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SHORT COMMUNICATION

Sika deer *Cervus nippon* out of the blue: a cryptic invasion in Italy

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Abstract

Hybridization between native and introduced species is one of the most important reasons underlying the impact on biodiversity exerted by biological invasions and also one of the hardest to be detected. The East-Asian sika deer *Cervus nippon* has been introduced to several European countries, where it is known to generate fertile offspring with the congeneric native red deer *C. elaphus*, threatening its local populations with genetic pollution. Most studies on hybrid populations have been carried out in northern and eastern Europe, whereas introductions of this deer and their effects are still poorly known in Southern countries. In this study, we updated the distribution of sika deer in Italy, where the species has been present since at least 25 years ago, but where no formal assessment has ever been conducted. We confirm the occurrence of sika deer genes in formerly pure red-deer populations in central Italy by means of genetic analyses. Further range expansion of invasive sika deer and red × sika deer hybrids is likely to lead to further hybridisations and introgression, with implications for the genetic integrity of native red deer populations.

Keywords Biological invasions · Distribution monitoring · Genetic analyses · Hybridization · Red deer

Introduction

The genus *Cervus* includes five species, mostly interfertile (Lorenzini and Garofalo 2015), with a Holarctic distribution: the red deer *C. elaphus* from Europe and the near East, the wapiti *C. canadensis* from North America and North-East Asia, the hangul or Tarim deer *C. hanglu* from Central Asia, the white-lipped deer *C. albirostris* from Tibet and the sika deer *C. nippon* from Eastern Asia. Since 1850s, sika deer has been released in Europe both for hunting purposes and for aesthetic reasons (Saggiomo et al. 2020; Putman et al. 2022). Currently, populations of sika deer are quite widespread in Europe (Fig. 1). Rare to moderate hybridisation between sika deer and red deer has been confirmed in European Russia, Poland, Lithuania, Ireland and United Kingdom (McFarlane et al. 2020; Putman et al. 2022). As to Southern Europe, information on sika deer distribution and hybridisation with red deer is scanty and mostly anecdotal. For instance, in Italy, Loy et al. (2019) report the sika deer as occurring in small groups, with no information available on their reproduction, population size, introduction history, actual distribution and potential hybridisation.

Hybridisation between wildlife and domestic or introduced species is a well-known issue (Donfrancesco et al.

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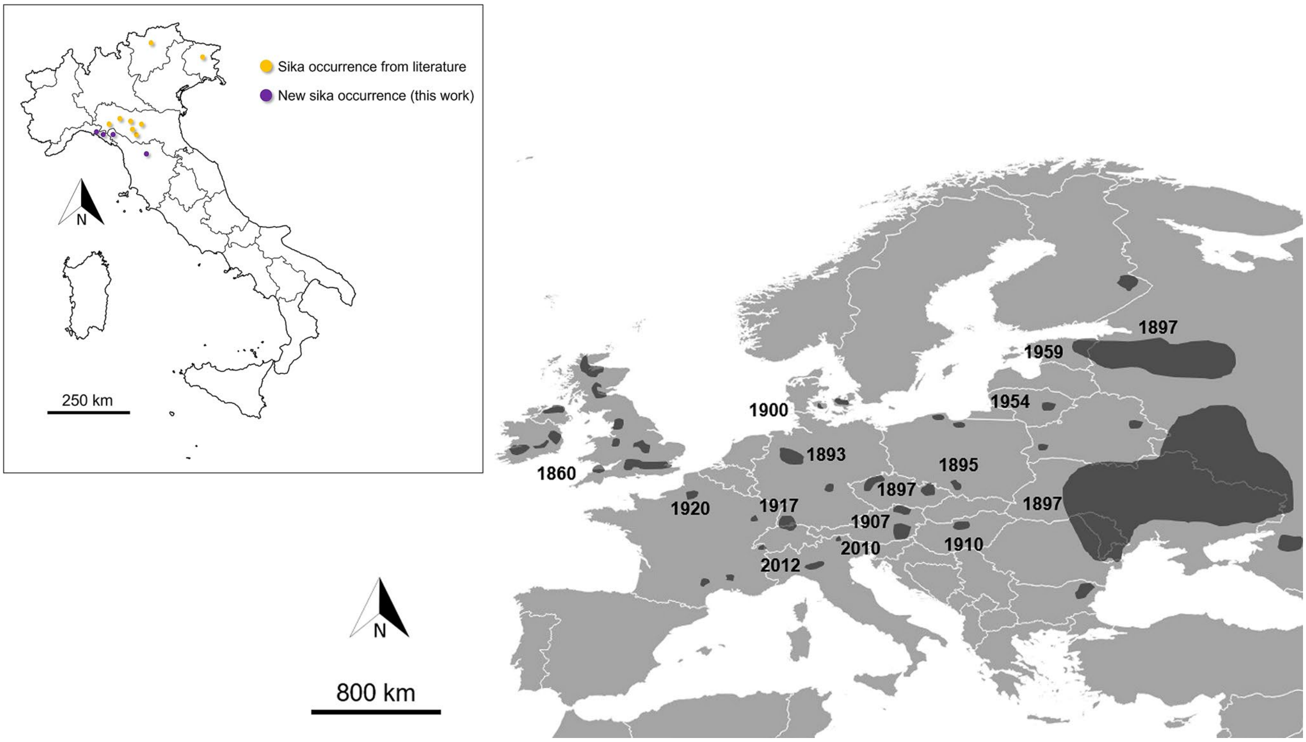

Fig. 1 Distribution of sika deer in Europe (dark grey areas) and years of first release in each country (from Putman et al. 2022, updated). Inset: current distribution of sika deer and sika × red deer in Italy

2019; Iacolina et al. 2019; Mattucci et al. 2019; Sainsbury et al. 2019) which underlies an important impact on biodiversity by determining genetic pollution (Iacolina et al. 2019). Several taxa are threatened or went locally extinct because of hybridisation and genetic pollution with closely-related introduced species (Canu et al. 2013; Iacolina et al. 2019; Putman et al. 2022), emphasizing the importance of assessing and dealing properly with it, at least at local scales. The identification of hybrids *C. elaphus* × *C. nippon* can be morphologically (e.g., via photos) and bio-acoustically possible only for the first hybrid generations, i.e., those still showing intermediate phenotypes between parental species, whereas the identification of deep backcrosses may require genetic analyses (Smith et al. 2018; Putman et al. 2022). However, hybrids can mainly be recognized by mitochondrial markers if mothers bring alien alleles. The Italian prioritization list of alien species (excluding those of European concern included in the EU Regulation 1143/2014) reports that the sika deer is, potentially, the second most harmful alien mammal to Italian native biodiversity (Bertolino et al. 2020) because it threatens the genetic identity of native deer through hybridisation (McFarlane et al. 2020) and it facilitates the transmission of allochthonous parasites (Andreani et al. 2019).

However, the current state of our knowledge on sika deer distribution in Italy is insufficient to provide clear indications for sound management in the near future. In this paper, we aimed at updating the current distribution of sika deer in Italy, with a special effort on the North-Eastern regions, focusing our attention to the potential risk of hybridization with red deer.

Materials and methods

We have reviewed the published literature on sika deer distribution in Italy between 1990 and 2023, to compile a dataset with all certified occurrences (i.e., morphologically confirmed through an expert-based approach). Particularly, we searched for the words "*Cervus nippon*", "Sika deer" and "Cervo sika" [in Italian] on Google Scholar, Scopus and ISI Web of Knowledge, as well as in the Zoological Records. We also searched for sika deer occurrences uploaded on citizen-science platforms and validated by experts (i.e., iNaturalist: www.inaturalist.org; Ornitho: www.ornitho.it, all accessed on 12.06.2023). The occurrence search was conducted in January–March 2023. We then conducted a data-mining campaign on social networks (Facebook, Twitter, TikTok and Instagram) to collect as many data as possible on the occurrence of sika deer in Italy. Records were considered as reliable only when confirmed, i.e., genetically identified or morphologically validated by experts, and when reported with geographical coordinates.

In 2022, we also collected seven tissue samples of sika-like individuals from three Italian regions, Emilia Romagna, Tuscany and Liguria as part of selective hunting programs and / or road kills. Samples were stored in absolute ethanol and kept in a freezer at − 20 °C. We opportunistically focused our sampling in the areas where the literature review identified sika presence and in neighboring regions: only individuals showing sika-compatible phenotypical traits (cfr. Ferri et al. 2014) were genetically analyzed.

DNA was extracted using the Qiagen Qiamp Blood and Tissue Kit (©Qiagen, Inc, Tokyo, Japan). We amplified a portion (738 bp) of the mitochondrial DNA control region (CR, D-loop), using a pair of deer specific primers already available in the scientific literature: CE-CR-FOR (5′-CAATACACTGGTCTTGTAAACC-3′) and CE-CR-REV (5′-TAATAGGAA GGCTGGGACC-3′: Giovannelli et al. 2022). The PCR was carried out with an Eppendorf MasterCycler X50s thermal cycler in 25 µl mix including 100 ng of each DNA sample, buffer 10×, 1.2 mM MgCl2, 200 µM dNTPs, 0.2 µM of each primer, and one unit of Taq polymerase (©Life Technologies, Waltham, Massachusetts, USA). PCR conditions included initial denaturing at 94 °C for 2 min, followed by 30 cycles of 94 °C for 45″, annealing at 50 °C for 30″, extending at 72 °C for 1 min, and a final extension to 72 °C for 10 min. PCR products were run by electrophoresis on 1.5% agarose gel, containing 05 mg/ml of SYBR green gel staining. Amplifications were then purified (ExoSAP-IT PCR clean-up Kit, ©Applied Biosystems, Foster City, California, USA) and sequenced via chain termination method at the BMR Genomics center (https://www.bmr-genomics.it/, Padua, Italy: accessed on 03.06.2023). Electropherograms were displayed on the Chromas 1.45 software (http://www.technelysium.com/au, accessed on 10.06.2023). Sequences were manually corrected and analyzed with the Mega XI software (Tamura et al. 2021). JModelTEST 304 (Posada and Crandall 1998) was used to test the most accurate model of substitution, using the Bayesian Information Criterion (BIC; Schwarz 1978) and the Akaike's Information Criterion (AIC), corrected for the heterogeneity between sites (gamma [G]). Deer sequences obtained in this study were aligned with previously published D-loop sequences available on GenBank (http://www.ncbi.nlm.nih.gov: accessed on 02.07.2023) (Table S1 in Supplementary Material 1). A first sequence dataset (Dataset 1) included long sequences of about 601 bp ($N=24$ sequences), and a second dataset (Dataset 2) consisted of short sequences (326 bp), but it included a higher number of European samples ($N=49$ sequences: Table S1 in Supplementary Material 1). We carried out a phylogenetic reconstruction by applying a Bayesian approach, through MrBayes v 3.2.6. (http://mrbayes.sourceforge.net/download.php: accessed on 11.06.2023). We ran four Markov chains in two independent analyses for 10 million generations, sampling every 1,000 generations. The first 25% of samples were discarded as burn-in. Sequences of *C. canadensis* (Table S1 in Supplementary Material 1) were used as outgroups. Support node values were given as posterior probability values.

Results and discussion

Our literature research confirmed six documents reporting on the occurrence of sika deer in Italy (Lapini 2009; Ferri et al. 2012, 2014, 2016, 2018; Loy et al. 2019). No sika deer record was available in online citizen-science platforms nor on social networks. The first female sika deer was road-killed in North-Eastern Italy in 1997, possibly as a local escape from enclosures (Lapini 2009). Afterwards, two male sika deer were killed and analyzed in October 2010 in the Bolzano District (Ferri et al. 2016). In March and October 2012, two adult males were shot near Modena, after being first observed in the wild in 2011 (Ferri et al. 2012, 2014). Another sika-like individual was killed in Parma District in 2014, where several phenotypically "pure" sika deer were observed since 2004–2005 (Ferri et al. 2016, 2018). In the same year and in 2016, several individuals with intermediate phenotype *C. elaphus* × *C. nippon* were recorded in the counties of Parma and Reggio Emilia (Ferri et al. 2016). Following unpublished microsatellite analyses (A. Barancekovà, Academy of Sciences of the Czech Republic, *in litteris*), these individuals were reported as *C. elaphus* × *C. nippon* hybrids, namely hybrids between red deer and the contested Ussuri sika deer subspecies *C. n. hortulorum* (Ferri et al. 2016). Amongst the seven sampled deer, five clustered with sika and two with red deer clades. The five sika deer samples analyzed by the D-loop gene portion, morphologically assigned to the sika phenotype, showed a close phylogenetic relationship with *C. nippon* (Fig. 2, S1 in Supplementary Material 1). The Bayesian trees obtained by both the datasets (Fig. 2, S1 in Supplementary Material 1) displayed a highly supported clade including the published *C. nippon* and sika deer sequences analyzed in this paper. All suspected sika deer showed haplotypes belonging to the clade *C. nippon.*

Conversely, the red deer samples (OQ627036 and OQ627037) clustered together with the eastern haplotype C group (lineages sensu Skog et al. 2009), naturally distributed in South-Eastern Europe (similar to the subspecies *C. e. brauneri*, from Romania). Both trees (obtained with Dataset 1 and Dataset 2) gave comparable results (Fig. 2, S1 in Supplementary Material 1). Since mitochondrial DNA (mtDNA) is regarded as having main maternal inheritance in animals (Vilaça et al. 2023), *C. nippon* individuals with a sika maternal ancestry can likely be determined. Conversely, the identification of hybrids cannot fully be excluded (particularly

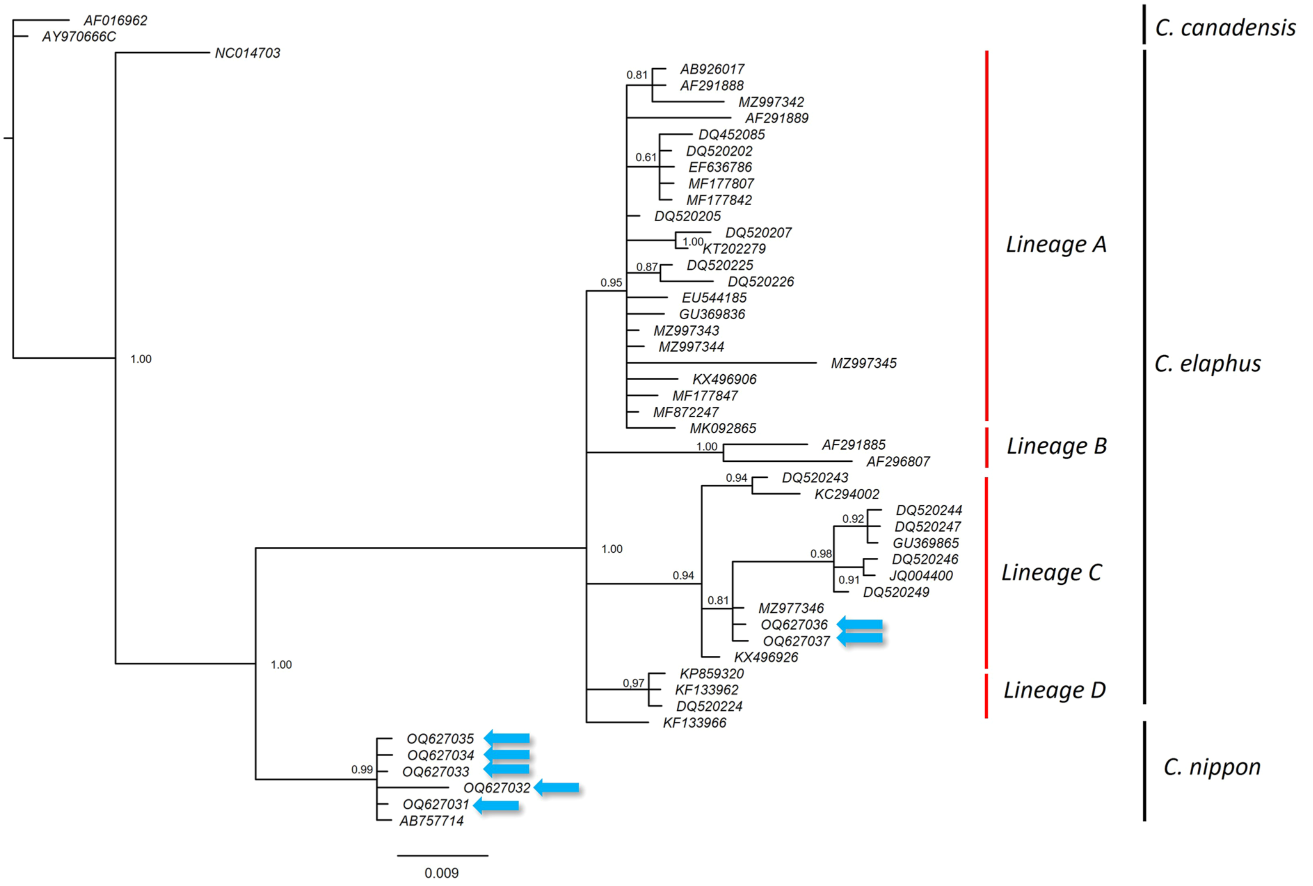

Fig. 2 Bayesian phylogenetic tree (MCMC = 10,000,000) of D-loop Dataset2 sequences. Posterior probability values are reported at nodes. Mitochondrial lineages are classified following Skog et al. 2009 and Giovanelli et al. 2022. Lineage A: Haplogroup A or Western European lineage; Lineage B: Haplogroup B or Mediterranean lineage; Lineage C: Haplogroup C or Eastern European lineage. Sample labels as in Table S1, Supplementary Material. Light blue arrows indicate genetic sequences generated in this study

in the case of phenotypically sika-compatible samples) in individuals with a paternal sika-ancestry. Therefore, even though our study represents first unequivocal data about the presence of sika deer in Italy, further investigations will have to be expanded to both nuclear and mitochondrial analyses.

Our study, although mostly based on opportunistic monitoring, confirmed the occurrence of sika deer in three Italian regions, therefore, suggesting a worse scenario compared to the one depicted by Loy et al. (2019) with data available at that time. According to the latter, *C. nippon* occurred in small groups in Northern Italy, suggesting potential hybridization with native red deer (*C. elaphus*) as already described in Great Britain by Smith et al. (2018). All the genetically supported *C. nippon* samples showed morphological features typical of sika deer.

Anthropogenic hybridisation, i.e., the one resulting from human actions, raises concern to conservation biologists. The speed of hybrid swarm formation and diffusion threatens parental population, particularly when alien species are involved (Grabenstein and Taylor 2018). Moreover, backcrosses with parental species limit the morphological identification success between pure and hybrid lines, making necessary the use of genetic analyses. In Scotland, for instance, the introgression of sika genes into red deer populations has been well-documented, but only the use of a high number of markers allows to identify them as *C. elaphus* × *C. nippon* hybrids (Smith et al. 2018; McFarlane et al. 2020), making hybrid discrimination relatively high demanding. Several red deer releases were conducted in Italy throughout the last century (e.g., in 1970s), and most of those in Central Italy included deer from Scotland (Ferri et al. 2016; Giovannelli et al. 2022). Furthermore, at least two escapes of 4–5 sika deer from enclosures and hunting preserves are known for Italy, both in Emilia Romagna, early in the 2000s (Ferri et al. 2016). Other red deer individuals of Scottish origin, locally bred for meat, accidentally escaped from enclosures in Emilia Romagna and other regions of Central-Northern Italy (where deer farming for meat was widespread in the 1970s and 1980s), which may have increased the distribution of genetically-polluted individuals (Ferri et al. 2016, 2018).

Moreover, sika deer were present in a local zoo of central Italy, which kept several animal species outside enclosures, although only hog deer *Axis porcinus* were observed in the wild in the surroundings of this area (S. Lovari, personal communication 2022). Therefore, it seems likely that populations of red deer from the Northern Apennines may be introgressed already with sika deer genes and that this introgression has been silent but continuous. It should be noted that the first sika introduction events occurred more than two decades ago, whereas our analyses have focused on individuals with sika-like phenotypes. Hence, it is extremely important that a broad-spectrum monitoring project be carried out on animals of the genus *Cervus* to understand the actual extent of this introgression in Italy. Red deer are hunted or at least culled throughout Italy (Mattioli et al. 2001). However, the Italian law does not list the sika deer amongst huntable species, thus determining a high risk of infringement. Therefore, an integration to the Italian law would be required as soon as possible to make culling sika deer and hybrids legally, as well as to eradicate all phenotypically compatible sika deer, as suggested by the European legislation on exotic species.

In conclusion, the sika deer has been present in Italy for at least 25 years, and—not surprisingly—its populations are apparently spreading. Since genetic tools play a fundamental role in the identification of sika deer and a high number of markers may be required to detect hybridisation (Smith et al. 2018; McFarlane et al. 2020), further studies should involve a substantial panel of mitochondrial and nuclear markers to identify hybrids *C. elaphus* × *C. nippon*. It would, therefore, be desirable that the local authorities set up a plan of intensive genetic surveys on the areas of hybridisation, in line of the one approved by the Emilia-Romagna regional council in 2020. Management programmes should also include the legal eradication of allochthonous or hybrid-genotype populations, to avoid genetic pollution of the endemic populations of red deer in Italy.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42991-023-00395-y.

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Data availability All data used in this study are available in the Electronic Supplementary Materials.

Declarations

Conflict of interest Authors declare no conflict of interest.

References

Andreani A, Sacchetti P, Belcari A (2019) Comparative morphology of the deer keds *Lipoptena fortisetosa* first recorded from Italy. Med Vet Entomol 33:140–153

Bertolino S, Ancillotto L, Bartolommei P, Benassi G, Capizzi D, Gasperini S, Lucchesi M, Mori E, Scillitani L, Sozio G, Falaschi M, Ficetola GF, Cerri J, Genovesi P, Carnevali L, Loy A, Monaco A (2020) A framework for prioritising present and potentially invasive mammal species for a national list. NeoBiota 62:31–54

Canu A, Scandura M, Luchetti S, Cossu A, Iacolina L, Bazzanti M, Apollonio M (2013) Influence of management regime and population history on genetic diversity and population structure of brown hares (*Lepus europaeus*) in an Italian province. Eur J Wildl Res 59:783–793

Donfrancesco V, Ciucci P, Salvatori V, Benson D, Andersen LW, Bassi E, Blanco JC, Boitani L, Caniglia R, Canu A, Capitani C, Chapron G, Czarnomska SD, Fabbri E, Galaverni M, Galov A, Gimenez O, Godinho R, Greco C, Hindrikson M, Huber D, Hulva P, Jedrzejewski W, Kusak J, Linnell JDC, Llaneza L, Lòpez-Bao JV, Mannil P, Marucco F, Mattioli L, Milanesi P, Milleret C, Myslajek RW, Ordiz A, Palacios V, Pedersen HC, Pertoldi C, Pilot M, Randi E, Rodriguez A, Saarma U, Sand H, Scandura M, Vik Stronen A, Tsingarska E, Mukherjee N (2019) Unravelling the scientific debate on how to address wolf-dog hybridization in Europe. Front Ecol Evol 7:175

Ferri M, Gelmini L, Rugnag L, Dall'Olio GL, Fontana R, Levrini M, De Pietri A (2012) Pathological findings on a sika deer *Cervus nippon* culled in the province of Modena, according to National Law on alien species. Hystrix 23:119

Ferri M, Dal Zotto M, Sala L, Todaro A, Barancekovà M, Fontana R, Lanzi A, Armaroli E, Musarò C, Andina L, Allegri M, Adorni PL, Peloso F, Gelmini L, Levrini M, De Pietri A (2014) Three sika deer *Cervus nippon* recently hunted in the Emilia-Romagna's area of A.C.A.T.E.R. west question the management of Italian *Cervus elaphus* population. Hystrix 25:104.

Ferri M, Fontana R, Lanzi A, Armaroli E (2016) Presenza di cervi sika (*Cervus nippon*) e loro ibridi in Italia. Una lunga indagine preliminare, tra ufficialità e "*citizen-science*". In: Angelici FM, Rossi L (eds) Atti del III Congresso Nazionale "Fauna Problematica". Cesena, Italy, 24–26/11/2016)

Ferri M, Fontana R, Lanzi A, Armaroli E, Musarò C, Andina L, Allegri M, Adorni PL, Peloso F (2018) Diffusione di cervi sika (*Cervus nippon*) in Italia, dagli Appennini alle Alpi. In: Guidarelli G, Sozio G, Preatoni DG (eds) Atti del XI Congresso Italiano di Teriologia (ATIt). Hystrix 29:65

Giovannelli F, Mori E, Zaccaroni M, Dessì-Fulgheri F, Baratti M (2022) Genetic insights into an Apennine population of the Italian red deer. Mammal Res 67:399–406

Grabenstein KC, Taylor SA (2018) Breaking barriers: causes, consequences, and experimental utility of human-mediated hybridization. Trends Ecol Evol 33:198–212

Iacolina L, Corlatti L, Buzan E, Safner T, Šprem N (2019) Hybridisation in European ungulates: an overview of the current status, causes, and consequences. Mammal Rev 49:45–59

Lapini L (2009) Micromammiferi della Riserva Naturale “Lago di Cornino” (Folgaria nel Friuli, Udine, Italia Nord-Orientale). Gortania 31:143–170

Lorenzini R, Garofalo L (2015) Insights into the evolutionary history of *Cervus* (Cervidae, tribe Cervini) based on Bayesian analysis of mitochondrial marker sequences, with first indications for a new species. J Zool Syst Evol Res 53:340–349

Loy A, Aloise G, Ancillotto L, Angelici FM, Bertolino S, Capizzi D, Castiglia R, Colangelo P, Contoli L, Cozzi B, Fontaneto D, Lapini L, Maio N, Monaco A, Mori E, Nappi A, Podestà M, Russo D, Sarà M, Scandura M, Amori G (2019) Mammals of Italy: an annotated checklist. Hystrix 30:87–106

Mattioli S, Meneguz PG, Brugnoli A, Nicoloso S (2001) Red deer in Italy: recent changes in range and numbers. Hystrix 12:27–35

Mattucci F, Galaverni M, Lyons LA, Alves PC, Randi E, Velli E, Pagani L, Caniglia R (2019) Genomic approaches to identify hybrids and estimate admixture times in European wildcat populations. Sci Rep 9:11612

McFarlane SE, Hunter DC, Senn HV, Smith SL, Holland R, Huisman J, Pemberton JM (2020) Increased genetic marker density reveals high levels of admixture between red deer and introduced Japanese sika in Kintyre, Scotland. Evol Appl 13:432–441

Posada D, Crandall KA (1998) Modeltest: testing the model of DNA substitution. Bioinformatics 14:817–818

Putman R, Krojerova-Prokešova J, Barančekova M (2022) Sika *Cervus nippon* Temminck, 1838. In: Corlatti L, Zachos F (eds) Terrestrial Cetartiodactyla. Handbook of the Mammals of Europe. Springer Editions, Cham, pp 87–114

Saggiomo L, Esattore B, Picone F (2020) What are we talking about? Sika deer (*Cervus nippon*): a bibliometric network analysis. Ecol Inform 60:101146

Sainsbury KA, Shore RF, Schofield H, Croose E, Campbell RD, Mcdonald RA (2019) Recent history, current status, conservation and management of native mammalian carnivore species in Great Britain. Mammal Rev 49:171–188

Schwarz G (1978) Estimating the dimension of a model. Ann Stat 6:461–464

Skog A, Zachos FE, Rueness EK, Feulner PGD, Mysterud A, Langvatn R, Lorenzini R, Hmwe SS, Lehoczky I, Hartl GB, Stenseth NC, Jakobsen KS (2009) Phylogeography of red deer (*Cervus elaphus*). J Biogeogr 36:66–77

Smith SL, Senn HV, Pèrez-Espona S, Wyman MT, Heap E, Pemberton JM (2018) Introgression of exotic *Cervus* (*nippon*and *canadensis*) into red deer (*Cervus elaphus*) populations in Scotland and the English Lake District. Ecol Evol 8:2122–2134

Tamura K, Stecher G, Kumar S (2021) MEGA11: molecular evolutionary genetics analysis version 11. Mol Biol Evol 38:3022–3027

Vilaça ST, Maroso F, Lara P, de Thoisy B, Chevallier D, Arantes LS, Mazzoni CJ (2023) Evidence of backcross inviability and mitochondrial DNA paternal leakage in sea turtle hybrids. Mol Ecol 32:628–643