivers with Beavers" project explored and deepened many ecological and behavioral aspects of reintroduced beavers in Central Italy (cf. Mori et al. 2022a, c; Viviano et al. 2022). In addition to behavioral studies and surveys on the impact of these animals on the environment, the project has also focused its attention on the possible implications of beavers on human and animal health. The main aim of this article was to define the first description of parasitic diversity of *C. fiber* in Italy, as well as the first health survey on a female individual found dead in Umbria, Central Italy.

Materials and methods

Analyses of feces

The first two fresh beaver fecal samples collected in April 2021 in La Befa, on the Ombrone river (Province of Siena,

Tuscany Region, Central Italy: 43.7°10.81″N-11.23°54.59″E) were analyzed for *Giardia duodenalis*, which was searched through rapid immunoassay commercial kits (Ridascreen, R-Biopharm, Darmstadt, Germany: Senini 2019). Helminthic eggs and protozoan cysts were also searched and molecularly identified (cf. Cavallero et al. 2021).

Other fecal samples ($n = 13$), collected in May–June 2021, in the same site described above ($N = 3$), along the Merse river near Orgia's bridge (Siena, $N = 3$) and at the confluence of Merse and Ombrone rivers ($N = 7$), were sent to the "Experimental Institute of Zooprophylaxis of Latium and Tuscany" for a parasitological investigation. We cannot rule out that feces collected at the same site may belong to the same individual, but we are confident that they may belong to three different family groups, composed by at least by 3–5 beaver individuals (see Mori et al. 2022a). The analyses included Flotac® test, immunofluorescence (IF) for *G. duodenalis* cysts and *Cryptosporidium* spp. oocysts (Merifluor® Cryptosporidium/Giardia direct fluorescence assay), and quali-quantitative evaluation of larvae/eggs/cysts/oocysts. The Flotac® test was performed according to Cringoli et al. (2010). Moreover, a general aerobic microbial screening for pathogens was carried out using Columbia blood agar and MacConkey agar incubated at 37 °C in O_2 for 24 h. Suspect colonies were sub-cultured on Columbia blood agar for further identification based on colony morphology, gram strain, catalase, and oxidase test. Feces were selectively screened for *Campylobacter* spp. and *Salmonella* spp. as described in WOAH Terrestrial Manual (World Organization for Animal Health 2017; 2022) and for *Yersinia* spp. (Carter and Cole 1990).

Necropsy and infectious diseases assessment

On October 9, 2022, an adult female beaver (total body length without the tail, 0.79 m; weight, 19 kg) was collected as roadkill in the surroundings of Promano (Province of Perugia, Umbria Region, Central Italy: 43.36°N-12.26°E). The beaver was stored at − 20 °C at the "Experimental Institute of Zooprophylaxis of Umbria and Marche" in Perugia, before the necropsy. Necropsy was carried out on October 26, 2022.

Before the necropsy, after defrosting, the beaver was assessed for ectoparasites by hair brushing throughout its body as suggested by Yakhchali et al. (2017), by dividing it into four parts: head, back, belly, and legs. Moreover, cotton swabs were used to search for parasites in each nostril and each ear. Swabs and brushed hairs were then stored in Falcon® tubes with 70% ethanol before lab analyses (Bochkov et al. 2012).

After the necropsy, we tested for a selection of viral, bacterial, and fungal diseases, as well as the analyses of the stomach and intestinal contents. In detail, a specific polymerase chain reaction (PCR) testing was performed for coronavirus and hepatitis E virus. For bacterial pathogens, samples from lungs, bronchi, and duodenum were cultured in blood agar plates (5%) at 37 °C. For *Salmonella* spp., gut samples were pre-enriched in buffered peptone water and then enriched in Rappaport broth. PCR analyses were conducted for *Coxiella burnetii*, *Chlamydophila* spp., *Leptospira* spp., *Brucella* spp., *Mycobacterium avium* ssp. *paratuberculosis*, *Toxoplasma gondii*, *Neospora caninum*, *Sarcocystis* spp., *Babesia* spp., and *Anaplasma* spp. For fungi, samples from oral cavity and rectum were cultured in Sabouraud Dextrose Agar at 37 °C and a skin brush test was performed and cultured in Dermasel Agar at 25 °C for 2 weeks. In addition, despite the poor state of preservation and the freezing, samples from liver, lung, stomach, duodenum, and tail tissue were taken and fixed in 4% formalin for histopathological examination.

Microscopical analysis of hair and skin samples

Samples containing hairs and fragments of skin were analyzed by stereomicroscope. The characterization of the mite specimens was carried out in the Acarology Laboratory in CREA-DC in Florence. The specimens were divided for samples and prepared in slides with Hoyer's medium (50 g distilled water, 30 g Arabic gum, 200 g chloral hydrate, 20 g glycerine). Then, slides were warmed up on a laboratory stove to lighten the integument of the mites. Afterwards, the specimens were compared with references on beaver parasites (Fain and Lukoschus 1985; Fain and Whitaker 1988; Bochkov and Dubinina 2011; Bochkov et al. 2012; Bochkov and Saveljev 2012).

The brushing sample from the ventral part included one larva of 0.9 mm in length. The larva was preserved on a slide with Hoyer's medium. A morphological analysis was also carried out for this insect, comparing it with reference atlases (Winter 1979; Peck 2006).

The microscopical analysis revealed also two larvae of a dipteran species in samples of the back and the belly, both in bad conservation conditions. Dipterans were molecularly analyzed (methodology described below) in the laboratory of the National Research Council of Italy in Sesto Fiorentino, Florence.

Molecular analysis on the dipteran specimen

Total genomic DNA was extracted from entire specimens through the Qiamp Tissue kit (Qiagen Inc., USA), following the manufacturer's protocol. Cytochrome C Oxidase Subunit I (COXI) PCR amplifications and sequencing were performed using the generic primers HCO 2198 (5′-TAA ACTTCAGGG-TGACCAAAAAATCA-3′) and LCO 1490 (5′-GGTCAACAAATCATAA-AGATATTGG-3′) (Folmer et al. 1994), following Mori et al. (2022b) for PCR

conditions. Chromatograms obtained from forward and reversal sequences were visualized by CHROMAS software version 2.6.6 (available at technelysium.com.au/wp/chromas, accessed on 29.01.2023). The sequence of the fly was blasted in the GenBank database to determine its affinities with deposited sequences (https://www.ncbi.nlm.nih.gov/). This approach allowed us to compare obtained amplicons using the basic local alignment search tool (BLAST), to scan for homologous sequences. The obtained sequence was deposited on GenBank (see "Results" for accession number). We looked at phylogenetic relationships by neighbor-joining analysis (NJ) (Saitou and Nei 1987) using MEGA XI software (Tamura et al. 2021) and the GTR distance model with 10,000 replications, corrected for rate heterogeneity among sites with a gamma distribution selected by jModelTest (Darriba et al. 2012). *Sarcophaga baranoffi*, *Calliphora vomitoria*, and *Calliphora vicina* were used as outgroups.

Analysis on collected ticks

Two ticks were found and collected from the ears of the road-killed beaver. Ticks were stored in vials with absolute ethanol and labeled with individual codes. Vials with ethanol and parasites were stored at − 20 °C at CNR-IRET laboratories in Sesto Fiorentino before analyses. Tick species identification was conducted using an optical microscope (×400, Leica), following Pérez-Eid (2007) and Guglielmone et al. (2010). The total genomic DNA was extracted from each tick using the NucleoSpin® Kit (Macherey Nagel, Duren, Germany) following the manufacturer's instructions. PCR of mtDNA COXI gene was performed to confirm molecularly the morphological species identification (Folmer et al. 1994). Extracted DNA samples were screened for the presence of *Rickettsia* spp., *Borrelia burgdorferi* s.l., *Anaplasma* spp., and *Babesia* spp. by standardized PCR protocols (Mori et al. 2018). Two PCR for *Rickettsia* spp. targeting the citrate synthase gene (gltA) were performed: a touchdown PCR using primers RpCS. 877p (5′-GGGGACCTGCTCACG GCGG-3′) and RpCS.1258n (5′-ATTGCAAAAAGTACA GTGAACA-3′) and a semi-nested PCR using the primers RICK F1 (5′-CCTATGGCTATTATGCTTGCGGC-3′), RICK R1 (5′-CATCTTTAAGAGCGA TAGCTTCAAG-3′), and RICK R2 (5′-GGTTCTCTTTCKGCATTTTATCC-3′) (Roux et al. 1997; Duron et al. 2017; Regnery et al. 1991). For the detection of *Borrelia*, a nested PCR targeting the 23S–5S rRNA spacer region was performed. The first set of primers being 5S rRNA (5′-CGACCTTCTTCGCCTTAA AGC-3′) and 23S rRNA (5′-TAAGCTGACTAATACTAA TTACCC-3′) (Schwartz et al. 1992). The second set of internal primers was primer 1 (5′-CTGCGAGTTCGCGGGAGA -3′) and primer 2 (5′-TCCTAGGCATTCACCATA-3′) and amplified a fragment of 226–266 bp of the intergenic region (Postic et al. 1994).

For *Anaplasma* spp., a semi-nested PCR was performed targeting a 546-bp region of the 16S rRNA gene using primers EHR 16SD (5′-GGTACCYACAGAAGAAGTCC-3′), EBR3 For (5′-TTGTAGTCGCCATTGTAGCAC-3′), and EBR3 Rev (5′-TTGTAGTCGCCATTGTAGCAC-3′) (Massung et al. 1998; Hornok et al. 2008; Teshale et al. 2018).

Finally, a portion of 408 bp of *Babesia* small subunit ribosomal gene was amplified using primers PIRO A (5′-AAT ACCCAATCCTGACACAGGG-3′) and PIRO B (5′-TTA AATACGAATGCCCCCAAC-3′) as described by Olmeda et al. (1997). Positive controls were available for all the testes pathogens. All the PCR products were visualized on 1.5% agarose gel. COXI PCR products were purified and sequenced for species identification (Eurofins genomic, Sanger Sequencing).

Results

Analyses of feces

Microscopical and molecular analyses on the first two collected feces confirmed the presence of several species of parasites, including *Giardia duodenalis* in one sample and *Trichuris* spp., in both samples. The nematode *Travassosius rufus*, found in both samples, is the first report within Italian territory (de Jong et al. 2014). Coccidia were not detected.

All the other 13 fecal samples were negative for *G. duodenalis* and *Cryptosporidium* spp. Two samples were positive for nematode larvae (which were not molecularly analyzed because of degraded DNA), whereas the other samples were negative at parasitological examination. We refrained from morphometric identification of the isolated larvae, due to the deterioration of caudal and rostral portions. All fecal samples were negative for *Campylobacter* spp., *Salmonella* spp., and *Yersinia* spp.

Necropsy

The animal was in good body condition, with sufficient perivisceral fat tissue, and showed severe traumatic injuries, suggestive of motor vehicle impact, with laceration of the left lateral abdominal muscles and rupture of the stomach and internal parenchymatous organs. The adult female showed evident nipples and uterine scars, suggesting recent suckling and confirming the recent event of reproduction. All molecular and bacteria culture tests were negative except the swabs from the bronchi, where bacterial colonies growth on blood agar plate were identified as *Bordetella bronchiseptica* by MALDI-TOF MS (Bruker Daltonics, Bremen, Germany). Histopathology revealed a mild form of chronic pneumonia of unknown origin.

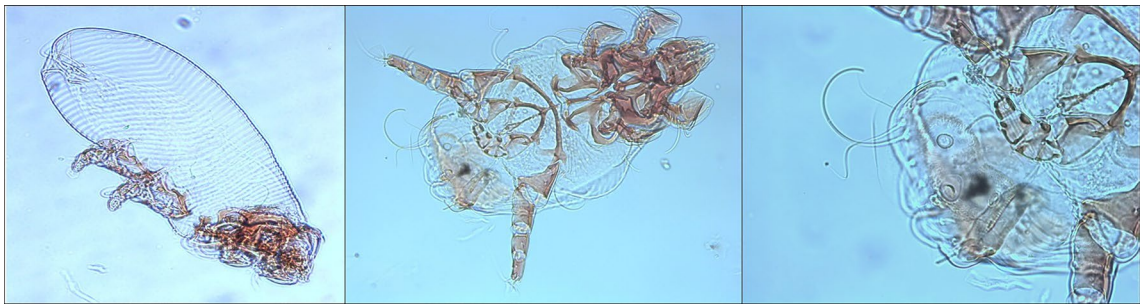

Fig. 1 (Left) female of *Schizocarpus* sp. (407 µm length), (center) male (375 µm length), (right) detail of the typical ventral pattern of a male

Microscopical analysis of hair and skin samples

Several mites were found in all hair samples [$n = 5$ from the back (1♂ and 4♀♀); $n = 8$ from the belly (3♂♂ and 5♀♀); $n = 5$ from nostrils (1♂ and 4♀♀); $n = 2$ from ears (2♀♀)]. Collected specimens were attributed to the parasitic mite of the genus *Schizocarpus*, commonly known as "fur mites." These determinations were confirmed through a comparison with slides from the Berlese Acaroteca (samples of *Schizocarpus mingaudi* from France; Castagnoli and Pegazzano 1985), currently available at the CREA DC Institute of Florence. However, our *Schizocarpus* mites could only be identified at the genus level (Fig. 1), as morphological characters did not correspond to any of the species described in available dichotomous keys (Fain and Lukoschus 1985; Bochkov 2012).

One larva at the first stage was found on the skin surface in the abdominal part, and it was morphologically identified as *Platypsyllus castoris*, a species of beetle previously never reported in Italy. The identification of the specimen (Fig. 2) was confirmed by the experts Stewart B. Peck and Sándor Szekeres.

Molecular analysis on the dipteran individual

We obtained a 686-bp sequence for COXI of the dipteran larvae. BLAST analyses confirmed it as a blowfly, *Chrysomya albiceps* (accession number, OQ181401), a pioneer colonizer of dead animals (Carvalho et al. 2004; Bugelli et al. 2015; Fig. 3).

Tick analysis

Both collected ticks were morphologically and molecularly identified as *Ixodes ricinus*. Concerning the targeted pathogen screening, no *Rickettsia* spp., *Borrelia* spp., *Anaplasma* spp., or *Babesia* spp. were detected.

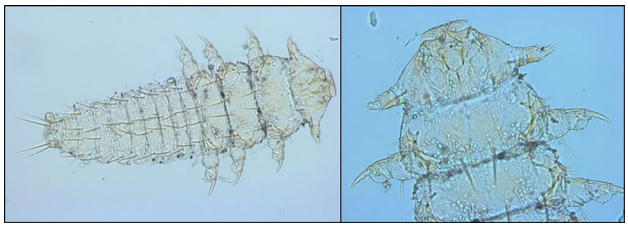

Fig. 2 Larva of *Platypsyllus castoris* (0.9 mm length), details of the anterior part on the right (ventral view)

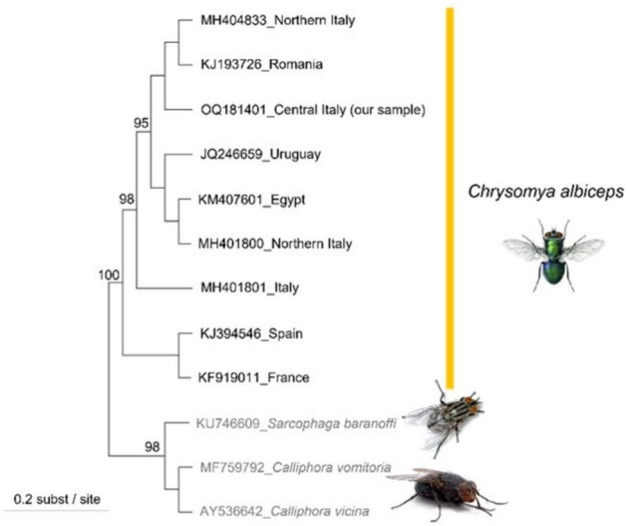

Fig. 3 NJ tree obtained from the analysis of COXI for 686 bp, with 10,000 replications. For each node, support values (bootstrap) were shown

Discussion

We recorded a diversified richness in beaver parasites in Central Italy, despite the small sample size (Åhlen et al. 2021; Benovics et al. 2022; Szekeres et al. 2022; Demiaszkiewicz et al. 2014). Concerning the findings of *Giardia duodenalis*, a modest number of oocysts of the parasitic

protozoan are sufficient to cause human gastroenteritis outbreaks. In the study conducted by Sroka et al. (2015), the impact of beavers on the spread of this protozoan was analyzed, and the largest densities of *Giardia* spp. cysts were found in samples taken in proximity to beaver lodges. Besides, humans may transmit giardiasis to wildlife including beavers through urban wastewater discharges (Sulaiman et al. 2004; Carmena et al. 2012).

The genus *Trichuris* is particularly well-known for being a common parasite of domestic animals, although specific determination is often limited by the low number of genetic sequences on GenBank (Ransom 1911; Cavallero et al. 2021). The nematode *Travassosius rufus*, recorded for the first time in Italy, might not represent a threat to local fauna after the introduction of beavers into new regions, because it is species-specific, and therefore infects only the *Castor* genus (Benovics et al. 2022).

The dipteran *Chrysomya albiceps* is widely distributed in several biomes of the world, including most of Europe and Italy (Hosni et al. 2022). It is the first time that a larva of *Ch. albiceps* is recorded on a beaver, although it is widespread on carcasses of several species (Vanin et al. 2011). This blowfly has major medical and veterinary importance (Bugelli et al. 2015), because it feeds on carcasses and feces and can be a vector of viruses, bacteria, and helminths. It can cause myiasis in livestock (Grassberger et al. 2003; Al-Shareef and Al-Qurashi 2016).

We also detected the first record of mites belonging to the *Schizocarpus* genus in Italy. The species identification in this group of mites is challenging, as it can be successfully performed exclusively on male individuals (Bochkov et al. 2012). Therefore, it has not been possible to identify the species, given the scarcity of male specimens.

The discovery of *Platypsyllus castoris* is of special entomological interest because this beetle is closely associated with beavers and it is one of the most modified and well-adapted ectoparasites among arthropods (Peck 2006). This species has been described mostly on beavers, although it may be detected also on the North American river otter *Lontra canadensis* (Peck 2006) and on the Caucasian otter *Lutra lutra meridionalis* (Pushkin 2014). Necropsies and further studies on parasite fauna should also be carried out on coexisting invasive coypu *Myocastor coypus* in Italy, to evaluate potential spill-over by this parasitic beetle.

The necropsy and application of the health-monitoring panel showed a healthy animal that had probably already reproduced. Fortunately, none of the investigated diseases of European importance were detected. The isolation of *Bordetella bronchiseptica* in a wild rodent is not surprising, because this gram-negative, aerobic strict, catalase, and oxidase-positive rod-shaped coccobacterium is widely described in wildlife (Wilson 1987). *Bordetella bronchiseptica* can colonize the respiratory tract of a variety of mammals and could represent a threat to wild and domestic animals and human health. Moreover, it is one of the main etiological agents of atrophic rhinitis and pneumonia in pigs, tracheobronchitis in dogs, rhinitis in rabbits, and respiratory tract diseases in cats (Corona et al. 2013). However, it has rarely been implicated as a cause of infection in humans, with most cases recorded in immunocompromised patients (Woolfrey and Moody 1991; Gupta et al. 2019). Despite all health monitoring programs on Eurasian beavers, this is the first report of *B. bronchiseptica* in this species, which has previously been reported only in *C. canadensis* (Petro et al. 2015). The histopathology showed a mild form of chronic interstitial pneumonia, not referable to the presence of *B. bronchiseptica* in bronchi, of unknown origin.

Conclusions

The high number of species found in this study should be considered in terms of risk of introducing significant diseases to humans, domestic animals, livestock, pets, and/or wildlife. However, the very low sample size, together with the actual impossibility to assess whether different feces belonged to different beaver individuals, prevented us to drive any conclusion on prevalence and incidence of such pathogens, which would require further studies.

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Author contribution F.T., E.M., A.V., C.P., and G.M. field work and sample collecting. F.T. and G.M. literature research. E.M., A.V., M.B., M.G., D.S., B.B., A.L., A.M., and M.G.D. laboratory analyses and data curation. F.T., E.M., M.G., and G.M. wrote the main manuscript text. G.M. supervision. All authors reviewed the manuscript and approved it.

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Data availability Research data is available upon receipt of a reasonable request.

Declarations

Ethical approval Not applicable.

Conflict of interest The authors declare no competing interests.

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