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New knowledge tools for crested porcupine (*Hystrix cristata* L., 1758) management in the wild

First census model, new behavioural ecology aspects and preliminary investigation on health status

Dottoranda

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Man is the most insane species. He worships an invisible God and destroys a visible Nature. Unaware that this Nature he's destroying is this God he's worshiping.

L'uomo è la specie più folle: venera un Dio invisibile e distrugge una Natura visibile. Senza rendersi conto che la Natura che sta distruggendo è quel Dio che sta venerando.

(Hubert Reeves)

Summary

The crested porcupine (*Hystrix cristata*) is a native African rodent and among European country is present as naturalized specie only in Italy.

In Italy the crested porcupine is a widespread species with a population trend that is in expansion. The porcupine is able to adapt itself to different habitat type where vegetation offers adequate woody and/or bushes covered and food availability. However, habitat diversity and environmental fragmentation are a key factor for the presence of this rodent. In the Italian agro-ecosystem the crested porcupine benefit from the increase in the production of agricultural crops leading to an increase of porcupine-human conflict. The crested porcupine is indeed considered a pest species due to the agricultural crop demage as well as damage performed to manmade riverbanks in which dig its burrows. For these reasons and also for its tasty meat the poaching of this rodent is widely diffused in Italy.

The crested porcupine is strictly protected by European Law with the Bern Convention of 1979 and by Italian Law since 1981 with the National Law n. 503 of 1981 and it is included in the IUCN Red List of threatened species among the “Least concern” species.

To date in Italy, despite the strict protectionist regime and the high conservation interest of the crested porcupine, there are not tools and plain for porcupine management. No data are indeed available concerning the density and abundance of the Italian porcupine population and no census method exist for this species. Moreover, very few knowledge are available on behavioural ecology and there is no data concerning the health status of porcupine population.

In this study, between 2016 and 2019, new behavioural ecology and health aspects of a porcupine population were investigated in two experimental areas in order to contribute to new knowledge tools potentially useful for the management and conservation of the species.

In both study areas geolocation and distribution analysis of ground entrance holes and settlements were performed. Among the geolocated settlements, 16 in the experimental area 1 (EA1) and 8 in the experimental area (EA2) were chosen as experimental settlements. All the experimental settlements were continuously monitored by camera-trapping in order to investigate the spatio-temporal use of settlements by porcupine and detect and describe new behavioural ecology aspects of this rodent.

Moreover, in order to better understand the spatio-temporal use of settlements porcupine capture-marking campaigns were performed. Each captured porcupine was individually marked in order to make it recognisable in the camera traps videos.

Biological samples (e.g. feaces, blood, kidney and intestine) from captured and road killed porcupines were collected for health status investigations. Faecal samples for parasitological analysis were also collected along transects.

The ground holes geolocated resulted clustered in well defined and circumscribable settlements with very variable sizes ranged from 2 to 20 ground holes. The settlements occurrence was mainly recorded in woody areas within 100 meters from feeding areas, showing a marked preference of the porcupines for ecotonal zones. The settlements are mostly distributed in binary systems called “stations”. Each station is characterized by the presence of two settlements near one to each other no more than 250 meters, opposite oriented and connected on the surface by a dense network of pathways. The settlements show a preferred orientation trend throughout the North-west and South-east axis. The Manly index shows a positive selection for South-east oriented ridges, a negative selection for those to North-east and absence of selection for North-west and South-west sector.

The crested porcupines resulted to be the main and faithful inhabitant of settlements and occasionally can co-habit with badger. The settlements inhabitation frequency by the porcupines on the total monitored settlements was about 70%.

Each settlement was always inhabited by only one porcupine family group ranged from 2 to 6 individuals.

Each porcupine family group always alternatively and complementarly inhabited two settlements belonging to a station with a different inhabitation pattern. The inhabitation of the two settlements resulted not season dependent while South-west and North-west oriented settlements were most frequently inhabited. New investigations concerning the factors influencing the use of settlements by the porcupine are neccessary.

Data obtained concerning the settlements inhabitation frequency and the spatio-temporal inhabitation of settlements by porcupines allowed us to elaborate for the first time the estimation of Porcupine Population Abundance (PPA) and a preliminary census model. The density of porcupine in the two experimental areas resulted to be 3.9 individuals/km^2 in EA1 and 3.6 individuals/km^2 in EA2.

The videos recorded by the camera-traps allowed us to observe and describe new behavioural ecology aspects of crested porcupine such as the reproduction behaviour in the wild and the pattern of first emerging time from burrow during the year. Moreover, two peculiar behaviours, the “sunbathing” and the scavenging on carcasses, are here describe for the first time.

Parasitological analysis allowed us to identified six different parasites: *Cryptosporidium* sp., *Trichuris* spp., *Capillaria* sp., gastrointestinal strongyles, coccidian oocysts and *Giardia duodenalis* assemblage *B*. Serological positivity for Leptospira with a prevalence of 58% (7 out of 12) was found and for the first time the isolation of *Leptospira interrogans* serogroup Pomona serovar Pomona on a road-killed porcupette was performed. In addition preliminary porcupine haematological and biochemical values were obtained.

The results obtained in this investigation provide a new set of knowledge tools on crested porcupine biology that could be usefull for the management of this precious rodent of the Italian fauna. Further investigations concerning some of the new behavioural ecology aspects observed in this study and on the health status of porcupines are desiderable.

1.Introduction

The Old World Porcupines

The Old World Porcupines belong to the order of Rodents, suborder Hystricomorpha or Hystricognata, superfamily Hystricoidea, family Hystricidae. The suborder Hystricomorpha or Hystricognata is assigned depending on whether zygomasseteric structure (anatomical arrangement of the masseter muscle of the jaw and the zygomatic arch of the skull) or lower jaw structure (*processus condylaris*) is respectively considered (Wood 1985). The superfamily Hystricoidea also includes the family of Erethizontidae called New World Porcupines (Dieterlen et al. 1974). The family of Hystricidae include three, all existing, genera: *Trichys*, *Atherurus* and *Hystrix* which are distinguished by different degrees of specialisation (Van Weers 1979, 1983, 2005; Jori et al. 1998, Rovi-Ryan et al. 2017).

The genus *Trichys*, long-tailed porcupine, is a monotypic genus with only one species (*T. fasciculata*) (Fig 1A). It is the least specialised genus and the only one that is limited to Southeast Asia (Malaya, Sumatra and Borneo) (Van Weers 1976, 1983, 2005). The porcupines of genus *Trichys* are characterized by short spines, long tactile bristle and long tail with bristles from 5.5 to 22cm and without brush (Dieterlen et al. 1974, Van Weers 1976, 1983).

The genus *Atherurus*, brush-tailed porcupine (Fig 1B), occurring in Africa as *Atherurus africanus* and is represented in Southeast Asia with *Atherurus macrourus* (Jori et al. 1998). The porcupines of this genus have the skin fully covered by long spines and a typical long tail-brush (up to 26 cm) covered with a mixture of hair and quills. The base of the tail is covered with a mixture of hair and quills, the middle part is scaly, hairless and it ends with a tuft of white bristles with the shape of a brush (Van Weers 1983, Jori et al. 1998). The *A. africanus* also present a small number of quills at the hind part of the back (Van Weers 1983).

Figure 1. (A) the long-tailed porcupine (*T. fasciculate*) and (B) the brush-tailed porcupine (*A. africanus*) (Photos by Klaus Rudloff)

The genus *Hystrix*, short-tailed porcupine, is the most specialised genus and includes three subgenera: *Acanthion*, *Thecurus* and *Hystrix* (Van Weers 2005) (Fig 2). The species of genus *Hystrix* present the body covered by quills that may be different in length and colouration and a short tail with rattle-quills. In most *Hystrix* species crest is also present (Van Weers 1976, 1983).

Figure 2. Porcupines of genus *Hystrix*: (A) porcupine of subgenus *Acanthion* (*H. (A.) brachyura*) (Photo by Sean Crane), (B) porcupine of subgenus *Thecurus* (*H. (T.) crassispinis*) (Photo by Animal Database) and (C) porcupine of subgenus *Hystrix* (*H. cristata*) (Photo by Felicioli)

The subgenus *Acanthion* occurs in Asia and includes two species, *H. (A.) brachyura* and *H. (A.) javanica*. In *H. (A.) brachyura* the subspecies *H. (A.) b. Brachyuran*, *H. (A.) b. subcristata* and *H. (A.) b. hodgsoni* are distinguished (Van Weers 1979, 2005).

The subgenus *Thecurus* also occur in Asia and includes three species: *H. (T.) sumatrae*, *H. (T.) crassispinis* and *H. (T.) pumila* (Van Weers 2005). However, study performed by Rovie-Ryan et al. (2017) suggests that *H. (T.) crassispinis* should be classified as *Hystrix crassispinis*.

The subgenus *Hystrix* is represented by three species *H. (H) indica* in Asia, *H. (H) africaeaustralis* in Africa and *H. (H) cristata* in Africa and Italy (Corbet and Jones 1964, Van Weers 1983, 2005).

Morphology and distribution of porcupines of subgenus *Hystrix*

The porcupines of the subgenus *Hystrix* are medium-sized rodents with a stocky and muscular body. The limbs are short and covered with bristles. The hand has four fingers with strong nails and the thumb is atrophied. The foot is longer than the hand with a developed big toe. Both hand and foot have a typical aspect of plantigrades (Toschi 1965) (Fig 3). The head is globular and stocky with skull convex and very elongated and enlarged nasal bones (Toschi 1965) (Fig 4). The nose is very large, S-shaped and with black whiskers (Dieterlen et al. 1974).

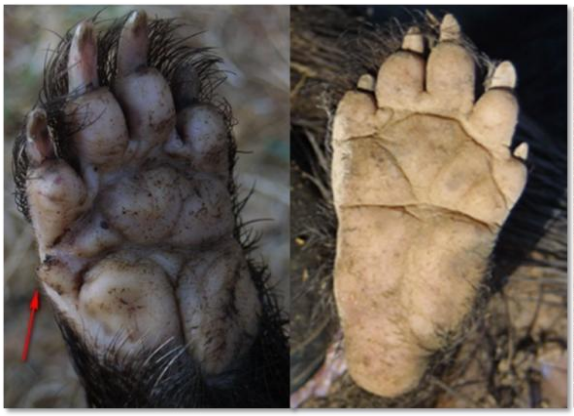

Figure 3. Hand (left) and foot (right) of porcupine, the atrophied thumb in the hand is visible (Photo by Felicioli)

Figure 4. The ear (left) and the nose (right) of porcupine (Photo by Felicioli).

The eyes are side, small, dark and have no eyelid (Tinelli and Tinelli 1983). The ear is characterized by a small but well-structured auricle (Felicioli 1991) (Fig 4). The incisor teeth (N=4) are continuously growing, robust and curved. The lower incisors are longer and less curved (Fig 5). Molars (N=6) and premolars (N=4) very similar to each other with an elliptical crown (Toschi 1965, Dieterlen et al. 1974, Angelici and Luiselli 1999). The body length ranged from 60 to 90 cm, including tail (Kingdon 1974, Van Weers 1983). The adult body weight is up to 18-20 Kg with a mean of 15 Kg (Santini 1980, Pigozzi 1987, Corbet and Van Aarde 1996). The sexual dimorphism in porcupines is still debated. Investigation performed by Pigozzi (1987) on *H. cristata*, by Alkon and Saltz (1985a) and Tohmè and Tohmè (1980) on *H. indica* and by Gaigher and Currie (1979) on *H. africaeaustralis* reported weight and size significantly greater in the female than in the male. Conversely Lovari et al. (2013a) and Mori and Lovari (2014) deny the presence of differences either between male and female adult body sizes of *H. cristata* and in body weights. Females and males do not present

other striking secondary sexual characteristics (Alkon and Saltz 1985a, Pigozzi 1987, Mori and Lovari 2014).

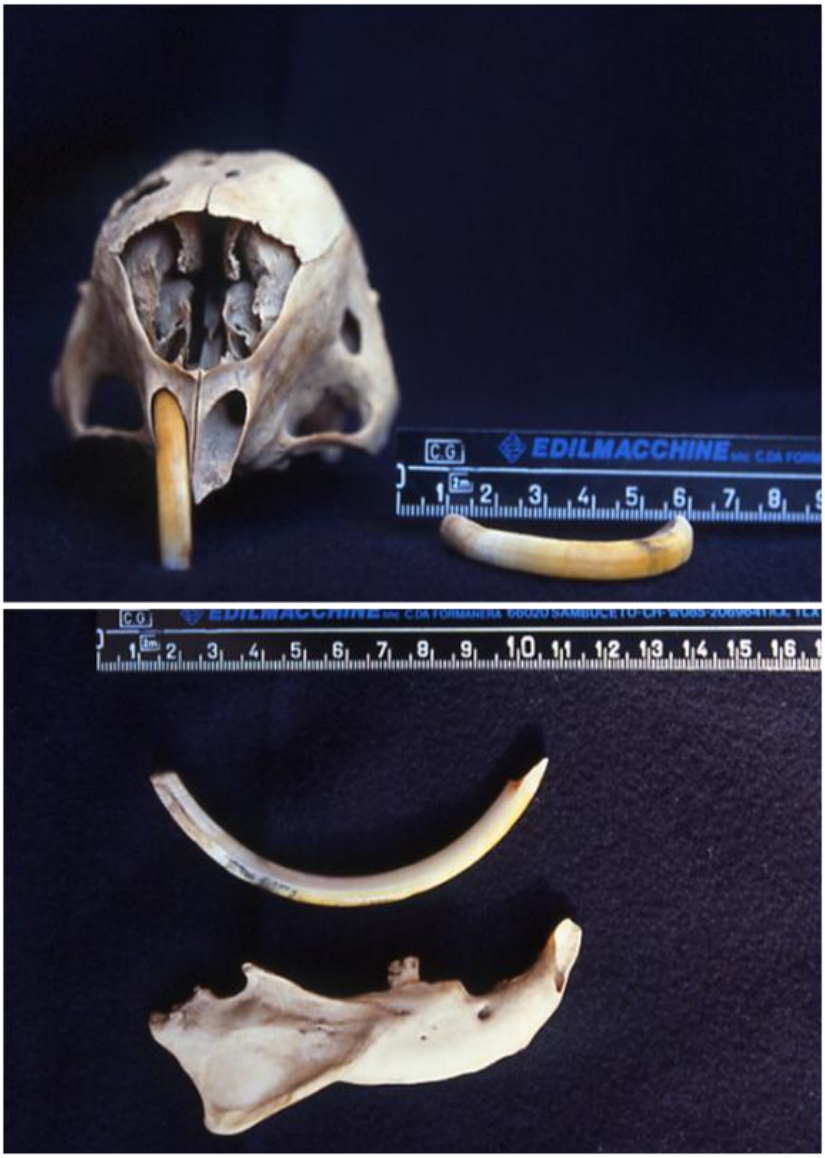

Figure 5. Skull of porcupine, the upper incisors are visible (up) and a jaw portion with a lower incisor (down)
(Photo by Felicioli)

The male has no scrotum; the penis retractile is located in the uro-genitalis region, is caudally directed and hidden in a deep crease in soft leather (Weir 1974, Tohmè and Tohmè 1981, Atalar and Ceribasi 2006) (Fig 6). The penis of the porcupines is considered in "vascular type penis", which also includes humans, equidae and carnivores and present two small nails (Atalar and Ceribasi 2006). The testicles are retained in the abdominal cavity (Tohmè and Tohmè 1981). The female present an internal vaginal membrane that opens only during

parturition and oestrus (Weir 1974). The nipples are located in lateral position (Fig 7). On each side may be 2 or 3 nipples. Often the number of nipples is different on the two sides. The nipples are present also in the male. Therefore the number of nipples may be odd (Weir 1974).

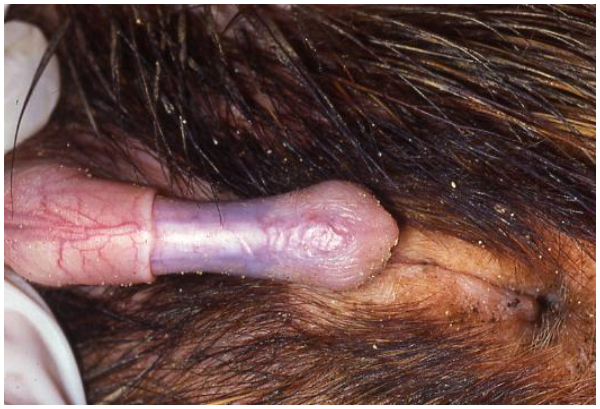

Figure 6. Porcupine penis (Photo by Felicioli)

Figure 7. Porcupine nipples (Photo by Felicioli)

The head, shoulders and limbs are covered by bristle. In the neck region the bristles are more thin and long and form a crest (Toschi 1965). Immediately below the throat a collar of white bristles is present (Tinelli and Tinelli 1983). The back, the rump and the hips are covered with long erectile quills mainly with white and black pigmented bands (Findlay 1977, Van Weers 1983). However there are totally black quills not specifically localized and totally white in the tail region (Van Weers 1983). The quills are modified hairs different in length, diameter and flexibility (Chernova and Kuznetsov 2001). Van Weers (1983) classified the quills in six types (Fig 8):

Spines: stiff, flat and grooved bristles with a sharp point, covering the greater part of the body

Quills: thickest modified hairs, very little flexible, circular in cross-section with the largest diameter about mid-length and with a very sharp point; the principal weapons of porcupines; length from a few cm to some tens cm.

Tactile bristles: very flexible bristles, circular in cross-section whit the largest diameter near the base; some very short, some of these bristles in each skin considerable longer than the longest quill; diameter on an average smaller than that of the quills.

Transitional quills: Intermediate forms between quills and tactile bristles as for length, diameter and flexibility

Rattle-quills: hollow capsule-like structures, open at end, secured to the terminal part of the tail by a thin stalk which produces a loud rattling sound as they strike against one another

Crest: Bundle of hairs on head and nape of the neck

Figure 8. The four types of porcupines quills (Van Weers 1983) (left) and rattle-quills of porcupine (right) (Photo by the Author)

However this classification is not complete and further investigations are needs. The three species of subgenus *Hystrix* present some phenotypic characters that allow distinguishing one from each other. The length and the diameter of the rattle-quills, colour of the tail, colour of the quills in median tract of rump and the crest are the clearly distinct characters between the three species (Corbet and Jones 1964, Van Weers 1983).

H. indica have brown crest, short rattle-quills (< 6 cm) with a dimeter between 4 to 7 mm, white quills in mid-line of rump and white ventral surface of the quills of the tail (Corbet and Jones 1964, Van Weers 1983) (Fig 9A).

H. africaeaustralis have white crest, long rattle-quills (> 6 cm) with a dimeter between 5 to 7 mm, white quills in mid-line of rump and ventral surface of the quills of the tail (Corbet and Jones 1964, Van Weers 1983) (Fig 9B).

H. cristata have white crest, short rattle-quills (< 5 cm) with a dimeter between 2 to 5 mm, black or mottled quills in mid-line of rump and white quills of the tail sometimes with some black quills below (Corbet and Jones 1964, Van Weers 1983) (Fig 9C).

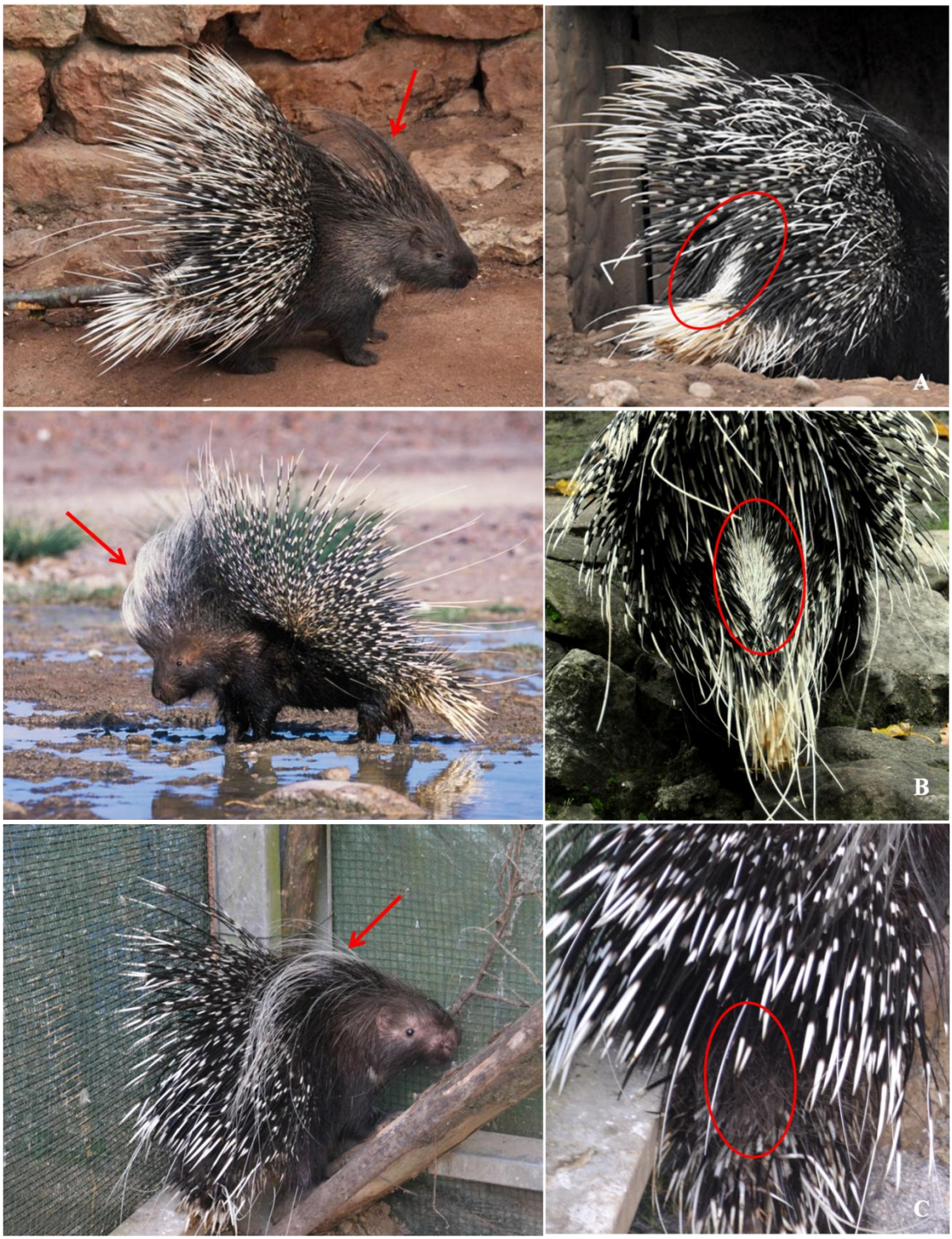

Figure 9. (A)The Indian crested porcupine (*H.indica*) the red arrow indicate the brown crest (Photo by Klaus Rudloff) and in detail the white qills in mide-line of rump (Photo by Lovech Zoo), (B) the cape porcupine (*H. africaeaustralis*) the red arrow indicate the whitw crest (Photo by Wildmoz) and in detail the white qills in mide-line of rump (Photo by Klaus Rudloff) and (C) the crested porcupine (*H. cristata*) the red arrow indicate the whitw crest and in detail the black qills in mide-line of rump (Photos by the Author)

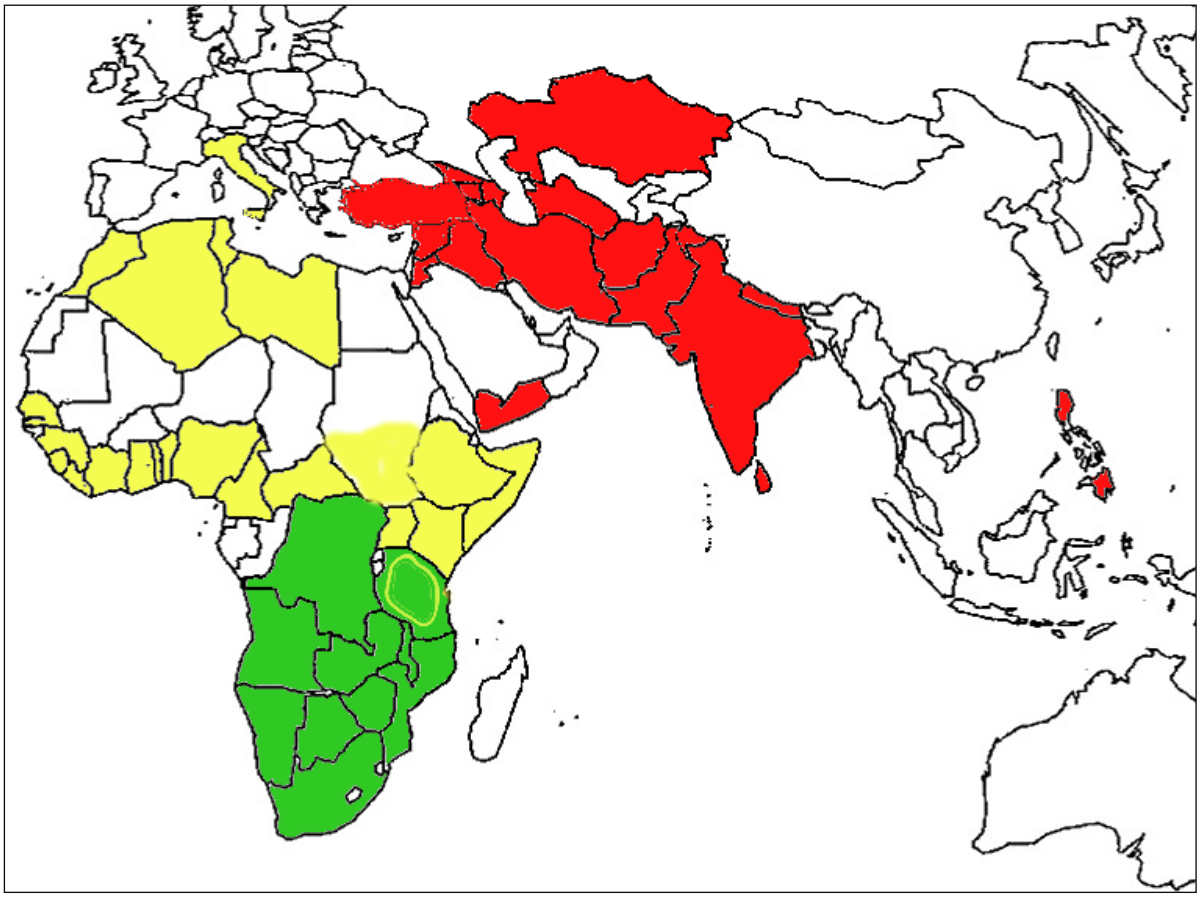

Figure 10. The distribution range of Indian crested porcupine (red), cape porcupine (green) and crested porcupine (yellow) (Source Google modified by the Author)

The Indian crested porcupine (*H. indica*) is widely distributed in the semi-arid areas of South-western Asia (Fig 10). The distribution range of *H. indica* include Turkey, Syria, Palestine, Iraq, Iran, Israel, Lebanon, Jordan, Pakistan, Turkestan, Nepal, Kashmir, Southern Arabia, Eastern Russian, Transcaucasia, Turkey, India and Sri-Lanka (Ellerman and Morrison-Scott 1951, Corbet and Jones 1964, Van Weers 1983, Kadhim 1997, Arslan 2008, Yurumez and Uluturk 2016).

The cape porcupine (*H. africaeaustralis*) is a southern African species that lives in semi-desert areas, in forests, in savannah and was found up to 3500 m a.s.l. in Kilimanjaro (Kingdon 1974, Barthelmess 2006) (Fig 10). In East Africa *H. africaeaustralis* are sympatric with *H. cristata* without no intermediate forms discovered (Kingdon 1974, Van Weers 1983).

The African distribution range of *H. cristata* includes parts of north-western Africa from Morocco to Libya and Sub-Saharan Africa from Gambia to Tanzania (Kingdon 1974, Van Weers 1983) (Fig 10).

In Europe the distribution of *H. cristata* is limited to Italy (Ghigi 1911, 1917, 1947, Toschi 1959, Santini 1980, Amori and Angelici 1992, Amori and Capizzi 2002, Capizzi and Santini 2008, Mori et al. 2013) although the presence of porcupine in Balkans is still debated (Von Wettstein 1941, Toschi 1959, Spitzemberg 1978, Santini 1980, Amori and Angelici 1992, Prigioni 1996, Amori and Capizzi 2002, Masseti 2003, 2009, 2012, Masseti and Sarà 2003, Krystufek et al. 2009, Masseti et al. 2010).

Origin and distribution of *Hystrix cristata* in Italy

The origin of the Italian populations of *H. cristata* has long been debated (Pigozzi 1992). Recent molecular investigation performed by Trucchi and Sbordoni (2009) evidenced the invasive origin of the Italian population of *H. cristata* from North Africa (Tunisia) introduced during Roman Age as hypothesised by Riquelme and Morales-Muniz (1997) and Petronio et al. (2007). Independent analyses of archaeological evidence and iconographic documentation performed by Masseti et al. (2010) prove the presence of the species in continental Italy from late Antiquity/early medieval times. Cranial morphometric variability undertaken by Angelici *et al*. (2003), suggest that the porcupines living in continental Italy have distinct characteristics from the animals living in Sicily and North Africa, which, conversely, seem to be related to each other. Angelici *et al*. (2003) also suggest that the morphometric differences between the Italian continental porcupines on the one hand and the Sicilian and African ones on the other are too large to have developed in the last few millennia. Masseti et al. (2010) therefore do not exclude the possibility that the crested porcupine only became an integral part of the Italian fauna, with viable and self-sufficient populations, in later times probably as sequence of several introduction events. New molecular analysis performed by Trucchi et al. (2016) confirmed a multiple introduction events where many small propagules were introduced over time by continuous commercial trading.

To date the crested porcupine is a common species in mainland Italy (Fig 11), although not evenly distributed. The presence of this rodent is reported since historical times throughout Veneto (Naccari 1818), Emilia Romagna (Ginanni 1774, Zangheri 1946, 1957, 1969, 1970, Zavalloni et al. 1991, Zavalloni and Castellucci 1994), Liguria (Balletto 1969, 1977, Lavezzi 1999, Ghezzi and Lavezzi 2004), Umbria and Marche (Orsomando and Pedrotti 1976, Pandolfi 1986), Molise and Abruzzi (Altobello 1920, Tassi 1971, De Santis 2011), Tuscany, Latium, Campania, Basilicata, Calabria and Apulia (Ghigi 1911, Toschi 1965, Santini 1980, Scaramella 1981, Mori et al. 2013). In the Veneto region after the early 1800s (Naccari 1818), the presence of porcupine was recorded only from the early 2000s (Bon 2001, Bon et al. 2006, Spada et al. 2008). Since 1980s, the distribution of crested porcupine has undergone rapid expansion northward and eastward, extending its range to Lombardy (Bollin and Leo 2013), Piedmont (Dutto et al. 2014, Chiodo and Mori 2015) and Trentino Alto Adige (Mori et al. 2013).

In the Italian islands porcupines are historically well documented in Sicily (Ghigi 1911, 1917, 1947, Toschi 1965, Santini 1980, 1983, Scaramella 1981, Amori and Angelici 1992, Amori

and Capizzi 2002, Capizzi and Santini 2008, Mori et al. 2013). In Sardinia, recently, Angelici et al. (2009a) reported that porcupines were illegally introduced in 2001 in Ogliastra Province. Furthermore, between 2005 and 2008 the discovery of dead porcupines and other signs (scats, quills, sightings by hunters, etc.) confirmed the presence of this rodent in the Island (Angelici et al. 2009a). There are no porcupines in the other Italian islands. In Elba, a large island of Tuscan Archipelago, the presence of porcupines is debated since 1808. Thiebaut de Bernaud (1808), for the first time reported the presence of porcupine in the Island of Elba in 1808, as a result of sightings by hunters. After the 1808 report there has been an ongoing debate between those who do not include Elba Island in the porcupine distribution range (Ghigi 1911, Toschi 1965, Orsomando and Pedrotti 1976, Santini 1980, Scaramella 1981, Amori and Angelici 1992, Mori et al. 2013) and those who include Elba Island in the porcupine distribution range (Lovari 1993, Agnelli 1996, De Marinis et al. 1996, Amori and Capizzi 2002, Bon et al. 2006, Capizzi and Santini 2008, Spada et al. 2008, Angelici et al. 2009a, Angelici et al. 2009b, Masseti et al. 2010). In 2018 the presence of crested porcupine was detected and documented also in Elba Island (Vecchio et al. 2018) (Fig 12). Further genetic investigation and comparison with the results obtained by Trucchi et al. (2016) are desirable and which may offer interesting facts on the origin of this rodent in the Island of Elba.

Figure 11. Distribution range of crested porcupine in Italy. Black triangles represent the hypothetical historical range, white circles the recent expansion. Introduced nuclei are circled: arrows represent the link with the source population, where known (Mori et al. 2013)

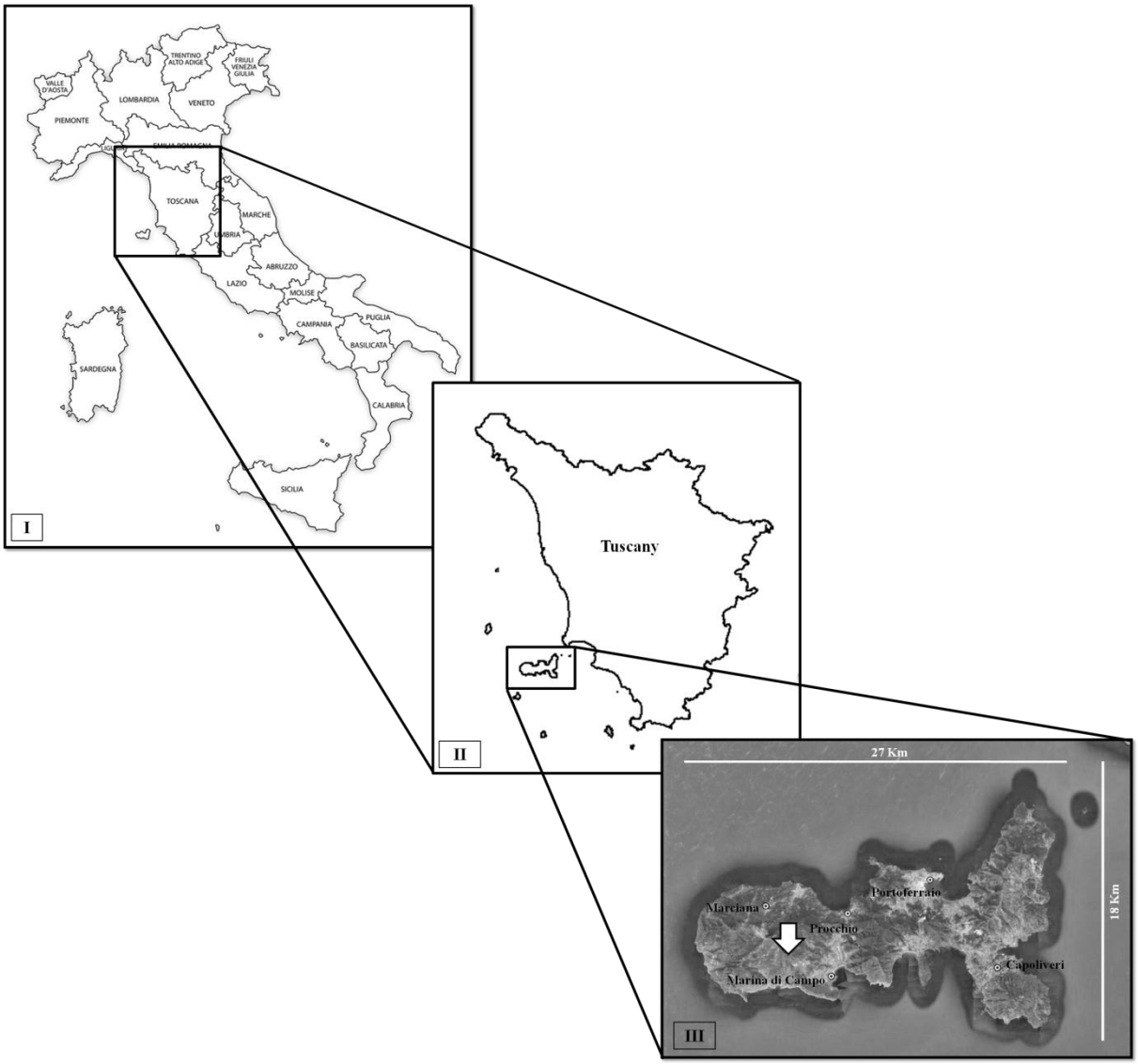

Figure 12. (I) Map of Italy, (II) Enlarged view of Tuscany Region showing the Island of Elba and (III) Details of the Island of Elba, the white arrow indicate the Monte Perone area where the porcupine was caught on camera in 2016 (Vecchio et al. 2018 modified by the Author)

Habitat

Figure 13. Frontal view of a black locust wood (*Robinia pseudoacacia*), in the foreground is visible a field of chicory and a strip of sorghum sown as environmental improvement (Photo by the Author)

Figure 14. Panoramic view of a fragmented environment, in the background is visible a black locust wood and an arable area with a small bushes covered area and in the foreground a vineyard (Photo by the Author)

Environmental fragmentation is a key factor for the presence of the porcupine (Fig 13 and Fig 14). In Italy the porcupine is mainly recorded at medium-low altitudes between 600 to 900 m a.s.l. (Orsomando and Pedrotti 1976, Santini 1980, 1983). The porcupine is able to adapt itself to different habitat type as woods, Mediterranean scrubwood and cultivated open plains where live along the banks of rivers and canals, where vegetation offers adequate woody and/or bushes covered (Fig 15) (Santini 1980, 1983, Felicioli 1991). Rarely can it be also found in woods of *Pinus pinea*, *P. pinaster* and *P. halepensis* (Tinelli and Tinelli 1980, Filibeck et al. 1981, Franconi et al. 2003).

Figure 15. Panoramic view of a bank of a river with a good vegetation covered near a cultivated area. In detail a burrow digged in the riverbank is visible (Photos by Felicioli)

The deciduous forests (e.g. *Robinia pseudoacacia*, *Quercus cerri*, *Fraxinus ornus*, *Ostrya carpinifolia*, *Quercus pubescens*, *Castanea sativa*, *Ulmus minor*, *Sorbus domestica*) and Mediterranean scrubwood (e.g. *Quercus cerri*, *Quercus ilex*, *Hedera helix*, *Pinus pinea*, *Pinus pinaster*, *Pinus halepensis* and other deciduous species) are the more suitable habitats for porcupine due to the presence of a thick vegetation that ensure food and an excellent coverage throughout the year (Fig 16) (Orsomando and Pedrotti 1976, Tinelli and Tinelli 1980, Felicioli 1991). The undergrowth of deciduous forest and Mediterranean scrubwood are mainly characterized by *Ceratonia siliqua*, *Cistus* sp., *Erica scoparia*, *Juniperus oxicedrus*, *Spartium junceum*, *Laurus nobilis*, *Rhamnus alaternus*, *Arundo donax*, *Cornus mas*, *Ruscus*

aculeatus, *Arbutus unedo*, *Rubus ulmifolius* and *Sambucus nigra*). In the herbaceous layer we can find *Myrtus communis*, *Asparagus* acutifolius, *Cyclamen* sp., *Tamus* communis, *Arum italicum*, *Rumex* sp. and *Orchis provincialis* (Polunin and Walters 1987).

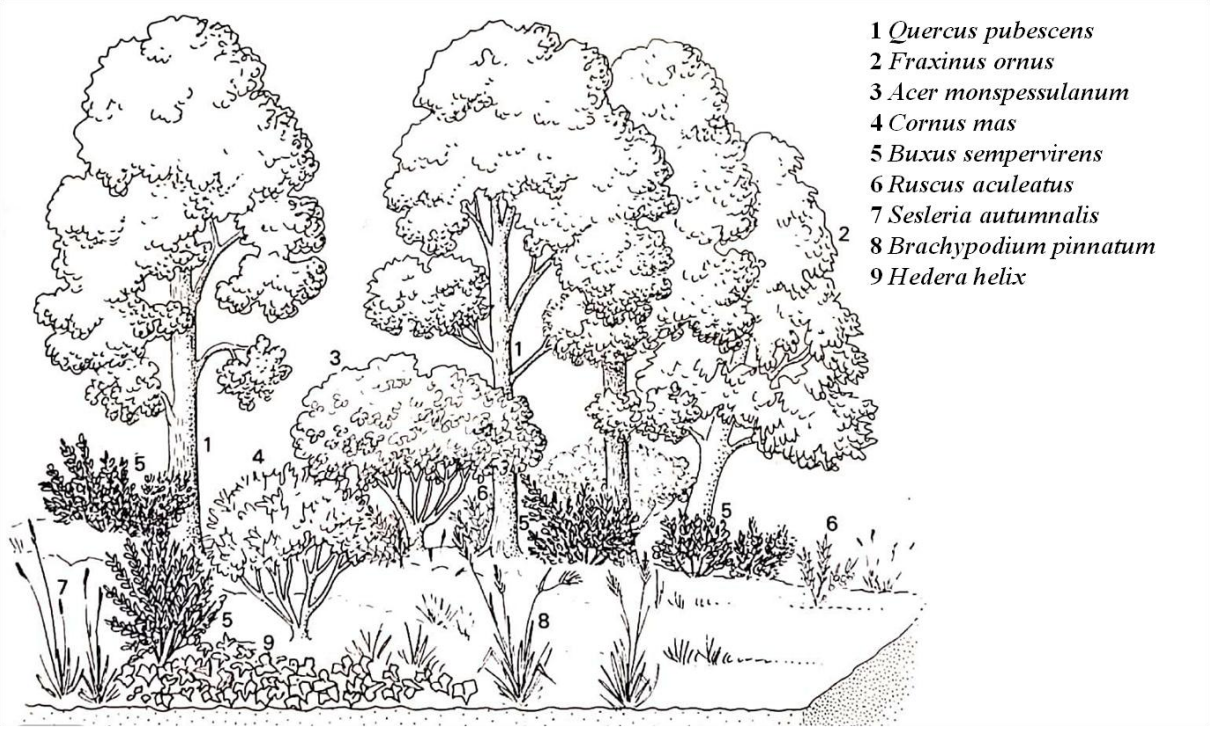

Figure 16. Sub-Mediterranean oak forest with some characteristic species (Polunin and Walters 1987)

The spatio-temporal habitat selection in crested porcupines was widely described in many studies using the radio-telemetry by Sonnino (1998), Borger (2002), Lovari et al. (2013a), Mori et al. (2014a). The same Authors report a marked preference of covered habitats and a reduction in the home-range size of porcupines in habitat with a high environmental fragmentation and food availability.

The home-range size seems to have a seasonal variability with maximum sizes in spring and summer and minimum in winter (Sonnino 1998, Borger 2002, Mori et al. 2014a). Significant seasonal changes in home-range sizes were also observed by Saltz and Alkon (1989) in *Hystrix indica* in the Negev desert and by Corbet and Van Aarde (1996) in *Hystrix africaeaustralis* in South Africa with an increase in home-range size as consequence of the reduction of food resources. This variability was more evident in animals that used only natural forages compared to those who also used the crops forages (Saltz and Alkon 1989, Sonnino 1998, Lovari at al. 2013a).

Differently environment temperature, sex and body size does not seem to influence the use of the spaces of this rodent (Borger 2002, Lovari et al. 2013a, Mori et al. 2014a).

The most suitable habitat is therefore characterized by high biodiversity and woody fragmentation in which small wood areas are interspersed with marginal areas, cultivated and uncultivated areas (Toschi 1965).

In these environments the porcupines dig its burrows (Tinelli and Tinelli 1980, Monetti et al. 2005) and coexist with two other semi-fossorial mammals: European badger (*Meles meles*) and red fox (*Vulpes vulpes*) (Tinelli and Tinelli 1980, 1983, Pigozzi 1986, Mori et al. 2015a).

Settlements: structure and use

Figure 17. Crested porcupine settlement. Two burrow entrance holes with spoil heap are visible (Photo by Coppola and Felicioli)

Figure 18. Natural cave use by crested porcupine as refuge

The crested porcupine is a semi-fossorial (not spends all of its life underground), nocturnal rodent and live in reproductive pair or family group in hypogeus burrow (Kingdon 1974, Santini 1980, Felicioli 1991, Monetti et al. 2005) (Fig 17). Occasionally the crested porcupine use natural shalters and caves as site of refuges (Felicioli 1991, Corsini et al. 1995) (Fig 18).

The hypogeous burrows are the main site for breeding, sleeping and refuges during the daylight hours (Santini 1980). Hypogeus burrows are digged in sandy soil with a good slope (10 to 40°) in wooded areas with a good vegetation cover and near to cultivated or uncultivated areas (Tinelli and Tinelli 1980, Felicioli and Santini 1994, Monetti at al. 2005). The porcupine settlements are characterized by the presence of a large spoil heap in front of each ground hole as consequence of the digging activity (Monetti at al. 2005) (Fig 19).

Figure 19. Large spoil heap in front of a burrow entrance hole (Photo by Coppola and Felicioli)

The burrows could be a very complex system of underground tunnels and chambres that show huge variation in both size and complexity (Tinelli and Tinelli 1980). Felicioli (1991) defined the burrow and/or burrow system as a set of one or more underground chambers communicating in series or in parallel which are accessed throughout one or more tunnels that open individually or after anastomosis to the outside by a ground level hole. Felicioli (1991) and Felicioli and Santini (1994) also defined:

Hole: opening in the ground that through one or more tunnels is in communication with the underground chambers

Settlement (Sett): cluster of ground holes detectable on the surface and connected to one or more hypogeous burrows that are generally not individually recognisable from each other

Therefore only the settlements are detectable on ground surface as a cluster of holes (Santini 1980, Felicioli 1991, Felicioli and Santini 1994).

Felicioli (1994) and Felicioli and Santini (1994) hypothesized a binary system distribution of settlements. According to Felicioli and Santini (1994) the settlements seems to be distributed in pair within the territory and oriented opposite one another in an about 150 m area on the Northwest-south axis. However, this hypothesis has never been confirmed and no other data on the spatial distribution of settlements is available.

The inhabitation of settlements by the crested porcupine are yet unknown. According to Santini (1980) and Monetti et al. (2005) each settlement is inhabit by only one reproductive pair that shows a greater site fidelity than unpaired animals. Felicioli (1991) and Felicioli and Santini (1994) by observing the signs of presence in the settlements suggest a seasonal preference for different burrow orientation throughout the four seasons. In winter the porcupine seems to choose south-oriented burrows while in summer a north-west orientation are preferred. In spring and autumn no preference was observed (Felicioli and Santini 1994). No other data are available about the inhabitation of settlement by crested porcupines and further investigations are desirable. Moreover, to date there are no data if the burrows are dug by porcupines, or are made by other semi-fossorial burrowing mammals, such as badgers (*Meles meles*) and red fox (*Vulpes vulpes*). No data are also available on how this three species use the settlements present within the territory.

Among mammals, at least 58% are known to exhibit burrowing behaviour (Kinlaw 1999). Given the energetic costs associated with digging and limitations in availability of burrowing

sites, many species share same den sites (Vleck 1979, Mukherjee et al. 2019). The coexistence of unrelated species and sharing of resources in a region has always been intriguing for ecologists to study the interactions between individuals of these species. Italy is the only European country where the crested porcupines, the European badger and the red fox coexist sharing the same hypogeous burrows (Pigozzi 1986, Mori et al. 2015a). Therefore, Italy provides an ideal natural set up of coexisting of these three species in a small area sharing common resources. For all these three species the burrows are the preferred sites for breeding, sleeping and hiding during daylight hours (Santini 1980, Kuuk 1989, Roper 1992a). The badger settlements show the same structural and habitat characteristics than the porcupine's ones (Dunwell and Killingley 1969, Kruuk 1978, 1989, Pigozzi 1986, Roper 1992a, b, Broseth et al. 1997, Bianciardi et al. 2014). Therefore the settlements of badgers are not recognizable from those of porcupines.

Otherwise the burrows of red fox are mainly located in wooded areas with a low vegetation and shrubby cover but very often use rocky outcrops or natural refuges as burrow (Meriggi and Rosa 1991). The red fox is also frequently reported to be an opportunistic user of badgers or porcupines burrows mainly in spring for breeding (Dunwell and Killingley 1969, Tinelli and Tinelli 1983, Kowalczyk et al. 2000, Goszczynski and Wòjtowicz 2001).

Observation of traces like footprints, hairs, quills and feaces of porcupine, red fox and badger in the same settlement indicate that porcupines and badgers traces are often found in the same settlement, but generally not at the same time (Tinelli and Tinelli 1983, 1988, Pigozzi 1986). Conversely, porcupines and red foxes traces have never been found simultaneously and the presence of red foxes in settlements has been detected only occasionally and always in spring for breeding (Tinelli and Tinelli 1988). Therefore, Tinelli and Tinelli (1983, 1988) and Pigozzi (1986) have hypothesized a sharing of the same settlement (sett-sharing) between crested porcupines, badgers and red foxes. The settlement-sharing (co-use of the same settlement not simultaneously) by crested porcupines, badgers and red foxes was subsequently confirmed from observations performed by Mori et al. (2015a) using camera-trapping

However no data are available whether the three species cohabit (contemporary inhabitation) in the same settlements.

Badger and fox are known to be predators (Kruuk 1989, Diaz-Ruiz et al. 2016). However, despite sett-sharing between porcupines and its two potential predators, no acts of predation have ever been observed in Italy.

Predation on speciemens of genus *Hystrix* has only been observed by Mills and Shenk (1992) on three young and twelve adult African crested porcupines by lions (*Panthera leo*). Acts of predation have been recorded in *Hystrix indica* by Alkon and Saltz (1988a) citing the personal communication of G.Ilani and by Kingdon (1974) on *Hystrix cristata* and *africaeaustralis* in Africa by the common leopard (*Pantera pardus*). Lovari et al. (2013b) in a review on the food habits reported that in Asia, *Hystrix* spp. is one of the most frequent used wild prey of the common leopards. However Loveri et al. (2013b) did not rule out the possibility of the scavenging of carcasses of some species in the diet of the common leopard. Porcupine remains were occasionally fuond also in other large carnivors like African wild dogs, lions and spotted hyenas (Breuer 2005).

According to Monetti et al. (2005) and Capizzi and Santini (2008) today in Italy the crested porcupine does not have natural predators able to kill adult specimens. The predation of porcupettes is also improbable because they are always protected by at least one parent (Mohr 1965, Morris and Van Aarde 1985, Sever and Mendelssohn 1988a, Felicioli et al. 1997a, b).

In Italy, porcupine remains were occasionally found in the feces of the wolf (*Canis lupus*) (Battochino et al. 2017), wild boar (*Sus scrofa*) (Massei et al. 1996) and red fox (Fais et al. 1991; Lucherini et al. 1995). Conversely, no evidence of porcupine in in the diet of badgers was reported (Ciampalini and Lovari, 1985, Melis et al. 2002, Balestrieri et al. 2004). Fais et al. (1991) and Lucherini et al. (1995) hypothesised that red foxes are an occasional porcupine predators. However predation on porcupines by red foxes has never been documented and Mori et al. (2014c) hypothesized mutual avoidance behaviour among porcupine, badger and red fox. The above factors could have implications on sett-sharing or cohabitation and need to be more investigated.

Temporal motor activity pattern

Crested porcupine is active throughout the year and is reported to be a strictly nocturnal mammal (Santini 1980).

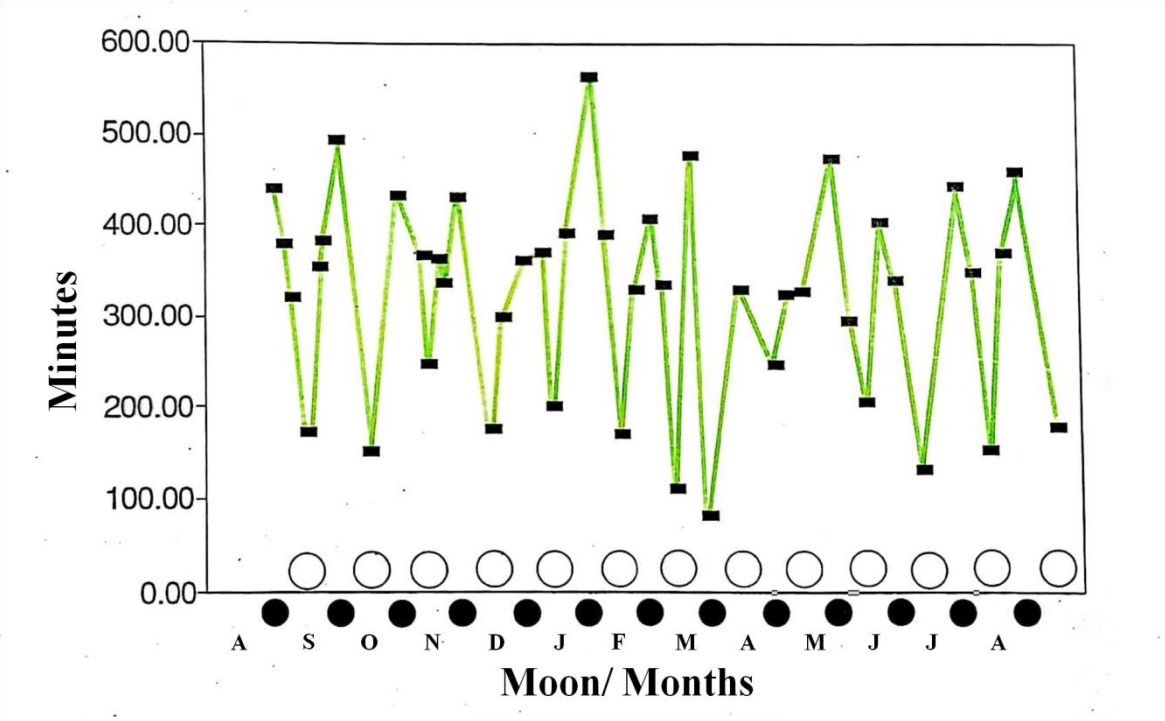

Figure 20. Variation of quantity of motor activity with moon phases in semi-captive crested porcupine (Felicioli 1991)

The porcupine motor activity is light-dependent (Felicioli 1991, Felicioli and Santini 1991). The porcupine shows a rhythmic variation of the quantity of motor activity that corresponds to the synodic lunar cycle (Felicioli 1991) (Fig 20). The nocturnal motor activity is more intense during the new-moon phases and a less intense one during the full-moon phases (moonlight avoidance) throughout the all year (Felicioli 1991, Mori et al. 2014b). The moonlight avoidance behaviour was also observed in *H. indica* by Alkon (1987) and by Alkon and Saltz (1983, 1986, 1988a). In this species the moonlight avoidance is reported to be maximum in winter and absent in late summer (Alkon and Saltz 1988a). Conversely moonlight avoidance seems not be present in *H. africaeaustralis* (Corbet 1991).

Crested porcupine mostly leave its burrow after sunset and stop their activity before sunrise (Felicioli 1991, Corsini et al. 1995). Felicioli (1991) in two semi-captives and two wild porcupine families observed that the first emergences time from burrow in winter occurs later than time of sunset (Fig 21). Differently in summer the first emergence occur near to sunset time (Felicioli 1991). The results obtained by Corsini et al. (1995) using radio-telemetry on

four wild porcupines confirm what observed by Felicioli (1991). Corsini et al. (1995) also reported that the ending of motor activity in crested porcupine occur before sunrise from September to March while from April to October the porcupines came back to their burrow significantly more often later in the morning (Fig 22). No informations are available concerning the possible factors affecting the patterns of first emergence time from burrow and last entrance and further investigation are desirable.

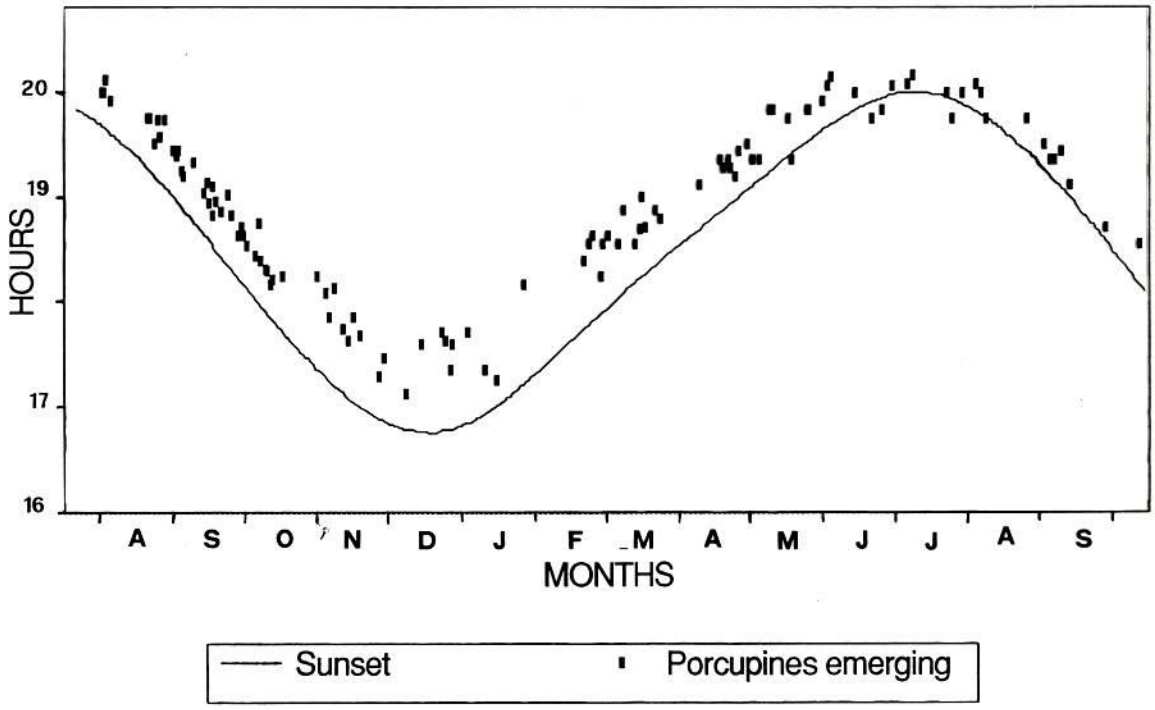

Figure 21. Pattern of first emerging time from burrow observed in semi-captive crested porcupines by Felicioli (Felicioli 1991)

The porcupine nocturnal motor activity regularly occurs during all year. This rodent spends most of the nocturnal time in search of food and in the social activities within its family group (Felicioli 1991). In autumn and winter when the nights are longer the activity interruption with resting periods and returning to the burrows was observed (Felicioli 1991, Corsini et al. 1995). The crested porcupine is mainly active in the first part of the night (from 8:00 p.m.to 11 p.m.) but show a high activity also in crepuscular interval (Corsini et al. 1995). Conversely Fattorini and Pokheral (2012) observed that *H. indica* is more active in the central part of the night (from 11:00 p.m. to 2 a.m.) than in crepuscular and daylight periods. Few data are available concerning motor activity in cape porcupine. This species seems to be more active in the central part of the night (after the 22:00 p.m.) whit a decreasing

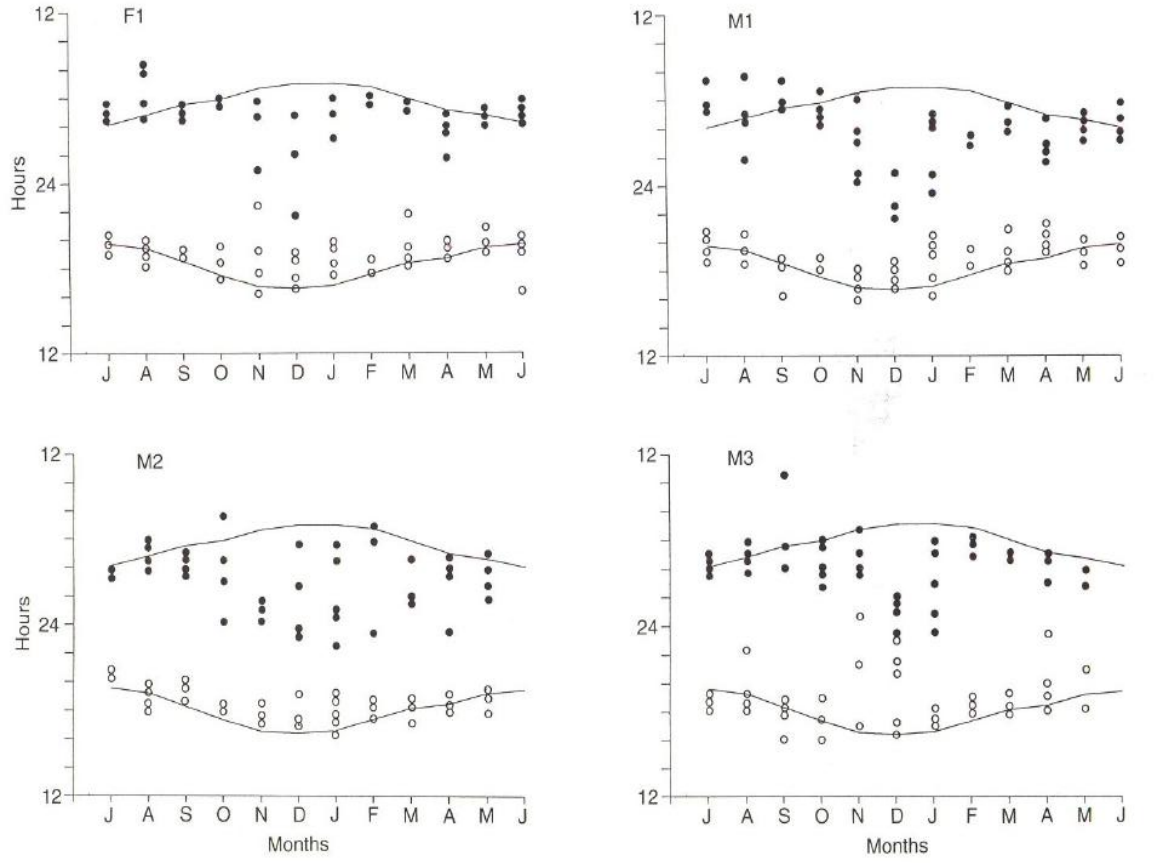

Figure 22. Pattern of first emerging time-from (white dots) and last returning time to the burrow (black dots) recorded by Corsini et al. in four different specimens of free-ranging porcupines. Upper solid line indicate the sunrise while lower solid line the sunset (Corsini et al. 1995)

of the activity towards dawn and little crepuscular activity (Corbet 1991). Corsini et al. (1995) have also observed the presence of an irregular diurnal motor activity throughout the year of *H. cristata* with a peak in spring (Fig 23). Daylight movements of crested porcupines were usually recorded to the surrounding of burrow, within 100 m from den (Corsini et al. 1995). Conversely no diurnal activity was recorded in *H. indica* (Fattorini and Pokheral 2012) and no data are available for *H. africaeaustralis*. Moreover Kingdon (1974) reported for the first time its observation in Africa concerning two adults and two young porcupines "enjoying a siesta" under a shady bush close to a large hole. Kingdon (1974) therefore hypothesised that porcupine exhibit the sunbathing behaviour. However the same author does not report on which of the two sympatric species of porcupines (*H. cristata* or *africaeaustralis*) the sunbathing behaviour was observed. Yellen (1991) also suggested the possibility of the presence of this behaviour in *H. africaeaustralis* without any support data. Subsequently no other observation and data are available concerning the sunbathing behaviour in other porcupine species of sub-genus *Hystrix* (*H. cristata* and *H. indica*) neither if it is a specie-

specific behaviour. Therefore further investigation in order to verify the presence of this behaviour in crested porcupine is desirable.

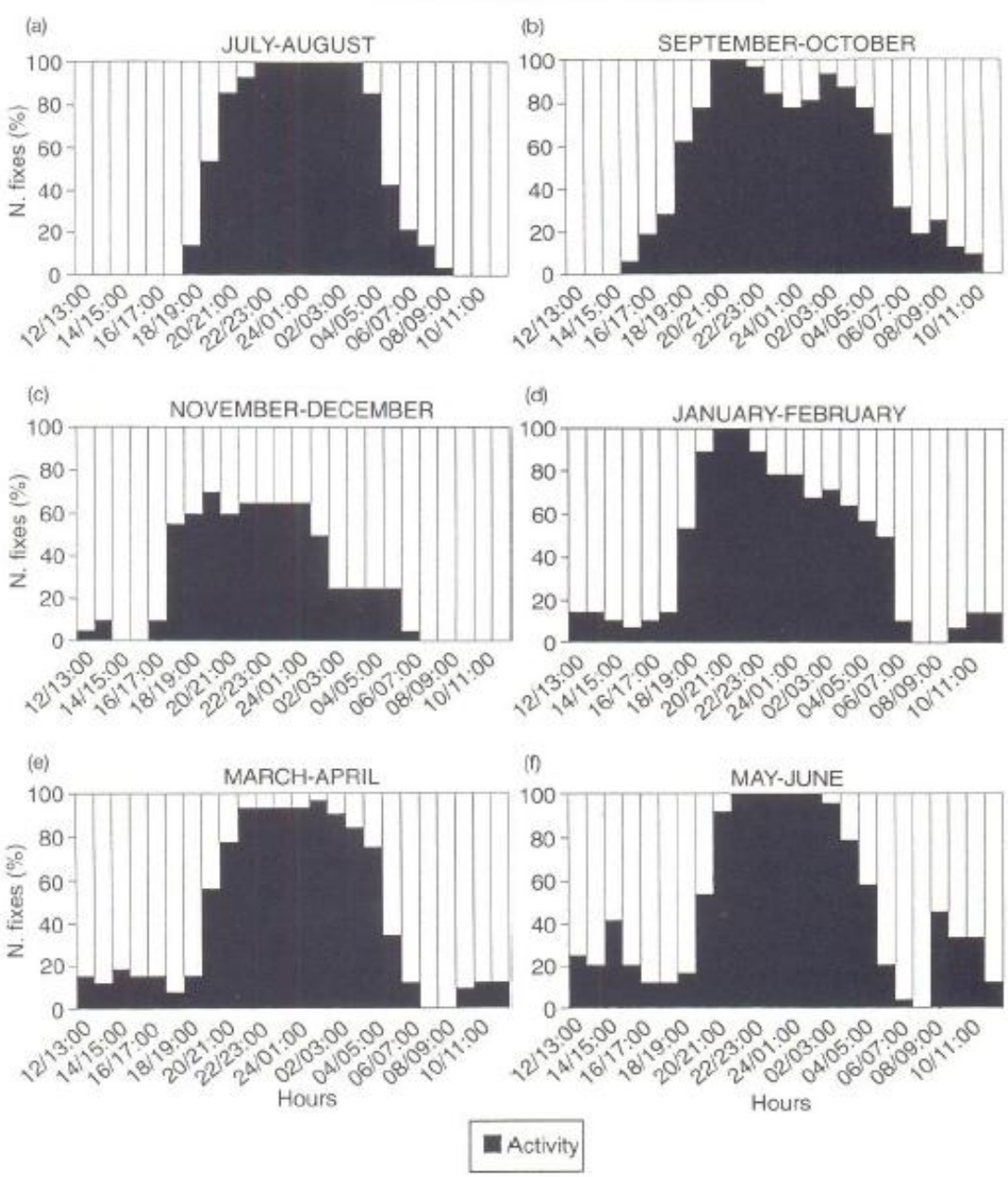

Figure 23. Bi-monthly activity rythms of four free-ranging radiotagged porcupines (Corsini et al. 1995)

Reproduction

Among the hystricomorph Rodents, the genus *Hystrix* shows a social organization based on the formation of the pair, on small family groups or clans (Mohr 1965, Kingdon 1974, Santini 1983, Morris and Van Aarde 1985, Saltz and Alkon 1992) in which monogamy appears to be a fundamental mating system (Mohr 1965, Weir 1974, Van Aarde 1987a, Sever and Mendelssohn 1988a, b). In *H. cristata* the season seems not to affect breeding which was observed throughout the year (Santini 1980, 1983, Grazzini 1992, Mori et al. 2016). In *H. cristata* peak of births were recorded in February and October (Mori et al. 2016), in *H. africaeaustralis* in summer between August and March (Kingdon 1974, Van Aarde 1984, 1985) while in *H. indica* between April and September (Kadhim 1997, Tohmè and Tohmè 1980). The duration of oestrus cycle in *H.cristata* is 35 days (Weir 1974) and the mounting and copulation behaviour were observed also out of oestrus state (Felicioli 1997a, b). The mounting and copulation are distinct behaviours. The mounting behaviour do not include the intromission (insertion of the penis into the vagina) and the thrusting (pelvic movements during intromission) while in the copulation intromission and thrusting occurs (Felicioli et al. 1997a, b). The crested porcupines performs copulation independently of the oestrus state with a night rhythm of multiple mounting also after or before births and even in presence of porcupettes (Grazzini 1992, Felicioli et al. 1997 a, b) as also reported for *H. indica* by Sever & Mendelsshon (1988a). Coversely in *H. africaeaustralis* night rhythm of mounting with copulation was observed only in the oestrus state (Morris and Van Aarde 1985). The vaginal membrane located in a deeper position than in other hystricomorph rodents could explain the different copulation rhythm in these three different species (Weir 1974). The mounting and copulation behaviours in *H. cristata* seem to follow a typical scheme that was described by Felicioli (1997a, b) in semi-captive porcupines. No data about the mounting and copulation behaviours in wild crested porcupines as well as in *H. indica* and *H. africaeaustralis* are available and should be more investigated. According to Felicioli (1997a, b) the mounting sequence begins either with both members of the couple sitting in contact or alongside each other facing in the same or opposite directions (*Resting*) (Fig 24A). In the resting mode, if one of the members of the pair rises up it is immediately followed by the partner (*Following*). When both partners are standing, different behavioural patterns can be observed:

- **olfactive (S*niffing*)**, occur only in male, is carried out either in front or at the side of the female and it is often associated with stepping (Fig 24B);

- **tactile (*Grooming*)** occur in male and female with act of self, allo or mutual grooming such as licking the tail, licking the head or the neck respectively (Morris and Van Aarde 1985, Sever and Mendelsshon 1990) (Fig 24C);
- **acoustic (*Sound*)**, occur in male and female alternatively with short and rapid vocal sounds emitted through the nostrils (snorts) and closely tied to male-female interactions;
- **Stepping** occur in male and consists in preparatory movements by stepping with hind legs on the spot.
- **Following** act of following each other. Outside the burrow the male follows close behind the female, while inside the burrow this behaviour becomes circular (Morris and Van Aarde 1985) (Fig 24D);

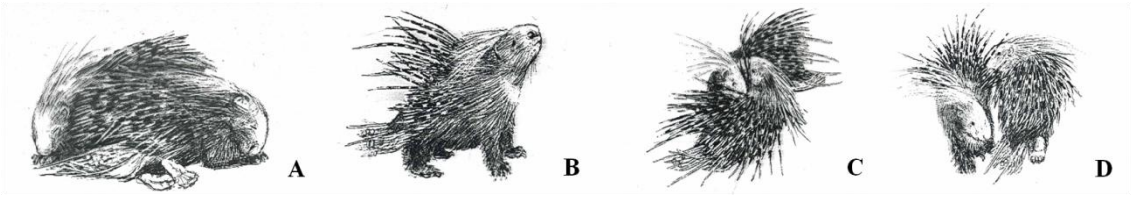

Figure 24. (A) Resting behaviour, (B) sniffing behaviour, (C) allo-grooming behaviour and (D) following behaviour (Drawing of Pat Gordon reported in Felicioli et al. 1997)

The behaviour patterns are variably interconnected with mutual precedence and without strict rigidity in the possible, alternative sequences performed. This early phase of the sequence leads to a peculiar behaviour named *Nose-Quill* contact (Fig 25A). In this behavioural pattern the male behind the female, with his head raises one of the long and completely white back facing quills which are located in each side of the tail region. These quills appear highly sensitive to contact.

The nose-quill contact is followed by the presenting of the female with erection of the back quills, rising of the tail on to her back and exposure of the ano-genital region for mounting next step (Fig. 25B).

Spontaneous presenting by the female is adopted by the genus *Hystrix* as an indication of the oestrus state (Kleiman 1974). The mounting and copulation occur with the male in bipedal position. The male lifts his body in an upright position, extroflects the penis and advances towards the female until the underside part of her raised tail makes contact with his belly. During the mounting the forelegs are put on female's back but do not hold her (Fig. 25C).

Figure 25. (A) Nose-quill contact behaviour, (B) presenting behaviour and (C) mounting and copulation behaviour (Drawing of Pat Gordon reported in Felicioli et al. 1997)

Even if the presenting of the female, the mounting, the intromission or the thrusting do not occur the mounting behaviour sequence do not stop but began again from one of the behavioural patterns above describe (e.g. following, sniffing or nose-quill contact) in order to repeat the sequence. After copulation the sequence can be repeated, otherwise it continue either with exploring, which ended the sequence, or with grooming and then resting.

The average time length of copulation in *H. cristata* is 8.3 SD 3.9 seconds and 5.2 SD 4.4 thrustings (Felicioli et al. 1997). In *H. africaeaustralis* the duration of copulation ranged from 2 to 3 min with an average of 38 thrustings (Morris and Van Aarde 1985). Felicioli et al. (1997) observed that in *H. cristata* copulation occur also in the burrows.

The gestation length in porcupines (*H. cristata*, *indica* and *africaeaustralis*) it is yet unknown as well as the number of births in the year. Kingdon (1974) report a gestation length for *H. cristata* and *H. africaeaustralis* from 42 and 112 days. Santini (1980, 1983) in *H. cristata*, Tohmè and Tohmè (1980) in *H. indica* and Van Aarde (1985) in *H. africaeaustralis* suggest a gestation length of about three month. Conversely Grazzini (1992) suggest that the length of gestation in crested porcupine is about 106 days. According to Santini (1980) for *H. cristata*, Kingdon (1974) for *H. cristata* and *H. africaeaustralis* and Kadhim (1997**)** for *H. indica* the births occur in the burrows probably one or two time *per* year.

The litter size in *H.cristata* as well as in *H. africaeaustralis* and *H. indica* range from one to two and only occasionally three porcupette (Fig 26A) (Weir 1974, Santini 1980, Van Aarde 1985, Grazzini 1992, Kadhim 1997, Mori et al. 2016). Only Tohmè and Tohmè (1980) observed litters of four porcupette in *H. indica*.

Figure 26. (A) Porcupine litter of three porcupette (Photo by Santini) and (B) pocupette just born (Photo by Biliotti)

The weight at births of *H. cristata* ranged from 260 to 400 g (Grazzini 1992) similarly to in *H. africaeaustralis* (300 to 440 g) (Van Aarde 1985). The porcupette are well developed at birth with open eyes, the back already covered with soft quills and move independently (Santini 1980, Grazzini 1992) (Fig 26B).

According to observations performed by Grazzini (1992) in semi-captive porcupines the porcupette leave the den for the first time when about 10 days old. Conversely Mori et al. (2016) reported the first observations of porcupette outside the den into the wild at about 45-60 days old.

The porcupettes spend most of the time outside the burrow playing, exploring and showing the "frisky-hop dance" behaviour (Kleiman 1974). The "frisky-hop dance" is characterized by small jumps and fast and unexpected runs interspersed by resting which may also involve adults or sub-adults of the same family.

The female suckles the porcupettes for about 40-50 days and the weaning occurs at around 3 months (Santini 1980, Grazzini 1992). However the porcupettes already start to feed with solid foods at one month old (Grazzini 1992).

Both male and female equally perform the parental cares (Fig 27) (Sever and Mendelssohn 1989a, 1991a, Grazzini 1992). Male and female alternatively remain with porcupettes for monitoring them (*Baby-sitting*) and frequently show allo- and mutual-grooming with porcupettes (Grazzini 1992). The *Baby-sitting* is also performed by sub-adults of the same family as helper in the porcupettes weaning (Grazzini 1992).

The sex maturity in *H. cristata* seems to be reached at about 9 months, when body weight is about 8-9 kg (Santini 1980). Van Aarde (1987b, c) reported the age of sexual maturity in *H. africaeaustralis* is of 52 week while Tohmè and Tohmè (1980) in *H. indica* of 2 years. The sub-adults seem to remain in the family group up to 1 or 2 years (Van Aarde 1987b, Grazzini 1992) and no data are available concerning their dispersal.

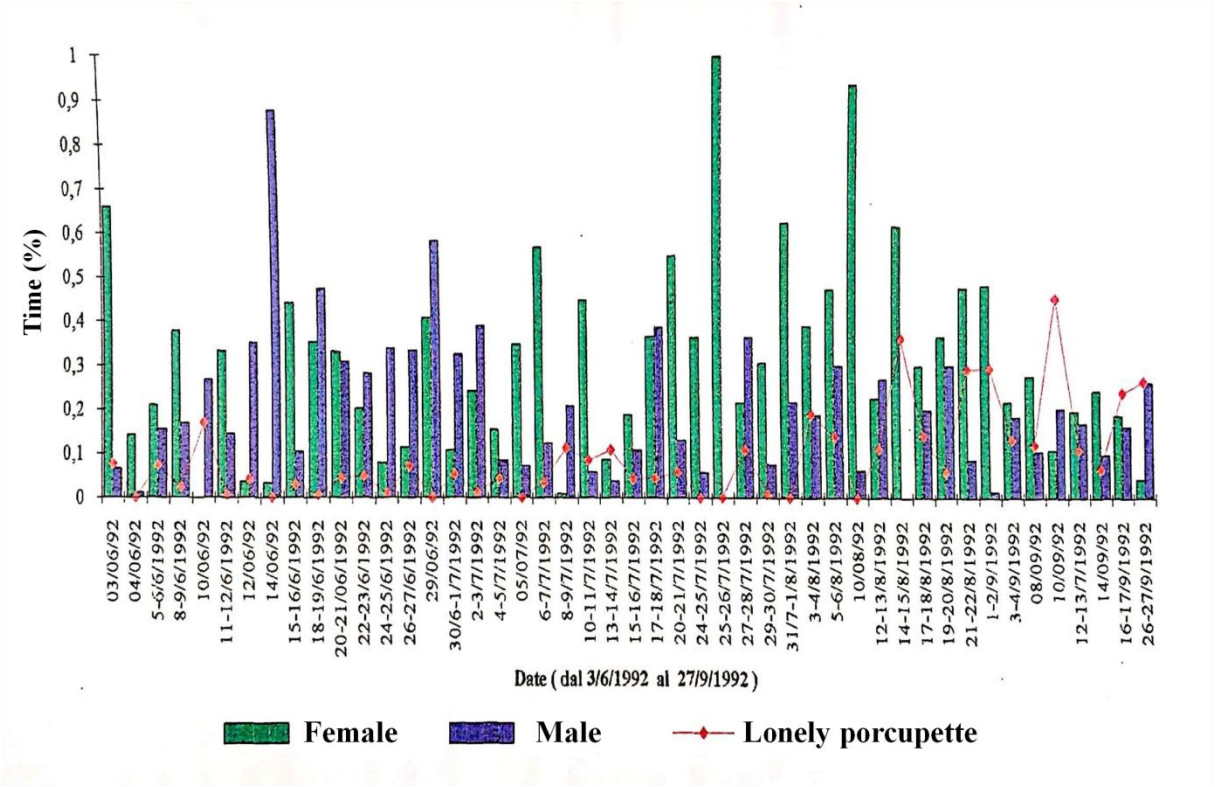

Figure 27. Percentage of time spent by the male and by the female with porcupette and by lonely porcupette in semi-captivity (Grazzini 1992)

Feeding behaviour

The crested porcupine is reported to be polyphagous, but strictly herbivorous, with a marked fondness for roots, bulbs, tubers (also truffles) and rhizomes of many wild and cultivated herbaceous plants which are dug up (Fig 28 and Fig 29) (Santini 1980, Alkon and Olsvig-Whittaker 1987, Pigozzi and Patterson 1990 Ori et al. 2018). It also feed on epigeal parts, leaves, flowers, buds, grass, inflorescences and fallen fruit (Santini 1980, Bruno and Riccardi 1995).

Figure 28. Porcupine digs in a food-patch area (Photo by the Author)

Porcupine foraging generates a network of direct and indirect impacts on ecological processes. Loose soil excavated by porcupines enhances soil erosion and transport, and may substantially enhance ecosystem flows of water, soil, and nutrients (Alkon 1999).

Porcupine often bites only partially its food, chewing it loudly and then discards it after just few mouthfuls (Chevalier and Ashton 2006).

The crested porcupine, as well as Cape porcupine and Indian crested porcupine, also perform osteophagia (consume bones) (Kingdon 1974, Duthie and Skinner 1986, 1989, Felicioli 1991, Robinovich and Horwitz 1994, Kiibi 2009, Akram et al. 2017, Mori et al. 2017a, 2018). Porcupines have long been known to collect and gnaw bones inside and outside their burrow (Rosevear 1969, Kingdon 1974, Santini 1980; Felicioli 1991, O'Regan et al. 2011, Horwitz et al. 2012). The reason for osteophagia in porcupine is still not clear and two main reasons have been hypothesised: the need to hone the open-rooted incisor teeth and/or gnawing bones for calcium and phosphorus assumption (Rosevear 1969, Kingdon 1974, Santini 1980, Duthie and Skinner 1986, 1989, Akram et al. 2017).

Porcupines have a simple stomach, a small intestine, a voluminous caecum (Van Jaarsveld 1983, Van Jaarsveld and Knight-Eloff 1984) and a peculiar furrow in the large intestine (Gorgas 1967). This anatomic feature allowed to hypothesising the presence of a caecotrophy practice (eating cecotropes or "soft feces" directly from anus) (Hagen et al. 2019) even if is not ever observed.

Roth (1964) and Kingdon (1974) observed porcupine (*H. cristata* and/or *H. africaeaustralis*) eating flesh of dead mammals or birds. Roth (1964) also report an eight-ten week old captive cape porcupine fed on boiled ham and fried meat. No other data or observation concerning this unexpected feeding behaviour in the other two believed herbivorous porcupine (*H. cristata* and *H. indica*) are available and further investigation are desiderable.

The crested porcupine forages singly and/or in pair, except when the young (up 6-8 months) are accompanied by the parents (Santini 1980, Van Aarde 1987c). When foraging, they may cover a long distance (up to 10-12 km) from their burrow and each time they follow the same path and visit the same biotopes (Santini 1980, Pigozzi and Patterson 1990).

Wild crops		
Scientific name	**Family**	**Part eaten**
Arum maculatum L.	Aracee	rhizome
Tamus communis L.	Discoreaceae	tuber
Iris germanica L.	Iridaceae	rhizome
Allium orsinum L.	Liliaceae	bulb
Asparagus officinalis L.	Liliaceae	rhizome, sprout
Rumex crispus L.	Poligonaceae	tap-root
Cyclamen europaeus L.	Primulaceae	tuber
Fraxinus ornus L.	Oleaceae	bark
Cultivated crops		
Common name	**Family**	**Part eaten**
Corn	Graminaceae	seeds
Potato	Solanaceae	tuber
Sugar beet	Chenopodiaceae	tap-root
White cabbage	Crucifereae	central sprount
Chickpea	Leguminosae	seeds
Watermelon	Cocurbitaceaea	fruit, seeds
Melon	Cocurbitaceaea	fruit, seeds
Onion	Liliaceae	bulb
Pumpkin	Cocurbitaceaea	fruit, seeds
Vine	Ampelidaceae	fruit

Figure 29. List of wild and cultivated plants most palatable by the crested porcupine (Santini 1980)

The season, the habitat richness and ecological features of the place where the porcupine live strongly influence the diet (Saltz and Alkon 1989, Pigozzi and Patterson 1990, Brown and Alkon 1990, Bruno and Riccardi 1995, Oussou et al. 2006, Fattorini and Pokheral 2012, Lovari et al 2013a, Akram et al 2017, Lovari et al 2017, Mori et al 2017b). Overall porcupines are considered generalists herbivorous able to reach a high fiber digestibility with a average intake of 308g of fruit and 575g of barks through a time of 48 and 24 hours respectively (Riccardi and Bruno 1996). Porcupines change their feeding habits according to food availability, showing a wide ecological tolerance and fitness maximization (Brown and Alkon 1990; Pillay et al. 2015). *Hystrix* porcupines are capable of substantial formation and mobilization of fat reserves and this is a useful adaptation in environmental seasonally fluctuating (Alkon et al. 1986). Large inverse changes in the proportional composition of body water and body solids are likely such that total body mass is an inadequate index to body condition.

In Italy the crested porcupines adopt a generalist strategy, consuming a variety of food resources according to their seasonal availability (Bruno and Riccardi 1995, Lovari et al. 2017). Ranging movements seasonal changes are due to a sensitive response in food availability (Mori et al. 2014a).

Ranging movements of porcupines are determined by habitat richness (Lovari et al. 2013a). Accordingly, the distribution of food resources is the main determinant of habitat selection of this rodent (Mori et al. 2014a). The habitat selection reflects the food resources distribution also in *H. indica* (Fattorini and Pokheral 2012) and in *H. Africaeaustralis* (De Villiers et al. 1994).

In Mediterranean scrubwood roots, tubers and wild fruits are available almost throughout the year and are mostly eat by porcupines between October and March. During the warm season the Mediterranean scrubwood is a poor source of food, the fruit production reaches its yearly minimum (Lucherini and Lovari 1996, Massei et al. 1996, Lovari et al. 2013a) and crested porcupines range towards agricultural areas (Santini 1980, Mori et al. 2014a). Therefore in Mediterranean scrubwood underground storage organs of wild plants made up the staple of the diet of the porcupine throughout the year, followed by wild fruits in the cold period and by agricultural products in the warm months (Mori et al. 2017b). Conversely, porcupine select deciduous woodland for feed during both the cold and the warm periods and in this habitat seems to avoid the agricultural areas (Mori et al. 2014a). Investigation performed by Bruno and Riccardi on the diet of *H.cristata* in deciduous woodland show that roots are the main

food in the porcupine diet and are consumed at a similar frequency all year round. In winter and spring in deciduous woodland food availability is generally poorly and the consumption of storage organs and herbs increase as consequence of a decrease in the availability of other food categories, such as grass inflorescences and fruits (Bruno and Riccardi 1995). Conversely grass inflorescences are an important diet component in summer while porcupines fed on fruits mainly in summer and autumn (Bruno and Riccardi 1995). The consumption of herbs seems to be a feeding compensatory strategy to the variation of seasonal availability of the others food sources (Bruno and Riccardi 1995).

Aggressive behaviour and territoriality

The body of porcupine is characterized by the presence of erectile quills covering the back, hips and the back region of the body. Quills are the main and efficient defensive-offensive weapon in the inter-specific interactions (Findlay 1977, Van Weers 1983, Zherebtsova 2000).

The defensive behaviour of porcupine is characterized by many behavioural displays: crest and quills erection, tail rattling, hind foot stamping, growling and backwards or sideways attack. These displays are shown in different combinations with the different aggressiveness degree (Rosevear 1969, Felicioli 1991, Mori et al. 2014c). In most cases quills erection and the tail rattle are enough to get away single opponent. Only if in extreme difficulty violent fights with often lethal consequences for aggressors occur (Kingdon 1974, Mori et al. 2014c). The defensive behavioural displays can also be observed in intra-specific interactions. In this case the main offensive weapons are the incisors. The attack is frontal with rapid accelerations to pierce the back region of the opponent with the teeth (Felicioli 1991). As in inter-specie specific aggressiveness the combination of behavioural displays change with the different aggressiveness degree. Territoriality, establishment or the pair defence are the main reasons for lethal attacks occurs (Felicioli, 1991).

Figure 30. The adult porcupine male dead after a fight with the female porcupine. The wounds caused by the incisors teeth are visible (Photo by Felicioli)

Intra-specific aggressiveness in crested porcupine was observed by Felicioli (1991) in two individuals (a male and a female) kept in captivity between September and November. In these occasions the two individuals have peacefully cohabited for 7 months in the same enclosure. Subsequently the male has been moved in another enclosure and the female has established a pair relationship. Accidentally, the male entered in the enclosure of the pair and fighting between the male and the female occurred with lethal consequence for the male (Fig 30). This event allowed to Felicioli (1991) to hypothesise the presence of territoriality in crested porcupine. However, territorial behaviour in wild crested porcupines has never been demonstrated (Massolo et al. 2009, Mori and Lovari 2014) and Mori et al. (2014a) refers to crested porcupine as non-territorial rodent. Territoriality behaviour was recorded in captive *H. asfricaeaustralis* by De Villiers et al. (1994) and Corbet and Van Aarde (1996) and hypothesised in free-ranging cape porcupines (Corbet and Van Aarde 1996). Alkon and Saltz (1983) suggest the presence of well-defined territories in free-ranging Indian crested porcupines with stable and largely exclusive home-ranges serving as feeding territories. Morris and van Aarde (1985), De Villans et al. (1994) and Corbet and Van Aarde (1996) observed that captive males of *H. africaeaustralis* scent-marked the perimeter of feeding sites with the peri-anal glands as territory maintenance. Scent-marking was also observed in captive *H. indica* by Sever and Mendelssohn (1989b).The territoriality seems to constrain the use of habitat in the Indian and cape porcupine (Sever and Mendelssohn 1989b). Investigation performed by Corbet and Van Aarde (1996) on free-ranging porcupines show that porcupines utilize the same area throughout the year and maintain a small, exclusive territory within a larger, non-exclusive home range. Moreover the encounter between neighbour porcupine families is rarely recorded. Therefore acccording to Corbet and Van Aarde (1996) is probably that the territory in cape porcupine is maintained by scent-marking rather than through direct interactions.

The scent-marking behaviour in crested porcupine has been observed only by Felicioli (personal communication) in semi-captive specimens. The individuals in the same night frequently walked on the stones present along the perimeter of the enclosure. On the stones presence of marking was visibile during the enclosure inspection. Felicioli et al. (1993) demonstrate the presence of eight new proteins (odorant-binding proteins) in the nasal tissue of crested porcupines suggesting a discriminating function of these proteins in the process of odour perception to mediate inter and intra-specific chemical communication. It cannot be excluded the involving of the peri-anal glands secretions and urin in scent-marking territory

and chemical communication. Behavioural data and bioassays are lacking and more investigation is desiderable.

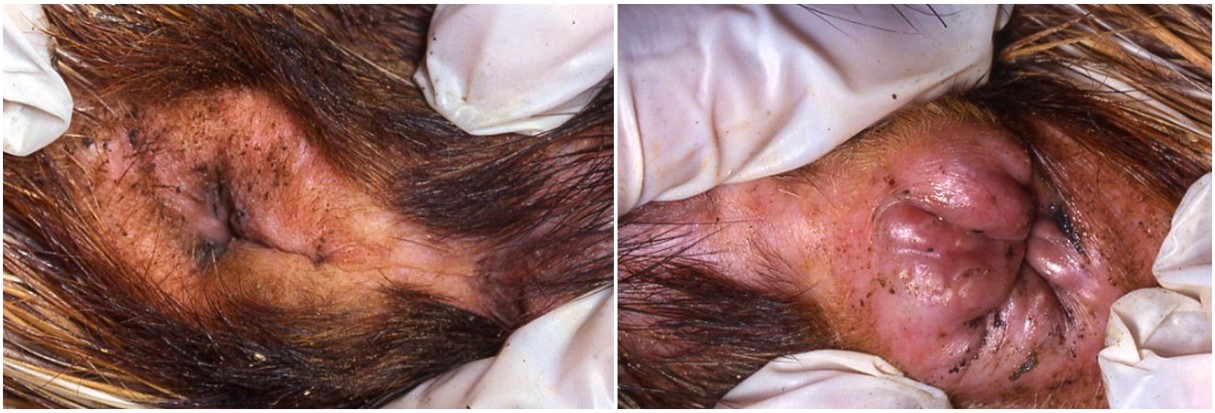

Figure 31. Anal region (left) and extroflected peri-anal glands (right) of the crested porcupine (Photos by Felicioli)

The peri-anal glands are well developed in the crested porcupine (Fig 31) and their waxy secretion has a peculiar odour that is reported to be perceivable around the dens (Massolo et al. 2009). Massolo et al. (2009) suggest that the peri-anal secretions may play a role in individual recognition. Despite the reasonability of the above mentioned hypothesised, further research is strongly needed to assess the role, or roles, of the urine and peri-anal glands in crested porcupines scent-marking and individual recognition.

Porcupine-human conflicts

The porcupines are considered as agricultural pests throughout its distribution range: *H. indica* (Greaves and Khan 1978, Moran 1981, Alkon and Saltz 1985b, c, 1988b, Alkon 1987, Hafeez 2011, Khan et al. 2014), *H. africaeaustralis* and *H. cristata* (Kingdon 1974, Skinner and Chimimba 2005). Also in Italy *H. cristata* is commonly believed as a pest species (Laurenzi et al. 2016). According to Skinner and Chimimba (2005) the cape porcupine is one of the species that have benefitted from an increase in the production of agricultural crops leading to a porcupine-human conflict. As natural food source have become limited, porcupine have turned to a larger and much more accessible and varied food source as agricultural crops (Khan et al. 2014). Trophic activity of the crested porcupine in Central Italy causes damage to crops in cultivated areas due to animal preference for maize, potatoes, sunflowers, pumpkin and melon (Santini 1980, Bertolino et al. 2015) and it is also reported to be responsible of trees debarking (Santini 1980). The crested porcupine crop damages occur mainly in private vegetable gardens in the surroundings of human settlements (Laurenzi et al. 2016) and this has increasing the conflict with humans.

Figure 32. Porcupine damage on pumpkin and melon in vegetable gardens
(Photos by Vecchio and Coppola)

The conflicts with this rodent are locally increased also due to wounds occur in hunting-dogs as well as damage performed to manmade riverbanks in which dig its burrows. An investigation performed by Laurenzi et al. (2016) show that in Central Italy the porcupine damage is about 3-4% of the overall total damage of wildlife in agricultural crops. The higher damage occurs in crop as melon, pumpkin, watermelon, sunflower and onion (Fig 32). Generally the crested porcupines damage are low even if may occasionally account for up to 50% or more the overall agricultural loss. Heaviest damage occurs to iris bulb (up to 100%) and potatoes (up to 90%). Conversely bark-stripping/gnawing in crested porcupine is a marginal problem and occurs only occasionally mainly on *Fraxinus ornus* and *Pyrus pyraster* (Laurenzi et al. 2016). All crop damages by porcupines are limited to small single vegetable gardens. Despite the losses are high it became negligible if compared to damages recorded on a large scale for other wildlife species as wild boar (Laurenzi et al. 2016). Moreover the porcupines crop damage in vegetables garden may be easily avoid by using simple 50 cm height fences partially buried (Sforzi et al. 1999).

The presence of porcupine burrow along the manmade riverbanks frequently causes the collapse of the banks with consequent flood events. The management of this species as well as that of other semi-fossorial species (e.g. badger and coypu) along the banks is a key factor for the human-safety. The investigations performed by Felicioli between 1996 and 1999 on the management of porcupine burrows in 6 rivers in the Province of Pisa show that the presence of porcupine burrow in these habitat are related to the manmade riverbanks vegetation cover (Santini 1999). The cut of the vegetation cover in the manmade riverbanks has resulted in the disappearance of some previous identified burrow and a high reduction of the fossorial activity in the others. Therefore maintaining the manmade riverbanks free from vegetation cover is a simple method in order to limit the presence of porcupines and increase the safety of the manmade riverbanks from possible collapses (Santini 1999). Conversely animal's removal without vegetation cover managements could cause a new re-colonization of the manmade riverbanks.

Status of crested porcupine in Italy

The crested porcupine is strictly protected by European Law with the Bern Convention of 1979 and by Italian Law since 1981 with the National Law n. 503 of 1981. Despite this law protection the porcupines is a widely poached species for its status of pest and for its tasty meat (Amori and Capizzi 2002, Chevallier and Ashton 2006, Lovari et al. 2017). Lovari et al. (2017) report a poaching rate over 70% on individually marked porcupines (n=11) during first three month of study.

The presence of crested porcupine in wildlife rescue centers in Italy in the last 10 years has undergone a strong increase with high mortality rates during hospitalization. The main causes of hospitalization are impacts with cars or finding of porcupettes. In the most cases the porcupines die for the severity of injuries and the adults that survive as well the porcupettes are inadequately managed. To date there are no guidelines for the management, rehabilitation and release in the wild of this rodent. The porcupines are generally released near the site of occurrence without any kind of post-liberation monitoring. Moreover for this species there are no scientific evidences about the efficacy of the rescue process made in wildlife rescue centres as well as the liberation success.

Despite the strong expansion of this rodent recorded in Italy (Mori et al. 2013a, Vecchio et al. 2018) there are no data concerning the density and the consistency of the Italian porcupine population. Niethammer (1982) refer to a crested porcupine population density of 2-4 individuals/km^2. Trucchi and Sbordoni (2009) hypothesised that the population living in North-Central Italy can be estimated as about 80.000-120.000 individuals. However to date no census of the species was even performed and there are no a census methodology. The IUCN Red List of threatened species includes the crested porcupine among the “Least concern” species. However, it cannot be excluded that the threats reported above could be an obstacle to the conservation of this precious rodent of the Italian fauna.

Lacks of knowledge on the health aspects of crested porcupine are available. At the best of literature analysis the only data available refer to one case of Pulmonary Adiaspiromycosis by *Emmonsia crescens* in a young porcupine found dead (Morandi et al. 2012) and one cases of *Toxoplasma gondii* infection in a captive adult female specimens with neurologic signs (Harrison et al. 2007). Presence of *Bacillus thuringiensis* was detected in captive *H. cristata* (Lee et al. 2002). Shaftenaar (2001) and Mouchantat (2003) also report one case of infection of Foot-and-Mouth Disease (FMD) in *H. cristata* in a zoo. *Salmonella* in cretsetd porcupine

feaceas in Nigeria was also searched by Falade and Durojaiye (1976) without obtain any result.

Ectoparasites in crested porcupine are rarely recorded and the most frequent species found are flea *Pulex irritans* and hard tick *Ixodes ricinus* (Monetti et al. 2005, Mori et al. 2015b). Parasitological positivities for *Trichuris infundibulum* and *T. hystricis* have been reported in *H. cristata* by Pavlov (1957). Among the other species of genus *Hystrix* parasitological positivity for *Giardia* sp. in Indian crested porcupine (Chakraborty et al. 2015) and serological positivity to *Leptospira* serovars Javanica, Hurstbridge, Ballum, Celledoni and Hardjoprajitno in captive Malayan porcupine (*Hystrix brachyura*) were found (Siti-Nurdyana et al. 2016).

No data are available concerning haematological and biochemical parameters in crested porcupine. Among hystricomorph rodent haematological and biochemical parameters were investigated in coypu (*Myocastor coypus*) (Martino et al. 2012) and capybara (*Hydrochoerus hydrochaeris*) (Arouca et al. 2000, Di Chiacchio et al. 2014) while among porcupine only few data are reported in some species of new world porcupines by De Almeida et al. (2011) in captive bristle-spined porcupine (*Chaetomys subspinosus*) and by Moreau et al. (2003) in Brazilian porcupine (*Coendou prehensilis*) and black-tailed hairy dwarf porcupine (*Coendou melanurus*). Therefore new investigations concerning the density, behavioural ecology and health aspects are desirable in order to increase the knowledge on this Italian rodent.

2.Aim of the work

The aim of this study was to investigate the behavioural ecology and health aspects of crested porcupine in order to contribute to the knowledge of porcupine biology for the set up of new potential tools useful for the management and conservation of the species.

The spatial distribution of settlements was here analysed for the first time in order to verify its binary distribution by using cluster analysis.

The occurrence of co-habitation of the same settlement by crested porcupines, European badgers and red foxes in a condition of a high density of available settlements was investigated by camera-trapping in order to assess the prediction that: I) porcupines co-habit in the same burrow with badgers and/or foxes independently from settlements availability, II) the lack of aggressiveness and/or predation between these three species in order to permit co-habitation.

Also the frequency and the spatio-temporal inhabitation of settlements by crested porcupine were investigated in order to assess the prediction that: I) porcupines inhabit the settlement with high frequencies than other burrowing species, II) exclusive use of one settlement by each porcupine family thoughout the year, III) presence of porcupine intraspecific territoriality for settlement defence.

Data collected on the spatio-temporal use of settlement were used for elaborate an estimation of porcupine population abundance and perform a first attempt to develop a porcupine census model.

The camera trapping monitoring was also used to collect new other behavioural ecology informations concerning the motor activity rythm of porcupine, feeding habit and reproduction.

Biological samples (e.i. feaces, blood and organs) from dead and captured porcupines were collected in order to preliminary investigate some health aspects of porcupine. The health monitoring focused on the evaluation of haematological and blood biochemical profile, detection of ectoparasite and zoonotic parasitic and infectious diseases, in particular Giardiasis and Leptospirosis, of crested porcupine.

3.Materials and Methods

3.1.Study Areas

A 3 years investigation has been performed in the years 2016-2019 within two experimental areas.

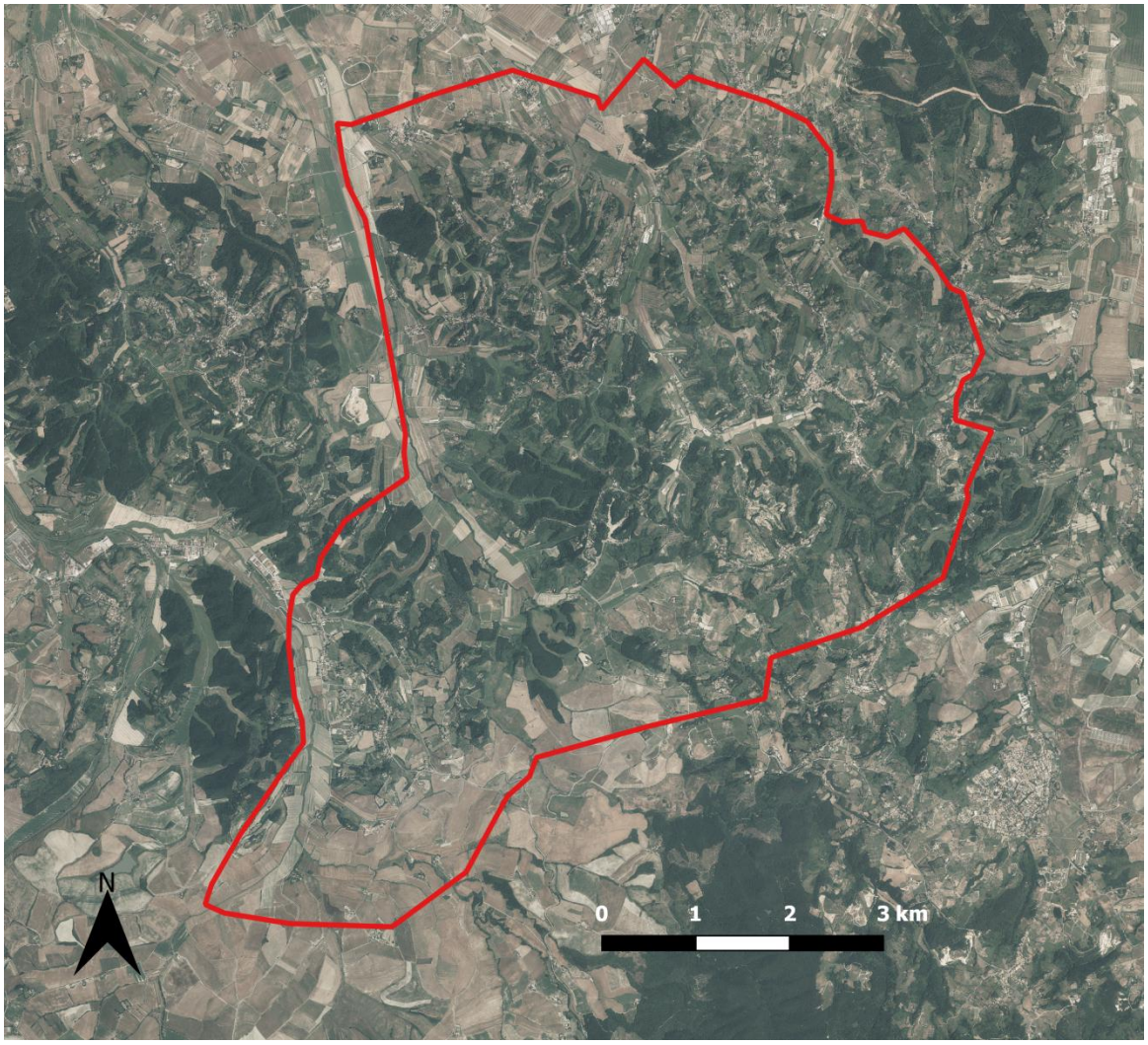

Figure 33. Experimental area (EA1) of 4.476 ha in Crespina-Lorenzana and Casciana Terme Lari

The first study area (EA1) was located in a hilly area (86 m a.s.l) of 4.476 ha in Crespina-Lorenzana and Casciana Terme- Lari (43.55943 Long - 10.56684 Lat) in the province of Pisa (Tuscany, Central Italy) (Fig 33). The study area is characterized by a high biodiversity and environmental fragmentation in which small woody areas are interspersed with uncultivated and/or cultivated areas and rivers. The woody cover is characterized by deciduous forest mainly of *Robinia pseudoacacia*, *Quercus cerris*, *Q. pubescens* and *Q. ilex* with a thick and thrive undergrowth. The undergrowth are composed by a large variety of shrubs and herbaceous plants such as *Sambucus nigra*, *Rubus ulmifolius*, *Laurus nobilis*, *Ruscus*

aculeatus, *Juniperus oxycedrus*, *Asparagus acutifolius*, *Cyclamen* sp., *Tamus communis* and *Orchis provincialis*. The agricultural activities include vineyards, olive groves, corns crops, alfalfa crops, sorghum crops, sunflower crops, wheat and barley crops, open field and private vegetable crops.

In the study area live a wide variety of wildlife mammals such as the crested porcupine, european hedgehog (*Erinaceus europaeus*), wild boar (*Sus scrofa*), roe deer (*Capreolus capreolus*), pine marten (*Martes martes*), stone marten (*Martes foina*), skunk (*Mustela putorius*) badger (*Meles meles*), hare (*Lepus europeus*), eastern cottontail (*Sylvilagus floridanus*), wild rabbit (*Oryctolagus cuniculus*), red fox (*Vulpes vulpes*), and wolf (*Canis lupus*). The climate is temperate with hot summers (average 23.1 °C in August) and rainy and mild winters (average 6.8° C in January). The average annual rainfall is 842 mm with peaks between October and December (Climate-Data. Org, Regional Hydro-geological section of Tuscany Region).

Since 2018 the investigation was extended to a second experimental area (EA2) of 514 ha was located in the wildlife hunting reserve "Camugliano" (621 ha) (43.60305 Long- 10.64909 Lat) in Ponsacco-Capannoli in the Province of Pisa at 24 m a.s.l. (Tuscany, Central Italy) (Fig 34).

The study area is characterized by a low environmental biodiversity and fragmentation. The woody area is evenly distributed for a total of about 100 ha. The woody cover is characterized by cops of *Q. cerri, Q. pubescens* and *Q. ilex* while the undergrowth is mainly composed by *Ruscus aculeatus*. The agricultural activities include mainly alfalfa, wheat and sunflower crops but also vineyards, olive groves and vegetable crops. In the study area a mixed crops of chicory, forage cabbage and sorghum are available for the exclusive use of the wildlife.
The mammals fauna of this area include the crested porcupine, european hedgehog, wild boar, roe deer, fallow deer (*Dama dama*), pine marten, stone marten, badger, hare, eastern cottontail and red fox. The climate is temperate with hot summers (average 23.4 °C in July) and rainy and mild winters (average 6.7° C in January). The average annual rainfall is 848 mm with peaks between October and December (Climate-Data. Org, Regional Hydro-geological section of Tuscany Region).

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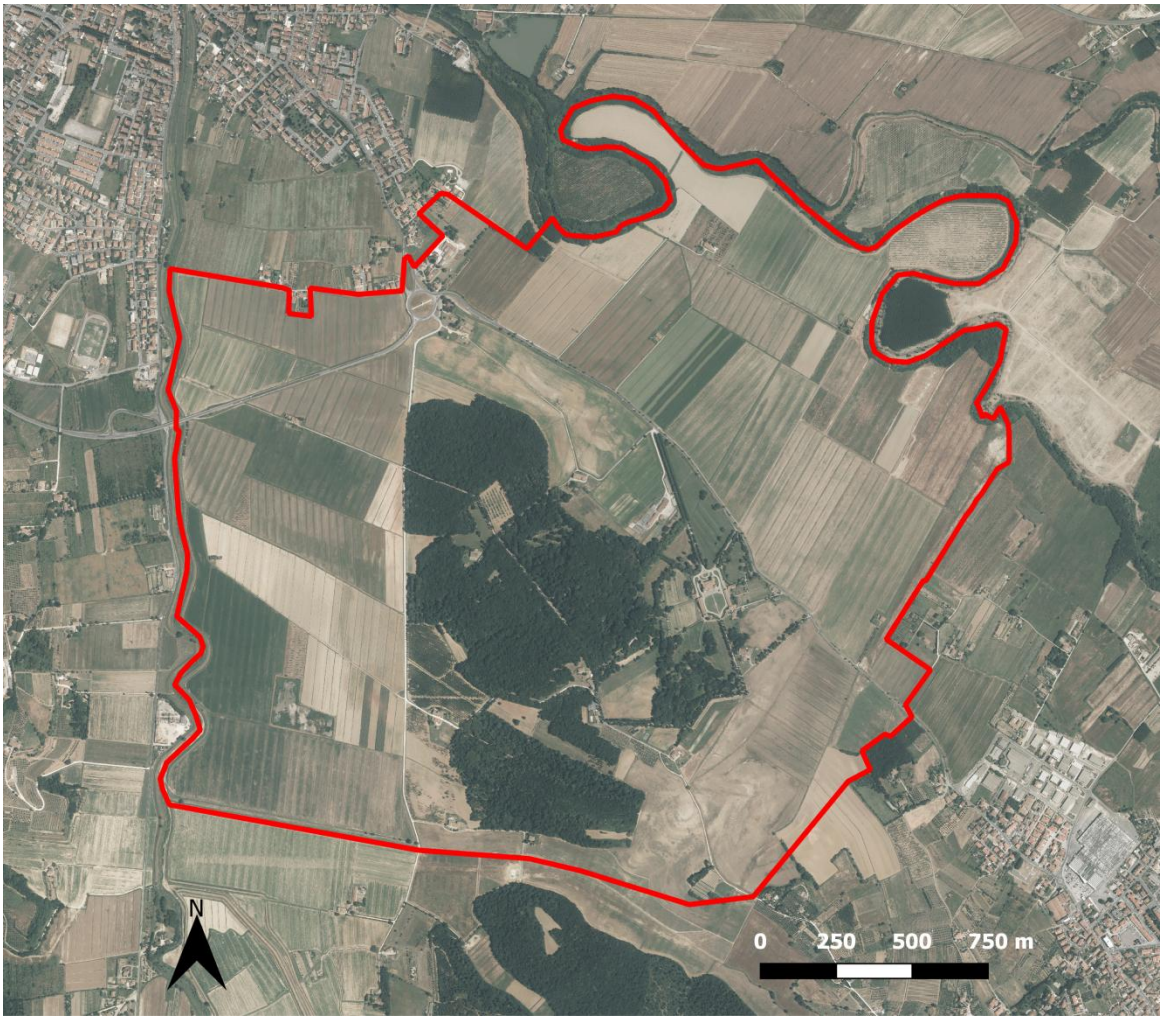


Figure 34. The Experimental area (EA2) of 621 ha in the wildlife hunting reserve “Camugliano”

This second study area was also used as control area for replicate and verifies the validity of the population estimate processed. In this area the porcupines population and all settlements present was already known.

3.2. Geolocation of settlements

Since 2017 the largest number of settlements in the study area EA1 was searched and geolocated.

The settlements ("Setts") were identified as a set of ground entrance holes detectable on the surface. Sets of ground entrance holes continuously detected were assigned as real settlements. Conversely, the sets of ground holes continuously detected but not clearly circumscribable were assigned as putative settlements. The detection of settlements was performed through active research in the area and in collaboration with hunting associations and citizens. Within the study area EA2 all settlements were previously known and only geo-location was performed in 2018. The settlements in both study areas were geolocated using GPS trackers such as Locus Map 3.38 android application and Garmin Oregon® 550 (Fig 35).

Figure 35. Garmin Oregon ® 550 (left) and Locus map 3.38 android application (right) used for settlements geolocation

Within each study area a unique progressive number was assigned to each settlement. Among the detected settlements the following data were collected:

- Geographical coordinates of the settlements
- Geographical coordinates of all ground entrance holes
- Habitat (wood, riverbanks, uncultivated areas)
- Soil type (sandy or clay)
- Presence of patch areas with detectable porcupine digs in the vicinity of settlements (cultivated and/or uncultivated areas)

- Orientation of settlement by using compass
- Number of ground entrance holes
- Orientation of each ground entrance holes by using compass (Fig 36)
- Status of settlement (used or abandoned)

Figure 36. Detection of burrow entrance hole orientation with compass (Photo by the Author)

The status of settlement was evaluated considering the following parameters:

- Presence/absence of fresh spoil heap in front of ground entrance holes
- Presence/absence of leaves and branches inside the visible part of the tunnels
- Presence/absence of cobwebs in front of ground entrance holes
- Presence/absence of signs of presence (quills, faeces, footprint, hairs) of burrowing species (red fox, badgers, porcupines).

Spatial-distribution analysis of settlements

Within each study area all detected ground holes and settlements were mapped using QGis 2.18 software. The spatial-distribution of both the ground holes and the settlements were analysed by Cluster analysis in R 3.6.1 software using 'cluster', 'factoextra', 'fcp' and 'NbClus't packages (Kassambara 2017). The clustering model that fit better for ground holes and settlements distribution analysis was assessed using the set of ground holes of the real settlements geo-located in EA1. Two clustering methods were used for a total of four clustering models tested:

1. **Hierarchical Clustering**
    - Agglomerative clustering, average linkage
    - Agglomerative clustering, Ward.D linkage
    - Agglomerative clustering, complete linkage

2. **Partitioning Clustering**
    - K-medoids algorithm

The agglomerative clustering is the most common type of hierarchical clustering use to group objects in clusters based on their similarity (Kassambara 2017). Each type of linkage uses a different method for computing distance between clusters.
The average linkage: define the distance between two clusters as the average distance between the elements in cluster 1 and the elements in cluster 2.
The Ward's minimum variance method (Ward.D): minimizes the total within-cluster variance.
The complete linkage: define the distance between two clusters as the maximum value of all pairwise distances between the elements in cluster 1 and the elements in cluster 2.
The k-medoids algorithm is a clustering approach for partitioning a data set into clusters in which each cluster is represented by one of the objects in the cluster named cluster medoids. The term medoid refers to an object within a cluster for which average dissimilarity between it and all the other members of the cluster is minimal. It corresponds to the most centrally located point in the cluster (Kassambara 2017). The Euclidean distance between geographical coordinates of ground holes was used to calculate the objects similarity.
External cluster validation was performed in order to quantify the agreement between the identified clusters obtained from each clustering models tested and the real settlements using

both the corrected *Rand index* and *Meila's variation index* (VI). The corrected *Rand index* varies from -1 (no agreement) to 1 (perfect agreement).
The clustering model with *Rand index* nearest to 1 and lowest *Meila's variation index* was choose as model for clustering the ground holes of the putative settlements both in EA1 and in EA2.
The agglomerative clustering with average and Ward.D linkage model were used as models to evaluate clustering of the ground holes of the putative settlements.

The internal cluster validation was performed in order to evaluate the goodness of a clustering structure. The correlation between the cophenetic distances (height of the dendrogram where the two branches that include the two objects merge into a single branch) and the original distance data was used to verify the validity of clustering. The linkage model with value of correlation > 0.75 was chosen for clustering the ground holes of the putative settlements.

The optimal number of cluster was defined using the average Silhouette method.

The agglomerative clustering with the same linkage model used for ground holes clustering was applied for the settlements spatial-distribution analysis in both study areas. In this case the Euclidean distance between the geographical coordinates of the settlements centroids was used. The geographical coordinates of the centroids were calculated as mean of the geographical coordinates of the all ground holes of each settlement. The value of maximum distance recorded between two settlements inhabited by only one porcupine family group was used to cut the dendogramm and clustering the settlements.

The ground entrance holes and settlements orientations in both study areas were analysed by the Circular Statistic in R 3.6.1 software using 'circular' package. The mean vector was calculated both for ground holes and settlements orientation. For settlements the longest vector between unimodal and bimodal was used. The mean vector length 'r' is the measure of dispersion of points around the mean angle 'm' (Batschelet, 1965) and its length ($0 < r < 1$) varies according to the concentration of data around the mean angle, where $r = 1$ indicates minimum dispersion and $r = 0$ means maximum dispersion (Batschelet, 1965; Maia-Carneiro and Rocha, 2013). To test the uniformity of compass orientation of settlements the Rayleigh test was performed.

Moreover, the frequencies of settlements and ground entrance holes detection (Xi) for each exposition sector (i = North-east, South-east, North-west and South-west) in both study areas have been analysed by Manly selection index (w) in R 3.6.1 software using 'raster', 'sp',

'rgdal' and 'adehabitatHS' packages (Manly et al. 1993, 2002). The selection index was computed as the ratios between used and available exposures proportion. The available exposures proportions were obtained by the exposure raster provided by the Tuscany Region and processed using the Q-Gis software.

The frequency of settlements detection in different habitats (wood, riverbanks and cultivated areas) and soil types (sandy or clay) were analysed using chi-square test (χ^2).

The resulted linear distance from patch and/or coultivated areas was measured using QGis "line measurement" tool in both study areas. Moreover, the settlements density on woody perimeter kilometre (Setts/km) was determined. The woody perimeter was extrapolated from layer of land use of Pisa province using QGis.

3.3. Porcupines capture and marking

The capture-marking activity of resident porcupines in EA1 and EA2 was approved by the Italian Institute for Environmental Protection and Research (ISPRA) with protocol number 22584 of the 8 May 2017 and protocol number 150071 of the 16 March 2018 respectively. The capture activity was also approved by Tuscany Region with the Decree n. 14235 of the 3 October 2017 and n. 4842 of the 6 April 2018 respectively for EA1 and EA2.

The capture-marking activity was performed between 2018 and 2019 in both study areas.

In EA1 in 2018 four capture campaigns between March and June were performed. From March to May 2018 the traps were activated for 10 days per month while in June 2018 for 30 consecutive days.

In 2019 a single capture campaign of 30 days between February and March was carried out. In the experimental area EA2 two capture campaigns of 30 days were performed between February and March in 2018 and 2019.

Within study area EA1 the capture-marking activity was performed only in Crespina-Lorenzana.

Within each experimental area, in each capture session, six traps were placed in the most used pathways (Fig. 37 and Fig. 38).

Wire mesh traps with double entrance (110 x 42 x 42 cm) baited with corn and potatoes were used for trapping porcupines (Fig 39).

Each capture trap was equipped with a numbered identification plate supplied by the Province of Pisa (Fig 40) and was monitored by a camera-trap set to record 20s videos.

The capture traps were checked two times a day, one after sunrise and one before sunset. The no-target species captured were immediately released.

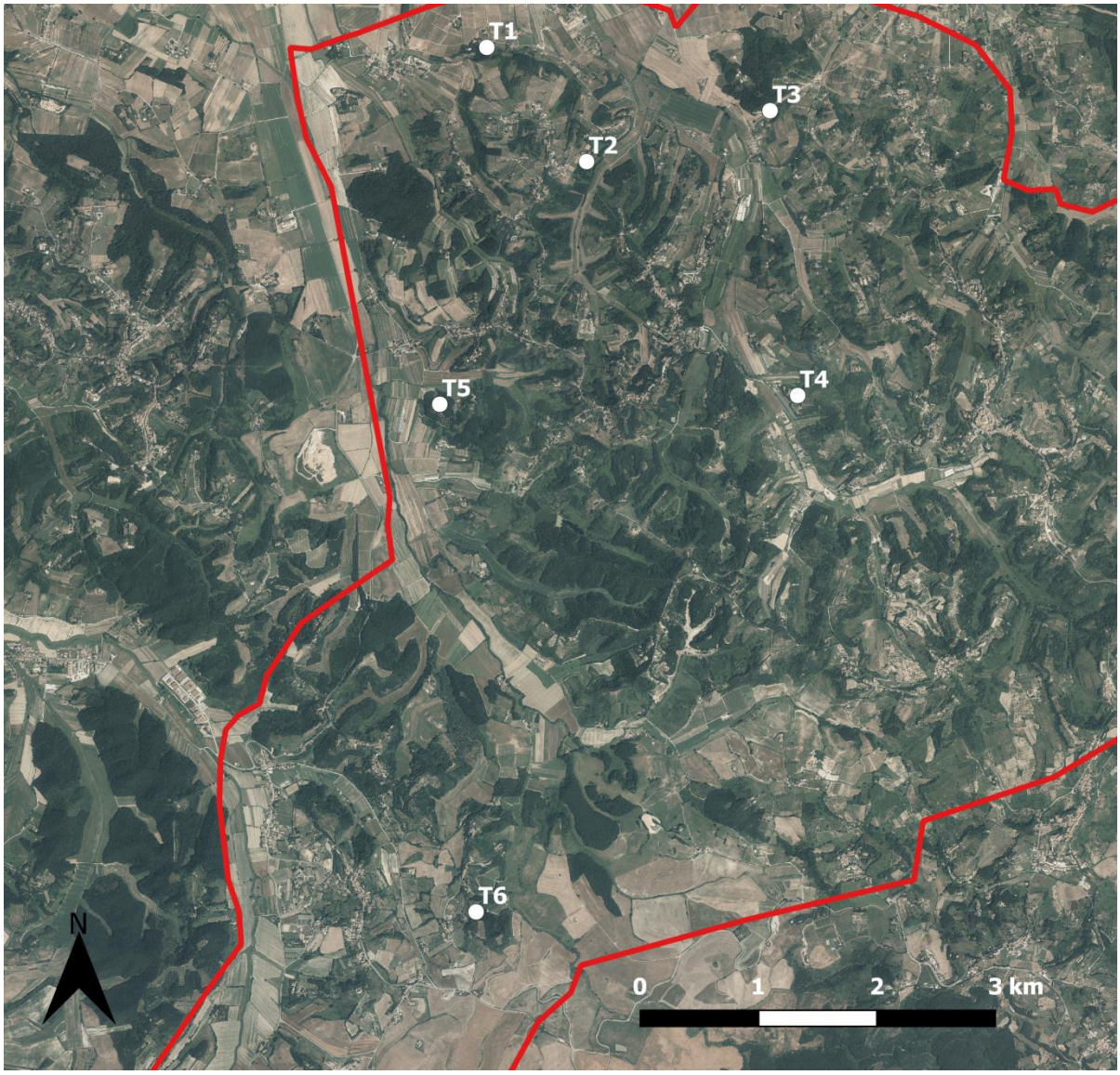


Figure 37. The 6 strap sites in EA1.Image elaborate using Q-Gis

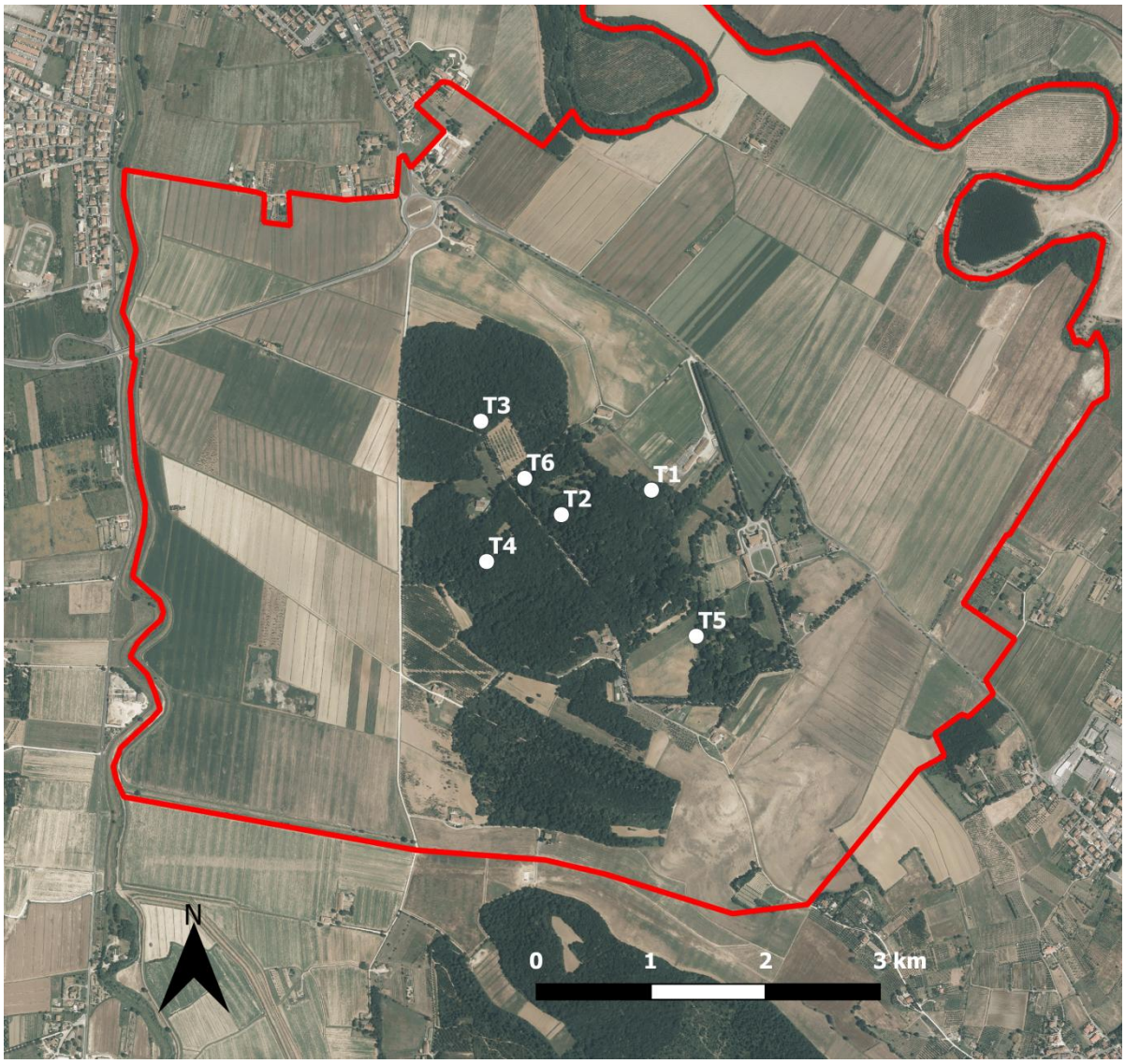


Figure 38. The 6 trap sites in EA2.Image elaborate using Q-Gis

Figure 39. Capture trap foraged with corn and potatoes and monitored by camera-trap (Photo by the Author)

Figure 40. Plate with the identification number supplied by the Province of Pisa located on a capture trap (Photo by the Author)

Porcupines handling procedures and sampling protocol

The handling of the captured animals was performed under the surveillance of the veterinarian Enrico D'addio, as expected in the authorization procedures. The whole trap with the trapped porcupine was weighed by an electronic dynamometer (Fig 41). The weight of each cage has been previously measured and the animal weight was obtained by difference.

The age class of each captured porcupine was estimated on the basis of the weight (Table 1).

Table 1. Ages range (month) and weight (kg) parameters used to assess the age class of porcupine

Age class	Ages range	Weight
Porcupettes	<5	<5
Sub-adults	5-11	5-11
Adults	>12	>11

Porcupines were anaesthetized with Zoletil 100® (Massolo et al. 2003) by intramuscular injections in the lumbar region using an air-compressed syringe (Mini-ject 2000, 2 ml) darted by a blow-pipe (Fig. 42 and Fig 43). The injection dose was to 0.05 ml/kg of live weight.
After sedation the animals were removed from the capture traps and a wet cloth on the eyes was applied (Fig 44). For each captured porcupine the sex was detected, the age class estimated and the body temperature measured (Fig 45). The presence of ectoparasite, scars and/or injuries were also recorded.

Each porcupines was individually marked by colored adhesive tapes apply on the quills, by white or black paint sprayed on the crest and/or on the tail or else by a combination of these latest (Fig 46). The marking was applied in order to individually recognise the porcupines recorded in camera-traps videos.

Figure 41. Weighing operation of captured porcupines. In the left two operators while raise the capture trap with the porcupine inside and in the right the detail of electronic dynamometer using for weigh the animals (Photo by Coppola and Felicioli)

Figure 42. (A) Preparation of the air-compressed syringe for sedation and (B) dard-syringe used for animals immobilization (Photo by Coppola and Felicioli)

Figure 43. Porcupine sedation using a blow-pipe (upper picture). In the lower picture the air-compressed syringe in the porcupine lumbar region is visible (Photo by Coppola and Felicioli)

Figure 44. Porcupine removing from capture trap after sedation (left) and immobilization of the animal after application of a wet cloth on the eyes (right) (Photos by Felicioli)

Figure 45. Sex assessment of porcupine (A:male and B:female), measuring of the body temperature (C) and detection of injuries (D) in captured porcupines ((Photo by Coppola and Felicioli)

Figure 46. Porcupines marking. In the upper, the application of colored adhesive tape on the quills and in the lower, the porcupine marking with a combination of adhesive tape on quills and white paint on the crest (Photo by Felicioli and Coppola)

For each captured porcupine the following sampling protocol was also applied:

- 1 ml of blood with anticoagulant (EDTA) (if possible)
- 3-4 ml of blood without anticoagulant
- Ectoparasites (if presents)
- 1 faecal samples (if present in the capture cage) (Fig.47)

Blood samples were collected from the femoral vein (Fig 47) into tubes and each blood sample without EDTA was subsequently divided in two sub-samples, one for biochemical analysis and one for microbiological investigation (Fig 48).

Figure 47. Porcupine blood sampling (left) and faecal sample collected from capture cage (right)
(Photos by Felicioli and Coppola)

Figure 48. Porcupine blood samples and faecal samples
(Photo by Felicioli and Coppola)

After awakening from sedation the animals were immediately released. In some cases the animals were released directly in burrow (Fig 49 and Fig 50). For each capture animal, the date, the number of capture cage, the post-anaesthesia immobilization and awakening time, the all detected parameters, the collected samples and the marking apply were reported in a redact form (Fig 51).

Figure 49. Liberation of a marked porcupette in front of the burrow entrance hole. In the left, the operator with the jute bag with the animal inside and in the right the marked porcupette while enter in the burrow (Photo by Felicioli)

Figure 50. A marked porcupette (left) and a marked adult porcupine while enter in their burrow (Photo by Coppola and Felicioli)

CAPTURE FORM

CAPTURE N°: _____

N° CAPTURE CAGE: _____

DATE: _____

WEIGHT: _____

ANESTHETIC INJECTION DOSE: _____

POST-ANAESTHESIA IMMOBILIZATION TIME: _____

POST-ANAESTHESIA AWAKENING TIME: _____

MORPHOMETRIC DATA:

SEX : **FEMALE** ☐ **MALE** ☐

AGE CLASS: **ADULT** ☐ **SUB-ADULT** ☐ **CUB** ☐

BODY TEMPERATURE: _____

ECTOPARASITES: **YES** ☐ **NO** ☐ **TYPE:** _____

SCARS / INJURIES: : **YES** ☐ **NO** ☐ **TYPE:** _____

SAMPLES:

BLOOD WITH ANTICOAGULANT: **YES** ☐ **NO** ☐ **Quantity:** _____

BLOOD WITHOUT ANTICOAGULANT: **YES** ☐ **NO** ☐ **Quantity:** _____

FAECAL SAMPLES: **YES** ☐ **NO** ☐ **Number:** _____

ECTOPARASITES: **YES** ☐ **NO** ☐

TYPE OF MARKING:

_____

_____

_____

_____

_____

Figure 51. Capture form which has been filled out for each captured porcupine

3.4. Camera-trapping

In study area EA1 the camera-trapping activity was performed in the settlements recorded in Crespina-Lorenzana between January 2017 and July 2019. All detected settlements were first alternatively monitored for 60-90 days. This preliminary monitoring allowed us to assess which settlements was mostly used by crested porcupine and between these 16 experimental settlements were chosen (Fig 52).

Each experimental settlement was monitored at different time range between 12 to 24 months by using two camera-traps. The number of camera traps in each settlement was chosen in order to monitor the greatest number of holes. In S2, S3, S4, S5, S7, S8, S10, S11, S12, S13 and S15 given their small size (from 2 to 6 holes), all ground holes have been monitored. Conversely, 4 out of 8, 3 out of 8, 3 out of 13, 3 out of 7 and 4 ou of 12 ground holes have been respectively monitored in S1, S6, S9, S14 and S16 due to their big size (more than 6 holes). Ten experimental settlements were continuously monitored for 12 months (S6, S7, S8, S9, S10, S11, S12, S13, S14, and S16), two for 15 months (S3, S15), two for 22 months (S4, S5) and two for 30 months (S1, S2). Each camera trap was sited at 2 m distance and 1 m high from entrance hole where clear signs of activity were presents (e.g. footprints, quills, scats, fresh spoil heap).

In study area EA2 the camera-trapping monitoring started in March 2018 and all settlements were preliminarily monitored as in EA1. The preliminary monitoring in EA2 lasted 30-60 days. Also in this case, within the settlements mostly used by the porcupines 8 experimental settlements were chosen (Fig 53). Each experimental settlement was continuously monitored for 1 year using one camera-trap sited in front of a burrow ground hole where clear signs of activity were presents.

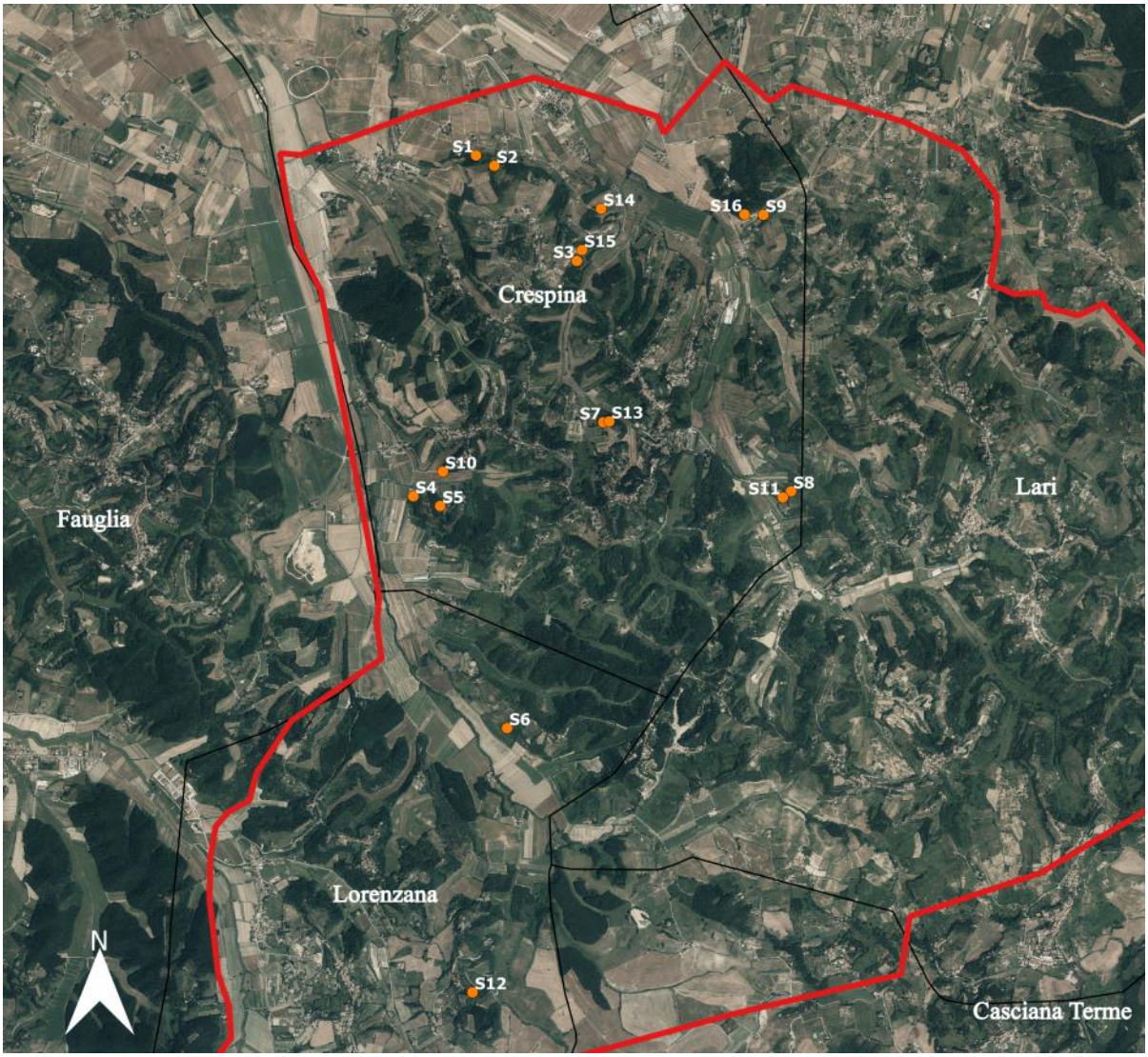


Figure 52. The 16 experimental settlements in EA1. The black line indicates the border of the municipalities. Image elaborate using Q-Gis

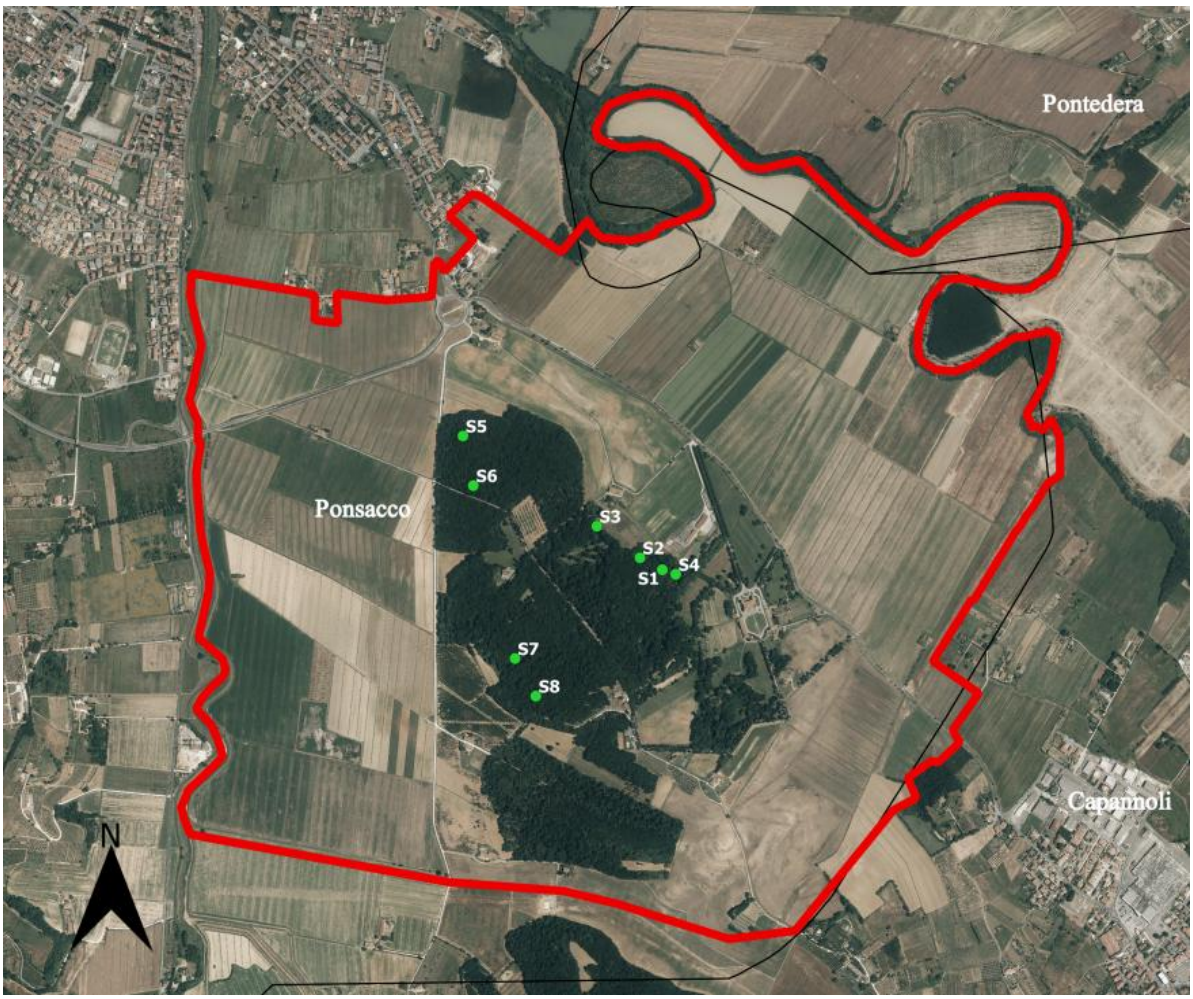


Figure 53. The 8 experimental settlements in EA2. The black line indicates the border of the municipalities. Image elaborate using Q-Gis

Forty five digital pocket cameras (Boskon Guard BG520, BG530 and Num'axes PIE1009) with passive infrared sensor (PIR) were used. The camera traps were deployed at a minimum distance of 1.5–2.5 m at a height of 1.5 m and set to record 20 s long video clips without time lapse and to stamp date and hour in each video recorded. Video trapping success was expressed as percentage of total number of useful videos in which animal were detect.

Camera-traps video recordings were checked and filed on a weekly basis. The videos recorded by camera-traps were used to investigate the inhabitation of settlements and interaction between crested porcupine, badgers and red foxes, the spatio-temporal inhabitation of settlements by crested porcupine and to detect, verify and describe the diurnal motor activity and sunbathing behaviour, the scavenging behaviour, the reproductive behaviour and the pattern of first daily emergence time from burrow in crested porcupine.

Individual recognition of the animals in camera-traps videos

The porcupines recognition in the recorded videos was possible for the presence of the specimens individually marked.

In addition other specimens were individually recognisable by the presence of phenotypic peculiarities (e.g. blindness, presence of injuries). The presence of the specimens marked and/or individually recognisable allowed us to identify the families group.

For each recognised porcupine family the number of the individuals was recorded.

Whenever possible, the individual recognition for both badgers and red foxes in EA1 were made by detecting the presence of some phenotypic peculiarities (e.g. blindness, presence of wounds or injuries, particularity in the coat coloration).

Co-habitation of settlements and interactions between crested porcupines, badgers and red foxes

The co-habitation of settlements and interactions between crested porcupines, badgers and red foxes was investigated in the 16 experimental settlements in EA1 due to the impossibility of individually recognize badgers and red foxes in EA2.

The use of settlements by each of the three investigated species was assessed by using the time of permanence in the burrow. The permanence is defined as the time continuously spent by the species inside or in front of the burrow (e.g. performing sunbathing and/or lactation) during daytime being longer than the minimum recorded time interval between the last coming in and the first coming out from the burrow itself.

Three possible types of settlement use were established: (I) inhabitation, (II) co-habitation and (III) visit or exploration. The settlements were considered inhabited if the permanence of a monitored species in the burrow was confirmed. Similarly, the settlement was considered co-habited when at least two individuals of different species simultaneously inhabited the same settlement. Conversely, the settlement was indicated as visited or explored if the permanence in burrow was not recorded.

Co-habitation in the same settlement between porcupine and badger (P-B), porcupine and red fox (P-F), badger and red fox (B-F) and porcupine, badger and red fox (P-B-F) was investigated. To each co-habitation event, the simultaneous time of permanence in a settlement was measured. In addition, the use of the same and/or different burrow entrance holes during emerging-from and returning-to the burrow were assessed. The correspondence between early or late emergence of the species with the respective early and late return to the settlement on the same day were also assessed. The exclusively inhabitation of porcupine's temporarily uninhabited burrows by red foxes and badgers were tested using chi-square test (χ^2).

Presence of free settlements within 1 km of those co-habited was checked in order to assess if co-habitation occur only in limited availability of settlements.

All the videos in which at least two species were detected together were analyzed for the interaction description. The interactions were classified into two types: avoidance (AV) and aggressive interactions (AI). The AV interaction was assigned when the two species showed changing direction associated to quiet behaviour and absence of aggressiveness. The AI was

assigned when at least one of the two species showed signs of aggression (e.g. quill erection, tail rattling, attempt to attack, approaching and biting).

Differences in the number of avoidances and aggressive interactions recorded in the dyads P-F and P-B in not co-habited settlements were analysed by using chi-square test ($\chi 2$). Moreover, differences in the number of avoidances and aggressive interactions in co-habited and not co-habited settlements between P-B, were also analysed by using chi-square test ($\chi 2$).

Spatio-temporal inhabitation of settlements by crested porcupine

The spatio-temporal inhabitation of settlements was investigated in 6 recognisable porcupine families, 5 in EA1 and 1 in EA2, in 12 settlements. The inhabitation of settlements by each family group was determined by assessing the time of permanence in the burrow.

The settlements were considered inhabited if the permanence of a porcupine family in the burrow during the daytime was confirmed. Those days when it was not possible to determine the permanence in the settlements were excluded from the analysis. For each porcupine family the number of settlement inhabited and the days of inhabitation in each settlement during the whole monitoring were recorded. In addition the number of settlement changes for each family was recorded. For each event of settlement change, the occurrence of human disturbance and/or activity by other species (e.i. badger and red fox) in the days before was assessed.

The overall inhabitation pattern of each investigated family in both study areas and the inhabitation pattern between July 2018 and July 2019 of the 5 family in EA1was build and analysed. The difference in the inhabitation frequencies (days of inhabitation in each sett/total days of monitoring) in each settlement by each porcupines family were analysed using chi-square test (χ^2).

Moreover, the differences in the inhabitation frequencies were also analysed on the basis of settlements exposure to North-west (N-W), South-west (S-W), North-east (N-E) and South-east (S-E) using chi-square test (χ^2). For S1 and S2 in EA1 the frequencies of inhabitation of the two setts in 2017 and 2018 and in each settlement in 2017 and 2018 were also analysed. Similarly, for S4 and S5 the frequencies of inhabitation of both settlement between January and July in 2018 and 2019 and in each settlement in the period January -July 2018 and 2019 were also analysed.

First daily emergence time of crested porcupine from burrow

The first daily emergence time was defined as the time of the first observation of recognisable porcupines while coming out from the burrow.

The time of first observation was obtained from the recorded camera-traps videos. Data concerning the first daily emergence time of porcupines from burrow were collected in all monitored settlements in both study areas.

The times of first daily emergence recorded were graphically represented in relation to the time of sunset throughout the year. The average and maximum delay of porcupines emergence from burrow compared to the time of sunset was computed throughout the year, during the cold period (September to February) and warm period (March to August).

In addition the average and maximum advance of porcupine emergence events recorded was calculated.

Diurnal motor activity and sunbathing in crested porcupine

The diurnal motor activity and sunbathing were investigated in EA1 within three porcupine age classes: porcupettes (< 5 month old), youngsters (5 to 12 month old) and adults (>12 month old). The porcupine youngsters included juveniles (5 to 8 month old) and sub-adults (8 to 12 month old). In order to distinguish porcupettes from juveniles we considered the weight and body length parameters reported in Table 1. The sub-adults and adults, due to very similar body size, were recognized by individual markings or phenotypic peculiarities (e.g. blindness, presence of injuries).

Diurnal motor activity was assessed when returning to or emerging from the burrow and when transits of porcupines in the settlements area during daylight hours were observed. All those videos recorded during daylight time from 60' after sunrise to 60' before sunset were considered for the analysis of diurnal motor activity. In addition those videos of emerging from burrow starting from 30' before sunrise as well as all those of returning to the burrow within 30' before sunset were also included. Consecutive videos clearly attributable to the same specimen and/or family group were considered as a single event. All events of diurnal motor activity in relation to sunrise and sunset times throughout the year were recorded in a graph. For each diurnal event the date, time and age class of detected porcupines were reported and, where possible, the outside permanence was also calculated. The frequency of diurnal motor activities in the settlements permanently and occasionally inhabited were analysed using chi square test (χ^2).

Sunbathing behaviour was assessed by recording porcupines pausing in front of the burrow for at least 1 minute. Even in this case consecutive videos clearly attributable to the same specimen and/or family group were considered as a single episode. The length of each sunbathing episode was established considering the hour of the first and the last observation of the same specimens and/or family group. For each sunbathing episode the settlement, date, time, duration, number and age class of detected porcupines were reported.

The frequency (number of events/episode for each porcupines age/total numbers of events/episode) of diurnal motor activity and sunbathing in different porcupine age were analyzed using chi-square test (χ^2).

Scavenging behaviour in crested porcupine

The presence of scavenging behaviour in crested porcupine was preliminary investigated only in study area EA2 between June and August 2019. Porcupine population in EA2 benefit of a high food availability due to the presence of cultivated crops for exclusive use of wildlife and artificial feeders with corn provided by operator throughout the year.

From June to August a pigeon carcass together whit corn and/or potatoes were placed in 7 porcupine monitored settlements (S1 to S7) and near to 2 monitored capture traps (T2 and T4) (Fig 54). Pigeon carcasses came from planned and authorized killing plans.

Figure 54. Pigeon carcasses and corn placed in Sett S6 (Photo by the Author)

The settlements chose for this investigation were well known to be inhabited and/or frequented by 4 different porcupine families. The porcupine families were recognisable due to the presence of specimens individually marked and/or recognisable in each family. The individual recognition of some specimens was possible due to the presence of phenotypic peculiarities such as blindness or scars and injuries.

Both settlements and capture traps were continuously monitored by a camera-trap set to record 20s long videos without time lapse.

Every two days the pigeon carcasses were replaced in all site and the videos recorded by the camera-traps were checked.

Scavenging of pigeons by porcupines was attributing if tear of the meat from carcass was clearly observed. The scavenging behaviour was assessed within three porcupine ages class: porcupettes (< 5 month old), youngsters (5 to 12 month old) and adults (>12 month old). For each pigeon scavenging event, the date, hour, specie, age class and sex (when possible) were recorded.

Reproduction in wild crested porcupine

The reproductive behaviour was investigated in each recognisable monitored reproductive porcupine pair in both study areas. Videos recording the mounting and copulation events were used for describe the reproduction behaviour in wild porcupines.

The mounting and copulation behaviour observed were compared with the observations reported by Felicioli et al. (1997a, b) in semi-captive porcupines. For each reproductive pair the nights in which single and multiple mounting and copulation events were performed were analysed. In accordance to Felicioli et al. (1997a) the mounting behaviour was assessed if intromission (insertion of the penis into the vagina) and thrusting (pelvic movements during intromission) not occurred. Conversely, the copulation was assessed if intromission and thrusting occurred. The duration of copulation and number of thrusting when possible were recorded.

For the monitored reproductive porcupines pairs the numbers of births throughout the all period of monitoring were also recorded. For each births event, the month and the litter size were recorded. The first emergence of porcupettes from burrow after birth was recorded and in addition the age of porcupettes at the time of their first emergence outside were estimated by observing the appearance of peculiar behaviours in the pair (e.i. Increase in the number of entrance of the adults in burrow during the night, bring food into the burrow). Whenever possible it was recorded the time interval between the first coming out of the porcupettes from burrow and the first time in which they were observed eating independently.

Moreover, the time spent with porcupettes (i.e. parental cares) by adult male and adult female was also analysed whenever possible in the recognisable families. The parental care by sub-adults and adult male and female togher were also recorded. The parental cares were assessed by using the camera-traps videos in wich porcupettes with adults or sub-adults were simultaneously present. The frequency of occurrence of parental care by adult male, adult female, male and female together and sub-adults was calculated as percentage of the total number of video in wich parental cares were recorded. The differences of occurrence of parental care by male and female in each family were analysed using chi-square test (χ^2).

The minimum time of permanence of marked and/or recognisable sub-adults within the family was also detected. The minimum permanence was assessed considering the last time in which the sub-adults were detected in camera traps with the family.

3.5. Porcupines Population Abundance estimate (PPA) and first hypothesis of census model

The porcupine population abundance estimate (PPA) was processed using the settlement as census unit and considering the following parameters:

- Number of settlements geo-located
- Number of geo-located settlements inhabited by crested porcupine
- Spatio-temporal inhabitation analysis of settlements by each recognisable porcupines family groups
- Average size of porcupines family group
- Total area of investigation

The porcupine population abundance was calculated in Crespina-Lorenzana area in EA1 and in EA2. The average porcupine population size was computed as a product of porcupine population abundance estimate and the total areas.

The census model was proposed and tested starting from the result obtained in this study concerning the spatial-distribution of settlements and the spatio-temporal use of settlements by crested porcupine in EA1. The model provides guidelines for the estimation of settlements density and porcupine population abundance. The census model was preliminary tested in EA2 that was used as internal control area. The estimated settlements density and porcupine population abundance obtained by apply the census model were then compared with those computed in the same area starting from geolocation and camera-traps data collected.

3.6. Health monitoring of crested porcupine

The health monitoring of crested porcupine included investigation of parasitological analysis on faecal samples and ectoparasites detection, haematological and blood biochemical parameters, antibody detection and isolation of *Leptospira* sp.. The biological samples were collected from both captured and road-killed porcupines. The faecal samples were also collected along transect in both study areas.

Parasitological analysis

Parasitological investigation was performed in collaboration with the parasitological section of the Department of Veterinary Science of Pisa University and the Department of Clinical Science and Translational Medicine, University of Rome "Tor Vergata".

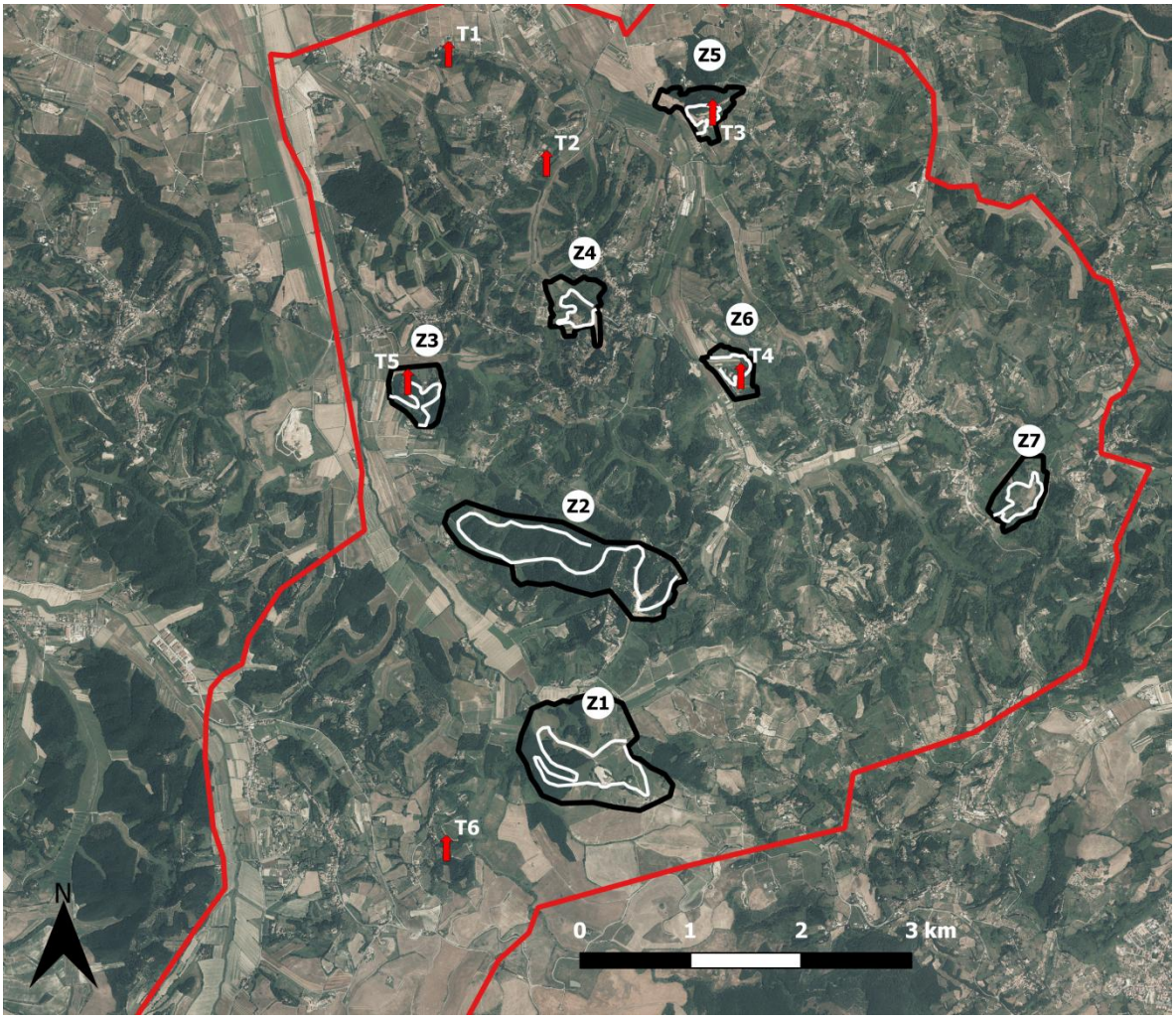


Figure 55. The7 zones (close areas in black, Z1-Z7), transects (white lines), and trap-sites (red arrows, T1-T6) in EA1 where faecal samples were collected

The faecal sampling were performed along 7 transects 1.8 SD 1.2 km average long in EA1 and in the feeding area near experimental settlements in EA2. Each transect in EA1 was embedded in a zone. The average distance between transects was 2.9 ± 1.14 km. The seven zones (Z1-Z7) were chosen in order to be at a distance compatible with the crested porcupine clan average home-range (100 ha) (Lovari et al. 2013a) (Fig 55).

Each transect was monitored every 48 hours and all the faecal samples found were removed even if they were not used in this study, as an attempt to ensure faecal samples were relatively fresh (max 48 hours from faecal deposition) when collected for parasitological analysis (Fig 56).

Fig 56. Fresh porcupines feaces found along a transect (Photo by Baldanti)

Faecal samples were also collected from road-killed and captured porcupines. Faecal samples were considered belonging to single individuals if taken from captured and road-killed animals, and if only a single faecal sample was collected from a transect during the whole sampling period. Therefore, only a partial number of collected faecal samples were considered attributable to different individuals.

All faecal samples collected were stored at -4°C and analysed within 24 hours.

Faecal samples were examined by using a commercial rapid immunoassay to detect *Giardia* spp. and *Cryptosporidium* spp. faecal antigens (Rida Quick® Cryptosporidium/Giardia Combi, R-Biopharm, Darmstadt, Germany). For the identification of helminthic eggs and protozoan cysts/oocysts, all faecal samples were analysed also microscopically by the Mini-FLOTAC technique (Cringoli et al. 2017) on 2 g-faecal samples by using satured sodium chloride as flotation solution (specific gravity 1.2). Magnifications of 100x and 400x were used to identify helminth eggs and protozoan cysts/oocysts. The results were expressed as the arithmetic mean number of eggs/oocysts per gram (EPG/OPG) of faeces.

Molecular investigation was performed to identify the species and genotypes of *Giardia* in samples found positive for *Giardia* spp. at parasitological analysis.For DNA extraction, samples were processed by a commercial kit (QIAamp DNA Stool Mini Kit, QIAGEN,

Valencia, CA, USA). A nested PCR protocol was applied to amplify a fragment of the small subunit ribosomal RNA (SSUrDNA, 130 bp, Read et al. 2002), of glutamate dehydrogenase (gdh, 432 bp, Read et al. 2004) and of triose phosphate isomerase (tpi, 530 bp, Sulaiman et al., 2003) genes. Positive amplicons were purified using mi-PCR Purification Kit, Metabion International AG.

Amplification products were sent to an external laboratory for sequencing (Bio-Fab Research, Rome, Italy). Forward and reverse sequences were manually checked using FinchTV. The obtained consensus sequences were then compared with those available in GenBank database by using the Standard Nucleotide BLAST search and aligned by Clustal Omega implemented in MEGA7.0 with representative sequences for the three loci and used as reference.

Ectoparasites

The presence of ectoparasites was checked in all capture porcupines and in two road-killed porcupines at post-mortem examination. All the body regions were examined for arthropods detection. The age class and sex of the porcupine in which ectoparasites were found were recorded. Collected fleas were fixed in 70% ethanol mounted with the Hoyer's solution and microscopically identified with optical microscope. Species identification was based on the morpho-anatomical characteristics and referred to descriptions reported by Berlinguer (1964) and Manfredini (2005).

Haematological and blood biochemical profile of crested porcupine

The haematological and blood biochemical profile of captured crested porcupine was investigated in collaboration with the didactic veterinary hospital of the Department of Veterinary Science of Pisa University. The blood samples with EDTA collected were analysed for blood count within 90 minute from sampled using automatic cell counters. The haematological values analysed were:

- Red blood cells (RBC)
- Haemoglobin (Hgb)
- MCV: mean corpuscular volume
- MCH: mean corpuscular haemoglobin
- MCHC: mean corpuscular haemoglobin concentration
- Platelets (PLT)
- White blood cells (WBC)
- Number and % of neutrophils
- Number and % of eosinophils
- Number and % of basophils
- Number and % of monocytes
- Number and % of lymphocytes

The blood samples without EDTA were centrifuged at 3.000 rpm for 10 min to separate the serum from blood. The serum obtained was used for assessed the biochemical profile using spectrophotometric, colorimetric and automated kinetic methods. The biochemical values analysed were:

- Total Proteins (TP)
- Total and direct bilirubin
- Urea
- Creatinine
- Glycemia
- Alanine aminotransferase (ALT)
- Aspartate transaminase (AST)
- Gamma-glutamyl transpeptidase (GGT)
- Creatine kinase (CK)

- Sodium (Na^{+})
- Potassium (K^{+})
- Calcium (Ca^{++})
- Phosphates
- Lactate

For each biochemical and haematological value obtained the mean, standars deviation and median were computed.

Leptospirosis in crested porcupine

The investigation was performed in collaboration with the infectious diseases and general pathology and veterinary pathological anatomy sections of the Department of Veterinary Science of Pisa University.

***Antibodies detection of* Leptospira**

For the detection of *Leptospira* antibodies, all blood samples collected from captured porcupines were centrifuged at 10.000 rpm for 10 min to separate the serum from blood. The sera were collected in a 1.5 ml microtube and were kept at -20° C until analysis occur.

Leptospira antibodies were detected in the serum by Microscopic Agglutination Test (MAT). Antibody titre of at least of 1:100 was considered positive.

The *Leptospira* live suspension employed for the MAT were: Icterohaemorrhagiae (serogroup Icterohaemorrhagiae, strain Bianchi), Canicola (serogroup Canicola, strain Alarik), Pomona (serogroup Pomona, strain Mezzano), Grippotyphosa (serogroup Grippotyphosa, strain Moskva V), Tarassovi (serogroup Tarassovi, strain Mitis Johnson), Bratislava (serogroup Australis, strain Riccio 2), Hardjo (serogroup Sejroe, serovar Hardjoprajitno), Castellonis (serogroup Ballum, strain Castellon 3), Copenhageni (serogroup Icterohaemorrhagiae, strain Wijmberg), Bataviae (serogroup Bataviae, strain Pavia), Australis (serogroup Australis, strain Ballico), Zanoni (serogroup Pyrogenes, strain Zanoni), Saxkoebing (serogroup Sejroe, strain Mus 24), Sejroe (serogroup Sejroe, strain Topo 1), Poi (serogroup Javanica, strain Poi), Mini (serogroup Mini, strain Sari), Lora (serogroup Australis, strain Riccio 37), Hardjo (serogroup Sejroe, strain Farina), Autumnalis (serogroup Autumnalis, strain Akiyami A), Hebdomadis (serogroup Hebdomadis, strain Hebdomadis).

Isolation of **Leptospira**

The investigation was performed on two dead porcupines, an 11kg adult female and a porcupette female of 1.4 kg., following an impact with a car in Grosseto province (Tuscany, Italy). From each carcass both kidneys and 1 ml of blood from heart cavity were collected during necroscopy.

The blood samples were centrifuged at 10.000 rpm for 10 min to separate the serum. The sera samples obtained were tested to detect *Leptospira* antibodies by using Microscopic Agglutination Test (MAT) (OIE 2014) as describe in the previous chapter (Antibodies detection of *Leptospira*).

The kidney samples were cultured in Ellinghausen-McCullough-Johnson-Harris (EMJH) medium (Difco, Detroit, MI, USA). A representative portion of 10 cm^3 from each kidney was homogenized with 5 ml of sterile water. One ml of homogenate was cultured in 5 ml of EMJH. Cultures were incubated at 30 °C ± 1 °C for 120 days and checked every 10 days under dark-filed microscopy to assess bacterial growth. Subcultures were arranged to maintain the isolated strain alive, in case of positive cultures.

Isolated *Leptospira* were genotyped using a Multilocus Sequence Typing (MLST) scheme encompassing housekeeping genes (Ahmed et al., 2006; Boonsilp et al., 2013; Varni et al., 2014). The amplification of each target gene was realized with HotStarTaq Master Mix Kit (Qiagen, Hilden, Germany) and further sequenced (BMR Genomics, Padova, Italy) using the same amplification primer sets and analysed using BioEdit Software (Hall, 1999).

The DNA was extracted from each kidney using the Quick-DNA Plus Kits (Zymo Research, Irvine, CA, USA) according to the manufacturer's instructions. The *lipL32* gene Taqman RealTime PCR was performed to detect pathogenic leptospires (Stoddard et al., 2009). The RealTime PCR assay was performed on a Rotorgene Corbett 6000 (Corbett Research, Sidney, Australia) with the following thermal conditions: a holding stage of 95 °C for 5 min, and 45 cycles of 95 °C for 15 sec and 60 °C for 30 sec. Sample with Ct *lipL32* < 35 was considered as positive.

Representative portions of kidneys collected during necropsy were routinely processed, paraffin-embedded and 5-µm thick sections were stained with haematoxylin and eosin, Masson trichrome Goldner and Warthin Starry stains. Tissue sections were also submitted to immunohistochemistry. Antigen retrieval was achieved on the slides by placing them in a bath

of 10 mmol/L citric acid (pH 6) and boiling for 16 min in an 800-watt microwave oven. The slides were dried at room temperature and washed with running tap water. A peroxidase block was performed and the slides were incubated with specific rabbit antisera against *Leptospira* serogroup Pomona and *Leptospira* serogroup Grippotyphosa. The primary antibodies were diluted 1:300 in a buffer solution (PBS) prior to incubation. A polyclonal horse serum (1 drop diluted in 1ml of PBS) was used as a secondary antibody for 30 min at room temperature (Vector Laboratories, CA USA). Antibody binding was detected using a streptavidin-biotin-peroxidase kit (Vector Laboratories, CA USA). The enzymatic reaction was developed with the use of 3-1- diaminobenzydine (Sigma Chemical, MO, USA) as a substrate. Stained slides were subsequently counter-stained in hematoxylin for 40 sec followed by a wash in tap water, dehydration in graded alcohols (70, 90, and 100%), and clearance with xylene. Sections were mounted in DPX (08600E; Surgipath Europe, UK). As a positive control a kidney of a PCR positive mouse was used.

4.Results

4.1. Geolocation of settlements

Within the study area EA1 were geolocated a total of 446 ground holes of which 434 attributable to 41 real settlements and to 48 putative settlements and 12 detected in 8 war-shelters (Fig 57). Within EA2, 97 ground holes distributed in 17 putative settlements were geo-located (Fig 58).

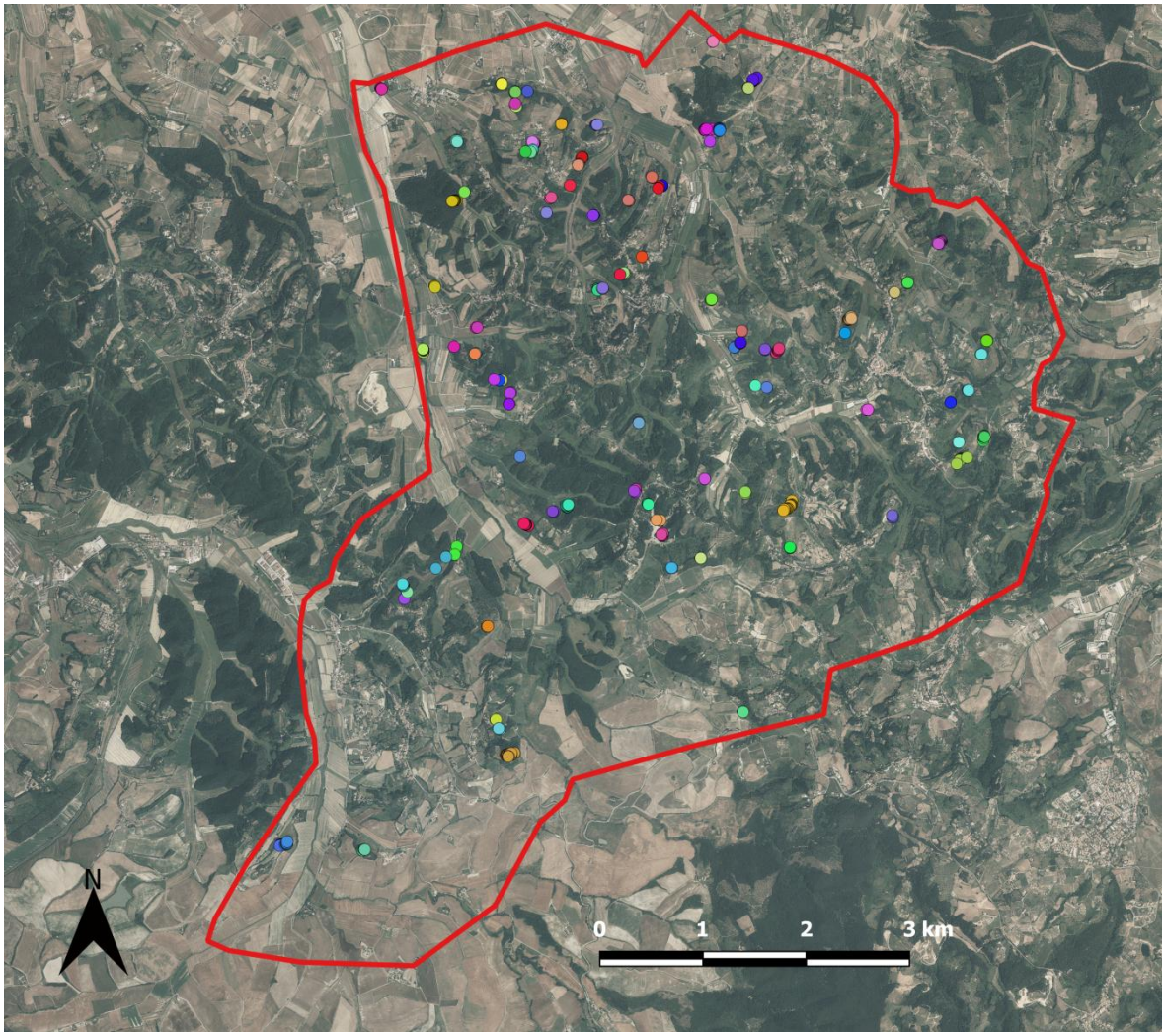


Figure 57. The 446 ground holes geolocated in EA1. The ground holes assigned to a real or putative settlement are represented with spots of the same colour. Image elaborate using Q-Gis

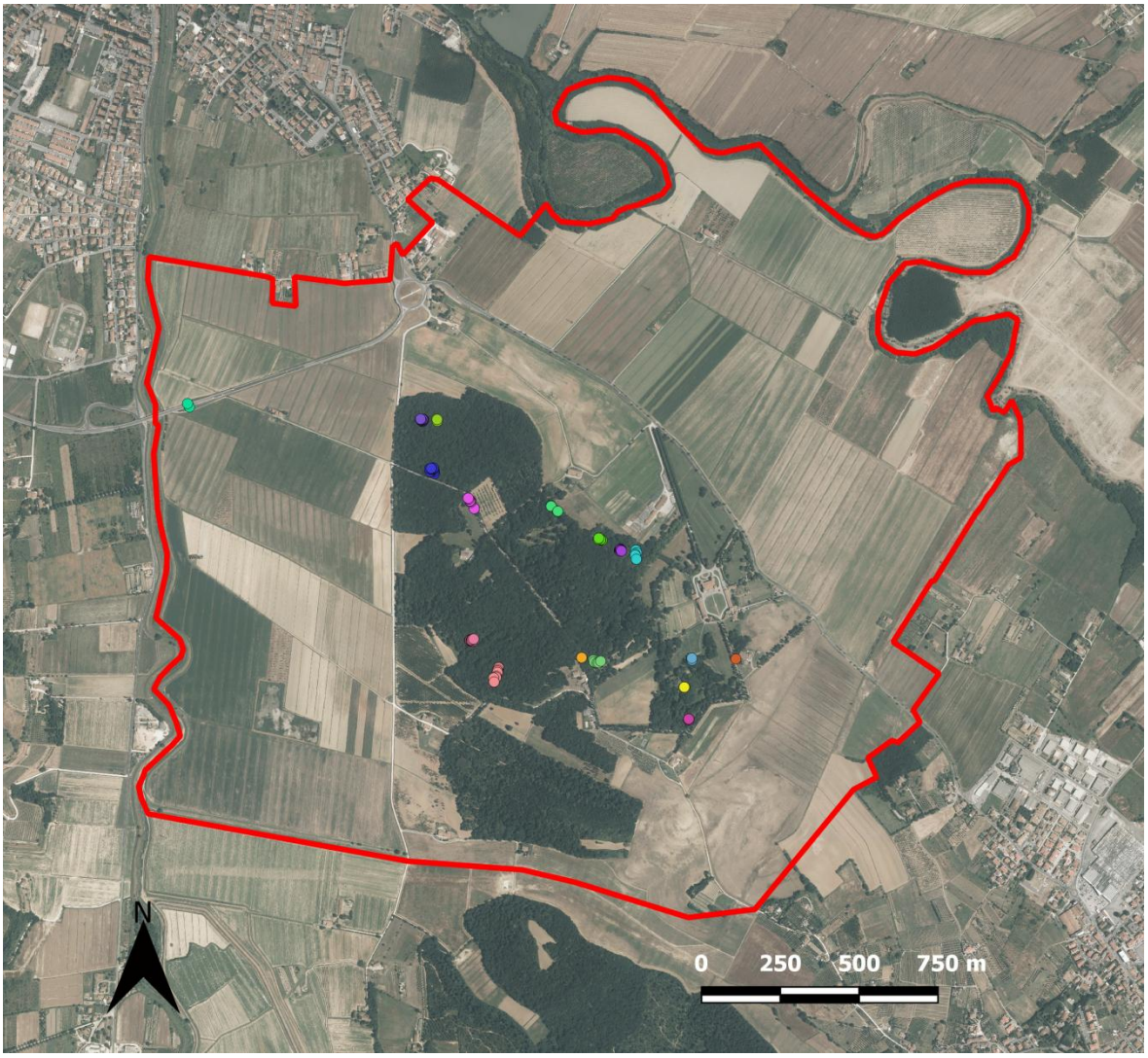


Figure 58. The 97 ground holes geolocated in EA2. The ground holes assigned to each putative settlement are represented with spots of the same colour. Image elaborate using Q-Gis

Spatial-distribution analysis of settlements

Among the tested clustering models both the agglomerative clustering average linkage and Ward.D linkage are resulted to be suitable model for settlements discrimination. For both methods the *Rand index* resulted 1 (perfect agreements) and the *Meila's variation index* (VI) resulted 8.9^{e-16}. Conversely the *Rand index* and the *Meila's variation index* (VI) was respectively 0.952 and 0.072 for agglomerative clustering complete linkage and 0.954 and 0.065 for k-medoids model.

The agglomerative clustering average linkage showed the highest correlation value (value=0.86). This model was used for clustering the ground holes of the putative settlements and for spatial-distribution analysis of the obtained settlements. The maximum distance recorded between two settlements inhabited by only one porcupine family group resulted 250 meters.

In EA1 the cluster analysis showed a distribution of the 434 geolocated ground holes in 85 settlements. The assignement of the 263 ground holes to the 41 real settlements was confirmed by the cluster analysis. Among the 171 ground holes assigned to putative settlements 126 (73.6%) were confirmed by the cluster analysis to belong to 33 settlements while the other 45 ground holes were clustered in 11 new settlements (Fig 59).

The average number of ground holes per settlement resulted 4.5 holes (SD = 4.4 holes) and the settlements density was 2 settlements/km^2.

In EA2 the 97 geolocated ground holes resulted clustered in 15 settlements. The assignement of the 77 (n=97, 79.4%) ground holes to 13 putative settlements was confirmed by the cluster analysis while the other 20 ground holes were clustered in 2 new settlements (Fig 60).

In EA2 the settlements density resulted to 2.4 settlements/km^2 and the average number of ground holes per settlement was 5.7 holes (SD = 4.5 holes).

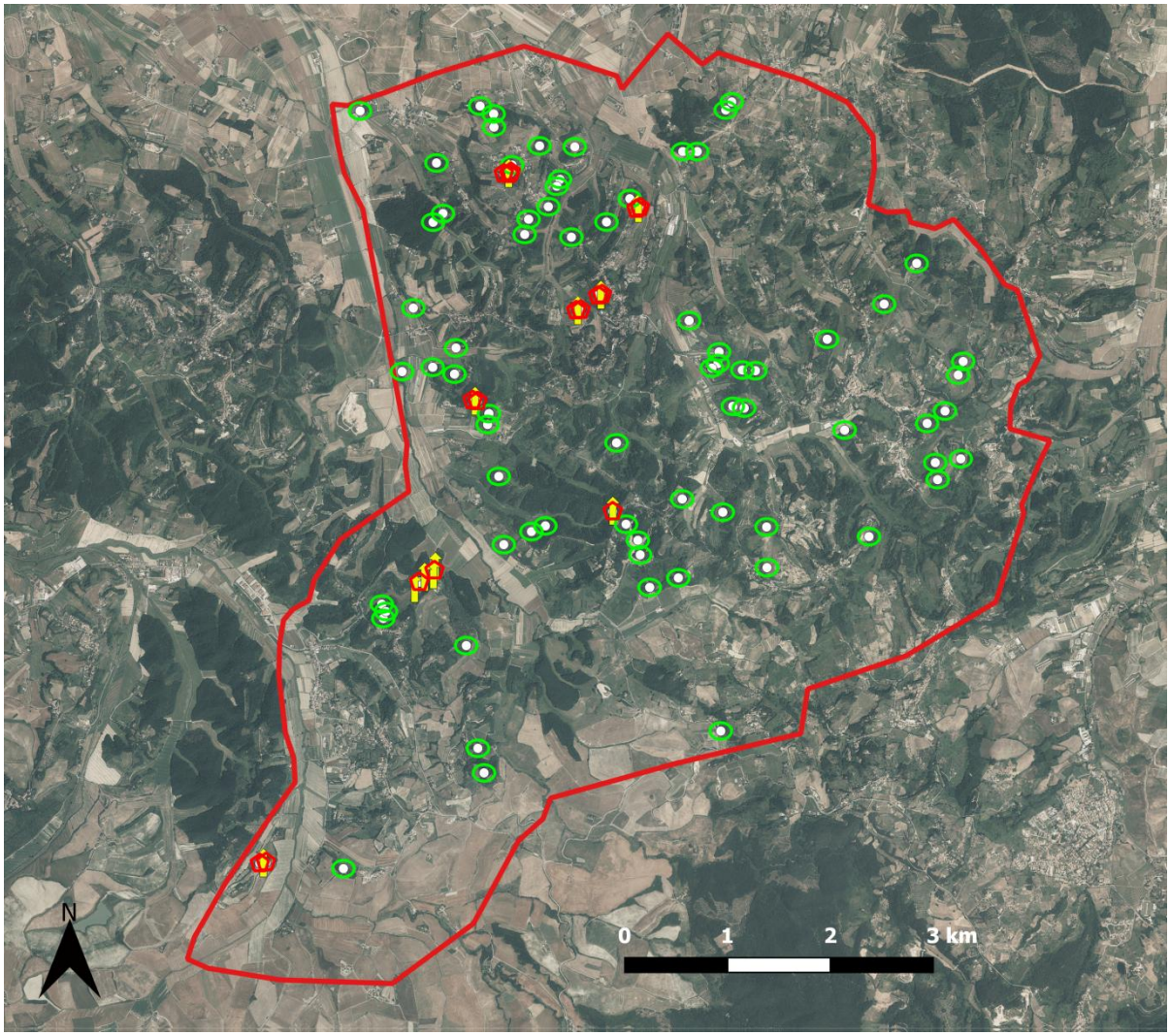


Figure 59. Identified settlements by cluster analysis in EA1. The white spots indicate the real and putative settlements analysed, the green circle the confirmed settlements, the red pentagon the no-confirmed settlements and the yellow arrow the new settlements identified.
Image elaborate using Q-Gis

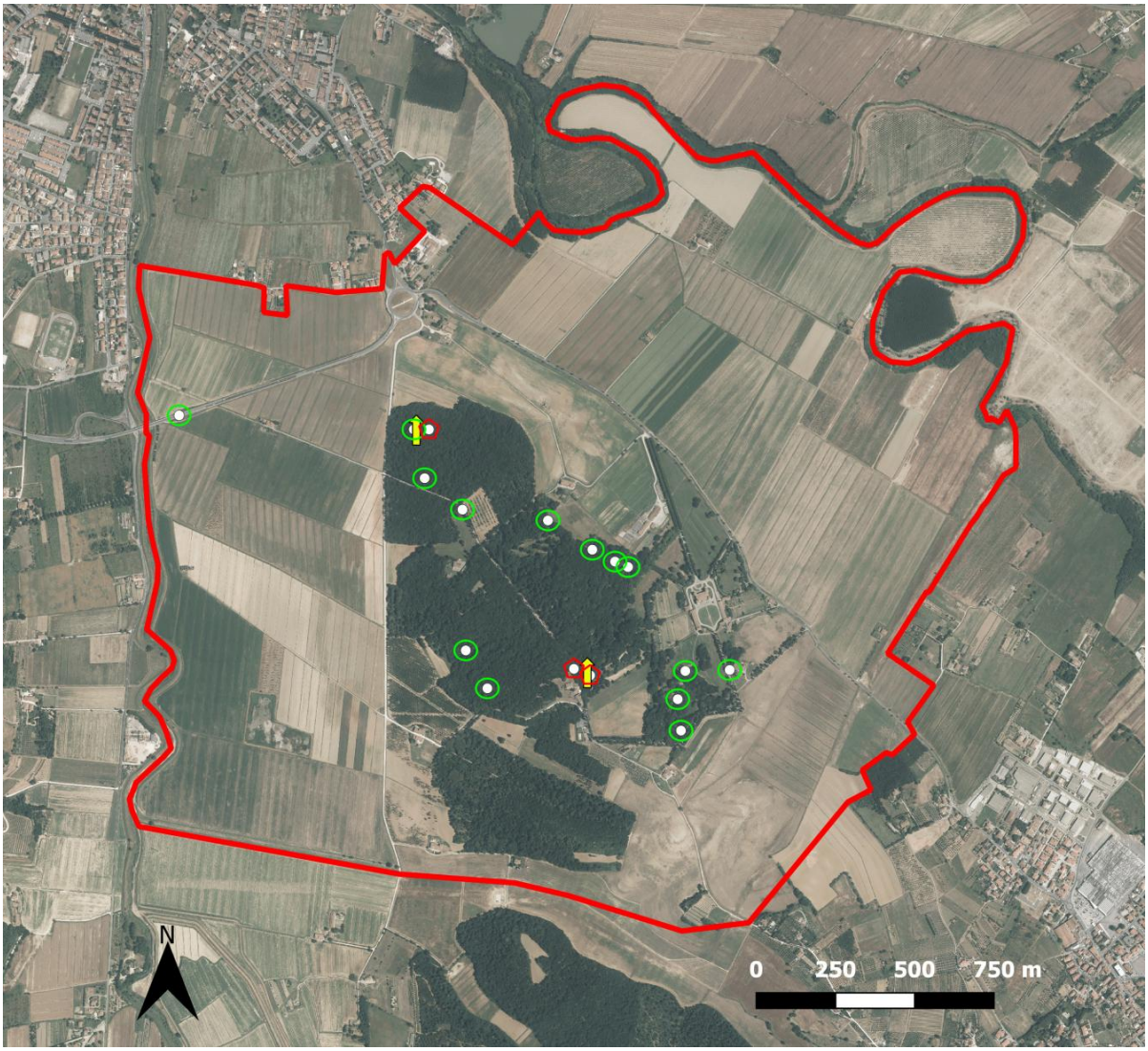


Figure 60. Identified settlements by cluster analysis in EA2. The white spots indicate the real and putative settlements analysed, the green circle the confirmed settlements, the red pentagon the no-confirmed settlements and the yellow arrow the new settlements identified.
Image elaborate using Q-Gis

The 67% (n=57) and 86.6% (n=13) of settlements in EA1 and EA2 respectively resulted distributed in settlements systems named here “Stations”. The settlements belonging to a station resulted opposite exposed and connected one to each other by a dense network of pathways. Overall 24 stations in EA1 and 4 in EA2 were identified (Fig 61 and Fig 62). In particular in the study area EA1 the 37.6% (n=32) of settlements resulted distributed in binary stations, the 24.7% (n=21) in ternary stations and the 4.7% (n=4) in quaternary stations. In the study area EA2 the 13.3% (n=2) of settlements resulted distributed in binary stations, the 20% (n=3) in ternary stations and the 53.3% (n=8) in quaternary stations. In the 71.4% (n=5) of ternary stations in EA1 and the 50% (n=1) of quaternary stations in EA2 the settlements systems were characterized by the presence of two main settlements and outlier settlements with one or two ground holes digged in war-shelters or in grooves created by rainwater.

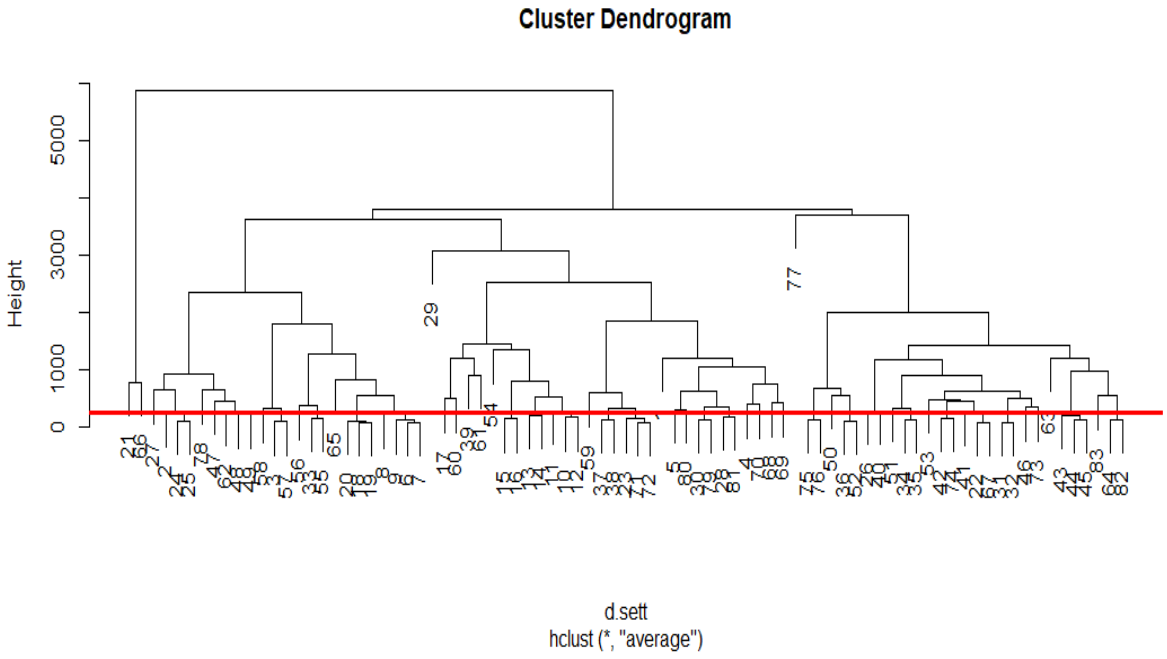


Figure 61. Cluster dendrogram of the 24 stations identified in EA1. The red line is the cut line at 250 meters used for settlements spatial-distribution analysis.

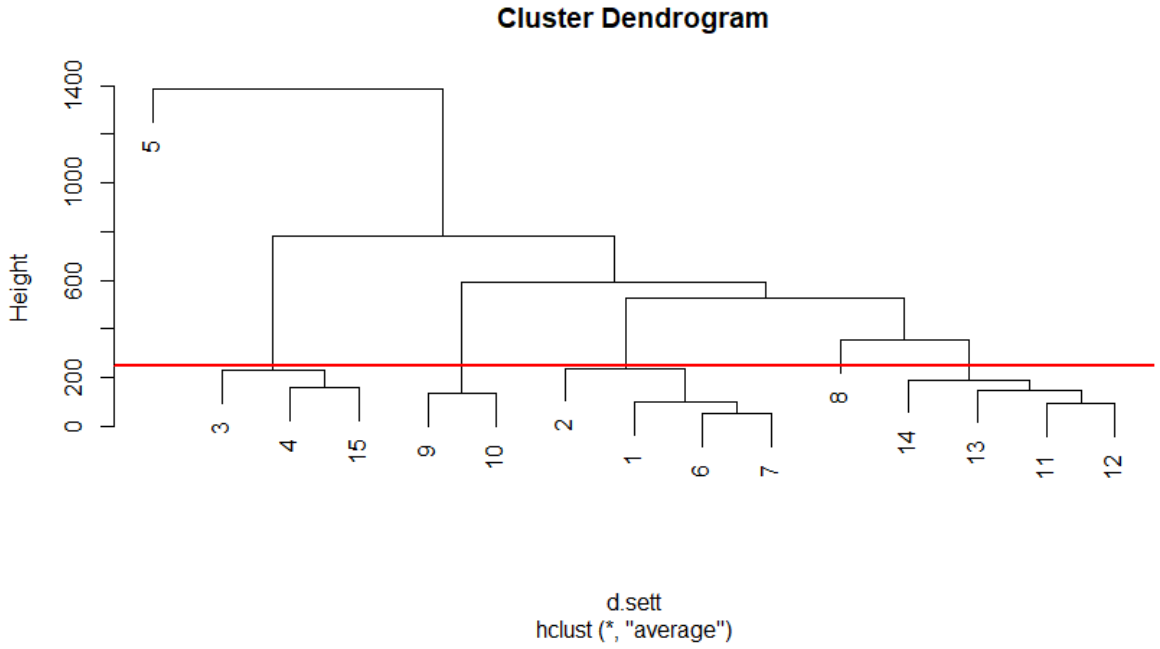


Figure 62. Cluster dendrogram of the 4 stations identified in EA2. The red line is the cut line at 250 meters used for settlements spatial-distribution analysis.

The ground holes orientation did not present a sector preference in the orientation in both the study areas. The mean vector of ground holes in EA1 resulted 164° with a mean vector length (r) of 0.062 (Fig 63A) while in EA2 242° with a mean vector length (r) of 0.074 (Fig 63B).

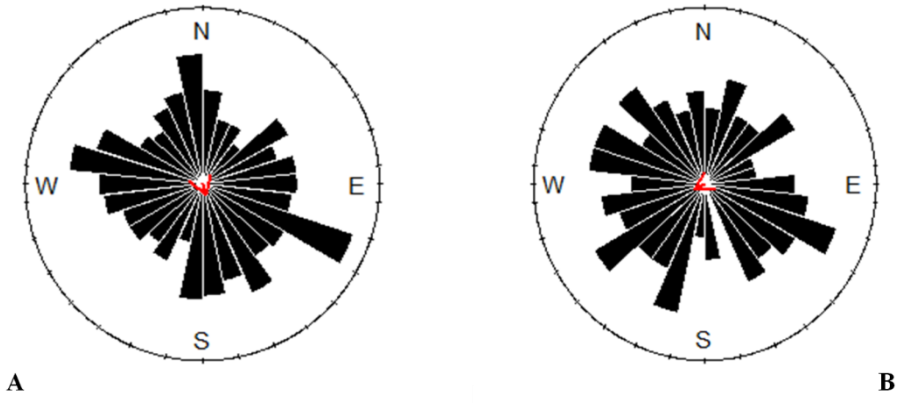


Figure 63. Circular distribution of the 434 ground holes in EA1 (A) and of the 97 ground holes in EA2 (B). The red arrows indicate the mean vector

The bimodal mean vector length (r) of settlements in EA1resulted 0.29 while in EA2 was 0.4. The unimodal mean vector length was 0.093 in EA1 and 0.1 in EA2. The Rayleigh test showed a highly significant bimodal distribution of the settlements orientation in EA1 ($P<0.001$) and a significant bimodal distribution of settlements orientation in EA2 ($P<0.05$). In EA1 the average settlements orientation trend resulted 122° South-east-302° North-west with confidence intervals ranges from 288° to 318° North-west and from 108° to 132° South-east. In EA2 the average settlements orientation trend resulted 130° South-east – 310° North-west while the confidence intervals ranges from 107° to 155° South-east and from 288° to 335° North-west (Fig 64).

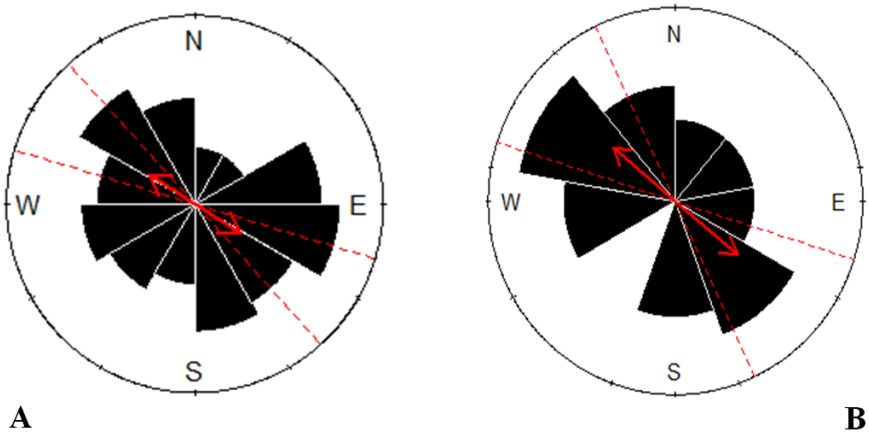


Figure 64. Bimodal circular distribution of the 85 settlements in EA1 (A) and of the 15 settlements in EA2 (B). The red arrows indicate the mean vector and the red dashed lines the confidence intervals.

Manly selection index for ground holes occurrence showed positive selection for the South-east and a negative selection for the North-east in both the study areas. Conversely no selection was obtained for South-west and North-west exposures sectors (Fig 65). Similarly in EA1 the Manly index showed a positive selection for settlements occurrence to South-east and a negative selection to North-east while no selection was resulted in EA2 (Fig 66).

In EA1 the 95.3% (n=81) of settlements were detected in woody areas, the 3.5% (n=3) in uncultivated areas and the 1.2% (n=1) in woody covered riverbanks. In EA2 the 80% (n=12) of settlements were presents in woody areas, the 13.3% (n=2) in uncultivated areas and the 6.7% (n=1) in woody covered riverbanks. The frequency of settlements occurrence was significantly higher in woody areas than in uncultivated areas ($P<0.001$, $\chi^2 = 143.2$) and woody covered riverbanks ($P<0.001$, $\chi^2 = 150.77$) in EA1 Similarly, in EA2, the settlements occurrence was significantly higher in woody areas than in uncultivated areas ($P<0.001$, $\chi^2 =$

13.39) and woody covered riverbanks (P<0.001, χ^2 = 16.42). All settlements in both study areas were detected in sandy soil.

In the study area EA1 the average distance of detected settlements from cultivated or uncultivated areas resulted 32.4 meters (SD = 18.2) and the maximum distance recorded was 77.5 meters. In study area EA2 the average distance between settlements and from cultivated or uncultivated areas was 37.3 meters (SD = 22.7) with a maximum distance recorded of 86.5 meters.

In EA1 the settlements average density on perimeter kilometre resulted 0.97 (SD = 0.37) settlements/km while in resulted 2.08 (SD = 0.006) settlements/km in EA2.

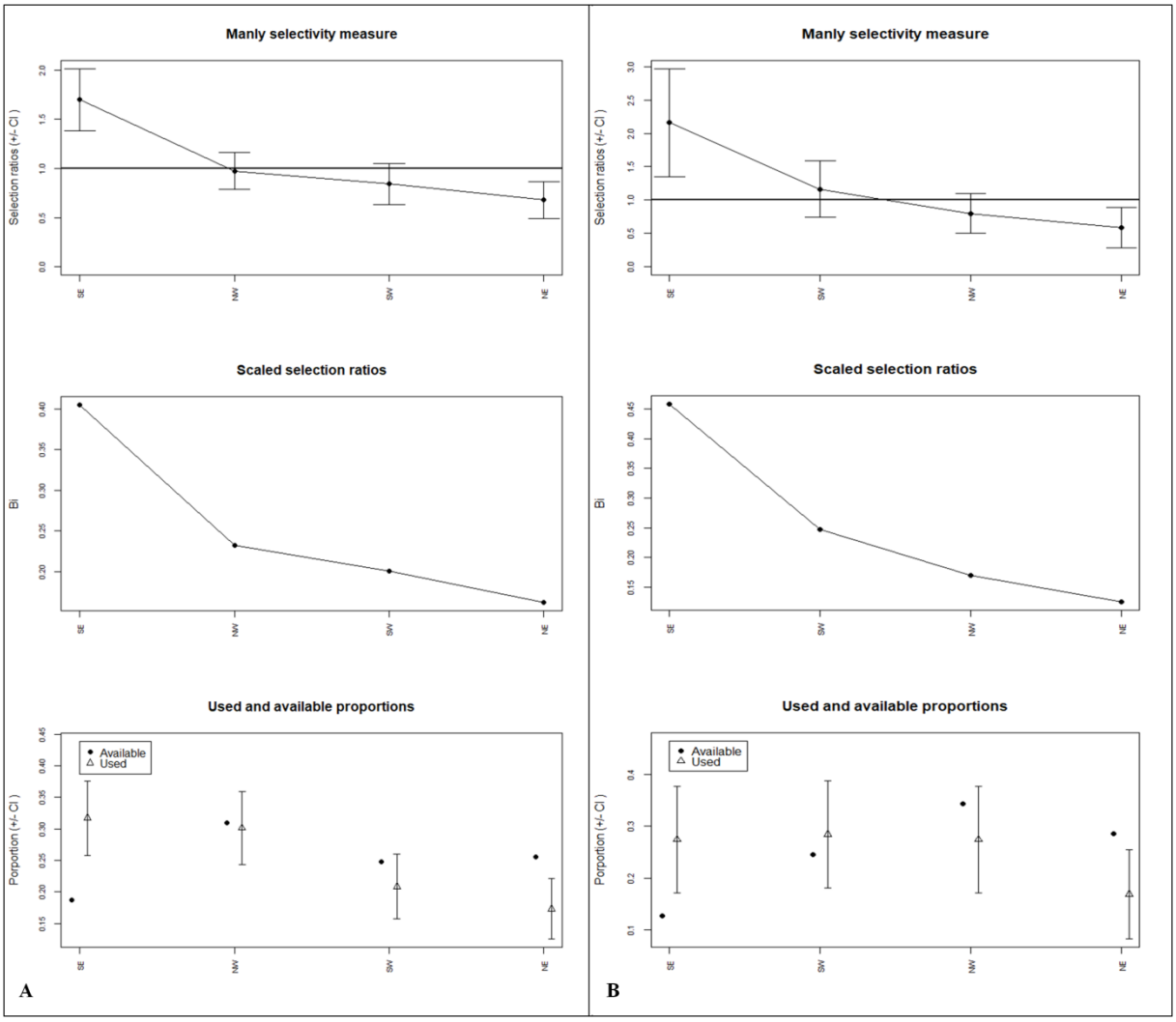


Figure 65. Sectors exposure selection for the ground holes occurrence in EA1 (A) and EA2 (B) using Manly selection index.

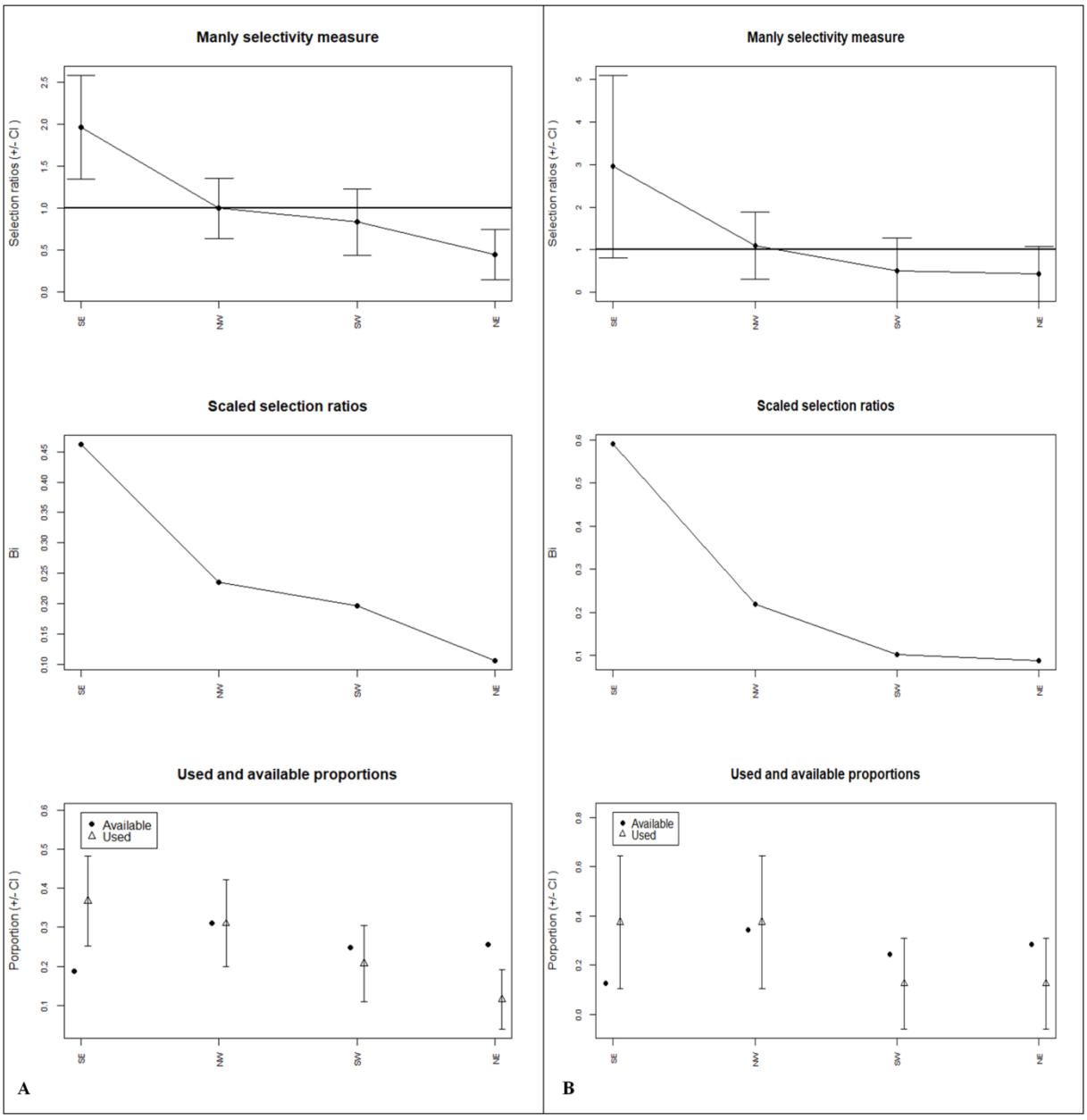


Figure 66. Sectors exposure selection for settlements occurrence in EA1 (A) and EA2 (B) using Manly selection index

4.2. Porcupines capture and marking

Overall 14 porcupines were captured and marked (Fig 67), 8 in EA1 and 6 in EA2. In Table 2 and Table 3 the sex, age class, weight of each capture porcupine and the samples collected for each specimen are reported.

Table 2. Sex, age class and weight of the 8 captured porcupines in EA1. For each porcupine the date and site of capture and the collected sample (blood, ectoparasite and feaces) are also reported

Sex	Age class	BW	Date	TS	BS	BS EDTA	EP	FS
Male	Porcupette	3.1	17/04/2018	T1				X
Male	Adult	11.6	13/06/2018	T1	X			X
Female	Porcupette	3.5	10/02/2019	T2	X			X
Male	Adult	14.5	14/02/2019	T5	X			X
Male	Adult	11.4	19/02/2019	T3	X			X
Female	Adult	12	28/02/2019	T5	X	X		X
Male	Adult	14.5	01/03/2019	T2	X	X		
Female	Sub-adult	11	01/03/2019	T1	X	X	X	X

BW= body weight (kg), TS= trap site, BS= blood samples, BS EDTA= blood samples with EDTA, EP=ectoparasite, FS= faecal samples

Table 3. Sex, age class and weight of the 6 captured porcupines in EA2. For each porcupine the date and site of capture and and the collected sample (blood, ectoparasite and feaces) are also reported

Sex	Age class	BW	Date	Trap	BS	BS EDTA	EP	FS
Female	Adult	13.4	20/02/2018	T2	X			
Female	Adult	11	22/02/2018	T2		X		
Female	Sub-adult	5.6	21/05/2018	T5	X	X		X
Female	Sub-adult	7.2	14/02/2019	T3	X			X
Female	Porcupette	1.9	02/03/2019	T3	X	X	X	X
Female	Sub-adult	9.5	02/03/2019	T4	X	X		X

BW= body weight (kg), TS= trap site, BS= blood samples, BS EDTA= blood samples with EDTA, EP=ectoparasite, FS= faecal samples

Figure 67. Specimens of porcupine captured and marked in EA1 and EA2: (A) adult male in EA1 marked with black tapes on the quills and black paint on the tail, (B) porcupette female in EA2 marked with black tapes on the quills, (C), sub-adult female in EA1 marked with a red taps on quills and black paint on the crest, (D) sub-adult female in EA2 marked with white tapes on the quills and white paint on the crest, (E) sub-adult female in EA1 marked with red tapes on the quills and white paint on the tail and (F) adult female in EA2marked with red tapes on the quills and white paint on the crest (Photos by the Author)

4.3. Camera-trapping

Overall, in EA1 68 out of 89 geolocated settlements, were monitored for a total of 58.347 (TV) video collected. The camera trapping success was 48% for a total of 28.050 useful video in which target and no-target animals were present (UV). In Table 4 the number of videos recorded for each observed species is reported.

Table 4: List of the species observed in the collected videos in EA1. For each specie the total number of videos recorded, the percentage on the total video collected during the monitoring (%TV) and the percentage on the useful video collected during the monitoring (%UV) are reported.

Scientific name	Common name	N° videos	%TV	%UV
Hystrix cristata	Crested porcupine	20.038	34.3	71.4
Meles meles	European badger	5.224	8.9	18.6
Vulpes vulpes	Red fox	1.046	1.7	3.7
Sus scrofa	Wild boar	267	0.4	0.9
Capreolus capreolus	Roe deer	271	0.5	1
Martes martes/foina	Pine marten/ Stone marten	45	0.08	0.2
Oryctolagus cuniculus	Wild rabbit	458	0.8	1.65
Sylvilagus floridanus	Eastern Cottontail	522	0.9	1.9
Lepus europeaus	European hare	6	0.01	0.02
Sciurus vulgaris	Red squirrel	26	0.04	0.09
Phasianus colchicus	Pheasant	17	0.02	0.06
Garrulus glandarius	Jay	76	0.1	0.3
Erinaceus europeus	Hedgehog	11	0.02	0.04
Upupa epops	Hoopoe	1	0.002	0.003
Picus viridis	Green woodpecker	29	0.05	0.1
Dendrocopos major	Great spotted woodpecker	3	0.005	0.01
Cyanistes caeruleus	Blue tit	1	0.002	0.003
Scolopax rusticola	European woodcock	1	0.002	0.003
Canis lupus	Wolf	1	0.002	0.003
Mustela putorius	Skunk	1	0.002	0.003
Mustela nivalis	Weasel	3	0.005	0.01

Among the monitored settlements the 69% (n=47) resulted inhabited by porcupines while the 31% (n=21) resulted abandoned and/or only visited.

In EA2 all geolocated settlements (n=17) were monitored for a total of 10.511 video recorded. The camera trapping success was 54.6 % for a total of 5747 useful video in which target and no-target animals were present. In Table 5 the numbers of videos recorded for each observed species are reported. The 65% (n=11) of monitored settlements resulted inhabited by porcupines while the 35% (n=6) were only visited.

Table 5: List of the species observed in the collected videos in EA2. For each specie the total number of videos recorded, the percentage on the total video collected during the monitoring (%TV) and the percentage on the useful video collected during the monitoring (%UV) are reported..

Scientific name	Common name	N° videos	% TV	%UV
Hystrix cristata	Crested porcupine	3815	36.3	66.4
Meles meles	European badger	626	6	11
Vulpes vulpes	Red fox	50	0.5	0.9
Sus scrofa	Wild boar	3	0.03	0.05
Capreolus capreolus	Roe deer	63	0.6	1.1
Dama dama	Fallow deer	53	0.5	0.9
Martes martes/foina	Pine marten/Stone marten	24	0.2	0.4
Lepus europeus	European hare	170	1.6	3
Oryctolagus cuniculus	Wild rabbit	432	4.1	7.5
Phasianus colchicus	Pheasant	485	4.6	8.4
Buteo buteo	Buzzard	26	0.2	0.4

Individual recognition of the animals in camera-traps videos

Overall, in EA1, in addition to the 8 marked porcupine, 5 no marked porcupines were recognisable by detecting the presence of phenotypic peculiarities. In this way 5 porcupine families were identified (Tab 6 and Fig 68, 69, 70, 71 and 72). The average size of the family group was 4.1 SD 1.5 individuals. In addition 9 badgers and 5 red foxes were individually recognised (Tab 7 and Fig 73).

Table 6. Porcupines individually marked and/or recognisable due to the presence of phenotypic peculiarities in each identified porcupine family groups in EA1. For the individuals phenotypically recognisable the phenotypic characteristic is reported.

	Specimens	**Marked**	**Recognisable**
Family 1	Adult male	X	White tapes on the quills Blindness left and right
	Sub-adult female	X	Red tapes on the quills Black paint on the crest
	Porcupette male	X	White paint on the tail
Family 2	Adult male	X	Black tapes on the quills Blindness left
	Adult female	NO	Blindness left
	Porcupette female	X	White tapes on the quills
	Sub-adult	NO	Absence of crest
Family 3	Adult female	NO	Blindness left
	Adult male	NO	Blindness right
Family 4	Adult male	X	Black tapes on the quills Black paint on the tail
	Sub-adult female	X	Red tapes on the quills White paint on the tail
Family 5	Adult male	X	White tapes on the quills Injury on the nose
	Adult female	NO	Crest carried on the left side

Table 7. Phenotypic characteristics of the 6 badgers and 5 red foxes identified in EA1

		Blindness right	Blindness left	Injuries	Particularities in coat
Badger	**1**	X			
	2		X		
	3	X	X		
	4			X	
	5		X		
	6	X			
Red fox	**1**	X			
	2		X		
	3	X	X		
	4				X
	5			X	

In EA2, in addition to the 6 marked porcupines, only 2 porcupines with individual phenotypic peculiarities were observed and 3 families groups were identified (Tab 8 and Fig 74, 75 and 76). The average size of the family group resulted 4.1 SD 1.6. No badger and red fox were identified.

Table 8. Porcupines individually marked and/or recognisable by the presence of phenotypic peculiarities in each porcupines family groups identified in EA2. For the specimens phenotypically recognisable the phenotypic characteristic is reported.

	Specimen	Marked	Recognisable
Family 1	Adult female	X	Black tapes on the quills
	Adult male	NO	Injury in the rump (left) Blindness right
Family 2	Adult female	X	Red tapes on the quills White paint on the crest
Family 3	Sub-adult female	X	White tapes on the quills
	Porcupette female	X	Black tapes on the quills
	Sub-adult male	NO	Injuries in the rump (left)

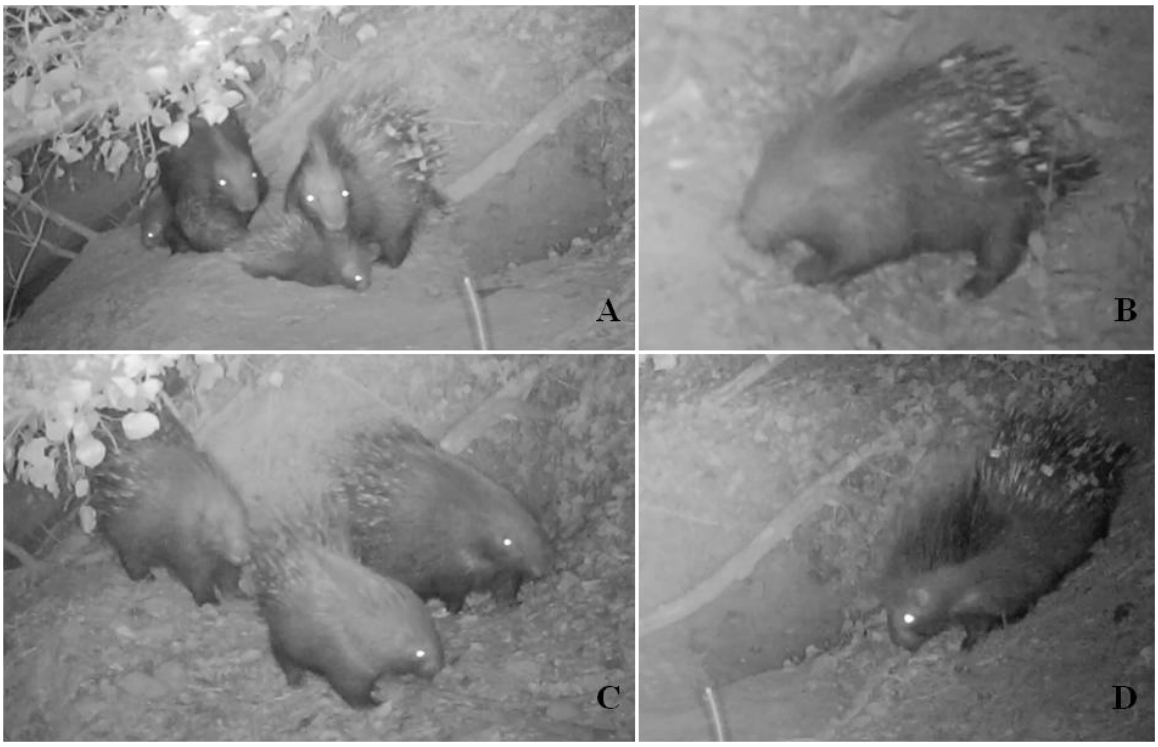


Figure 68. Marked and individually recognisable individuals in the family 1 of EA1: (A) the marked sub-adult female with red tapes on the quills and black paint on the crest with the adult female and two 1 month old porcupettes, (B) the marked adult male with white tapes on the quills, the blindness of the left eye is visible, (C) the marked adult male with white tapes on the quills, the blindness of the right eye is also visible, with the adult female and the marked male porcupette with white paint on the tail, (D) the marked sub-adult female with red tapes on the quills and black paint on the crest.

Figure 69. Marked and individually recognisable individuals in the family 2 of EA1: (A) the marked adult male with black tapes on the quills, (B) the left eye blind adult female, (C) the marked porcupette female with white tapes on the quills and (D) the without crest sub-adult.

Figure 70. Individually recognisable specimens in the family 3 of EA1: (A) the blind left eye adult male with the blind right eye adult female, a sub-adult and a porcupette and (B) the blind right eye adult female with blind left eye adult male and a sub-adult.

Figure 71. Marked and individually recognisable specimens in the family 4 of EA1: (A) the marked adult male with black tapes on the quills and black paint on the tail, (B) the marked adult male with black tapes on the quills and black paint on the tail with the adult female of the pair (C) the marked adult male with black tapes on the quills and black paint on the tail with the adult female of the pair and a porcupette and (D) the marked sub-adult female with red tapes on the quills and white paint on the tail with another sub-adult individual.

Figure 72. Marked and individually recognisable specimens in the family 5 of EA1: (A) the recognisable adult female with the crest carried on the left side (B) the marked adult male with white tapes on the quills with the recognisable adult female with the crest carried on the left side, the injury on the adult male nose is visible (C) the marked adult male with white tapes on the quills an recognisable for the presence of an injuries on the nose with the recognisable adult female with the crest carried on the left side, a sub-adult and a porcupette and (D) the marked adult male with white tapes on the quills with a sub-adult individual.

Figure 73. (A) An adult badger blind from right eye and (B) an adult female of red fox blind from its left eye both detected in EA1.

Figure 74. Marked and individually recognisable specimens in the family 1 of EA2: (A) the marked adult female with black tapes on the quills with the recognisable adult male, the injury on the male rump is visible and (B) the the recognisable adult male, the blind from the right eye is visible, with the marked adult female with black tapes on the quills and a porcupette.

Figure 75. Marked specimens in the family 2 of EA2: (A) the marked adult female with red tapes on the quills and (B) the marked adult female with red tapes on the quills with the adult male of the pair.

Figure 76. Marked and individually recognisable specimens in the family 3 of EA2: (A) the marked sub-adult female with white tapes on the quills, (B) the recognisable sub-adult male with a injury in the rump, (C) the marked sub-adult female with white tapes on the quills with the marked porcuppette female with black tapes on the quills and (D) the marked porcuppette female with black tapes on the quills with an adult individual.

Co-habitation of settlements and interaction between crested porcupines, badgers and red foxes

The inhabitation of settlements and interaction between crested porcupines, badgers and red foxes were assessed in 12 out of 16 settlements. The investigated settlements were often explored by crested porcupines, badgers and red foxes within the same night but at different times (Fig 77).

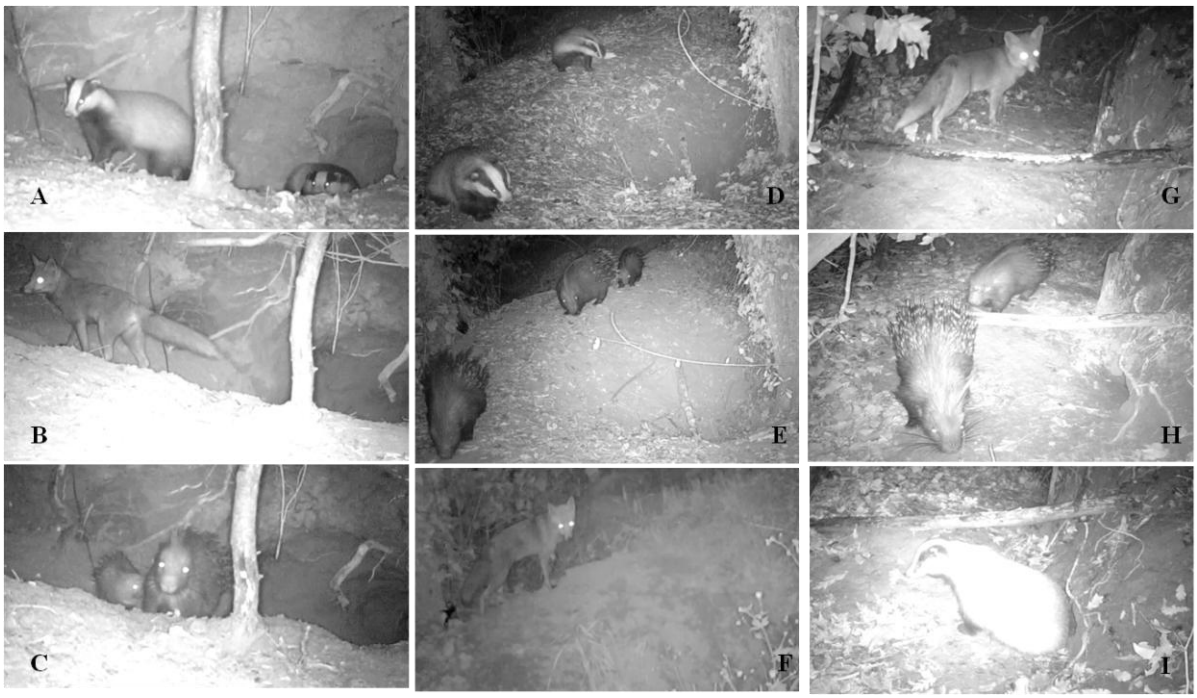


Figure 77: Sharing of the same settlements at different time by crested porcupines, badgers and red foxes observed in S8 (A, B, C), in S1 (D, E, F) and in S9 (G, H, I).

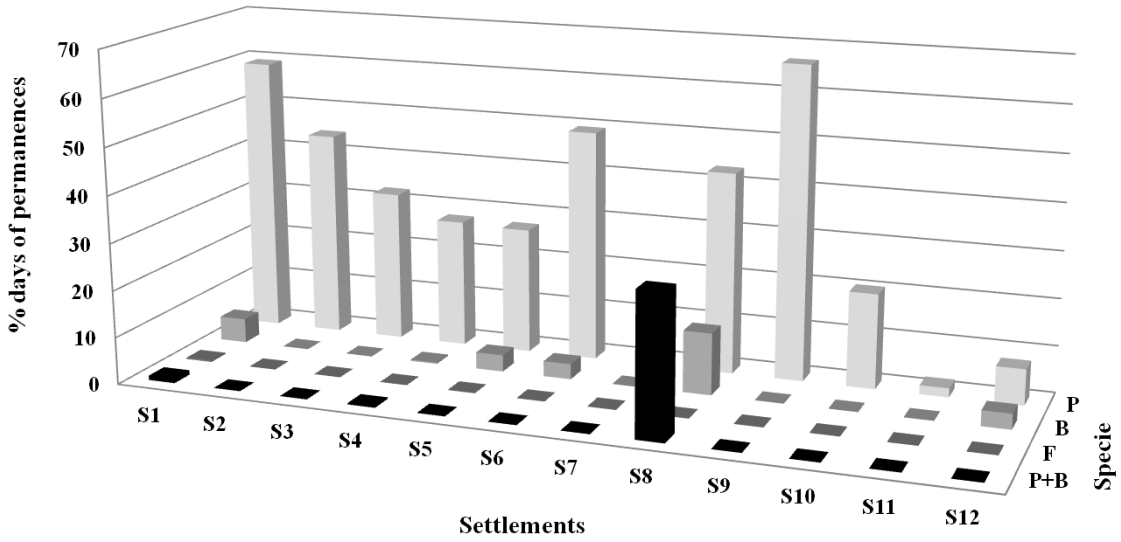
The minimum time of permanence resulted 10 hours and 11 minutes while the average time was 13 hours and 44 minutes (SD = 1 hour and 26 minutes). Porcupines inhabited not permanently 12 monitored settlements, the red foxes inhabited only S1 and S2 while the badgers inhabited 7 out of 16 settlements, four of them (S2, S5, S6, S12) in absence of porcupine and three (S1, S4, S8) in presence of porcupines (Fig 78).

In each settlement, the porcupine's permanence ranged from 1 day to 10 consecutive months. The longer permanence of porcupines in one settlement was related to the presence of porcupettes.

The badgers had inhabited S1, S2, S4, S5 and S12 for short and reiterate periods (1 to 6 days) in autumn and winter. Only in S8 a social group of three badgers have permanently inhabited

the settlemenet from September 2018 to February 2019 and from June to July 2019. The same social group of badgers inhabited S8 for short and reiterate periods (1 to 4 days) between March and June 2019. In the others settlements, events of inhabitation by badgers in spring and summer were rarely recorded and only for very short periods (1 to 2 days).

The red foxes inhabited S1 on two occasions in spring (April 2018 and April 2019). In both cases, red fox exploratory activity in the settlement inhabited by porcupine family 1 with porcupettes, was recorded. After 6 days, the resident porcupine family moved to S2.



	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
P+B	1,0	0,0	0,0	0,3	0,0	0,0	0,0	30,1	0,0	0,0	0,0	0,0
F	0,3	0,00	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
B	5,2	0,0	0,0	0,3	3,6	3,3	0,0	13,2	0,0	0,0	0,0	3,3
P	59,2	44,1	32,3	27,4	27,0	49,3	1,4	43,0	66,6	20,3	1,9	7,7

Figure 78. Settlements inhabitation. Percent of days of inhabitation of the 12 settlements by crested porcupines (P), badgers (B), red foxes (F) and cohabitation between porcupines and badgers.

The red fox immediately occupied S1 and gave birth to four pups in April 2018 and five pups in April 2019 (Fig 79). The red fox and its pups inhabited the settlement for one month in springs in both years. After the red fox and its pups left the settlement, the porcupine family 1 returned.

Figure 79. The adult red fox in April 2018 while nursing its pups in S1 (A) and the red fox while resting with its pups (B) in S1 in April 2019

The co-habitation was only recorded between badgers and porcupines in S1 (n=7 events), S4 (n=2 events) and S8 (n=110 events) (Fig. 78), each inhabited by a different recognisable porcupine family, for a total of 119 events. The 5% (n=6) of co-habitation events were recorded in 2017, the 43.7% (n=52) in 2018 and the 51.3% (n=61) in 2019.

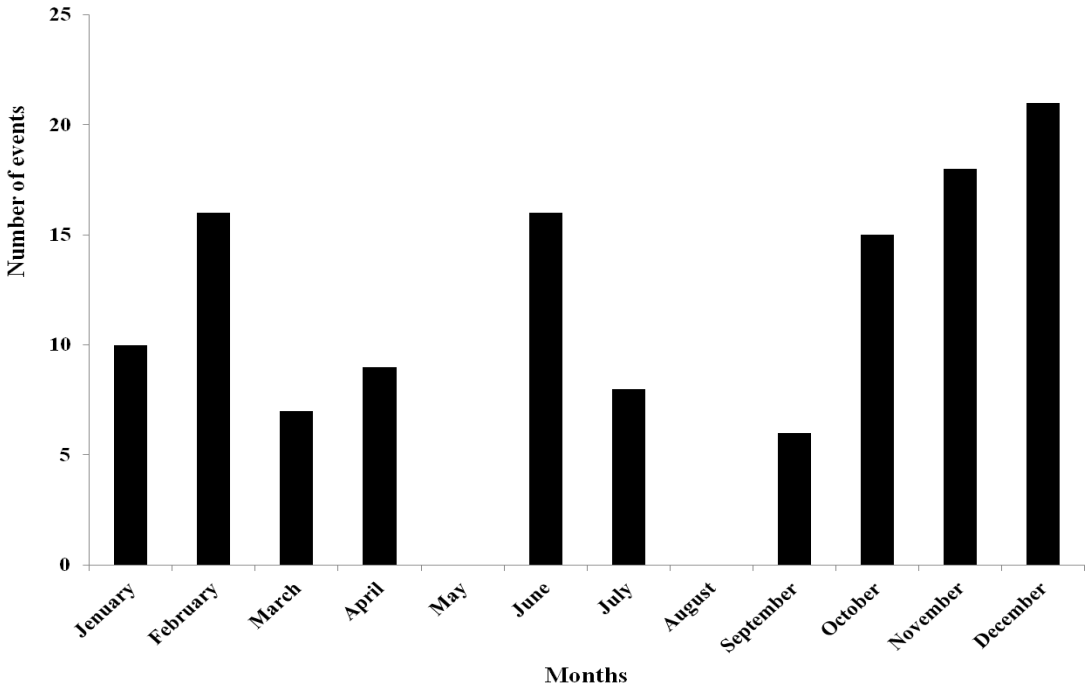


Figure 80. Number of monthly co-habitation events recorded during the whole period of monitoring

The co-habitation between porcupines and badgers resulted distributed throughout the year (Fig 80). The S1 and S4 were both co-habited by a single badger and a porcupine family

group for no more than 1 day. S8 was co-habited by a social group of three badgers and a family group of five porcupines (two adults, one young and two porcupettes). Badgers and porcupines in 2018 co-habited in the S8 for 26, 24 and 23 day intervals from October to December and for 14 and 16 day intervals in February and June-July 2019 respectively.

In the 77.3% of co-habitation days (n=92) the co-habitation was performed in the presence of porcupettes. Co-habitation between badgers and porcupines with porcupettes was recorded in all settlements in which co-habitation occurred.

Porcupines and badgers in S1 only used 2 out of 4 monitored ground holes, 1 out of 2 in S4 and 2 out of 5 in S8 for emerging-from and returning-to the burrow. In Table 9 are reported the percentage of use of the same or different burrow entrance holes during emerging-from and returning-to the burrow. Usually each species used separate entrance. In some cases the two species used the same entrance hole without any specific priority in the entrance order of one species over the other. Only in one case in S8 the two species use both entrance holes without priority in the entrance order of one species over the other.
Within the co-habitation days (n=119), badgers emerged from and returned to the burrow after the porcupines 30 times (25.2%),they emerged after and returned before the porcupines in 62 times (52.1%), emerged before and returned after the porcupines 14 times (11.7%) and emerged and returned before the porcupines 18 times (15.1%). The average delay of first emergence between the two species was 46 minutes (SD = 38 minutes). The last returning home was recorded with an average delay between the two species of 41 minutes (SD = 37 minutes). The average co-habitation time between the two species was 12 hours and 40 minutes (SD = 1 hour and 6 minutes), from 10 hours and 11 minutes to 16 hours.

The exclusively inhabitation of porcupines temporarily uninhabited burrows by red foxes and badgers resulted significantly different ($p<0.001$, $X^2 = 52.26$). The foxes exclusively inhabited the settlements in the 100% of cases while badgers both exclusively inhabited and co-habited with porcupines in 57% and 43% of cases respectively. Within a settlement, all the co-habitation events always happen in the vicinity (no more than 250 meters) of another available free/empty settlement.

Table 9. Percentage of use of the same and different burrow entrance holes during emerging-from and returning-to the burrow by porcupines and badgers in S1, S4 and S8. The single event of co-use of the ground holes by both species is not here reported.

	S1	**S4**	**S8**
Same hole	2 (14.3%)	2 (100%)	23 (21.3%)
Different holes	12 (85.7%)	0	84 (77.7%)

Totally 44 interaction events were recorded of these the 86.4 % (n=38) were detected between porcupines and badgers in S1, S8 and S9 (Fig 81), the 11.4 % (n=5) between porcupines and foxes in S1, S2, S9 and S10 (Fig 82A) and the 2.3 % (n= 1) between badgers and foxes only in S1 (Fig 82B). In Table 10 the number and percentage of both types of interactions between P-B, P-F and F-B in each settlement are reported.

In 63.2% (n=24 events) of porcupines and badgers interactions, a mutual avoidance behaviour was observed. Events of aggressive interactions occurred in 36.8% (n=14 events). Aggressive interaction between badgers and porcupines always starts with the two animals staring at each other then, suddenly the badger would move forward while the porcupine would attack sideways. Erection of quills, rattling of tail, snorting and grumbling from the porcupine would usually be enough to end the interaction.

In three occasions, in S8 interactions between adult badger and a four-month old porcupette were recorded. The porcupette always drove away the badger by sideways attack, quills erection and tail rattling.

Figure 81. Interactions between crested porcupines and badgers: (A) Aggressive interaction between adult badger and a three months old porcupette of family 3 in S8, (B) Aggressive interaction between an adult badger and the adult male porcupine of family 5 in S9, (C) Avoidance interaction between a sub-adult with a porcupette of family 3 and an adult badger in S8 and (D) Avoidance interaction between an adult badger and the adult male porcupine of family 1 in S1.

Figure 82: Avoidance interactions between the adult male porcupine of family 1 (in the circle) and a young red fox (A) and between an adult red fox and an adult badger in S1

Table 10. Number and percent of avoidance (AV) and aggressive interactions (AI) recorded between porcupines and badgers (P-B), porcupines and foxes (P-F), badgers and foxes (B-F) in those settlements in EA1 in which interactions occurred during the monitoring period.

	Settlement	AI	AV	AI+AV
P-B	S1	0	8 (100%)	8
	S8	12 (40%)	14 (60%)	26
	S9	2 (50%)	2 (50%)	4
P-F	S1	0	1 (100%)	1
	S2	2 (100%)	0	2
	S9	0	1 (100%)	1
	S10	1 (100%)	0	1
B-F	S1	1 (100%)	0	1

In 40% (n=2) of porcupines and red foxes interactions a mutual avoidance behaviour was also recorded. Aggressive interactions between porcupines and red foxes were always recorded as consequence of an intense exploratory activity of the red fox just after the birth of porcupettes in S2 and in S10. The explorative behaviour of the red fox was always the same: it approached the entrance hole, sniffed, tried to enter and then walked away. In two cases, an adult porcupine drove away the red fox while it was sniffing in front of the entrance hole where the porcupettes were present. After this, the red fox activity immediately stopped.

No statistical differences were recorded in the number of avoidances and aggressive interactions between P-F and P-B in not co-habited settlements while statistical differences ($p<0.01$, $\chi^2 =7.27$) resulted in the number of avoidances and aggressive interactions between P-B in the co-habited and not co-habited settlements.

Predation events on porcupine by both badger and red fox have never been observed.

The scavenging of porcupine carcasses by red foxes was recorded in October 2018 in S1 and in November 2019 in S2 (Fig 83). In both cases the recorded videos clearly show the red fox sniffing and yelping before partially enter the ground hole and pulling out a dead porcupine from the burrow without any display of predatory movements. In both cases, the fox ate the innards of the dead porcupine and decapitated the carcass. The fox then moved the remains a few meters away (Fig 84).

Furthermore, in the first case, a badger's exploratory activity was also recorded a few minutes before the red fox arrival. In this occasion, the badger stopped and sniffed in front of the burrow where the dead porcupine was present and then moved away.

Figure 83. Scavenging of porcupine carcass by red fox. (A) The red fox while extracting the dead porcupine from burrow. (B) The red fox while scavenging porcupine carcass after extracting it from the burrow

Figure 84. Carcass of decapitated porcupine found near the burrow from where it was extracted (Photo by the Author)

Spatio-temporal inhabitation of settlements by crested porcupine

All the 12 investigated settlements resulted inhabited always by only one porcupines family without cohabitation with other porcupines. In four occasions, 2 in S1 and 1 in S2 and S5, encounters between porcupines that inhabited the settlement and porcupines visiting the settlement were recorded and in all cases fighting occurred. The family 3 in EA2 and 4 out of 5 porcupine families in EA1 alternatively and complementarily inhabited two settlements belonging to a station while the family 5 in EA1 stably inhabited only one settlement. Each porcupine family show a different inhabitation pattern of the two inhabited settlements during the whole period of monitoring (Fig 85). Different inhabitation patterns were also observed between the 5 porcupine families in EA1 during the same year of monitoring (Fig 86).

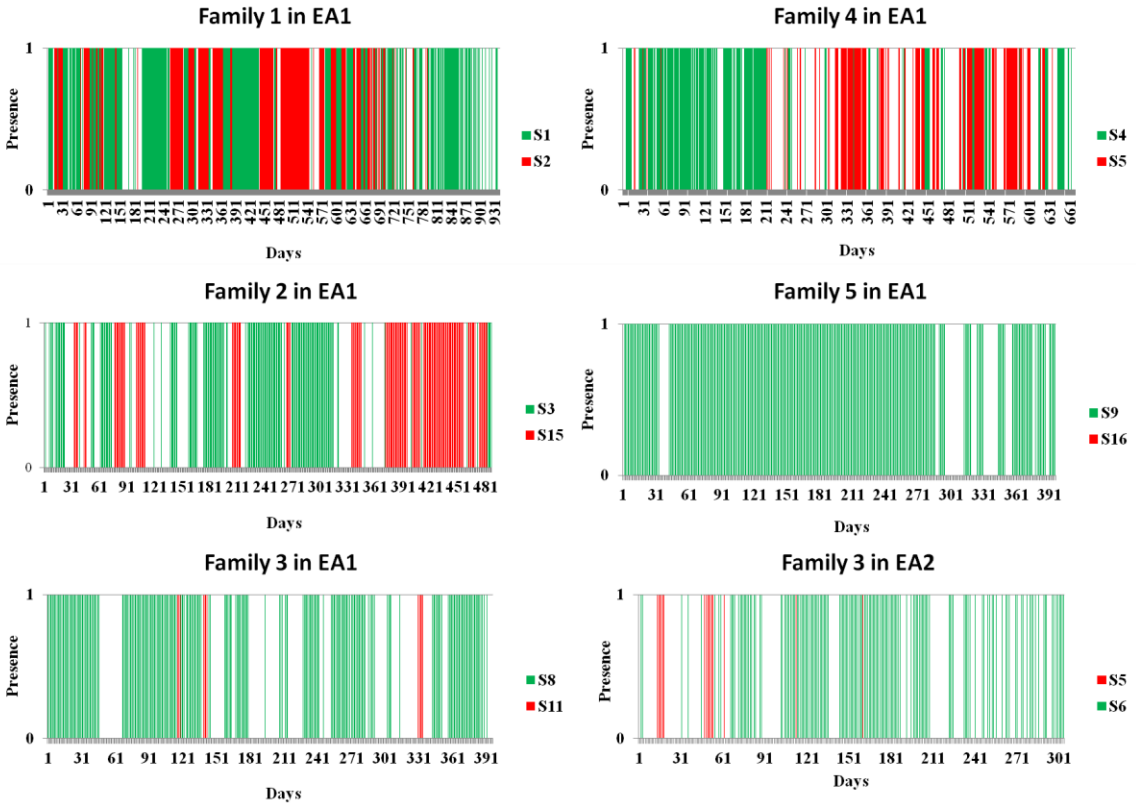


Figure 85. Inhabitation pattern of each porcupines family group in the two inhabited settlements (green and red colored) belonging to a station during the whole monitoring period. White space indicates the days in which it was not possible to assess the inhabitation. Green colour always refer to the mostly used settlement

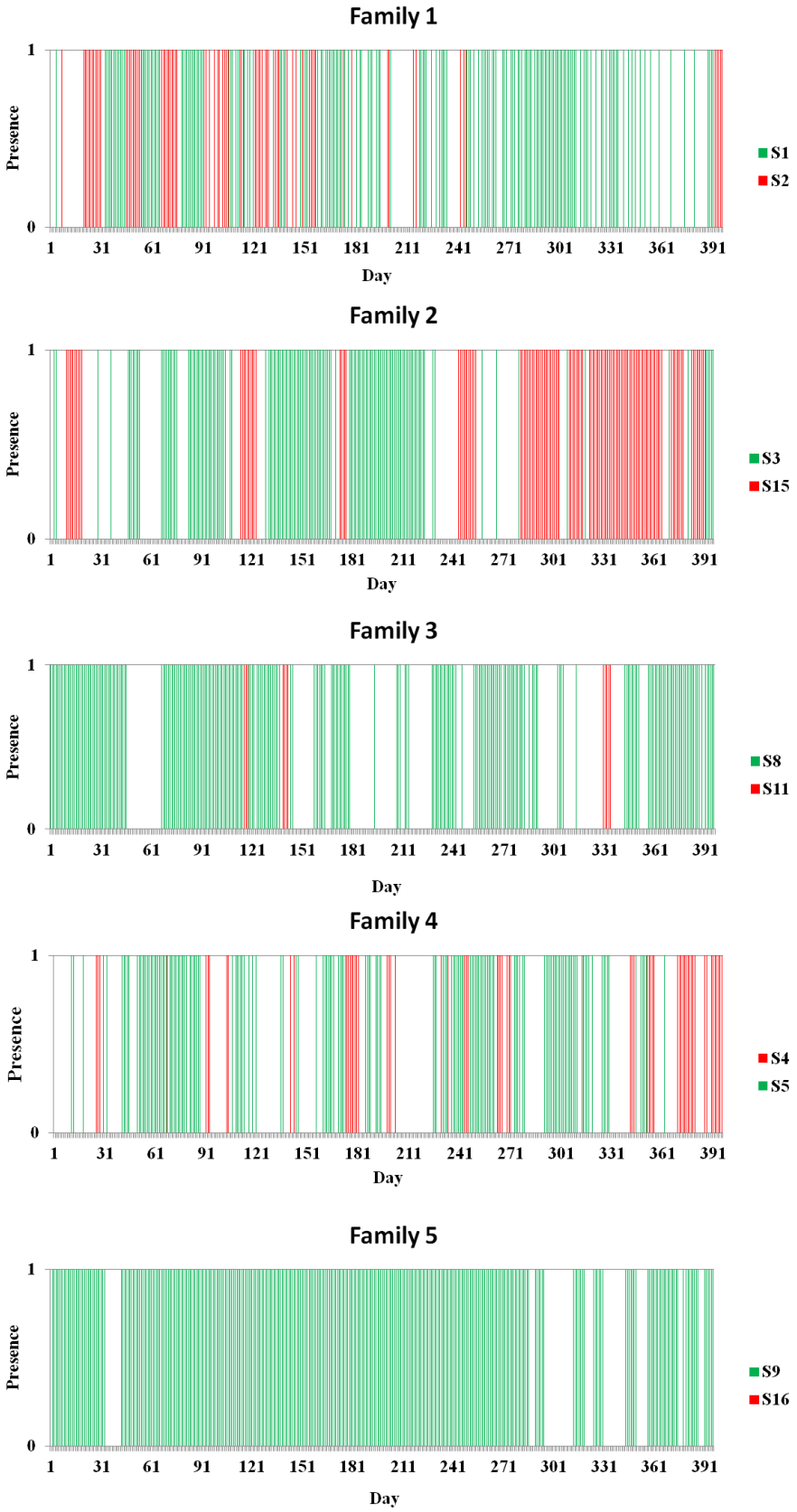


Figure 86. Inhabitation patterns of the 5 porcupines family in their two inhabited settlements (green and red colored) belonging to a station in EA1 during the same period of monitoring (July 2018 and July 2019). White spaces indicates the days in which it was not possible to assess the inhabitation Green colour always refers to the mostly used settlement.

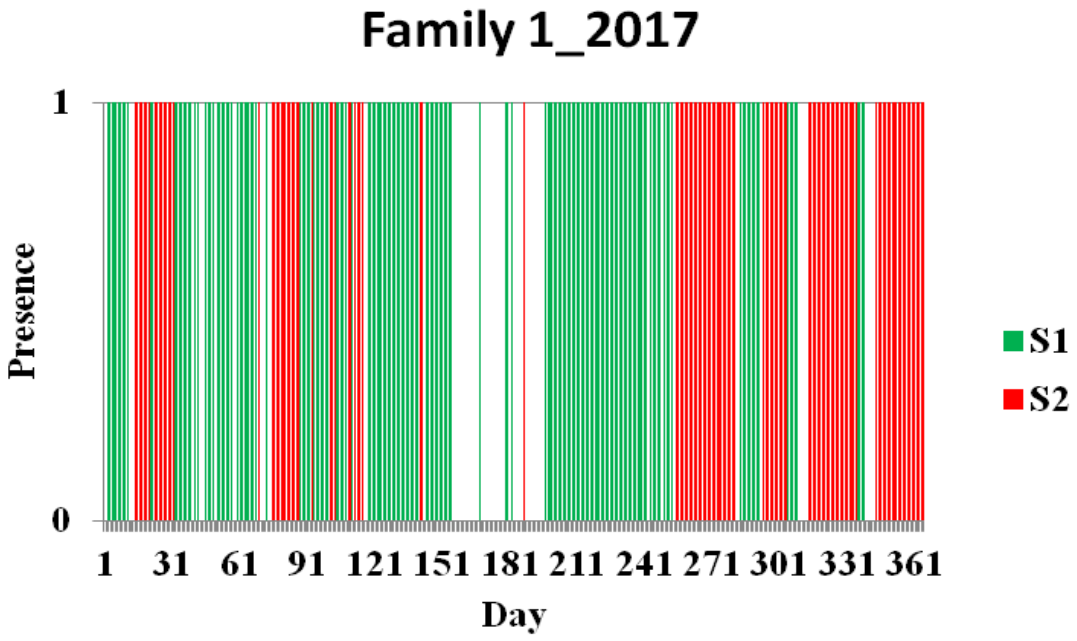
In EA1 the settlements S1, S11and S16 were oriented to South-west, the settlements S2, S3 and S5 to South-east, the settlements S4, S8 and S9 to North-west and S15 to North-east. In EA2 the settlement S5 was oriented to North-west while S6 to South-west. Inhabitation of the station by other porcupines families other than the "owner" was never been observed not even when the "resident" family was not present in one of the two settlements.

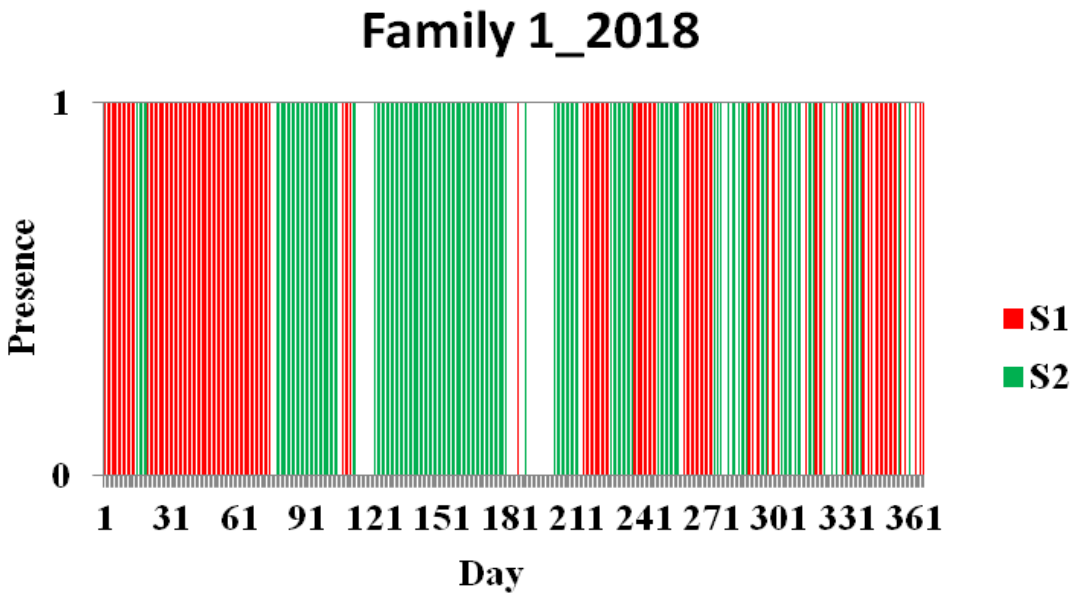
In EA1 the family 1 inhabited the S1 and S2, the family 2 the S3 and S15, family 3 the S8 and S11, family 4 the S4 and S5 while, family 5 always inhabited only S9. In EA2 the family 3 inhabited S5 and S6. In EA1 the frequencies of inhabitation of the families 1, 3, 4, 5 in S1 (n=421 days), S8 (n= 239 days), S4 (n=230 days) and S9 (n=331 days) during the whole period of monitoring was significantly higher ($P<0.001$) than in the S2 (n=297 days), S11 (n=12 days), S5 (n=165 days) and S16 (n=0) respectively. The family 2 inhabit significantly more frequently ($P<0.05$, $\chi^2 = 4.42$) S3 (n=181 days) than S15 (n=150 days). The family 3 in EA2 inhabited the S6 (n=158 days) significantly more frequently ($P<0.001$, $\chi^2 = 174.05$) than the S5 (n= 12 days).

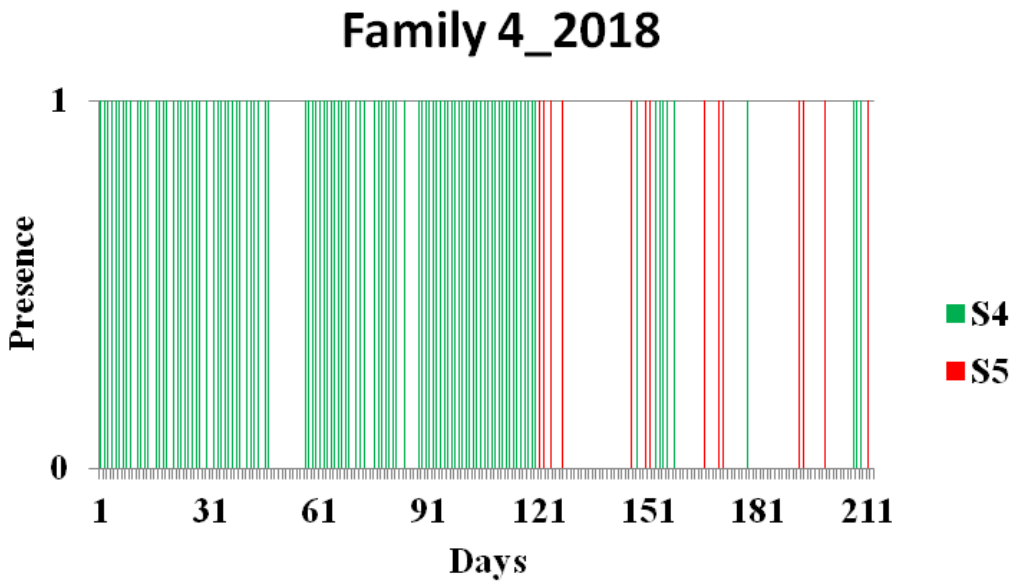
Overall the inhabitation frequencies resulted significantly higher ($P<0.001$) in N-W and S-W oriented settlements than in S-E and N-E oriented settlements and in S-E oriented settlements than those to N-E. No statistical differences were recorded between the inhabitation frequencies in N-W and SW oriented settlemements.

In EA1 the family 1 inhabited S1 and S2 with a clearly different pattern in 2017 and 2018 (Fig 87). The S1 (n=173) was significantly more frequently inhabited ($P<0.001$, $\chi^2 = 13.06$) than S2 (N=125) in 2017 while no statistical difference were recorded in the inhabitation of S1 (n=147) and S2 (n=160) in 2018. The frequencies of inhabitation of S1 by family 1 in 2017 (n=173) and 2018 (n=147) do not show statistical differences while the inhabitation of S2 was significantly higher ($P<0.01$, $\chi^2 = 7.05$) in 2018 (n=160) than in 2017 (n=125). As observed for family 1, also the family 4 showed a different inhabitation pattern in the two settlements in the period January-July 2018 and 2019 (Fig 88). The S4 (n= 123) was significantly more frequently inhabited ($P<0.001$, $\chi^2= 10.82$) than S5 (n=83) between January and July 2018. Conversely, between January and July 2019 the frequency of inhabitation of family 4 was significantly higher ($P<0.001$, $\chi^2= 14.95$) in S5 (n=92) than in S4 (n=53). The inhabitation of S4 was significantly higher ($P<0.001$, $\chi^2= 39.42$) between January and July 2018 (n=107) than in January-July 2019 (n=45). The S5 was inhabited by Family 4 significantly more frequently ($P<0.001$, $\chi^2= 58.19$) in January-July 2019 (n=79) than in January-July 2018 (n=14).

Overall 133 events of settlement change were recorded and only in the 2.3% (n=3) the increase of the exploratory activity by badger and/or red fox in the days before the settlement change were recorded. In the 97.7% (n=130) the settlement change occurs without any detectable disturbance and no settlement change was documented even if in presence of high human disturbance for hunting. The inhabitation of the settlements by each family ranged from 1 to 244 consecutive days with an average time of permanence in each settlement of 7 SD 16.6 days. Long periods of permanence in the same settlement (> 20 consecutive days) were recorded in 24 occasions and in the 50% of these (n=12) porcupettes were present.







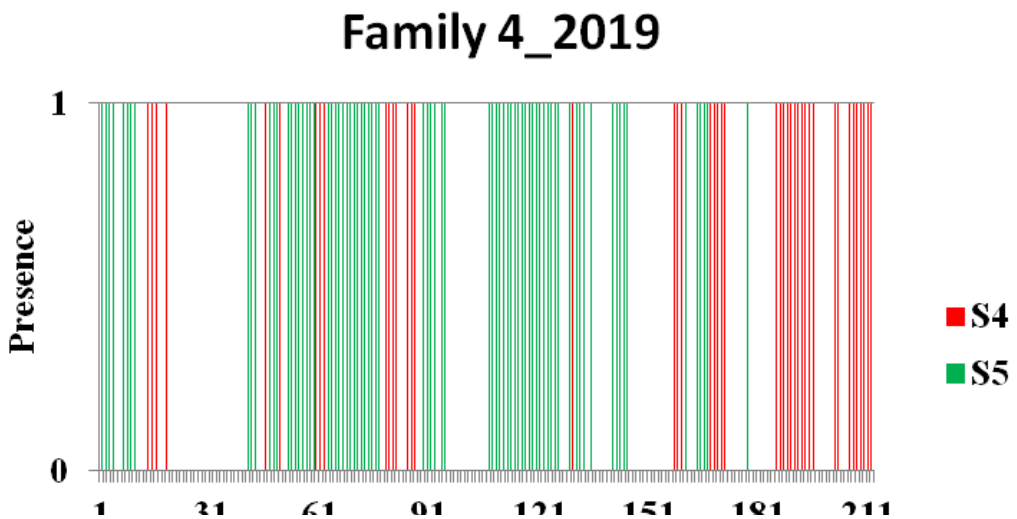


Figure 87. Inhabitation pattern of porcupine family 1 in EA1 in S1 and S2 belonging to a station in 2017 and 2018. White spaces indicates the days in which it was not possible to assess the inhabitation Green colour refer to the mostly used settlement

Figure 88. Inhabitation pattern of porcupine family 4 in EA1 in S4 and S5 (green and red colored) belonging to a station in the period January-July in 2018 and 2019. White spaces indicates the days in which it was not possible to assess the inhabitation Green colour refer to the mostly used settlement

First daily emergence time of crested porcupine from burrow

Overall 1077 events of first emergence of porcupines from burrow were recorded from all the monitored settlements of both the study areas during all the monitoring period (Fig 89 and Fig 90).

The average delay of emergence of porcupine from burrow compared to the time of sunset throughout the years resulted to be 1 hour and 23 minutes (SD = 1 hour and 1 minute. In the cold period (September to February) the average delay of emergence resulted 1 hour and 54 minutes (SD = 1 hour and 4 minutes) with a maximum delay of 6 hours and 18 minutes. In the warm period (March to August) the average delay of emergence resulted 45 minutes (SD = 27 minutes). In this period the maximum delay of emergence resulted 3 hours and 36 minutes. The first emerging from burrow before sunset was recorded mainly between March and August for a total of 36 events while only one event was recorded in cold period. The average advance of emergence resulted 24 minutes (SD = 21 minutes) with a maximum advance of 1 hour and 22 minutes.

Figure 89. (A) the adult pair and two porcupettes of family 2 in S3, (B) the adult male and a sub-adult of family 5 in S9, (C) the adult pair and two porcupettes of family 1 in S2 and (D) the adult female and a sub-adult of family 3 in S8 during their first daily emergence from burrows in EA1

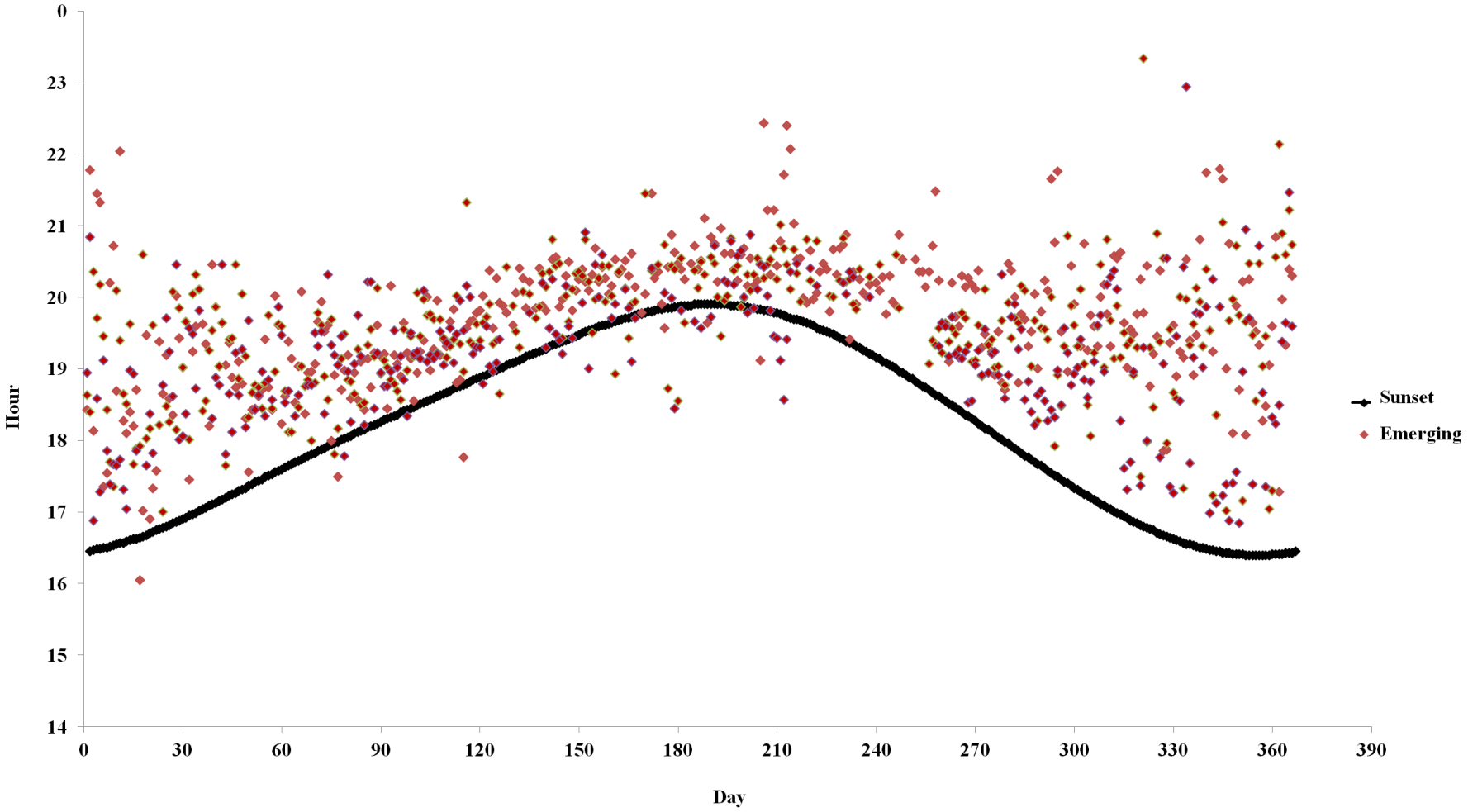


Figure 90. Graphical representation of the 1077 events of first daily emergence of porcupines from burrows compared to the time of sunset throughout all the year (21 December - 20 December)

Diurnal motor activity and sunbathing in crested porcupine

Diurnal motor activity was recorded in 10 out of 16 settlements for a total of 148 events of diurnal motor activity recorded in 214 videos (1.06% of videos where porcupines were present) (Fig 91A). Times of permanence outside the burrow were obtained from 11 diurnal motor activity events (Fig 91B).

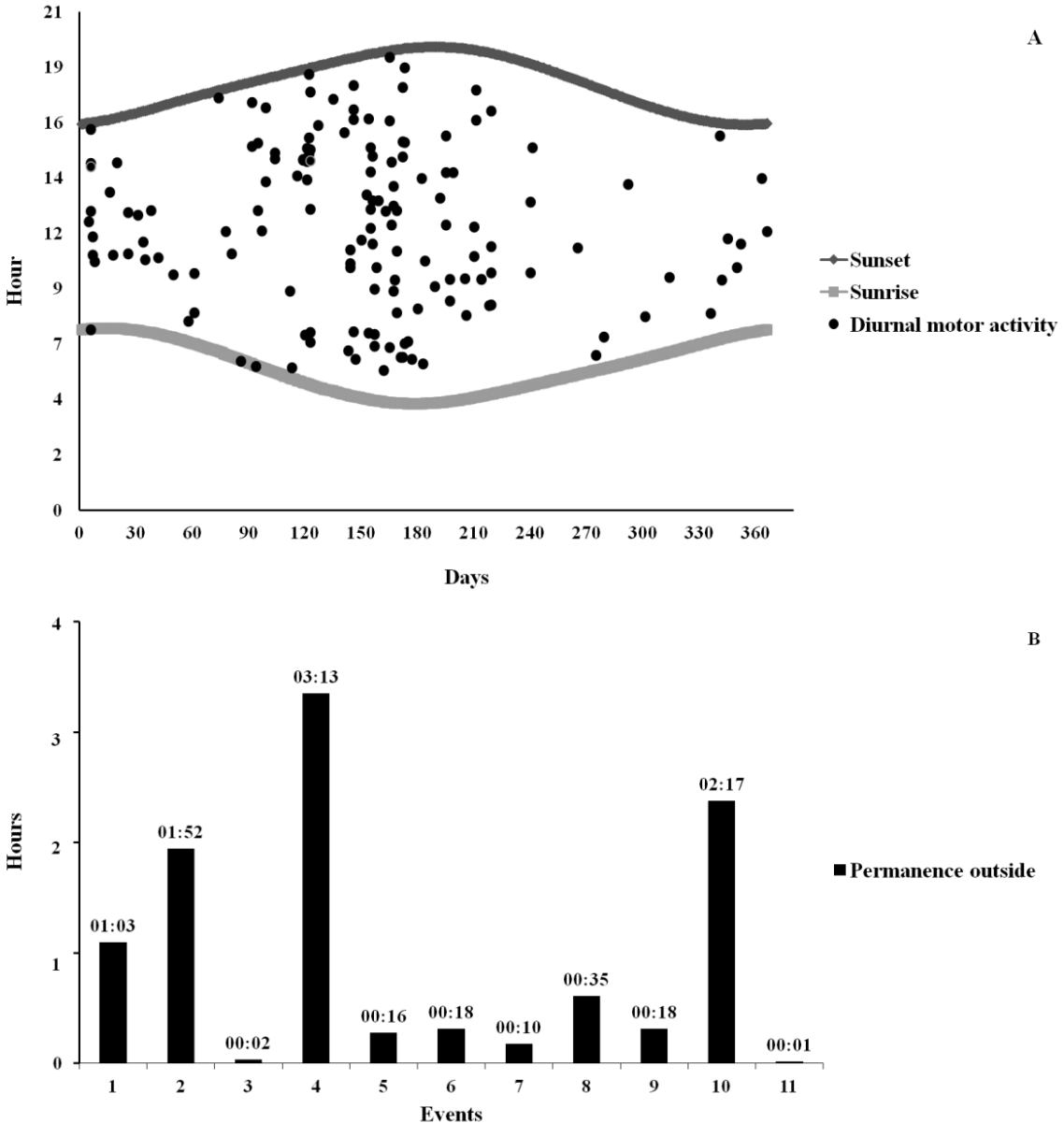


Figure 91. (A) Graphical representation of the 148 diurnal motor activity events recorded in the monitored settlement in relation to the time of sunset and sunrise during the year (21 December - 20 December) and (B) times of permanence outside the burrow recorded in 11 diurnal motor activity events.

The average time of permanence outside the burrow resulted in 55 minutes SD 1 hour and 4 minutes. The minimum permanence time was up to 1 minute and the maximum time up to 3hours and 13 minutes.

A diurnal motor activity irregular pattern was observed within each porcupine family throughout the year (Fig 92).

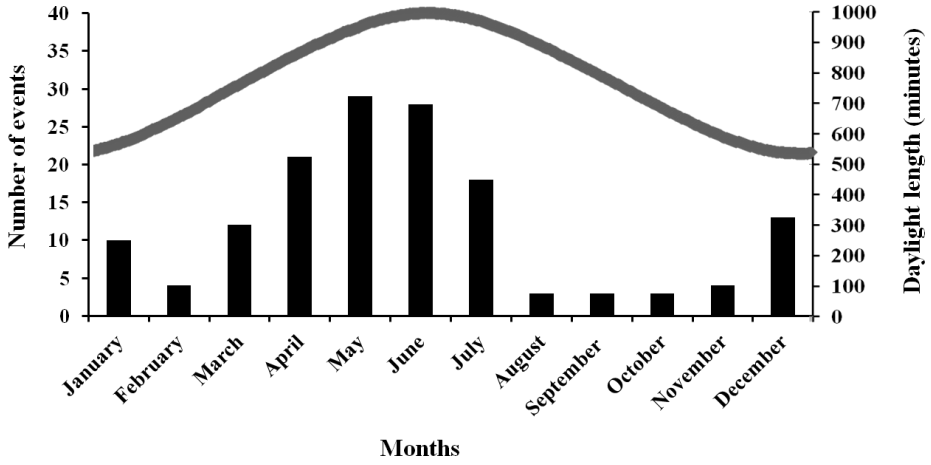


Figure 92. Total number of monthly diurnal motor activity events recorded in the monitored settlement in relation to daylight hours during the year.

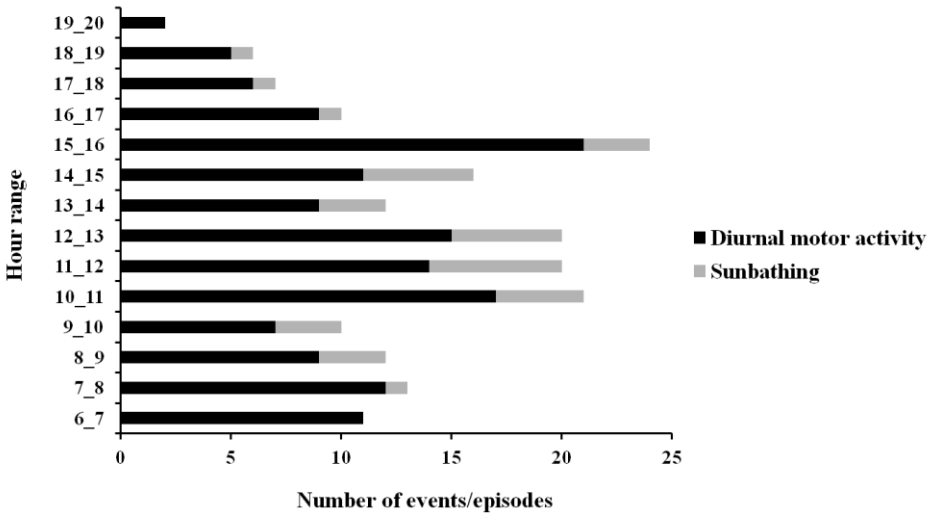


Figure 93. Number of hourly events of diurnal motor activities events and sunbathing episodes recorded in the monitored settlement during the camera-traps monitoring.

The 92.2% (n=135 events) of porcupine diurnal movements were recorded between December and July with a peak between April and June (n=78 events). The 8.7% (n=13 events) of diurnal motor activity was detected between August and November. The diurnal motor activity was recorded at all hours in daytime with a peak between 15:00 to 16:00 (Fig 93).

The 28.3% of daylight events were performed by porcupettes (n=46 events), the 24.3% by youngsters (n=36 events), the 29.7% by adults (n=44 events) and the 14.8% by family groups (n=22 events) (Fig 94, 95 and 96). The diurnal motor activity resulted significantly lower in family groups compared to lonely youngsters ($P<0.05$), porcupettes and adults ($P<0.001$). No significant differences were recorded among the porcupettes, youngsters and adults daylight movements. The occurrence of diurnal motor activity resulted significantly higher in the settlements permanently inhabited ($P<0.001$) than in those only occasionally inhabited by porcupines (Fig 97).

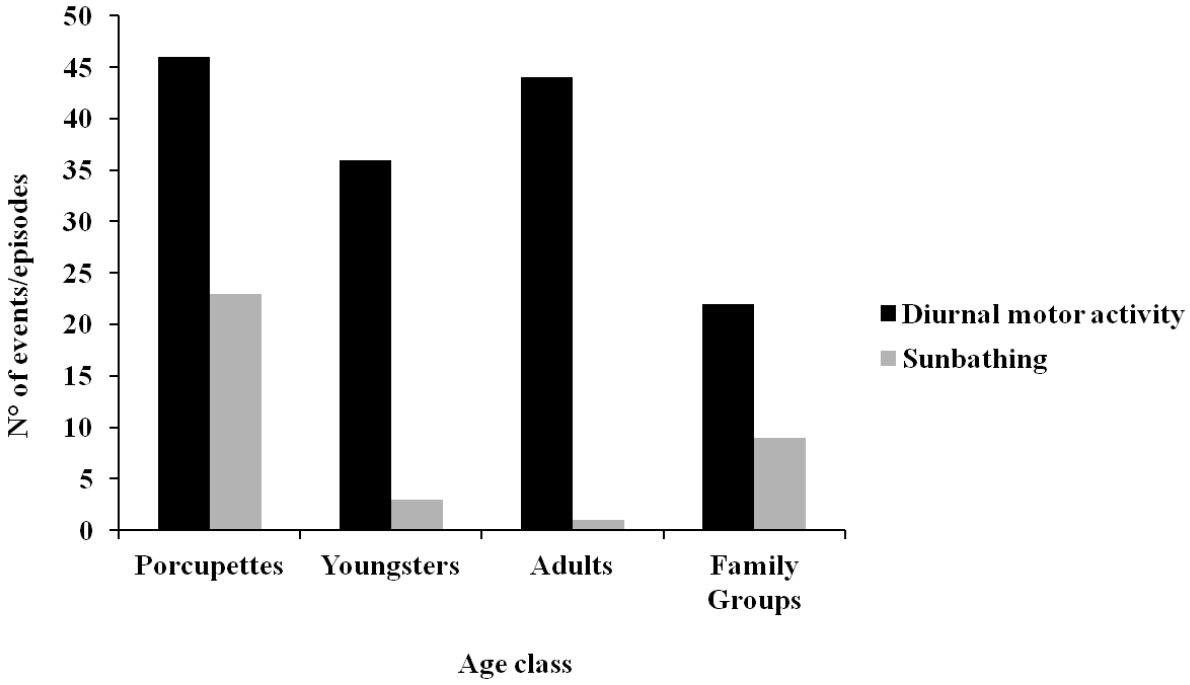


Figure 94. Total numbers of events of diurnal motor activity and sunbathing recorded in the monitored settlement in porcupettes, youngsters, adults and family groups.

Figure 95. (A) The adult male and female of family 5, recognisable by the presence of the injury on the nose and of the crest carried on the left side respectively, with two porcupettes in S9 and (B) the blind left eye adult female of family 2 with a porcupette and a sub-adult in S3 outside the burrow during the daylight hours

Figure 96. (A) The blind left eye adult female of family 2 in S3, (B) the marked adult male with white tapes on the quills (the injury on the nose is also visible) of family 5 in S3, (C) a sub-adult of family 1 in S1and (D) a porcupette of family 3 in S8 outside the burrow during the daylight hours

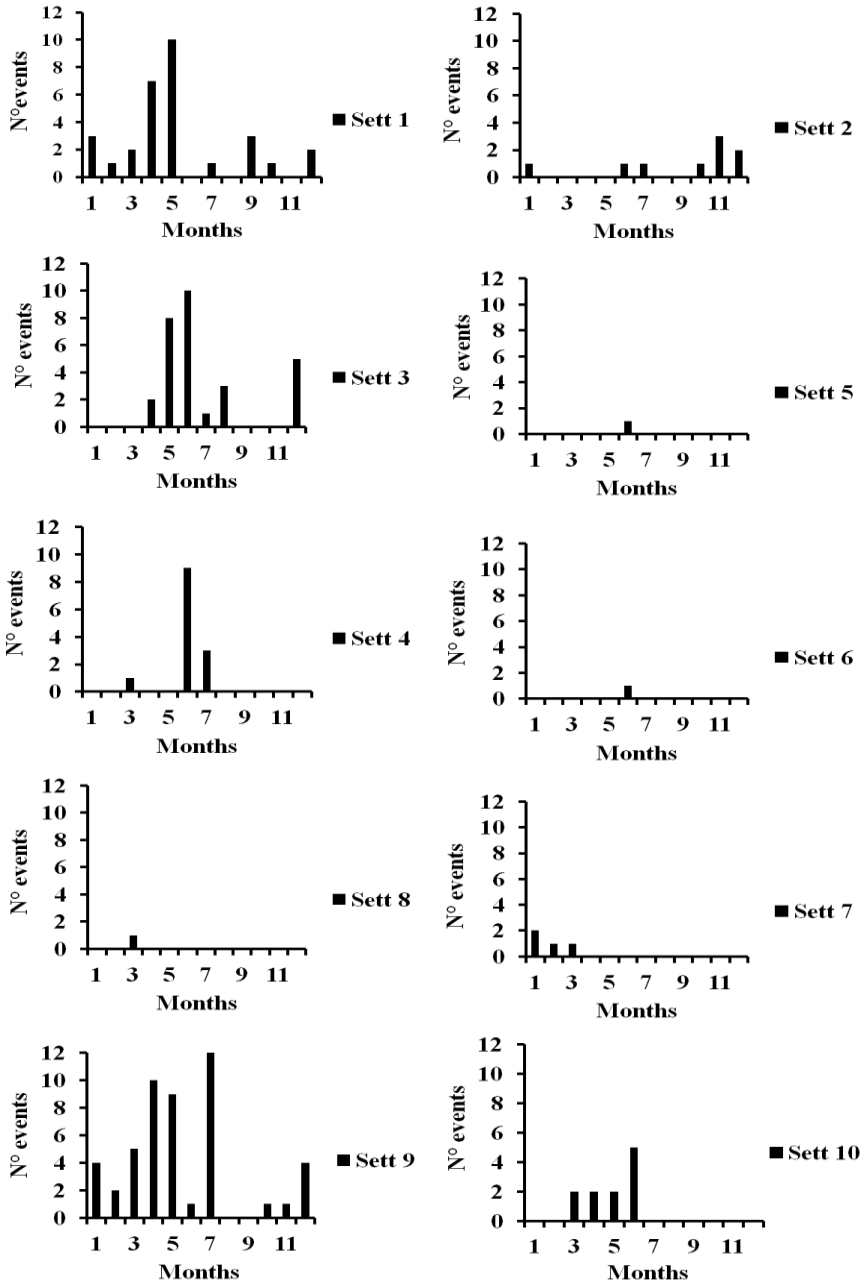


Figure 97. Number of monthly diurnal motor activity events recorded in the settlements permanently (S1, S3, S4, S8, S9) and in those occasionally (S2, S5, S6, S7, S10) inhabited by porcupines.

The sunbathing behaviour was detected in 0.8% (n=160) of videos with porcupines for a total of 36 episodes (Fig 98). The sunbathing was observed only in porcupines belonging to 5 family groups in S1, S3, S6, S9 and S10 always in the vicinity of the ground holes entrance of the burrow (Fig 99).

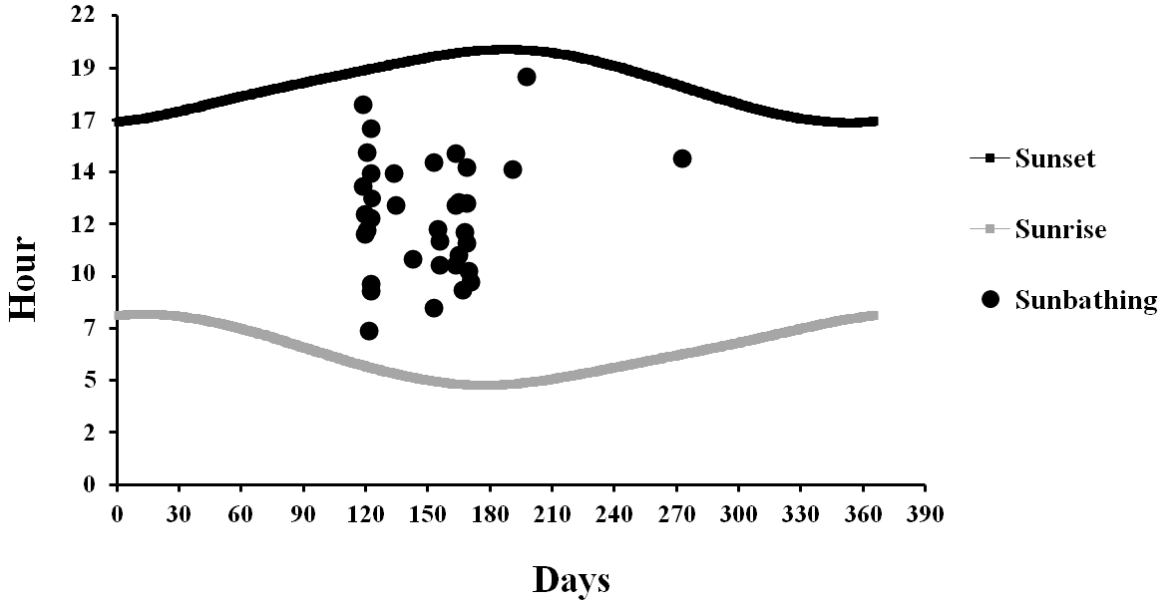


Fig 98. Graphical representation of the 36 episodes of sunbathing recorded in the monitored settlement in relation to the time of sunset and sunrise during the year (21 December - 20 December).

In the 64% of recorded episodes the sunbathing behaviour was performed by porcupettes (n=23 episodes), in the 8.3% by youngsters (n=3 episodes), in the 2.7% (n=1 episode) by adults and in the 25% (n=9 episodes) by porcupettes with adults or youngsters of the same family (Fig 94).

The occurrence of sunbathing behaviour was significantly higher in porcupettes (64%) ($P<0.001$) compared to youngsters (8.3%), adults (2.7%) and porcupettes with adults or youngsters (25%). The sunbathing performance in porcupettes with adults or youngsters was higher than in youngsters only ($P<0.05$) and adults only ($P<0.01$). No statistical difference resulted between adults and youngsters sunbathing performances.

Figure 99. Porcupines performing sunbathing. (A) Two porcupettes of family 2 in S3, (B) the adult female of family 1 in S1, (C) the adult female recognisable by the crest carried on the left side and a porcupette of family 5 in S9 and (D) a sub-adult in S6.

The 72.2% of sunbathing episodes was recorded between April and June mainly from 11:00 to 12:00 (Fig 93).

The average duration of the sunbath resulted to be 20 minutes SD 28 minutes with the duration range between 1 to 123 minutes (Fig 100).

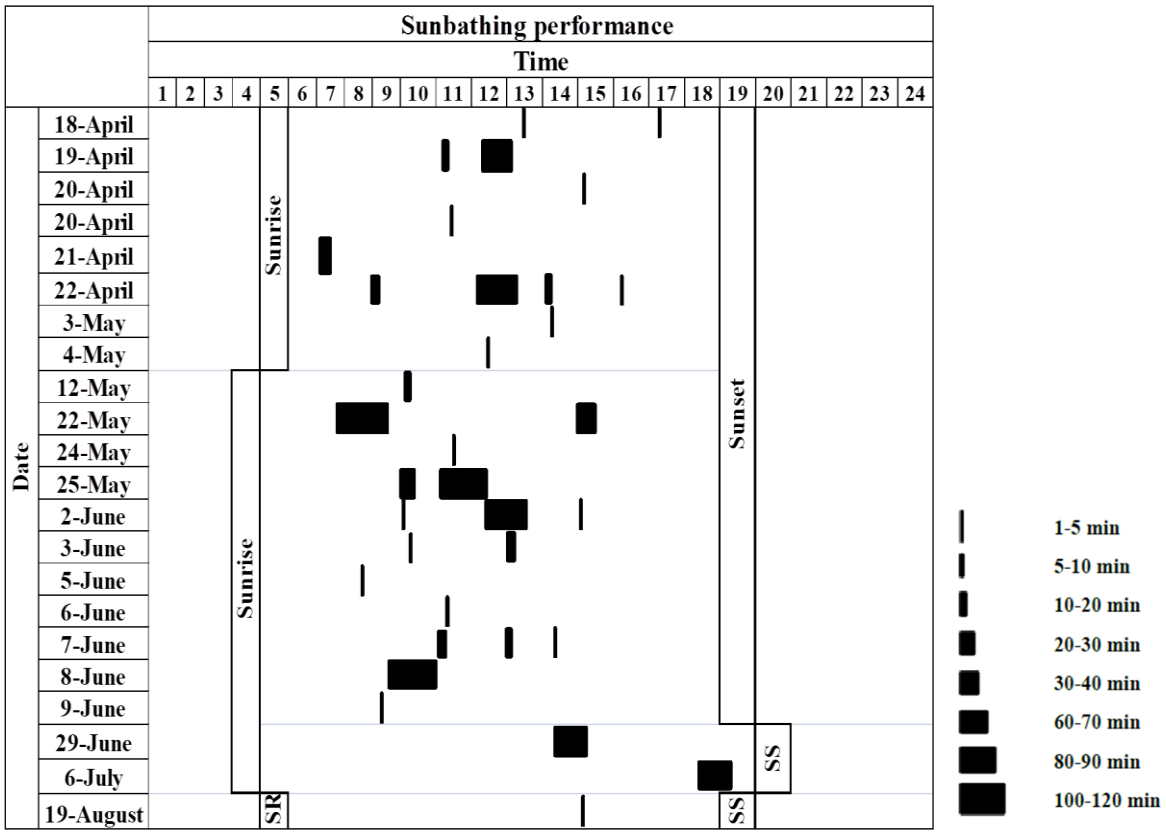


Figure 100. Graphical representation of the length of sunbathing episodes recorded. For each sunbathing episode the date, the time, the duration (min), the time of sunset (SS) and sunrise (SR) are indicated.

Scavenging behaviour in crested porcupine

Overall a total of 4 scavenging events by crested porcupines were recorded. The scavenging of pigeon carcass was observed in the adult female of family 2 in S1, in a no-identified adult in S7 and in one occasions in adult porcupine near capture cage T2 and T4 respectively (Fig 101). In the scavenging event recorded near T2 another porcupine specimen, probably a sub-adult, was present with the adult porcupine without show any interest to the carcass. The same lack of interest was observed in the adult male present with adult female of family 1. In the specimens observed near the capture cage it was not possible to assess the marking; however in one case we cannot exclude that it could be the adult female of family 1. In 4 occasions 2 sub-adults, a male and a female, of family 3 sniffed the pigeon but didn't proceed to scavenging.

Figure 101. Adult porcupine while eat the pigeon carcass near T4

Reproduction in wild crested porcupine

The reproductive behaviour was investigated in the pairs of porcupines belonging to the 5 recognisable families in EA1 (Pair 1 to 5) and to the family 1 (Pair 6), family 2 (Pair 7) and family 3 (Pair 8) in EA2. Overall 813 videos recording the porcupines mounting and copulation behaviour were collected for a total of 217 events of mounting behaviour and 2 copulation events within all monitored pair recorded. In the 80.2% (n=174) single events of mounting were observed while in the 19.8% (n=43) multiple events of mounting in the same night were recorded. In Table 11 are reported the number of nights in which mounting, single mounting (SM), multiple mounting (MM) and copulation for each investigated porcupine pair were performed.

Table 11: Total number of nights in which mounting and/or copulation events were recorded in each monitored porcupine pair. For each pair of porcupine the number of events of single mounting (SM) and multiple mounting (MM) observed were reported.

	Mounting nights (n)	**SM nights (n)**	**MM nights (n)**	**Copulation nights (n)**
Pair 1	76	56	20	0
Pair 2	21	18	3	0
Pair 3	5	5	0	0
Pair 4	12	9	3	1
Pair 5	52	41	11	1
Pair 6	8	8	0	0
Pair 7	8	8	0	0
Pair 8	35	29	6	0

The mounting behaviour was performed throughout the year even after births, in presence of porcupettes and during lactation (Fig 102 and 103).

In the 83.8% (n=182) of the recorded mounting events the mounting sequence started with the nose-quill contact, followed by the presenting of the female with erection of the back quills, raising of the tail on to her back and exposure of the ano-genital region for mounting next step (Fig 104). In the 16.1% (n=35) of mounting events spontaneous presenting of the female were observed. The behavioural pattern of grooming, sniffing, sound, stepping and following were always performed in different sequences during the mounting events observed.

Figure 102. Mounting behaviour in Pair 2 within S3 (A), in Pair 4 within S9 (B), in Pair 1 within S1 (C) in Pair 5 within S4 (D) of the study area EA1 and in Pair 7 within S1 (E) and in Pair 8 within S6 (F) in the study area EA2

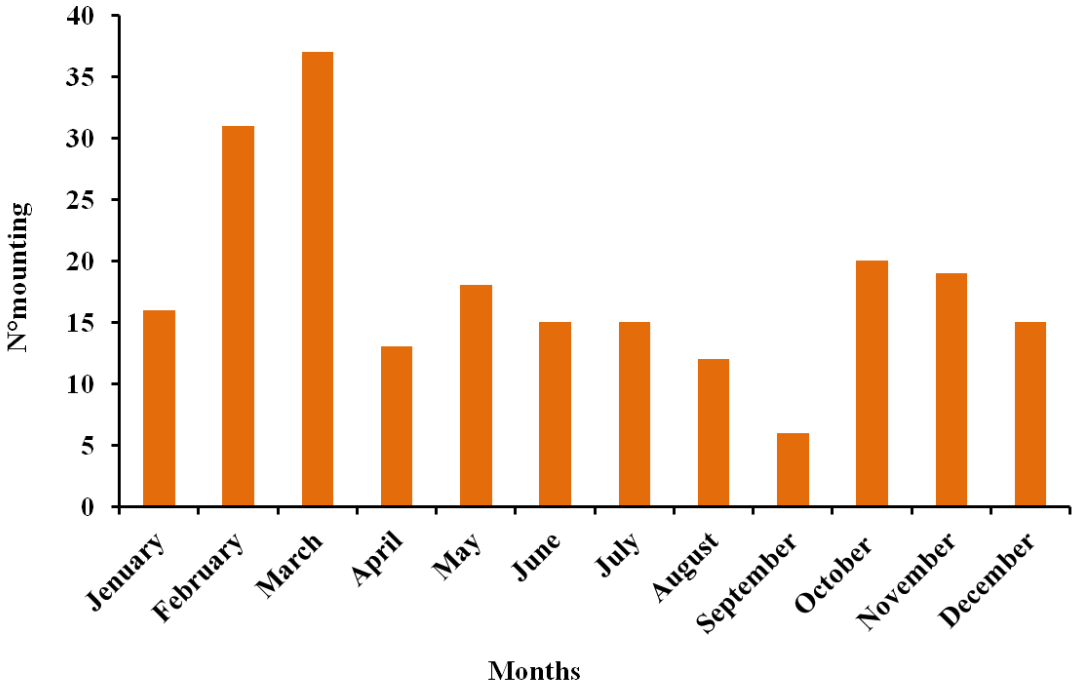


Figure 103. Total number of monthly mounting events recorded in 8 monitored porcupine pair during the whole period of monitoring

Figure 104. Mounting sequence.observed in Pair 7 within S1 of EA2 (A) Resting behaviour, (B) Allo-grooming behaviour, (C) Contact nose-quills and (D) presenting behaviour and mounting.

Copulation was recorded only in 2 nights of the monitoring period in Pair 4 and 5. In Pair 5 a single copulation were observed in January 2018 while in Pair 4 multiple copulations in the same night were recorded in November 2018. The average duration of copulation resulted to 24 seconds (SD = 7 seconds) with an average of 17 thrusting (SD = 5.5 thrusting).

Birth data were collected from the 8 recognisable porcupine pairs and from other 3 porcupine pairs inhabiting the experimental S10, S12 and S14 of EA1. Overall 21 births for a total of 35 porcupettes were recorded during the all period of monitoring. The porcupine births occurred throughout the year (Fig 105) with an average of 1.7 births (SD = 0.7 births) (from 1 to 3) *per* pair *per* year. The average size of porcupine litters resulted to 1.6 porcupettes (SD = 0.5 porcupettes) (from 1 to 2). No litters of 3 porcupettes were recorded.

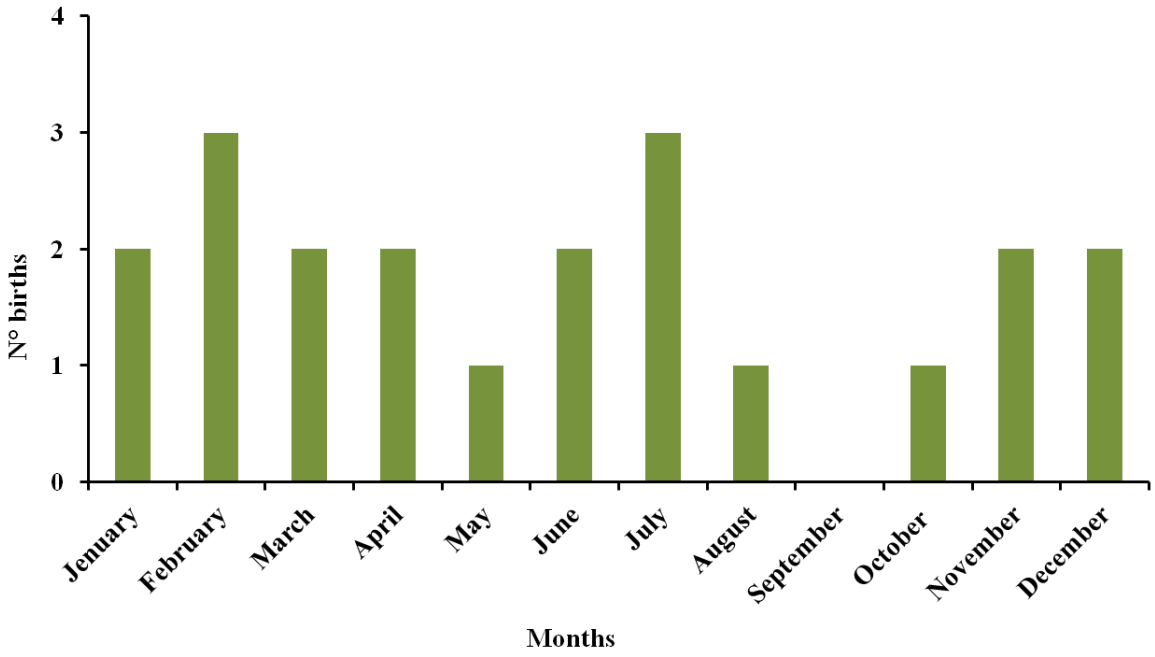


Figure 105. Total number of monthly births recorded in 11 porcupines pair during the whole period of monitoring

The age of first emergence of porcupettes from burrow was estimated in a range between 10 and 15 days after birth. In the previously days of porcupettes emergences, in all porcupines pairs, male and female alternately emerged from burrow and increased the number of entrance in burrow during the same night. In the same period a low activity of the females outside the burrow was recorded. Moreover, in 6 occasions, the adult male brings food and/or bones into the burrow during the days before the first detection of porcupettes outside the burrow was observed.

Figure 106. Adult female of pair 4 in S9 (A), of pair 1 in S1 (B), of pair 2 in S3 (C) and of pair 3 in S8 (D) while their nursing its porcupettes

The porcupettes began to eat independently after 5-10 days after their first coming out from the burrow but nursing events were recorded up to 2 months after the first emergence of porcupettes from burrow (Fig 106).

The parental care by adult male, adult female, sub-adults and by adult male and female togher was investigated in the 5 recognisable porcupine families in EA1 and in Family 3 in EA2.

Overall 576 events of parental cares were recorded in Family 1, 433 in Family 2, 548 in Family 3, 102 in Family 4, 236 in Family 5 of EA1 and 144 in Family 3 of EA2.

In all porcupines families the frequency of occurrence of parental cares by the male and by the female did not show any statistical differences. The parental cares by sub-adults and adult male and female together were recorded in all the investigated porcupine families. In Table 12 are reported the percentage of time spent performing parental cares by adult male, adult female, sub-adults and male and female together recorded in each porcupine family.

The minimum time of permanence of the sub-adults within the family resulted to be at least 1 year.

Table 12: Percentage of time spent performing parental cares by adult male (Male), adult female (Female), sub-adults and male and female together (Male + Female) recorded in each investigated porcupine family

	Male	**Female**	**Male +Female**	**Sub-adults**
EA1				
Family 1	17.4	17.7	59.5	0.5
Family 2	16.8	17.5	24.4	27.5
Family 3	15.1	16.6	28.8	17.8
Family 4	18.6	15.7	30.4	6.8
Family 5	14.8	19.9	38.5	16.5
EA2				
Family 3	12.5	10.4	59.0	9.0

4.4. Porcupines Population Abundance estimate (PPA) and first hypothesis of porcupine census model

The porcupines population abundance estimate (PPA) was computed as the ratio between the number of estimated porcupines (EP) and the surface of the investigated area (SUP).

PPAI = EP/SUP

The number of estimated porcupines (EP) was computes we the following formula:

EP = USp/2*FG

where:
USp: is the number of settlements used by porcupines among the whole geolocated and monitored settlements;
USp/2: number of stations, considering that each porcupine family group alternatively used two settlements (station)
FG: average size of porcupine family group recorded

The porcupine population abundance is expressed as number of porcupines for kilometre square (individuals/km^2).
In Crespina-Lorenzana area (2458 ha) in EA1 47 out of 68 geolocated and monitored settlements resulted used by porcupines and the average number of porcupines per family resulted 4.1 individuals (SD = 1.5 individuals). In EA2 (612 ha) 11 out of 15 settlements resulted used by porcupines and the average size of family group was 4.1 individuals (SD = 1.6 individuals).
The porcupine population abundance in Crespina-Lorenzana resulted 3.9 individuals /km^2 (SD = 1.4 individuals /km^2) for an average porcupine population size in EA1 of 174.56 individuals (SD = 67.14) individuals. In EA2 the porcupine population abundance was 3.6 individuals /km^2 (SD = 1.4 specimens/km^2) for an average porcupine population size of 22.3 individuals (SD = 8.7 individuals).

The porcupine census model was made up by 6 steps:

1. Assess the environmental suitability for the presence of the species:
    - Presence of woody areas with a good vegetation covered
    - Presence of sandy soil with a good slope (5-10%)

2. Verify the presence of the specie in the investigation area by:
    - Detection of the signs of presence like quills and faeces
    - Detection of road killed specimens
    - Camera-trapping on sampling areas
3. Estimate the number settlements (ES) as the product of the average settlements density and the total area in kilometre square (SUP) of investigation zone

ES (setts/km^2) = 2.2 * SUP

4. Estimate the number of settlements used by porcupine (USp)

USp = 70% ES

5. Estimate the number of porcupine (EP) using as average size of porcupine family group (FG) the value 4.1 SD 1.4.

EP = USp/2*FG

6. Estimate the abundance of porcupine population (PPA) and the average porcupines population size as a product of PPA and the total areas of the investigation zone.

The PPAI in EA2 obtained by applying the census model resulted 3.2 specimens/km^2 for a porcupine population size estimated ranged from 20 to 27 individuals compared to 3.6 specimens/km^2 and a population size from 22 to 31 computed.

4.5.Health monitoring of crested porcupine

Parasitological analysis

Overall, a total of 52 porcupine faecal samples were collected and analysed, of which 45 in EA1 and 7 in EA2 (Fig 107 and Fig 108).

The 84.6% (n=44) of the faecal samples were detected along transects in the seven different zones in EA1 and feeding area in EA2. The 1.9% (n=1, adult male) and the 13.5% (n=7, 2 adult males, 3 sub-adult female and 2 porcupette females) of the faecal samples were collected from road-killed and captured crested porcupines, respectively. In four of the seven zones in EA1, only a single faecal sample was found and collected during the whole investigation period.

Totally, 12/52 faecal samples resulted to be attributable to individual animals.

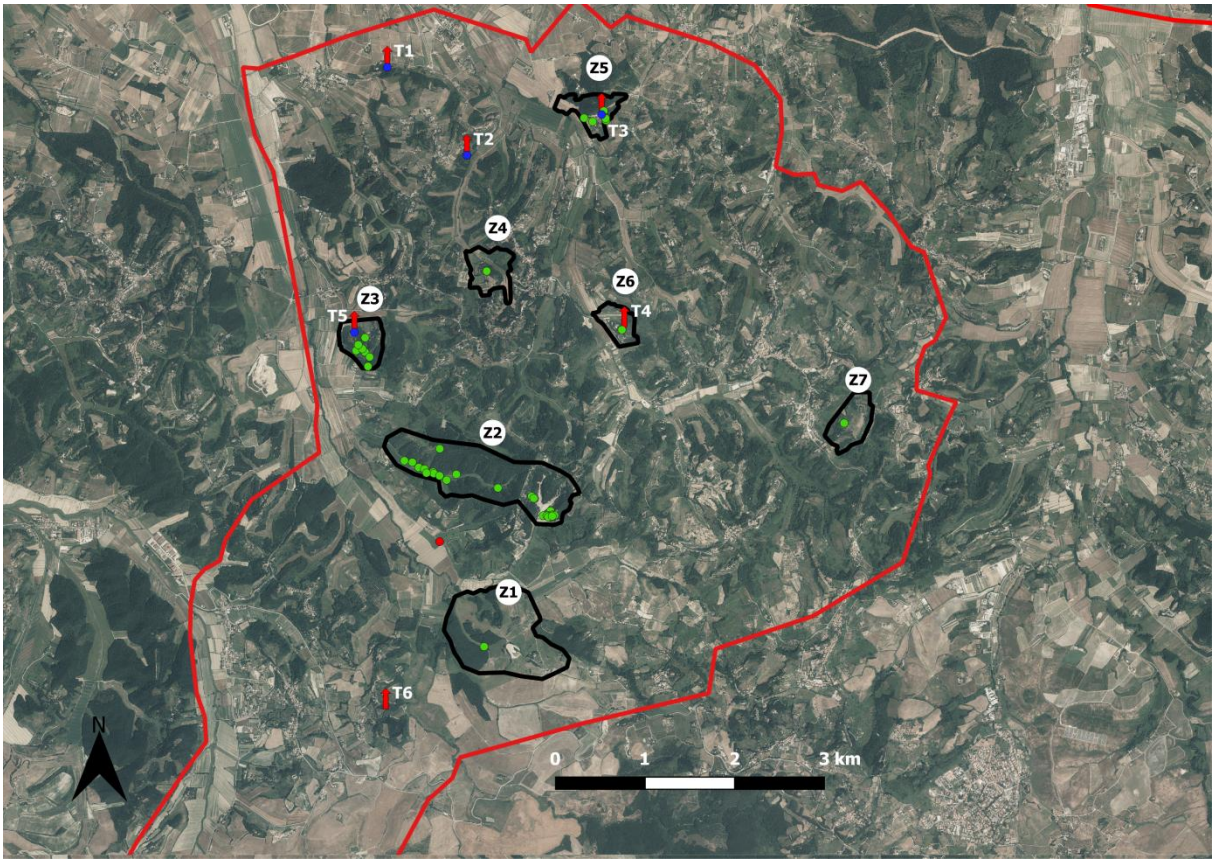


Figure 107. Location of faecal samples collected in each zone along transect (green dots), from captured (blue dots) and road-killed (red dot) porcupines in EA1

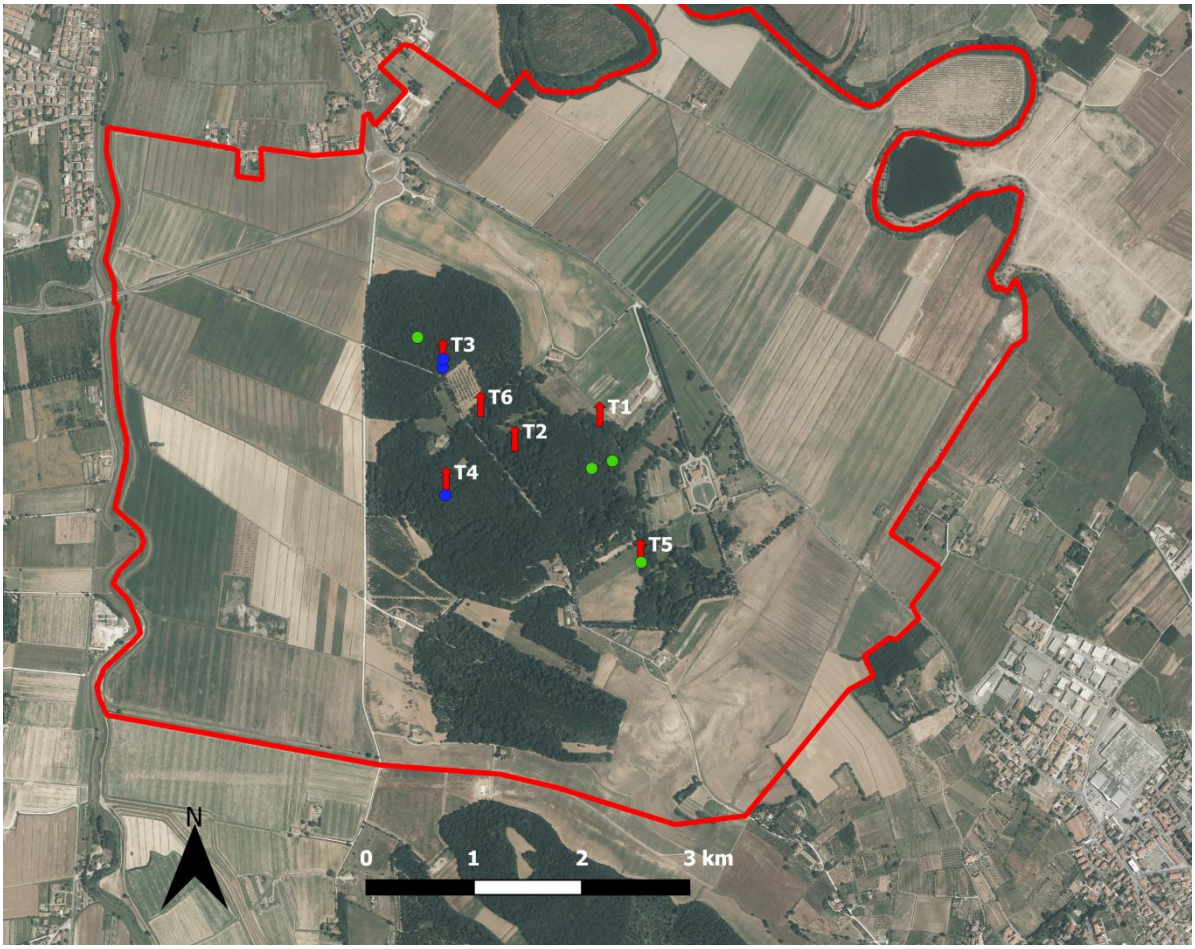
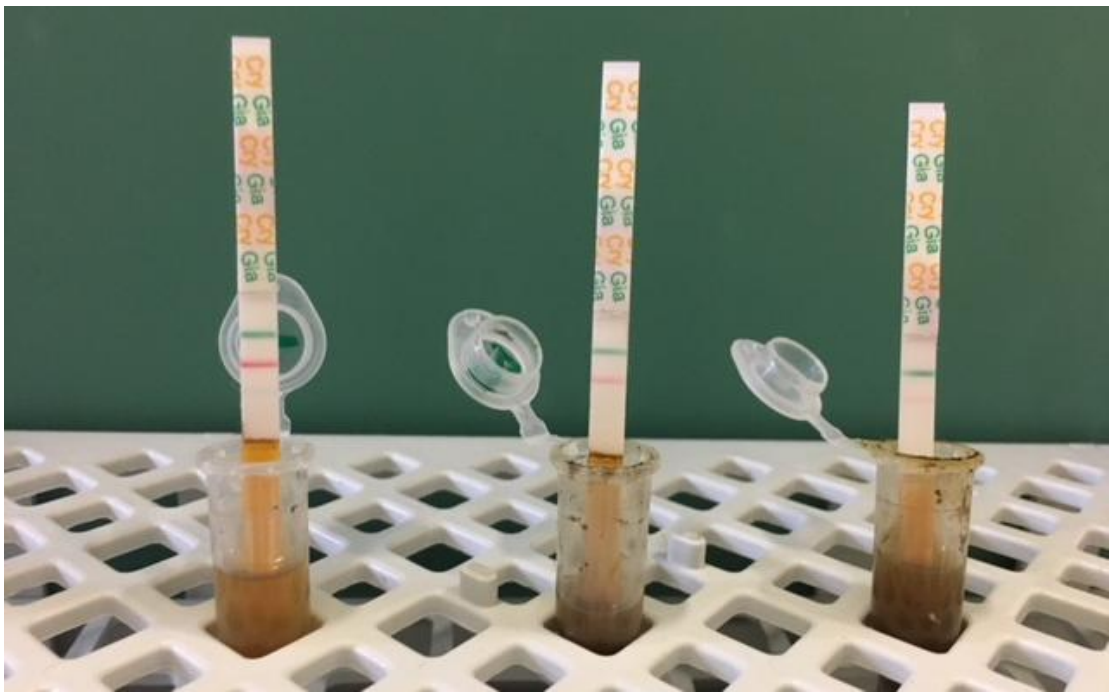


Figure 108. Location of faecal samples collected in feeding areas (green dots) and from captured porcupines (blue dots) in EA2

Overall, 39 out of 52 samples were found positive for at least one parasite taxa at parasitological analysis and six different parasites were identified (Table 13).

The 48% (25/52) of analysed faecal samples were found positive for *Giardia* spp. and the 1.9% (1/52 in EA1) for *Cryptosporidium* spp.by the immunoassay only (Table 13 and Fig 109).

The 96% (24/25) of *Giardia* positive faecal samples were collected in EA1 (Fig. 110) while in EA2 only the faecal samples belonging to the sub-adult female captured in T3 resulted positive.



Figurre 109. Immunoassay: *Giardia* sp. positive faecal samples

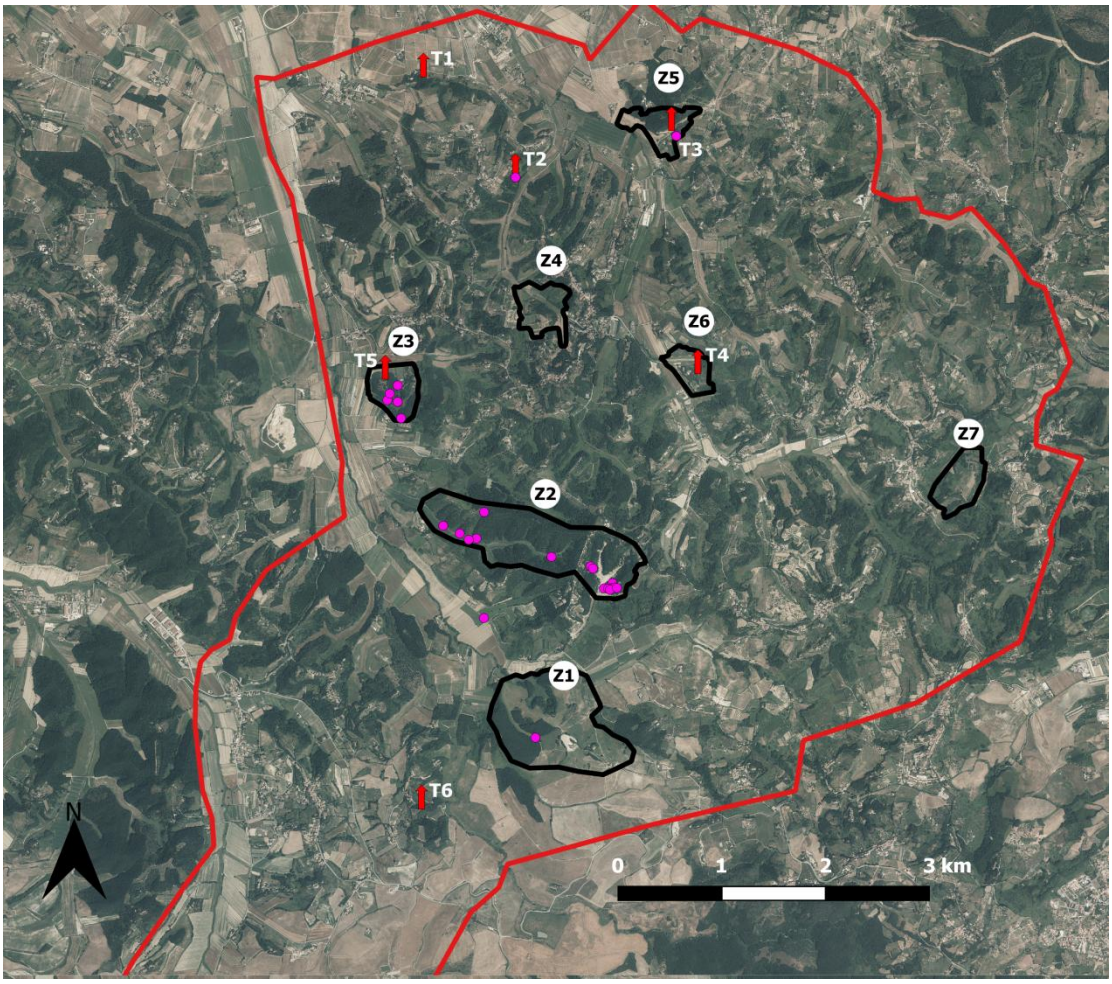


Figure 110. Location of *Giardia* spp. positive faecal samples (pink dots) in EA1

Table 13. Number of total positive faecal samples collected from road killed and captured crested porcupine and sampled in transectsor feeding areas for each type of detected parasites

Parasites	**N. positive (%)**	**Road killed**	**Captured**	**Faecal samples**
Helminths				
GI* Strongyles	17/52 (32.7)	1	4	12
***Trichuris* sp.**	17/52 (32.7)	0	2	15
***Capillaria* sp.**	2/52 (3.8)	0	0	2
Protozoa				
***Giardia* sp.**	25/52 (48)	1	2	22
Coccidia	1/52 (1.9)	0	1	0
***Cryptosporidium* sp.**	1/52 (1.9)	0	0	1

*Gastrointestinal

Table14**.** Multiple parasite infections found in 39 out of 52 crested porcupine positive faecal samples

Parasites	**N. positive (%)**
***Trichuris* spp. + *Giardia* spp.**	7/39 (17.9)
GI* Strongyles + *Giardia* spp.	5/39 (12.8)
***Capillaria* spp. + *Giardia* spp. + *Trichuris* spp.**	1/39 (2.6)
GI* Strongyles + *Giardia* spp. + *Trichuris* spp.	1/39 (2.6)
***Cryptosporidium* spp. + *Giardia* spp. + *Trichuris* spp.**	1/39 (2.6)
GI* Strongyles + *Trichuris* spp.	3/39 (7.7)
GI* Strongyles + *Capillaria* spp.	1/39 (2.6)
***Trichuris* spp. + Coccidia**	1/39 (2.6)

*Gastrointestinal

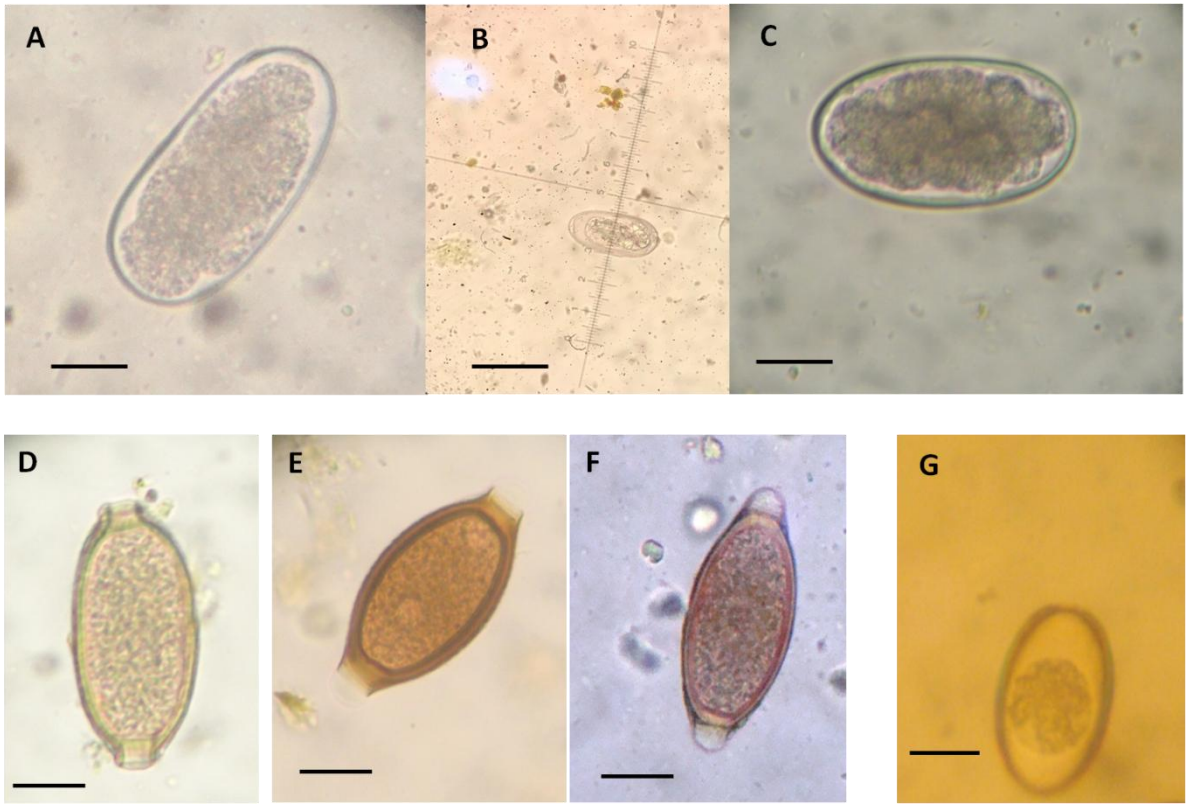


Figure 111. Parasites identified in 52 crested porcupine faecal samples: **A-C**).Gastrointestinal strongyle eggs (59.8-78 µm x 28.6-44.2 µm) (A and C 400X, scale bar 15 µm; B 100X, scale bar 60 µm); **D**). Capillariid egg measuring 59.8 µm x 26 µm (400X, scale bar 15 µm); **E, F**). *Trichuris* sp. eggs of 60 x 28.6-40 µm in dimensions (400X, scale bar 15 µm); **G**). Coccidian oocyst measuring 36.4 µm x 23.4 µm (400X, scale bar 15 µm).

Among the 12 faecal samples belonging to different individuals, four samples (33.3%, 4/12) were positive for *Giardia* spp..

At microscopical examination, positivity for *Trichuris* spp. eggs (32.7%, 17/52), gastrointestinal strongyle eggs (32.7%, 17/52), capillariid eggs (3.8%, 2/52) and coccidian oocysts (1.9%; 1/52) was also evidenced (Table 13 and Fig 111).

Multiple parasite infections were found in 20/39 positive samples (51.28%). *Giardia* plus other parasites coinfections were detected in 15/39 (38.46%) positive samples (Table 14).

Molecular analysis was performed on 17 out of the 25 *Giardia*-positive isolates. At the *SS rDNA* locus, expected bands were achieved for 12/17 isolates. All isolates were assigned to assemblage B. Only 3 out of 17 samples were successfully amplified at the *tpi* locus. Sequencing confirmed assemblage BIV for one isolate and revealed assemblage AII for the other two samples. However, no heterozygous positions (double peaks) were detected during chromatogram inspection of these two isolates at both loci. Attempts to further subtype these samples at gdh locus were not successful.

Ectoparasites

A total of 2 out of 14 (14.3%) captured porcupines and 1 out of 2 road-killed porcupines (50%) examinated were found infested by parasitic arthropods species (n=16, 18.8%). All ectoparasited detected were fleas. The fleas collected in the capture porcupines (an adult and a porcupette) were identified as *Pulex irritans* (Fig 112) while those found in the road-killed (porcupette) porcupine was *Ctenocephalides felis*. All porcupines infested by fleas were female.

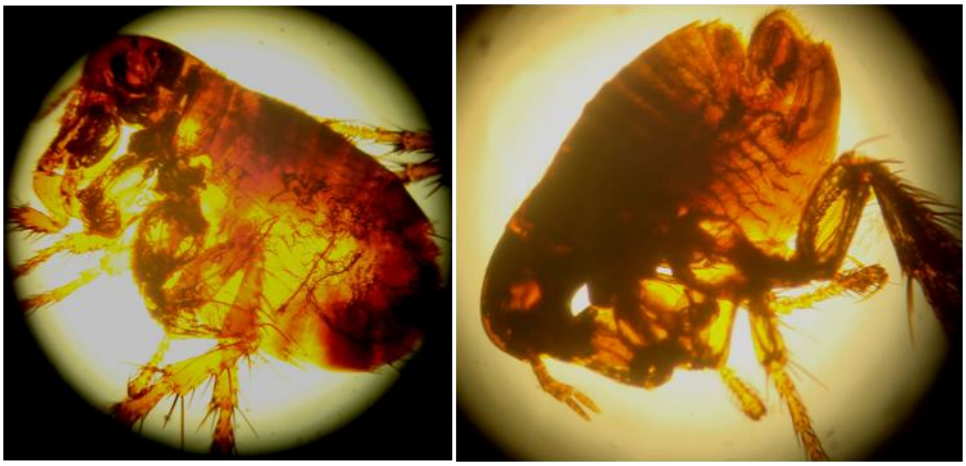


Figure 112. Female (left) and male (right) specimen of *Pulex irritans* detected in the captured porcupines

Haematological and blood biochemical profile of crested porcupine

Blood samples for haematological and biochemical analyses were collected from 7 out of 14 captured porcupines: 4 adult female, 1 adult male, 1 sub-adult female and 1 porcupette female. All the captured porcupines were in good physical condition while no data were available on the health status. Out of 7 collected samples only 5 were used for haematological and/or biochemical analysis. On the other samples it was not possible to perform the analysis beacouse one was haemolytic and the other badly preserved. Biochemical profile was obtained for 5 out of 7 (71%) blood samples while the haematological values only for 2 out of 7 (0.3%) samples.

In table 15 are reported the age class and sex of porcupines for each blood sample collected and the analysis performed.

In table 16 and table 17 the biochemical and haematological values obtained for each samples are reported.

Table 15: Age class and sex of porcupines for each blood sample analysed. For each blood samples the analysis performed (CBC= Complete blood count or Biochemical) are indicated

Blood Sample	Age class	Sex	CBC	Biochemical
1	Adult	Female	X	X
2	Sub-adult	Female		X
3	Adult	Male		X
4	Adult	Female		X
5	Sub-adult	Female	X	X

Table 16: Biochemical values of crested porcupine blood. For each parameter the mean, the standard deviation (SD) and the median are reported.

	Urea (mg/dL)	**Creat (mg/dL)**	**Bil tot (mg/dL)**	**Bil Dir (mg/dL)**	**Glic (mg/dL)**	**TP (g/dL)**	**GGT (U/L)**	**AST (U/L)**	**ALT (U/L)**	**CK (U/L)**	**Na^+ (mmol/L)**	**K^+ (mmol/L)**	**Ca^{++} (mg/dL)**	**Phosph (mg/dL)**	**Lactate (mmol/L)**
1	19	1,1	0,5	-	-	-	6,0	90,0	-	426	-	-	-	-	-
2	22	1,2	0,7	-	-	-	37,0	235,0	-	1003	-	-	-	-	-
3	41	11,2	-	-	-	7,3	8	261	-	230	-	-	-	-	-
4	20	1	-	-	-	9,3	36	201	-	684	-	-	-	-	-
5	9	0,7	0,2	-	-	6	5	282	-	4243	-	-	-	-	-
Mean	**22,2**	**3,04**	**0,47**	-	-	**7,53**	**18,4**	**213,8**	-	**1317,2**	-	-	-	-	-
SD	**16,7**	**4,6**	**0,25**	-	-	**1,7**	**16,56**	**75,53**	-	**1661**	-	-	-	-	-
Median	**20**	**1,1**	**0,5**	-	-	**7,3**	**8**	**235**	-	**684**	-	-	-	-	-

Creat=Creatinine, Bil Tot= Total bilirubin, Bil Dir=Direct bilirubin, Glic= Glycemia, TP=total proteins, GGT= Gamma-glutamyl transpeptidase, AST= Aspartate transaminase, ALT= Alanine aminotransferase, CK= Creatine kinase, Na^+= Sodium, K^+= Potassium, Ca^{++}= Calcium, Phosph= Phosphates

Table 17. Haematological profile of crested porcupine. For each parameter the mean and the standard deviation (SD) are reported.

	RBC (M/µL)	Hgb (g/dL)	Hct (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	PLT (K/µL)	WBC (K/µL)	LYM (K/µL)	LYM %	NEU (K/µL)	NEU %	MON (K/µL)	MON %	EOS (K/µL)	EOS %	BAS (K/µL)	BAS %
1	5,5	14,1	39,4	71,5	25,6	35,8	226,0	7,5	2,0	26,1	5,1	68,7	0,1	1,3	0,2	3,2	0,1	0,7
5	6,2	15,5	51,9	82,6	25,7	29,9	90	9,3	2,8	30	5,6	60	0,9	10	0,0	0,0	0,0	0,0
Mean	5,9	14,8	45,7	78,9	25,7	32,9	158	8,4	2,4	28,1	5,4	64,4	0,5	5,7	0,1	1,6	0	0,4
SD	0,49	0,99	8,84	10,4	0,07	4,17	96,17	1,29	0,59	2,76	0,3	6,15	0,59	6,15	0,17	2,3	0,04	0,5

RBC= Red blood cells, Hgb= Haemoglobin, Hct= hematocrit, MCV= mean corpuscular volume, MCH= mean corpuscular haemoglobin, MCHC= mean corpuscular haemoglobin concentration, PLT= Platelets, WBC= White blood cell, LYM= lymphocytes, NEU= neutrophils, MON= monocytes, EOS= eosinophils, BAS= basophils

Leptospirosis in crested porcupine

Antibodies detection of Leptospira

A total of 12 blood samples were collected and analysed. In Table 18 the sex, age class and MAT results for each captured porcupine are reported. Totally 7 out of 12 sera (58%) resulted positive to MAT. The serogroup Icterohaemorrhagiae was recorded in 4 out of 7 sera (57%). In one case (sub-adult female) Icterohaemorrhagiae was found associated to Sejroe with antibody titer of 1:200 and 1:100 respectively. Pomona serogroup positivity was detected in 2 out of 7 sera (28.5%) and in one of these was found associated with Australis, both with antibody titer of 1:100. The serogroup Australis was also found in the serum of a sub-adult female with the higher antibody titer recorded (1:1600).

Table 18: Sex, age class and MAT results for each captured porcupine. For each MAT positive sample the serogroup and titer is reported.

Samples	Sex	Age class	MAT result	Serogroup	Titer
1	Female	Adult	Positive	Pomona	400
2	Female	Sub-adult	Positive	Australis	1600
3	Male	Adult	Negative	-	-
4	Female	Porcupette	Positive	Pomona	100
				Australis	100
5	Male	Adult	Positive	Icterohaemorrhagiae	400
6	Female	Sub-adult	Positive	Icterohaemorrhagiae	200
				Sejroe	100
7	Male	Adult	Positive	Icterohaemorrhagiae	100
8	Female	Adult	Positive	Icterohaemorrhagiae	800
9	Male	Adult	Negative	-	-
10	Female	Sub-adult	Negative	-	-
11	Female	Sub-adult	Negative	-	-
12	Female	Porcupette	Negative	-	-

Isolation of Leptospira

Both sera samples collected resulted negative to MAT for all Leptospira serovar tested. Only in porcupette kidney the *Leptospira* DNA was detected and the *Leptospira* isolation was performed. The strain was identified as *L. interrogans* serogroup Pomona serovar Pomona, showing Sequence Type (ST) 140 for scheme 1, ST 4 for scheme 2 and ST 58 for scheme 3.

The kidney of porcupette showed small grey-white focal lesions, mainly located in the renal cortex and varying from 1 to 2 mm in diameter. Microscopically a mild chronic interstitial nephritis was present, characterized by vacuolar degeneration of the tubular epithelium and scattered interstitial foci consisting of lymphocytes and plasma cells (Figure 113A), accompanied by interstitial fibrosis (Figure 113B). In silver-stained sections, leptospires were never detected in the tubular lumen adhering to the luminal surface of tubular cells, while Intracytoplasmic spherical bodies within cells of a tubule undergoing regressive changes were observed. In immunoperoxidase-stained sections, using antisera against *Leptospira* serogroup Pomona an intense immunoreactivity for leptospiral antigen was detectable within the tubular epithelia cells and in cellular debris in tubular lumen (Figure 113C), while absence of immune-labelling was observed when the antiserum against *Leptospira* serogroup Grippotyphosa was used.

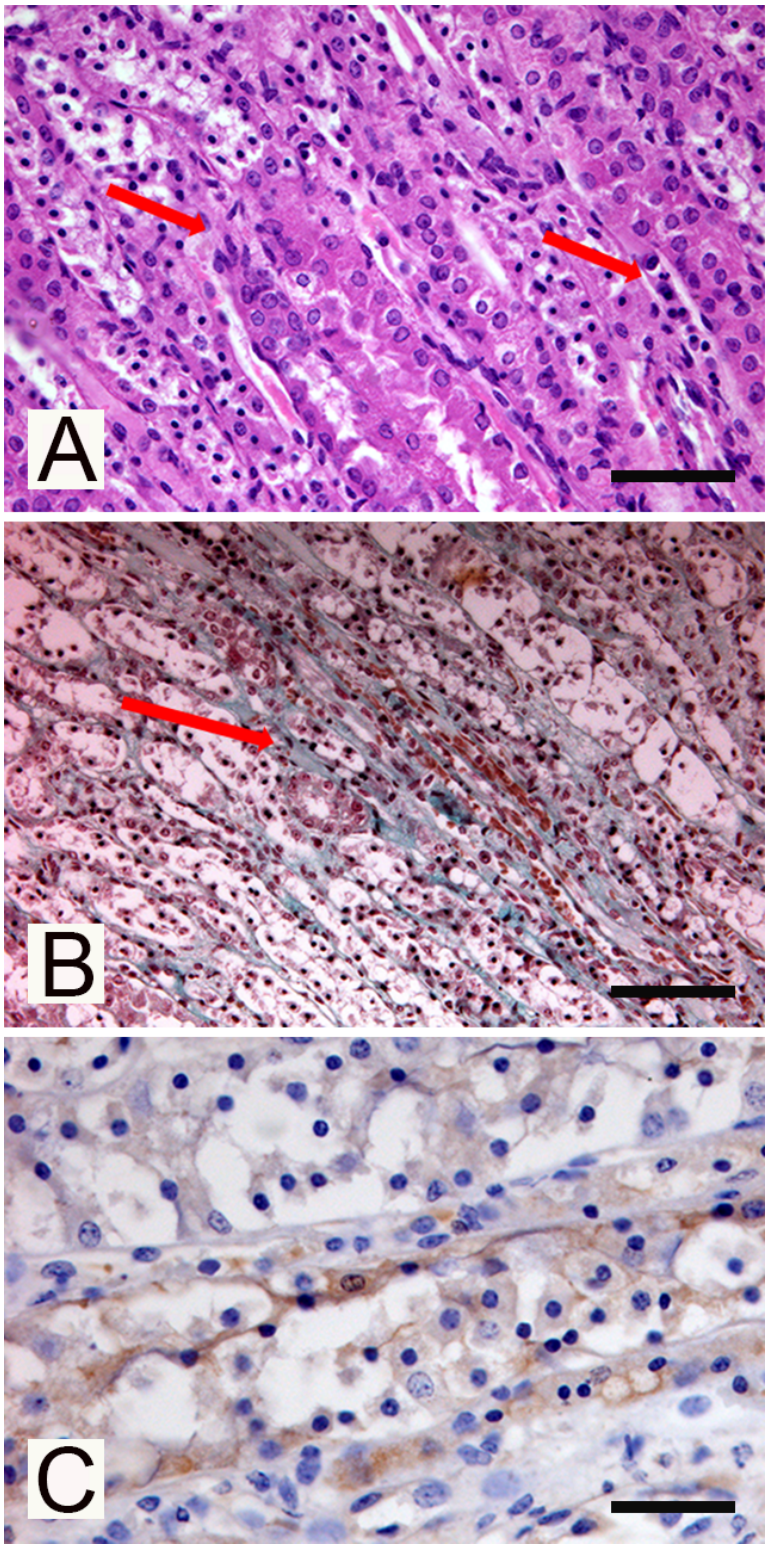


Figure 113. Crested porcupine kidney. Renal alterations associated with *Leptospira* infection. A) mild interstitial nephritis characterized by lymphocyte and plasma cell infiltration (arrows) (H-E; Bar =50 µm). B) Mild interstitial fibrosis (arrow) surrounded by scattered inflammatory cells (Masson trichrome Goldner stain; Bar = 50 µm). C) Leptospiral antigen is present within tubular epithelial cells undergoing regressive changes (Immunohistochemical staining using anti L. Pomona antiserum and hematoxylin counterstain; Bar = 30 µm).

5.Discussion

5.1. Geolocation of settlements

Spatial-distribution analysis of settlements

In this study the spatial distribution analysis of porcupine settlements was performed for the first time. At the best of our knowledges, to date the only available data on the spatial distribution of settlements are those reported by Felicioli (1991) and Felicioli and Santini (1994) basing on field observations.

The cluster analysis here performed show that the 434 ground holes in EA1 and the 97 ground holes in EA2 are distributed in 85 and 15 ground holes cluster respectively. This result confirms that the settlements are made up by a cluster of ground holes detectable on the surface as defined by Felicioli (1991) and Felicioli and Santini (1994). The settlements are charecterized by a high variability in the number of ground holes that ranges from 2 to 20. The average settlements size recorded in this study (4.5 SD 4.4 in EA1 and 5.7 SD 4.5 in EA2) are greater than those reported by Mukherjee et al. (2017a) in India for *H. indica* (2.36 SD 1.4). Conversely, similar settlement sizes to those detected in this study have been reported by Tinelli and Tinelli (1988) and Felicioli (1991) in Italy and by Kruuk (1978) for the badger settlements in England. Italy is the only European country in which the crested porcupine and the badger coexist and share the same settlements (Pigozzi 1986, Tinelli and Tinelli 1980, 1983, Mori et al. 2015a). Both badger and crested porcupine are burrowing mammals and excellent diggers (Kruuk 1978, 1989, Santini 1980) but there is no evidence on who digs the settlements. Since most of the settlements are known to be older than 50 years, therefore we can not exclude that settlements included in this study may be digged by the badgers and than occupied by the crested porcupines or *viceversa*.

The cluster analysis have also showed that the 67% and 87% of settlements in the two study areas are clustered in setts-system called “Station” confirming the Felicioli (1994) and Felicioli and Santini (1994) hypothesis. In accordance with Felicioli (1994) and Felicioli and Santini (1994) the settlements belonging to a station are detectable within a distance ranging from 100 to 250 meters, are characterized by different exposure and are connected on the surface by a dense network of pathways. Monetti et al. (2005) reported to have never found two settlements closer than 150 meters. Howerever, despite the same Authors do not refer to the spatial organization of settlements this result is also in accordance with Felicioli (1994) and Felicioli and Santini (1994) data and with the results obtained in this investigation.

The cluster analysis showed that in the most cases, the settlements resulted distributed in binary stations as previously suggested by Felicioli and Santini (1994). However, ternary and

quaternary stations in this study are also detected. In these last cases the stations was characterized by the presence of two main settlements and outlier settlements digged in war sheters or in grooves created by rainwater. This allows hypothesising that the presence of ternary and quaternary stations may be an alteration of the binary station due to the presence of outlier refuges and that the binary system could be the rule.

In accordance with the results obtained by Mukherjee et al. (2017a) and the observations performed by Felicioli and Santini (1994) the ground holes orientations in both study areas did not present a sector preference. The settlements orientation in both study area shows a bimodal distribution with an orientation trend to South-east to North-west in accordance with Felicioli and Santini (1994). The selection pattern of exposure sectors resulted to be the same for the ground holes and the settlements in both study areas. The selection pattern for settlements occurrence shows a marked preference for South-east sector and avoidance for North-east while no selection was obtained for North-west and South-west exposure sector. This result confirm the negative selection of slopes exposed to North-east reported by Monetti et al. (2005) and the hypothesis of south exposure selection (Felicioli and Santini 1994). The highest porcupine inhabitation frequency in this study was recorded in settlements in North-west and South-west exposure sector indicating that the settlements inhabitation is not exposure related.

The results obtained in this study confirm that the presence of settlements is stricty linked to the woody habitat that provide good vegetation coverage and food availability and to the presence of soils easy to digging (Tinelli and Tinelli 1980, 1988, Monetti et al. 2005) as also reported by Mukherjee et al. (2017b) in *H. indica* and by Marina and Subaid (2015) in *Hystrix brachyura* and *Hystrix crassispinis* in Malaysia. In woody habitat settlements occurred within 100 meter from feeding areas such as arable or uncultivated areas suggesting ecotonal preferece of the crested porcupine.

The settlement densitity ranged between 2 to 2.4 settlements for kilometer square. This result suggests that there are no differences between areas with differend woody extension and distribution. Conversely, significantly difference resulted in this study concerning the settlements density on woody perimeter kilometer with higher values in big continuous wooded areas than in small fragmented woody areas. This result is probably consequence of ecotonal habit of crested porcupines. Data on settlement density are not available in other areas of distribution of crested porcupine in Italy. Mukherjee et al. (2018) reported similar value of Indian porcupine settlement density in India.

5.2. Camera-trapping

Co-habitation of settlements and interactions between crested porcupines, badgers and red foxes

The result obtained in this study clearly indicates that red foxes always inhabited in exclusive mode the settlements while both crested porcupines and badgers could inhabit the same settlement in exclusive mode as well as together.

The crested porcupines are the main stable and faithful inhabitants of settlements as observed by Monetti et al. (2005). The badgers and red foxes, during the whole year, are the most common visitors or explorers of the settlements inhabited by the porcupines. Camera-trap videos recorded during this investigation, show that badgers inhabit a single settlement only for short periods and seems to confirm the reluctant habit of badgers to live in a stable settlement as previously reported (Kruuk 1978, Roper 1992). The inhabitation of settlements by badgers was mainly recorded in autumn-winter months and it is possible that badgers inhabited unmonitored settlements within the territory in spring and summer. Data collected in this investigation reinforced the observations of Tinelli and Tinelli (1988) concerning the opportunistic occupation of settlements for breeding by red foxes, as also hypothesized in studies performed in Poland and Belarus (Kowalczyk et al. 2000, Goszczynski and Wòjtowicz 2001).

The potential co-habitation of settlements between red foxes and porcupines could arise with foxes density increasing.

The same settlement is usually shared at different times by crested porcupines, badgers and red foxes. This result confirms the settlement-sharing between these three species as previously hypothesized by Pigozzi (1986) and Tinelli and Tinelli (1980, 1983) and observed also by Mori et al. (2015a). However, Mori et al. (2015a) defined the simultaneous use of the same settlement during a surveyed period of 12-14 days as “sett-sharing”. The same Authors consider a settlement in use whenever an animal is detected entering and emerging from the same burrow without discriminating between exploration activity and inhabitation. Furthermore, Mori et al. (2015a) report that the same ground holes of the same settlement could be co-used by porcupines and badgers but no co-using by porcupines and red foxes was detected. Even in this case no information on the time spent together inside the burrow is available.

In the present study sett-sharing and co-habitation have different meanings. Sett-sharing refer to the co-use by more than one species of the same settlement not simultaneously. Co-habitation refers to simultaneous permanence of more than one species in the same burrow.

The co-habitation between porcupines and badgers occurred in several period throughout whole the year also when porcupettes are present. The availability of not inhabited settlements in the vicinity of the co-habited ones allows hypothesizing that co-habitation is not due to lack of settlements. .Although the density of porcupines and badgers population in the study area is not known, co-habitation by these two species is intriguing and overcrowding seems no to be the reason for co-habitation. Co-habitation between porcupines and badgers seems to not be due also to peculiar physiological state of the badgers as overwintering. In the winter cohabitation periods recorded in this investigation, the badger did not reduce its nocturnal activity indicating no overwintering at this latitude and altitude. In the study area in the cold season the average nocturnal temperature resulted 6.8°C and occasionally reached the minimum of 3°C. According to Goszczyński et al. (2001) the time spent by badgers in their settlements was closely dependent on the outside temperature.

The co-habitation of porcupines and badgers in the same burrow is probably due to the possibility of using different rooms. The use of the same and/or different burrow entrances by both porcupines and badgers at different times during emerging-from and returning-to the burrow, confirm the existence of an underground connection between the ground holes and indicate the use of different rooms by the two species. Moreover, the correspondence between early or late emergence of either of the species with the respective early and late return to the settlement on the same day, do not follow a rigid scheme. Within these three large settlements (S1, S6 and S9) the co-habitation was recorded only in S1. In this case 4 out of 8 ground entrance holes were not monitored. So in this case there could be an underestimation of co-habitation occurrence. In the other two settlements no co-habitation has been recorded and also in this case there is a possibility of underestimation.

A general non aggressive coexistence of porcupine, badger and red fox has been observed as hypothesized by Mori et al. (2015a). The same peaceful behaviour was observed between badgers and red foxes inside the settlements in England (Macdonald et al. 2004).

Both aggressive interaction (AI) and avoidance behaviour (AV) between porcupines and badger resulted significantly higher in the co-habited settlements compared to those exclusively inhabited ones. Conversely, no significantly differences resulted in aggressive

interaction in the dyads porcupines-badgers compared to porcupines-foxes. These results surprisingly disregard the prediction that absence of aggressive interactions permits co-habitation

According to Monetti et al. (2005), predation represents a significant risk for both porcupette and juveniles. The results obtained in this investigation indicate for the first time that porcupettes at least of four month of age when necessary, show effective defence ability. Porcupette and juveniles show the whole aggressive set of display previously described in the adults (Rosevear 1969, Felicioli 1991, Mori et al. 2014c).

To the best of our knowledge, scavenging of porcupine carcasses by red foxes is documented here for the first time. This result suggests that the occasional presence of porcupine remains found in red fox faeces (Fais 1991, Lucherini et al. 1995) is more likely to be a consequence of scavenging on carcasses rather than of predation. It is therefore possible to assume that the red fox is not a natural predator of the porcupine but scavenges on carcasses that it occasionally finds. Conversely, the badger seems not to be a scavenger of porcupine carcasses and porcupine remains were never found in badger faeces (Melis et al. 2002, Balestrieri et al. 2004).

New investigation are needed in order to assess the settlements features that determine the choice by porcupines and badgers as well as individuate the potential advantages of co-habitation for both the species.

Spatio-temporal inhabitation of settlements by crested porcupine

Each settlement was inhabited by only one porcupines family group and no cohabitation between more porcupines families was never been documented. This result indicate the exclusive use of a settlement by only a porcupines family group and the strong site fidelity in paired crested porcupine as previously recorded by Monetti et al. (2005). In four occasions encounters between porcupines that inhabited the settlement and porcupines visiting the settlement were recorded and in all cases aggressive interactions or fighting occurred. As observed by Corbet and Van Aarde (1996) in free-ranging cape porcupines (*H. africaeaustralis*) also in *H.cristata* the family group use exclusive territory and the occurrence of fighting observed were probably due to territory defence. The territorial behaviour was observed in semi-captive crested porcupine (Felicioli 1991) and in captive cape porcupine (De Villiers et al. 1994, Corbet and Van Aarde 1996). Moreover Corbet and Van Aarde (1996) hypothesised the presence of territorial behaviour also in wild cape porcupines. The territoriality in free-ranging crested porcupines has never been demonstrated (Massolo et al. 2009, Mori and Lovari 2014) and despite this some Authors refer to *H. cristata* as a non territorial rodent (Mori et al. 2014a). However, the observations performed in this study allow to suggest the presence of intraspecific territoriality in the crested porcupine.

The 83.3% (5 out of 6) of the monitored porcupine families alternatively and complementarily inhabited always the same two settlements belonging to a station during the whole period of monitoring. The two settlements used by the monitored porcupines families were never been inhabited by other porcupines not even when the resident family was not present. These results suggest that the intraspecific territoriality is not strictly linked only to a settlement but can be extended to a station.

The alternate use of settlements by crested porcupines was previously reported by Felicioli (1991) and Felicioli and Santini (1994). The same Authors hypothesised the presence of a relationship between burrows orientation and seasonal use by crested porcupines on the basis of signs of presence. According to Felicioli (1991) and Felicioli and Santini (1994) the porcupine prefer south-oriented burrows in winter and north-west orientation in summer while no orientation preference was observed in spring and autumn. Conversely, the results obtained in this study using camera-trapping show a very complex and different inhabitation pattern for each monitored family throughout the year. Data collected in this study show that each porcupine family present a different inhabitation pattern of the two settlements during the whole period of monitoring without significant preference in settlements orientation. Different patterns of inhabitation were also recorded between the 5 porcupines families in EA1 during

the same year of monitoring. This result is not in acoordance with what hypothesised by Felicioli (1991) and Felicioli and Santini (1994) and suggests that the settlements inhabitation is not season drive. In addition the same families in different years of monitoring showed a significantly different use of the two settlements with a different pattern, confirming the absence of relationship between settlement inhabitation and seasons. During the year, change of settlements by each porcupine family occurred without any apparent reason. The analysis of overall inhabitation frequencies of settlements show that crested porcupines prefer North-west and South-west oriented settlements than those oriented to North-east and South-east. This result partially disagrees with the pattern of exposure selection obtained in this study (Chapter 4.1). Conversely, the negative selection of North-east exposure recorded in this study seems to be confirmed by the settlements frequencies inhabitation analysis. However, only one North-east oriented settlements was analysed and further investigation are necessary. Settlements inhabitation changes occur without any detectable reason and the time of permanence in each settlements show a high variation range. The alternative inhabitation pattern of settlements by crested porcupine seems not to be affected by the season and/or by the presence of human disturbance. The presence of porcupettes resulted to be a factor determining long periods of permanence in a settlement. Despite this, long periods of permanence in the settlements were recorded also in absence of porcupettes and further investigations are necessary in order to assess the factors that can be influencing the settlement changes. Despite the crested porcupine seems to prefer the North-west and South-east exposure settlements this can not to be considering the only factor affecting the settlement change. The lack of data concerning the inhabitation of settlements by the crested porcupines does not allow us to compare our results and further investigation are needed.

First daily emergence time of crested porcupine from burrow

Data collected in this investigation confirm that usually the first daily emergence of crested porcupine from burrow occur after sunset as reported by Felicioli and Santini (1994) and Corsini et al. (1995).

The average delay time of emergence recorded during the year was 1 hour and 23 minutes SD 1 hour and 1 minute. However the pattern of first emergence from burrow resulted different between cold and warm period. As observed by Felicioli (1991) and Felicioli and Santini (1994) in semi-captive porcupines, in the cold period, the first emergence time from burrow occurs with a higher delay and lower accuracy compare to the time of sunset. Conversely, in the warm period a lower delay and a higher accuracy in the first emergence time occur. In cold period the average delay resulted 1 hour and 54 minutes (SD = 1 hour and 4 minutes) while in the warms period resulted 45 minutes (SD = 27 minutes). Moreover, in accordance to Corsini et al. (1995), in the warm period porcupines emergences from burrow before sunset has been often observed in this study with a maximum advance recorded of 1 hour and 22 minutes. The different pattern of first emergence from burrows between cold and warm period is probably due to the combined effect of the reduction of: I) seasonal natural food availability and II) darkness hours necessary for feeding (Fig 114).

The crested porcupine is a generalist herbivorous consuming different food resource according to their seasonal availability (Bruno and Ricciardi 1995, Lovari et al. 2017)**.** The crested porcupine show a marked fondness for roots, bulbs, tubers (also truffles) and rhizomes of many wild and cultivated herbaceous plants that are mainly available in late summer, autumn and early winter (Santini 1980, Pigozzi 1980, Pigozzi and Patterson 1990, Felicioli 1991, Bruno and Riccardi 1995, Lovari et al. 2017, Mori et al. 2017a). However, it also feed on epigeal parts, leaves, flowers, buds, grass, inflorescences, vegetables and fallen fruit (Santini 1980, Bruno and Riccardi 1995). The increasing of consumption of these foods during warm period is considered a feeding compensatory strategy due to the variation of seasonal availability of the others food sources (Bruno and Riccardi 1995).

Therefore, the most probable hypothesis is that at our latitude (45° north) the variations in first emergence time of porcupine from burrow in the warm period is due to contemporary reduction of night hours and of natural food more fondness and readily available. This hypothesis could be reinforced by the increasing of porcupine diurnal motor activity resulted

in this study and also previously reported by Corsini et al. (1995) as well as by the home-range increasing in the warm period (Sonnino 1998, Borger 2002, Mori et al. 2014a).

Investigations concerning the first daily emergence time of porcupines from burrows in equatorial countries, where night length variation during the year are absent, could be provide useful information on the porcupine daily emergence pattern and in particularly on role of food availability on this behaviour.

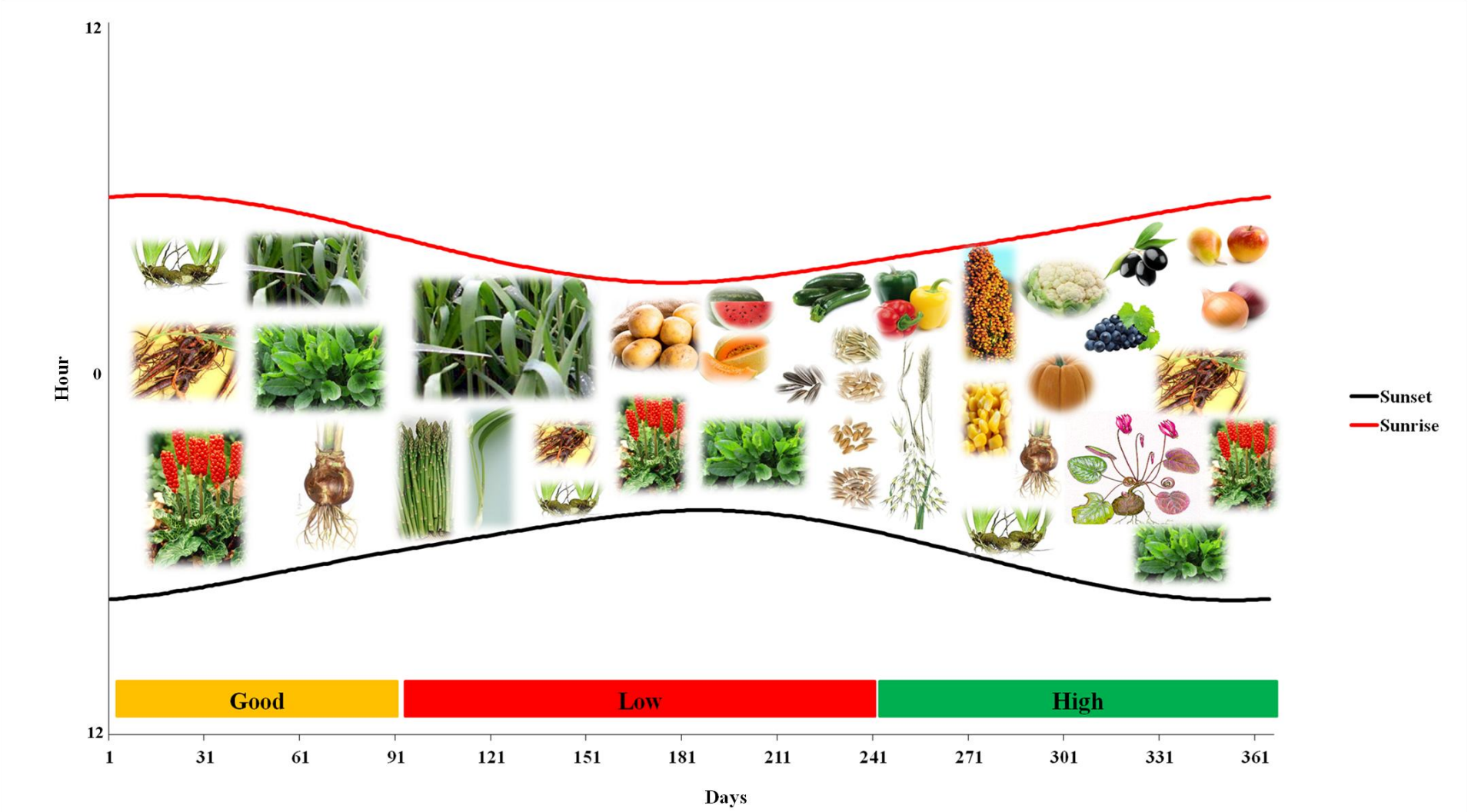


Figure 114. Food availability during the year (21 December – 20 December) at the latitude of 45°north in relation to the reduction of darkness hours

Diurnal motor activity and sunbathing in crested porcupine

Crested porcupine spends most of nocturnal hours searching for food (Pigozzi 1980, Felicioli 1991, Lovari et al. 2017). Corsini et al. (1995) also described the presence of some diurnal activity in the crested porcupine and hypothesised that this behaviour maybe due to an extension of their night-time foraging behaviour or thermoregulation strategy. The diurnal motor activity in crested porcupines was observed in all the monitored settlements and shows an irregular pattern throughout the year. The diurnal motor activity events were recorded mainly between December and June with a peak from April to June in accordance to Corsini and colleagues (1995). The diurnal outdoor permanence ranged from 1 minute to 3 hours and 13 minutes. The increasing of occurrence of the daytime activity between December and June could be due to the combined effect of I) reduction of darkness hours necessary for feeding II) reduction of favourite food due to season changes. Most of porcupines favourite food (roots, bulbs, tubers and rhizomes of many wild and cultivated herbaceous plants) is mainly available in late summer, autumn and early winter (Santini 1980, Pigozzi 1980, Felicioli 1991, Bruno and Riccardi 1995, Lovari et al. 2017, Mori et al. 2017a).

According to Corsini and colleagues (1995) crested porcupines seem to show an irregular diurnal motor activity with a peak in spring when the night becomes progressively shorter and a lack of diurnal activity during summer nights. These Authors do not explicit whether this behaviour is the result of anticipation of emerging and/or delay in returning to the burrow. The same Authors speculate that this behaviour could be either a feeding and/or thermoregulation strategy. In this investigation the porcupine motor activity was performed throughout all daytime hours with a preference for central hours therefore it cannot be connected to anticipation of emerging or delay of returning to the burrow. In accordance to Corsini and colleagues (1995), these results support that motor diurnal activity could be linked to feeding strategy. The presence of diurnal motor activity also in summer days has been observed thereby excluding thermoregulation needs, as hypothesised by Corsini and colleagues (1995). The Italian population of crested porcupines seems to have African origins (Trucchi et al. 2016) where this species lives in desert areas of northern Africa and Sub-Saharan Africa overlapping its distribution range with *Hystrix africaeaustralis* (Kingdon 1974). Study performed in captivity shows that Cape porcupines (*H. africaeaustralis*) acclimated to 25°C can regulate their body temperature between 13° and 30°C (Haim et al. 1990). No data are available for both crested porcupine (*H. cristata*) and *H. indica*. However it can be assumed that also *H. cristata* may have similar ranges of thermoregulation.

Moreover no information is available concerning the thermoregulation ability of this species at low temperatures. In Italy porcupines live in temperate areas with hot summers and mild winters (Mori et al. 2013, Vecchio et al. 2018). Therefore it is more likely that these environmental conditions can be widely tolerated by porcupines without significant thermoregulation need.

Family group daylight movements resulted significantly different from lonely specimens of all ages. On the other hand, no difference was recorded in diurnal motor activity between lonely specimens (porcupettes, youngsters or adults). This result suggests that daylight behaviour could not be related to sub-adults dispersion although no information is available on the dispersal of sub-adults. The only information available is that the youngsters seem to remain with the parents until they reach the adult weight size (10 kg, from 1 to 2 years old) (Van Aarde 1987b, Grazzini 1992).

Diurnal motor activity resulted significantly higher in the settlements permanently inhabited by porcupines while sporadic diurnal transits were recorded in settlements occasionally inhabited. This result may exclude a connection between diurnal motor activity and exploration of the environment and thereby supporting the linkage of diurnal movements to feeding strategy.

The sunbathing behaviour was observed in five porcupine families. 32 out of 36 episodes show only porcupettes or porcupettes with adults or youngsters. These observations confirm that porcupines perform sunbathing, as hypothesized by Kingdon (1974) and suggested by Yallen (1991), and prove for the first time the presence of sunbathing behaviour in crested porcupine. Sunbathing (direct exposure to maximum sun) is a common thermoregulation behavioural mechanism both in ecto and endotherms animals that live in desert and arctic areas (Morse 1980).

Sunbathing also occurs in a wide range of bird species (Kennedy 1969, Cade 1973, Yavad et al. 2018) and it has been frequently observed in primates (Hanya et al. 2007, Danzy et al. 2012, Lubbe et al. 2014), felines (Smith and Kok 2006) and some small mammals (George and Crowther 1981). Among rodents the sunbathing behaviour is normally exhibited in some small diurnal species such as round-tailed ground squirrel (*Spermophilus tereticaudus*) (Walsberg 1988) and gundi (*Ctenodactylus gundi*) (Honigs and Greven 2003). No data are indeed available for nocturnal rodents. The sunbathing behaviour in crested porcupine seems to be somehow related to the presence of porcupettes (Coppola et al. 2019a). The presence of adult and young of the same family with the porcupettes during sunbathing may be due to

porcupettes protection. So it is possible to hypothesise that the porcupettes need to perform sunbathes for thermoregulation and/or synthesis of metabolites such as vitamins (Vitamin D) necessary for growth. As hypothesised for diurnal motor activity, thermoregulation may not be a necessity for adults. However, considering the lack of information on the ability of thermoregulation of the species it cannot be excluded that the porcupettes may have some difficulties in thermoregulation. The Vitamin D is necessary for the normal skeletal development in the young as well as maintenance of calcium and phosphate homeostasis in the adult (DeLuca 1967). Reduction in growth and bone deformities were reported to be the main consequences of vitamin D deficiency in diets or of lack of natural sunlight in young animals (Miller et al. 1983, El Shorafa et al. 1979, Kenny 1998). Exposure to sunlight initiates the formation of vitamin D and studies performed on ponies (El Shorafa et al. 1979) and primates in zoos (Kenny 1998) reported that dietary vitamin D is not needed for normal bone development when there is abundant sunlight. No data are available concerning vitamin D requirement in nocturnal mammals and among these, porcupines. In this investigation sunbathing behaviour was recorded between April and June mainly in the hot hours of the day. The sunlight exposure in hottest period of the year and during hottest hours of the day could reinforce the hypothesis of sunbathing as thermoregulation strategy or sunlight absorption for Vitamin D production. For all diurnal events/episodes, the porcupines showed no changes in their usual nocturnal activity pattern the previous and successive nights. In this case the permanence of porcupines near the burrow during daylight hours seems to be probably due to metabolic necessities. In conclusion the crested porcupine seems not to be a strictly nocturnal mammal. Porcupines show peaks of diurnal activity, probably as feeding strategy, mainly in late winter and spring when the hours of darkness necessary for feeding decrease and there is a reduction of food sources preferred by porcupines. Crested porcupine porcupettes perform sunbathing in spring. The permanence under sunlight seems to be a behaviour probably regulated by metabolic necessities.

At the light of the results obtained in this investigation, feeding strategy is not the exclusive reasons of the occurrence of diurnal activity in porcupine as predicted in this study. Further investigations concerning the pattern of diurnal activity and physiological aspects of the species are desirable in order to explain the diurnal behaviour of this nocturnal rodent.

Scavenging behaviour in crested porcupine

The scavenging behaviour in crested porcupines was here observed for the first time in four occasions. This result clearly shows that also the crested porcupine eats flesh as previously reported in *Hystrix africaeaustralis* by Roth (1964) and Kingdon (1974).

The scavenging behaviour of crested porcupine was recorded in presence of available fresh food provided by the operators and in high food availability habitat. This condition indicate that feeding on carrion flesh by crested porcupine is not related to lack of food and it is more probably due to physiological and biochemical requirement. The vegetarian diet supplemented with animal protein is common in many frugivorous and granivorous rodents, especially in growing young and breeding females (White 2007, 2011). The animal protein supplementation in vegetarian diet seems to be the result of behavioural and physiological adaptation in order to increase the reproduction success (White 2011).

In this investigation the eating on carrion flesh was clearly observed only in adult specimens, one of which was a female and the other was not sex determined. The observation of this feeding behaviour in a porcupine female suggest that eating animal protein could be a feeding strategy for the assumption of key proteins necessary for breeding as indicated by White (2011).

The Authors are aware of the small number of events recorded in this investigation however this result disclosed new important information concerning the feeding behaviour of this believed strictly herbivorous rodent. Further investigations are necessary in order to assess the occurrence of the scavenging behaviour in crested porcupine in relation to age, sex and physiological state.

Reproduction in wild crested porcupine

The mounting behaviour in free-ranging crested porcupines was observed throughout the year independently from births, lactation and presence of porcupettes.

Nightly rhythm of single mounting regularly occurs and multiple mountings per night were also observed. This result is in accordance with the observations performed by Felicioli et al. (1997a, b) in semi-captive crested porcupines and by Sever and Mendelsshon (1988a) in semi-captive Indian crested porcupine (*H. indica*). The crested porcupine is a monogamous rodent living in pair, small family group (Mohr 1965) or clan where sub-adults of previously litters behave as helper. The oestrus cycle of *H. cristata* is 35 days (Weir 1974). Therefore, the observation of nightly mounting indicates that this behaviour is not linked to the oestrus state and it's probably performed as a mechanism for pair maintaining. The sequence of mounting observed in free-ranging porcupines is the same described by Felicioli (1997a, b) in semi-captivity and also into the burrow. In the 83.8% of mounting events the sequence starts with the nose-quill contact by the male followed by the presenting of the female while in the 16.1% spontaneous presenting by the female was observed. According to Keliman (1974) the spontaneous presenting could be an indication of oestrus state. The behavioural display as grooming, sniffing, following, sound and stepping, observed in semi-captive porcupines (Felicioli 1997a, b), are also recorded in free-ranging porcupines. Moreover as reported by Felicioli (1997a, b), in each mounting event the behavioural pattern are performed with different combination and no standard sequence in the performing of these is recorded.

Copulation in free-ranging crested porcupine was detected in only two occasions. The average duration of copulation recorded was 24 SD 7 seconds with a mean of 17 SD 5.5 thrusting. Conversely, Felicioli et al. (1997) reported lower copulation time (8.9 SD 3.9 sec) in semi-captive crested porcupines while longer copulation time (2-3 minutes) was observed by Morris and Van Aarde (1985) in *H. africaeaustralis*.

Porcupine births were observed thuoghtout the year as previously reported by Santini (1980, 1983) and Mori et al. (2016) in free-ranging porcupines and by Grazzini (1992) in semi-captive porcupines. Mori et al. (2016) also reported the presence of two significantly births peaks of in February and October. However, our observations indicate the absence of seasonal effect in *H. cristata* reproduction (Santini 1980, Grazzini 1992).

The number of births recorded in wild crested porcupine in this study ranged from 1 to 3 *per* year. This result is in accordance with results obtained by Grazzini (1992) in semi-captive porcupine while no other data are available on free-ranging porcupines.

The size of porcupine litters recorded in this investigation ranged from 1 to 2 porcupettes *per* biths and no litters of 3 or 4 porcupettes has been recorded. The porcupines litters of three porcupettes are an extremely occasional event and only Santini (unpublished data) and Mori et al. (2016) in free ranging porcupines have both documented in one occasion this event. Porcupine litters of four porcupettes have been reported only by Tohmè and Tohmè (1980) in *H. indica*. Despite these exceptions, in the most cases the litter's size recorded is the same obtained in this study (Weir 1974, Santini 1980, Felicioli 1991, Grazzini 1992, Mori et al. 2016).

Mori et al. (2016) in free ranging porcupines reported that porcupettes leave the burrow for the first time when about 40-50 days. The age of first emergence of porcupettes from burrow in this study was estimated between 10 and 15 days after birth. The same interval time between birth of porcupettes and their first emerging from artificial burrow was recorded by Grazzini (1992) in semi-captive porcupines. Considering that birth porcupettes are well developed with open eyes and at 30 days start to eat solid food (Santini 1980, Grazzini 1992), the interval between birth and first emergence of porcupettes from burrow reported in this study is reliable.

In the interval between birth and emergence of porcupettes, adult male and female in all monitored pair, started to alternate into the burrow and increased the number entrance in burrow during the same night. This behaviour was also oserved by Felicioli (1991) in semi-captivity and by Mori et al. (2016) in free-ranging crested porcupines. Before first emergence from burrow of porcupettes a lower activity of the females outside the burrow has been also recorded and adult males while brings food and/or bones into the burrow were detected. The combined observation of these two behaviours allows us to hypothesise that during the first days after porcupettes birth the females mostly remain in burrow for porcupettes care and nursing while the males look after to its partner. The observations performed in this study confirm that porcupettes start to eat solid food at about 30 days after birth while the nursing following up to 2-3 months (Grazzini 1992).

In accordance with the observations performed by Grazzini (1992) in semi-captive crested porcupines also in free-ranging porcupines both adult male and female equally perform

parental cares and also the sub-adults actively take parte to the care of the new born porcupettes.

The minimum time of permanence of the sub-adults within the family resulted 1 year as hypothesised by Grazzini (1992) on *H.cristata* and by Van Aarde (1987b) on *H. africaeaustralis*. The achievement of sex maturity seems to occur at around 9-10 months in *H. cristata* (Santini 1980) and at 1 year in *H. africaeaustralis* (Van Aarde 1987b). Therefore, is possible that the departure of sub-adult from the family is linked to the the achievement of sex maturity. However no information is available concerning the dispersal of porcupines sub-adults and futher investigation are necessary.

5.3. Porcupine Population Abundance estimate (PPA) and first hypothesis of census model

In both study areas, the porcupine population abundance provided very similar result. In EA1 (Crespina-Lorenzana) the average porcupine density resulted 3.9 specimens/km^2 (SD = 1.4 specimens/km^2) while in EA2 (Camugliano) was 3.6 specimens/km^2 (SD = 1.4 specimens/km^2).

Despite the distribution expansion of this rodent in Italy (Mori et al. 2013, Vecchio et al. 2018) there are no data concerning the density and abundance of crested porcupine and this lack of information do not allow comparing the results obtained in this study.

The porcupine populations are self regulatory (Van Aarde 1998). The density of porcupines varies primarily according to food availability and in areas in the surrounding of agricultural fields increases the likelihood of a relatively high density of porcupines (Horwitz et al. 2012). The sociality induced delay in reproduction limiting population growth, while a relaxation of reproductive suppression, through the creation of dipersal opportunities, enhances population growth rate (Van Aarde 1998). Study performed by Van Aarde (1987c) suggests a low mortality rate in the first two months of porcupine life which mostly increase up to 70% during the first year of life. Moreover, porcupine females have the ability to compensate for the loss of litters by conceiving within a few days of such loss (Van Aarde 1998). Therefore, the resistance and resilience of porcupine populations may be explained in terms of reproductive plasticity, where changes in the social environment induce a response on the reproductive axis.

Alkon and Saltz (1985a) in a semi-desert area and Sever and Mendelssohn (1991b) in coastal plain of Israel reported a population density of *H.indica* of 4 porcupines/Km^2, basing on the home-range sizes. Conversely, Alkon (1999) estimates in Desert Negev for *H. indica* a population density of 2.1 porcupines/Km^2 for animals utilizing only natural habitats and 7.5 animals/km^2 for “agricultural” porcupines.

Sharma (2001) using the pellets count method and direct counts of animals along transects obtained a porcupine density for *H. indica* in a semi-arid zone in wester India of 12.4 animals/Km^2 and 8.8 animals/Km^2 respectively. Despite these two methods are widely used for wildlife census (Eberhardt 1978, Plumptre 2000, Lioy et al. 2015) there are not suitable for porcupine estimate density and determine an overestimation of population abundance. The porcupine is suggested to be a territorial rodent (Felicioli 1991, De Villiers 1994, Corbet and

Van Aarde 1996) that use and maintain an exclusive territory whitin a large, non-exclusive home range (Corbet and Van Aarde 1996). Moreover, when foraging the porcupines follow the same path and visit the same biotopes (Santini 1980, Pigozzi and Patterson 1990).

More recently, Mukherjee et al. (2018) estimated a density of Indian crested porcupine in a semi-arid zone of India (3.2 SD 1.3 individuals/Km^2) similar to those obtain in this investigation using a very similar aproach.

The difference between the method conceived in this study and the one used by Mukherjee et al. (2018) is the way to compute the number of settlement inhabited by porcupine. Mukherjee et al. (2018) computed the number of estimate inhabited settlements by using the inhabitation frequency recorded in the sampled settlements. Conversely, in our computation forecast the estimation of the number of inhabited settlements by using the frequency of settlements use by the porcupines and considering the spatio-temporal inhabitation of settlements by the crested porcupine. Although the application of the two methods gives the same results, the method developed in this study includes an additional data concerning the pattern of settlements inhabitation by the crested porcupine.

The preliminary census model developed in this study provides the guidline for the crested porcupines' estimation density without the necessity to search and monitor all settlements present in an area. The preliminary result obtained by testing the census method in Camugliano area, used in this case as a control area, show a good match with the result obtained by using the real data. Interestingly, if this method is applied to Mukherjee et al. (2018) data the same result can be obtained. However, despite this model is preliminary and not exhaustive, can represents a usefull and easy method to be replicated in other area of crested porcupine presence and probably also of the two other species of genus *Hystrix* (*H. indica* and *H. africaeaustralis*). At the current state of model development it is not possible to establish which can be the error range obtainable from its application. Therefore, further applications of this method and obtained data collection are desirable in order to validate and assess the reliability of the model.

5.4. Health monitoring of crested porcupine

Parasitological analysis

G. duodenalis is a common parasite of the small intestine and one of the most important and frequent causes of human and animal diarrheal disease worldwide (Cacciò et al. 2018). Long-term health consequences of giardiasis have also been documented in humans (Lanata et al. 2013).

Among parasites identified in 52 porcupine faecal samples collected within the study area, *Giardia* spp. was the most prevalent. Almost half of the samples examined (48%) were found positive to *Giardia* by the immunoassay and *G. duodenalis* was identified in 12 out of 17 isolates examined at molecular analysis. However, the possibility that some of the analysed faecal samples were belonging to the same animals is high, especially for those samples collected within transects where more than one sample was found.

Nevertheless, within the 25 *Giardia* spp. positive faecal samples to the immunoassay also 4 out of 12 (33.3%) individual faecal samples were included. These four samples were belonging to two captured animals, one road-killed specimen and the single faecal sample collected in Z1 in EA1.

For the detection of *G. duodenalis*, *Cryptosporidium parvum* and *Cryptosporidium hominis*, a sensitivity and a specificity of 85-96% and 89%, 80-85% and ≥ 98%, 96% and 99% has been reported for the rapid immunoassay used in this study compared to conventional microscopy, direct fluorescent-antibody and PCR, respectively (Weitzel et al. 2006, Regnath et al. 2006, Chalmers et al. 2011). However, false negative results may be possible due to intermittent faecal excretion of these protozoans or if the amount of protozoan antigens in the examined samples is too small (RIDASCREEN® *Cryptosporidium*/*Giardia* Combi). Moreover, the sensitivity and the specificity of this commercial kit for the detection of other *Giardia* and *Cryptosporidium* species is unknown. Therefore, it is possible that some samples tested negative to these protozoa in the present study were instead positive.

Results from the immunoassay were not confirmed by PCR in all cases. As previously described, the difference between immunological and molecular results may be due to the presence of PCR inhibitors in faecal samples, with the amplification turning out unsuccessful due to the loss of pretreatment methods for removal of specific PCR inhibitors (Schrader et al. 2012).

Nevertheless, this study is the first report of *G. duodenalis* infection in the crested porcupine.

Indeed, *Giardia* spp. infection was detected also by Chakraborty et al. (2015) in the Indian crested porcupine (*H. indica*), but species, assemblage and subtype identification were not performed. In the present study, different combinations of *G. duodenalis* assemblages were identified in two isolates at *SSU rDNA* and *tpi* loci. The genotyping lack of concordance is quite recurrent in *G. duodenalis*; these discordant results can derive from mixed infections, subsequent infections or heterogeneity of allelic sequences (Sprong et al. 2009, Ryan and Cacciò 2013, Fahmy et al. 2015, Adell-Aledón et al. 2018). However, due to the small number of samples, it was difficult to draw any conclusion concerning the genotyping discordance.

The habitat diversity and environmental fragmentation linked to anthropic activities are key factors for the presence of crested porcupines (Toschi 1965). In the Italian agro-ecosystem, the crested porcupine is considered as a pest and it benefits from the increase in the production of agricultural crops (Santini 1980, Laurenzi et al. 2016). Therefore, contaminated anthropogenic settlements may represent a risk factor for the acquisition of *G. duodenalis* infection by crested porcupines in the examined area, as anthropogenic spread of *G. duodenalis* is well documented in other wildlife species and environments worldwide (Hillman et al. 2019, Thompson 2013). Conversely, land-use changes by humans for agricultural development and urbanization have been associated with higher risks of zoonotic pathogen transmission by potential reservoir animals, and the presence of crested porcupines in habitat characterised by anthropic activities may enhance the possibility for the spread of crested porcupine parasite infections that could affect also humans (Chakraborty et al. 2015, Otranto and Deplazes 2019, Mendoza et al. 2020). As assemblages AII and BIV are likely associated with human infections, results here obtained may suggest that *H. cristata* can act as source of *G. duodenalis* cysts potentially infectious to humans (Robertson 2019, Cacciò et al. 2018). In Europe, *G. duodenalis* infection has been reported in badgers, red foxes and wild boars (Hamnes et al. 2007, Cacciò et al. 2008, Barlow et al. 2010, Beck et al. 2011, Onac et al. 2015, Stojecki et al. 2015, Robertson et al. 2019). Interestingly, the majority of identifiable *G. duodenalis* DNA isolated from red fox and wild boar samples in previous European studies was found to be assemblage A or B, suggesting that these wild animals may play a role in the zoonotic transmission of *G. duodenalis* (Stojecki et al. 2015, Robertson et al. 2019). In the area here examined, wild boars, red foxes and badgers share the same porcupine habitat, with crested porcupines and badgers occasionally sharing and co-habiting the same settlements.

Moreover, the ingestion of porcupine carrions by red foxes may represent another source of infection in these animals (Fais 1991). Therefore, it is possible to hypothesise a putative circulation and transmission of potentially zoonotic *G. duodenalis* assemblages among crested porcupines, red foxes, wild boars and badgers in the study area with a potential impact on the population health of these wild animal species. Moreover, these wild species may act as reservoirs of *G. duodenalis* infections for humans (Hillman et al. 2019, Thompson 2013).

Concerning other parasites identified in this study, eggs of *Trichuris* spp. and of gastrointestinal strongyles were found in about 33% of examined samples, while capillariids, coccidia and *Cryptosporidium* spp. were much less frequent (about 2-4%). However, it should be considered that the Mini-FLOTAC technique used for the detection of most of these parasites has a sensitivity of 10 eggs-oo(cysts)/ gram of faeces (Cringoli et al. 2017). Therefore, it is possible that positivity was not evidenced for all those parasites present at lower quantities in examined faecal samples.

In previous studies, *Trichuris infundibulum* and *Trichuris hystricis* have been reported from *H. cristata* (Pavlov 1957), and *Trichuris landak* from *H. javanica* (Purwaningsih, 2013), while gastrointestinal strongyles (*Trichostrongylus colubriformis*) and unidentified *Trichuris* and *Capillaria* species have been reported from *H. indica* (Wertheim and Durette-Desset 1975, Mir et al. 2016). However, to the best of our knowledge coccidian and *Cryptosporidium* spp. infections have been never reported before in crested porcupines. About *Cryptosporidium*, it would be extremely interesting to perform further and more in-depth studies aimed to the identification of *Cryptosporidium* species infecting crested porcupines and to assess the molecular epidemiology of this protozoan species in the examined area. More specifically, it would be very interesting to know whether in the study area involved species/genotypes are shared with other wild mammals cohabiting with crested porcupines or specific for *H. cristata*, as host-adapted species/genotypes are frequently observed among wildlife (Thompson and Ash 2019). It would be also very interesting to know whether in the study area are involved zoonotic species, considering the recent detection of potentially zoonotic *Cryptosporidium* species in European red foxes and badgers (Mateo et al. 2017).

Further studies aimed to know the impact on the health of crested porcupines of the remaining parasites identified in this study are also needed, since data are lacking. In conclusion, results obtained in this investigation improved the knowledge on the parasite-fauna of crested porcupines in Italy and showed for the first time that *H. cristata* can be infected by *G. duodenalis*. More studies are needed on the epidemiology of *Giardia* and other parasite

species in crested porcupine populations, especially on *G. duodenalis* assemblages to better evaluate the zoonotic potential of *Giardia* in *H. cristata*.

Ectoparasites

Ectoparasites were collected in 3 out of 16 (18.8%) examinated porcupines. The ectoparasite prevalence obtained in this investigation is in accordance with those reported by Mori et al. (2015b). Despite the small sample size, our results seem to confirm a low ectoparasite infection in crested porcupines. Mori et al. (2015b) reported the presence of a higher abundance of arthropod parasite and parasitic load in porcupines living in richness habitat compared to those in densely wooded areas. This data seems not match with the results obtained in this study. In this study both porcupines capture areas are characterized by a high environmental richness but despite this, low variability and abundance of parasite was recorded. In the examinated porcupines only fleas were collected and the only detected specie in captured porcupine was *Pulex irritans*. The presence of this species in crested porcupines was frequently reported with values of prevalence above 20% (Monetti et al. 2005, Mori et al. 2015b). However, *P.irritans* is the most common flea in wild mammals such as red foxes and European badgers (Dominiguez 2004, Martinez-Carrasco et al. 2007). This suggest a relationship between the presence of fleas in crested porcupines and habitat and settlement sharing by crested porcupines, badgers and red foxes (Mori et al. 2015a, Coppola et al. 2019b). According to Mori et al. (2015b) also *Ixodes ricinus* result to be a dominant species in crested porcupine however, in this study no hard tick was ever been detected.

The only one road-killed porcupine examinated resulted infected of *Ctenocephalides felis. C. felis* is the most common flea on dogs and cats (Rust 2005) but it can also parasitize wild mammals including red foxes (Martinez-Carrasco et al. 2007). Therefore, the infection of examineted porcupine with *C. felis* could be related to environmental and settlements sharing with red fox (Mori et al. 2015a, Coppola et al. 2019b) or consequence of hospetalization in a veterinary clinic before the examination.

In accordance with Mori et al. (2015b) the parasitic arthropod fauna of crested porcupines seems to be composed by a relatively low number of species. All recorded ectoparasite in crested porcupine are sourced from other mammals known to be primary hosts, mainly red foxes and badgers and no specific taxa was never been recorded. At the best of our knowledge no information are available concerning the parasitic arthropod fauna of this rodent in their original country. However, a possible hypothesis is that, in Italy, the absence of specific taxa in crested porcupines may be due to its non native Italian specie status (Trucchi and Sbordoni 2009).

Haematological and blood biochemical profile of crested porcupine

Biochemical profile of crested porcupine was obtained for 5 out of 7 blood samples collected from captured porcupines while the haematological values only for 2 out of 7 samples. Author is aware of the small sample size nevertheless few data are available concerning the haematological and biochemical profile of old world porcupines and in particular on crested porcupines (Leonetti 2018). Therefore, the results obtained in this study may be used as a basis for futher haematology and biochemical studies in *H. cristata*.

The haematological values obtained in this investigation are in accordance with those reported in some species of new world porcupines by De Almeida et al. (2011) in captive bristle-spined porcupine (*Chaetomys subspinosus*) and by Moreau et al. (2003) in Brazilian porcupine (*Coendou prehensilis*) and black-tailed hairy dwarf porcupine (*Coendou melanurus*). Moreover, the same haematological values are also reported in coypu (*Myocastor coypus*) (Martino et al. 2012) and capybara (*Hydrochoerus hydrochaeris*) (Arouca et al. 2000, Di Chiacchio et al. 2014). These last two species are the only two wild rodents belonging to the same suborder of porcupines but not to the same familiy. *H. cristata*, *C. Subspinosus*, *C. Prehensilis*, *C. Melanurus*, *H. hydrochaeris* and *M. Coypus* all present similar values of haemoglobin (Arouca et al. 2000, Moreau et al. 2003, De Almeida et al. 2011, Martino et al. 2012, Di Chiacchio et al. 2014). However, the crested porcupine shows a higher mean value of red blood cells but a lower mean value of MCV (mean corpuscular volume) than the other ones.

In the biochemical profile it was not possible to obtained values for all the established parameters. Blood sampling in the captured porcupines has often been very difficult (e.g. vein collapse and rapid coagulation) and this factor can have influenced the samples analysis.
The mean value of TP in crested porcupine (7.5 SD 1.7) resulted in accordance with the values reported for capybara (Di Chiacchio et al. 2014), coypu (Martino et al. 2012), Brazilian porcupine and black-tailed hairy dwarf porcupine (Moreau et al. 2003). The same can be assumed for mean creatinine values recorded in 4 out of 5 examined crested porcupines (1.1 SD 0.2). In only one case value of creatinine of 11.2 mg/dL was recorded in an adult male associated with a high value of urea (41 mg/dL). Considering the high prevalence of leptospira antibodies detected and the isolation performed during this study, the high values of urea and creatinina could be due to kidney damage for *leptospira* infection. Unfortunately for this subject no MAT result was obtained due to aemolitic blood sample.

In the other porcupines the values of urea resulted similar to the mean value recorded in coypu (Martino et al. 2012) and black-tailed hairy dwarf porcupine (Moreau et al. 2003) while a lower and a nigher mean value was recorded in Brazilian porcupine and capybara respectively (Moreau et al. 2003, Di Chiacchio et al. 2014). In these individuals positivity for leptospira antibody was recorded only in the sample 2 with the highest titer detected. In this case the absence of alteration in urea and creatinine values could be do to the absence of kidney localization of *leptospira* or to the young ages of the individual.

Among the investigated serum enzymes results were obtained for GGT, AST and CK. The mean value of GGT recorded in crested porcupine resulted higher than capybara (Di Chiacchio et al. 2014), Brazilian porcupine and black-tailed hairy dwarf porcupine (Moreau et al. 2003). Despite this, the mean value is not to be considered reliable due to the presence of two samples with high GGT values (>30 U/L) probably due to the presence of pathological states. Conversely, in the other three samples similar values of GGT to those reported for the other previously mentioned species are recorded.

The values CK resulted extremely high in all analysed samples. As suggest in another similar study (Moreau et al. 2003) these results are most probably attributable to the excitation and stress state during the captures and handling. This hypothesis seems is reinforced also by the presence of AST very high values in 4 out of 5 porcupines blood samples. Moreover, extreme values of CK were recorded in two blood samples attributable to porcupines remain in the cage traps more than 12 hours.

Leptospirosis in crested porcupine

Antibodies detection of Leptospira

Humans, domestic animals and wildlife share Leptospirosis a zoonosis caused by the pathogenic spirochete of genus *Leptospira* (Adler and de la Peña Moctezuma 2010). Urine shedding and marsh water contact is the main cause of clinical disease (Adler and de la Peña Moctezuma 2010, Faine et al. 1999, Haake and Levett 2015). *Leptospira* epidemiology is strictly linked to the widespread of maintenance host species (Bertelloni et al. 2019, Cerri et al. 2003). Wildlife plays a pivot role in the epidemiology of leptospirosis. Leptospira maintenance host function has been described for some rodents in different countries, such as for *Apodemus* spp., *Bandicota* spp., *Delomys* spp., *Mus* spp., *Necromys* spp., *Oryzomys* spp., *Rattus* spp., *Thaptomys* spp., *Trinomys* spp. and also for *Myocastor coypus* and *Hydrochaeris hydrochaeris* (Levett 2001, Michel et al. 2001, Jorge et al. 2012, Cosson et al. 2014, Cortizo et al. 2015, Fratini et al. 2015, Haake and Levett 2015, Moreno et al. 2016a, Vieira et al. 2018, 2019, Krijger et al. 2019). Icterohaemorrhagiae and Ballum serogroup are the most widespread in rodents that contribute to the base of the trophic chain (preys) (Faine et al. 1999, Levett 2001). Conversely, several wild carnivores and scavengers are at the top of the feeding chain and are potentially exposed to the pathogen *Leptospira* through their diseased preys and carrion respectively (Ellis 2015). Italy is the only European country where four semi-fossorial mammals two mainly herbivorous rodents (porcupines and coypus) and two mainly carnivores (red foxes and badgers) potentially share both foraging areas and burrows (Pigozzi 1986).

The seroprevalence of leptospirosis in crested porcupine resulted 58% (7 out of 12). Authors are aware that the size of the samples is small nevertheless the crested porcupine belong to the rodents order that are well known to be important *Leptospira* carriers (Blasdell et al. 2019, Mori et al. 2017b). The crested porcupine is a widespread species in Italian agro-ecosystems and its distribution range is rapidly expanding (Mori et al. 2013, Vecchio et al. 2018). These three factors warrant to propose this preliminary report as a potential useful set of information for better understanding the epidemiology of leptospirosis in wildlife.

The seroprevalence in crested porcupine found in this investigation seems to be in accordance with the prevalence of *Leptospira* infection reported in the semi-fossorial Eurasian badger, red fox and coypus (Ayral et al. 2016, Fratini et al. 2015, Michel et al. 2001, Milas et al. 2006, Millán et al. 2009, Slavica et al. 2008, 2011, Vein et al. 2014). In badger a seroprevalence of

50% (n=2) was reported. (Millán et al. 2009). Moreover, a prevalence of 8.2% (n=316) of pathogenic *Leptospira* DNA in badger kidneys was detected by Ayral et al. (2016) in France. In the red fox, a seroprevalence of 5.3% (n=94) was reported in a study performed in Tuscany by Bertelloni et al. (2019), while seroprevalence of 33.8% (n=121), of 31.3% (n=112) and of 57.6% (n=59) were recorded in Croatia (Milas et al. 2006, Slavica et al. 2008) and of 47.1% (n= 17) in Spain (Millán et al., 2009). In addition, the 6.1% (n=362) of red fox kidney resulted positive to pathogenic *Leptospira* DNA in France (Ayral et al. 2016). Concerning coypu, MAT analysis scored positive of 27.9% (n=122) and 28.5% (n=70) in two Tuscanian investigation (Bertelloni et al. 2019, Fratini et al. 2015), while in two studies performed in France by Vein et al. (2014) and Michel et al. (2001) the seroprevalence resulted of 76% (n=50) and 64% (n=75) and between 16.5% (n=236) and 66% (n=100) respectively.

In the porcupine, the serogroup Icterohaemorrhagiae prevalence resulted 57% (4 out of 7 sera). A comparable value of prevalence of Icterohaemorrhagiae was also reported in red fox, badger (Millán et al. 2009) and coypu (Bertelloni et al. 2019, Fratini et al. 2015, Michel et al. 2001). In one porcupine the serogroup Icterohaemorrhagiae was found associated to Sejroe with antibody titer of 1:200 and 1:100 respectively. In this case, the low Sejroe titer, could be the consequence of a higher Icterohaemorrhagiae titer that lead to aspecific reaction between the sera antibody and Sejroe antigens (Faine et al. 1999). The serogroup Pomona was detected in 28.5% of sera (2 out of 7). Pomona seropositivity was never found in badgers in Spain and France (Ayral et al. 2016, Millán et al. 2009), in coypus in Italy and France (Fratini et al. 2015, Michel et al. 2001, Vein et al. 2014) and in red foxes in Italy (Bertelloni et al. 2019). Conversely Pomona seropositivity was found in red foxes in Croatia (Milas et al. 2006, Slavica et al. 2011). Further investigations are necessary to assess Pomona serogroup presence in foxes, badgers and coypus in the same porcupine area as well as establish if serogroup Pomona is shared among porcupine and these other three semi-fossorial mammals. Noteworthy the serogroup Pomona was found in high prevalence in the wild boar (Bertelloni et al. 2019, Boqvist et al. 2012, Castillo-Contreras et al. 2018, Tagliabue et al. 2016, Vale-Gonçalves et al. 2015; Żmudzki et al. 2016). In Italy wild boar and crested porcupines share the same habitat (Massei et al. 2015, Santilli and Varuzza 2013). Since wild boar is recognised as a Pomona *reservoir* (Ellis 2015) is reasonable to hypothesise a possible transmission of *Leptospira* Pomona from wild boar to porcupines. Further investigation would be useful to disclose the hidden relationship between the four semi-fossorial mammals (i.e. crested porcupines, red foxes, badgers and coypus) and wild boar in order to delineate the Pomona infection route throughout wildlife. In the crested porcupines, the highest titer

resulted the serogroup Australis (1:1600) from a serum of the female sub-adult (5.6 Kg) specimen. The serogroup Australis is also the most detected in red foxes (Bertelloni et al. 2019, Milas et al. 2006, Slavica et al. 2008, 2011) and coypus (Michel et al. 2001; Vein et al. 2014). Moreover, the serogroup Australis was isolated in red foxes and badgers in Grain Britain (Hathaway et al. 1983). The high titer found in the sub-adult porcupine could be due to: I) a recent *Leptospira* infection in the porcupette by environmental contamination, II) congenital route, III) a reaction caused by a new host-pathogen relationship (Adler and de la Peña Moctezuma 2010, Faine et al. 1999) between serogroup Australis and *Hystrix cristata*.

In one porcupine female porcupette Pomona and Australis serogroup were both found with a titer of 1:100. These two serogroup are known to not cross-react each other (André-Fontaine and Triger 2018, Houwers et al. 2011) so the co-presence could indicate a co-infection as well as that the low detected titer could suggest that one of the two is a false positive.

Among porcupines, *Leptospira* was found in *Erethizon dorsatum, Sphiggurus villosus* and *Hystrix brachyura* (Fornazari et al. 2018; Mitchell et al. 1966; Siti-Nurdyana et al. 2016). In a specimen of *E. dorsatum* Pomona serogroup was isolated (Mitchell et al. 1966). In *S. villosus* while MAT resulted negative, DNA of pathogen *Leptospira* was found in urine without serogroup specification (Fornazari et al. 2018). In *H. brachyura* seropositivity to Javanica, Hurstbridge, Ballum and Celledoni was found (Siti-Nurdyana et al. 2016). These results indicate that porcupines could be infected by several serogroup of *Leptospira*. The role of *reservoir* or accidental host need to be address. Further investigations are necessary to disclose the relationship and route of leptospirosis between porcupines, red foxes, badgers and coypus and their interaction with wild boar in order to clarify the leptospirosis – epidemiology – wildlife framework at the light of its potential zoonotic source.

Isolation of Leptospira

Leptospira interrogans serogroup Pomona, serovar Pomona, was isolated for the first time from a porcupette kidney. Both sampled sera resulted negative to MAT for all *Leptospira* serovars tested. The seronegativity of *Leptospira*-positive subjects has been previously reported in literature for other species (Merien et al. 1995; Agampodi et al. 2012; Hall and Lambourne 2014) and also for porcupine (*Sphiggurus villosus*) (Fornazari et al. 2018). The serological negativity of *Leptospira*-positive porcupette could be related to the bad quality of blood sample collected in the heart cavity or to a chronic or an early infection. The hypothesis of a chronic or early stage infection are both unlikely due to the young age of the individual and the stage of renal lesions characterized by the presence of chronic inflammatory infiltrates and fibrosis. Therefore, the most probable hypothesis could be that the absence of *Leptospira* antibodies in the *Leptospira*-positive porcupette may be related to the antibiotic treatment with Enrofloxacin performed during its hospitalization.
Activity of Enrofloxacin against *Leptospira in vitro* is documented. However, some studies suggest an increasing of the MIC values in recent isolates (Moreno et al. 2016b, Liegeon et al. 2018). Carrascosa et al. (2017) reported a low effectiveness of Enrofloxacin antibiotic *in vivo* in order to prevent renal colonization of *Leptospira* in hamster. In state of this, antibiotic treatment of *Leptospira*-positive porcupette could have affected the antibodies response, leading to the negative MAT result. The antibiotic treatment could have also determined the lack of leptospires in the renal tissue highlighted with specific Warthin Starry staining.

At the best of our knowledge, among Hystricomorph rodent, serological positivity for phatogenic Leptospire were detected only in *Sphiggurus villosus*, *Hystrix brachyuran* and in a North American porcupine without specified the species (Mitchell et al. 1966, Siti-Nurdyana et al. 2016, Fornazari et al. 2018). Isolation of *Leptospira* serovar Pomona from kidney, blood and urine was only performed in one individual of North American porcupine (Mitchell et al. 1966). No other *Leptospira* serovar have ever been isolated in the porcupines.

Wild and domestic rodents are well known to be important *Leptospira* carrier, involved in transmission to animals and humans (Mori et al. 2017b, Blasdell et al. 2019).

However, the *Leptospira* infection of crested porcupine documented in this investigation could be the result of porcupines habitat sharing with wild boar (*Sus scrofa*) that are present in Tuscany with an high density population (Santilli and Varuzza 2013, Massei et al. 2015). Wild boar plays a key role in the spreading of some *Leptospira* serovars, such as Pomona, in

the environment (Chiari et al. 2016, Bertelloni et al. 2019). All these factors could strongly increase and promote the possibility of *Leptospira* infection in crested porcupine.

Therefore, the isolation of *Leptospira* in the crested porcupine could indicate that *H. cristata* could be a new potential natural host for this bacterium.
This is a single case report of *Leptospira* serovar Pomona in crested porcupine, however, this result could be a useful contributes to the description of the epidemiology of leptospirosis. Further investigations in order to assess the real role of crested porcupine as accidental or as maintenance host for *Leptospira* are desirable.

6.Conclusions

The crested porcupine is a burrowing rodent and its presence is strictly related to the settlements availability and/or site for burrowing in the territory.

The settlements are distributed in ecotonal woody areas with sandy soil. The settlements density does not vary in areas with different woody distribution and extension (2-2.4 settlements/Km^2). The settlements density strongly increases in ecotones (settlements/ ecotonal kilometer) of big continuous and homogeneous woody areas rather in small fragmented ones.

The settlements are cluster of ground holes detectable on the surface and connected to a one or more hypogeus burrows in wich the porcupine spend most of the daylight hours.

The settlements have a bimodal distribution trend in orientation to the Northwest-Southeast axis and are distributed on the territory as binary systems named “Stations”.

The stations are characterized by the presence of two settlements detectable within a maximum distance of 250 meters, different oriented and connected on the surface by a dense network of pathways.

Settlemnets are usually shared by crested porcupine, badger and red fox at different time, for exploring or inhabit them. Red foxes always inhabited in exclusive mode the settlements while both crested porcupines and badgers could inhabit the same settlement in exclusive mode as well as together (co-habitation).

Co-habitation between crested porcupine and badger occur in the same settlements and also in the same burrow probably using different chambers and is not due to lack of un-inhabited settlements. Porcupines and badgers co-habit even in presence of porcupettes and the cohabitation is associated to an increase of aggressive interaction between the two species. New investigation are needed in order to assess the settlements features that determine the choice by porcupines and badgers as well as individuate the potential advantages of co-habitation for both the species.

The badger and the red foxes are not porcupine predators while the red fox is an occasional scavenger of porcupine carcasses.

The crested porcupine is the main and faithful inhabitant of settlements and using about the 70% of available settlements.

Each settlement is inhabited by only one porcupine family group ranging from 2 to 6 individuals.

Each porcupine family group alternatively and complementarly inhabit always the same two settlements belonging to a station, showing a very complex and different inhabitation pattern.

The alternate inhabitation pattern in the two settlements seems not to be affect by the season and N-W and S-W oriented settlements seems to be preferred. Further investigations are needed in order to identify the possible factors influencing the porcupine inhabitation pattern.

The fidel and exclusive inhabitation of the station by each porcupine family suggest the presence of intraspecific territoriality in this rodent. Territoriality in crested porcupine need to be assessed and investigated.

The first daily emerging of porcupine from burrow is sunset-light dependent. However, in the warm period emergence from burrow before sunset occur frequently probably due to the combinate effect of the reduction of both seasonal natural food availability and darkness hours available for feeding.

The crested porcupine is mainly a nocturnal mammal and regularly shows diurnal motor activity often performing a peculiar behaviour defined as “Sunbathing”.

Diurnal motor activity in crested porcupine occurs mainly in late winter and spring probably as a feeding strategy. The sunbathing behaviour is performed only in spring by porcupettes, lonely or with parents, probably due to metabolic necessities like thermoregulation and/or synthesis of metabolites such as vitamins (Vitamin D) necessary for growth. Further investigation concerning the pattern of diurnal motor activity and physiological aspects of the crested porcupines are desirable in order to explain the diurnal behaviour of this nocturnal rodent.

The crested porcupine is not a stricltly haerbivourous mammal and adult female eat on carrion flesh. This feeding behaviour is not linked to lack of food and could be a feeding strategy for the assumption of key proteins necessary for breeding. Investigation on the occurrence of the scavenging behaviour in crested porcupine in relation to age, sex and physiological state are necessary.

The reproduction of free-ranging crested porcupine is not season dependent. Nightly rhythms of single and/or multiple mounting are regularly performed, probably linked to to pair sability

and maintenance and the births occur in all months of the year, 1 to 3 times *per* year. The size of porcupine litters ranges from 1 to 2 porcupettes. The porcupine give birth in burrow and the first emergence of porcupettes from burrow occur 10-15 days after birth. Adult male and female but also the sub-adults of the family actively perform parental care. The sub-adults remain within the family at least for 1 year and further investigations are needed concerning the dispersal of the sub-adults.

The porcupine population density ranges from 3.6 to 3.9 SD 1.4 specimens/Km^2. The porcupine population abundance estimate (PPA) and the hypothesis of census model are elaborated and applied in this work for the first time. The census model, despite is still preliminary and not exhaustive, provide a useful and easy method for porcupine census to be replicate in other areas of porcupine presence. The model needs to be further tested in order to assess its reliability and establish the error range obtainable from its application.

The crested porcupine can be infected by *Trichuris* sp., *Capillaria* sp., gastrointestinal strongyles, coccidian, *Cryptosporidium* sp. and *Giardia duodenalis* assemblage BIV that is potentially zoonotic. More studies are needed on the epidemiology of *Giardia* and other parasite species in crested porcupine populations, especially on *G. duodenalis* assemblages to better evaluate the zoonotic potential of *Giardia* in *H. cristata*. Investigations in order to identify the *Cryptosporidium* species infecting the crested porcupine are also necessary due to its zoonotic potential.

Ectoparasites infections in crested porcupine are low. *Pulex irritans* is the most frequent ectoparasite found. No specific taxa has been detect in crested porcupines in Italy probably due to its non native Italian specie status.

The haematological and blood biochemical values of the crested porcupine are here investigated for the first time. The data obtained are preliminary and not exhaustive, however could represent a basis for further investigation.

The crested porcupine could be infected by several serogroup of *Leptospira*. For the first time the isolation of Leptospira serovar Pomona from a porcupine kidney was performed and this could indicate that *Hystrix cristata* could be a new potential natural host for this bacterium. The role of porcupine as *reservoir* or accidental host of *Leptospira* need to be addressed. Moreover, further investigations are necessary to disclose the relationship and the route of leptospirosis between porcupines, red foxes, badgers and coypus and their interaction with

wild boar in order to clarify the leptospirosis – epidemiology – wildlife framework at the light of its potential zoonotic source.

The results obtained in this study represent a new set of knowledges on the use of the space, time and feeding sources by the crested porcupine.

The use of the space by the crested porcupine is drived by environmental features as woody fragmentation and their shape as well as animal density and settlements availability. However, the exclusive and limited use of settlements by crested porcupines families indicate that high availability of settlements does not necessary implicates high porcupine population density. Moreover, environmental and settlements sharing by the crested porcupine with two other burrowing mammals can affect the porcupine space use and density but even the health status of their population. The space use of crested porcupine as well as population density and health status are also strongly determined by feeding source availability in space and time. The rythms of crested porcupine motor activity for searching food are indeed strictly related to the length of the night at different latitudes.

The crested porcupine is protected by Italian law, is included among the “Last concern” species by the IUCN Red list and its presence in Italy seems to increase its distribution range. Despite this no data are yet available on the Italian crested porcupine population density and abundance. Italy is the only European country in which the crested porcupine lives as naturalized species, however is a widely poaching species, road mortality is increasing and and results collected in this study suggest that it may still be vulnerable to environmental changes.

In conclusion, is desiderable that the results obtained in this study can be used as a basis set of knowledges that could be a useful tool for the development of crested porcupine management plans necessary to monitor, promote and ensure the conservation of this precious rodent in Italy.

7.References

Adell-Aledón M, Köster PC, de Lucio A, Puente P, Hernández-de-Mingo M, Sánchez-Thevenet P, Dea-Ayuela MA and Carmena D. **2018**. Occurrence and molecular epidemiology of *Giardia duodenalis* infection in dog populations in eastern Spain. BMC Vet Res, 14, 26. DOI: 10.1186/s12917-018-1353-z.

Adler B and de la Peña Moctezuma A. **2010**: Leptospira and leptospirosis. Vet. Microbiol. 140: 287–296.

Agampodi SB, Matthias MA, Moreno AC and Vinetz JM. **2012**: Utility of quantitative polymerase chain reaction in leptospirosis diagnosis: Association of level of leptospiremia and clinical manifestations in Sri Lanka. Clin. Infect. Dis. 54: 1249–1255. DOI: 10.1093/cid/cis035.

Agnelli P. **1996**. I mammiferi. In Isola d'Elba: geologia, flora, fauna, storia, arte, ambiente (Ed. Giubelli G). PRO. GRA.MS, Italia, pp. 66-73.

Ahmed N, Devi SM, de los Á Valverde M, Vijayachari P, Machang'u RS, Ellis WA and Hartskeerl RA. **2006**: Multilocus sequence typing method for identification and genotypic classification of pathogenic Leptospira species. Ann. Clin. Microbiol. Antimicrob. 5: 28, DOI: 10.1186/1476-0711-5-28.

Akram F, Ilyas O, Haleem A. **2017**. Food and feeding habits of Indian crested porcupine in Pench Tiger Reserve, Madhya Pradesh, India. Amb Sci 4(1). DOI:10.21276/ambi.2017.04.1.ra02.

Alkon PU and Saltz D. **1983**. Studying the behavioral ecology of Negev porcupines. Land and Nature 9(2): 70-74.

Alkon PU and Saltz D. **1985a**. Investigating the field behavior of crested porcupines (*Hystrix indica*). In Noctural mammals: techniques for study (Ed. Brooks RP). School of Forest Resources, Research Paper n. 48. Pennsylvania State University, University Park, pp. 19-34.

Alkon PU and Saltz D. **1985b**. Potatoes and the nutritional ecology of crested porcupine in a desert biome. J Appl Ecol 22:727-737.

Alkon PU and Saltz D. **1985c**. Patterns of crested porcupine (*Hystrix indica*) damage to cultivated potatoes. Agric. Ecosyst. Environ. 14:171-183.

Alkon PU and Saltz D. **1986**. Crested porcupine activity patterns in the Negev highlands: Ecological and management implication. In Environmental quality and Ecosystem stability Vol.III(A), Proceedings of the third International Conference of the Israel society for Ecology and Environmental quality science (Eds. Dubinsky Z and Steinberger Y). Bar-Ilan University Press, pp. 159-167.

Alkon PU and Saltz D. **1988a**. Influence of season and moonlight on temporal-activity patterns of Indian crested porcupines (*Hystrix indica*). J. Mammal. 69(1): 71-80.

Alkon PU and Saltz D. **1988b**. Foraging time and the northern range limits of Indian crested porcupines (*Hystrix indica* Kerr). J Biogeogr 15:403-408.

Alkon PU, Degen AA, Cohen A and Pollak H. **1986**. Seasonal energy requirements and water intakes of Indian crested porcupines (*Hystrix indica*) in captivity. Journal of Mammalogy 67:333-342.

Alkon PU. **1987**. Moonlighting and Other insights into the behavioral ecology of Negev porcupines. In Progress in Desert Research (Eds. Berkofsky L. and Wurtele MG.), Rowman & Littlefield Publichers., pp. 118-129.

Alkon PU. **1999**. Microhabitat to landscape impacts: crested porcupine digs in the Negev Desert highlands. Journal of Arid Environments 41: 183-202.

Alkon PU. and Olsvig-Whittaker L. **1987**. Crested porcupine digs in the Negev desert highlands: patterns of density, size and longevity. Journal of Arid Environments 17:83-95.

Altobello G. **1920**. Fauna dell'Abruzzo e del Molise. Mammiferi III, I rosicanti (Rodentia: Simplicidentata e Duplicidentata). Campobasso.

Amori G and Angelici FM. **1992**. Note on the status of the crested porcupine *Hystrix cristata* in Italy. Lutra 35: 44-50.

Amori G and Capizzi D. **2002**. Istrice. In Mammiferi d'Italia (Eds. Spagnesi M and De Marinis AM). Quad. Cons. Natura, 14, Min. Ambiente - Ist. Naz. Fauna Selvatica, pp. 209-210.

André-Fontaine G and Triger L. **2018**. MAT cross-reactions or vaccine cross-protection: retrospective study of 863 leptospirosis canine cases. Heliyon 4:e00869. https://doi.org/10.1016/j.heliyon.2018.e00869.

Angelici FM and Luiselli L. **1999**. Extra teeth and dental anomalies in the crested porcupine *Hystrix cristata*, from Sicily. Acta Theriologica 44(2): 219-223.

Angelici FM, Cabras PA and Trucchi E. **2009a**. Records of crested porcupine, *Hystrix cristata*, in Sardinia. Mammalia. 73, 377-379.

Angelici FM, Capizzi D, Amori G and Luiselli L. **2003**: Morphometric variation in the skulls of the crested porcupine *Hystrix cristata* from mainland Italy, Sicily, and northern Africa. Mamm. Biol. 68: 165–173.

Angelici FM, Laurenti A and Nappi A. **2009b**. A checklist of the mammals of small Italian islands. Hystrix It. J. Mamm. 20 (1): 13-27.

Arouca ME, de Miranda LB, Lopes RS, Takahira RK, Kohayagawa,A, Ciarlini PC and Oba E. **2000**. Valores hematologicos de capivaras (*Hyrdochoerus hydrochaeris*) criadas am cativeiro no municipio de Botucatu, SP. Cienc. Rural 30 (5): 813-817.

AroucaME, de Miranda LB, Lopes RS, Takahira RK, Kohayagawa A, Ciarlini PC, Oba E. **2000**. Hematological values of captive capybara (*Hydrochoerus Hydrochaeris*) in Botucatu Country, Sao Paulo, SP, Brazil. Cienc. Rural 30 (5): http://dx.doi.org/10.1590/S0103-84782000000500012.

Arslan A. **2008**. On the Indian crested Porcupine, *Hystrix indica* (Kerr, 1972) in Turkey (Mammalia: Rodentia). Pakistan Journal of Biological Sciences 11(2): 315-317.

Atalar O and Ceribasi AO. 2006. The morphology of the penis in porcupine (*Hystrix cristata*). Veterinarni Medicina 51(2): 66-70.

Ayral F, Djelouadji Z, Raton V, Zilber AL, Gasqui P, Faure E, Baurier F, Vourc'h G, Kodjo A and Combes B. **2016**. Hedgehogs and Mustelid Species: Major Carriers of Pathogenic Leptospira, a Survey in 28 Animal Species in France (20122015). PlosONE 11:e0162549. https://doi.org/10.1371/journal.pone.0162549.

Balestrieri A, Remonti L and Prigioni C. **2004**. Diet of the Eurasian badger (*Meles meles*) in an agricultural riverine habitat (NW Italy). Hystrix 15(2): 3-12. DOI: https://doi.org/10.4404/hystrix-15.2-4332.

Balletto E. **1969**. Considerazioni sull'origine e la costituzione della fauna terrestre della Liguria.

Balletto E. **1977**. Analisi Faunistico-venatoria ed ecologica della regione Liguria.

Barlow AM, Mullineaux E, Wood R, Taweenan W and Wastling JM. **2010**. Giardiosis in Eurasian badgers (*Meles meles*). Wildlife disease. doi: 10.1136/vr.c7346

Barthelmess EL. **2006**. *Hystrix africaeaustralis*. Mammalian species 788: 1-7.

Batschelet E. **1965**. Statistical Methods for the Analysis of Problems in Animal Orientation and Certain Biological Rhythms. American Institute of Biological Sciences, Washington DC.

Battocchio D, Iacolina L, Canu A and Mori E. **2017**. How much does it cost to look like a pig in a wild boar group?. Behavioural Processes 138: 123-126. https://doi.org/10.1016/j.beproc.2017.03.001.

Beck R, Sprong H, Lucinger S, Pozio E and Caccio` SM. **2011**. A Large Survey of Croatian Wild Mammals for *Giardia duodenalis* Reveals a Low Prevalence and Limited Zoonotic Potential. Vector-Born and Zoonotic diseases 11(8): 1049-1055. DOI: 10.1089/vbz.2010.0113.

Berlinguer G. **1964**. Aphaniptera d'Italia. "Studio monografico". Il Pensiero Scientifico, Roma.

Bertelloni F, Cilia G, Turchi B, Pinzauti P, Cerri D and Fratini F. **2019**. Epidemiology of leptospirosis in North-Central Italy: Fifteen years of serological data (2002–2016). Comp Immunol Microbiol. Infect. Dis. 65:14–22.

Bertolino S, Colangelo P, Mori E and Capizzi D. **2015**. Good for management, not for conservation: an overview of research, conservation and management of Italian small mammals. Hystrix, the Italian Journal of Mammalogy 26(1): 25-35.

Bianciardi CM, Rigo V, Azzolini S and Gnoli C. **2014**. Eurasian badger (*Meles meles*) habitat and sett selection in the northern Appennines. Nat Hist Sci. 1(1): 41-48. DO1: 10.4081/nhs.2014.58.

Blasdell KR, Morand S, Perera D and Firth C. **2019**. Association of rodent-borne Leptospira spp. with urban environments in Malaysian Borneo. PLoS Negl.. Trop. Dis. 13:e0007141. https://doi.org/10.1371/journal.pntd.0007141.

Blasdell KR, Morand S, Perera D and Firth C. **2019**: Association of rodent-borne Leptospira spp. with urban environments in Malaysian Borneo. (Mathieu Picardeau, Ed.). PLoS Negl. Trop. Dis. 13: e0007141, DOI: 10.1371/journal.pntd.0007141.

Bollin E and Leo R. **2013**. Prima segnalazione di istrice *Hystrix cristata* L. in provincia di Brescia. Natura Bresciana. Ann. Mus. Civ. Sc. Nat., Brescia 38: 149.

Bon M, Latella L, Longo L and Salmaso R. **2006**. Status dell'istrice *Hystrix cristata* Linnaeus, 1758 nel Veneto (Mammalia, Rodentia). Boll. Mus. Civ. St. Nat. Verona 30: 293-296.

Bon M. **2001**. Segnalazione di *Hystrix cristata* L., 1758 in Provincia di Rovigo. Boll. Mus. Civ. St. Nat. Venezia 51: 157-158.

Boonsilp S, Thaipadungpanit J, Amornchai P, Wuthiekanun V, Bailey MS,. Holden MTG, Zhang C, Jiang X, Koizumi N, Taylor K, Galloway R, Hoffmaster AR, Craig S, Smythe LD, Hartskeerl RA, Day NP, Chantratita N, Feil EJ, Aanensen DM, Spratt BG and Peacock SJ. **2013**: A single Multilocus Sequence Typing (MLST) scheme for seven pathogenic Leptospira species. (Mathieu Picardeau, Ed.). PLoS Negl. Trop. Dis. 7, e1954, DOI: 10.1371/journal.pntd.0001954.

Boqvist S, Bergström K and Magnusson U. **2012**. Prevalence of Antibody to Six Leptospira Servovars in Swedish Wild Boars. J. Wildl. Dis. 48:492–496.

Borger L. **2002**. Uso dello spazio nell'istrice (*Hystrix cristata* L.,1758) in un'area costiera mediterranea". University of Pisa, Thesis.

Breuer T. **2005**. Diet choice of large carnivores in northern Cameroon. African Journal of Ecology 43: 181-190.

Broseth H, Bevanger K and Knutsen B. **1997**. Function of multiple badger *Meles meles* sett: distribution and utilisation. Wildlife Biol. 3(2): 89-96. DOI: 10.2981/wlb.1997.011.

Brown JS and Alkon PU. **1990**. Testing values of crested porcupine habitats by experimental food patches. Oecologia 83:512-518.

Bruno E and Riccardi C. **1995**. The diet of the Crested porcupine *Hystrix cristata* L., 1758 in a Mediterranean rural area. Z. Saugetierkunde 60:226-236.

Cacciò SM, Beck R, Lalle M, Marinculic A and Pozio E. **2008**. Multilocus genotyping of *Giardia duodenalis* reveals striking differences between assemblages A and B. International Journal for Parasitology 38: 1523–1531.

Cacciò SM, Lalle M and Svärd SG. **2018**. Host specificity in the *Giardia duodenalis* species complex. Infect. Genet. Evol. 66: 335-345.

Cade TJ. **1973**. Sun-bathing as a thermo-regulatory aid in birds. The Condor 75: 106-133.

Capizzi D and Santini L. **2008**. *Hystrix cristata* Linnaeus, 1758. In Fauna d'Italia, Mammalia II: Erinaceomorpha, Soricomorpha, Lagomorpha, Rodentia (Eds. Amori G, Contoli L and Nappi A). Edizione Calderini de il Sole 24 Ore, Milano, pp. 695-706.

Carrascosa A, Gutierrez L, De la Peña A, Candanosa IE, Tapia G and Sumano H. **2017**: Efficacy of a New Recrystallized Enrofloxacin Hydrochloride-Dihydrate against Leptospirosis in a Hamster Model. Antimicrob. Agents Chemother. 61, DOI: 10.1128/AAC.01285-17.

Castillo-Contreras R, Carvalho J, Serrano E, Mentaberre G, Fernández-Aguilar X, Colom A, González-Crespo C, Lavín S and López-Olvera JR. **2018**. Urban wild boars prefer fragmented areas with food resources near natural corridors. Sci. Total Environ. 615:282–288.

Cerri D, Ebani VV, Fratini F, Pinzauti P and Andreani E. 2003. Epidemiology of leptospirosis: observations on serological data obtained by a "diagnostic laboratory for leptospirosis" from 1995 to **2001**. New Microbiol. 26: 383–389.

Chakraborty D, Hussain S, Reddy DM, Raut S, Tiwari S, Kumar V and Umapathy G. **2015**. Mammalian gastrointestinal parasites in rainforest remnants of Anamalai Hills, Western Ghats, India J Biosci 40(2): 399-406.

Chalmers RM, Campbell BM, Crouch N, Charlett A and Davies AP. **2011**. Comparison of diagnostic sensitivity and specificity of seven *Cryptosporidium* assays used in the UK. J. Med. Microbiol. 60: 1598–1604.

Chernova OF and Kuznetsov GV. **2001**. Structural Features of Spines in Some Rodents (Rodentia: Myomorpha, Hystricomorpha). Biology Bulletin 28(4): 371-382.

Chevallier N and Ashton B. **2006**. A report on the porcupine quill trade in South Africa. http://media.withtank.com/23fd88d381/porcupine_quill_trade. Accessed 23 September 2019.

Chiari M, Figarolli BM, Tagliabue S, Alborali GL, Bertoletti M, Papetti A, D'Incau M, Zanoni M and Boniotti MB. **2016**: Seroprevalence and risk factors of leptospirosis in wild boars (*Sus scrofa*) in northern Italy. Hystrix, Ital. J. Mammal. 27, DOI: 10.4404/hystrix-27.2-11682.

Chiodo E and Mori E. **2015**. Nuove segnalazioni di istrice *Hystrix cristata* in Piemonte, con particolare riferimento alla provincia di Torino. Rivista piemontese di Storia Naturale 36: 247-252.

Ciampalini B and Lovari S. **1985**. Food habits and trophic niche overlap of the Badger (*Meles meles* L.) and the Red fox (*Vulpes vulpes* L.) in a Mediterranean coastal area. Z. Saugetierkd 50: 226-234.

Coppola F, Vecchio G and Felicioli A. **2019a**. Diurnal motor activity and "sunbathing" in crested porcupine (*Hystrix cristata* L., 1758). Scientific Reports 9:14283 https://doi.org/10.1038/s41598-019-50784-y.

Corbet GB and Jones LA. **1964**. The specific characters of the crested porcupines, subgenus *Hystrix*. Procedeeings of the Zoological Society of London 144(2): 285-300.

Corbet NU and Van Aarde RJ. **1996**. Social organization and space use in the Cape porcupine in a Southern African savanna. Afr. J. Eco1.34: 1-14.

Corbet NU. **1991**. Space use and group living in cape porcupine (Hystrix africaeaustrali Peters 1852). University of Pretoria, Thesis.

Corsini MT, Lovari S. and Sonnino S. **1995**. Temporal activity patterns of crested porcupine *Hystrix cristata*. J. Zool. Lond. 236: 43-54.

Cortizo P, Loureiro AP, Martins G, Rodrigues PR, Faria BP, Lilenbaum W and Deminicis BB. **2015**. Risk factors to incidental leptospirosis and its role on the reproduction of ewes and goats of Espírito Santo state, Brazil. Trop. Anim. Health. Prod. 47: 231–235.

Cosson JF, Picardeau M, Mielcarek M, Tatard C, Chaval Y, Suputtamongkol Y, Buchy P, Jittapalapong S, Herbreteau V and Morand S. **2014**. Epidemiology of Leptospira Transmitted by Rodents in Southeast Asia. PLoS Negl. Trop. Dis. 8:e2902. https://doi.org/10.1371/journal.pntd.0002902.

Courdurie C, Le Govic Y, Bourhy P, Alexer D, Pailla K, Theodose R, Cesaire R, Rosine J, Hochedez P and Olive C. **2017**: Evaluation of different serological assays for early diagnosis

of leptospirosis in Martinique (French West Indies). PLoS Negl. Trop. Dis. 11, e0005678, DOI: 10.1371/journal.pntd.0005678.

Cringoli G, Maurelli MP, Levecke B, Bosco A, Vercruysse J, Utzinger J, et al. **2017**. The Mini-FLOTAC technique for the diagnosis of helminth and protozoan infections in humans and animals. Nat Protoc 12(9): 1723-1732.

Danzy J, Grobler JP, Freimer N and Turner TR. **2012**. Sunbathing: A Behavioral Response to Seasonal Climatic Change among South African Vervet Monkeys (*Chlorocebus aethiops*). African Primates 7(2): 230-237.

de Almeida NHCC, Oliveira PA, Lima RBS, da Silveira JAG, Santos JLC and Chiarello AG. **2011**. Haematology and several health aspects of endangered free-ranging thin-spined porcupines, *Chaetomys subspinosus* (Olfers, 1818) (Erethizontidae: Chaetomyinae). Comp. Clin. Pathol. 21:1109–1113. DOI 10.1007/s00580-011-1241-4.

De Marinis AM, Masseti M and Sforzi A. **1996**. Note on the non-flying Terrestrial Mammals of the Tuscan Archipelago, Northern Tyrrhenian Sea (Italy). Boll. Mus. Reg. Sci. Nat. Torino 14 (1): 275-281.

De Santis F. **2011**. Distribuzione dell'Istrice in Abruzzo. University of Aquila. Thesis.

De Villiers MS, Van Aarde RJ and Dott HM. 1994. Habitat utilization by the Cape porcupine *Hystrix africaeaustralis* in a savanna ecosystem. J. Zool., Lond. 232: 539-549.

DeLuca FH. **1967**. Mechanism of Action and Metabolic Fate of Vitamin D. Vitam. Horm. 25:315-367.

Diaz-Ruiz F, Caro J, Delibes-Mateos M, Arroyo B. and Ferreras P. **2016**. Drivers of red fox (*Vulpes vulpes*) daily activity: prey availability, human disturbance or habitat structure?. J. Zool. 298: 128-138.

Di Chiacchio RG, Prioste FES, Vanstreels RET, Knobl T, Kolber M, Miyashiro SI and Matushima ER. **2014**. Health evaluation and survey of zoonotic pathogens in freeranging capybaras (*Hydrochoerus Hydrochaeris*). Journal of Wildlife Diseases 50(3):496–504.

Dieterlen F, Freye HA, Heinemann D. **1974**. Gli Istricomorfi. In Vita degli animali: Moderna Enciclopedia del Regno Animale (Ed. Grzimek B). Bramante Editore, pp. 465-478.

Dominiguez G. **2004**. North-Spain (Burgos) wild mammals ectoparasites. Parasite 11: 267-272.

Dunwell MR and Killingley A. **1969**. The distribution of badger sets in relation to the geology of the Chilterns. Journal of Zoology 158 (2): 204-208.

Duthie AG and Skinner JD. **1986**. Osteophagia in the Cape porcupine *Hystrix africaeaustralis*. S Afr J Zool 21(4):316-318.

Dutto M, Rinaudo S, Moino G, Mori E. **2014**. Primi dati sulla presenza di *Hystrix cristata* Linnaeus, 1758 (Mammalia, Rodentia, Hystricidae) nel Piemonte sud-occidentale (nord-ovest Italia). Biologia Ambientale 28(1): 90-93.

Eberhardt LL. **1978**. Transect methods for population studies. J. Wildlife management 42(1): 1-31.

El Shorafa WM, Feaster JP, Ott EA and Asquith RL. **1979**. Effect of Vitamin D and sunlight on growth and bone development of young ponies. J. Anim. Sc. 48(4):882-886.

Ellerman JR and Morrison Scott TCS. **1951**. Checklist of Palaearctic and Indian mammals (1758 to 1946). Printed by order of the trustees of the British Museum, London, pp. 810.

Ellis WA. **2015**. Animal Leptospirosis, in: Current Topics in Microbiology and Immunology. pp. 99–137. https://doi.org/10.1007/978-3-662-45059-8_6

Fahmy HM, El-Serougi AO, El Deeb HK, Hussein HM, Abou-Seri HM, Klotz C, Aebischer T and El Sayed Khalifa Mohamed K. **2015**. *Giardia duodenalis* assemblages in Egyptian children with diarrhea. Eur. J. Clin. Microbiol. Infect. Dis. 34: 1573-1581.

Faine S, Adler B, Bolin C and Perolat P. **1999**. Leptospira and leptospirosis. Medisci Press, Melbourne.

Fais I, Costanzo M. and Massa B. **1991**. First data on the feeding habits of the red fox (*Vulpes vulpes* L.) in Sicily. Hystrix, 3: 105-112.

Fattorini N and Pokheral CP. **2012**. Activity and habitat selection of the Indian crested porcupine.
Ethol. Ecol. Evol. 24: 377-387.

Felicioli A and Santini L. **1994**. Burrow entrance-hole orientation and first emergence time in the crested porcupine *Hystrix cristata* L.: Space-time dependence on sunset. Pol. Ecol. Stud. 20(3-4): 317-321.

Felicioli A, Ganni M, Garibotti M and Pelosi P. **1993**. Multiple types and forms of odorant-binding proteins in the Old-World Porcupine *Hystrix cristata*. Comp. Biochem. Physiol. 105B (3/4): 775-784.

Felicioli A, Grazzini A and Santini L. **1997a**. The mounting and copulation behaviour of the crested porcupine *Hystrix cristata*. Ital J Zool 64:155-161.

Felicioli A, Grazzini A and Santini L. **1997b**. The mounting behaviour of a pair of crested porcupine *H. cristata* L. Mammalia 61(1): 123-126.

Felicioli A. **1991**. Analisi spazio-temporale dell'attività motoria in *Hystrix cristata* L. Thesis, University of Pisa.

Felicioli A. **1994**. Motor activity in *H. cristata* L.:aspects of a spatio-temporal investigation. Ethology Ecology & Evolution 6:420-421.

Felicioli A. and Santini L. **1991**. Investigation on the relationship between the moon cycle and the motor activity of Hystrix cristata. I European Congress of mammalogy, Lisboa.

Filibeck U, Locasciulli O, Procacci M, Tinelli A and Tinelli P. **1981**. Il trappolamento come tecnica di ricerca per studi sulle popolazioni dell'istrice: sperimentazione ed osservazioni preliminari nel Parco Regionale della Maremma. Atti Soc. ital. Sci. nat. Museo civ. Stor. Nat. Milano 122 (3-4): 204-216.

Findlay GH. **1977**. Rhytmic pigmentation in porcupine quills. Zeitschrift für Säugetierkunde 42: 231–239.

Fornazari F, Langoni H, Marson PM, Nóbrega DB and Teixeira CR. **2018**. Leptospira reservoirs among wildlife in Brazil: Beyond rodents. Acta Trop. 178: 205–212.

Franconi N, Borger L, Lucherini M, Monetti L, Massolo A, Sforzi A and Lovari S. **2003**. Uso dell'habitat dell'istrice in due aree della Toscana Meridionale. In IV Congr. It. Teriologia, Hystrix, It. J. Mamm.

Fratini F, Turchi B, Ebani VV Bertelloni F, Galiero A and Cerri D. **2015**. The presence of Leptospira in coypus (Myocastor coypus) and rats (Rattus norvegicus) living in a protected wetland in Tuscany (Italy). Vet. Arh. 85: 407–414.

Gaigher IG and Currie MH. **1979**. Preliminary studies on the ecology of the southern African porcupine, *Hystrix africaeaustralis*. Department of Nature and Environmental Conservation, Provincial Administration of the Cape of Good Hope, South Africa, Research report, pp. 55-69.

George W and Crowther G. **1981**. Space partitioning between two small mammals in a rocky desert. Biol. J. Linn. Soc. Lond. 15:195-200.

Ghezzi D and Lavezzi F. **2004**. Recenti segnalazioni relative a cinque specie di mammiferi in provincia di Cremona. Pianura, scienze e storia dell'ambiente padano 18: 147-154.

Ghigi A. **1911**. Ricerche faunistiche e sistematiche sui mammiferi d'Italia che formano oggetto di caccia. Natura 2: 289-337

Ghigi A. **1917**. I mammiferi d'Italia considerati nei loro rapporti coll'agricoltura. Natura 8: 85-137.

Ghigi A**. 1947**. Fauna e Caccia, Ed. Agricole, Bologna.

Ginanni F. **1774**. Istoria civile, e naturale delle pinete ravennati. Stamperia Generoso Salomoni. Roma.

Gorgas M .**1967**. Comparative anatomical studies on the gastrointestinal tract of Sciuromorpha, Hystricomorpha and Caviomorpha (Rodentia). In German: Vergleichend-anatomische Untersuchungen am Magen-Darm-Kanal der Sciuromorpha, Hystricomorpha und Caviomorpha (Rodentia). Z Wiss Zool 175: 237–404.

Goszczynski J and Wòjtowicz I. **2001**. Annual dynamics of den use by red foxes *Vulpes vulpes* and badgers *Meles meles* in central Poland. Acta Theriol 46(4): 407-417. https://doi.org/10.1007/BF03192447.

Goszczynski J and Wòjtowicz I. **2001**. Annual dynamics of den use by red foxes *Vulpes vulpes* and badgers *Meles meles* in central Poland. Acta Theriol. 46(4): 407-417.

Grazzini MT. **1992**. Comportamento riproduttivo e accrescimento post-natale in *Hystrix cristata* L. (Rodentia, Hystricidae). University of Pisa, Thesis.

Greaves JH and Aziz Khan A. **1978**. The status and control of porcupines, genus *Hystrix* as forest pest. Commonw. For. Rev. 57(1): 25-32.

Haake DA and Levett PN. **2015**. Leptospirosis in humans. Curr. Top. Microbiol. Immunol. 387: 65–97.

Hafeez S. **2011**. Food habits, feeding seasonality and assessment of damage inflicted by the Indian crested porcupine, Hystrix indica, in different agro-forestry systems of the Punjab, Pakistan. Thesis, University of Faisalabad.

Hagen KB, Hammer S, Frei S, Ortmann S, Glogowski R, Kreuzer M and Clauss M. **2019.** Digestive physiology, resting metabolism and methane production of captive Indian crested porcupine (*Hystrix indica*). J Anim Feed Sci 28(1):69-77.

Haim A, Van Aarde RJ and Skinner JD. **1990**. Metabolism and Thermoregulation in the Cape Porcupine, *Hystrix africaeaustralis*. Physiological Zoology 63(4):795-802.

Hall C and Lambourne J. **2014**: The Challenges of Diagnosing Leptospirosis. J. Travel Med. 21:139–140. DOI: 10.1111/jtm.12095_1.

Hall TA. **1999**. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41: 95–98.

Hamnes IS, Gjerde BK, Forberg T and Robertson LJ. **2007**. Occurrence of *Giardia* and *Cryptosporidium* in Norwegian red foxes (*Vulpes vulpes*). Vet. Parasitol. 143: 347–353.

Hanya G, Kiyono M and Hayaishi S. **2007**. Behavioral Thermoregulation of Wild Japanese Macaques: Comparisons Between Two Subpopulations. Am. J. Primatol.69:802-815.

Harrison TM, Moorman JB, Bolin SR, Grosjean NL, Lim A and Fitzgerald SD. **2007**. *Toxoplasma gondii* in an African crested porcupine (*Hystrix cristata*). J.Vet. Diagn. Invest. 19: 191-194.

Hathaway S, Little T, Headlam S and Stevens A. **1983**. Infection of free-living carnivores with leptospires of the Australis serogroup. Vet. Rec. 113: 233–235.

Hillman AE, Ash AL, Lymbery AJ and Thompson RCA. **2019**. Anthropozoonotic significance, risk factors and spatial distribution of *Giardia* spp. infections in quenda (*Isoodon obesulus*) in the greater Perth region, Western Australia. Int. J. Parasitol. Parasites Wildl. 9: 42-48.

Honigs S and Greven H. **2003**. Biology of the gundi, *Ctenodactylus gundi* (Rodentia: Ctenodactylidae), and its occurrence in Tunisia. Kaupia 12: 43-55.

Horwitz LK, Cohen SL, Więckowski W, Mienis HK and Baker J. **2012**. Recent Indian Porcupine (*Hystrix indica*) burrows and their impact on ancient faunal and human remains: a case study from Tel Zahara (Israel). J. Taphon. 10:85-112.

Houwers DJ, Goris MGA, Abdoel T, Kas JA, Knobbe SS, van Dongen AM, Westerduin FE, Klein WR and Hartskeerl RA. **2011**. Agglutinating antibodies against pathogenic Leptospira in healthy dogs and horses indicate common exposure and regular occurrence of subclinical infections. Vet. Microbiol. 148: 449–451.

Jorge S, Monte LG, Coimbra MA, Albano AP, Hartwig DD, Lucas C, Seixas FK, Dellagostin OA and Hartleben CP. **2012**. Detection of virulence factors and molecular typing of pathogenic Leptospira from capybara (*Hydrochaeris hydrochaeris*). Curr. Microbiol. 65: 461–464. DOI: 10.1007/s00284-012-0169-5.

Jori F, Lopez-Béjar M and Houben P. **1998**. The biology and use of the African brush-tailed porcupine (*Atherurus africanus*, Gray, 1842) as a food animal. A review. Biodiversity and Conservation 7: 1417-1426.

Kadhim AHH. **1997**. Distribution and reproduction of the Indian Crested Porcupine *Hystrix indica* (Hystricidae: Rodentia) in Iraq. Zoology in the Middle East 15:1.

Kassambara A. **2007**. Practical guide to Cluster Analysis in R, Unsupervised Machine Learning. STDH.

Kennedy RJ. **1969**. Sunbathing behaviour of birds. Br. Birds 62(7): 249-258.

Kenny DE. **1998**. The Role of Sunlight, Artificial UV Radiation and Diet on Bone Health. In Zoo Animals in Biologic Effects of Light (Eds. Holick MF and Jung EG). Springer, pp.111-119.

Khan AA, Mian A and Hussain R. 2014. Deterioration Impact of Indian Crested Porcupine, *Hystrix indica*, on Irrigated Forest Plantations in Punjab, Pakistan. Pakistan J Zool 46(6):1691-1696.

Kiibi JM. **2009**. Taphonomic aspects of African porcupines (*Hystrix cristata*) in the Kenyan Highlands. Journal of Taphonomy 7(1):21-27.

Kingdon J. **1974**. Porcupines (*Hystrix*). In East African Mammals, An Atlas of Evolution in Africa, Vol. II Part B (Hares and Rodents) (Ed. Kingdon J.), Academic Press London, New York, pp. 689-695.

Kinlaw A. **1999**. A review of burrowing by semi-fossorial vertebrates in arid environments. J. Arid. Environ. 41: 127–145.

Kleiman DG. **1974**. Patterns of behaviour in Hystricomorph rodents. In The Biology of Hystricomorph Rodents (Eds. Rowlands IW and Weir BJ). Symposia of the Zoological Society of London 34, Academic Press, pp. 171-248.

Kowalczyk R, Bunevich AN and Jedrzejewska B. **2000**. Badger density and distribution of sett in Bialowieza Primeval Forest (Poland and Belarus) compared to other Eurasian populations. Acta Theriol 45(3): 395-408. DOI: 10.4098/AT.arch.00-39.

Krijger IM, Ahmed AAA, Goris MGA, Groot Koerkamp PWG and Meerburg BG. **2019**. Prevalence of Leptospira Infection in Rodents from Bangladesh. Int. J. Environ. Res. Public. Health 16: 2113.

Kruuk H. **1978**. Spatial organization and territorial behaviour of the European badger *Meles meles*. J. Zool. 184: 1-19.

Kruuk H. **1989**. The Social Badger: Ecology and Behaviour of a Group-living Carnivore (*Meles meles*). Oxford University Press, New York

Krystufek B, Vohralik V and Obuch J. **2009**. Endemism, vulnerability and conservation iusses for small terrestrial mammals from the Balkans and Anatolia. Folia Zool. 58 (3): 291-302.

Lanata CF, Fischer-Walker CL, Olascoaga AC, Torres CX, Aryee MJ, et al. **2013**. Global causes of diarrheal disease mortality in children <5 years of age: a systematic review. PLoS ONE 8: e72788 10.1371/journal.pone.0072788.

Laurenzi A, Bodino N and Mori E. 2016. Much ado about nothing: assessing the impact of a problematic rodent on agriculture and native trees. Mamm Res 61:65-72.

Laurenzi A, Bodino N, Mori E. **2016**. Much ado about nothing: assessing the impact of a problematic rodent on agriculture and native trees. Mammal Research 61: 65-72.

Lavezzi F. **1999**. Prima segnalazione di istrice (*Hystrix cristata*) in provincia di Cremona. Pianura, scienze e storia dell'ambiente padano 11: 177-179.

Lee DH, Machii J and Ohba M. **2002**. High frequency of *Bacillus thuringiensis* in feces of herbivorous animals maintained in a zoological garden in Japan. Appl. Entomol. Zool. 37: (4): 509-516.

Leonetti S. **2018**. Profili ematologici e biochimici in tasso (*Meles meles*) e istrice (*Hystrix cristata*) ricoverati in primo soccorso. Thesis, University of Pisa.

Levett PN. **2001**. Leptospirosis. Clin. Microbiol. Rev. 14: 296–326.

Liegeon G, Delory T and Picardeau M. **2018**: Antibiotic susceptibilities of livestock isolates of leptospira. Int. J. Antimicrob. Agents 51, 693–699, DOI: 10.1016/j.ijantimicag.2017.12.024.

Lioy S, Braghiroli S, Dematteis A, Meneguz PG and Tizzani P. **2015**. Faecal pellet count method: some evaluations of dropping detectability for *Capreolus capreolus* Linnaeus, 1758 (Mammalia: Cervidae), *Cervus elaphus* Linnaeus, 1758 (Mammalia: Cervidae) and *Lepus europaeus* Pallas, 1778 (Mammalia: Leporidae). Italian Journal of Zoology 82(2): 231-237.

Lovari S, Corsini MT, Guazzini B, Romeo G and Mori E. **2017**. Suburban ecology of the crested porcupine in a heavily poached area: a global approach. Eur J Wildl Res 63:10. DOI 10.1007/s10344-016-1075-0.

Lovari S, Sforzi A and Mori E. **2013a**. Habitat richness affects home range size in a monogamous large rodent. Behavioural Processes 99: 42– 46.

Lovari S, Ventimiglia M and Minder I. **2013b**. Food habits of two leopard species, competition climate change and upper treeline: a way to the decrease o fan endangered species?. Ethology Ecology & Evolution 25(4): 305-318.

Lovari S. **1993**. Evoluzione recente delle popolazioni di grandi mammiferi della fauna d'Italia. In Atti del XIX Seminario sulla Evoluzione Biologica e i grandi problemi della Biologia – Faune attuali e faune fossili. Accademia Nazionale dei Lincei, Contributi del Centro Linceo Interdisciplinare "Beniamino Segre", Roma, 86: 21-37.

Lubbe A, Hetem RS, McFarland R, Barrett L, Henzi PS, Mitchell D, Meyer LCR, Maloney SK and Fuller A. **2014**. Thermoregulatory plasticity in free-ranging vervet monkeys, *Chlorocebus pygerythrus*. J. Comp. Physiol. B. 184:799-809.

Lucherini M, Lovari S and Crema G. **1995**. Habitat use and ranging behaviour of the red fox (*Vulpes vulpes*) in a Mediterranean rural area: is shelter availability a key factor?. J Zool 237: 577-591. DOI: 10.1111/j.1469-7998.1995.tb05016.x.

Macdonald DW, Bueschin CD, Stopka P, Henderson J, Ellwood SA and Baker SE. **2004**. Encouters between two sympatric carnivores: red foxes (*Vulpes vulpes*) and European badgers (*Meles meles*). J. Zool., 263: 385-392.

Maia-Carneiro T and Rocha CFD. **2013**. The behaviour of orientation of openings of burrows by *Liolaemus lutzae* (Squamata: Liolaemidae): is it influenced by environmental factors? J. Nat. Hist. http://dx.doi.org/10.1080/00222933.2012.743612.

Manfredini L. **2005**. Insetti e acari di importanza veterinaria e medica. Mazzanti Editori Srl, Venezia.

Manly BFJ, MacDonald LL and Thomas DL. **1993**. Resource selection by animals. Chapman and Hall, New York.

Manly BFJ., MacDonald LL and Thomas DL, McDonald TL and Erickson WP. **2002**. Resource selection by animals, Statistical design and analysis for field studies. Second edition London, Kluwer Boston, Massachusetts.

Marina MT and Zubaid A. **2015**. Porcupine burrow distribution in relation to soil types in Sarawak, Malaysia. Malayan Nature Journal 67(1): 24-32.

Martinez-Carrasco C, Ruiz De Ybanez MR, Sagarminaga JL, Garijo MM, Moreno F, Acosta I, Hernandez S and Alonso FD. **2007**. Parasites of the red fox (*Vulpes vulpes* Linnaeus, 1758) in Murcia, southeast Spain. Revue Méd. Vét. 158 (7): 331-335.

Martino PE, Aráuz SM, Anselmino F, Cisterna CC, Silvestrini MP, Corva S and Hozbor FA. **2012**. Hematology and serum biochemistry of free-ranging nutria (*Myocastor coypus*). Journal of Zoo and Wildlife Medicine 43(2):240-247.

Massei G, Genovesi PV and Staines BW. **1996**. Diet, food availability and reproduction of wild boar in a Mediterranean coastal area. Acta Theriol 41: 307-320. DOI: 10.4098/AT.arch.96-29.

Massei G, Kindberg J, Licoppe A, Gačić D, Šprem N, Kamler J, Baubet E, Hohmann U, Monaco A, Ozoliņš J, Cellina S, Podgórski T, Fonseca C, Markov N, Pokorny B, Rosell C

and Náhlik A. **2015**. Wild boar populations up, numbers of hunters down? A review of trends and implications for Europe. Pest Manag. Sci. 71: 492–500.

Masseti M and Sarà M. **2003**. Non-volant Terrestrial Mammals on Mediterranean Isalnds: Tilos (Dodecanese, Greece), a Case Study. Bonner zoologische Beiträge 51 (4): 261-268.

Masseti M, Albarella U and De Grossi Mazzorin J. **2010**. The crested porcupine, *Hystrix cristata* L., 1758, in Italy. Anthropozoologica 45 (2): 27-42.

Masseti M. **2003**. Holocene endemic and non endemic mammals of the Aegean islands. British School at Athens Studies 9: 53-63.

Masseti M. **2009**. Protected areas and endemic species. In Biodiversity Conservation and Habitat Management I (Eds. Gherardi F, Corti C and Gualtieri M). Encyclopedia of life support systems, Oxford, pp. 118-127.

Masseti M. **2012**. Species dubiously and/or erroneously recorded from the Ionian and Aegean Island. In Atlas of terrestrial mammals of the Ionian and Aegean islands (Ed. Masseti M). Walter de Gruyter, Berlin/Boston, pp. 174-176.

Massolo A, Dani FR and Bella N. 2009. Sexual andindividualcuesintheperi-analgland secretum of crested porcupines (*Hystrix cristata*). Mammalian Biology 74: 488-496.

Massolo A, Sforzi A and Lovari S. **2003**.Chemical Immobilization of Crested Porcupines with Tiletamine HCl and Zolazepam HCl (ZoletilT) under Field Conditions. Journal of Wildlife Diseases 39(3):727–731.

Mateo M, de Mingo MH, de Lucio A, Morales L, Balseiro A, EspíA, Barral M, Lima Barbero JF, Habela MÁ, Fernández-García JL,Bernal RC, Köster PC, Cardona GA and Carmena D. **2017**. Occurrence and molecular genotyping of *Giardia duodenalis* and *Cryptosporidium* spp. in wild mesocarnivores in Spain. Vet Parasitol. 235: 86-93. doi: 10.1016/j.vetpar.2017.01.016.

Melis C, Cagnacci F and Bargagli L. **2002**. Food habits of the Eurasian badger in a rural Mediterranean area. Z Jagdwiss 48(Supp.): 236-246. https://doi.org/10.1007/BF02192413

Mendoza H, Rubio AV, García-Peña GE, Suzán G and Simonetti JA. **2020**. Does land use change increase the abundance of zoonotic reservoirs? Rodents say yes. Eur. J. Wildl. Res. 66: 6. https://doi.org/10.1007/s10344-019-1344-9.

Merien F, Baranton G and Perolat P. **1995**: Comparison of Polymerase Chain Reaction with Microagglutination Test and Culture for Diagnosis of Leptospirosis. J. Infect. Dis. 172: 281–285. DOI: 10.1093/infdis/172.1.281.

Meriggi A and Rosa P. **1991**. Fattori influenzanti la distribuzione delle tane di volpe (*Vulpes vulpes*) in Italia Settentrionale. Hystrix 3: 31-39.

Michel V, Ruvoen-Clouet N, Menard A, Sonrier C, Fillonneau C, Rakotovao F, Ganière JP and André-Fontaine G. **2001**. Role of the coypu (*Myocastor coypus*) in the epidemiology of leptospirosis in domestic animals and humans in France. Eur. J. Epidemiol. 17: 111–121.

Milas Z, Turk N, Janicki Z, Slavica A, Starešina V, Barbić LJ, Lojkić M and Modrić Z. **2006**. Leptospiral antibodies in red foxes (*Vulpes vulpes*) in northwest Croatia. Vet. Arhiv. 76: 51-57.

Millán J, Candela MG, López-Bao JV, Pereira M, Jiménez MÁ and León-Vizcaíno L. **2009**. Leptospirosis in Wild and Domestic Carnivores in Natural Areas in Andalusia, Spain. Vector-Borne Zoonotic. Dis. 9: 549–554.

Miller SC, Halloran BP, DeLuca FH and Jee WSS. **1983**. Studies on the Role of Vitamin D in Early Skeletal Development, Mineralization, and Growth in Rats. Calcif. Tissue Int. 35:455-460.

Mills MGL and Shenk TM. **1992**. Predator-prey relationships:the impact of lion predation on wildebeest and zebra populations. Juornal of Animal Ecology 61: 693-702.

Mir AQ, Dua K, Singla LD, Sharma S and Singh MP. **2016**. Prevalence of parasitic infection in captive wild animals in Bir Moti Bagh minizoo (Deer Park), Patiala, Punjab. Vet World 9(6):540-543. doi: 10.14202/vetworld.2016.540-543.

Mitchell D, Robertson A, Corner AH and Boulanger P. **1966**. Some observations on the diagnosis and epidemiology of leptospirosis in swine. Can. J. Comp. Med. Vet. Sci. 30: 211–217.

Mohr E. **1965**. Altweltliche Stachelschweine, Wittenberg Lutherstadt, Germany.

Monetti L, Massolo A, Sforzi A and Lovari S. **2005**. Site selection and fidelity by crested porcupines for denning. Ethology Ecology & Evolution 17: 149-159.

Moran S. **1981**. Damage by vertebrate sto plastic irrigation pipes in Israel. Phytoparasitica 9(3): 211-216.

Morandi F, Galuppi R, Buitrago MJ, Delogu M, Lowenstine LJ, Panarese S, Benazzi C and Sarli G. **2012**. Disseminated Pulmonary Adiaspiromycosis in a Crested Porcupine (*Hystrix cristata* Linnaeus, 1758). Journal of Wildlife Desease 48(2): 523-525.

Moreau B, Christophe Vié J, Cotellon P, De Thoisy I, Motard A and Raccurt CP. **2003**. Hematologic and Serum Biochemistry Values in Two Species of Free-Ranging Porcupines(*Coendou prehensilis*, *Coendou melanurus*) in French Guiana. Journal of Zoo and Wildlife Medicine 34(2): 159-162.

Moreno LZ, Miraglia F, Lilenbaum W, Neto JSF, Freitas JC, Morais ZM, Hartskeerl RA, da Costa BLP, Vasconcellos SA and Moreno AM. **2016b**: Profiling of Leptospira interrogans, L. santarosai, L. meyeri and L. borgpetersenii by SE-AFLP, PFGE and susceptibility testing--a continuous attempt at species and serovar differentiation. Emerg. Microbes Infect. 5, e17, DOI: 10.1038/emi.2016.16.

Moreno LZ, Miraglia F, Marvulo MFV, Silva JCR, Paula CD, Costa BLP, Morais ZM, Ferreira F, Neto JSF, Dellagostin OA, Hartskeerl RA, Vasconcellos SA and Moreno AM. **2016a**: Characterization of Leptospira santarosai Serogroup Grippotyphosa Serovar Bananal isolated from capybara (*Hydrochaeris hydrochaeris*) in Brazil. J. Wildl. Dis. 52, 688–693, DOI: 10.7589/2015-09-245.

Mori E and Lovari S. **2014**. Sexual size monomorphism in the crested porcupine (*Hystrix cristata*). Mammalian Biology 79: 157–160.

Mori E, Bozzi R and Laurenzi A. **2017b**. Feeding habits of the crested porcupine *Hystrix cristata* L. 1758 (Mammalia: Rodentia) in a Mediterranean area of Central Italy. Eur Zool J 84(1):261-265.

Mori E, Lovari S and Mazza G. **2017a**. Temporal patterns of bone gnawing behaviour by crested porcupine in central Italy. XXVII Convegno della Società Italiana di Etologia, Calci.

Mori E, Lovari S and Mazza G. **2018**. The bone collector: temporal patterns of bone-gnawing behaviour define osteophagia as a female prerogative in a large rodent. Behav Ecol Sociobiol 72:89. https://doi.org/10.1007/s00265-018-2504-8.

Mori E, Lovari S, Sforzi A, Romeo G, Pisani C, Massolo A and Fattorini L. **2014a**. Patterns of spatial overlap in a monogamous large rodent, the crested porcupine. Behavioural Processes 107: 112–118

Mori E, Maggini I and Menchetti M. **2014c**. When quills kill: the defense strategy of the crested porcupine *Hystrix cristata* L., 1758. Mammalia 78: 229-234.

Mori E, Menchetti M and Balestrieri A. **2015a**. Interspecific den sharing: a study on European badger sett using camera traps. Acta Ethol. 18: 121-126.

Mori E, Menchetti M, Lucherini M, Sforzi A and Lovari S. **2016**. Timing of reproduction and paternal cares in the crested porcupine. Mammalian Biology 81: 345-349.

Mori E, Nourisson DH, Lovari S, Romeo G and Sforzi A. **2014b**. Self-defence may not be enough: moonlight avoidance in a large, spiny rodent. Journal of Zoology 294: 31–40.

Mori E, Sforzi A and Di Febbraro M. **2013**. From the Apennines to the Alps: recent range expansion of the crested porcupine *Hystrix cristata* L., 1758 (Mammalia: Rodentia: Hystricidae) in Italy. Ital. J. Zool. 80: 469–480.

Mori E, Sforzi A, Menchetti M, Mazza G, Lovari S and Pisanu B. **2015b**. Ectoparasite load in the crested porcupine *Hystrix cristata* Linnaeus, 1758 in Central Italy. Parasitology Research 144(6): 2223-2229.

Mori M, Bourhy P, Guyader M, Le Esbroeck M, Van Djelouadji Z, Septfons A, Kodjo A and Picardeau M. **2017**. Pet rodents as possible risk for leptospirosis, Belgium and France, 2009 to 2016. Eurosurveill. 22. https://doi.org/10.2807/1560-7917.ES.2017.22.43.16-00792

Morris DJ and Van Aarde RJ. **1985**. Sexual behavior of the female porcupine *Hystrix africaeaustralis*. Horm Behav. 19: 400-412. https://doi.org/10.1016/0018-506X(85)90037-6.

Morse DH. **1980**. Behavioural mechanisms in Ecology. Hard University Press.

Mouchantat S, Haas B, Lutz W, Pohlmeyer K and Frölich K. **2003**. A serological survey for antibodies against foot-and-mouth diseases virus (FMDV) in free ranging roe deer (*Capreolus capreolus*) from selected areas of Germany. Verh.ber. Erkrg. Zootiere 41:141.

Mukherjee A, Arijit P, Velankar A, Kumara HN and Bhupathy S. **2019**. Stay awhile in my burrow! Interspecific associations of vertebrates to Indian crested porcupine burrows. Ethol. Eco. Evol. DOI:10.1080/03949370.2019.1594392.

Mukherjee A, Kumara HN and Bhupathy S. **2017b**. Determination of occupancy and burrow site selection by Indian crested porcupine in Keoladeo National Park, Bharatpur, Rajasthan, India. Curr. Sci. 112 (12): 2440-2448.

Mukherjee A, Kumara HN and Bhupathy S. **2018**. Environmental determinants of activity variation of an overlooked burrowing rodent: the Indian crested porcupine. Mammalia 82(5):449-459.

Mukherjee A, Pilakandy R, Kumara HN, Manchi SS and Bhupathy S. **2017a** Burrow characteristics and its importance in occupancy of burrow dwelling vertebrates in Semiarid area of Keoladeo National Park, Rajasthan, India. J.Arid Environ. 141: 7-15.

Naccari FL. **1818**. L'istrice. Fracasso, Chioggia.

Niethammer VJ. **1982**. *Hystrix cristata* Linnaeus, 1758. In Handbuch der Saugetiere Europas (Eds. Niethammer JV and Krapp F). Akademische Verlagsgesellschaft, Wiesbaden, pp.585-605.

O'Regan HJ, Kuman K and Clarke RJ. **2011**. The Likely Accumulators of Bones: Five Cape Porcupine Den Assemblages and the Role of Porcupines in the Post-Member 6 Infill at Sterkfontein, South Africa. Journal of Taphonomy 9(2):69-87.

OIE. **2014**. Leptospirosis. Man. Diagnostic. Tests Vaccines Terr. Anim.

Onac D, Oltean M, Mircean V, Jarca A and Cozma V. **2015**. Occurrence of *Giardia duodenalis* zoonotic assemblages in red foxes from Romania. Sci. Parasitol. 16: 177–180.

Ori F, Trappe J, Leonardi M, Iotti M and Pacioni G. **2018**. Crested porcupine (*Hystrix cristata*): mycophagist spore dispersers of the ectomycorrhizal truffle Tuber aestivum. Mycorrhiza 28:561-565. https://doi.org/10.1007/s00572-018-0840-1.

Orsomando E and Pedrotti F. **1976**. Notizie sulla presenza e sull'habitat dell'istrice nelle Marche e nell'Umbria. In SOS Fauna animali in pericolo in Italia (Ed. Pedrotti F). WWF Camerino, pp. 249-263.

Otranto D and Deplazes P. **2019**. Zoonotic nematodes of wild carnivores. Int. J. Parasitol. Parasites Wildl. 9: 370-383.

Oussou CTB, Mensah GA and Sinsin B. **2006**. Etude de l'écologie du porc-épic (*Hystrix cristata*) et de son régime alimentaire en milieu naturel. Bulletin de la Recherche agronomique du Benin 53:45-52.

Pandolfi M. **1986**. Modificazioni recenti dell'areale di *Hystrix cristata* Linnaeus, 1758, nell'Italia centrale Adriatica. Hystrix 1: 69-76.

Pavlov A. **1957**. Trichocephalidae and capillariidae of animals and man and the diseases caused by them. In Essentials of Nematodology (Eds. Skrjabin KI, Shikhobalova NP and Orlov IV). Russia: Izdatelstvo Akademii Nauk SSSR. Pp.261.

Petronio C, Di Canzio and Salari L. **2007**. The late Pleistocene and Holocene Mammals in Italy: new biochronological and paleoenvironmental data. Palaeontographica Abt. A 279: 147-157.

Pigozzi G and Patterson IJ. **1990**. Movements and diet of crested porcupines in the Maremma Natural Park, central Italy. Acta Theriol 35(3-4):173-180.

Pigozzi G. **1986**. Crested porcupines (Hystrix cristata) within badger setts (Meles meles) in the Maremma Natural Park, Italy. Saugetierk. Mitt. 33: 261–263.

Pigozzi G. **1987**. Female-biased sexual size dimorphism in the crested porcupine (*Hystrix cristata* L.) It. J. Zool. 54:255-259.

Pigozzi G. **1992**. On the distribution, management and conservation of the crested porcupine Hystrix cristata (L.) in Italy. Atti società Italiana Scienze Naturali e del Museo Civico di Storia Naturale di Milano 133(3):33-40.

Pillay KR, Wilson AL, Ramesh T and Downs CT. **2015**. Digestive parameters and energy assimilation of Cape porcupine on economically important crops. Afr Zool 50(4):321-326. DOI: 10.1080/15627020.2015.1116373.

Plumptre AJ. **2000**. Monitoring mammal populations with line transect techniques in African forests. Journal of Applied Ecology 37:256-368.

Polunin O and Walters M. **1987**. Guida alle vegetazioni d'Europa. Zanichelli.

Prigioni C. **1996**. Distribution of mammals in Albania. Hystrix 8 (1-2): 67-73.

Purwaningsih E. **2013**. The first report of new species: *Trichuris landak* n. sp. Asian Pac. J. Trop. Biomed. 3(2): 85-88. doi: 10.1016/S2221-1691(13)60029-5.

Rabinovich R and Horwitz LK. **1994**. An experimental approach to the study of porcupine damage to bones: a gnawing issue. In Taphonomie, bone modification. 6e table ronde. Outillage peu elabore en os et bois de cervides Vol. IV (Ed. Patou-Mayhis M), Edition du CEDARC, Bruxelles, pp. 97-118.

Read C, Walters J, Robertson ID and Thompson RCA. **2002**. Correlation between genotype of *Giardia duodenalis* and diarrhea. Int. J. Parasitol. 32: 229-231.

Read CM, Monis PT and Thompson RC. **2004**. Discrimination of all genotypes of *Giardia duodenalis* at the glutamate dehydrogenase locus using PCR-RFLP. Infect. Genet. Evol. 4: 125-130.

Regnath T, Klemm T and Ignatius R. **2006**. Rapid and accurate detection of *Giardia lamblia* and *Cryptosporidium* spp. antigens in human fecal specimens by new commercially available qualitative immunochromatographic assays. Eur. J. Clin. Microbiol. Infect. Dis. 25: 807–809.

Ricaldi JN, Swancutt MA and Matthias MA. **2013**: Current trends in translational research in leptospirosis. Curr. Opin. Infect. Dis. 26, 1, DOI: 10.1097/QCO.0000000000000001.

Riccardi C and Bruno E. 1996. Food intake of captive porcupines *Hystrix cristata* (Rodentia, Hystricidae). Atti Soc tosc Sci nat, Mem Serie B 103:81-83.

Riquelme JA and Morales-Muniz A. **1997**. A Porcupine find from Roman Africa with a review of archaeological data from circummediterranean sites. Archaeofauna 6: 91-95.

Robertson LJ, Clark CG, Debenham JJ, Dubey JP, Kváč M, Li J,Ponce-Gordo F, Ryan U, Schares G, Su C and Tsaousis AD. **2019**. Are molecular tools clarifying or confusing our understanding of the public health threat from zoonotic enteric protozoa in wildlife? Int. J. Parasitol. Parasites. Wildl. 13(9): 323-341. doi: 10.1016/j.ijppaw.2019.01.010.

Roper TJ. **1992a**. Badger *Meles meles* sett: architecture, internal environment and function. Mamm. Rev. 22(1): 43-53.

Roper TJ. **1992b**. The structural and function of badger setts. J Zool 227: 691-694. DOI:10.1111/j.1469-7998.1992.tb04425.x

Rosevear DR. **1969**. *Hystrix*. In The rodent of West Africa (Ed. Rosevear DR). British Mus. (Nat. Hist.), London, pp. 529-540.

Roth HH. **1964**. Note on the early growth development of *Hystrix africaeaustralis*. In Zeitschrift fur saugetierkunde (Eds. Mohr E and Rohrs). Verlag Paul Parey, Hamburg und Berlin, pp.313-316.

Rovie-Ryan JJ, Khan FAA, Zainuddin ZZ, Ahmad AH, Gani M, Julaihi AM and Saaban S. **2017**.Molecular phylogeny of the old world porcupines (Family Hystricidae) using mitochondrial cytochrome B gene. Journal of Sustainability Science and Management 12(1): 1-11.

Rust MK. **2005**. Advances in the control of *Ctenocephalides felis* (cat flea) on cats and dogs. TRENDS in Parasitology 21(5): 232-236.

Ryan U and Cacciò SM. **2013**. Zoonotic potential of *Giardia*. Int. J. Parasitol. 43: 943–956. https://doi.org/10.1016/j.ijpara.2013.06.001.

Saltz D and Alkon PU. **1989**. On the spatial behaviour of Indian crested porcupines *(Hystrix indica).* J. Zool., Lond. 217: 255-266.

Saltz D and Alkon PU. **1992**. Observations on den shifting in Indian crested porcupine in the Negev (Israel). Mammalia 56: 665-667.

Santilli F and Varuzza P. **2013**. Factors affecting wild boar (Sus scrofa) abundance in southern Tuscany. Hystrix Ital. J. Mammal. 24: 169–173.

Santini L. **1980**. The habits and influence on the environment of the old world porcupine *Hystrix cristata* L. in the northernmost part of its range. Proceedings of the 9th Vertebrate Pest Conference 34:149-153.

Santini L. **1983**. I roditori italiani di interesse agrario e forestale. C.N.R, Padova.

Santini L. 1999. Indagine conoscitiva sull'ecologia e sull'impatto ambientale dell'istrice (*Hystrix cristata* L.) nella Provincia di Pisa. Technical Report.

Scaramella D. **1981**. Istrice. In Distribuzione e biologia di 22 specie di mammiferi in Italia (Ed. Corpo forestale dello stato e delle regioni autonome). C.N.R., Roma, pp. 55-59.

Schaftenaar W. **2002**. Use of vaccination against foot and mouth disease in zoo animals, endangered species and exceptionally valuable animals. Rev. sci. tech. Off. int. Epiz. 21 (3): 613-623.

Schrader C, Schielke A, Ellerbroek L and Johne R. **2012**. PCR inhibitors - occurrence, properties and removal. J. Appl. Microbiol. 113: 1014-1026.

Sever Z and Mendelssohn H. **1988a**. Copulation as a possible mechanism to maintain monogamy in porcupines, *Hystrix indica.* Animal Behaviour 36 (5): 1541-1542. http://dx.doi.org/10.1016/S0003-3472(88)80225-2.

Sever Z and Mendelssohn H. **1988b**. Nightly copulations throughout the year in monogamous porcupines. 2nd International Conference on Behavioral Ecology, Vancouver, Canada.

Sever Z and Mendelssohn H. **1989a**. Paternal behavior in porcupines. Isr. J. Zool. 36: 172 − 173.

Sever Z and Mendelssohn H. **1989b**. Scent marking behaviour of monogamous porcupines. 5° International Theriological Congress, Rome.

Sever Z and Mendelssohn H. **1990.** Grooming behaviour in porcupines. Israel J. Zoology.

Sever Z and Mendelssohn H. **1991a**. Time budget of paternal behaviour in monogamous porcupines. Tertium International Colloquium "Rodent & Spatium III", Lugdunum (Gallia).

Sever Z and Mendelssohn H. **1991b**. Spatial movement patterns of porcupines (*Hystrix indica*). Mammalia 55:187-206. DOI: https://doi.org/10.1515/mamm.1991.55.2.187.

Sforzi A, Massolo A, Bozzi R and Lovari S. **1999**. L'istrice in provincia di Grosseto:aspetti biologici e gestionali. Technical Report to the Provincial Council of Grosseto, Grosseto, Italy.

Sharma D. **2001**. Estimating density of porcupinesin semi-arid Sariska Valley, Western India. Journal of the Bombay Natural History Society 98(2):161-168.

Siti-Nurdyana A, Bahaman A, Sharma R, Azlan C and Abdul Razak M. **2016**. Serological prevalence of leptospiral infection in captive Malayan porcupines (Hystrix brachyura). J. Vet. Malaysia 28: 1–3.

Skinner JD and Chimimba CT. **2005**. The Mammals of the Southern African Subregion. Third edition. Cambridge University Press, Cambridge, UK.

Skinner JD and Duthie AG. **1989**. Osteophagia in the cape porcupine *Hystrix africaeaustralis*. 5° International Theriological Congress, Rome.

Slavica A, Cvetnić Ž, Milas Z, Janicki Z, Turk N, Konjević D, Severin K, Tončić J and Lipej Z. **2008**. Incidence of leptospiral antibodies in different game species over a 10-year period (1996–2005) in Croatia. Eur. J. Wildl. Res. 54: 305–311.

Slavica A, Dezdek D, Konjevic D, Cvetnic Z, Sindicic M, Stanin D, Habus J and Turk N. **2011**. Prevalence of leptospiral antibodies in the red fox (Vulpes vulpes) population of Croatia. Vet. Med. 56: 209-213.

Smith Y and Kok OB. **2006**. A suggested Thermoneutral Zone for African Lions (*Panthera leo* Linnaeus, 1758) in the Southwestern Kalahari, Namibia. Pak. J. Biol. Sci. 9(13):2535-2537.

Sonnino S. **1998**. Spatial activity and habitat use of crested porcupine, Hystrix cristata L., 1758 (Rodentia, Hystricidae) in central Italy. Mammalia 62(2): 175-189.

Spada A, Bon M, Latella L and Salmaso R. **2008**. Primi indizi di riproduzione di istrice *Hystrix cristata*, in Veneto (Rodentia: Hystricidae). In Atti 5° Convegno Faunisti Veneti (Eds. Bon M, Bonato L and Scarton F). Boll. Mus. civ. St. nat. Venezia. Suppl. al vol. 58: 323-327.

Spitzenberger VF. **1978**. Die Säugetierfauna Zyperns Teil I: Insectivora und Rodentia. Ann. Naturhistor. Mus. Wien. 81: 401-441.

Sprong H, Cacciò S M and van der Giessen JW. **2009**. Identification of zoonotic genotypes of *Giardia duodenalis*. PLoS Negl. Trop. Dis. 3, e558.

Stoddard RA, Gee JE, Wilkins PP, McCaustland K and Hoffmaster AR. **2009**: Detection of pathogenic *Leptospira* spp. through TaqMan polymerase chain reaction targeting the LipL32 gene. Diagn. Microbiol. Infect. Dis. 64, 247–255, DOI: 10.1016/j.diagmicrobio.2009.03.014.

Stojecki K, Sroka J, Caccio SM, Cencek T, Dutkiewicz J and Kusyk P. **2015**. Prevalence and molecular typing of *Giardia duodenalis* in wildlife from eastern Poland. Folia Parasitol. (Praha) 62:042. doi:10.14411/fp.2015.042.

Sulaiman IM, Fayer R, Bern C, Gilman RH, Trout JM, Schantz PM, Das P, Lal AA and Xiao L. **2003**. Triosephosphate isomerase gene characterization and potential zoonotic transmission of *Giardia duodenalis*. Emerg. Infect. Dis. 9: 1444-1452.

Tagliabue S, Figarolli BM, D'Incau M, Foschi G, Gennero MS, Giordani R, Giordani R, Natale A, Papa P, Ponti N, Scaltrito D, Spadari L, Vesco G and Ruocco L. **2016**. Serological

surveillance of Leptospirosis in Italy: two-year national data (2010-2011). Vet. Ital. 52: 129–138.

Tassi F. **1971**. Parco Nazionale d'Abruzzo: importanza biogeografia problemi di conservazione. In Il popolamento animale e vegetale dell'Appennino centrale Vol. II (Ed. Lavori della Società Italiana di Biogeografia). Forlì, pp. 615-654.

Thiebaut de Bernaud A. **1808**. Voyage à l'île d'Elbe, suivi d'une notice sur les autres îles de la Mer Tyrrhenienne. D.Colas & Le Normant, Paris.

Thompson RCA. **2013**. Parasite zoonoses and wildlife: one health, spillover and human activity. Int. J. Parasitol. 43: 1079–1088.

Thompson RCA and Ash A. **2019**. Molecular epidemiology of *Giardia* and *Cryptosporidium* infections – What's new? Infect. Genet. Evol. 75: 103951. https://doi.org/10.1016/j.meegid.2019.103951

Tinelli A and Tinelli P. **1980**. Le tane di istrice e di tasso: censimento e densità delle tane nella Riserva Presidenza di Castelporziano per la conservazione dell'istrice e del tasso. Tenuta di Castel Porziano: Segretario Generale della Presidenza della Repubblica.

Tinelli A and Tinelli P. **1983**. L'istrice nel parco naturale della Maremma, fasi e risultati di una ricerca. Editori del Grifo.

Tinelli A and Tinelli P. **1988**. Struttura e caratteristiche di alcuni sistemi di tane di istrice (*Hystrix cristata* L.). In Atti del I convegno nazionale dei biologi della selvaggina Vol. XIV (eds. Spagnesi M. and Toso S.), Istituto nazionale di biologia della selvaggina "Alessandro Ghigi", pp. 375-388.

Tohmè G and Tohmè H. **1980.** Contribution à l'ètude du porc-èpic *Hystrix indica indica* Kerr, 1972(Rodentia). Mammalia 44(4): 523-529.

Tohmè H and Tohmè G. **1981**. Quelques donnèes anatomiques sur le porc-èpic *Hystrix indica indica* Kerr, 1972(Rodentia). Mammalia 45(3): 363-371.

Toschi A. **1959**. La Fauna. Conosci l'Italia Vol. III. Touring Club Italiano, Milano.

Toschi A. **1965**. Fauna d'Italia. Mammalia: Lagomorpha, Rodentia, Carnivora, Artiodactyla e Cetacea. Edizione Calderini, Bologna.

Trucchi E and Sbordoni V. **2009**. Unveiling an ancient biological invasion: molecular analysis o fan old European alien, the crested porcupine (*Hystrix cristata*). BMC Evolutionary Biology 9:109.

Trucchi E, Facon B, Gratton P, Mori E, Stenseth NC and Jentoft S. **2016**. Long live the alien: is high genetic diversity a pivotal aspect of crested porcupine (*Hystrix cristata*) long-lasting and successful invasion?. Molecular Ecology 25: 3527-3539.

Vale-Gonçalves HM, Cabral JA, Faria MC, Nunes-Pereira M, Faria AS, Veloso O, Vieira ML and Paiva-Cardoso MN. **2015**. Prevalence of Leptospira antibodies in wild boars (Sus scrofa) from Northern Portugal: risk factor analysis. Epidemiol. Infect. 143: 2126–2130.

van Aarde RJ. **1984**. Reproduction in the porcupine *Hystrix africaeaustralis* Peters. University of Pretoria, South Africa. Thesis.

van Aarde RJ. **1985**. Reproduction in captive female Cape porcupines *(Hystrix africaeaustralis).* J. Reprod. Fert. 75: 577-582.

van Aarde RJ. **1987a**. Reproduction in the Cape porcupine *Hystrix africaeaustralis:* an ecological perspective. South Afr. J. Sci. 83*:*605-607.

van Aarde RJ. **1987b**. Pre- and post natal growth of the Cape porcupine *Hystrix africaeaustralis*. J. Zool., Lond. 211:25-33.

van Aarde RJ. **1987c**. Demography of a Cape porcupine, *Hystrix africaeaustralis*, population. J. Zool., Lond. 213:205-212.

Van Aarde RJ. **1998**. An ecological perspective of reproduction in the Cape porcupine. Trans. Roy. SOC. S. Afr. 53 (2):237-243.

Van Jaarsveld AS and Knight-Eloff AK. **1984**. Digestion in the porcupine *Hystrix africaeaustralis*. S Afr J Zool 19(2):109-112. DOI: 10.1080/02541858.1984.11447867.

Van Jaarsveld AS. **1983**. Aspects of the digestion in cape porcupine. S Afr J Anim Sci 13(1):31-33.

Van Weers DJ. **1976**. Notes on Southeast Asian Porcupines (Hystricidae, Rodentia) I. On the taxonomy of the genus *Trichys* Günther, 1877. Beaufortia: a series of miscellaneous publications 319(25): 15-31.

Van Weers DJ. **1979**. Notes on Southeast Asian Porcupines (Hystricidae, Rodentia). IV. On the taxonomy of the subgenus *Acanthion* F. Cuvier, 1823 with notes on the other taxa of the family. Beaufortia: a series of miscellaneous publications, 356(29): 215-272.

Van Weers DJ. **1983**. Specific distinction in Old World Porcupines. Zool. Garten N.F. 53: 226-232.

Van Weers DJ. **2005**. A taxonomic revision of the Pleistocene *Hystrix* (Hystricidae, Rodentia) from Eurasia with notes on the evolution of the family. Contributions to Zoology 74(3/4): 301-312.

Varni V, Ruybal P, Lauthier JJ, Tomasini N, Brihuega B, Koval A and Caimi K. **2014**: Reassessment of MLST schemes for Leptospira spp. typing worldwide. Infect. Genet. Evol. 22: 216–222. DOI: 10.1016/j.meegid.2013.08.002.

Vecchio G, Coppola F, Scarselli D, Giannini F and Felicioli A. **2018**. Crested porcupine in the Island of Elba, Italy: native or alien?. Curr. Sc. 114(2): 246-247.

Vein J, Leblond A, Belli P, Kodjo A and Berny PJ. **2014**. The role of the coypu (Myocastor coypus), an invasive aquatic rodent species, in the epidemiological cycle of leptospirosis: a study in two wetlands in the East of France. Eur. J. Wildl. Res. 60: 125–133.

Vieira AS, D'Andrea PS, Vilela RDV, Loretto D, Jaeger LH, Carvalho-Costa FA and Lilenbaum W. **2019**. Pathogenic Leptospira species are widely disseminated among small mammals in Atlantic Forest biome. Transbound. Emerg. Dis. 66: 1195–1201.

Vieira AS, Pinto PS and Lilenbaum W. **2018**. A systematic review of leptospirosis on wild animals in Latin America. Trop. Anim. Health. Prod. 50: 229–238.

Vleck D. **1979**. The energy cost of burrowing by the pocket gopher *Thomomys bottae*. Physiol. Zool. 52: 122–136.

Von Wettstein O. **1941**. Die Säugerwelt der Ägäis, nebst einer Revision des Rassenkreises von *Erinaceus europaeus*. Annalen Des Naturhistorischen Museums in Wien. 52: 245-278.

Walsberg G E. **1988**. Consequences of skin color and fur properties for solar heat gain and ultraviolet irradiance in two mammals. J. Comp. Physiol. *B.* 158: 213-221.

Weir BJ. **1974**. Reproductive characteristics of Hystricomorph rodents. In The Biology of Hystricomorph Rodents (Eds. Rowlands IW and Weir BJ). Symposia of the Zoological Society of London 34, Academic Press, pp. 265-301.

Weitzel T, Dittrich S, Möhl I, Adusu E and Jelinek T. **2006**. Evaluation of seven commercial antigen detection tests for *Giardia* and *Cryptosporidium* in stool samples. Clin. Microbiol. Infect. 12: 656-659.

Wertheim G, Durette-Desset M. **1975**. Helminths of birds and mammals from Israel. VI. The taxonomy and ecology of Trichostrongylid Nematodes. Ann. Parasitol. Hum. Comp. 50(6):735-762.

White TCR. **2007**. Mast seeding and mammal breeding: can a bonanza food supply be anticipated? N Z J Zool 34: 179- 183. https://doi.org/10.1080/03014220709510076.

White TCR. 2011. The significance of unripe seeds and animal tissues in the protein nutrition of herbivores. Biol Rev 86: 217-224. Doi: 10.1111/j.1469-185X.2010.00143.

Wood AE. **1985**. The relationship, origin and dispersal of the Hystricognathous Rodents. In Evolutionary Relationships among Rodents (Eds. Luckett WP and Hartenberger JL). Springer Science+Business Media, New York, pp. 475-513.

Yavad R, Kumar A and Kanaujia A. **2018**. Sunbathing: A thermoregulatory behavior of wing stretching by Long-billed Vulture (*Gyps indicus*), at Bundelkhand Region, Madhya Pradesh, India. EJFW 6(4) & 7(1): 430-437.

Yellen JE. **1991**. Small Mammals: !Kung San Utilization and the Production of Faunal Assemblages. J. Anthropol. Archaeol. 10: 1-26.

Yurumez G and Uluturk S. **2016**. Distribution of Indian Crested Porcupine, *Hystrix indica* (Kerr, 1972) (Mammalia: Rodentia) in Batman Province. Journal of life Science 6(1):181-186.

Zangheri P. **1946**. Fauna di Romagna: L'istrice (*Hystrix cristata* L.) nel versante romagnolo dell'Appennino. Natura 37: 57-59

Zangheri P. **1957**. Fauna di Romagna, Mammiferi. Bollettino di Zoo1ogia 24 (1): 17-38.

Zangheri P. **1969**. Repertorio sistematico e topografico della flora e della fauna vivente e fossile della Romagna, IV. Memorie f. ser. Mus. civ. Stor . Nat. Verona 1: 1820.

Zangheri P. **1970**. Repertorio sistematico e topografico della flora e della fauna vivente e fossile della Romagna, V. Memorie f. ser. Mus. civ. Stor . Nat. Verona.

Zavalloni D and Castellucci M. **1993**. Analisi dell'areale dell'istrice (*Hystrix cristata* Linnaeus, 1758) in Romagna. Hystrix 5(1-2): 53-62.

Zavalloni D, Castellucci M and Tedaldi G. **1991**. Situazione attuale dell'istrice *Hystrix cristata* L., in Romagna. Atti Soc. It. Sc. Nat., Museo Civ. Stor. Nat. Milano 132:193-199.

Zherebtsova OV. **2000**. Spiny cover and defence strategy of mammals. Trans. Zool. Inst., Russian Ac. Sci. 286: 169−174.

Żmudzki J, Jabłoński A, Nowak A, Zębek S, Arent Z, Bocian Ł and Pejsak Z. **2016**. First overall report of Leptospira infections in wild boars in Poland. Acta Vet. Scand. 58: 3. https://doi.org/10.1186/s13028-016-0186-7.

Online Source

Climate-Data. Org https://it.climate-data.org/

Regional Hydro-geological section of Tuscany Region
http://www.sir.toscana.it/index.php?IDS=2&IDSS=6

RIDASCREEN® *Cryptosporidium*/*Giardia* Combi. https://clinical.r-biopharm.com/wp-content/uploads/sites/3/2017/05/C1121-RIDASCREEN-Cryptosporidium_Giardia Combi_lang-2017-04-20_EN.pdf. Last access 29 December 2019.

8.Scientifc production

Scientific paper

Published:

Vecchio G, **Coppola F**, Scarselli D, Giannini F, Felicioli A. **2018**. Crested porcupine in the Island of Elba, Italy: Native or alien?. Current Science, 114(2): 246-247

Coppola F, Vecchio G, Felicioli A. **2019**. Diurnal motor activity and "sunbathing" behaviour in crested porcupine (*Hystrix cristata* L., 1758). Scientific Reports 9:14283

Coppola F, Maestrinia M, Berrilli F, Procesi IG, Felicioli A, Perrucci S. **2020**. First report of *Giardia duodenalis* infection in the crested porcupine (*Hystrix cristata* L., 1758). IJP: Parasites and Wildlife 11:108-113.

Under review:

Coppola F, Dari C, Vecchio G, Scarselli D, Felicioli A. Co-habitation of settlements between Crested Porcupines (*Hystrix cristata*), Red Foxes (*Vulpes vulpes*) and European Badgers (*Meles meles*). Submitted to Current Science.

Cilia G, Bertelloni F, **Coppola F**, Turchi B, Biliotti C, Poli A, Parisi F, Felicioli A, Cerri D, Fratini F. Isolation of *Leptospira* serovar Pomona from a crested porcupine (*Hystrix cristata* L., 1758). Submitted to Veterinary Medicine and Science.

Coppola F, Cilia G, Bertelloni F, Casini L, D'addio E, Fratini F, Cerri D, Felicioli A. Crested porcupine (*Hystrix cristata* L.): a new potential host for pathogenic Leptospira among semi-fossorial mammals. Submitted to Comparative immunology, Microbiology and Infectious Deseases.

Coppola F, Guerrieri D, Simoncini A, Varuzza P, Vecchio G, Felicioli A. Scavenging behaviour in crested porcupine. Submitted to European Journal of Wildlife Research.

Poster and Oral presentations to Congress

Coppola F, Baldanti S, Vecchio G, De Santi L, Russo C, Casini L, Felicioli A. Wolves in a highly anthropic area in the Province of Pisa, Tuscany, Italy. XI International Symposium on Wild Fauna (ISoWiF), Viterbo, 25-28 Settembre 2019. Poster

Coppola F, Dari C, Vecchio G, Aloisi M, Romeo G, Biliotti C, Felicioli A. Liberazione in natura di istrici cresciute presso Centri di Recupero. Convegno Nazionale " La fauna selvatica nelle produzioni animali: aspetti gestionali e sanitari. Pisa, 27 Settembre 2019. Poster

Coppola F, Baldanti S, Vecchio G, De Santi L, Russo C, Casini L, Felicioli A. Prima segnalazione del lupo nei Comuni di Crespina-Lorenzana e Cascana Terme-Lari. Convegno Nazionale " La fauna selvatica nelle produzioni animali: aspetti gestionali e sanitari. Pisa, 27 Settembre 2019. Poster

Russo C, Cecchi F, **Coppola F**, Daghio M, Gatta D, Minieri S. Indagini preliminari sulla qualità della carne di capriolo abbattuto in Toscana. Convegno Nazionale " La fauna selvatica nelle produzioni animali: aspetti gestionali e sanitari. Pisa, 27 Settembre 2019. Poster

Perrucci S, Berrilli F, **Coppola F**, Maestrini M, Felicioli A. *Giardia* spp. infection in a population of crested porcupine (*Hystrix cristata* L., 1758) from central Italy. 7th International Giardia and Cryptosporidium Conference, Rouen-Francia, 23-27 Giugno 2019. Poster

Coppola F, Vecchio G, Scarselli D, Felicioli A. Settlement sharing by crested porcupines, badgers and red foxes. XI International Symposium on Wild Fauna (ISoWiF), Viterbo, 25-28 Settembre 2019. Presentazione orale

Cilia G, Bertelloni F, **Coppola F**, Biliotti C, Turchi B, Felicioli A, Cerri D, Fratini F. Pathogenic *Leptospira interrogans* Serogroup Pomona Isolated from a Kidney of a crested porcupine (*Hystrix cristata*) in Tuscany Region, Italy. 11th International Leptospirosis Meeting, Vancouver, 8-12 Luglio 2019. Presentazione Orale

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"L'unico modo di fare un buon lavoro è amare quello che fai"….Steve Jobs