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# Splitting hairs: How to tell hair of hares apart for predator diet studies

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ABSTRACT

Hares are a major prey for many carnivorous vertebrates worldwide. Their occurrence in the diet of predators is mostly assessed through the analysis of indigested remains (especially hair) in faeces or pellets. In Italy, four hare species are present, locally occurring in sympatry, and several studies confirmed they are preyed upon by 15 carnivorous vertebrates, overall. A reliable identification of hare species in their diet is only possible if specific diagnostic keys of their hair are available. To provide diagnostic features of the four Italian hare species, we collected 218 hair samples from 37 individuals belonging to 13 hare populations. Samples were measured and analysed at the microscope; five indices were assessed. Hair indices of morphology differed significantly across the four species, both in the cortex and in medulla structure. Species discrimination through hair may be crucial especially if the range overlap among hare species will increase, due to environmental/climatic changes and/or human management actions (e.g. restocking).



## Introduction

Food habits of vertebrate species are mainly assessed through the analysis of food remains from faeces (i.e., seeds, stems, hair and bones: e.g. Putman, 1984; Reynolds and Aebischer, 1991; Klare et al., 2011) or egested pellets (Yalden and Yalden, 1985; Johnstone et al., 1990; Votier et al., 2003). Food remains can be determined through specific reference atlases (e.g. Mayer, 1952; Teerink, 1991; De Marinis and Agnelli, 1993; Oli, 1993; Nappi, 2001; De Marinis and Asprea, 2006) or ad hoc reference collections. Mandibular structures and hair of prey of carnivores often show species-specific diagnostic features (Hausman, 1920; Faliu et al., 1980; Teerink, 1991). When the size of the prey is relatively large with respect to that of predator, teeth are rarely found in faeces (Nappi, 2001), and, thus, researchers have to rely on hair analysis; accordingly, macroscopical and microscopical analyses of mammal hair may represent a valuable tool for ecological research, including food habits of carnivores and raptors (e.g. Yalden and Yalden, 1985; Oli, 1993; Redpath et al., 2002; Kerley et al., 2015).

Hares *Lepus* spp. are widespread worldwide, but for Antarctica, and are important prey species for many vertebrates (e.g. felids: Lovari et al., 2013; Apostolico et al., 2016; canids: Hayward et al., 2014; Pagh et al., 2015; Newsome et al., 2016; mustelids: Macdonald et al., 2000; Posłuszny et al., 2007; raptors: Kopij, 2016; Rehnus et al., 2016). In the specific case of Italy, it has been reported that hare species are present in the diet of 7 species of mammalian carnivores, 6 species of raptors, the wild boar *Sus scrofa* and the carrion crow *Corvus corone* have been reported to feed on hare species (Appendix I in Supplementary material). Effective species determination from undigested hairs is needed to estimate the actual role of hares in the diet of predators (Temple and Terry, 2009), as well as to obtain sound information on mortality/limiting factors for conservation/management of wild hare populations. De Marinis and Agnelli (1993) reported that hair of all species belonging to the genus *Lepus* show a multicellular column-shaped medulla in the shield, a concave cross section and a pale bar. Furthermore, hairs of *Lepus* species show a diagnostic pale (light brown to red) bar near the tip, which allow researchers to distinguish them from those of other mammal species (Teerink, 1991). Diagnostic keys are required to identify hair at the specific level when two or more hare species co-occur.

Italy represents a useful case study for setting up species-specific diagnostic keys to identify hair of hare species. In this

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**Table 1**
Percent overlap in the Italian range of *Lepus europaeus* with *L. corsicanus* and *L. timidus*.

| SPECIES 1 → SPECIES 2 ↓ | *L. europaeus* | *L. corsicanus* | *L. timidus* |
|---|---|---|---|
| *L. europaeus* | – | 40.0 | 9.3 |
| *L. corsicanus* | 64.1 | – | 0.0 |
| *L. timidus* | 47.4 | 0.0 | – |

country, 4 out of 6 European hare species are present (Aulagnier et al., 2010). Mainly due to restocking events for hunting purposes (Santilli, 2007; Ferretti et al., 2010), the European brown hare *L. europaeus* is the most widespread species, and it is the only one coexisting with other species (Table 1). The Sardinian hare (*L. capensis mediterraneus*, hereafter *L. capensis*: Slimen et al., 2005) has been released in historical times to Sardinia from North Africa. Concerning the other species, the Apennine hare *L. corsicanus* is endemic to central and southern Italy (Scalera and Angelici, 2003), whereas the mountain hare *L. timidus* is widely distributed throughout the northern Palearctic and the Alps (Aulagnier et al., 2010). The latter species is listed within the European Union Habitat Directive, Annex V. Thus, both the Apennine hare and the mountain hare are species of conservation concern (Amori et al., 2008) and their distribution ranges partially overlap with that of the brown hare (Aulagnier et al., 2010; www.iucnredlist.org, accessed on 04th May 2017: Table 1).

A morphological characterization of hair is only available for the mountain- and European brown hares (Teerink, 1991). Rugge et al. (2009) found that coat colouration is an effective feature for discriminating between *L. corsicanus* and *L. europaeus* in Southern Italy, suggesting that differences in hair morphology may also occur. Thus, our aim was to identify diagnostic features to distinguish the hairs of the four hare species, as to provide researchers with an effective key for this genus.

## Material and methods

### *Collection and measurements of hair samples*

We collected guard hair samples from 13 populations (*L. corsicanus*, N = 3; *L. timidus,* N = 3; *L. capensis*, N = 3; *L. europaeus*, N = 4) of all the 4 hare species, from a total of 37 individuals (*L. corsicanus*, N = 8; *L. timidus*, N = 8; *L. capensis*, N = 6; *L. europaeus*, N = 15). Hairs were taken from individuals of both sexes. Coat colouration and morphology (i.e. hair length) do not change throughout the year for most species adapted to live in areas with limited annual climatic fluctuations (Stoner et al., 2003). We decided to use only hair collected between September and November to avoid the winter-early spring white coat of the mountain hare. Furthermore, most captures for behavioural and parasitological studies, as well as hunting, mainly occur in these months or early after (e.g. Santilli, 2007; Ferretti et al., 2010; Zaccaroni et al., 2013), therefore increasing the success of hair collection. We assumed that no age difference in hair ultrastructure would occur in hares (cf. Faliu et al., 1980; Teerink, 1991). We also have no evidence of any age class being more susceptible to predation. Since most restocked European hares are adult (Amori et al., 2008; Sokos et al., 2015), we only sampled hairs of subadult/adult individuals.

Hairs were taken from killed and live captured individuals, from both the rump and hind legs. A total of 218 hair samples was analysed, between 4 and 6 hairs per individual. The total hair length, pale bar width (hereafter "bar width"), and distance between the bar and hair tip ("bar-tip distance": Fig. 1) were measured by a precision calliper (0.1 mm in sensitivity: © DIN 862, Würth, Germany). In turn, we used five indices of hair morphology, some of which can be obtained even from hairs partially destroyed by digestive processes: i) hair length; ii) bar width; iii) bar-tip distance; iv) "bar width: bar-tip distance" ratio; v) "bar width: hair length" ratio. Hairs were prepared through standard protocols (Mori et al., 2016). The analysis of the cortex and cuticula was carried out by placing each hair on a glass slide, on a layer of nail polish. When polish was completely dry, the hair was removed, and the mould observed at the microscope and described according to the categories reported by Teerink (1991). The analysis of the medulla was carried out with the hair longitudinally sectioned and wet with cedar oil at the section level. Slides were observed under the microscope to compare the structure of the medulla with patterns reported by Teerink (1991).

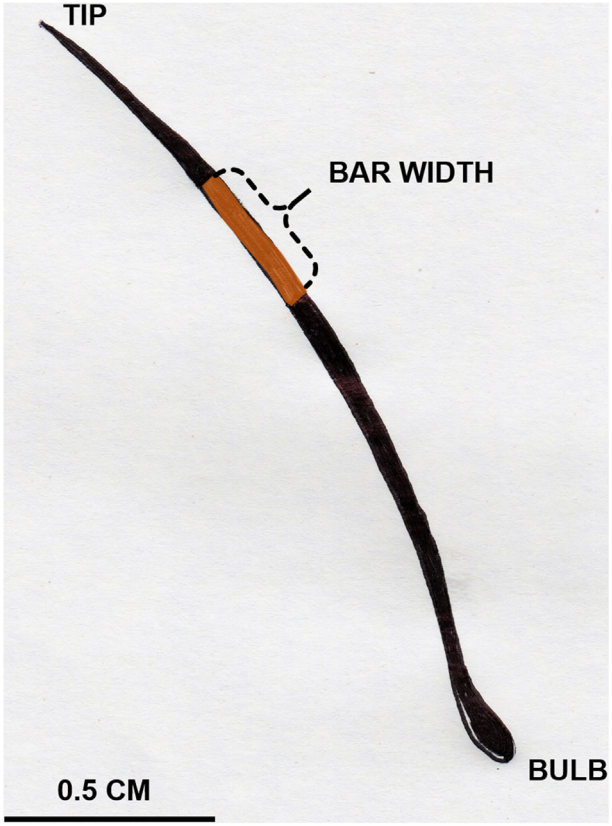


**Fig. 1.** General structure of hair of hare species in Italy.

### *Statistical analyses*

We used Fisher's linear discriminant analysis (LDA: Rencher, 1995) to assess the separation among species hair morphology. LDA replaced the values of original variables (i.e. hair indices) with the scores of three discriminant functions, i.e. linear combinations of the variables providing the greatest separation between species. We applied a non-parametric adaptation of the multivariate analysis of variances based on Euclidean distances between LDA scores to test for the statistical significance of differences between species (NPMANOVA; Anderson, 2001). The significance was computed by class permutations, using 99,999 replicates. The pairwise comparison of species was used as a *post-hoc* test using Bonferroni's correction. These statistical analyses were performed through the software PAST (Hammer et al., 2001).

We evaluated whether indices of hair morphology differed among species through generalised linear mixed models (GLMMs; Zuur et al., 2009). Being continuous, positive variables, indices were modelled through an inverse Gaussian error distribution and an