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## Hair identification key of wild and domestic ungulates from southern Europe

Anna Maria De Marinis & Alessandro Asprea

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We analysed macro- and microscopic features of dorsal guard hairs in 105 specimens of 10 wild and five domestic ungulates from southern Europe to work out a dichotomous key with a photographic reference system of diagnostic hair features. We integrated and extended the available data on hair morphology of wild ungulates and provide a first comparative analysis of hair structure of domestic forms. To develop the key, we used clearly recognisable qualitative characters of cuticle and medulla. The techniques used in this study can be easily, quickly and economically applied in routine investigations, keeping the time required to identify a sample at a minimum. The accuracy of the key was assessed through a blind test carried out by four trained observers. We describe the effects of age and season on the microscopic structure of hair, which have not yet been described in European literature. A review of all the available data on hair morphology of wild ungulates is presented and the relevant differences between domestic forms and their relative wild ancestors that have arisen during the domestication process are described. A hair identification key has a wide range of practical applications in biology, such as the study of carnivore feeding habits through scat analysis.

Key words: bovids, deer, domestic, guard hairs, identification key, southern Europe, suids

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Identification of hair of mammalian species has practical applications in forensic medicine, taxonomy, palaeontology, zooarchaeology, anthropology and ecology. In particular, the study of predator feeding habits from the analysis of prey hairs found in scats has been widely used for describing the diet of mammalian carnivores, because this technique is non-destructive and scats are easy to collect throughout the year. In southern Europe, several studies on the feeding habits of the wolf *Canis lupus* have been carried out during the last two decades

(cf. Meriggi & Lovari 1996, Ciucci & Boitani 1998a). Wild and domestic ungulates represent the main component of wolf diet in relation to their local abundance and/or local accessibility (Meriggi & Lovari 1996). Predation on livestock is the crucial socio-economic factor promoting wolf persecution (cf. Ciucci & Boitani 1998b, Boitani 2003). Therefore the knowledge of wolf diet plays a critical role in terms of conservation of one of the last large carnivores in southern Europe. An identification key to the hairs of wild and domestic ungu-

lates has a valid practical application in studies on wolf feeding habits.

Several atlases (Faliu et al. 1980, Debrot et al. 1982) and identification keys (Day 1966, Dziurdzik 1973, Keller 1978, 1980, 1981a, 1981b, 1992, Teerink 1991, Meyer et al. 2002) have been published on European mammal hairs. Only some of them deal with ungulates, but none includes all the species occurring in southern Europe. For example, the hairs of wild goat Capra aegagrus and southern chamois Rupicapra pyrenaica have not been described previously. The description of the hair structure of the different species is not always linked to dichotomous keys (Debrot et al. 1982, Faliu et al. 1980). Existing keys do not allow identification at the species level (Dziurdzik 1973, Meyer et al. 2002) or concern only a few species (Keller 1981b, Teerink 1991). Moreover the study of hair morphology of domestic ungulates (Abad 1955, Dziurdzik 1973, Faliu et al. 1980, Keller 1992) is not exhaustive. Only Keller (1992) compared wild equids with domestic forms. The effects of age and season on hair structure of ungulates have not been investigated in these studies. Last, but not least, each of these manuals is based on different techniques, characteristics and nomenclature. Since in many cases it is necessary to consult and compare several works at once, some confusion can arise during identification. Moreover, some of these studies require special techniques and equipment such as scanning electron microscopes and microtomes which, in turn, require skilled labour, thus slowing down the analysis.

The aims of our paper are 1) to integrate and extend the available data on hair morphology of wild ungulates occurring in southern Europe, 2) to present the first comparative analysis of hair structure of domestic forms, and 3) to provide an unambiguous discrimination tool between hairs of ungulates, based on a dichotomous key and a photographic reference system.

#### Material and methods

We studied the hair structure of 10 wild ungulate species: wild boar *Sus scrofa*, fallow deer *Dama dama*, red deer *Cervus elaphus*, roe deer *Capreolus capreolus*, southern chamois (Apennine population) *Rupicapra pyrenaica*, alpine chamois *R. rupicapra*, alpine ibex *Capra ibex*, Spanish ibex *Capra pyrenaica*, wild goat *Capra aegagrus* and mouflon *Ovis orientalis*. Animals sampled came from the Italian peninsula, Sardinia and Corsica, except for *Capra pyrenaica* (Spain) and *Capra aegagrus* (Montecristo Island and Crete). We compared the hair structure of these wild ungulates with that of 12

breeds and cross-breeds of five domestic ungulates (cow, sheep, goat, horse and donkey). Our identification key does not include ungulate species recently introduced in southern Europe which have only formed very small populations at geographically restricted ranges, such as e.g. axis deer Axis axis occurring in the Brijuni Islands, Croatia (Mitchell-Jones et al. 1999). The names of wild species follow Wilson & Reeder (1993), and the taxonomy has not been updated to be in line with the new accounts prepared for Wilson & Reeder (2005) to make easier the analysis of our review and the comparison with previous works. Terrestrial mammals are covered with two distinct types of hairs: long, thick, pigmented guard hairs, determining the general colour of the coat, and short, thin, less pigmented and more numerous fine hairs, supplying insulation. Only guard hairs are important in species identification as they exhibit diagnostically reliable features. Since the hair structure of fresh and tanned specimens is identical (Mayer 1952, Perrin & Campbell 1980, Hess et al. 1985), we collected hair tufts from live or freshly hunted animals, and dry skins housed in Italian mammal collections (Natural History Museum, Zoological Section, Florence; Italian Wildlife Institute, Bologna; Regional Museum of Natural Science, Chateau de Saint-Pierre). Hair samples were taken from 105 specimens (75 wild and 30 domestic). The hairs were collected from not less than five spots in the dorsolateral body region of each specimen. Hairs of other body regions generally show similar characteristics, but they are often less marked and thus hardly identifiable (Amerasinghe 1983, Teerink 1991). We did not include in this key specialised kinds of guard hairs, i.e. bristles. Previous studies reported that the first moult in young cervids and bovids of some European and North American species coincides with the development of adult hair characteristics (cf. Scott & Shackleton 1980, Kennedy & Carbyn 1981, Jedrzejewski et al. 1992, Gade-Jørgensen & Stagegaard 2000). As far as we know, no data are available on domestic ungulates. To study the effect of age on hair structure of wild and domestic ungulates, we defined two age classes: young (prior to the first moult) and adult. We sampled 28 young of known age (< 4 months for wild species, < 2 months for domestic forms) and 69 adults. To study the effect of season on hair structure of wild ungulates, we collected hair tufts from winter (N =25) and summer (N = 15) coats.

Pigmentation and hair dimension are variable characters and their usefulness in an identification key is correspondingly limited. They change with age, season and body region. Moreover, pigmentation can be deteriorated, e.g. under the action of the digestive enzymes, while hair dimension can be modified, e.g. because of frag-

mentation during digestion (Kennedy & Carbyn 1981, Amerasinghe 1983). Only hair profile and microstructure have diagnostic value and are species representative. The guard hairs of ungulates do not show any expanded and flattened regions (Kennedy & Carbyn 1981). We distinguished two general regions along the hair, each one corresponding to half a hair, i.e. the lower and upper shaft (Moore et al. 1974). Hair microstructure is described referring to these regions, firstly because microstructure changes along the hair (Wallis 1993, Oli 1993) and lastly because hairs can be fragmented and only lower or upper shafts can be examined.

Taking into account the nomenclature currently used to describe hair profile and microstructure (cf. Kennedy & Carbyn 1981, Teerink 1991), we selected only a few descriptive categories, easily identifiable without misunderstandings. We distinguished two types of hair profile: undulated and straight. The hair tip is described as split or not split. Hair microstructure is composed of three layers of keratin: medulla, cortex and cuticle, from the innermost to the outermost. The medulla is composed of loosely packed cells with air spaces in the cells themselves or between them. It is described (Fig. 1) by composition (unicellular irregular and multicellular), structure (amorphous, uniseriate, multiseriate, vacuolated, filled lattice and partially filled lattice), pattern (continuous and fragmental) and margin form (irregular, straight and scalloped). The cortex, composed of cells coalesced into a hyaline mass, does not exhibit characters which could be used as criteria for identification because of its nearly homogeneous structure. However, there is a considerable interspecific variation in the cortex width and thus we included this parameter in the key. The cortex width was estimated by eye, taking the width of the medulla as a reference unit. The cuticle con-

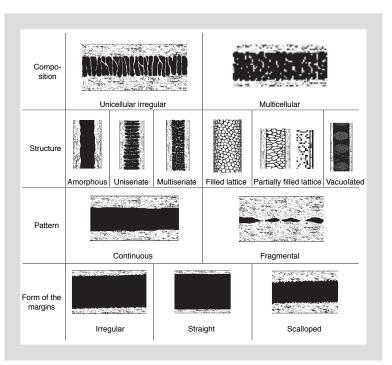


Figure 1. Medulla classification system used in our hair key to identify wild and domestic ungulates from southern Europe.

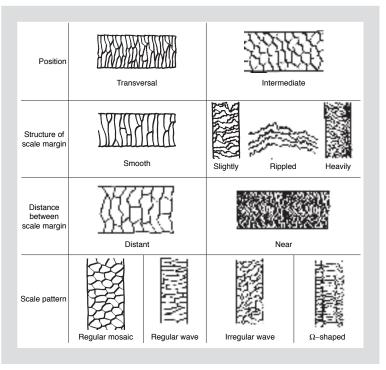


Figure 2. Cuticle classification system used in our hair key to identify wild and domestic ungulates from southern Europe.

sists of overlapping scales. We considered (Fig. 2) the position of the scales in relation to the longitudinal axis of the hair (transversal and intermediate), the structure of scale margins (smooth and rippled), the distance between scale margins (distant and near) and the scale pattern (regular mosaic, regular and irregular wave,  $'\Omega'$ -shaped).

Microscopic hair preparations were made following De Marinis & Agnelli (1993). Hair cross-sectioning is the most complicated step of the whole microscopic analysis. The shapes of cross-sections change along the hair and only the sequence of the shapes along the hair, rather than the shape at any particular point, is important for the identification (Teerink 1991). Furthermore, cross-sections of hairs of closely related species, such as those included in this key, appear very similar to each other, without diagnostic value (Kennedy & Carbyn 1981). Practicability is an important factor to take into account when selecting diagnostic criteria, thus hair cross-sections have not been included in this key. Microphotographs were taken using a light microscope fitted with a digital camera. Only microphotographs of representative and predominant patterns were included in the key in order to provide a valid help during the successive identification steps (see microphotographs in Appendix I).

The accuracy of the key developed in this study was assessed through a blind test on a sample of 260 hairs randomly taken from our reference collection. Four trained observers identified 65 samples each, to the level of species, age class and season.

#### Results and discussion

#### Macroscopic hair description

Among wild ungulates, only wild boar hairs can be surely identified without the aid of microscopic analysis. The hair of the wild boar can be easily identified by eye on the basis of the general appearance of the hair, which is split at least once. Piglets do not show split tips. However, they have bristly hairs which can be distinguished from those of other ungulates even if the observer is not highly skilled in hair identification (Table 1). Frayed

guard hairs also characterise domestic and feral swine and hybrids (Marchinton et al. 1974, Hess et al. 1985, Mayer & Brisbin 1991). Boar subspecies and other Suidae species have bristles with typically frayed ends (Koppiker & Sabnis 1977, Amerasinghe 1983, Hess et al. 1985). Moreover, the Tayassuidae species, ecological equivalents of suids in the New World, have guard hairs split at the tip (Hess et al. 1985). Degree of fraying might be correlated with chronology of hair replacement (Hess et al. 1985). The adaptive significance of this character of hair morphology is not known. Adults of cervids and wild bovids can be macroscopically distinguished from domestic forms because of their shiny appearance and undulated profile (see Table 1). Hairs of young other than sheep can be distinguished from those of adults because they are thin and straight, in wild and domestic forms as well (see Table 1). However, young of wild species have a shiny appearance as do adults. Adults and young of sheep show undulated and dull hairs (see Table 1). The macroscopic analysis of the guard hairs represents only the first step in the identification process of an unknown hair sample. Moreover, the macroscopic analysis can hardly be carried out when examining fragmented hairs, such as those found in carnivore scats.

#### Microscopic hair description

#### Medulla features

We identified two different medullary structures in wild ungulates: amorphous in wild boar and lattice in the others (Table 2). We did not find the medullary lattice structure in wild boar hairs, as previously reported by Faliu et al. (1980) and Keller (1981b). This structure has been observed by Amerasinghe (1983) only in the bristles of the mid-dorsal line of the wild pig in Sri Lanka, however, it was much reduced and fragmental. The medulla structure per species is constant throughout the hair length, and it does not change with age or season. A strong structural homogeneity in medullary pattern can be observed in the whole Cervidae family throughout the world. A review of 21 studies carried out on the microscopic features of deer hairs revealed that all the species have a medullary structure which is filled lattice (Appendix II). As far as it is currently known, a similar struc-

Table 1. Macroscopic characters distinguishing hairs of wild and domestic ungulates.

Ungulate	Family	Age class	Profile	General appearance	Tip
Wild	Suidae	Adult and piglet	Straight	Bristly	Split, except in piglet
	Cervidae/Bovidae	Adult	Undulated	Thick and shiny	Not split
	Cervidae/Bovidae	Young	Straight	Thin and shiny	Not split
Domestic	Bovidae/Equidae	Adult and young	Straight (undulated in sheep)	Thick (thin in young) and dull	Not split

Table 2. Medulla features of wild ungulates according to age class.

		Medulla						
Age class	Taxon	Composition	Structure	Pattern	Form of the margins	Cortex width		
Adult	Sus scrofa	Cells not clearly visible	Amorphous	Continuous, fragmental at the base	Irregular, especially in the upper shaft	Wider than medulla in the lower shaft, more and more narrow in the upper shaft		
	Dama dama Cervus elaphus Capreolus capreolus	Multicellular	Filled lattice with	Continuous, suddenly interrupted at the base	Medulla fills the entire	Very narrow or not visible		
	Rupicapra pyrenaica R. rupicapra Ovis orientalis	Municentiar	polygonal cells	Continuous, fragmental at the base	width of the hair			
	Capra ibex C. pyrenaica C. aegagrus	Multicellular	Partially filled lattice with elongated cells	Continuous, fragmental at the base	Scalloped	Clearly visible		
Young	Sus scrofa		As in		As in the adult			
	Dama dama Cervus elaphus Capreolus capreolus Rupicapra pyrenaica R. rupicapra Ovis orientalis	Multicellular	Partially filled lattice with polygonal cells	Continuous, fragmental at the base	Scalloped	Clearly visible		
	Capra ibex C. pyrenaica C. aegagrus		As in		As in the adult			

tural homogeneity is found in bovids but only at the subfamily level. For example, re-examining 17 studies on the hairs of Caprinae and Bovinae, the former shows a medullary lattice structure while the latter presents a multiseriate structure (Appendix III). Different patterns of medullary cells might express the evolutionary history of the species (Sheng et al. 1993, Chernova 2001), although ecological factors almost certainly play an important role in determining hair morphology (*cf.* Perrin & Campbell 1980).

The domestic ungulates showed multiseriate and uniseriate medullary structures (Table 3). Sheep and goats have a medullary structure that differs from that of their relative wild ancestors, i.e. *Ovis orientalis* and *Capra aegagrus*, respectively. During the domestication process important structural modifications in the architectonics of medulla were induced by artificial selection as

affected by economic, cultural and aesthetic reasons (Clutton-Brock 1999, Meyer et al. 2002). These variations are especially prominent in mammals that have undergone a long domestication process, such as sheep and goats. Differences in hair structure are less obvious in species that have been bred less intensively (Meyer et al. 2002). Our hair samples of Ovis orientalis and Capra aegagrus came from animals living respectively on Corsica and Sardinia, and on Montecristo and Crete. Generally speaking, Mediterranean islands often represent natural enclosures where goats and sheep have been kept and bred since prehistory in a free ranging state (Masseti 1998). Therefore our samples are not representative of the true wild ancestor of sheep and goats; they come from relic populations of animals that were introduced on the Mediterranean islands and now live as wild populations (Clutton-Brock 1999, Masseti 2002). Never-

Table 3. Medulla features of young and adult domestic ungulates.

Medulla							
Domestic form	Composition	Structure	Pattern	Form of the margins	Cortex width		
Cow	Multicellular	Multiseriate, appearing vacuolated	Continuous	Straight or irregular	Wide as medulla or ½,⅓ of medulla		
Goat	Multicellular and unicellular irregular	Multiseriate and uniseriate	Continuous or fragmental	Scalloped and irregular	Very narrow but well recognisable if medulla is multicellular, wider than medulla if medulla is unicellular		
Sheep	Multicellular	Multiseriate	Continuous	Scalloped	Very narrow, but well recognisable		
Horse	Multicellular and unicellular irregular	Multiseriate and uniseriate	Continuous or fragmental	Scalloped and irregular	½ of medulla if medulla is multicellular, wider than medulla if medulla is unicellular		
Donkey	Multicellular	Multiseriate	Continuous	Straight and irregular	Very narrow, but well recognisable		

Table 4. Cuticle features of wild ungulates according to age class and season.

				Lowe	r shaft			Upper	shaft	
Age class	Taxon	Season	Scale position	Scale margin	Scale mar- gin distance	Scale pattern	Scale position	Scale margin	Scale mar- gin distance	Scale pattern
Adult	Sus scrofa		Transversal	Smooth	Near	Regular wave	Transversal	Heavily rippled	Near	Regular wave
	Cervus elaphus	Winter	Transversal	Smooth	Distant	Regular wave	Transversal	Generally smooth	Distant	Regular wave
	etaphus	Summer						Generally rippled	Distant or near	
	Capreolus capreolus	Winter	Intermediate	Smooth	Distant	Regular mosaic	Transversal	Slightly rippled	Distant	Regular wave
		Summer	Transversal			Regular wave				
	Dama dama	Winter	Transversal	Smooth	Distant	Regular	Transversal	Generally slightly rippled	Distant	Regular wave
		Summer				wave		Slightly or heavily rippled	Distant or near	
	Rupicapra pyre R. rupicapra Capra ibex	naica	Transversal	Smooth	Distant	Irregular wave	Transversal	Generally rippled	Distant	Regular wave
	C. aegagrus C. pyrenaica		Transversal	Smooth	Distant	Irregular wave	Transversal	Generally rippled	Distant	Regular wave and 'Ω'-shaped
	Ovis orientalis		Intermediate	Smooth	Distant	Regular mosaic	Transversal	Generally rippled	Distant	Regular wave
<b>V</b>	Sus scrofa		Transversal	Smooth	Distant	Regular wave	Transversal	Rippled	Distant	Regular wave
Young	Cervidae Bovidae		Transversal	Smooth	Distant	Irregular wave	Transversal	Smooth	Distant	Irregular wave

theless, the medullary structures are strongly different between these 'wild' forms and their domestic counterparts, because domesticated animals that return to the wild, will usually revert by natural selection to a physical form that is closer to the wild species (Clutton-Brock 1999). In fact *Capra aegagrus* and *Ovis orientalis* kept the identical medullary structure of the relative species of the same genus (see Table 2) which have never been domesticated (Clutton-Brock 1999).

#### Cuticle features

We did not observe a clear differentiation in the cuticle features between domestic and wild ungulates, as found for the medulla (Tables 4 and 5). The cuticle was a useful tool to discriminate species among wild ungulates and to distinguish young from adult and winter from summer coat in deer (see Table 4). Meyer et al. (2001) already differentiated three subfamilies within Cervidae in a comparative structural analysis of hair cuticular pat-

tern. In their study, the subgroup differentiation was obtained by calculating quantitative parameters such as the ratio of scale width to scale height. These groupings corroborated the zoosystematical relationships that have been achieved using modern molecular genetic techniques.

We could not use cuticle to distinguish domestic ungulates from each other, because of high overlap among cuticular patterns (see Table 5). Domestication induced changes in several cuticular features of the hair shaft, and the resulting structural homogeneity in the cuticle compromises species identification (Meyer et al. 1997, 2000). Sheep have a cuticular structure different from their relative wild ancestors, i.e. *Ovis orientalis*, while goats do not show clear differences in the cuticle from their relative wild ancestors, i.e. *Capra aegagrus* (see Tables 4 and 5). According to Meyer et al. (2001) there is a relationship between the cuticle scale parameters and the coat structure and function. Therefore, the strong arti-

Table 5. Cuticle features of domestic ungulates.

			Lower shat	ìt	Upper shaft				
Domestic form	Scale position	Scale margin	Scale margin distance	Scale pattern	Scale position	Scale margin	Scale margin distance	Scale pattern	
Cow	Transversal	Smooth	Distant	Regular and irregular wave	Transversal	Smooth and rippled	Distant	Regular wave	
Goat	Transversal	Smooth	Distant	Irregular wave	Transversal	Smooth and rippled	Distant and near	Regular wave and $'\Omega'$ -shaped	
Sheep	Transversal	Smooth	Distant	Irregular wave	Transversal	Generally rippled	Distant	Irregular wave and $'\Omega'$ -shaped	
Horse	Transversal	Smooth	Distant	Irregular wave	Transversal	Generally smooth	Distant	Regular wave	
Donkey	Transversal	Smooth	Distant	Regular wave	Transversal	Rippled	Near	Regular wave	

Table 6. Key to identifying wild and domestic ungulates from southern Europe based on hair samples. The figure numbers given below refer to Fig. 1 - Fig. 24 in Appendix I.

1.	Hairs bristly, split at tip usually more than once Sus scrofa (adult)  Hairs other than above
2.	Medullary structure amorphous (Fig. 2). Sus scrofa (piglet) Medullary structure other than above. 3
3.	Medullary structure lattice (Figs. 3 and 4).4Medullary structure other than above.5
4.	Medullary cells polygonal, practically filling the entire width of the hair so that the cortex is very narrow or not visible (filled structure) (Fig. 3)
5.	Medullary structure vacuolated (Fig. 5)       cow         Medullary structure other than above (Figs. 6, 7, 8 and 9)       .8
6.	Basal part of the hair wine-glass shaped (Fig. 10)
7.	Cuticular scale pattern irregular wave and scale margins smooth throughout the hair length (Fig. 12)
8.	Medullary composition only multicellular (Figs. 6, 7 and 8)       12         Medullary composition multicellular and unicellular irregular (Fig. 9)       13
9.	Cuticular scale pattern regular mosaic in the basal part of the lower shaft of the hair (Fig. 13)
10.	Cuticular scale pattern regular mosaic in the basal part of the lower shaft of the hair (Fig. 15).  Cuticular scale pattern irregular wave throughout the lowershaft of the hair (Fig. 16).  Rupicapra sp.
11.	Cuticular scale pattern generally regular wave in the upper shaft of the hair (Fig. 17)
12.	Medulla margins scalloped (Fig. 6)       sheep         Medulla margins straight and irregular (Fig. 7)       donkey
13.	Very narrow cortex, where the medulla has a multicellular composition (Fig. 6); cuticular scale pattern       'Ω'-shaped in some tracts of the upper shaft of the hair (Fig. 19).       goat         Cortex ½ of medulla, where the medulla has a multicellular composition (Fig. 8); cuticular scale pattern other than above       horse
14.	Cuticular scale margins generally smooth in the upper shaft of the hair (Fig. 20). Cervus elaphus (winter) Cuticular scale margins generally rippled in the upper shaft of the hair (Fig. 21)
15.	Cuticular scales margins also heavily rippled and near in the upper shaft of the hair (Fig. 22)

ficial selection for wool production may have transformed the hair structure of the sheep but not that of the goat.

#### Hair identification key

Our key (Table 6) is based on three main steps: 1) macroscopic analysis permitting the separation of adult wild boar from all other ungulates; 2) analysis of medulla and cortex features allowing the separation between wild species and domestic forms, and the identification of domestic ungulates; 3) analysis of cuticular features in wild ungulates leading to the determination of age class and species, and the distinction between winter and summer coats.

Keller (1981b) separated deer from wild bovids on the basis of cross-sections. We distinguished these *taxa* by the shape of the basal part of the hair (see Appendix I, App. Figs. 10 and 11), without hair cross-sectioning, which is a complicated and time consuming step of the laboratory procedures.

In a few cases it was not possible to identify to species level. The cuticular features of the species belonging to the genus *Rupicapra* are practically identical. Difficulty

was encountered in differentiating the species of the genus *Capra*; only *Capra ibex* can be separated on the basis of the scale pattern of the upper shaft. However, the range of these species does not overlap at all in southern Europe (Mitchell-Jones et al. 1999).

The variability observed in the hair cuticular features of the winter and summer coats of deer makes the species identification more complicated. The species can be separated from each other only when comparing winter hairs. In summer, the differences between species are less pronounced, and only fallow deer hairs are recognisable. 'V' shaped incisions in the cuticular pattern of the upper shaft have been reported as a diagnostic feature to distinguish roe deer from other deer (Lomuller 1924, Keller 1981b, Teerink 1991). We frequently observed these 'V' shaped incisions in almost all wild and domestic ungulates (see Appendix I, App. Fig. 1A), so they should not be considered a reliable character for hair identification.

We observed cylindrical root shape (see Appendix I, App. Fig. 23) in horses and donkeys (90 and 82.6% respectively, N = 53), and conical root shape (see Ap-

pendix I, App. Fig. 24) in sheep and goats (90.5 and 100%, respectively, N = 53). It is not common to find hairs with roots in the scats; when it happens, the root shape can help in hair identification of goats and horses (see point 13 of the key in Table 6).

Young of deer and wild bovids can be distinguished from adults because they have an irregular wave cuticular scale pattern throughout the hair length (see Table 4). All the hair samples of young animals that we examined showed this feature from birth until 3-4 months of age. Young change their birth coat into a coat with hairs similar to those of adults during their first moult (*cf.* Ryder 1960, Johnson & Hornby 1980, Jedrzejewski et al. 1992). Young of the domestic forms showed the same microscopic hair features as adults.

We did not observe relevant differences within the breeds and cross-breeds examined.

Macroscopic and microscopic features used in this key allowed a correct identification with an accuracy that averaged  $97.7 \pm 0.9\%$  at the species level (in wild ungulates: 98.1%, N = 157; in domestic ungulates: 99%, N = 98), 100% (N = 154) at the age class level and 96.6% (N = 29) in the recognition of winter and summer coat of deer. The misidentifications concerned mostly (50%) the genus Capra. Generally, the mistakes occurred in the identification of the  $'\Omega'$ -shaped cuticular pattern, the distinction between slightly and heavily rippled cuticular patterns and the recognition of the partially filled structure of medulla. The experience of the observer affected the correct identification of the cuticular patterns. The possible degradation of strongly pigmented hairs, occurring during the depigmentation process, may lead to the misidentification of the medullary structure.

#### Conclusion

Our dichotomous key allows hair identification based on characters which are clearly recognisable, considering the effects of age and season on hair structure. The techniques used in our study can be easily, quickly and economically applied in routine investigations keeping the time required to identify a sample at a minimum, but yielding accurate identifications.

However, some suggestions have to be kept in mind before using our key:

- Prepare a reference collection including samples of all the species that are going to be investigated. This is particularly recommended when examining domestic ungulates.
- Work preliminarily with known samples taken from

- the reference collection until good confidence with the identification criteria is gained. Use blind tests to determine proficiency of identification.
- Analyse several tufts of hairs of the same individual because of the considerable variety of hair types encountered in different body regions and even within the same body region (see Appendix I, Fig. 1). Nonetheless no general statement can be made on the number of hairs that constitute an adequate sample for identification (Mayer 1952, Day 1966, Meyer et al. 2002).
- Compare always a set of features and do not rely on a single character, considering the appreciable degree of interspecies overlap in certain characteristics.

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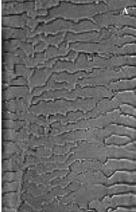
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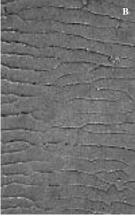
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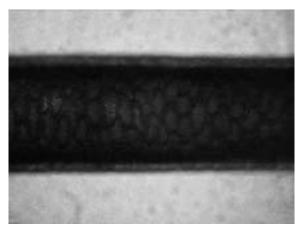
### Appendix I

Microphotographs of representative and predominant patterns used in our hair key to identify wild and domestic ungulates from southern Europe.

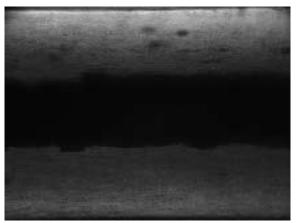




Appendix Figure 1. *Cervus elaphus* (adult), variation of cuticular scale pattern in the upper shaft of the hair in the same individual (400x); 'V' shaped incisions are visible in A.



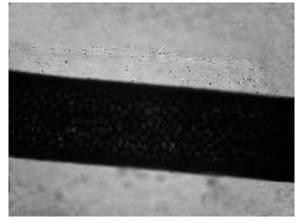
Appendix Figure 4. Capra ibex (young), medulla; upper shaft of the hair (400x).



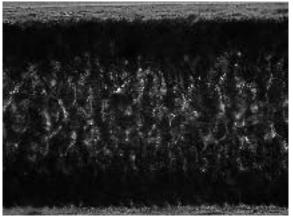
Appendix Figure 2. Sus scrofa (piglet), medulla; upper shaft of the hair (400x).



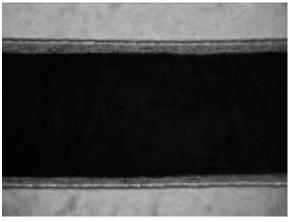
Appendix Figure 5. Cow (adult), medulla; lower shaft of the hair (400x).



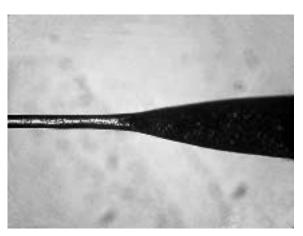
Appendix Figure 3. Cervus elaphus (adult), medulla; upper shaft of the hair (100x).



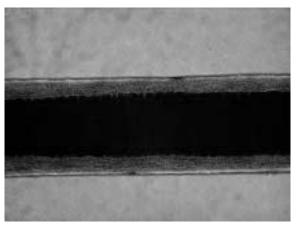
Appendix Figure 6. Goat (adult), medulla; upper shaft of the hair (400x).



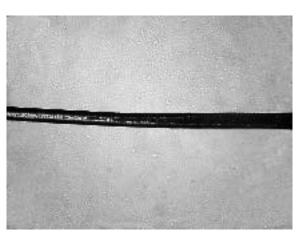
Appendix Figure 7. Donkey (adult), medulla; upper shaft of the hair (400x).



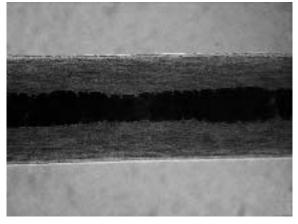
Appendix Figure 10. Capreolus capreolus (adult); basal part of the hair (100x).



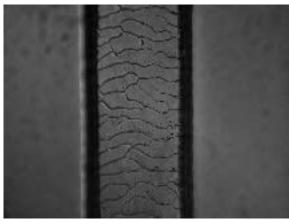
Appendix Figure 8. Horse (adult), medulla; upper shaft of the hair (400x).



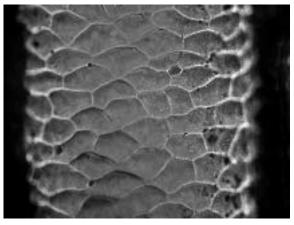
Appendix Figure 11. Rupicapra pyrenaica (adult); basal part of the hair (100x).



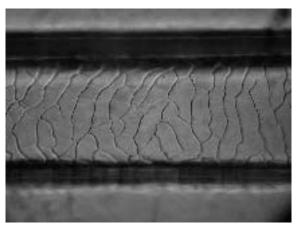
Appendix Figure 9. Horse (adult), medulla; upper shaft of the hair (400x).



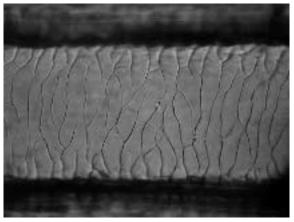
Appendix Figure 12. Capreolus (young); cuticle, upper shaft of the hair (400x).



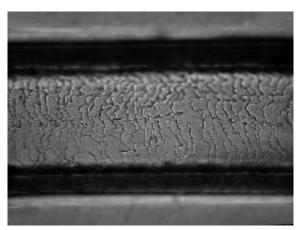
Appendix Figure 13. Capreolus capreolus (adult, winter); cuticle, lower shaft of the hair (400x).



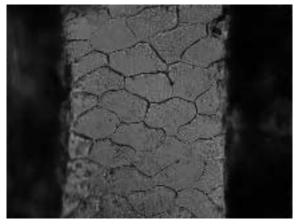
Appendix Figure 16.  $Rupicapra\ pyrenaica$  (adult); cuticle, lower shaft of the hair (400x).



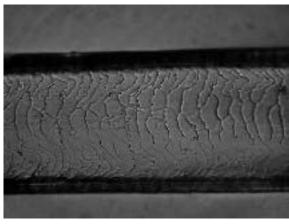
Appendix Figure 14. Dama dama (adult), cuticle; lower shaft of the hair (400x).



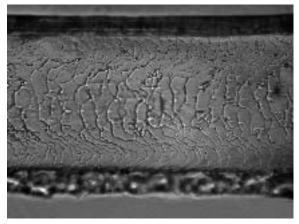
Appendix Figure 17.  $Capra\ ibex$  (adult), cuticle; upper shaft of the hair (400x).



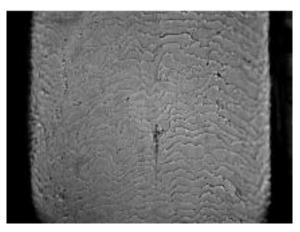
Appendix Figure 15. Ovis orientalis (adult), cuticle; lower shaft of the hair (400x).



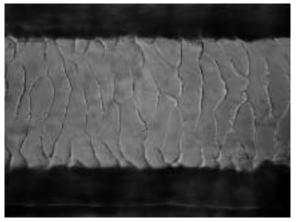
Appendix Figure 18. Capra aegagrus (adult); cuticle, upper shaft of the hair (400x).



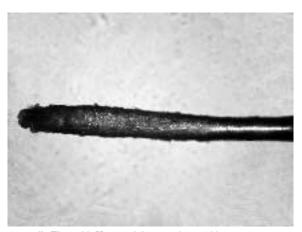
Appendix Figure 19. Goat (adult), cuticle; upper shaft of the hair (400x).



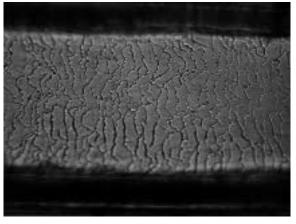
Appendix Figure 22. *Dama dama* (adult, summer); cuticle, upper shaft of the hair (400x).



Appendix Figure 20. *Cervus elaphus* (adult, winter); cuticle, upper shaft of the hair (400x).



Appendix Figure 23. Horse (adult); root shape (100x).



Appendix Figure 21.  $Dama\ dama$  (adult), cuticle; upper shaft of the hair (400x).



Appendix Figure 24. Goat (adult); root shape (100x).

## Appendix II

Review of 21 studies on medullary structure of Cervidae. All the species listed have a filled lattice structure. Classification according to Wilson & Reeder (1993).

Subfamily	Species	Reference			
Cervinae	Axis axis	Lomuller (1924), Amerasinghe (1983), Chehébar & Martin (1989), Mukherjee et al. (1994)			
	A. porcinus	Amerasinghe (1983), Sheng et al. (1993)			
	Cervus albirostris	Sheng et al. 1993			
	C. elaphus	Present study, Lomuller (1924), Jullien (1930), Lochte (1938), Mayer (1952), Dziurdzik (1973), Moore et al. (1974), Kennedy & Carbyn (1981), Keller (1981b), Debrot et al. (1982), Chehébar & Martin (1989), Teerink (1991), Sheng et al. (1993), Meyer et al. (2002)			
	C. eldii	Sheng et al. (1993)			
	C. nippon	Sheng et al. (1993), Meyer et al. (2002)			
	C. unicolor	Koppiker & Sabnis (1976), Amerasinghe 1983, Sheng et al. (1993), Mukherjee et al. (1994)			
	Dama dama	Present study, Jullien (1930), Lochte (1938), Dziurdzik (1973), Debrot et al. (1982), Chehébar & Martin (1989), Teerink (1991), Meyer et al. (2002)			
	Elaphurus davidianus	Sheng et al. (1993)			
Hydropotinae	Hydropotes inermis	Sheng et al. (1993)			
Muntiacinae	Elaphodus cephalophus	Sheng et al. (1993)			
	Muntiacus crinifrons	Sheng et al. (1993)			
	M. feae	Sheng et al. (1993)			
	M. muntjak	Amerasinghe (1983), Sheng et al. (1993)			
	M. reevesi	Sheng et al. 1993)			
Capreolinae	Alces alces	Jullien (1930), Lochte (1938), Dziurdzik 1973, Moore et al. (1974), Kennedy & Carbyn (1981), Debrot et al. (1982), Sheng et al. (1993), Wallis (1993), Meyer et al. (2002)			
	Capreolus capreolus	Present study, Lomuller (1924), Jullien (1930), Lochte (1938), Dziurdzik (1973), Faliu et al. (1980), Keller (1981b), Debrot et al. (1982), Teerink (1991), Sheng et al. (1993), Meyer et al. (2002)			
	Hippocamelus antisensis	Vazquez et al. (2000)			
	H. bisulcus	Chehébar & Martin (1989)			
	Mazama americana	Vazquez et al. (2000)			
	M. guazoubira	Vazquez et al. (2000)			
	Odocoileus hemionus	Mayer (1952), Moore et al. (1974), Kennedy & Carbyn (1981)			
	O. virginianus	Mayer (1952), Moore et al. (1974), Kennedy & Carbyn (1981), Tumlison (1983), Wallis (1993)			
	Pudu puda	Chehebar & Martin (1989), Feder & Arias (1992)			
	Rangifer tarandus	Lomuller (1924), Jullien (1930), Kennedy & Carbyn (1981), Debrot et al. (1982), Sheng et al. (1993)			

## **Appendix III**

Review of 17 studies on medullary structure of Caprinae and Bovinae. All the species listed have a structure which is either lattice (Caprinae) or multiseriate (Bovinae). Classification according to Wilson & Reeder (1993).

Subfamily	Species	Reference			
Caprinae	Capra aegagrus	Present study			
	C. caucasica	Jullien (1930)			
	C. ibex	Present study, Lomuller (1924), Jullien (1930), Mandelli (1960),			
		Couturier (1962), Keller (1981b), Debrot et al. (1982), Meyer et al.			
		(2002)			
	C. pyrenaica	Present study, Jullien (1930)			
	Hemitragus jemlahicus	Oli (1993)			
	Oreamnos americanus	Jullien (1930), Moore et al. (1974), Kennedy & Carbyn (1981)			
	Ovis canadensis	Mayer (1952), Moore et al. (1974), Kennedy & Carbyn (1981)			
	O. dalli	Kennedy & Carbyn (1981)			
	O. orientalis	Present study, Lomuller (1924), Jullien (1930), Lochte (1938),			
		Dziurdzik (1973), Debrot et al. (1982), Teerink (1991), Meyer et al.			
		(2002)			
	Pseudois nayaur	Oli (1993)			
	Rupicapra rupicapra	Present study, Jullien (1930), Lochte (1938), Couturier (1938),			
		Mandelli (1960), Dziurdzik (1973), Faliu et al. (1980), Keller			
		(1981b), Meyer et al. (2002)			
	R. pyrenaica	Present study			
Bovinae	Bison bison	Mayer (1952), Moore et al. (1974), Kennedy & Carbyn (1981)			
	B. bonasus	Dziurdzik (1978), Meyer et al. (2002)			
	Boselaphus tragocamelus	Mukherjee et al. (1994)			
	Tetracerus quadricornis	Mukherjee et al. (1994)			