



Short Note

Reappearance of the Eurasian beaver *Castor fiber* L. in Tuscany (Central Italy): the success of *unauthorised* releases?

Chiara Pucci¹, Davide SENSERINI², Giuseppe MAZZA³, Emiliano MORI⁴

¹Free-lance wildlife technician, Str. di Pilli, 1 - 53035 Monteriggioni - Siena, Italy

²Free-lance wildlife technician, Loc. Defizio, 58036, Roccastrada, Grosseto, Italy

³CREA Research Centre for Plant Protection and Certification, Via di Lanciola 12/a, Cascine del Riccio, 50125, Firenze, Italy

⁴CNR-IRET, Consiglio Nazionale delle Ricerche – Istituto di Ricerca sugli Ecosistemi Terrestri (CNR-IRET), Via Madonna del Piano 10, 50019, Sesto Fiorentino (FI), Italy

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Abstract

In this short report, we documented the reappearance of the Eurasian beaver *Castor fiber* L. for the first time in Tuscany (Central Italy). After the detection of unequivocal signs of presence, we confirmed the occurrence of beavers through camera trapping. Genetic analyses (cytochrome-b mitochondrial gene) and hair microstructure confirmed it as the Eurasian species. The reports given here extend the current known range about 530 km south to the known extent of occurrence of this species. The presence of a relict population in this area is almost unlikely, despite being quite far from the nearest village; we therefore may suggest that the individuals occurring in Tuscany may be the result of local unauthorized releases. A precise number of free-ranging beavers remain unknown though at least two individuals are suspected to occur, based on body size. Apart from records of adult individuals, the presence of at least one juvenile suggests that reproduction in the wild may have occurred in 2020. Immediate actions should be promoted to monitor potential expansion, preserve this population, and/or to limit impacts on ecosystems and conflict with human activities.

The Eurasian beaver, *Castor fiber* Linnaeus, 1758 was once present in a large part of the Palaearctic, from Portugal to Eastern Russia and northwestern China, throughout several riparian habitat types including forests, tundra, and steppe (Salari et al., 2020; Halley et al., 2021). Afterward, the species underwent population decline after medieval times, mostly because of hunting for fur and meat, habitat loss, and demand for castoreum, a secretion from the scent glands particularly appreciated in the pharmacopeia and perfume industry (Halley and Rosell, 2002; Halley et al., 2021). At the start of 1900, the range of the Eurasian beaver was limited to scattered *refugia* between France and Mongolia, hosting less than 1200 individuals. Since 1920, legal protection together with reintroduction events and natural spread triggered the recovery of the species in most of its original range, up to a current minimum population estimate of about 1.5 million individuals worldwide (Rosell et al., 2005; Dewas et al., 2012; Smeraldo et al., 2017; Bouroş et al., 2021; Halley et al., 2021). In some countries (e.g. Finnish and Russian fur industry) the North American beaver *Castor canadensis* Kuhl 1820 has been released before it was identified as a different species in the 1950s, thus resulting in several alien populations in North-Eastern Europe (Parker et al., 2012). Further escapes from local zoos in Europe have resulted in the establishment of North American beavers (Kuehn et al., 2000). No evidence of hybridisation between *C. fiber* and *C. canadensis* is documented, due to differences in number of chromosomes. North American beavers disappeared or were proactively eradicated from most areas where released, remaining only with some substantial populations in Finland and Russia (Halley et al., 2021). Currently, the Eurasian beaver shows reproductive populations in most of its original range, apart from Portugal, Italy, and the southern Balkans

(Gabryś and Ważna, 2003; Halley et al., 2011; Wróbel, 2020; Halley et al., 2021). Subspecific taxonomy is quite slapdash and possibly related to the result of fur trade *refugia* rather than to actual genetic differences due to biogeography (Senn et al., 2014).

Disentangling *C. fiber* from *C. canadensis* only through outer morphology is very difficult and impractical. For instance, *C. fiber* tails are usually proportionately narrower than *C. canadensis* ones but some overlap may occur (Aulagnier et al., 2010). Furthermore, anal gland secretions are different between beaver species, but their sampling may require animal captures (Rosell and Sun, 1999). Hair cuticular and medullar structures show some differences between *Castor* species (Keller, 1983; Chernova and Tselikova, 2018), but molecular analyses using Single Nucleotide Polymorphisms or the mitochondrial cytochrome-b are the most reliable method to differentiate amongst beavers (Kuehn et al., 2000; McEwing et al., 2014).

As to Italy, Salari et al. (2020) summarised that beavers occurred in the Late Miocene (fossils of *Castor prefiber*), whereas *C. fiber* appeared in Early Pleistocene in central and northern regions, whereas its distribution was limited to northern Italy and Po plain in Late Pleistocene and Holocene. In his “*Naturalis Historia*”, Pliny the Elder also reported the presence of the beaver in Italy in the Roman Age (Salari et al., 2020), which also occurred in the early Medieval times as supported by bone records in northern and in central Italy (Salari et al., 2020). The Eurasian beaver may have persisted in the eastern Po forests up to 1500s (Pontarini et al., 2019; Salari et al., 2020), apart from single individuals locally observed as a result of escapes from captivity (i.e. an individual detected and poached in early 2000s in Northern Tuscany, near the Foreste Casentinesi National Park). Reintroduction

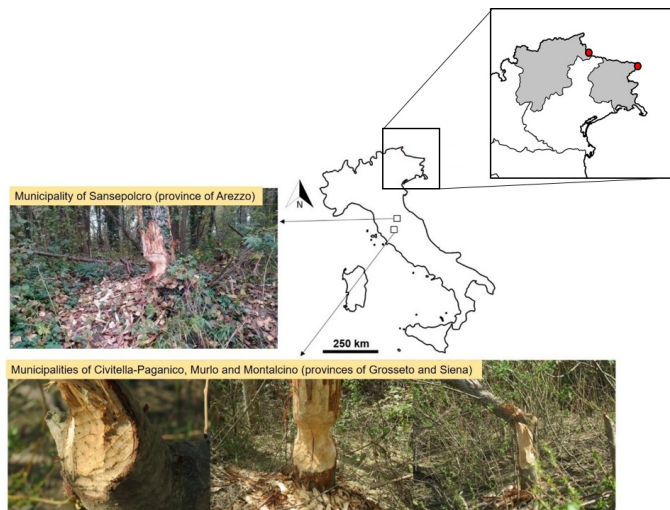


Figure 1 – Gnawed-trunks observed in Tuscany and reported as signs of presence of the beaver. Red dots in the inset show the known occurrences of the Eurasian beaver (N=2 individuals) in Italy.

programs occurred in the last century in neighboring countries (Austria and Switzerland) between the 1970s and the 1990s have promoted a recolonisation of many areas of these countries Pontarini et al. (2019); Halley et al. (2021). In October 2018, a male Eurasian beaver was identified in the municipality of Tarvisio (province of Udine, northern Italy) probably as a result of natural dispersal from Austria (Loy et al., 2019; Pontarini et al., 2019). In November 2020, another individual has been camera-trapped in Val Pusteria (in the surroundings of Sesto Pusteria, province of Bolzano), near the Austrian border, where the last record of this species dated back to 1594 (Versciaco di Sopra, province of Bolzano). Both of these beaver individuals detected in Italy since 2018 have been classified as *C. fiber* following the tail shape and as being in continuity with the Austrian population of this species, but no genetic confirmation is available. Reporting the occurrence of beavers in Italy is mandatory for several reasons. First, the Eurasian species is listed within the Annexes of the Habitats Directive (92/43/EEC) and any natural range expansion in our country would require the completion of the standard forms provided for each six-year reporting (Genovesir et al., 2014; Stoch and Genovesi, 2016). Furthermore, both beaver species are reported as “ecosystem engineers” as they may modify the ecosystem where they live in terms of geomorphology, vegetal assembly composition, and hydrological characteristics of the landscape (Rosell et al., 2005). The recent Italian Legislative Decree 19/2021 (“Rules for the protection of plants from harmful organisms”), which entered

into force on 13 March 2021, imposes that any previously unrecorded species should be immediately signaled to the National Phytosanitary Service before any publication (both scientific and newspaper articles).

In early 2021, members of the provincial police noted some unequivocal signs of beaver presence (i.e. deeply debarked trees of *Salix alba* L. and *Populus* spp., which could not be attributed to any other species) in Tuscany (Central Italy), in two areas far from the native known distribution of this species. The first area included the municipalities of Civitella-Paganico, Murlo, Monticiano, and Montalcino (provinces of Grosseto and Siena), the second one was placed in the surroundings of Sansepolcro (province of Arezzo). These areas are separated by one another by over 110 km in a straight line. Dispersal distances can range from 5–10 km to over 25–85 km, depending on several factors e.g. population density, habitat quality, and food availability (Saveljev et al., 2002; Campbell-Palmer et al., 2021 for a summary). Therefore, a single individual may also travel the straight distance occurring between the two study sites in Tuscany along a river with no obstacles, although most individual movements occur in 3–4 km of river (e.g. Saveljev et al., 2002; Campbell-Palmer et al., 2021). However, the two areas were also located in different, well-separated river basins, suggesting that at least two groups of individuals occur in this region. We observed signs of presence for at least 3 km on the Tevere river from Sansepolcro area, whereas addressed surveys confirmed that signs of presence occurred for at least 15 km long trait encompassing both the Ombrone and Merse rivers in the municipalities of Civitella-Paganico, Monticiano, Murlo, and Montalcino. This second area, where signs of beaver activity (i.e. gnawing) were detected on over 1000 trunks (about 85% on *Salix alba*, 15% on *Populus alba* and *Populus nigra*: Fig. 1), is located at the confluence of the Farma, Merse, and Ombrone rivers and its surroundings. The area is covered with a deciduous riparian woodland mostly composed by *Salix alba* Linnaeus, 1753, *Populus nigra* Linnaeus, 1753, *Populus alba* Linnaeus, 1753 and *Acer negundo* Linnaeus, 1753 as an alien species. Beaver individuals have been present in the area for at least two years, given the age of the vegetation regrowth over gnawed trunks and that first signs of presence date back to 2019.

We placed four camera traps (IR PLUS® BFHD) in the surroundings of partially gnawed trunks for 60 nights in March–May 2021. Camera-trapping confirmed the presence of at least three individuals of beaver in the study area, recorded in two areas separated one another by at least 7 km of river, with at least one juvenile individual, remarkably smaller than the adult ones (Fig. 2). This suggests that at least one reproductive event may have occurred. Given the smaller size of the juvenile and the fact that beavers reach the adult body size in about 18–20 months, we suggest that reproduction may have occurred. Therefore, at least three individuals (the mother, the father and at least one kit) should be present. Lack of sexual size dimorphism prevented us to assess sex and the exact number of adult individuals.

Beavers forage around thick bramble bushes, which allowed us to collect also beaver hairs. Hair samples were used to determine the beaver species both through morphological micro-structure (different between *C. fiber* and *C. canadensis*: Chernova and Tselikova, 2018), and through molecular analyses (cytochrome-b: Kuehn et al., 2000). Presumed beaver guard hairs were collected on bramble thorns and stored at -20°C in labeled plastic bags (Herr and Schley, 2009). Beaver guard hairs are easily identified according to colour and morphology, as being flat, shine and beige-to brown coloured, with a conspicuous narrowing of the diameter in the middle portion of each hair (Keller, 1983). Once in the lab, three hair roots were plucked from the fur and prepared following Kuehn et al. (2000). DNA was extracted following a phenol-chloroform purification protocol: hair roots were grinded using liquid nitrogen, mortar, and pestle (Nerva et al., 2016). DNA pellets were re-suspended in 150 µl of elution solution and then cleaned with the DNA Clean and Concentration kit (ZymoResearch, CA, USA) to separate them from PCR-inhibiting molecules. We amplified a 450 bp fragment of the mitochondrial cytochrome-b gene by PCR using primers developed for rodents (Kuehn et al., 2000), through a 2720 Thermal Cycler (Applied Biosystems). The electrophoresis was run for 35' on a 1% agarose gel, and sequenced using the dideoxy-chain



Figure 2 – a-d) Beavers camera-trapped on the Ombrone and Merse riversides in April–May 2021. A comparison between c) and d) allows to distinguish between an adult and a juvenile individual, respectively.

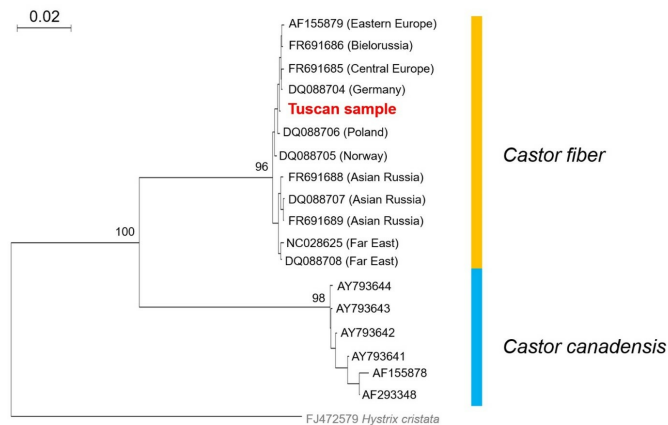


Figure 3 – Neighbour-Joining tree on mtDNA sequence of the cytochrome-b of beavers including all available sequences from GenBank and the one from our Italian sampling area (red); a sequence of *Hystrix cristata* is also included as an outgroup (grey).

termination method (Sanger et al., 1997) with the forward primer. The genetic sequence was cleaned at the 5' and 3' ends by looking at the chromatogram. At the end, to confirm the correct origin of the obtained sequences, we compared our sequences to the ones deposited in the NCBI database using BLASTn (Altschul et al., 1990). Alignments of cytochrome-b sequences were performed through the software ClustalW (Higgins and Sharp, 1988); the best evolutionary model used for phylogenetic analyses was selected through the software MEGA7 (Kumar et al., 2016). Tamura-Nei model with Invariant sites (TN93+I) resulted to be the best model for our data. The rest of the hair apart from the root was used to analyse the microscopical structure. To analyse the cuticula, the hair was placed on a glass slide, on a layer of transparent nail polish. After the slide was completely dry, the hair was removed with steel tweezers. The mould was observed at the binocular microscope (B-192s Optika 600) and compared with a specific atlas, showing the differences between *C. fiber* and *C. canadensis* (Chernova and Tselikova, 2018). To analyse the medullar structure, the hair was cross-sectioned and wet with bergamot oil at the section level. The prepared slide was observed under the microscope, so to compare the structure of the medulla with the atlas tables (Chernova and Tselikova, 2018).

The genetic sequence of cytochrome-b (Accession Number: <https://www.ebi.ac.uk/ena/browser/view/OD934229OD934229>) clustered within the variability of *C. fiber* (Fig. 3); accordingly, microscopical hair structure confirmed the species as *C. fiber*, with a scaly cuticula and a continuous medullar pattern throughout the hair (Keller, 1983; Fig. 4).

The Eurasian beaver is protected by the Habitats Directive, therefore requiring updated population and distribution monitoring (Genovesi et al., 2014; Stoch and Genovesi, 2016). Furthermore, the species is fast expanding in Europe, following legal protection, habitat recovery, and reintroduction events, reaching areas where it went extinct several centuries ago, with over 1.5 million individuals currently present (Wróbel, 2020; Bourouš et al., 2021; Halley et al., 2021). The species has been recorded for the first time in Italy in 2018, with a single individual in North-Eastern Italy, and with one other individual present

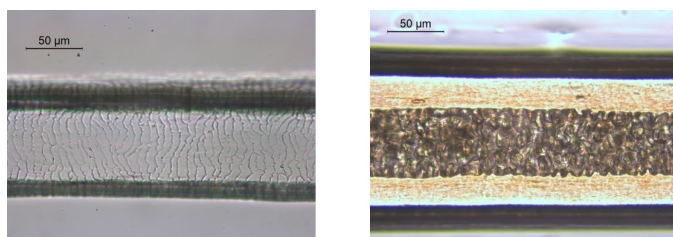


Figure 4 – Cuticular (right) and medullar (left) patterns of the hair of *Castor fiber* collected in Tuscany in April 2021.

in Alto Adige since late 2020. Despite having recorded in Tuscany up to medieval times (Salari et al., 2020), it is quite unlikely that the occurrence of the beaver in the Ombrone river basin represents a relict population, as this ecosystem engineer rodent would have been detected before. However, both hair morphology, tail shape, and molecular analyses confirmed it as the Eurasian beaver species. Assessing the origin of animals currently present in the provinces of Grosseto and Siena is challenging. Molecular analyses showed that the sampled individual was similar to Central European beavers. However, the distribution of different subspecies has been altered by reintroduction programs, thus making not possible to assess the origin of a beaver only from molecular similarities. Halley et al. (2012) reported that several populations of beaver in Europe have originated by escapes from local zoos. As to Italian zoos and game parks, the Eurasian beaver is only reported for Poppi (province of Arezzo, <http://www.zootierliste.de/en>, accessed on 15.04.2021), which is located over 100 km north to the Ombrone-Farma-Merse river basins. Therefore, the return of the Eurasian beaver in Tuscany may be due to unofficial and unauthorized reintroduction events, as it happened for Flanders in Belgium (Verbeylen, 2003). The activity of beavers is known to change vegetation structure to a great extent, in turn influencing other components of the ecosystems, including the diversity and abundance of invertebrates, amphibians, and wading birds (Bashinskiy, 2020). Beaver alteration of heterogeneity and connectivity of habitats needs further research in our study area (Bashinskiy, 2020). No reliable evidence on the number of free-ranging beavers in Italy is available (Wróbel, 2020), although we are confident that at least three individuals are present in our study site, which may represent the first reproductive area for this species in Italy after medieval times. Given the availability of molecular markers, genetic analyses on further samples should be necessary both to determine population numbers and to assess the potential population origins. Immediate actions for detailed distribution assessment and breeding evidences of the species would be necessary to assess its potential expansion and actual conservation status. ☞

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