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The otter population of the River Ticino (N Italy) 20 years after its reintroduction

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ABSTRACT

On the River Ticino (Piedmont and Lombardy regions, N Italy), the Eurasian otter *Lutra lutra* became extinct in the 1980s and was reintroduced in 1997. Since then, the status of the reintroduced population has been assessed only occasionally, in 2008 and 2010. Between 2016 and 2018, we conducted an extensive survey for otter signs along the whole Italian stretch of the River Ticino, following the 'Standard Method' for otter surveys. In 2016–2017, we found 101 spraints (mean marking intensity: 0.40 spraints/100 m), spread over a 97-km long stretch of the river. In 2018 only five spraints were collected, the two furthermost marking sites being 32 km apart. Genotyping of nDNA extracted from 21 faecal samples enabled the identification of six different individuals.

The surveys led to drawing a reliable picture of otter distribution and population size, with evidence of otter occurrence on a longer than previously recorded stretch of the river. The results of the 2018 survey suggest that stochastic factors may still threaten the survival of reintroduced otters and would suggest a reinforcement of the population is required to increase its genetic diversity.

Keywords: *Lutra lutra*, standard survey, genotyping, population viability, inbreeding, marking intensity.

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INTRODUCTION: OTTER REINTRODUCTION ON THE RIVER TICINO

With a catchment of 7,228 km^2, the River Ticino is the largest tributary of the River Po. The overall length of the watercourse is 248 km: 91 km in Switzerland, upstream of Lake Maggiore (47 km), and 110 km in Italy. The sublacual portion of the river is still characterized by large riparian woodlands and alluvial forests, forming one of the best preserved plain-fluvial ecosystems in Europe and a natural corridor racing off southwards from the Alps. The area is protected by two regional Parks (the river marks the border between Piedmont and Lombardy regions) and several Site of Community Importance and Special Protection Areas (Figure 1). The ecological condition of the river is generally good, excluding its southernmost stretch, downstream Pavia, where the concentration of hexachlorocyclohexanes (HCHs) exceeds EU limits (2000/60/EU Water Framework Directive; Dotti et al., 2014).

At the beginning of the last century, otters were still widespread on the river, as, generally, throughout Italy. Following the sharp decline that has occurred since the 1970s (Prigioni et al., 2007), on the river the last individual was killed in 1974, while the last reliable sign of presence dates back to 1980 (Gropello Cairoli, Pavia

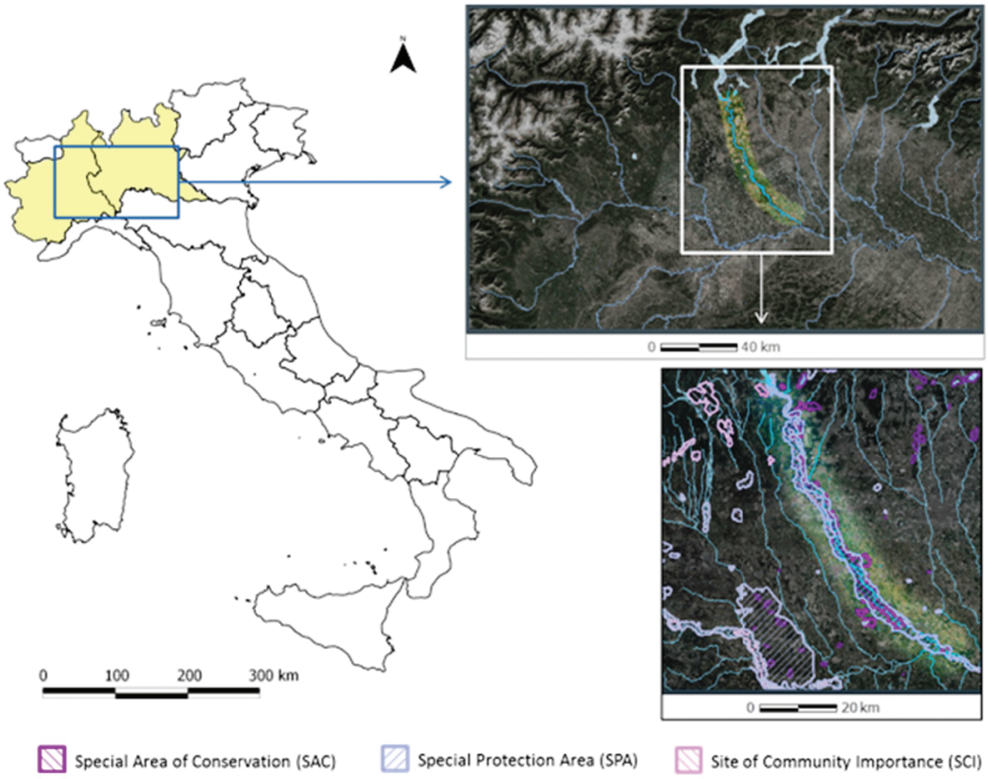

Figure 1 Study area.

province; Galeotti, 1981). In 1984–1985, during the WWF national otter census, no sign of the species was found (Cassola, 1986; Prigioni, 1986), and the otter was declared locally extinct.

The reintroduction of the otter in the Ticino valley has been proposed since the late 1970s (Prigioni et al., 1979), and the river was identified as a potential reintroduction area in the *Action Plan for the Conservation of European Otters* (Macdonald and Mason, 1990). In 1978, the 'European Captive Reproduction Programme' (Europäisches Erhaltungszucht Programm, EEP; Vogt, 1995) was launched with the collaboration of zoos and European breeding centres. At the beginning of the 1990s, almost 200 animals born and bred in captivity were available for reintroduction plans. Unfortunately, the analysis of mitochondrial DNA (Randi et al., 2001) of *ca*. 40 EEP otters showed evidence of the occurrence of some founders of the Asiatic subspecies *Lutra lutra barang* (the so-called genetic B-line). In addition, the otters hosted in the Italian breeding centres showed high levels of inbreeding (Randi et al., 2001).

At the end of the 1980s, the Ticino Park (Piedmont) started an otter reintroduction project, creating a breeding enclosure in the 'Bosco Vedro' natural reserve, in the northern part of the river (Cameri, Novara province) (Montanari and Boffino, 2000). The first pair of otters, a two-year-old female from the Norfolk Wildlife Park and a one-year-old male sold from the Zurich Zoo, was accommodated into the enclosure in March 1989. After the death of the male, a second individual, a 2.5-year-old male from Norfolk, was introduced in March 1990. The couple reproduced successfully from the first year (a female in 1990, two males in 1991 and two females in 1992, one male and one female in 1994). Both founders died in 1997 and were replaced by a three-year-old male from 'La Torbiera' Wildlife Centre (Agrate Conturbia) and a two-year-old female from the Caramanico Terme centre (Pescara), the daughter of one of the two males born at Bosco Vedro in 1991. Despite the founder stock being confirmed as belonging to the B-line (Randi et al., 2005; Panzacchi et al., 2010), the reintroduction took place on 22 August 1997, with the release of a male from 'La Torbiera' and a young female, born from the second pair of founders (Montanari and Boffino, 2000).

In parallel, but independently, the Park of the Ticino Valley (Lombardy) activated a second breeding centre, located in 'La Fagiana' natural reserve in the central part of the river, about 15 km downstream of 'Bosco Vedro' centre. During a flooding episode that occurred in 2001, an adult female and a puppy escaped from this centre.

In 2007, the carcass of a juvenile female was found near 'La Fagiana'. In addition, some footprints were found at the end of the same year (Bellani A., pers. com.). During a survey conducted between June and September 2008, the otter was recorded in only three sampling stations, corresponding to a 2.6-km stretch of the river including the release site (Prigioni et al., 2009). In 2010, an otter survey was carried out on a 35-km long stretch of the river and otter spraints were found on a 7-km long stretch, mainly on canals and secondary arms (Prigioni and Balestrieri, 2011). In late summer 2012, an adult otter was recorded about 30 km downstream of the release site (Meriggi and Bellati, pers. com.), while in winter

2013 two individuals were seen a further 10 km downstream (Cavalleroni, pers. com.). In March 2016, a young female was road-killed next to Gropello Cairoli.

THE 2016–2017 OTTER SURVEY

From March 2016 to March 2017, a survey for otter signs (spraints, footprints) was conducted on 32 sampling stations (mean length ±SD = 567 ± 263 m; min–max = 100–1,120 m) along the whole sublacual stretch of the River Ticino, following the 'Standard Method' for otter surveys recommended by the IUCN/SSC Otter Specialist Group (Reuther et al., 2000). Shoreline areas were searched by walking on both riversides and around small islands, looking for typical otter sprainting sites (e.g. large stones, bridges, pool banks, confluences), following Macdonald and Mason (1983) and Prigioni (1997).

Each station was surveyed, on average, 1.9 ± 1.5 times (min–max = 1–9). A total of 101 spraints was collected in 16 different stations (50%) and 41 marking sites. The distance between two adjacent positive stations ranged between 8.5 and 18 km, while the two furthermost positive stations were 97 km apart, from Golasecca (Province of Varese) in the north to Pavia in the south (Figure 2). Based on the number of signs and positive surveys, otter presence could be considered stable on a *ca*. 65-km long stretch of the river, from Cameri to Bereguardo (Pavia province).

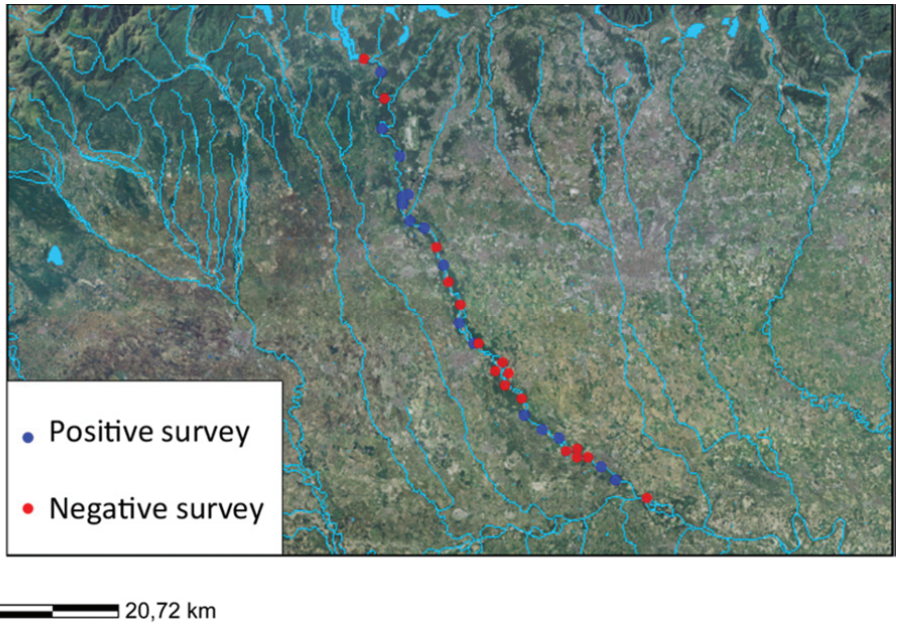

Figure 2 Results of the otter survey carried out in 2016–2017 along the River Ticino.

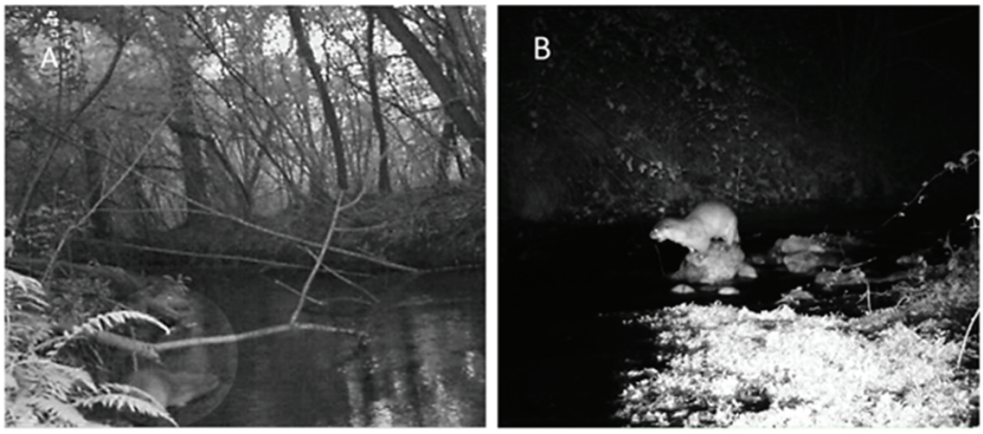

Figure 3 Otters recorded by camera-traps in 'Bosco Vedro' (Cameri): (A) 01/08/2016 at 21:03; (B) 06/01/2017 at 21:58 (Courtesy of Paola Trovò and Alberto Mantellino).

Average marking intensity (within positive stations) was 0.40 spraints/100 m. In the same period (June 2016–March 2017), a camera-trap (model UM595-GSM) deployed by P. Trovò (Natural Park of the Ticino Valley) and A. Mantellino on a side-arm of the river in 'Bosco Vedro' provided three otter records (Figure 3).

THE 2018–2019 OTTER SURVEY

In 2018, a further otter survey was granted by the Park of the Ticino Valley. The whole Italian stretch of the river was surveyed four times between January 2018 and February 2019, using the 'standard method'. Surveys were carried out within a grid (Figure 4) superimposed on the kilometric grid of digitalized, 1:10,000 Regional Technical Maps.

For each mesh of the grid, each visit implied the survey of 1–4 transects (mean length ±SD = 1,081 ± 453 m; min–max: 300–2,400 m). We applied a 'hybrid' sampling design, i.e., throughout the study period, some transects were surveyed only once, while others were visited up to four times; for large study areas, this design represents a good compromise between the robustness of standard designs (all sites surveyed K times) and cost efficiency (Mackenzie and Royle, 2005).

A total of 77 km of watercourses was surveyed. Five spraints were found in winter 2017/2018, on side arms and canals flowing in 4 out of the 9 river sections (44%). The distance between the two furthermost positive stations was 32 km apart, from Cassolnovo (Pavia province) in the north to Bereguardo in the south (Figure 4). Mean marking intensity was 0.16 ± 0.03 spraints/100 m.

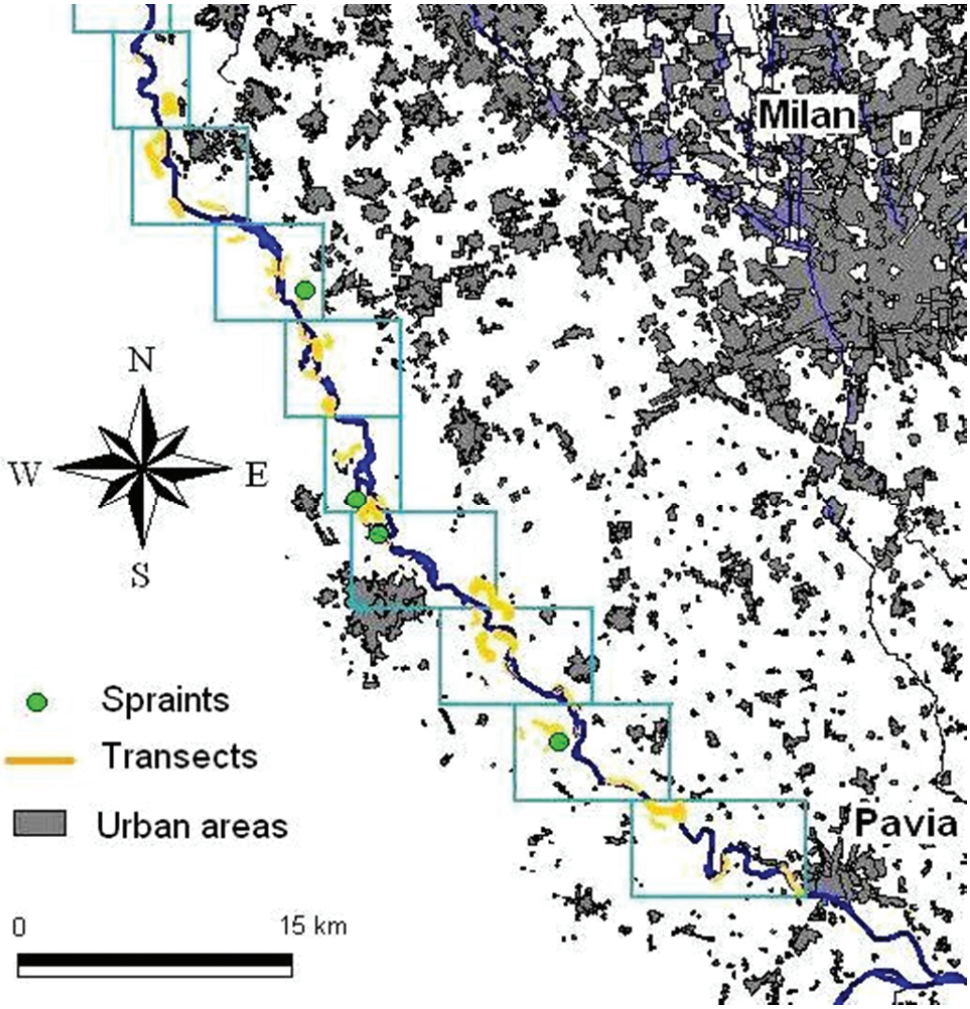

Figure 4 Sampling stations and otter signs on the River Ticino in 2018–2019 (mesh height = 5 km).

GENETIC ANALYSIS OF FAECAL DNA

Twenty-one samples collected in 2016–2017 and four in 2018 were preserved in ethanol at –40°C for genetic analyses. DNA was extracted with the Qiagen QIAamp® DNA Stool Mini Kit and amplified by multiplex PCR, using the QIAGEN® Multiplex PCR kit. Individual genotypes were determined based on 11 polymorphic autosomal microsatellite *loci*: Lut 435, Lut 453, Lut 701, Lut 715, Lut 818, Lut 833, Lut 902 (Dallas and Piertney, 1998; Dallas et al., 1999), OT-04, OT-14, OT-17 and OT-19 (Huang et al., 2005). The 11 primer pairs were split into two multiplex PCRs, in order to distinguish the amplified fragments using

different dyes and according to the expected length of PCR products. The analysis of PCR fragments was performed using a commercial service provided by Macrogen (Seul, South Korea). Electropherograms were analysed using GeneMarker V.2.2.0 (SoftGenetics, State College, PA, USA). We carried out four independent replicates and, to lower the probability of retaining false homozygotes or false alleles, constructed consensus genotypes using an approach commonly used in non-invasive genetic analyses, that is we accepted heterozygotes if the two alleles were seen at least in two replicates and homozygotes if a single allele was seen at least in three replicates (Frantz et al., 2003; Balestrieri et al., 2016).

Following these criteria, eight samples were amplified for ≥6 loci, providing a total of 32 alleles in 67 loci (mean amplification success of 76%), with a heterozygosity of 47.8% (Table 1). Two *loci* were monomorphic and 9 polymorphic, with a maximum of 5 alleles per *locus* (OT-04).

Table 1 Number (N_A) and size of the alleles found in the genotyped otter DNA samples. The amplification success (% Ampl) of each locus is also shown.

Locus	N_A	Size (BP)	% Ampl
OT-14	1	129	100
OT-04	5	166, 173, 175, 177, 190	87.5
LUT 453	4	123, 127, 129, 131	100
OT-17	3	147, 151, 172	75.0
LUT 833	4	154, 163, 165, 175	87.5
LUT 701	2	207, 247	50.0
LUT 818	4	140, 156, 160, 170	62.5
OT-19	3	211, 215, 218	62.5
LUT 435	3	123, 129, 143	100
LUT 715	1	203	75.0
LUT 902	2	145, 166	37.5
MEAN	2.9	–	76.1

By comparing the allelic profiles of the 8 samples, 6 different genotypes were identified (five in 2016–2017 and one in 2018), spreading on the river stretch between Cameri in the north and Vigevano (Pavia province) in the south (Figure 5).

DISCUSSION

Since the very start of the reintroduction, the status of otter population on the River Ticino has been assessed only by a couple of incomplete surveys, while its stable occurrence in the area was occasionally attested by the recording of road-kills or tracks. Despite the unreliability of available information, the prevalent impression was that the reintroduced population, 10–13 years after the first release, was still on the edge of extinction, probably consisting of a handful of

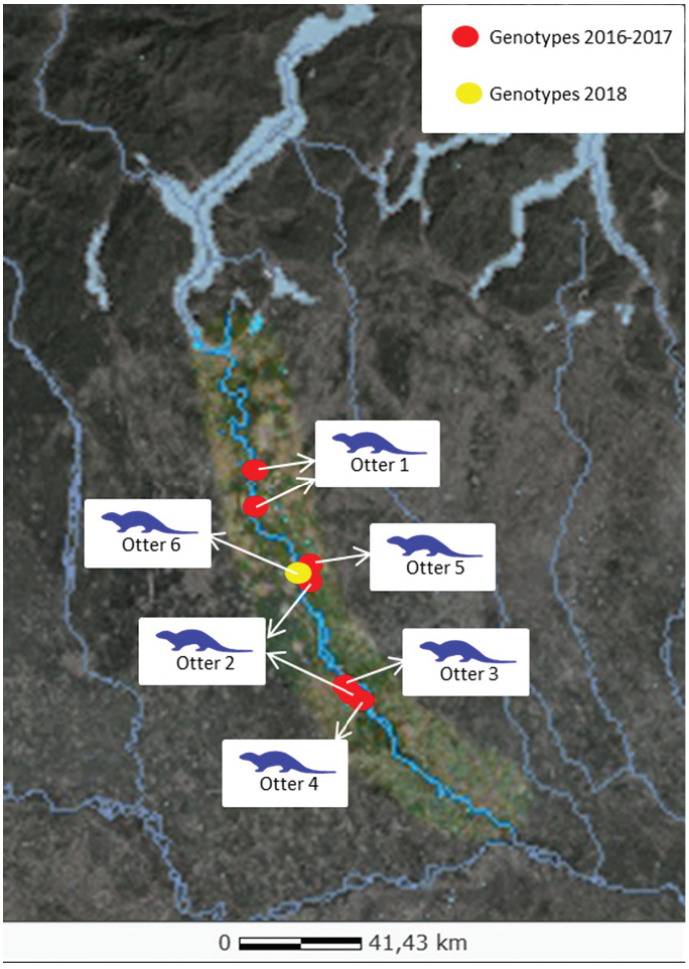

Figure 5 Distribution of the six genetically identified otters.

individuals Prigioni and Balestrieri, 2011). The estimate of population size had been based on marking intensity – 1–4 spraints per km of watercourse vs. 32–38 in the core area of the Italian otter range – but, although we may call it rough, was consistent with population numbers in areas recently recolonized by the otter in central and southern Italy (Prigioni et al., 2006, 2009).

The surveys carried out in 2016–2018 have allowed us to draw a more reliable picture of otter distribution in the protected area, with evidence of otter occurrence on a longer than previously recorded stretch of the river, confirming the most recent available assessment of its suitability for otters (Prigioni and Balestrieri, 2011).

Genetic analyses, although hindered by the difficulty of collecting fresh faecal samples due to low population density and adverse climatic conditions, have been confirmed as an effective method to investigate the status of this elusive mustelid

(Verduci, 2018). The development of a successful protocol for faecal-DNA analysis is a major goal *per se*, and, eventually, will enable us to carry out a more regular monitoring of otter status on the River Ticino. Minimum population size, as assessed in 2016, was probably underestimated but still larger than previous estimates. In contrast, the results of the successive survey were by far less positive, suggesting that otter population may rise and fall in the short period due to stochastic factors. Although rainfall and river discharge probably affected the 2018 survey, the disappearing of otter signs from sites where it had always been recorded since its reintroduction (e.g. the river stretch up- and downstream of the release site) suggests that the assessment of otter abundance was not completely biased by lowered otter detectability.

Why is it that, in the past two decades, the otter has not spread throughout the valley of the River Ticino, and its population still does not seem capable of approaching the carrying capacity?

An *a posteriori* attempt to assess the dynamics of the otter population since the reintroduction has been made using Vortex 10 (Lacy and Pollack, 2018), an individual-based stochastic simulation model developed to analyse population viability. We parameterized the model using available published data and ran several different models to take due account of uncertainty in both population parameters and actual number of released animals. All models showed that the number of founders was too small to ensure the long-term survival of the population, mainly as a consequence of inbreeding depression and loss of genetic variation (Boggioni, 2018). In this context, stochastic events, such as road mortality or exceptional floods, may have dramatic consequences.

All things considered, the reinforcement of otter population on the River Ticino, which has been the object of debate since 2008 (Conroy, unpub. workshop report, Cameri 1–2 April 2008), may represent the only effective measure to secure the success of otter reintroduction. With respect to the 1980s, nowadays the ongoing recovery of the otter in several European countries would enable us to dismiss wild animals as founders, e.g. from neighbouring Austria, where conflict with fish farmers has arisen (Kranz, 2000). Currently, the main impediment to population reinforcement is the genetic origin of reintroduced animals, which should belong to the so-called B-line (resulted from crossbreeding with the Asian subspecies *L. lutra barang*), as also suggested by the amplification of a 175-bp allele at locus Lut 833 (Mucci, pers. com.; Verduci, 2018). Further analyses, based on mtDNA primers, are needed to confirm the occurrence of B-line otters conclusively.

Phylogenetic analysis of mtDNA sequences is also recommended to ascertain the origin of otters recently recorded in the Swiss stretch of the River Ticino. Currently, Swiss rivers seem to have been recolonized by otters from both French and Austrian populations (Angst, 2018), thus we cannot exclude the crossing of Lake Maggiore by otters from the reintroduced population.

In the near future, we suggest that the major management goals should be the regular monitoring of the otter population and the updating of available data on habitat suitability for otters on the whole course of the river Ticino, including Lake Maggiore and tributaries.

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