BMC Veterinary Research

CASE REPORT Open Access

Free-ranging Eurasian otter (*Lutra lutra*) with rare osteoblastic osteosarcoma: a forensic and genetic investigation from central Italy

Francesca Maccagnan[1], Lorena Di Benedetto[1*], Claudia Eleni[2], Valentina Galietta[2], Amaranta Biancheri Mantovani[3], Emiliano Mori[4,5], Mariella Baratti[4], Leonardo Ancillotto[4,5], Andrea Viviano[4], Leonardo Leonardi[6] and Giulia Rosa[1]

Abstract

Background The Eurasian otter *Lutra lutra* experienced a dramatic decline in Europe during the 20th century. The urbanization of human population following the abandonment of countryside as well as reintroduction plans led to a recent recovery in many areas.

Case presentation We investigated both cause of death and genetic origins of a recently discovered dead otter specimen in Tuscany, a region of central Italy where the species disappeared three decades ago. The necropsy was performed using a forensic approach. We also sequenced three gene fragments [cytochrome-oxidase subunit I (COXI), cytochrome-b (cytb), control region (D-loop)] of the mitochondrial DNA (mtDNA), to infer to which populations the rescued individual was genetically closest to. Then, we compared these sequences with those retrieved from fecal samples collected in north east Italy as well as with many others downloaded from the GenBank.

Conclusion This is the first case of a neoplastic lesion in a bone tissue of wild Eurasian otter, with morphological features compatible with osteoblastic osteosarcoma. Molecular analyses revealed a high affinity of the individual with European *L. l. lutra* specimens, particularly with those from England (COXI) and Italy (D-loop and cytb).

Keywords Eurasian otter, *Lutra lutra*, Osteosarcoma, Histology, Genetics, Forensic veterinary medicine

*Correspondence:
Lorena Di Benedetto
lorena.dibenedetto@izslt.it
[1]Centro di Referenza Nazionale per la Medicina Forense Veterinaria, Istituto Zooprofilattico Sperimentale del Lazio e della Toscana M. Aleandri, Viale Europa 30, Grosseto 58100, Italy
[2]Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", via Appia Nuova 1411, Roma 00178, Italy
[3]Istituto Zooprofilattico Sperimentale del Lazio e della Toscana M. Aleandri, Viale Europa 30, Grosseto 58100, Italy
[4]Research Institute on Terrestrial Ecosystems IRET, CNR, Via Madonna del Piano 10, Sesto Fiorentino – Firenze I- 50019, Italy
[5]National Biodiversity Future Center, Palermo 90133, Italy
[6]Dipartimento di Medicina Veterinaria, Università degli Studi di Perugia, Via San Costanzo, 4, Perugia 06126, Italy

Background

Apex predators, such as otters (Mammalia: Mustelidae), play a crucial role in maintaining the delicate balance of river ecosystems [1]. Their genetic variability is pivotal for the local adaptation of these species [2], allowing them to respond to environmental challenges, including changes in food availability, habitat loss, and emerging diseases [3–5].

Otters comprise 13 species in 8 genera, distributed across the Americas, Eurasia, and Africa [6]. Except for the North American river otter *Lontra canadensis*, all these taxa are listed as "Near Threatened" (including the

Eurasian otter *Lutra lutra*) or "Threatened" by the International Union for Conservation of Nature [7]. The Eurasian otter ranges from Portugal to Japan and Indochina, including southern India, Sri Lanka and Sumatra (Indonesia), with 12 morphological subspecies ([7, 8], 8: Fig. 1).

Despite the huge range, the species is listed as "Near Threatened" [8]. In Europe, the decline of the native populations (*L. l. lutra*) was mainly due to habitat destruction, pollution, and overfishing [6–9].

In protected wildlife species, forensic necropsy represents a valuable tool for determining the cause of death, particularly when illegal killing or other non-natural causes are suspected [10, 11]. Indeed, forensic methodology can provide reliable evidence on mortality causes, helping to identify anthropogenic threats—such as poaching, trauma of suspicious origin, or improper handling—that may otherwise go undetected. This information is essential for wildlife authorities and conservation managers to adopt appropriate mitigation measures, improve surveillance, and support law enforcement actions aimed at protecting recovering populations. In the literature, a documented case has shown that the application of a forensic protocol was decisive in confirming the unlawful killing of a Eurasian river otter (*Lutra lutra*) [12]. A *post-mortem* study conducted on this species between 2009 and 2017 has led to identify road traffic collisions as the major cause of death, followed by blunt chest trauma of uncertain origin, attacks by dog and conspecific and, more rarely, diseases [9]. Nonetheless, neoplastic diseases are rarely reported in Eurasian otters with a few cases described only in captive individuals [13–17] and to the best of our knowledge no case of osteoblastic osteosarcoma has ever been documented.

Low genetic variability in the Eurasian otter is linked to the species decline across Europe [18–20]. Italian museum specimens holds two mitochondrial DNA (mtDNA) haplotypes only [21], whereas modern individuals from England display a higher diversity, which is most likely due to multiple releases [22, 23]. Individuals from Russia and Transcaucasia also show a great variability, with unique haplotypes and endemic subspecies *L. l. meridionalis* and *L. l. seistanica* (Fig. 1 [8, 22]),.

Since the 1990 s, national action plans, reintroduction projects, and international protection laws such as the Berne Convention and the Habitats Directive 92/43/EEC have supported the recovery of several European otter populations, including those from southern and central Italy [9, 24, 25]. The increasing concentration of people in urban areas has also favored the natural re-expansion of relict populations [24], though long-term monitoring is still required to track population trends [25].

Fig. 1 Distribution of subspecies of *Lutra lutra* in Eurasia and northern Africa(source: www.iucnredlist.org (Accessed on 20.12.2025); [8]

In Italy, otters showed the same decline–recovery pattern as that observed in conspecific populations across Europe [21, 24–26]. Up to the 1970 s, the Eurasian otter was widespread across the Italian peninsula [21], but in the 1980 s numbers fell sharply, with the species surviving only in the southernmost regions with an estimated number of 100–130 individuals [21]. In the late 1990 s, releases were carried out in the Ticino River (north-western Italy) using otters of genetic lineage "B" (*L. l. barang*), introgressed with the Indochinese subspecies and therefore unsuitable for conservation programs [24–26]. Following a legal protection under the Italian national law, a slow but steady recovery began, and otters recolonized most of the river basins in southern Italy and expanded towards central regions [24–28].

Since 2011, recolonization of northern Italy has occurred via natural dispersal from populations of neighboring countries [29–31], with rivers in a natural state acting as ecological corridors [32]. A recent national monitoring, combining spraint analysis, camera-trap records, and environmental DNA, has confirmed the occurrence of otters in parts of central Italy where they were formerly extinct [33], as well as in north-western areas near the border with France [25, 34, 35].

The Italian population is currently estimated at about 1,000 individuals, a size far below the 5,400 which is considered as the minimum viable threshold [36, 37], with the species being still absent in large parts of its historical range [25]. Eurasian otters are not confirmed in Aosta Valley, Umbria and Emilia-Romagna, although in the latter some reports are under verification [25]. Isolated findings from northern Tuscany, Piedmont and the Po River delta (southern Veneto), such as road-killed or deceased individuals, remain unexplained (EM and LA, personal observation 2024; [38].

In Tuscany (central Italy), the last records for the occurrence of otters date back to late 1980 s - early 1990 s [39–41]. In 2024, after a survey with no evidence for the occurrence of the species conducted between 2022 and 2023, a male otter was found dead in the province of Massa Carrara, suggesting a recent recolonization of the northern part of this region [25]. Considering the unknown origin of this individual and the suspicious context of the finding, we wanted to investigate the cause of death also to exclude potential anthropogenic or illegal causes through a forensic necropsy and to assess the genetic identity of the otter in point by means of a comparison with available datasets from conspecifics sampled across the species' range.

Case presentation

A dead adult male otter was found on the banks of the Magra River (locality La Barca, province of Massa Carrara) in March 2024. This place was approximately 170 km away from the northernmost (Ticino River), 150 km from the westernmost (Liguria), 250 km from the easternmost (Veneto), and 410 km from the southernmost (Latium) known Italian populations [25, 38]. Although a few evidences suggest the occurrence of some otter movements along the coastline [42], the origin of this individual remains uncertain, also because there are no nearby enclosures from where it could have escaped [25, 38].

The specimen was delivered to the laboratory of the Centro di Referenza Nazionale per la Medicina Forense Veterinaria at the Istituto Zooprofilattico Sperimentale del Lazio e della Toscana (IZSLT) in Grosseto, for a *post-mortem* examination.

Post-mortem examination

The necropsy was conducted by forensic pathologists strictly adhering to veterinary forensic guidelines [10]. The corpse was initially kept at −20 °C and then maintained for 7 days at a refrigeration temperature (5 °C) to warrant for a gradual and non-traumatic thawing process. Tissue and organs samples were collected for supplementary laboratory exams. Bacteriological cultures were performed after necropsy on lungs, kidney, liver, heart, and brain using standard aerobic methods [43]. Virological analyses on lungs, kidney, liver homogenates were conducted through cell culture isolation; intestinal samples were examined using transmission electron microscopy [44]. For parasitological screening, fecal samples were analyzed using flotation and the FLOTAC® technique [45]. Samples from thigh muscle and femoral bone with macroscopic lesions were collected, fixed in 10% buffered formalin, decalcified with a commercial mixture of hydrochloric acid and EDTA for 48 h and then routinely processed for histopathological examination. The entomological fauna was collected for species identification through a macroscopic examination.

Genetic analyses

DNA was extracted from 30 mg of tissue sample (lateral body muscular part) using a Qiagen Blood and Tissue kit (©Qiagen, Inc, Aarhus, Denmark). Moreover, we extracted DNA from three spraints collected in north-eastern Italy (Friuli Venezia Giulia) in the framework of the national monitoring program [25], using the GenEluteTM Stool DNA Isolation Kit (©Merck, Burlington, Massachusetts, USA). Both fluorometry (Qubit) and Nanodrop™ spectrophotometer (Thermo Fisher Scientific Inc.) were used to measure the amount of DNA obtained from the Magra River otter sample (191 ng/µl), and the absorbance ratio, respectively A260/A280 = 1.9, and A260/A230 = 1.9. We amplified three mitochondrial DNA fragments of the otter individual: a 5' portion of cytochrome-b (hereafter, cytb, 308 bp) using the universal primers CYTB L14841 (5'-AGAACACCCATTC

ATCATTATGC-3′) and CYTB H15149 (5′-AAACTG CAGCCCCTCAGAATGATATTTGTCCTCA-3′) [46] the 3′ region of the cytb, tRNAPro, tRNAThe, the whole control region (D-loop), and the 5′ region of 12 S rRNA using LutbF_15201 (5′-AGAACACCCATTCATCATTA TCG-3′) and LLU12SH91_620 (5′-CTAGAGGGATGTA AAGCACCG-3′) [47] and a minibarcode, a small portion (169 bp) of the cytochrome oxidase I (hereafter, COXI, see below). PCR reactions were carried out in an Eppendorf MasterCycler X50 thermal cycler with a 25 µL mix including 100 ng of DNA, 2.5 µL of 10X reaction buffer, 1 µL of 2 mM MgCl2, 1 µL of each 200 µM dNTPs, 1 µL of each 0.2 µM primer, and one unit of Taq DNA polymerase (©Life Technologies, Waltham, Massachusetts, USA). For both the cytb and the D-loop, the PCR thermal profile included an initial denaturation at 95 °C for 2 min, followed by 35 cycles of 95 °C for 50 s, annealing at 50 °C (for cytb)/55°C (for D-loop) for 45 s, extension at 72 °C for 1.5 min, and a final extension phase at 72 °C for 10 min (modified from [48]).

Since the DNA extracted from the spraints was of low quality (quantity: < 1ng/µl, absorbance ratios: A260/A280 and A260/A230 < 1.8), we amplified the minibarcode through the universal primers UniMiniBarF1_13906 (5′-TCCACTAATCACAARGATATTGGTAC-3′) and UniMiniBarR1_14075 (5′-ACTATAAAGAAGATTATT ACAAAGGC-3′) [49]. The gene fragment was amplified through a touch-up PCR profile: initial denaturation at 95 °C for 2 min, followed by 5 cycles at 95 °C for 1 min, 46 °C for 1 min, and 72 °C for 30 s, followed by 30 cycles at 95 °C for 1 min, 56 °C for 1 min, and 72 °C for 30 s, and a final extension at 72 °C for 5 min.

Amplicons were purified with the ExoSAP-IT PCR clean-up Kit (©Applied Biosystems, Foster City, California, USA) and sequenced on both DNA strands via Sanger chain termination method at the BMR Genomics (Padua, Italy).

Then, sequences were corrected manually and aligned with MEGA XI [50], including COXI and D-loop sequences of conspecifics downloaded from the GenBank (http://www.ncbi.nlm.nih.gov. Accessed on 23 September 2024) (Table 1).

We organized four datasets including modern and museum samples (Table 1) as it follows: the first one comprising 960 bp-long D-loop sequences (dataset I, number of sequences: 50), the second one with 249 bp-long D-loop sequences (dataset II, number of sequences: 93), the third one with 169 bp-long COXI sequences (dataset III, number of sequences: 58) and the fourth one with 249 bp-long cytb sequences (dataset IV, number of sequences: 64).

Overall genetic diversity through the different datasets (number of haplotypes (Nh), haplotype diversity (*h*), and nucleotide diversity (π)) was calculated with DNaSP 5.10.1 software [51]*h* is the probability that two haplotypes randomly chosen from a population are different and π is the average number of nucleotide differences per site between all possible pairs of DNA sequences in a sample population. To visualize the genealogic relationships among haplotypes, a network using the TCS algorithm [52] was constructed in Hapsolutely [53].

Genetic distances among samples were calculated from the four DNA sequence datasets. Jmodeltest 2.1.10 was used to identify the best-fit substitution model based on Akaike (AIC) and Bayesian (BIC) information criteria. The best model selected for COXI data set was JC [66], for Dloop dataset HKY + I [67] and for cytb TPM1uf [68]. The resulting distance matrices were exported in CSV format [50]. A Principal Coordinates Analysis (PCoA) was performed in R (version 4.1.2) for all mtDNA fragments, using the *cmdscale()* function. The first two principal coordinates (PCo1 and PCo2) were extracted and visualized in a scatter plot. The proportion of genetic variability explained by each principal coordinate was calculated from the eigenvalues obtained from the analysis. We applied PERMANOVA to formally test whether the group-level patterns observed in the PCoA ordinations reflected statistically significant differences in multivariate distances among geographic groups.

Necropsy and histological findings

Gross examination and histological results were partially affected by the state of preservation of the carcass, which was in advanced stage of decomposition [69], as well as freezing. Although a gradual thawing protocol was implemented, freeze–thaw cycles can still induce artefacts and reduce diagnostic capacity, particularly in specimens that were already decomposed.

Gross findings

Abundant cadaveric entomofauna was observed, predominantly in the oral cavity and in the left thigh and tibial region, where lacerations and larval feeding tracks were identified. The detection of specimens from the order Diptera, family Calliphoridae, genus *Calliphora* at the third stage of development as well as adults from the order Coleoptera, family Staphylinidae and Silfidae, confirmed the *post-mortem* stage [70]. Moreover, eye retraction was evidenced.

The state of the hair coat was poor with wide areas of detachment from the skin, which exhibited a greenish coloration. Poor body condition associated with a generalized muscle atrophy and prominent bone exposure were evident. A severe swelling on the left knee and thigh was noticed. In this area three lacerations were detected, two in the left thigh and one in the left hip. The latter was the only one affected by tissue reaction and bone

Table 1 Accession numbers of Eurasian otters involved in this study. *amplified in this work (sequences will be available on GenBank upon publication or after the 10 April 2026). NA, not available. (L), long; (S), short. "Direct submission to GenBank" means sequences which have been directly uploaded on GenBank, without any related reference paper

D-loop	COXI	cytb	Country of origin	Reference
NA	PQ359396*	NA	Codroipo, northern Italy	This work
PQ367294 (L) *	PQ359395*	PQ438381*	Gemona, northern Italy	This work
PQ367293 (L) *	PQ359397*	PQ438382*	Magra River, central Italy	This work
NA	PQ359394*	NA	Tarvisio, northern Italy	This work
NA	KY754512	NA	Austria	[54]
AY860334 (S)	NA	NA	central Italy	[21]
AY860335 (S)	NA	NA	central Italy	[21]
AY860336 (S)	NA	NA	central Italy	[21]
AY860337 (S)	NA	NA	central Italy	[21]
AY860338 (S)	NA	NA	central Italy	[21]
AY860339 (S)	NA	NA	central Italy	[21]
AY860340 (S)	NA	NA	central Italy	[21]
AY860341 (S)	NA	NA	central Italy	[21]
AY860342 (S)	NA	NA	central Italy	[21]
AY860343 (S)	NA	NA	central Italy	[21]
AY860344 (S)	NA	NA	central Italy	[21]
AY860345 (S)	NA	NA	central Italy	[21]
AY860346 (S)	NA	NA	central Italy	[21]
AY860347 (S)	NA	NA	central Italy	[21]
AY860348 (S)	NA	NA	central Italy	[21]
AY860349 (S)	NA	NA	central Italy	[21]
AY860350 (S)	NA	NA	central Italy	[21]
LC049377 (L)	LC049377	LC049377	China	[55]
LC049378 (L)	LC049378	LC049378	China	[55]
LC049952 (L)	LC049952	LC049952	China	[55]
LC049953 (L)	LC049953	LC049953	China	[55]
MW344881 (L)	MW344881	MW344881	China	[56]
NA	NA	OL814571	China	Direct submission to GenBank
OR655422 (L)	OR655422	OR655422	China	[22]
BK064833 (L)	BK064833	BK064833	Denmark	[23]
NA	MN122838	MN122838	Denmark	Direct submission to GenBank
HQ113947 (S)	NA	NA	Germany	[57]
NA	PP527131	NA	Iran	Direct submission to GenBank
NA	NA	LT593913	Iraq	[58]
NA	NA	LT593914	Iraq	[58]
NA	NA	LT593915	Iraq	[58]
NA	NA	LC006975	Japan	[59]
LC049955 (L)	LC049955	LC049955	Japan	[55]
LC050126 (L)	LC050126	LC050126	Japan	[55]
NA	LC094961	LC094961	Laos	Direct submission to GenBank
OP554563 (L)	OP554563	OP554563	Laos	[60]
OP554564 (L)	OP554564	OP554564	Laos	[60]
OP554565 (L)	OP554565	OP554565	Laos	[60]
NA	OP236725	NA	Mongolia	Direct submission to GenBank
AY860329 (S)	NA	NA	northern Italy	[21]
AY860330 (S)	NA	NA	northern Italy	[21]
AY860331 (S)	NA	NA	northern Italy	[21]
AY860332 (S)	NA	NA	northern Italy	[21]
AY860333 (S)	NA	NA	northern Italy	[21]
NA	NA	EF689067	Portugal	[61]
BK064834 (L)	BK064834	BK064834	Russia	[23]

Table 1 (continued)

D-loop	COXI	cytb	Country of origin	Reference
LC049954 (L)	LC049954	LC049954	Russia	[55]
PP199409 (L)	NA	NA	Russia	[62]
PP199414 (L)	NA	NA	Russia	[62]
PP199415 (L)	NA	NA	Russia	[62]
PP199416 (L)	NA	NA	Russia	[62]
PP199417 (L)	NA	NA	Russia	[62]
PP199418 (L)	NA	NA	Russia	[62]
PP199419 (L)	NA	NA	Russia	[62]
PP199420 (L)	NA	NA	Russia	[62]
BK064835 (L)	BK064835	BK064835	Norway	[23]
NA	HM380218	NA	Norway	Direct submission to GenBank
NA	HM380219	NA	Norway	Direct submission to GenBank
KC823049 (S)	NA	NA	Sweden	[63]
NA	NA	X94923	Sweden	[64]
NA	EF672696	NA	South Korea	[18]
FJ236015 (L)	FJ236015	FJ236015	South Korea	[19]
NA	KP992963	NA	South Korea	[20]
NA	KP992965	NA	South Korea	[20]
NA	NA	KU953399	South Korea	[59]
NA	NA	KU953400	South Korea	[59]
NA	NA	KU953401	South Korea	[59]
NA	NA	KU953402	South Korea	[59]
NA	NA	KU953403	South Korea	[59]
NA	NA	KU953404	South Korea	[59]
MW573979 (L)	MW573979	MW573979	South Korea	[56]
OR655425 (L)	OR655425	OR655425	South Korea	[23]
OR655426 (L)	OR655426	OR655426	South Korea	[22]
OR655427 (L)	OR655427	OR655427	South Korea	[22]
NA	NA	LT593916	southern Italy	[58]
AY860320 (L)	NA	NA	southern Italy	[21]
AY860321 (S)	NA	NA	southern Italy	[21]
AY860322 (S)	NA	NA	southern Italy	[21]
AY860323 (S)	NA	NA	southern Italy	[21]
AY860324 (S)	NA	NA	southern Italy	[21]
AY860325 (S)	NA	NA	southern Italy	[21]
AY860326 (S)	NA	NA	southern Italy	[21]
AY860327 (S)	NA	NA	southern Italy	[21]
AY860328 (S)	NA	NA	southern Italy	[21]
AY860351 (S)	NA	NA	southern Italy	[21]
AY860352 (S)	NA	NA	southern Italy	[21]
AY860353 (S)	NA	NA	southern Italy	[21]
AY860354 (S)	NA	NA	southern Italy	[21]
NA	BK064847	NA	Sumatra Island, Indonesia	[22]
NA	NA	OQ885370	Switzerland	[65]
MW316682 (L)	MW316682	MW316682	Taiwan	[56]
NA	NA	MW464990	Taiwan	[56]
NA	NA	MW465928	Taiwan	[56]
NA	OM220055	NA	Taiwan	Direct submission to GenBank
LR822067 (L)	LR822067	LR822067	England	Direct submission to GenBank
OR633269 (L)	OR633269	OR633269	England	[23]
OR633270 (L)	OR633270	OR633270	England	[23]
OR633271 (L)	OR633271	OR633271	England	[23]
OR633272 (L)	OR633272	OR633272	England	[23]

Table 1 (continued)

D-loop	COXI	cytb	Country of origin	Reference
OR633273 (L)	OR633273	OR633273	England	[23]
OR633274 (L)	OR633274	OR633274	England	[23]
OR633275 (L)	OR633275	OR633275	England	[23]
OR633276 (L)	OR633276	OR633276	England	[23]
OR633277 (L)	OR633277	OR633277	England	[23]
OR633278 (L)	OR633278	OR633278	England	[23]
OR633279 (L)	OR633279	OR633279	England	[23]
OR633280 (L)	OR633280	OR633280	England	[23]
OR633281 (L)	OR633281	OR633281	England	[23]
OR633282 (L)	OR633282	OR633282	England	[23]
OR633283 (L)	OR633283	OR633283	England	[23]
OR633284 (L)	OR633284	OR633284	England	[23]
OR633285 (L)	OR633285	OR633285	England	[23]
OR633286 (L)	OR633286	OR633286	England	[23]
OR655423 (L)	OR655423	OR655423	England	[23]
OR655424 (L)	OR655424	OR655424	England	[23]

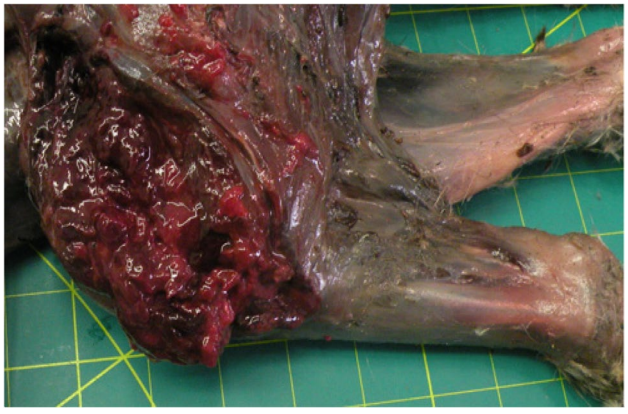

Fig. 2 Left knee and thigh haemorrhagic muscles

Table 2 Morphometric measurements of the left and right femurs

	Left femur	Right femur
Proximal epiphysis	28.32 mm	22.78 mm
Diaphysis	18.26 mm	12.41 mm
Distal epiphysis	22.16 mm	12.80 mm

exposure. Another reactive skin laceration was noticed on the left elbow.

A main lesion, which clearly involved the left limb - specifically the thigh - was identified. In this area, the subcutaneous tissue revealed extensive and severe bruising. The left knee and thigh muscles were intensively hemorrhagic (mostly affecting the dorsal part of the bundles) and softened with loss of the entire macro architecture of the muscles (Fig. 2). Numerous bone fragments (various shapes and sizes) were embedded to the hemorrhagic and softened muscles, especially in the left coxofemoral joint.

The left femur showed increased volume (Table 2) and an irregular, thickened periosteum with proliferative and infiltrative changes. However, no bone fractures were observed.

The abdominal cavity and abdominal wall exhibited a greenish discoloration consistent with putrefactive changes, which extensively involved the internal organs and precluded their reliable assessment. A slight emoabdomen was detected. Cadaveric entomofauna was found in the esophagus. The stomach was empty, with only a moderate amount of blood and brown liquid. Catarrhal jejunitis and presence of blood in colon and rectum were detected. The gut was also examined for the presence of parasites, however none was detected. All internal organs were severely affected by putrefactive changes, preventing reliable evaluation. Several parenchymal nodules were detected in the lungs, where also a hemorrhage to the right caudal lobe was found. Pathogenic bacteria (*Moellerella wisconsensis*, *Gemella haemolysans* and *Aerococcus viridans*) were detected in the lungs, kidneys, liver, heart, and brain. No viruses and parasites were revealed (Table 3). The micro-organisms detected are pathogenic bacteria. These bacteria may have contributed to a secondary septicemic process, although definitive conclusions are limited by the advanced state of decomposition and we cannot exclude a contamination phenomenon.

Microscopic findings

Histological examinations of femur and of thigh muscle revealed presence of neoplastic tissue with cellular and nuclear pleomorphism, frequently associated with bizarre mitosis, and markedly hyperchromatic chromatin. The tumoral osseous matrix detected in both samples investigated presents a pattern of small islands. In

Table 3 Results of laboratory diagnostic tests ("-" test not performed)

Organ	Viruses	Bacteria	Parasite
Brain	-	*Moellerella wisconsensis*, *Aerocccous viridans*	-
Heart	-	*Moellerella wisconsensis*	-
Kidney	Not detected	*Moellerella wisconsensis*, *Aerocccous viridans*	-
Liver	Not detected	*Moellerella wisconsensis*, *Aerocccous viridans*	-
Lungs	Not detected	*Moellerella wisconsensis*, *Gemella haemolysans*	-
Intestine	Not detected	-	Not detected

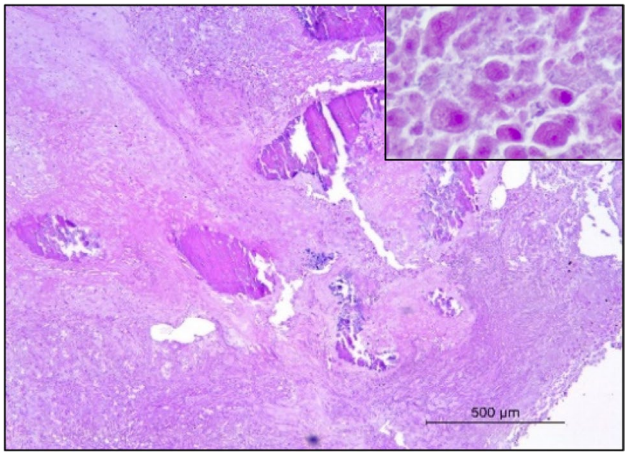

Fig. 3 Femur bone. Neoplastic tissue with osteoblastic differentiation. HE; 5x inset: neoplastic cells with eccentric nuclei and moderate cellular pleomorphism

Table 4 Genetic diversity in terms of number of haplotypes, nucleotide and haplotype diversity for all gene fragments. SD, standard deviation

	Dataset I (D-loop long)	Dataset II (D-loop short)	Dataset III (COXI)	Dataset IV (cytb
Number of haplotypes	21	13	5	14
Haplotype diversity h (SD)	0.802 (0.058)	0.688 (0.030)	0.329 (0.061)	0.786 (0.043)
Nucleotide diversity π (SD)	0.005 (0.001)	0.005 (0.001)	0.002 (0.001)	0.007 (0.001)

the samples of neoplastic tissues, clear signs of prevalent osteoblastic differentiation have been detected in focal path and frequently mixed with foci of collagen (Fig. 3). An admixture of two prevailing elements represents, in different proportion, the common histologic feature of osteosarcoma [71]. Pleomorphism and atypia involved nuclei and cytoplasm represented key diagnostic elements. Osteosarcomatous cells were characterized by hyperchromatic nuclei, frequently central or eccentric in the cell soma. Mitotic bizarre figures and prominent nucleoli were not very common.

Genetic findings

Number of haplotypes, nucleotide and haplotype diversity for the four datasets were reported in Table 4.

Looking at the haplotype networks obtained for the different datasets, the sample from central Italy (Magra River, red color) showed close genealogic relationships with samples from England and southern Italy (Fig. 4).

In the dataset II network the Italian samples, including museum samples, belonged to one of the two more frequent haplotypes, with a smaller sample from southern Italy included in the other more frequent haplotype together to the otter newly analyzed (Magra River, central Italy). Magra River sample did not share the same haplotype of all other Italian samples (including spraints analyzed in this paper). The PCoA plot revealed distinct clusters corresponding to geographic origin. The Magra River sample was always positioned within European clusters (Fig. 5).

Across all four PCoA analyses, the visual clustering patterns were supported by formal statistical tests. PERMANOVA confirmed significant differences among geographic groups in every dataset, with R^2 values ranging from 0.18 to 0.29 and all p-values ≤ 0.002, suggesting that the separation observed in the ordinations reflects genuine structure rather than random scatter. Pairwise PERMANOVA further indicated that the strongest differentiation consistently occurred between European and East Asian groups ($p < 0.001$). Within Europe, most comparisons were nonsignificant, although Dataset III revealed a distinct separation between most of UK samples *plus* Magra River and the rest of the European cluster ($p = 0.015$). (Fig. 5). Tests of multivariate homogeneity of group dispersions showed that European samples generally exhibited lower within-group variance than non-European groups; dispersion differences reached significance in Datasets I and IV but did not alter the overall direction of the PERMANOVA results, indicating that group separation was not driven solely by heteroscedasticity.

Discussion and conclusions

Cause of death

Based on the gross and histopathological findings and considering decomposition-related artifacts, a diagnosis of primary osteoblastic osteosarcoma was considered plausible in this Eurasian otter. As a matter of fact, osteosarcoma is a well-documented malignant tumor in domestic carnivores, known for its high metastatic potential, poor prognosis and ability to induce systemic effects such as cachexia [71–73]. In our case, the absence of evident metastatic foci could be attributed to the advanced state of post-mortem autolysis, which may have obscured secondary lesions. However, no data are currently available on the metastatic behavior and systemic

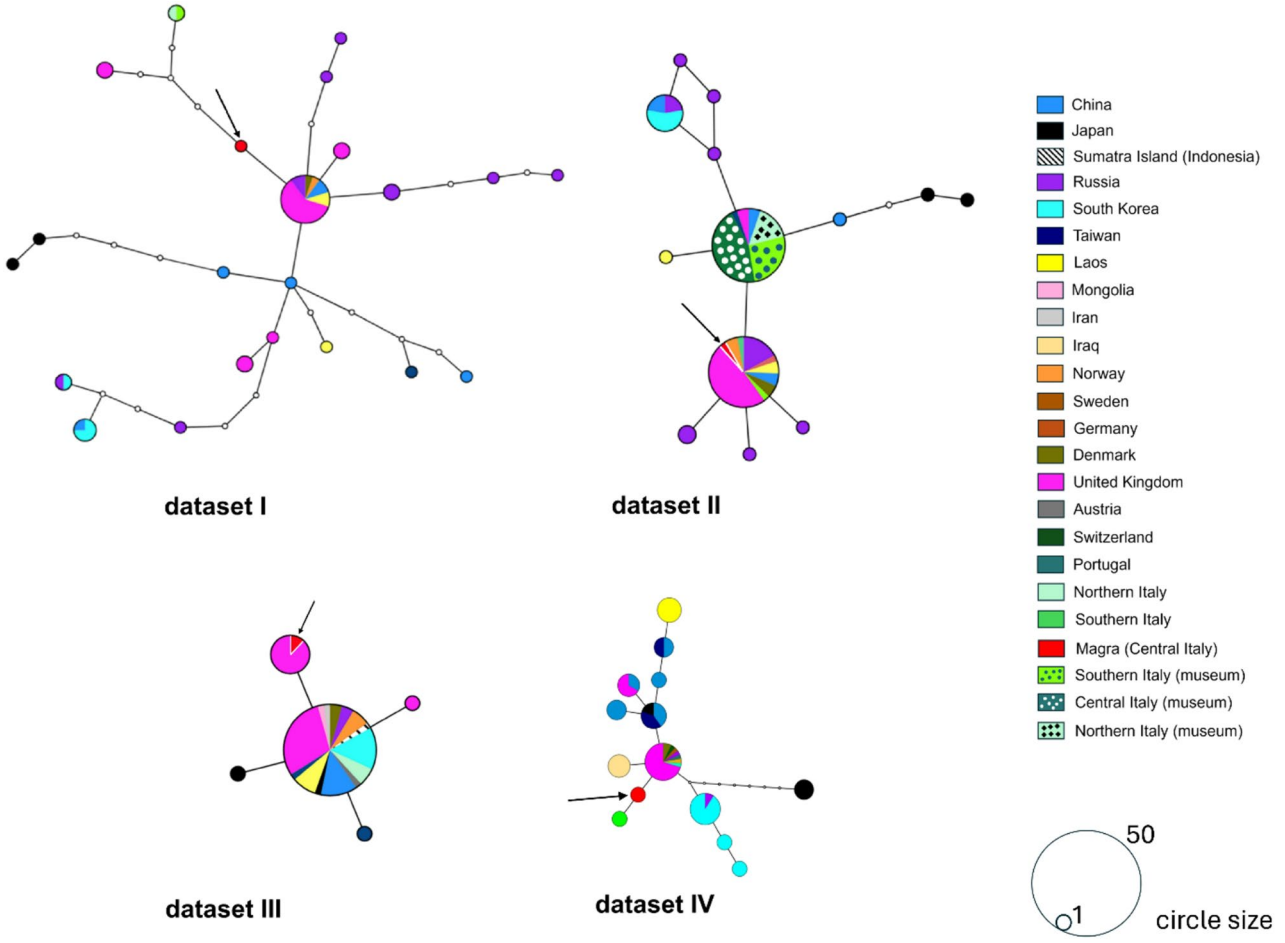

Fig. 4 Haplotype networks for all mtDNA fragments analyzed in this work. The circle size is proportional to the number of sequences for each haplotype (see legend). White dots indicate more than one mutational step between linked haplotypes. dataset I: D-loop sequences (960 bp, number of sequences: 50), dataset II: short D-loop sequences (249 bp, number of sequences: 93), dataset III: COXI sequences (169 bp, number of sequences: 58) and dataset IV: cytb data (249 bp, number of sequences: 64). mus, museum. The black arrow shows the sample from Magra River in Tuscany (central Italy)

effects of osteosarcoma in Eurasian otters, as no studies have specifically investigated this neoplasm in the species in point.

The cause of death was attributed to severe malnutrition and cachexia, due to the progressive functional impairment caused by an advanced osteoblastic osteosarcoma in the left femur. The extensive neoplastic infiltration presumably impaired mobility, significantly reducing the otter's ability to hunt and feed, eventually leading to starvation. In addition, the occurrence of pathogenic bacteria in multiple organs was proved, this suggesting a concomitant septicemic process that may have further aggravated the deteriorating health condition and contributed to the animal's death. Importantly, the forensic investigation played a key role to exclude anthropogenic or illegal causes of death. Given the suspicious context of the discovery—an otter found in a region where the species had been absent for over three decades—the forensic approach allowed us to exclude trauma, violence, or other anthropogenic causes, thus confirming that the death was attributable to a natural pathological condition. This highlights the relevance of forensic necropsy in wildlife conservation, particularly for protected and recovering species. The uniqueness of this research lies in the detection of a bone neoplastic lesion showing features compatible with osteosarcoma in a species where tumors are rarely reported, especially in free-ranging specimens. In fact, most of the neoplastic diseases - including malignant melanoma, mammary adenoma and fibrosarcoma - previously detected in otters deal with captive individuals [15–17]. Only a single case of osteosarcoma affecting the maxilla has been documented in a southern sea otter (*Enhydra lutris nereis*) [74]. Additionally, Burek-Huntington and colleagues [75] described three cases of sarcomas in free-ranging northern sea otters (*Enhydra lutris kenyoni*), including a chondrosarcoma and two low-grade fibrosarcomas. These findings suggest that, although rare, sarcomas may occur in wild mustelids, particularly in marine species.

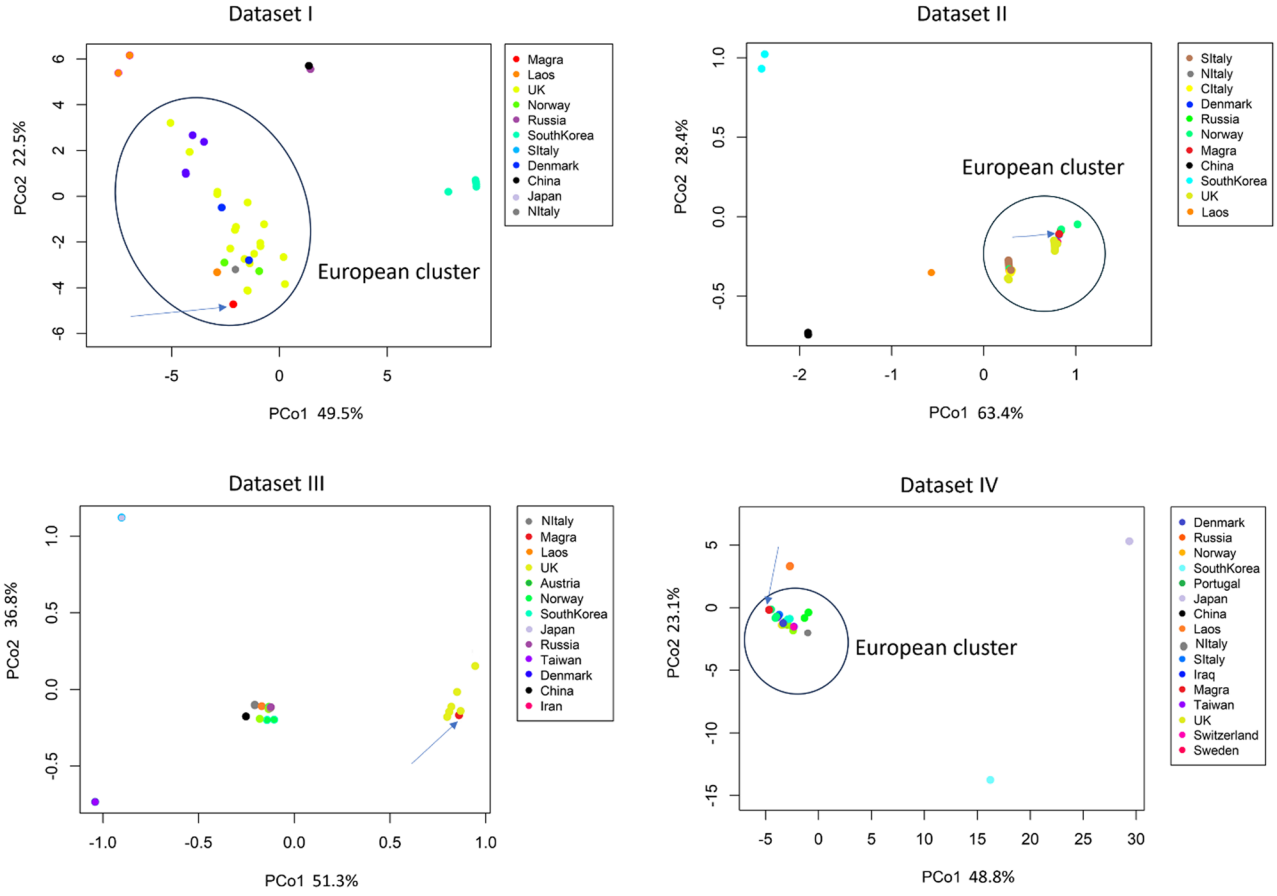

Fig. 5 Principal Coordinates Analysis (PCoA) of all samples based on the genetic distances. The total variance explained is shown in percentage at each axis. Colors represent geographic origin. The Magra River sample is always highlighted in red and by the arrow. The clustering pattern reflects the same relationships observed in the networks

Overall studies on neoplastic diseases in Eurasian otters and other mustelids are needed to assess their prevalence, potential environmental risk factors, and long-term implications for conservation.

Genetic assessment

Our study confirms the low genetic variability of the Eurasian otter across its entire range (nucleotide diversity = 0.004–0.007), which is similar to or slightly higher than that reported in previous studies [76, 77], but lower than that detected in Scandinavia [63]. Conversely, the unexpected high genetic diversity recorded in Eurasian otters from England might be related to the admixture between regional populations which survived the local crash in the 1950–1980 s and non-British individuals released to overcome the population bottleneck [23, 76]. Likewise, Asian populations included the highest number of morphological subspecies and haplotypes [18–20, 56, 59, 60], e.g., the Indochinese *L. l. barang* from Laos.

Based on the limited data from museum specimens, modern Italian samples are close to those from England as well as historical samples from the Italian Peninsula [21]. Additionally, according to D-loop analysis, the Magra River specimen clustered together with museum samples from southern Italy. Analyses of cytb and COXI genes also show that it is similar to modern southern Italian otters, even though it has a unique haplotype.

Population isolation has reduced gene flow across Europe [77–79], producing genetic structuring among localities [80]. Yet European bottlenecks seem to have only marginally influenced the genetic diversity of the Eurasian otter [81]. At least three genetic groups are currently present in Italy: one from southern Italy with individuals recolonizing northern regions [5, 21], the Indochinese haplotype in the Ticino River [26], and the "new" haplotype identified in the otter found in the Magra River. The PCoA analyses provided a coherent view of the geographic structure underlying our datasets. In all cases, except dataset III, the European samples consistently built up a homogeneous and statistically reliable cluster. The disclosure of a fine-scale structure within Europe in Dataset III (COXI), particularly the pronounced isolation of the samples from England, may be related to the high sample size for this gene fragment.

Conversely, the main limitation of our study was the small sample size for the Italian population, which included a few individuals only. Hence, further samplings in both northern and central regions will provide data useful to disentangle the relationships between the Italian and other European conspecifics. In addition, the amplification of nuclear genes would allow a more reliable assessment of the gene flow between the otters from central Italy and those occurring in other regions.

The reduction of threats and habitat fragmentation of otter populations have promoted a slow recovery of the species throughout its European range [82, 83], thus triggering a potential genetic admixture of different populations [84].

After decades of local extinction, the Eurasian otter is slowly recovering in Italy, both from southern relict populations and through natural expansion from neighboring countries (e.g., France, Switzerland, Austria and Slovenia: [19]). In this context, unauthorized releases or escapes should be strictly avoided, as they may introduce non-native genotypes with negative consequences for native populations [5].

Abbreviations

mtDNA	Mitochondrial deoxyribonucleic acid
DNA	Deoxyribonucleic acid
cytb	Cytochrome b
tRNAPro	Transfer ribonucleic acid for proline
tRNAThe	Transfer ribonucleic acid for the amino acid threonine
rRNA	Ribosomal ribonucleic acid
PCR	Polymerase chain reaction
Taq DNA Polymerase	(Thermus aquaticus) thermostable deoxyribonucleic acid polymerase
ExoSAP-IT	Exonuclease I – Shrimp Alkaline Phosphatase – IT
EDTA	Ethylenediaminetetraacetic acid
COXI	Cytochrome c oxidase I
D-loop	Control region

Acknowledgements
Authors would like to thank Giuseppe Negrari, who found the first otter in Tuscany after 35 years of local extinction, as well as Tiziano Fiorenza and Renato Pontarini for samples from North-Eastern Italy. The authors express their gratitude to the Istituto Zooprofilattico Sperimentale del Lazio e della Toscana diagnostic, virological and parasitological laboratories involved.

Authors' contributions
GR, FM, LDB, EM and AV conceived this work; GR, FM, EM, AV and LA collected samples; MB and EM performed genetic analyses; GR and FM performed the necropsy; CE, VG, and LL carried out the histological exams. FM, LDB, MB and EM wrote the first draft of the MS. All authors participated in preparing the final draft. Approval from all authors was obtained before the submission of the manuscript.

Funding
Otter monitoring was financed and coordinated by WWF Italia and Università del Molise. LA and EM were funded by the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4 - Call for tender No. 3138 of 16 December 2021, rectified by Decree n.3175 of 18 December 2021 of Italian Ministry of University and Research funded by the European Union – NextGenerationEU; Project code CN_00000033, Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research, CUP B83C22002930006, Project title "National Biodiversity Future Center - NBFC".

Data availability
Sequences obtained during the current study are available on GenBank upon publication or after the 10 th April 2026 (http://www.ncbi.nlm.nih.gov) (Accession numbers: PQ359394, PQ359395, PQ359396, PQ359397, PQ367293, PQ367294, PQ438381, PQ438382).

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 16 September 2025 / Accepted: 23 January 2026
Published online: 07 March 2026

References

1. Ruiz-Olmo J, Calvo A, Palazón S, Arqued V. Is the otter a bioindicator? Galemys. 1998;10:227–37.
2. Hagen SB, Kopatz A, Aspi J, Kojola I, Geir Eiken H. Evidence of rapid change in genetic structure and diversity during range expansion in a recovering large terrestrial carnivore. Proc R Soc Lond B Biol Sci. 2015;22:282.
3. Larson S, Monson D, Ballachey B, Jameson R, Wasser SK. Stress-related hormones and genetic diversity in sea otters (*Enhydra lutris*). Mar Mamm Sci. 2009;25(2):351–72.
4. Tison JL, Blennow V, Palkopoulou E, Gustafsson P, Roos A, Dalén L. Population structure and recent temporal changes in genetic variation in Eurasian otters from Sweden. Conserv Genet. 2014;16(2):371–84.
5. Balestrieri A, Gianfranceschi L, Verduci F, Gatti E, Boggioni P, Mucci N, et al. Genetic and viability assessment of a reintroduced Eurasian otter *Lutra Lutra* population on the river Ticino, Italy. Oryx. 2022;56(4):617–26.
6. De Ferran V, Vieira Figueiró H, Trinca CS, Hernández-Romero PC, Lorenzana GP, Gutierrez-Rodríguez C, et al. Genome-wide data support recognition of an additional species of Neotropical river otter (Mammalia, Mustelidae, Lutrinae). J Mammal. 2024;105(3):534–42.
7. de Ferran V, Figueiró HV, de Jesus Trindade F, Smith O, Sinding MHS, Trinca CS, et al. Phylogenomics of the world's otters. Curr Biol. 2022;32(16):3650–8.
8. Hung N, Law CJ. <Emphasis Type="Italic">Lutra lutra</Emphasis> (Carnivora: Mustelidae). Mamm Species. 2016;48(940):109–22.
9. Fusillo R, Romanucci M, Marcelli M, Massimini M, Della Salda L. Health and mortality monitoring in threatened mammals: a first post mortem study of otters (*Lutra lutra L.*) in Italy. Animals. 2022;12(5):609.
10. Paciello O, Fico R. Linee guida nazionali per le autopsie a scopo forense in medicina veterinaria. 2018. Available from: https://www.izslt.it/medicinaforense/wp-content/uploads/sites/12/2019/06/Linee-guida-nazionali-per-le-autopsie-a-scopo-forense-in-medicina-veterinaria.pdf. Accessed on 01.12.2025.
11. Beiglböck C, Walzer C. Standard operating procedures for forensic investigations of suspected illegal killing of wildlife. 1st ed. Research Institute of Wildlife Ecology (FIWI) of the University of Veterinary Medicine Vienna; 2019.
12. Miller I, Gutleb AC, Kranz A, Gemeiner M. Forensics on wild animals: differentiation between otter and pheasant blood using electrophoretic methods. Electrophoresis. 1995;16(5):865–8.
13. Capber F. Veterinary care of Eurasian otters (*Lutra lutra*) at the otter breeding centre of Hunawihr (France). IUCN Otter Spec Group Bull. 2007;24(1):47–62.
14. Rohner S, Wohlsein P, Prenger-Berninghoff E, Ewers C, Waindok P, Strube C, et al. Pathological findings in Eurasian otters (*Lutra lutra*) found dead between 2015–2020 in Schleswig-Holstein. Ger Anim. 2022;12(1):59.
15. Domenis L, Pezzolato M, Biasibetti E, Spedicato R, Robetto S. Gallbladder carcinoma in a Eurasian otter (*Lutra lutra*). Animals. 2025;15(17):2484.
16. Bae IH, Pakhrin B, Jee H, Shin NS, Kim DY. Hepatocellular adenoma in a Eurasian otter (*Lutra lutra*). J Vet Sci. 2007;8(1):103–5.
17. Fuke N, Diep N Van, Hirai T, Suwanruengsri M, Izzati UZ, Yamaguchi R. Three neoplasms in a eurasian otter (*Lutra lutra*): Malignant melanoma, trichoblastoma and mammary gland adenoma. J Vet Med Sci. 2021;83(11):1661–5.

18. Ki JS, Hwang DS, Park TJ, Han SH, Lee JS. A comparative analysis of the complete mitochondrial genome of the Eurasian otter *Lutra Lutra* (Carnivora; Mustelidae). Mol Biol Rep. 2010;37(4):1943–55.
19. Jang KH, Ryu SH, Hwang UW. Mitochondrial genome of the Eurasian otter *Lutra Lutra* (mammalia, carnivora, mustelidae). Genes Genom. 2009;31(1):19–27.
20. Hong S, Gim JS, Kim HG, Cowan PE, Joo GJ. A molecular approach to identifying the relationship between resource use and availability in Eurasian otters (*Lutra lutra*). Can J Zool. 2019;97(9):797–804.
21. Ketmaier V, Bernardini C. Structure of the mitochondrial control region of the Eurasian otter (*Lutra lutra*; Carnivora, Mustelidae): patterns of genetic heterogeneity and implications for conservation of the species in Italy. J Hered. 2005;96(4):318–28.
22. Du Plessis SJ, Hong S, Lee B, Koepfli KP, Chadwick EA, Hailer F. Mitochondrial genome-based synthesis and timeline of Eurasian otter (*Lutra lutra*) phylogeography. Anim Cells Syst. 2023;27(1):366–77.
23. Du Plessis SJ, Blaxter M, Koepfli KP, Chadwick EA, Hailer F. Genomics reveals complex population history and unexpected diversity of Eurasian otters (*Lutra lutra*) in Britain relative to genetic methods. Mol Biol Evol. 2023;40(11):msad207.
24. Balestrieri A, Remonti L, Prigioni C. Towards extinction and back: decline and recovery of otter populations in Italy. In: Angelici FM, editor. Problematic wildlife: A Cross-Disciplinary approach. London: Springer; 2016. pp. 91–105.
25. Canu A, Galaverni M, Di Febbraro M, Giovacchini S, Loy A. La Lontra in Italia. Un Nuovo censimento nell'areale storico. Roma, Italy: WWF Editions; 2024.
26. Prigioni C, Smiroldo G, Remonti L, Balestrieri A. Distribution and diet of reintroduced otters (*Lutra lutra*) on the river Ticino (northern Italy). Hystrix. 2009;20(1):45–53.
27. Marcelli M, Striglioni F, Fusillo R. Range reexpansion after long stasis: Italian otters (*Lutra lutra*) at their northern edge. Ecol Evol. 2023;13(1):e9726.
28. Remonti L, Prigioni C, Balestrieri A, Sgrosso S, Priore G, Remonti L. Distribution of a recolonising species may not reflect habitat suitability alone: the case of the Eurasian otter (*Lutra lutra*) in Southern Italy. Wildlife Res. 2008;35(8):798.
29. Lapini L, Pontarini R, Molinari P, Cantarutti G, Dorigo L, Pecorella S, et al. The return of the Eurasian otter in north-eastern Italy. New challenges for biological conservation from Friuli Venezia Giulia region. J Mt Ecol. 2020;13:41–50.
30. De Nadai G, Cassol M, Lapini L. First data on the natural recovery of the Eurasian otter (*Lutra l. lutra* Linnaeus, 1758) in Veneto Region (north-eastern Italy). Habitat Online. 2022;1:1.
31. Stokel G, Franchini M, Frangini L, Pizzul E, Filacorda S. Has the recolonization of the Po plain begun? Updates regarding the presence of the Eurasian otter (*Lutra lutra*) in north-eastern Italy. IUCN Otter Spec Group Bull. 2022;39(2):90–101.
32. Van Looy K, Piffady J, Cavillon C, Tormos T, Landry P, Souchon Y. Integrated modelling of functional and structural connectivity of river corridors for European otter recovery. Ecol Model. 2014;273:228–35.
33. Cervoni F, Giardini M, D'Urbano S, Marini D, Grispigni Manetti C. A Eurasian otter *Lutra Lutra* (Linnaeus 1758) (Carnivora: Mustelidae) in the Tiber basin near rome: relict or range expansion? Nat Hist Sci. 2024;11(2).
34. Ballini L, Ottonello D, Repetto V, Natali C, Chini G, Tolve L, et al. Early detection of rare and elusive endangered species using environmental DNA: a case study for the Eurasian otter and the white-clawed crayfish in northwestern Italy. Conserv Genet. 2024;25(4):999–1005.
35. Leoncini F, Semenzato P, Di Febbraro M, Loy A, Ferrari C. Come back to stay: landscape connectivity analysis for the Eurasian otter (*Lutra lutra*) in the western Alps. Biodivers Conserv. 2023;32(2):653–69.
36. Loy A, Diamente S, Di Febbraro M. Population viability analysis of the Italian otter population. Proc 8th Eur Congress Mammalogy Warsaw Pol. 2019;8:23–7.
37. Giovacchini S, Antonucci A, Bartolomei R, Bandini M, Caldarella M, De Castro G, et al. Conservation status of Eurasian otter *Lutra Lutra* in Italy. Eur Otter Workshop. 2021;1:10.
38. Loy A, Bon M, Di Febbraro M, Baisero D, Amori G. Atlas of Mammals in Italy. Latina, Italy: Edizioni Belvedere; 2025.
39. Sforzi A, Ragni B. Atlante dei mammiferi Della provincia Di Grosseto. Atti Mus Stor Nat Maremma. 1997;16:153–5.
40. Reggiani G, Raganella Pelliccioni E, Bianco P, Bargagli R. Studio sull'ittiofauna, La Lontra e l'ambiente acquatico Nelle Valli Del Farma e Del Merse. Rapporto tecnico, amministrazione provinciale Di Siena. Roma, Italy: Istituto Ecologia Applicata; 1995.
41. Reggiani G, Pittiglio C, Zini R, Boitani L. Progetto Lontra Grosseto. Rapporto finale, amministrazione provinciale Di Grosseto Editions, Giugno 2001. Italy: Grosseto; 2001.
42. Buglione M, Petrelli S, Troiano C, Notomista T, Petrella A, De L, et al. Spatial genetic structure in the Eurasian otter (*Lutra lutra*) meta-population from its core range in Italy. Contr Zool. 2020;90(1):70–92.
43. Quinn PJ, Markey BK, Leonard FC, Hartigan PJ, FitzPatrick ES, Fanning S. Veterinary microbiology and microbial disease. 2nd ed. Wiley, editor. 2011: 928.
44. Maclachlan NJ, Dubovi EJ. Fenner's Veterinary Virology. 5th ed. Press A, editor. 2010: 534.
45. Cringoli G, Rinaldi L, Maurelli MP, Utzinger J. FLOTAC: new multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. Nat Protoc. 2010;5(3):503–15.
46. Kocher TD, Thomas WK, Meyer A, Edwards SV, Paabo S, Villablanca FX, et al. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc Natl Acad Sci U S A. 1989;86(16):6196–200.
47. Pertoldi C, Breyne P, Cabria MT, Halfmaerten D, Jansman HAH, Van Den Berge K, et al. Genetic structure of the European polecat (*Mustela putorius*) and its implication for conservation strategies. J Zool. 2006;270(1):102–15.
48. Ruiz-González A, Madeira MJ, Randi E, Abramov AV, Davoli F, Gómez-Moliner BJ. Phylogeography of the forest-dwelling European pine marten (*Martes martes*): new insights into cryptic Northern glacial refugia. Biol J Linn Soc. 2013;109(1):1–18.
49. Meusnier I, Singer GAC, Landry JF, Hickey DA, Hebert PDN, Hajibabaei M. A universal DNA mini-barcode for biodiversity analysis. BMC Genom. 2008;12;9.
50. Tamura K, Stecher G, Kumar S. MEGA11: molecular evolutionary genetics analysis version 11. Mol Biol Evol. 2021;38(7):3022–7.
51. Librado P, Rozas J. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics. 2009;25(11):1451–2.
52. Templeton AR, Crandall KA, Sing CF. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics. 1992;132(2):619–33.
53. Vences M, Patmanidis S, Schmidt JC, Matschiner M, Miralles A, Renner SS. Hapsolutely: a user-friendly tool integrating haplotype phasing, network construction, and haploweb calculation. Bioinformatics Adv. 2024;4(1):vbae083.
54. Schäffer S, Zachos FE, Koblmüller S. Opening the treasure chest: a DNA-barcoding primer set for most higher taxa of central European birds and mammals from museum collections. PLoS ONE. 2017;12(3):e0174449.
55. Waku D, Segawa T, Yonezawa T, Akiyoshi A, Ishige T, Ueda M, et al. Evaluating the phylogenetic status of the extinct Japanese otter on the basis of mitochondrial genome analysis. PLoS ONE. 2016;11(3):e0149341.
56. Jang-Liaw NH, Tan YC, Chang CJ, Juan CH, Hou HY, Chung LW, et al. Genetic diversity and structure of Eurasian otters on Kinmen Island. Conserv Genet. 2023;24(5):589–606.
57. Honnen AC, Petersen B, Kaßler L, Elmeros M, Roos A, Sommer RS, et al. Genetic structure of Eurasian otter (*Lutra lutra*, carnivora: Mustelidae) populations from the Western Baltic sea region and its implications for the recolonization of north-western Germany. J Zool Syst Evol Res. 2011;49(2):169–75.
58. Moretti B, Al-Sheikhly OF, Guerrini M, Theng M, Gupta BK, Haba MK, et al. Phylogeography of the smooth-coated otter (*Lutrogale perspicillata*): distinct evolutionary lineages and hybridization with the Asian small-clawed otter (*Aonyx cinereus*). Sci Rep. 2017;7:41611.
59. Park HC, Kurihara N, Kim KS, Min MS, Han S, Lee H, et al. What is the taxonomic status of East Asian otter species based on molecular evidence? Focus on the position of the Japanese otter holotype specimen from museum. Anim Cells Syst. 2019;23(3):228–34.
60. Coudrat CNZ, Chutipong W, Sukmak M, Sripiboon S, Klinsawat W. Taxonomic status of otter species in Nakai-Nam Theun National Park, Lao PDR, based on DNA evidence. Ecol Evol. 2022;12(12):e9601.
61. Fernandes CA, Ginja C, Pereira I, Tenreiro R, Bruford MW, Santos-Reis M. Species-specific mitochondrial DNA markers for identification of non-invasive samples from sympatric carnivores in the Iberian Peninsula. Conserv Genet. 2008;9(3):681–90.
62. Sokolova NA, CH A, Korablev NP, КН П, Korablev PN, КП Н, et al. Mitochondrial genetic diversity of Eurasian otter (*Lutra lutra*) from European part of Russia and Transcaucasian countries. Genetika. 2023;59(7):804–12.
63. Honnen AC, Roos A, Stjernberg T, Zachos FE. Genetic analysis of Eurasian otters (*Lutra lutra*) reveals high admixture in Finland and pronounced differentiation in Sweden. Mamm Biol. 2015;80(1):47–53.

64. Ledje C, Arnason U. Phylogenetic analyses of complete cytochrome b genes of the order carnivora with particular emphasis on the caniformia. J Mol Evol. 1996;42(2):135–44.
65. Ruedi M, Manzinalli J, Dietrich A, Vinciguerra L. Shortcomings of DNA barcodes: a perspective from the mammal fauna of Switzerland. Hystrix. 2023;34(1):54–61.
66. Jukes TH, Cantor CR. Evolution of Protein Molecules. In: Mammalian Protein Metabolism. Academic Press; 1969. p. 21–132.
67. Hasegawa M, Kishino H, Yano TA. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J Mol Evol. 1985;22(2):160–74.
68. Tamura K, Nei M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol. 1993;10(3):512–26.
69. Galloway A, Birkby WH, Jones AM, Henry TE, Parks BO. Decay rates of human remains in an arid environment. J Forensic Sci. 1989;34(3):607–16.
70. Von Hoermann C, Jauch D, Kubotsch C, Reichel-Jung K, Steiger S, Ayasse M. Effects of abiotic environmental factors and land use on the diversity of carrion-visiting silphid beetles (Coleoptera: Silphidae): a large scale carrion study. PLoS One. 2018;13(5):e0196839.
71. Leonardi L. Bone tumors in domestic animals: comparative clinical pathology. Springer, editor. 2022: 173.
72. Simpson S, Dunning MD, de Brot S, Grau-Roma L, Mongan NP, Rutland CS. Comparative review of human and canine osteosarcoma: morphology, epidemiology, prognosis, treatment and genetics. Acta Vet Scand. 2017;59(1):71.
73. Bryan JN. Updates in osteosarcoma. Vet Clin North Am Small Anim Pract. 2024;54(3):523–39.
74. Rodriguez-Ramos Fernandez J, Thomas NJ, Dubielzig RR, Drees R. Osteosarcoma of the maxilla with concurrent osteoma in a Southern sea otter (*Enhydra Lutris nereis*). J Comp Pathol. 2012;147(2–3):391–6.
75. Burek-Huntington KA, Mulcahy DM, Doroff AM, Johnson TO. Sarcomas in three free-ranging northern sea otters (*Enhydra lutris kenyoni*) in Alaska. J Wildl Dis. 2012;48(2):483–7.
76. Stanton DWG, Hobbs GI, Chadwick EA, Slater FM, Bruford MW. Mitochondrial genetic diversity and structure of the European otter (*Lutra lutra*) in Britain. Conserv Genet. 2009;10(3):733–7.
77. Mucci N, Arrendal J, Ansorge H, Bailey M, Bodner M, Delibes M, et al. Genetic diversity and landscape genetic structure of otter (*Lutra lutra*) populations in Europe. Conserv Gen. 2010;11(2):583–99.
78. Hájková P, Pertoldi C, Zemanová B, Roche K, Hájek B, Bryja J, et al. Genetic structure and evidence for recent population decline in Eurasian otter populations in the Czech and Slovak republics: implications for conservation. J Zool. 2007;272(1):1–9.
79. Geboes AL, Rosoux R, Lemarchand C, Hansen E, Libois R. Genetic diversity and population structure of the Eurasian otter (*Lutra lutra*) in France. Mamm Res. 2016;61(2):121–9.
80. Randi E, Davoli F, Pierpaoli M, Pertoldi C, Madsen AB, Loeschcke V. Genetic structure in otter (*Lutra lutra*) populations in Europe: implications for conservation. Anim Conserv. 2003;6(2):93–100.
81. Dallas JF, Bacon PJ, Carss DN, Conroy JWH, Green R, Jefferies DJ, et al. Genetic diversity in the Eurasian otter, *Lutra lutra*, in Scotland. Evidence from microsatellite polymorphism. Biol J Linn Soc. 1999;68:73–86.
82. Mason CF, Macdonald SM. Growth in otter (*Lutra lutra*) populations in the UK as shown by long-term monitoring. Ambio. 2004;33(3):148–52.
83. Thomas NE, Hailer F, Bruford MW, Chadwick EA. Country-wide genetic monitoring over 21 years reveals lag in genetic recovery despite spatial connectivity in an expanding carnivore (Eurasian otter, *Lutra lutra*) population. Evol Appl. 2022;15(12):2125–41.
84. Pigneur LM, Caublot G, Fournier-Chambrillon C, Fournier P, Giralda-Carrera G, Grémillet X, et al. Current genetic admixture between relictual populations might enhance the recovery of an elusive carnivore. Conserv Genet. 2019;20(5):1133–48.

Publisher's Note