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DISTRIBUTION AND GENETIC ANALYSIS OF WILD-LIVING EURASIAN BEAVERS IN CENTRAL ITALY

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Mori E., Viviano A., Brustenga L., Olivetti F., Peppucci L., Pucci C., Senserini D., Sergiacomi U., Spilinga C., Roversi P.F., Mazza G.- Distribution and genetic analysis of wild-living Eurasian beavers in Central Italy.

The presence of the Eurasian beaver *Castor fiber* L. has been recently confirmed with two separated populations in Tuscany (Central Italy) and probably represents the result of an unofficial release. In late spring and summer 2021, seven reliable records of Eurasian beaver have been collected in Umbria and other neighbouring regions, implying that the distribution of this large rodent is even wider than previously reported. In this short work, we updated the distribution of this protected species in Central Italy, by collecting and mapping all the confirmed occurrences. Beavers were proved to be present throughout the Tiber (Tevere) river basin in both provinces of Umbria, and another individual has been road-killed in the Marche region, near the border with Tuscany. Other single signs of presence occurred in Emilia Romagna and Latium. The only hair sample we were able to collect confirmed it as the Eurasian beaver species. No reliable evidence is available on the number of free-ranging beavers in Central Italy, and systematic monitoring is needed. Before any management and conservation action, further data are required concerning distribution range, potential origin, social perception, and the effects on the ecosystems.

KEY WORDS: *Castor fiber*; distribution assessment; riverine ecosystems; Rodentia; species release.

INTRODUCTION

Within the dynamics of ecological systems, assessing species distribution, the modification of habitat types, and the interactions with human activities are pivotal for conservation and management purposes.

The variety of life at every hierarchical level and spatial scale of biological organisation from genes to ecosystems is widely known as "biodiversity". The Anthropocene is the historical period we are currently living in, characterised by a phase of severe decline of biodiversity worldwide, mostly due to habitat loss and fragmentation, and biological invasions (DIRZO *et al.*, 2014; BELLARD *et al.*, 2016). As a response, the European Union Biodiversity Strategy, adopted by the European Commission, has set out several targets and actions to limit the loss of biodiversity by 2030: all European countries should maintain and restore biodiversity (MAMMOLA *et al.*, 2020). In this context, reintroductions, i.e. translocations of individuals of a species in an area where the species is extinct but it was present in historical times, could be a useful tool to face the biodiversity crisis. The International Union for the Conservation of Nature (IUCN) has designed detailed guidelines applying to the whole spectrum of conservation translocations, including legal permissions, selection of stocks, and stock health status (IUCN/SCC, 2013). Furthermore, all extinction causes should have been removed from the release-area, to increase the success of the translocation (IUCN/SSC 2013; DROUILLY & O'RIAIN, 2021). Official and unofficial releases have helped the range expansion of several threatened or locally extinct species in many areas of the world (CROMSIGT *et al.*, 2018; ZIELKE *et al.*, 2019; BODE,

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2021). Amongst the most reintroduced species in Europe, the Eurasian beaver *Castor fiber* Linnaeus, 1758 underwent a severe population decline during Medieval times (e.g. SALARI *et al*., 2000) because of hunting for fur and meat, as well as because of habitat loss, with only 1200 individuals remained between France and Mongolia at the beginning of 1900 (HALLEY & ROSELL 2002; HALLEY *et al*., 2021). Since then, reintroduction events followed by natural spread triggered the recovery of the species in most of its original range, up to a current global population estimate of 1.5 million individuals (WRÓBEL, 2020; BOUROȘ *et al*., 2021; HALLEY *et al*., 2021).

Together with legal reintroductions conducted following IUCN guidelines, several unauthorised releases of Eurasian beavers of unknown origins have occurred in Europe, e.g. in Belgium and Spain (DEWAS *et al*., 2012; GIRLING *et al*., 2019). Most of these releases have been successful and, after reintroductions, Eurasian beavers have rapidly expanded their ranges to new areas where they were historically extirpated (DEWAS *et al*., 2012). The Eurasian beaver survived in northern Italy to the early 1800s (HAINARD, 1949; PSENNER, 1971). After reintroduction programs conducted in Austria and Switzerland from the 1970s - 1990s, at least two individuals of this rodent crossed the Italian borders in 2018 while spreading (PONTARINI *et al*., 2019; PUCCI *et al*., 2021). In both cases, the involved streams flowed out of Italy into the Danube watershed, on the northern side of the Alps.

Later on, in early 2021, some wildlife technicians and members of the provincial police noted some unequivocal signs of beaver presence in two areas of Tuscany (Central Italy), one in the municipalities of Civitella-Paganico, Murlo, Montalcino, Buonconvento, and Monticiano (provinces of Grosseto and Siena), and the other near Sansepolcro (province of Arezzo). In the first area, over 10 km of rivers (Ombrone river basin with tributaries, i.e. Merse, and possibly Farma) were characterised by the beaver presence; in the second one (Tevere, i.e. Tiber river basin with its tributary, Cerfone), signs of presence covered at least 6-7 km of the river (PUCCI *et al*., 2021). These areas are separated by one another by over 110 km in a straight line. After the publication of PUCCI *et al*. (2021) and the media echo, further data on beaver presence have been collected in Umbria and other regions. In this note, we aimed to determine beaver distribution in Central Italy through on-site investigations in areas characterised by signs of the presence of this species (i.e. gnawed trunks).

METHODS

Monitoring of beaver occurrences has been carried out in April-July 2021, and the authors verified all the received reports through direct on-site visits to assess the presence of signs of the presence of the Eurasian beaver. We also checked on social networks and websites for wildlife occurrence data collection (i.e. iNaturalist: www.inaturalist.org, Ornitho: www.ornitho.it), but no further record was found on these platforms.

Beaver guard hairs were found on a bramble bush between Sansepolcro and Città di Castello and stored at -20°C in labeled plastic bags (HERR & SCHLEY, 2009). A further beaver hair sample was collected at the Poppi Zoo Park (Arezzo) from the only individual still present (i.e., an adult male born in 2011). The Poppi Zoo Park is the only one officially recognized structure in Italy hosting Eurasian beavers together with the "Oasi di Sant'Alessio" in Pavia (PUCCI *et al*., 2021). Once in the lab, hair roots were plucked from the fur, and DNA was extracted following a phenol-chloroform purification protocol: hair roots have been grinded using liquid nitrogen, a mortar, and a pestle (NERVA *et al*., 2021). DNA pellets were re-suspended in 150 µL of elution solution and cleaned with the DNA Clean and Concentration kit (Zymoresearch, CA, USA). A 450 bp fragment of the mtDNA cytochrome-b gene was amplified by PCR using beaver-specific primers (KUEHN *et al*., 2000), through a 2720 Thermal Cycler (Applied Biosystems). Sequencing was conducted using the dideoxy-chain termination method (SANGER *et al*., 1977) with the forward primer. Genetic sequences were cleaned at the 5' and 3' ends by looking at the chromatogram. To conclude, the obtained sequence was compared to the ones deposited in the NCBI database using BLASTn to confirm the correct origin of the obtained sequences (ALTSCHUL *et al*., 1990). Alignments of cytochrome-b sequences were performed through ClustalX and Mega7 softwares (HIGGINS & SHARP, 1988; KUMAR *et al*., 2016).

RESULTS

We found a total of 12 occurrence points (i.e. coordinates confirmed by photos of gnawed trunks and/or photos of individual beavers) from Umbria. Amongst those, seven belonged to the Eurasian beaver, two to the coypu *Myocastor coypus* (Molina, 1782), and 3 were unidentifiable signs of presence. Direct visits to all of these sites confirmed the presence of the Eurasian beaver in the Val Tiberina (i.e. the Tevere river valley) in Umbria (Fig. I), both in Perugia (municipalities of Città di Castello and Deruta) and Terni (municipalities of Guardea and Alviano: see Figure 1a). A further road-killed individual was observed near Mercatello sul Metauro (province of Pesaro Urbino, Marche region) in late June 2021, and a sign of presence (i.e. a gnawed trunk) was recorded in Porretta (Bologna, Emilia Romagna region) in August 2021, with no other evidence of presence (Fig. I, 2). Occurrences of Eurasian beaver from Latium were only confirmed at the immediate borders with Umbria, thus requiring further research downstream.

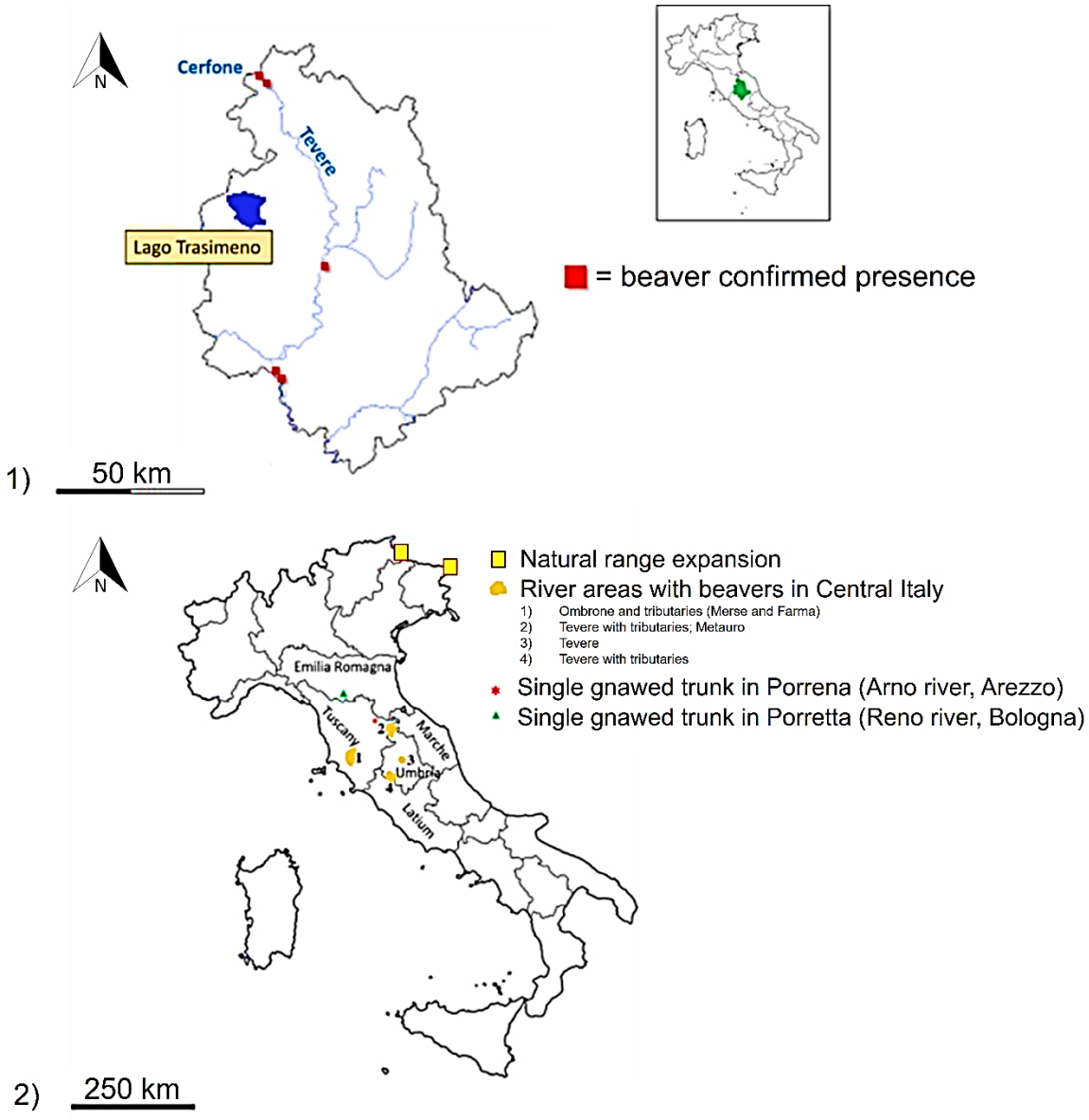

Fig. I - 1) Map of the confirmed occurrences of the Eurasian beaver in Umbria. 2) Summary of beaver distribution in Central Italy.

Individuals and signs of presence were observed in the abovementioned sites (Fig. II). Areas of beaver presence are covered with deciduous riparian woodlands composed by *Salix alba* Linnaeus, 1753, *Populus nigra* Linnaeus, 1753 and *Populus alba* Linnaeus, 1753. Beavers have been present in Umbria for at least one year, given the age of the vegetation regrowth over gnawed trunks. We detected 55 gnawed trunks (about 60% on *Salix alba*, 40% on *Populus* spp.), with an average diameter of 15±5 cm.

The genetic sequence of cytochrome-*b* from Sansepolcro (Val Tiberina, province of Arezzo) clustered within the variability of the Western clade of *C. fiber*, whereas the sample of the only individual currently present in the Poppi Zoo Park clustered within the Eastern clade of the same species (Fig. III).

DISCUSSION

The Eurasian beaver may attract human and media attention more than other smaller, cryptic mammal species which have recently been (re)discovered in Italy (DONDINI *et al.*, 2014; MORI *et al.*, 2020). The appeal of this large rodent to humans may be due to its size, morphology, and famous cartoons that may have stimulated empathy towards this species (e.g. “Don Chuck Monogatari” and “Papa Beaver’s Story”).

Fig. II - Signs of presence and individual of Eurasian beaver in Val Tiberina, Umbria (photos A. Viviano, S. Galletti, F. Olivetti, and L. Peppucci).

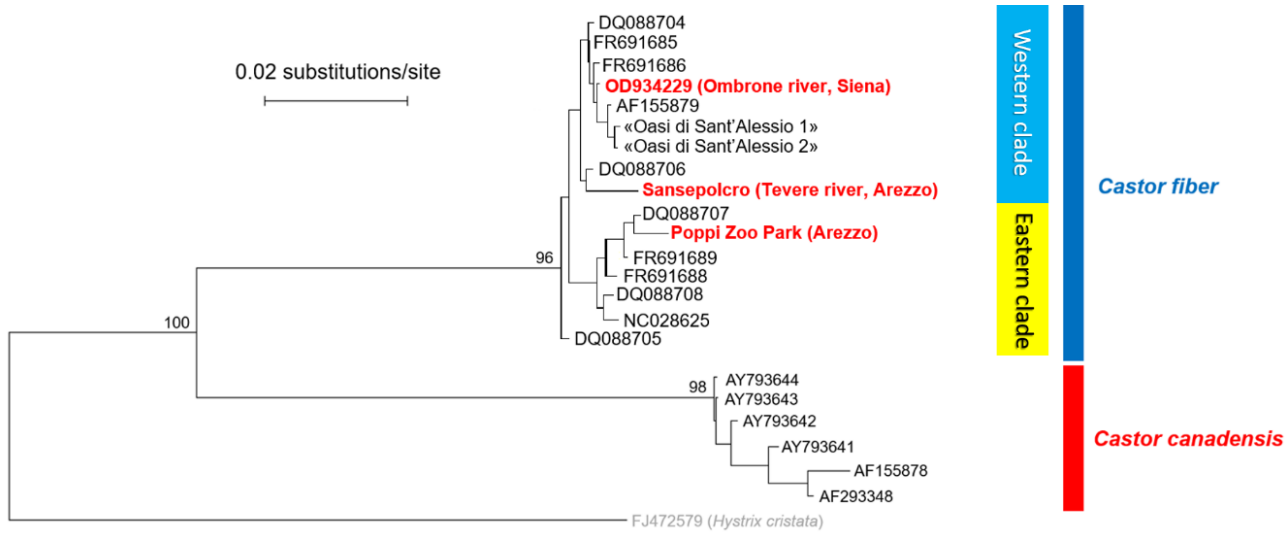

Fig. III - Neighbour-Joining tree on mtDNA sequence of the cytochrome-*b* of beavers including all available sequences from GenBank and our samples. Italian sequences are shown in bold; a sequence of *Hystrix cristata* is also included as an outgroup (grey).

In this brief report, we reported the presence of the Eurasian beaver throughout the Umbria region, with evidence of occurrences in four municipalities out of both Perugia and Terni provinces. Further records from North-Eastern Tuscany (Porrena, Arno river), North-Eastern Latium (Tevere river), and Emilia-Romagna were only based on single signs of presence (i.e. one gnawed trunk per area) and require further researches, possibly during the cold months to confirm the beaver presence discard the hypothesis of a local "native" population. In fact, a genuinely native Italian population would not be expected to show a so close similarity to the Western clade, which derived from an Ice Age refuge in Iberia (ELMEROS *et al.*, 2003). All new records confirmed in this work come from the Tevere river basin, being one record near the confluence with the Paglia river and one other in Cerfone river. The origin of these populations of Eurasian beaver in Central Italy, where the original population became extinct in Medieval times (SALARI *et al.*, 2019), is still unknown. The simultaneous appearance of beavers in different areas of Central Italy after centuries being unnoticed, and molecular data bring us to

(MARR *et al*., 2018). Most likely, given the known Ice Age vegetation of Italy, beavers survived the last Ice Age in an Italian refuge, possibly with a genotype similar to the extinct Danube clade (MARR *et al*., 2018). On the other hand, it is not possible to establish whether they represent an unofficial release, an escape from confined areas, or a natural population. However, the individual still present in the only zoo park hosting beavers in Central Italy belonged to a different clade with respect to those sampled in the wild in Central Italy (see MARR *et al*., 2018). Moreover, it is impossible to report the actual number of individuals occurring in these areas. However, we can confidently believe that populations of beavers in Central Italy will increase (HALLEY & ROSELL, 2002); the rivers they are on are relatively small compared to those in Central Europe, so there may be little or no 'lag' time before the period of exponential population growth begins (HALLEY & ROSELL, 2002).

The IUCN strongly discourages introductions or reintroductions conducted without feasibility studies and no molecular analysis of released stocks, because of the damage they may cause to native ecosystems (KLEIMAN, 1989; HALLEY *et al*., 2009). The activity of beavers could locally alter the riparian vegetation structure, in turn influencing other components of the ecosystems, including the diversity and abundance of invertebrates, amphibians, and wading birds (NOLET *et al*., 1994; ROSELL *et al*., 2005; BASHINSKIY, 2020). For this reason, beaver alteration of heterogeneity and connectivity of habitats needs further research in our study area (BASHINSKIY, 2020). Where released, if impacts are evident and affect the native biodiversity directly or through the interaction with other native/alien species (CAMPBELL-PALMER *et al*., 2016), management strategies should also consider drastic actions including individual removal (cf. GARGIONI *et al*., 2021).

Conversely, where reintroduced, Eurasian beavers are reported to improve the hydrogeological status of European rivers, to increase local species richness, and to mitigate environmental pollution (ROSELL *et al*., 2005; KEMP *et al*., 2012; PUTTOCK *et al*., 2017; CAMPBELL-PALMER *et al*., 2021). If on one side the Eurasian beaver is protected by the Habitats Directive (1992/43/EC, Annex IV) and several national laws, human-wildlife conflict may arise when releases are not authorised and, sometimes, even where these operations are authorised, at least in public perception (HALLEY & ROSELL, 2002). Although crop damages by Eurasian beavers are negligible in Europe (MIKULKA *et al*., 2020), particularly when compared to those exerted by the wild boar *Sus scrofa* Linnaeus, 1758 and the red deer *Cervus elaphus* Linnaeus, 1758, illegal removal of animals perceived as causing a nuisance may occur. However, from the legal point of view, species protected according to the Habitats Directive (92/43/EEC) should be treated as alien species if released by humans where they are not naturally present (for example the common chameleon *Chamaeleo chamaeleon* Linnaeus, 1758 in Southern Italy: BASSO *et al*., 2019). Natural range expansion to new areas, and any official reintroductions, require completion of the standard forms required for each six-year reporting period (GENOVESI *et al*., 2014; STOCH & GENOVESI, 2016). In the case of Eurasian beaver in Central Italy, conservation and restoration of riparian vegetation and physical protection systems for trees may be used to prevent damage (HALLEY & ROSELL, 2002). Unauthorised (re)introductions are not to be condoned (IUCN/SCC, 2013) and should be reprobated. In most cases, however, introduced populations of protected mammal species in Italy, including those listed in the Annexes of the Habitats Directive as well as the Eurasian beaver, have been left unmanaged (e.g. mouflons *Ovis aries* Linnaeus, 1758 in continental Italy: LOY *et al*., 2019; Alpine marmots *Marmota marmota* (Linnaeus, 1758) in the Apennines, both of them originally not native of the study area: LOY *et al*., 2019; Eurasian otters *Lutra lutra* Linnaeus, 1758 where originally native, i.e. in Valsavarenche and, introgressed with the Asian subspecies *L. l. barang* F.G. Cuvier, 1823, in the Ticino Valley: PRIGIONI *et al*., 2009; FERRARI *et al*., 2017; alien red deer in Monte Penna - Castell'Azzara, Grosseto and in several other areas in Italy; alien crested porcupines *Hystrix cristata* Linnaeus, 1758 in the Varese province, Western Liguria and Sardinia regions: MORI *et al*., 2013). Removal strategies for unofficially released Eurasian beavers have, where attempted, been both ineffective and expensive (e.g. experience from Spain: HALLEY *et al*., 2020), and should be considered only in case of undeniable proofs of human-mediated releases or, by way of derogation to the Habitats Directive, in case of severe and proven crop damage. As to Spain, 15 years after the introduction, the Eurasian beaver is considered as naturalised and, following the decision of the European Commission, it cannot be removed anymore and it requires monitoring following the Habitats Directive. However, no complaint linked to this increasing, unofficially-released population (occurring throughout Ebro River up to Zaragoza) is recorded yet, with only minor damages to trees in river groves (Eugenio Fernandez, personal communication 2021). For Central Italy, it would be important to currently concentrate on how to manage the species, considering also human attitudes. Different countries in Europe with similar cultural landscapes have very different public attitudes towards beavers, i.e. from very negative to very positive, with little relationship to local beaver ecology (CURRY-LINDAHL, 1967; SIEMER *et al*., 2013; AUSTER *et al*., 2021). Imposing strong management actions even if beavers have been unofficially released could thus trigger a chronic, expensive, and emotionally tiring problem.

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