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Population survey of European beaver (*Castor fiber* Linnaeus, 1758) in the western Iberian Peninsula: insights from camera trapping and non-invasive genetics

Estudio poblacional de castor europeo (*Castor fiber* Linnaeus, 1758) en el oeste de la península ibérica: resultados mediante fototrampeo y análisis genéticos no invasivos

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Abstract

The numerous reintroductions of European beaver (*Castor fiber*) over the last two centuries have played an important role in the recovery of the species across most of its native range, despite many of these efforts have not been official. In the Iberian Peninsula, where beaver was extinct for several centuries, this species was illegally released in 2003 in the north-eastern region and, since then, the species has increased its distribution along the Ebro basin. Two decades later, in 2022, new evidences of the species were reported in the Douro River basin (Salamanca, Spain), close to the Portuguese border and apparently resulting from another illegal release. Herein, we present the results of a beaver population survey in two sites of this new area in the Douro basin, using sign surveys, direct observations, camera trapping and genetic non-invasive sampling. We confirmed the presence of a minimum of 10 individuals in two tributaries of the Douro River, including two family groups with reproduction in 2023 and a potential third one. Genetic analyses of a non-invasive sample (hair) using mitochondrial DNA control region suggest that the individuals belong to the European beaver species, specifically to the western clade (subspecies *C. fiber albicus-galliae-fiber*). The findings of this work provide relevant information on the current status of European beaver in a transboundary area of western Iberia, contributing to support management actions that should target population monitoring, public awareness and ecological modelling to predict their future distribution.
Keywords: Arribes del Duero Nature Park, illegal releases, reproduction, taxonomic assignment.

Resumen

Las numerosas reintroducciones de castor europeo (*Castor fiber*) a lo largo de los dos últimos siglos han desempeñado un papel fundamental en la recuperación de la especie en la mayor parte de su área de distribución nativa, a pesar de que muchos de estos esfuerzos no han sido oficiales. En la península ibérica, donde el castor estuvo extinguido durante varios siglos, esta especie fue liberada ilegalmente en 2003 en la región nororiental y, desde entonces, la especie ha aumentado su distribución a lo largo de la cuenca del Ebro. Dos décadas después, en 2022, se reportaron nuevas evidencias de la especie en la cuenca del río Duero (Salamanca, España), cerca de la frontera portuguesa y, aparentemente, como resultado de otra liberación ilegal. En este trabajo, presentamos los resultados del estudio de la población de castor en dos lugares de esta nueva área en la cuenca del Duero, a través del reconocimiento de

signos de presencia, observaciones directas, fototrampeo y muestreo genético no invasivo. Confirmamos la presencia de un mínimo de 10 individuos en dos afluentes del río Duero, incluyendo dos grupos familiares con reproducción en 2023 y un posible tercero. Los análisis genéticos de una muestra no invasiva (pelos) utilizando la región control del ADN mitocondrial sugieren que los individuos son castores europeos, concretamente del clado occidental (subespecie *C. fiber albicus-galliae-fiber*). Los resultados de este trabajo aportan información relevante sobre el estado actual del castor europeo en una zona transfronteriza del oeste de la península ibérica, contribuyendo a apoyar acciones de gestión que deberían dirigirse al seguimiento de la población, la concienciación pública y la modelización ecológica para predecir su distribución futura.
Palabras clave: Muestreo genético no invasivo, introducciones ilegales, Parque Natural Arribes del Duero, reproducción.

Introduction

The current population of the European beaver (*Castor fiber* Linnaeus, 1758) is estimated at more than 1 million individuals and now covers almost all countries of its former European range, with some exceptions such as Portugal (Halley *et al.* 2020). This outcome is the result of the combined action of natural dispersal from the few population nuclei that persisted after its disappearance from most original range (Nolet & Rosell 1998) and, especially, from the numerous reintroductions carried out during the last century (Halley & Rosell 2002, Halley *et al.* 2012). Reintroductions or translocations (a common strategy in rewilding initiatives) are frequently carried out to restore a particular species to its original range. The reintroduction of European beavers is not only promoted by conservation purposes to restore their original range in Europe and Asia (Djoshkin & Safonov 1972, Campbell-Palmer *et al.* 2016) but also due to its foreseeable positive impact on the natural environment. Because of its role as an ecosystem engineer (Brazier *et al.* 2021), beaver is a key species in mitigating human impacts on the environment (Puttock *et al.* 2017) and improving species richness locally (Nummi *et al.* 2019, Wojton & Kukuła 2021, Andersen *et al.* 2023).

To provide a basis for the design and management of beaver translocations in the European territory, the International Union for Conservation of Nature (IUCN) published general guidelines (IUCN/SSN 2013). However, many of the translocations of beavers in Europe are being carried out through illegal releases of specimens without any conservation or scientific criteria. This is how the species returned to the Iberian Peninsula in 2003, after the unofficial release in the Ebro basin (Navarre province, Spain) of 18 individuals from Germany (Ceña *et al.* 2004). Due to the illegal nature of this introduction in Iberia, the European Commission initially granted authorization to eradicate the population (Guerrero-Campo *et al.* 2009) by capturing specimens (Halley *et al.* 2020). However, eradication did not succeed and, in 2018, taking into account the historical data on its Iberian presence, the European beaver came to be considered a native species in Spain and was included in the national List of Species under the Special Protection Regime (BOE 2020). Since then, beaver has expanded its distribution in NE Spain, along the Ebro Basin, and, more recently, also appeared in other distant areas located in the western Iberian Peninsula. In fact, almost two decades after the introduction in the Ebro Basin, the presence of the species was documented in 2022 in the Douro Basin, within Spanish territory (province of Salamanca) but close to the Portuguese border (Calderón *et al.* 2022), and approximately 600 km from the known distribution area in the Peninsula. Considering the dispersal rate of the species in the Ebro basin, Calderón *et al.* (2022) estimated that it would take more than 40 years to cover the long distance of riverbanks that separate the Ebro Basin from the new presence site in Douro Basin. Therefore, the presence of beaver in this area of the Douro Basin is probably the result of another illegal release.

Initially, the occurrence of beaver in this new area in western Iberia was identified by Calderón *et al*. (2022) based on typical presence signs (e.g. tree trunks with teeth marks and gnawed bark) detected along a small segment of the Tormes River (Douro Basin), located inside a Spanish protected area (Arribes del Duero Nature Park) and approximately 5 km from the border with Portugal. However, important information to support adequate management was still lacking. For example, the distribution and number of individuals in the area were unknown, as well as the occurrence of reproduction. Besides, there is no data confirming whether the new population belonged to the western or eastern mitochondrial lineage of the native European beaver (Frosch *et al.* 2014, Senn *et al.* 2014, Durka *et al.* 2005) nor whether the evidence belonged to the sister species from the American continent (*Castor canadensis*), considered as an invasive species in Europe (Halley *et al.* 2020).

The main objective of this work is to expand knowledge on those first beaver records in Douro basin, western Iberia, by (1) providing data on its distribution and population status based on field sampling by sign surveys, direct observations and camera-trapping, as well as (2) identifying the beaver species and its population of origin based on genetic analysis of non-invasive samples. These findings will improve the knowledge about the species in a transboundary area of western Iberia, which will provide baseline information to support adequate management actions related to population monitoring, public awareness and ecological modelling to predict their future distribution.

Material and methods

Field sampling for beaver monitoring was conducted in 2023 between April and November in two Spanish tributaries of the Douro River, in western Iberia (Fig. 1A). One of them is the Tormes River, where the first beaver records were detected by Calderón *et al.* (2022). After surveys in the neighbouring watercourses (based on field sampling and information from local people), we found a second area with beaver presence, in the Uces River. Both areas are included within the Arribes del Duero Nature Park (province of Salamanca, Spain) and are separated 18 km by land (the shortest distance in a straight line) and 30 km by waterway (Douro River). The Tormes River area shows a practically continuous water regime, since it is located below a dam (Almendra dam) that regulates its flow (Fig. 1B). In contrast, the Uces River area is a seasonal river with a significant reduction of its flow during the summer, resulting in ponds connected by small streams. However, during the rainy season, the river exhibits a continuous and high flow, with waterfalls of up to 40 m (Confederación Hidrográfica del Duero). The riverbank vegetation in both areas is characterized by the presence of ash groves (*Fraxinus angustifolia*) to some extent anthropized, willow groves (*Salix fragilis, Salix atrocinerea, Salix salviifolia, Salix purpurea* and *Salix triandra*) and some wild and cultivated poplars (*Populus nigra*) (Marino *et al.* 2014).

Data collection in both rivers was carried out using several methods and different sampling areas in order to assess distribution (e.g., occupied area), population status (e.g., family groups and reproduction) and taxonomic assignment (e.g., genetic confirmation of species and subspecies).

Distribution (sign surveys and direct observations)

We conducted field surveys along the banks of both rivers in order to find signs of beaver presence (mainly trees with gnawed bark, burrows, scats and scent mounds; Fig. S1) to determine the approximate size of the occupied range. At Tormes River, the steep terrain prevented us from carrying out linear transects along some areas of the banks, and in those cases, transects were made along the riverbed by using a raft. In addition, direct observations were made at accessible points along Tormes river (in sites Tormes3 and Tormes4; see next section on camera-trapping), and also Uces River, always at a prudent distance to not interfere with animal behaviour. The observations were carried out during the daytime, at dusk, and also at night, using night vision devices (Bushnell Equinox Z).

Population status (camera-trapping)

Four camera traps (Browning Patriot, Browning Dark Ops) were placed at 4 different points of the Tormes River (Fig. 1C) during different sampling periods (Fig. 2). The locations were named Tormes1, Tormes2, Tormes3, and Tormes4 following a chronological order (i.e. Tormes1 was the first location to be monitored while Tormes4 was the last one). The Uces River was not included in the camera-trapping survey. Cameras were placed facing beaver trails at a height of ca.1.50 metres above the ground, without using any attractant. To standardize data collection, cameras were programmed to take 20-second videos. In this study, we only considered videos that recorded the presence of medium to large sized mammals, excluding rodents other than beavers. We calculated the effort for a single detection (i.e., the number of sampling days necessary for detecting a species) by dividing the number of sampling days by the number of records obtained for each species. We also calculated the detection rate (i.e., the number of detection records of a given species considering an interval of 100 sampling days).

Regarding the beaver-specific data, the information recorded with camera trapping was categorized as: location, video ID, date, solar time, time interval (00:00h to 3:59h; 4:00h to 7:59h; 8:00h to 11:59h; 2:00h to 15:59h; 16:00h to 19:59h; 20:00h to 23:59h), number of individuals, age (K: kit, with < 1 year old; A: adult), sex (Unk: unknown; M:

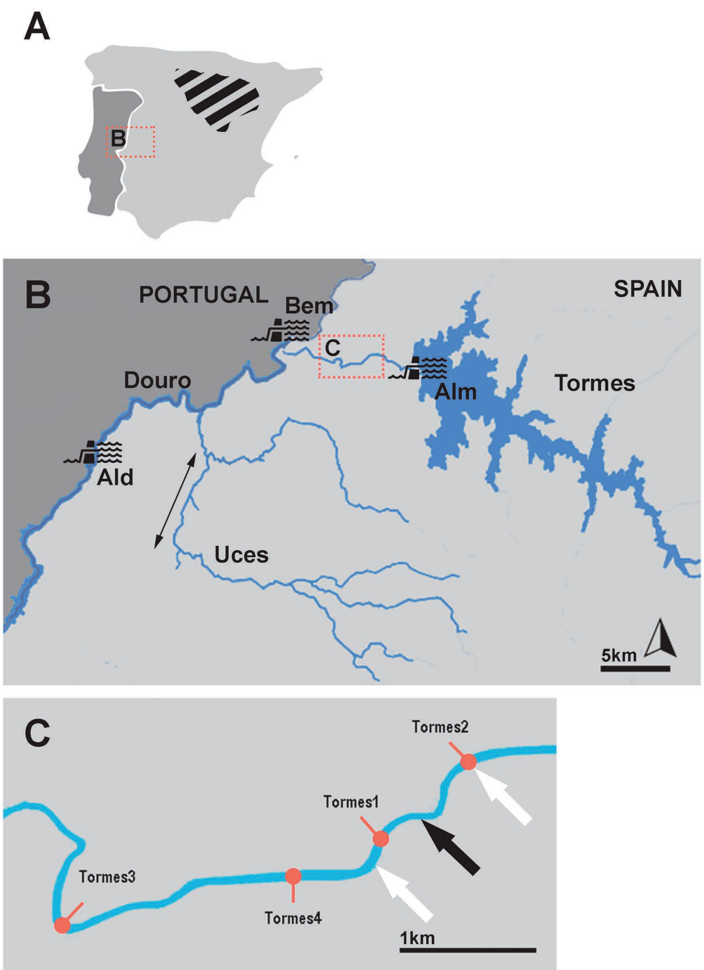

Figure 1. A) Spatial representation of the known beaver distribution in the Iberian Peninsula before 2022 (dashed area) and the location of the new findings in 2022/2023 in Douro basin, Western Iberia (red square). **B)** Location of the Tormes, Uces and Douro rivers with the position of the Almendra (Alm), Bemposta (Bem) and Aldeadávila (Ald) dams as well as the sampled segment in Uces River (line with double arrowhead) and the sampling area in Tormes River (red square). **C)** Location of the camera traps in the Tormes River sampling area, with the area between the white arrows indicating the location of presence signs previously documented by Calderón *et al.* (2022), while the black arrow indicates the location of the hair sample collected for genetic analysis.

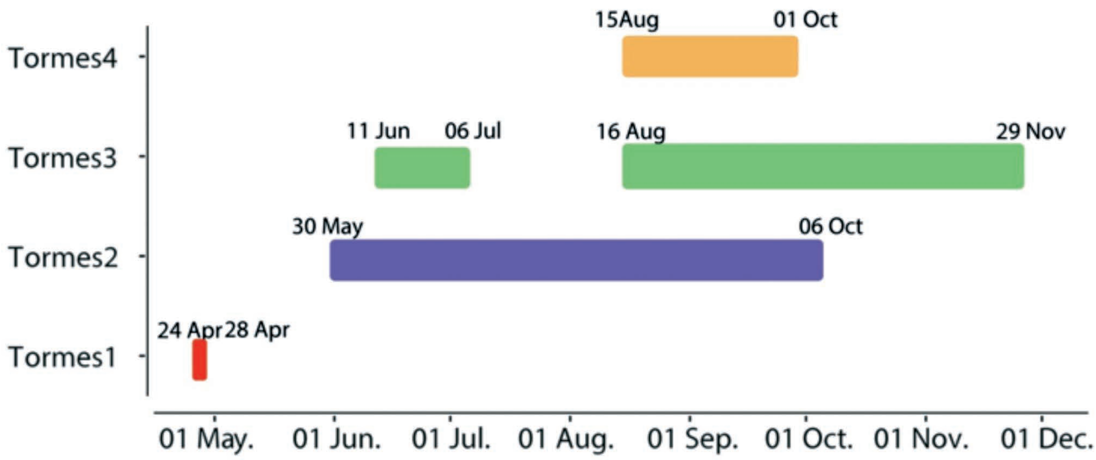

Figure 2. Periods of data collection by camera trapping in 2023 in the 4 sampling locations of the Tormes River (Douro basin, Salamanca province, Spain).

male; F: female) and behaviour (*Grooming*: individual(s) cleaning their fur; *Feeding*: individual(s) feeding either directly from a tree trunk or from previously cut branches; *Resting*: individual(s) sleeping/resting; *Transporting material*: individual(s) moving around carrying branches; *Walking*: individual(s) moving around without carrying material; *Escaping*: individual(s) moving quickly without carrying material). In addition, the tail scar pattern was used to identify different beaver individuals (Mayer *et al.* 2020, Dytkowicz *et al.* 2024) while the presence of adults with evident mammary glands was considered evidence of reproduction (Rosell & Campbell-Palmer 2022).

Taxonomic assignment (Genetic non-invasive sampling)

A single hair sample collected in a non-invasive manner was used for the genetic analysis. The hair sample was collected by setting an arch formed with barbed wire in one of the paths used by beavers in Tormes River. We could not find biological samples in Uces River. The collected material was kept in a paper envelope until processing in a dedicated laboratory used exclusively for low-quality DNA samples in CIBIO (Research Centre in Biodiversity and Genetic Resources, Portugal). DNA extraction was performed using the commercial E.Z.N.A. tissue DNA kit (Omega Bio-Tek, GA, USA), with an additional initial step of digestion with a lise wash buffer described in Maudet *et al.* (2004) for 15 min at 56ºC. A 500 base pair fragment of the D-loop gene (or control region) of the mitochondrial DNA was amplified by PCR, using the oligonucleotides Thr-L15926(5′-CAATTCCCCCCGGTCTTGTAACC-3′) and DL-H16340 (5′-CCTGAAGTAGGA-ACCAGATG-3′), according to the conditions described in Durka *et al.* (2005). A negative control was always included during DNA extraction and PCR to monitor for potential contamination. Subsequently, exonuclease I and alkaline phosphatase enzymes were used to purify the PCR product, and then sequenced with BigDye chemistry (Applied Biosystems), using the Thr-L15926 oligonucleotide and following the BigDye Terminator v3.1 cycle sequencing protocol (Applied Biosystems). The electropherogram was visually inspected and aligned using the GENEIOUS program (https://www.geneious.com). In addition, the obtained sequence was compared with sequences available in the GenBank database (Clark *et al.* 2016) of European beaver and American beaver for the same fragment (see Durka *et al.* 2005 and Table S1), allowing identification of species and lineage. First, a BLASTn was performed against the database to determine whether the obtained sequence belonged to European or American beaver. Next, the sequence obtained was aligned with those available using the Mafft program (Katoh & Standley 2013). Finally, a Neighbor-Joining Haplotype Network (Bandelt *et al.* 1999) and a phylogenetic maximum likelihood tree using the ungapped alignment, a GTR substitution model, GAMMA model of rate heterogeneity and 100 randomized starting trees (RAxML; Stamatakis 2014) were reconstructed to clarify the mitochondrial lineage of origin.

Results

Distribution

During the sampling period in the Douro basin located in the western part of the Iberian Peninsula, presence signs of beavers were found along the banks of two rivers: Tormes and Uces (Fig. 1B). In the case of Tormes River, the beaver signs extended along 15 km of river, from the Almendra Dam to its mouth in the Douro River, just a few meters of distance from the Portuguese border. In the case of Uces River, presence signs were identified along 2 km of the river, most of them concentrated in a pond during the summer, as well as in some isolated points of tributary banks.

The main evidence of beaver presence in both rivers consisted mainly of gnawed logs, with the main tree species affected being *Populus* sp. and *Salix* sp. In addition, we also identified some *Quercus pyrenaica* and *Arbutus unedo* trees with bite marks in the Uces River area (Fig. S1). Three den burrows were found in the Uces River banks (two of them collapsed) and in the Tormes River was found one additional den burrow as well as a small cave/shelter accessible only from the water. Several scent mounds (i.e., small piles of mud near the water's edge with castoreum, a substance from the castor sacs used for scent marking; Rosell & Campbell-Palmer 2022) were observed on the banks of Tormes River.

Direct observations were made on both rivers, with individuals being only observed at dusk and during the early night hours (20:00h to 23:59h), except for one individual that was sighted at midday (12:00h to 15:59h) in Tormes River. All direct observations involved a single beaver, except: i) two adult individuals observed in Tormes River using a burrow and transporting plant material; and, ii) an adult individual together with a kit born that year observed in Uces River during night observation (Table 1).

Population status

In 4 locations at Tormes River, we conducted an overall camera trapping effort of 310 days (187 calendar days, as some cameras made recordings in different locations on the same day, see Figure 2) at different sampling intervals depending on the location. A total of 1547 records of mammals were obtained, 1344 (88%) of which corresponded to beavers. The species identified, as well as the detection effort and detection rates by species and location are presented in Table S2.

During the sampling period with camera-trapping in Tormes River, there was evidence of one family group with reproduction, and a possible second group downstream (Table 1). One group was detected in 3 sampling locations (Tormes1, Tormes2 and Tormes4) based on the following evidences: i) An adult individual with a prominent abdomen, compatible with pregnancy, was first detected by camera-trapping in Tormes1 in April 2023; ii) Later, two different adult individuals were identified by camera-trapping in Tormes2 and Tormes4 based on the tail pattern (presence of distinctive scars on the tail) (Fig. 3A-B); iii) One of those adult individuals exhibited evident mammary glands in spring-summer 2023, compatible with nursing (Fig. 3C-D); iv) a kit with less than 6 months accompanied

Table 1. Summary information on the breeding groups found in the study area. *Indicates the non-confirmed, reproductive group.

Area	Family group	Members	Sampling location
Uces River	1	1 Adult 1 Kit	NA
Tormes River	2	2 Adults 4 Kits	Tormes1 Tormes2 Tormes4
	3*	2 Adults 1 Kit	Tormes3

by the mother was first identified in Tormes2, on August 21st; and v) thereafter, numerous records of up to four kits (Fig. 4A) together with the tail-marked adult pair were obtained in Tormes2 and 4. Additionally, another potential family group composed of two adult individuals without tail scars and accompanied by one kit (which could not be differentiated from the other 4 kits found upstreaming in the Tormes River), was detected in Tormes3 from July onwards (Table 1)

Overall, we identified two family groups with reproduction during 2023, one in Uces River (based on direct observation of 1 adult with a kit, see above) and other one in Tormes River (based on camera trapping of 2 adults and 4 kits), and another potential reproductive group in Tormes River (based on camera trapping of 2 adults and possibly one different kit), comprising a minimum of 10 individuals, including 5 adults and 5 (possibly 6) kits (Table 1).

Furthermore, the data collected in the four camera trap locations in Tormes River allowed the identification of different types of behaviour, with some differences found between locations (Fig. 4B). In Tormes1 and Tormes2 the most recorded activity was *feeding,* while in Tormes3 the predominant behaviours were *walking* and *transporting material*, and in Tormes4, most of the records showed *resting* behaviour.

Finally, regarding the period of activity based on camera trapping, most of beaver records were obtained during night (interval*:* 00:00h to 03:59h) in Tormes1, 2 and 3, while in Tormes4 the main activity was recorded at dawn and during the daytime (intervals 04:00h to 07:59h and 16:00 to 19.59h, respectively). It is also noteworthy the high activity detected during the central hours of the day in Tormes4 (08:00 to 11:59h and 12:00 to 15.59h) in contrast to the scarce daytime activity in the other three locations (Figure 3C).

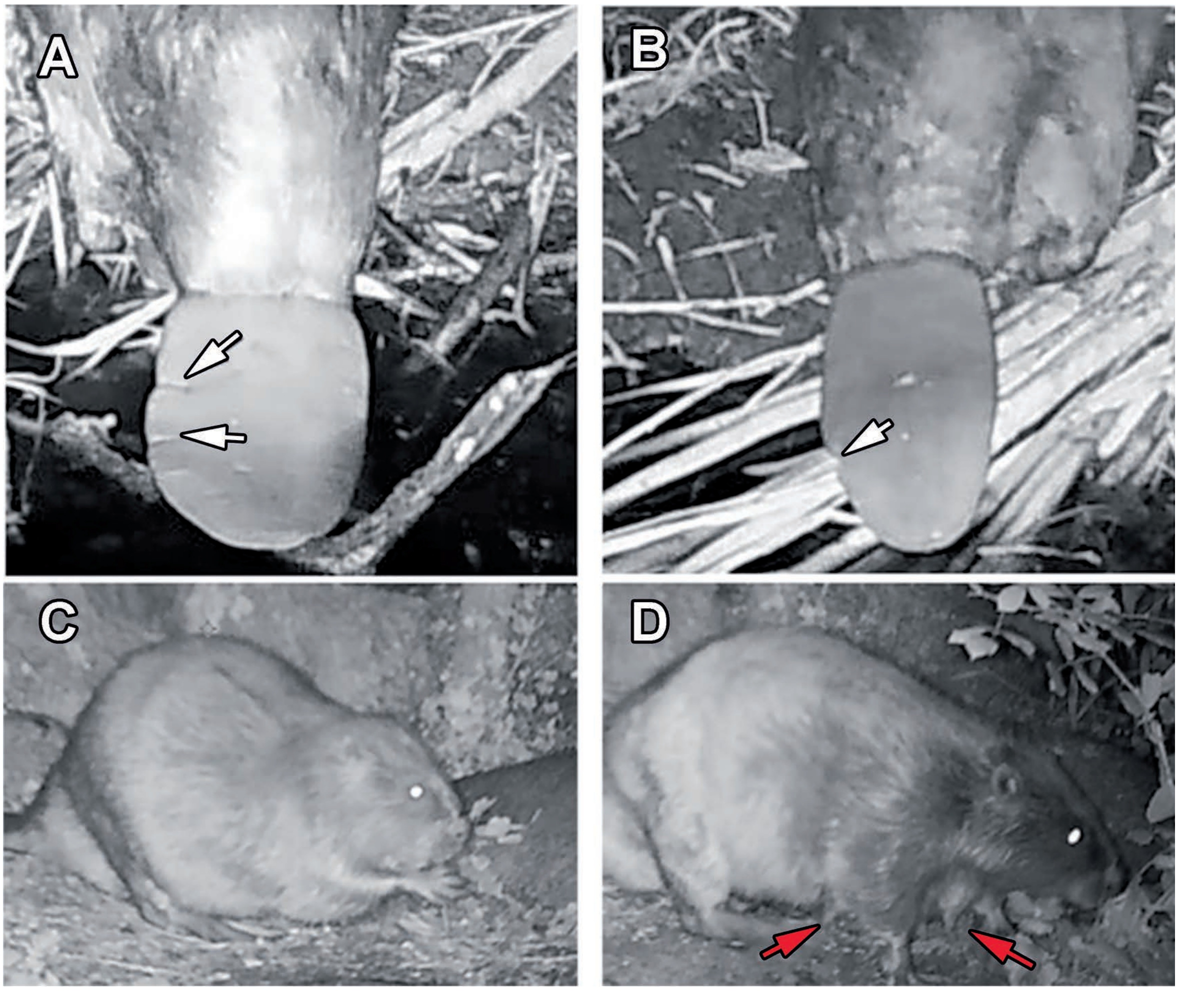

Figure 3. Camera trap images showing (A, B) the identification of tail patterns in two different individuals (white arrows) and (C, D) the absence and presence of mammary glands in adult individuals (red arrows).

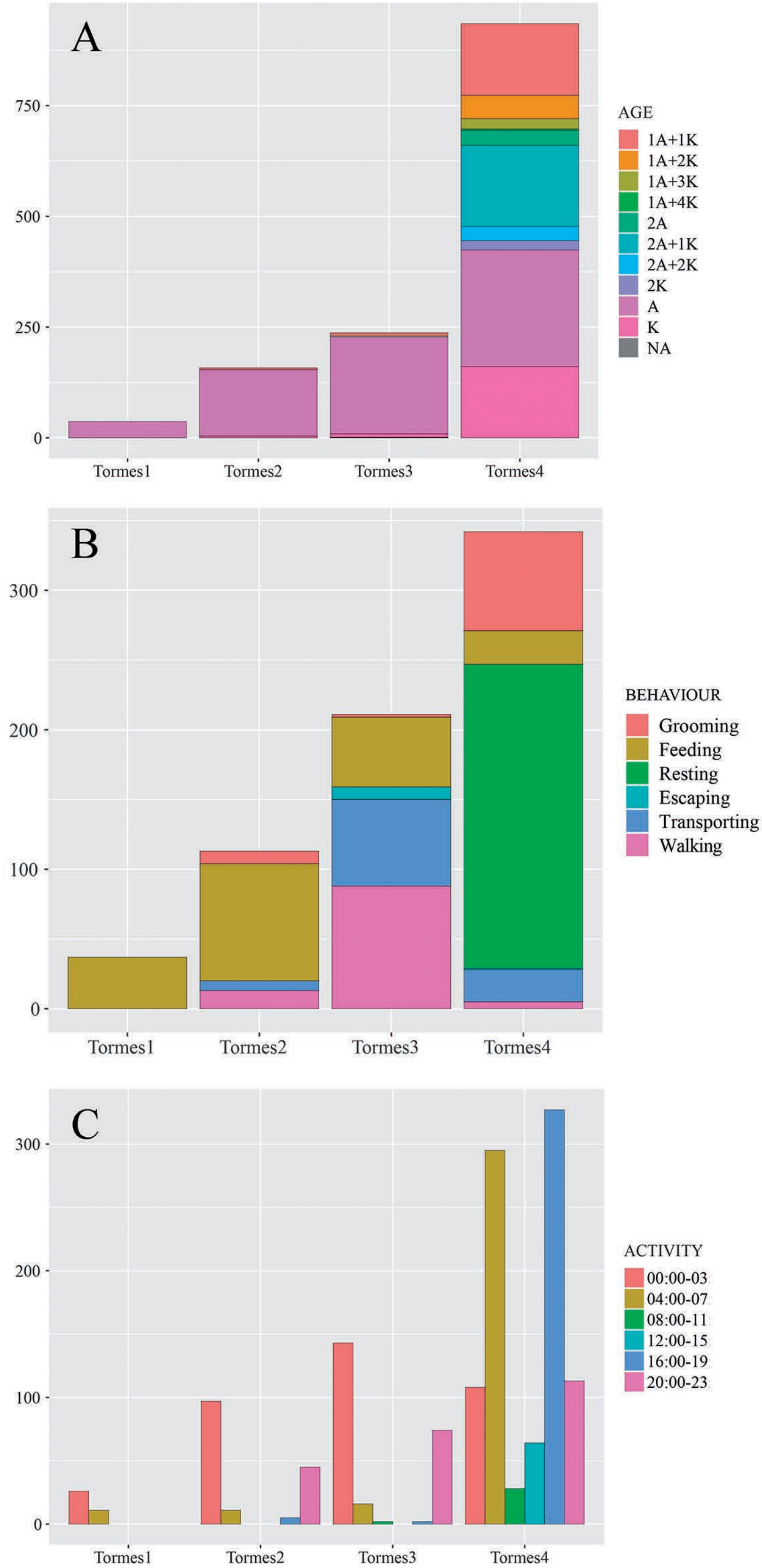

Figure 4. Number of camera trapping records of beaver obtained in each area of Tormes River, according to **(A)** nº of detected individuals per age class (A: Adult; K: kit), **(B)** type of recorded behaviour, and **(C)** activity period.

Taxonomic assignment

Genetic confirmation of the species and subspecies of the beavers found in Douro basin was achieved by successfully extracting DNA from a hair sample collected in Tormes River, amplifying it for the D-loop fragment and successfully sequencing it. BLAST analysis confirmed the sequence from our sample belonging to the European beaver (529 base pairs with 100% Query Cover, e-value of 0 and ~100% identity in significant alignments; GenBank accession number: PV449090). Specifically, in the RAxML maximum likelihood tree, our sample clusters within the Western European mitochondrial lineage defined by Durka *et al.* (2005) (Fig. 5A). The same result is observed in the NJ haplotype network including sequences available in GenBank (see Table S1), although the assignment of samples to the respective eastern and western mitochondrial lineages is not fulfilled for all geographical areas (see Frosch *et al.* 2014, Senn *et al.* 2014) (Fig. 5B).

Discussion

This study confirmed the presence of a small breeding population of the western lineage of European beaver in two Spanish tributaries of the Douro Basin, the Tormes and Uces rivers, located next to the border with Portugal. The relatively large distance (~20 to 30 km) and the topography barriers (i.e., great cascades) between the two rivers may suggest two independent release sites rather than natural dispersal. These findings add additional knowledge in relation to previous studies (Calderón *et al.* 2022) and provide crucial information for adequate management at a transboundary level.

The confirmed presence of beaver in Uces River is first reported in this study, comprising at least one family group apparently with reproduction in the sampling year (2023), based on the direct observation of an adult with a kit. Regarding the Tormes River, where beaver presence was already reported by Calderón *et al.* (2022), data gathered by camera-trapping confirmed the presence of one family group of 6 individuals (two breeding adults identifiable by tail scars and 4 offspring born in 2023). The presence of at least two more adults in the Tormes3 location (without tail scars) accompanied by a kit born in 2023 suggests the presence of a second breeding pair downstream in the same river and approximately 1 km apart. However, it is important to note that it was not possible to confirm if the kit from Tormes3 was different from the ones detected in the family group upstream. Typically, a beaver family group is composed of the breeding adults, the previous year's offspring, plus the same year's kits (Busher 2007). Given the presence of beaver in the Tormes River, at least since the summer of 2022 (Calderón *et al.* 2022), there is a possibility that some individuals detected in Tormes3 could be the previous year's offspring accompanied by one of the kits detected in the group upstream, resulting in just one breeding pair in Tormes River. However, considering the average territory size of 3 km for a beaver family (Rosell & Campbell-Palmer 2022), it is likely that at least, two family groups may exist in the sampled area of river Tormes, one group encompassing the locations Tormes1, 2 and 4, below the Almendra dam, and another group extending from location Tormes3 towards the mouth into Douro River, in the Portuguese border. This hypothesis can also be supported by the several scent mounds found along Tormes River, since it is reported as a scent marking behaviour for territory defence (Dewas *et al.* 2012, Rosell & Campbell-Palmer 2022). Nevertheless, territory size is conditioned by numerous factors that may cause large variations (Campbell *et al.* 2005), rendering the need for further research to estimate more accurately the number of breeding pairs and beaver population size in both Tormes and Uces rivers.

The activity patterns of beavers in Tormes River showed wide variation, being mainly nocturnal in locations Tormes1, Tormes2, and Tormes3 and mostly diurnal in Tormes4. This pattern can be related to the use of this last location as a resting area, due to limited human disturbance. In general, the species usually dens in burrows in riverbanks, which are unnoticeable from outside the water unless they are collapsed or have a hole covered with small branches (Rosell & Campbell-Palmer 2022), as found in Uces River. However, in cases where the terrain prevents the creation of these burrows, beavers can take advantage of natural refuges such as caves or cavities between rocks (Rosell & Campbell-Palmer 2022), such as the one found in Tormes River. In fact, the presence of this resting cave in location Tormes4 explains the frequent records of resting and grooming behaviour obtained in this location compared to the other ones.

Differences were also found in the number of individuals detected in the different locations along Tormes River. While in Tormes4, there are

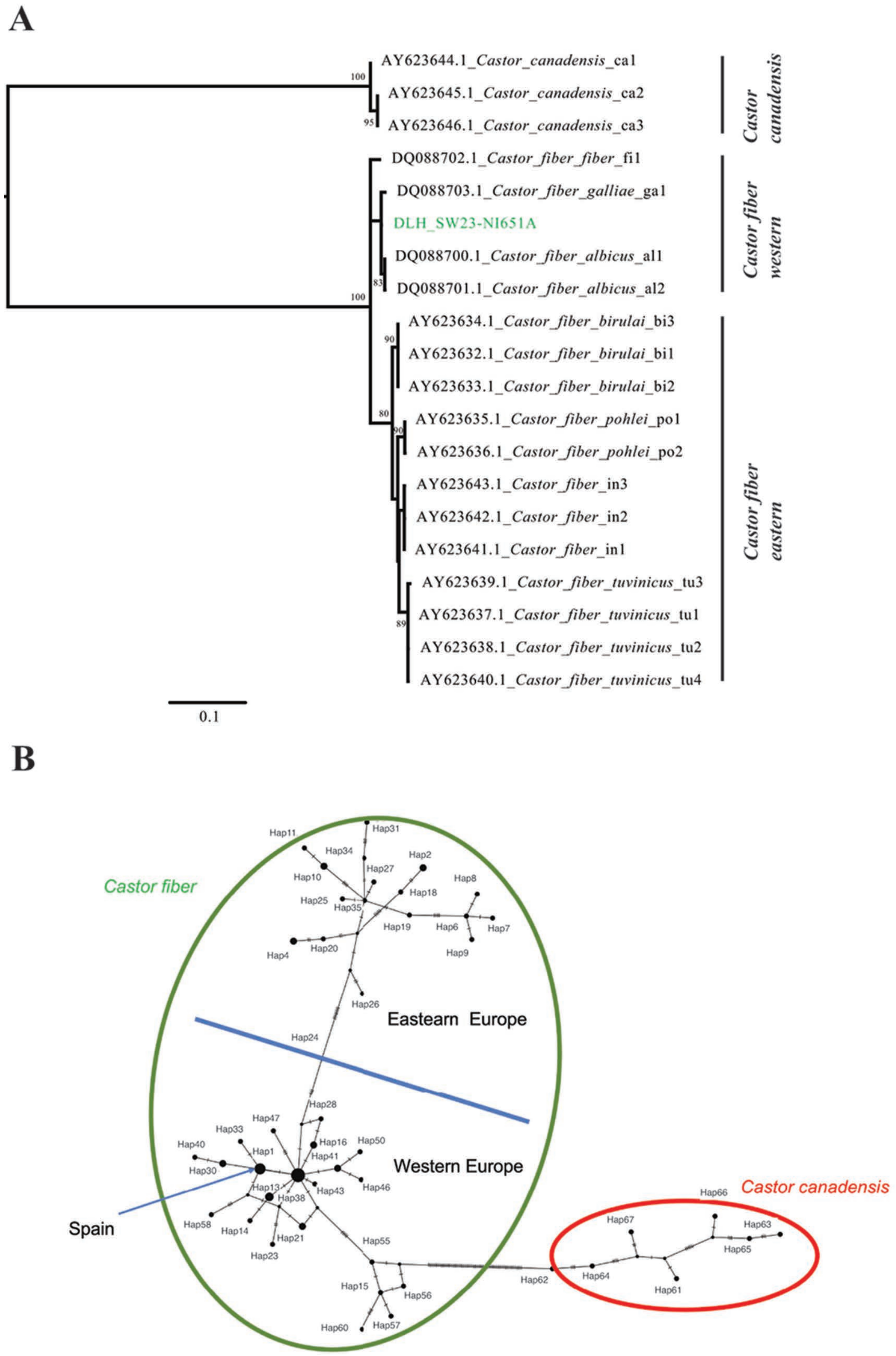

Figure 5. A) Phylogenetic reconstruction from the 468 base pairs ungapped alignment of the D-loop based on the RAxML method, with 100 initial trees, showing the inclusion of the hair sample collected in Tormes River (highlighted in green) in the western clade of European beaver formed by the two subspecies in southwestern Europe (*Castor fiber albicus* -al- and *C.f. galliae* -ga-). Also included are the remaining subspecies (western: *C. f. fiber* -fi- and eastern *C. f. tuvinicus* -tu-, *pohlei* -po-, *birulai* -bi-) considered in Durka *et al.* (2005) rooted with the Canadian beaver (*Castor canadensis*). Bootstrap values are shown for nodes above 70%. The scale is given in substitutions per position. **B)** Neighbour-Joining haplotype network including available D-loop sequences from the GenBank database, showing the sample from Tormes River (marked as Spain) included as *Castor fiber* from Western Europe.

numerous records of two breeding adults with up to 4 kits, in the other locations, adults tend to appear individually, which could indicate a strategy to reduce resource competition (Rosell & Campbell-Palmer 2022). This behaviour was observed in a beaver population in Norway (MCClanahan 2020), and could also be the case in the Tormes River bank since the presence of areas with poplars and willows is quite scarce (Marino *et al.* 2014). Nevertheless, the frequent detection of adults with kits in location Tormes4 can also be related to other characteristics found in this important site, probably used for denning, such as low human disturbance, presence of a resting cave and a dominant daily activity with resting and grooming behaviours, as mentioned above.

Taxonomic affinity

Non-invasive genetic analysis confirmed that this new population (or at least the one from the Tormes River) consists of European beavers from the same Western European lineage previously identified in the Ebro River basin in north-eastern Spain, suggesting that the Iberian individuals likely originated from the same source population (Gómez-Moliner & Ruiz-González 2008). However, the use of a single mitochondrial DNA sample hinders the possibility of drawing more robust conclusions, since it could be expected that a mix of east and west genetic material will be found in this Iberian population in line with what has been described in the Ebro basin population, with a potential source from Germany (Ceña *et al.* 2004, Frosch *et al.* 2014, Senn *et al.* 2014). Therefore, considering that these Iberian populations result from unauthorized reintroductions of few individuals with unknown genetic diversity, more comprehensive genetic studies combining information from other markers including nuclear (e.g., microsatellites or SNPs) are needed to better clarify the origin, size and genetic fitness of this population in Douro River along with the rest of the populations found in the Iberian Peninsula (Frosch *et al.* 2014, Senn *et al.* 2014). This molecular approach using nuclear markers in non-invasive samples would allow to identify different individuals, evaluate the degree of kinship between them, determine their genetic diversity or inbreeding, and, combined with other capture-recapture statistical methods, to accurately estimate the population size and survival (Carroll *et al.* 2020). This information is highly relevant and necessary to carry out appropriate management and conservation measures given the low degree of knowledge on this species in Spain. Furthermore, the development of global management tools aimed at monitoring beaver populations in Europe is a challenge that has to incorporate genetics to properly evaluate the phylogeographic history shaped by glacial refuges and natural dispersal, and, most importantly, the origin and impact of human translocations carried out so far.

Future trends and management recommendations

Our short but effective survey based on a multi-method approach has unveiled crucial and innovative information regarding the presence of European beavers in the western Iberian Peninsula, apparently as a result of an unauthorized release (Calderón *et al.* 2022). A similar methodological approach should be promoted to monitor areas of known occurrence in the Iberian Peninsula (e.g., Ebro and Douro basin) or new sites with documented presence of beaver, such as the recent sightings in Tagus River (Ansón & García-Prendes 2024) and in Guadalquivir River (Burón *et al.* 2023, Bogallo *et al.* 2025), located in central and southern Spain, respectively.

The current population in the Douro basin was estimated in approximately 10 individuals, with two different areas of occurrence and at least two breeding pairs with reproduction in 2023. However, it was not possible to assess the exact date and original number of individuals released in this area, where beaver presence was first reported in summer 2022 (Calderón *et al.* 2022). Generally, when beaver become established in a new territory, the population growth rate is usually very slow since the dispersing individuals are juveniles and their mating probabilities are low (Rosell & Campbell-Palmer 2022). However, the Tormes River and Uces River populations consist of breeding adults and their offspring, so the population growth rate is expected to be faster. However, range expansion may not follow a regular pattern as, according to the findings from Fustec *et al.* (2001), distant suitable sites could be occupied before the nearer sites with lower habitat quality, which would be progressively occupied by dispersal individuals from previously settled sites.

Beaver populations in the Uces and Tormes rivers face important dispersal challenges, as there are several dams in the area, which may act as barriers to the dispersal of this species (Halley & Rosell 2002, Falaschi *et al.* 2024, Serva *et al.* 2024). In the case of Tormes River, dispersal is unlikely to occur upstream due to the presence of the Almendra dam (ca. 200 metres height). Downstream, the Tormes River flows into the international section of the Douro River, which constitutes the border between Portugal and Spain, and where there are several dams for hydroelectric power. The nearest is the Bemposta dam (ca. 90 metres height), located in the Douro River immediately upstream from the Tormes River mouth. In addition, the Aldeadávila hydroelectric dam (ca. 150 metres height) is located ca 30 kilometres downstream from the mouth of Tormes River and 15 kilometres from the mouth of Uces River. Therefore, these barriers, especially downstream, would restrict the potential expansion of beavers along the Douro River, as the Aldeadávila dam is enclosed in a rock canyon. Moreover, in most of the international Douro River, banks are quite steep and unvegetated (the rock canyon meets the water), which makes the main river less suitable to beavers settle in. Notwithstanding, the Douro River may act as a potential corridor to high-quality sites in the main tributaries.

Considering the proximity of the two presence areas described here, and the fact that beaver individuals come from illegal introductions (Calderón *et al.* 2022), it is possible that the locations of release were chosen to maximize the viability of the species in the territory. Moreover, the rough topographic conditions make the area suitable for a cryptic population, difficult to detect as it may live in burrows and rarely felling trees (i.e., feeding mainly on grasses, forbs and small twigs), rendering the possibility that there may exist other undetected family groups or introduction sites along Douro Basin. This phenomenon of illegal beaver introductions spread throughout Europe, has been an increasing trend also in Spain, where beavers were recently reported in Guadalquivir and Tagus rivers (Burón *et al.* 2023, Ansón & García-Prendes 2024). The scattered distribution of these illegal releases in Iberia suggests the purpose of encouraging the wide settlement of this species to establish a viable population. Regarding Portugal, where the species was extinct and no illegal introductions are documented, beaver presence was recently confirmed in the Portuguese bank of the international section of Douro River, near the mouth of Tormes River, based on camera-trapping records of one individual in June 2025 (Couto *et al.* 2025), after the sampling period of our study but quite expected given our results in Tormes River.

Considering the expected wide return of the European beaver to Iberian rivers, it becomes mandatory to define trans-border management guidelines and actions for this native species, particularly in Portugal, where it is considered extinct since historical times (although still unclear during which period; Román & Aguilar-Gómez 2023) but is not yet assessed in the national red data book (Álvares *et al.* 2019). Detailed data on beaver populations, as the one reported in this study, will contribute to monitoring demographic parameters, habitat selection, distribution trends and environmental effects, as well as to allow ecological modelling to predict expansion routes and future range. Besides research efforts on population status and ecology, field conservation actions are urgent to create public awareness on the relevance of this key species in ecosystems and to prevent potential conflicts such as damage to tree crops and forestry (Campbell-Palmer 2016), as this can generate a negative social perception towards beavers (Taylor *et al.* 2017).

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