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The breaking news on distribution of the Italian hare (Lepus corsicanus) in Cilento, Vallo di Diano e Alburni National Park

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Abstract

The Italian hare (*Lepus corsicanus*) is an endemic species of the Central-Southern Italy and Sicily, classified as vulnerable by the International Union for Conservation of Nature because of the impact of human habitat alterations, the low density and fragmentation of sub-populations and the ecological competition with the European hare (*Lepus europaeus*), intensively restocked as a game species.

The Cilento, Vallo di Diano e Alburni National Park is one of the most important area of occurrence of the Italian hare in Southern Italy, where it live in sympatry with the European hare. However, from 2010 there are no updated information about the distribution of the Italian hare in this important basin. Here, we provide the "breaking news" on its distribution in the National Park using non-invasive genetically validated data. Our findings could give an effective contribution for management strategies aiming at the conservation of this endemism since that the clear knowledge of the distribution pattern of a species is the first stage required in all ecological studies and management planning.

Keyword: Distribution, faecal pellet, conservation, genetic assignment, non-invasive genetics

Riassunto

La lepre italica (*Lepus corsicanus)* è una specie endemica dell'Italia centro-meridionale e della Sicilia, classificata come vulnerabile dall'Unione Internazionale per la Conservazione della Natura, a causa dell'impatto delle alterazioni antropiche dell'ambiente, la bassa densità e la frammentazione delle sotto-popolazioni e la competizione ecologica con la lepre europea (*Lepus europaeus*), intensamente introdotta a scopo venatorio.

Il Parco Nazionale del Cilento, Vallo di Diano e Alburni è uno dei principali bacini di presenza della lepre italica, che qui si trova in simpatria con la lepre europea in molte aree. Tuttavia, dal 2010 non sono disponibili informazioni aggiornate sulla distribuzione della lepre Italica in questo importante zona di presenza. Qui, noi forniamo le notizie più recenti sulla distribuzione della lepre italica in questo Parco Nazionale usando dati geneticamente validati. Queste informazioni potrebbero rappresentare un contributo concreto per la conservazione di questo endemismo dato che la precisa conoscenza della distribuzione di una specie è il primo step richiesto in tutti gli studi ecologici e piani di gestione.

Parole chiave: Distribuzione, campione fecale, conservazione, assegnazione genetica, genetica non invasiva

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Introduction

The Italian hare (*Lepus corsicanus*) is an endemism of the Central-Southern Italy and Sicily (Trocchi & Riga 2005; Amori & Castiglia 2018; Lo Valvo 2007) and it was introduced in Corsica in XVI century (Vigne 1988).

The species has experienced considerable contraction of distribution range during the past several decades, and, to date, it is classified as vulnerable (VU - A2bcde) in the Red List of the International Union for Conservation of Nature (Randi and Riga 2019). The major threats that affect the status of the species are the human habitat alterations, the low density and fragmentation of sub-populations, the poaching/hunting activities and the ecological competition with native and exotic lineages of the European hare (*Lepus europaeus*), intensively restocked as a game species (Pierpaoli et al., 2003; Kasapidis et al., 2005; Angelici et al., 2008; Fulgione et al., 2009).

While Sicilian sub-population of the Italian hare shows a continuous range (Lo Valvo et al., 1997), on the Italian peninsula the species is widespread as small and fragmented nuclei (Angeli & Luiselli, 2001; Trocchi & Riga 2005; Angelici & Luiselli 2007; de Filippo et al., 2007; Fulgione et al., 2009; Angelici et al., 2010; Scarselli et al., 2016; Dori et al., 2018; Mori et al., 2020). In

particular, the Cilento, Vallo di Diano e Alburni National Park could be considered one of the most important area of occurrence of the species in southern Italy. Previous studies reported a sub-population showing the highest density among Southern Italian peninsular ones on some Apennine mountain areas including in this National Park (de Filippo et al., 2007; Fusco et al. 2007; Fulgione et al., 2009), however, from 2010 there are no updated information about the distribution of the Italian hare in this important basin.

Here, we provide the "breaking news" on distribution of the Italian hare in the Cilento, Vallo di Diano e Alburni National Park using non-invasive genetically validated data.

A clear knowledge of the distribution pattern of a species is the first stage required in all ecological studies (Palomares et al., 2002; Measey et al., 2011) and our data could effectively support management actions in conservation planning for the Italian hare.

Material and Methods

Study area

The study was carried out in the Cilento, Vallo di Diano e Alburni National Park (PNCVDA, 40° 17′ N, 15° 19′ E - Salerno, Campania) (Fig.1). The landscape shows extremely mosaicized structure typically of the Mediterranean basin, with small urban areas alternated with agricultural and natural ones. At higher altitudinal level, it is dominated by Fagus sylvatica forests interspersed with grasslands of secondary origin composed mainly by *Poaceae*, *Fabaceae* and *Cyperaceae*. The underlying altitudinal range is covered by forest of *Alnus cordata* and *Castanea sativa*, followed by deciduous oaks (*Quercus cerris, Quercus pubescens*) and maple trees (*Acer* sp.) in the medium-lower altitude level (Pignatti et al., 1998).

Samples collection

From 2014 to 2019, we conducted a wide field survey to record the presence of the Italian hare, including all four seasons.

The entire protected area was divided into 2.5 km2 squares, in which sampling was performed in medium and high habitat suitability areas for the Italian hare (de Filippo et al., 2007; Fulgione et al., 2009), between 100 and 1900 meters a.s.l. (Fig. 1).

These areas were identified as stable territories of presence for both the Italian hare and the European one (de Filippo et al., 2007; Fulgione et al., 2009; Buglione et al., 2018). To discern between the Italian hare and the European hare using their phenotype (i.e. body measurements and coat color shades, Riga et al., 2001; Trocchi & Riga 2005) is not easy in field and/or without close contact with these species. Thus, in order to use only reliable data, minimising the impact on the vulnerable species, we use only faecal pellets genetically characterized (see below).

The non-invasive sampling was performed by the operators walking along transects of 5 - 8 km, chosen in order to be representative of the different environmental conditions present in the sampled area and in order to cover the largest possible area.

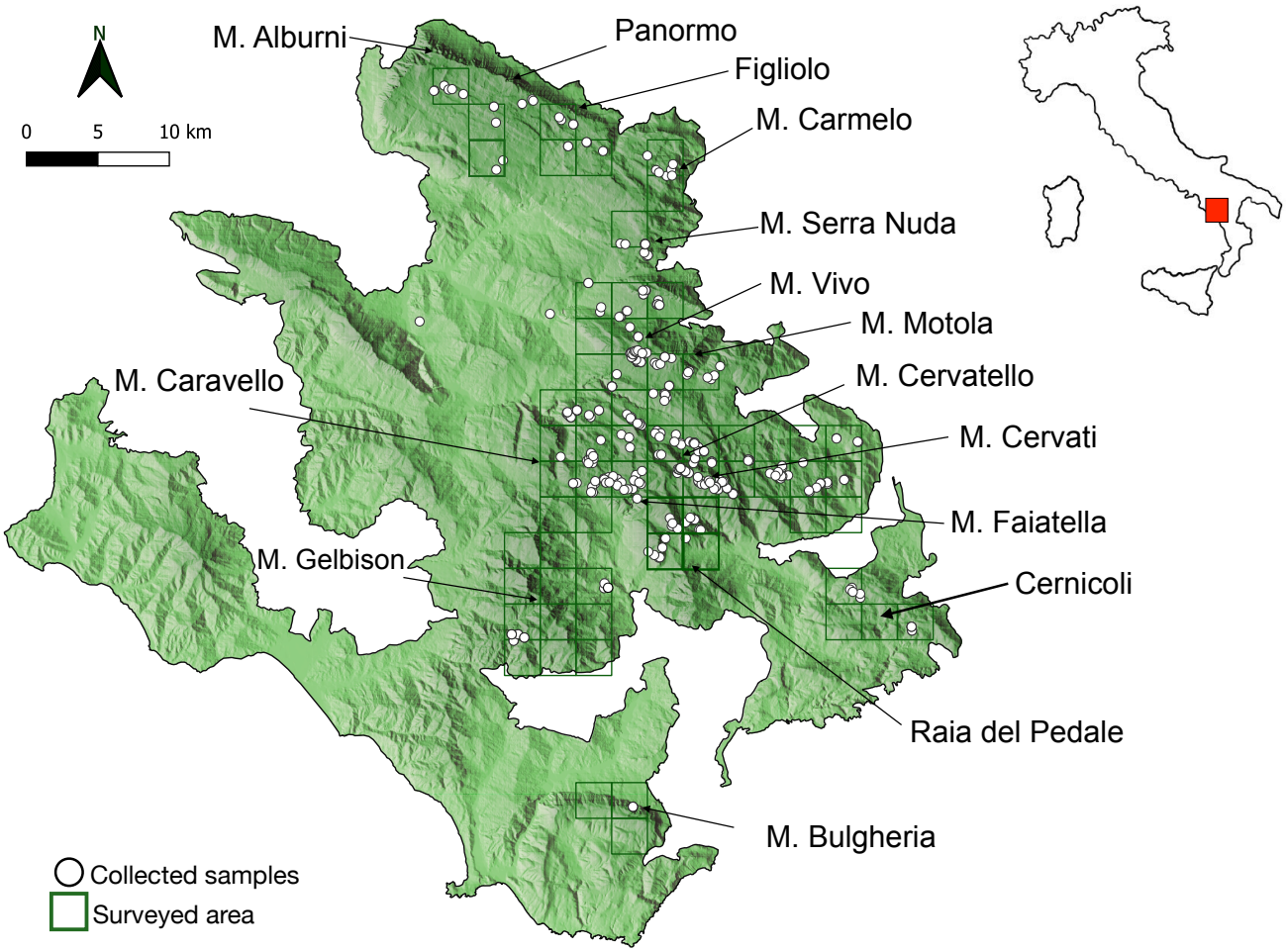

Figure 1: Study area. Cilento, Vallo di Diano e Alburni National Park (PNCVDA). Green squares of 2.5 km2 represent the surveyed areas. White spots indicate collected faecal pellets.

The DNA fragmentation increases with age of samples (Taberlet et al., 1996) and with exposure to environmental agents (Jansman et al., 2001), and depends on way of collection and preservation, leading to limitation in subsequent laboratory procedures. Therefore, to reduce interval from defecation to collection and to minimize efforts using samples to yield high-quality DNA, we performed the sampling of faecal pellet in early morning (Dallas et al., 2000; Goossens et al., 2000), collecting only fresh samples (0-2 day old), aged by skilled field collectors using aspect patterns (Piggott 2005; Santini et al., 2007). All samples were handled with sterilized equipment and preserved in sterile Falcon® tubes with silica desiccant granules (Wasser et al., 1997). Then stored at 4°C (during transport) or -20°C (in laboratory) until their processing.

Species assignment

No studies report difference in the faecal pellet morphology between the Italian hare and the European hare and this made molecular characterization of faecal DNA necessary.

Total genomic DNA was extracted from the external surface of faecal pellet using QIAamp DNA Fast Stool Mini Kit (QIAGEN, Valencia, CA), according to manufacturer's instructions. Blank extractions were included in each extraction to exclude potential contaminations. Integrity of DNA was verified using agarose-gel electrophoresis run while quality and concentration were checked with Nanodrop ND-2000 (Nanodrop, Wilmington, DE, USA) and Qubit Fluorometer 3.0 (Thermo Fisher Scientific).

Species assignment was developed using HRM analysis (Farrar & Wittwer 2017) with experimental protocol and raw data analyses performed according to Buglione et al., 2020. The comparison between the melting profile of the faecal DNA from known Italian hare and European hare samples and that of unknown samples was used to assign these latter to corresponding hare species.

Species distribution map

All records were geo-referenced (by GPS; UTM-WSG 84) and software QGIS 3.4.1. was used to elaborate species-specific distribution map (scale 1:320,000) using geographical coordinate of genetically validated data.

Results

We collected 408 faecal pellets in total, of which 101 were assigned to the Italian hare and 181 to the European hare. For 126 faecal pellets, HRM did not allow the species assignment.

Assuming that the method of collection was the same for the two species, and that, less likely, the species are evenly distributed throughout the National Park, we can say that if a hare pellet is found, there is a 25% probability that it is from the Italian hare and 44% that it was deposited by an European hare. Geographical spazialization of genetically characterised samples showed that the Italian hare was distributed in most of the internal mountain areas of the PNCVDA, including the Mount Alburni, M. Serra Nuda, M. Motola, M. Vivo, M. Faiatella, M. Caravello, Raia del Pedale, M. Cervati, M. Cervatello, M. San Giacomo M. Cernicoli, M. Gelbison and M. Bulgheria (Fig. 2A), spreading in an altitudinal range from 630 to 1848 m a.s.l. The major number of samples was widespread between 1000 and 1300 m a.s.l, with a main peak at about 1150 m a.s.l (Fig. 2B).

The habitat of these locations, mainly on M. Cervati and M. Alburni, was represented by wooded areas with beech, oak and chestnut forests, interspersed with open grassland dominated by the red clover (*Trifolium pratense*) and meadow brome (*Bromus erectus*), that outlines environments with marked ecotonal characteristics and high environmental diversity (Fig. 2C and D).

We found the species also in highland pastures of secondary origin of Festuco-Brometalia and, at higher altitude (>1500 m a.s.l.) on almost all mountain massifs (M. Cervati, M. Alburni, M. Motola, M. Serra Nuda and M. San Giacomo), the Italian hare was

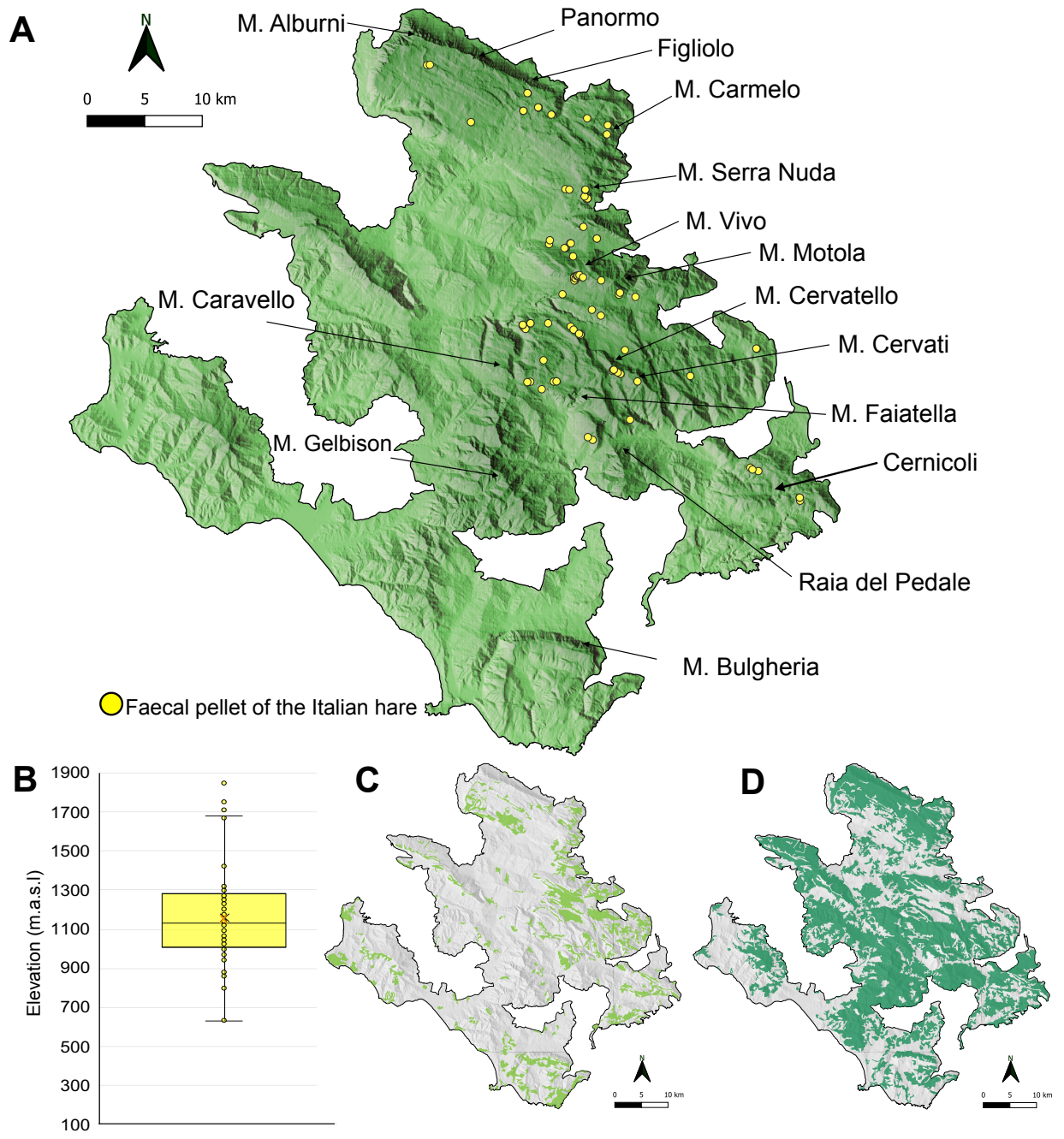

Figure 2: A) Distribution of the Italian hare (*Lepus corsicanus*) in Cilento, Vallo di Diano e Alburni National Park (PNCVDA) based on genetic validated data. Yellow spots represent the faecal pellets assigned to the Italian hare. B) The Italian hare (*Lepus corsicanus*) distribution related to the elevation. Distribution of C) pastures and grassland and D) woods and forests in PNCVDA according to CORINE Land Cover 2000, Class 3.

localised in dry grasslands with low vegetation, interrupted by rocks, stones, shrubs and low scrub with scattered conifers. In addition, in particular on the M. Vivo, the Italian hare faecal pellets were sampled also in very small residual cultivated areas.

Discussion

We provide updated information on the distribution of the Italian hare in the PNCVDA, about which little was known since 2010.

Our data confirm a fragmented distribution of the Italian hare in internal mountains of the National Park (M. Alburni, M. Motola, M. Cervati and M. Faiatella (de Filippo et al., 2007; Fulgione et al., 2009) and define new areas of occurrence (i.e. M. Cernicoli, M. Caravello and Raia del Pedale).

On most of the territory, the Italian hare is in sympatry with the European hare (data not shown), even if we found some areas of exclusive presence of the endemic species, for example on M. Serra Nuda, where, however, it is less abundant. This could depend on low population density, that reduce the probability to localise faecal samples. However, these territories could be consider as suitable sites for reintroduction of new individuals aiming to revert population decline induced by the low densities.

Another factor affected the probability to found the faecal pellets could be the characteristics of the habitat. This, in some areas, made sampling difficult, as on Monte Gelbison, where most of territories are dominated mainly by the common bracken (*Pteriudium aquilinum*), probably due to the lithological nature of mountains, where overgrazing and subsequent abandonment of the areas promoted the growth of this fern.

The *Lepus* sp. samples collected on M. Gelbison did not provide yield high-quality DNA suitable for species characterization and further studies should be performed to validate the absence/presence of the Italian hare in these territories.

On M. Bulgheria, we collected only one faecal sample ascribable to the Lepus sp. As before, this result could depend on low population density of the hare, and furthermore, this condition could become worse because of the geographical isolation of M. Bulgheria. Indeed, the massif shows a high degree of isolation from the rest of mountain system of the Cilento, as emerged by analyses of habitat connectivity for the PNCVDA territories (data not shown). Also here other deepened analyses need to define what hare species live in these territories.

The major number of samples assigned to the Italian hare was collected at mean at 1150 m a.s.l., in wooded areas interspersed with open grassland and in mountain highland pastures. These habitats mirror the environmental preferences of the species in the PNCVDA (de Filippo et al., 2007; Fulgione et al., 2009) and in other localities (Trocchi & Riga 2005; Angelici & Luiselli 2007; Angelici et al., 2010), and agree also with its trophic requirements (Freschi et al., 2016; Buglione et al., 2018).

The next steps should be to continue the monitoring of the species to keep information on its distribution up to date and to extend the survey to other areas of the PNCVDA that have not yet been investigated, both in the mountainous internal territories and on the coast. Indeed, the Italian hare in other Italian regions (i.e. in Latium) or in Corsica was reported also at

low altitude and close to coastline (Trocchi & Riga 2001; Petri 2015), meanwhile for coasts of the Cilento there are only few and old reports attributable to the European hare (de Filippo et al., 2000).

Our data on precise distribution of the Italian hare in PNCVDA represent an effective support for management strategies. Indeed, they suggest what could be the useful areas for reintroductions or for definition of ecological corridors aimed at connecting isolated sub-populations or, considering the ecological requirement of the Italian hare, for environmental improvements aiming at the conservation of this important tile of global biodiversity.

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Author contributions

Conceptualization: Maria Buglione and Domenico Fulgione; Data curation: Maria Buglione, Domenico Fulgione, Claudia Troiano and Gabriele de Filippo. Formal analysis: Maria Buglione, Domenico Fulgione, Claudia Troiano; Investigation: Maria Buglione, Domenico Fulgione, Claudia Troiano, Simona Petrelli, Gabriele de Filippo, Tommaso Notomista, Valeria Maselli. Methodology: Maria Buglione, Domenico Fulgione and Gabriele de Filippo; Project Administration: Maria Buglione and Domenico Fulgione; Resources: Domenico Fulgione, Gabriele de Filippo and Romano Gregorio; Writing – original draft: Maria Buglione and Domenico Fulgione; Writing – final draft preparation: Maria Buglione, Domenico Fulgione, Claudia Troiano, Simona Petrelli, Gabriele de Filippo, Tommaso Notomista, Valeria Maselli and Romano Gregorio.

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