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Factors affecting the (in)accuracy of mammalian mesocarnivore scat identification in South-western Europe

P. Monterroso[1,2,3], D. Castro[1], T. L. Silva[1,2], P. Ferreras[3], R. Godinho[1] & P. C. Alves[1,2,4]

1 CIBIO/InBio, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Vairão, Portugal
2 Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Porto, Portugal
3 Instituto de Investigación en Recursos Cinegéticos (IREC, CSIC-UCLM-JCCM), Ciudad Real, Spain
4 Wildlife Biology Program, College of Forestry and Conservation, University of Montana, Missoula, MT, USA

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Correspondence
Pedro Seabra Monterroso, Campus Agrário de Vairão, 4485-661 Vairão, Portugal.
Email: pmonterroso@cibio.up.pt

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Abstract

Research on terrestrial carnivore ecology frequently relies on scat identification and analysis. However, species assignment is commonly based on scat morphology. Potential errors in scat identification are rarely accounted for and might contribute to substantial bias of the final results. Using molecular methods, we evaluate the accuracy of species identification based on morphological characteristics of mammalian mesocarnivore scats collected in two areas in the Iberian Peninsula. Our results revealed that error rates in species assignment of scats based on morphology were highly variable, ranging from 14%, for putative red fox *Vulpes vulpes* samples, to 88%, for putative wildcats *Felis silvestris*. The developed models revealed that putative species, season, study area and target species abundance are among the factors involved in identification accuracy. However, the low variability explained suggests that unaccounted factors also had significant effects on accuracy rates. The error rates in scat species assignment constitute a potential source of bias in ecological studies, with serious consequences for the management of threatened species, as unrealistic estimates of status and distribution are prone to occur. Our results suggest that scat identification accuracy rates are circumstance-specific and therefore should not be transferred or extrapolated. We suggest that scat-based studies should implement measures (molecular or others) that allow researchers to determine their own circumstance-specific error rates in scat identification, which should be incorporated in subsequent analyses, ensuring reliable ecological inferences.

Introduction

Adequate and adjusted conservation planning relies on the collection, analysis and interpretation of field data. For this reason, the accuracy and reliability of data collected in the field assumes a crucial role in wildlife conservation. Data collection on mammalian carnivores is particularly challenging because they typically occur in low densities, are crepuscular and/or nocturnal, and elusive (Wilson & Delahay, 2001). As a result, knowledge on these species frequently relies on indirect methods, namely on species presence signs rather than on the observation or capture of the animals themselves (Heinemeyer, Ulizio & Harrison, 2008). Among the indirect field methods employed for carnivores, scat searching is one of the most frequently used (Davison *et al*., 2002). This method has been argued as being one of the most efficient methods for the detection and monitoring of European mammalian mesocarnivores (Sadlier *et al*., 2004; Barea-Azcón *et al*., 2006; Rosellini *et al*., 2008). Moreover, scat analysis has the potential to provide information on many other ecological aspects (e.g. Trites & Joy, 2005; Janko *et al*., 2011; Asa, 2012). However, all the potential information retrieved from carnivore scats can only be useful upon correct species identification. During recent years, advances in non-invasive molecular methods have allowed the extraction and amplification of fragmented and degraded DNA (Broquet, Ménard & Petit, 2007; Beja-Pereira *et al*., 2009) and species-specific markers have been developed (Livia *et al*., 2006; Oliveira *et al*., 2010). The application of genetic scatology has highlighted the fact that the evaluation of scat morphology alone is prone to misidentifications among sympatric carnivore species, even when evaluated by experienced field technicians (Davison *et al*., 2002; Janecka *et al*., 2008; Harrington *et al*., 2010). Regardless, monitoring programmes and ecological research on carnivore species are still mainly carried out based on morphologically identified scats, without acknowledging potential biases induced by misidentifications. However, morphology-based scat searching methods are often the only available alternative for conducting large-scale surveys on carnivore species because of the reduced costs and labour when compared with other methods (Wilson & Delahay, 2001; Barea-Azcón *et al*., 2006). Moreover, information on the diet of species as elusive as most carnivores

can only be accessible through scat analysis (Janecka *et al.*, 2008; Napolitano *et al.*, 2008). For these reasons, scat-based methods cannot be readily discarded; however, potential biases should be acknowledged and accounted for.

The red fox *Vulpes vulpes*, the European wildcat *Felis silvestris* and the stone marten *Martes foina* are three mammalian mesocarnivores whose distribution areas overlap in Europe (Mitchell-Jones *et al.*, 1999), occurring in sympatry in the Iberian Peninsula (Palomo, Gisbert & Blanco, 2007). These species' similar size leads to potential misidentifications of their scats, particularly when their scats dimensions and diets overlap significantly (Farrell, Roman & Sunquist, 2000; Posluszny *et al.*, 2007).

In this work, we evaluate the accuracy of species identification of mammalian mesocarnivore scats collected in the field in two study areas during two different seasons. An evaluation of potential factors that affect scat identification accuracy is also implemented. This evaluation provides a glimpse on some factors affecting the accuracy of scat morphological identification and thus allows the implementation of measures that minimize (or at least account for) scat misidentification rates.

Methods

Study areas

Samples were collected in two Iberian Mediterranean protected areas: the Guadiana Valley Natural Park (GVNP, south-east Portugal) and the Cabañeros National Park (CNP, Central Spain). These two areas belong to the Mediterranean pluviseasonal continental bioclimate region (Rivas-Martínez, Penas & Díaz, 2004). A study area of approximately 6000 ha within each of the protected areas was selected based on the criteria of ecosystem conservation status and logistic factors.

The landscape at GVNP is highly fragmented with cereal croplands and agroforestry systems ('Montado') of stone pine *Pinus pinea* L. and holm oak *Quercus ilex* L. Scrubland patches are mainly associated with steeper slopes and elevation ridges. The red fox, stone marten, Egyptian mongoose *Herpestes ichneumon* and European wildcat are the most common mammalian mesocarnivore species present, despite the presence, in lower densities, of Eurasian badger *Meles meles* and common genet *Genetta genetta* (Monterroso *et al.*, 2009; Monterroso, Alves & Ferreras, 2011). Predator control directed towards red fox and Egyptian mongoose is legally allowed.

The landscape at CNP is dominated by *Pyro-Quercetum rotundifoliae* series and other sub-serial stages (Rivas-Martinez, 1981), especially associated with the steeper slopes, higher elevations and main water bodies. The landscape at the central lower part of this study area constitutes a savannah-like system, with holm oak trees scattered within a grassland matrix (García-Canseco, 1997). The red fox, stone marten and common genet are the most abundant mammalian carnivore species, while wildcats and Eurasian badgers are also found but in lower densities (Guzmán, 1997; Monterroso *et al.*, 2011). Neither hunting activity nor predator control is allowed.

Field sampling

Both study areas were sampled in two distinct seasons: summer/autumn (July–October), when the offspring of most medium-sized carnivores from that year become independent, and winter/spring (February–April), during these species breeding season (Blanco, 1998).

Within each study area, 10 transects, 3 km long each, were designed along unimproved roads or trails for active searching of carnivore signs. Each transect was sampled twice per season: once at the beginning of the sampling campaign and again after approximately 20 days (20.25 ± 3.16 days; mean $\pm$ SD). Transects were spatially distributed in order to adequately sample all existing habitats. They were surveyed on foot by trained field technicians who collected all carnivore scats within a bandwidth of 2 m to each side of the transect line. Scats were identified based on their location, morphology, dimensions, colour and odour, with the aid of specific field guides (Bang, Dahlstrom & Mears, 2007; Iglesias & España, 2010). Scats were collected, taking all precautions to prevent contamination from the collector or cross-contamination from other samples. All scats estimated to be over 1-month old, or for which species assignment was doubtful, were discarded from further procedures. Selected samples identified as belonging to the European wildcat, red fox or stone marten were preserved in plastic vials in ethanol (96%) until DNA extraction. Additional opportunistically collected scats, from the same study areas and seasons, were also included in this study.

As a measure of carnivore-relative abundance, we used data obtained from camera trapping (see details in Monterroso *et al.*, 2011). The trap success estimated for each of the target species followed the methods described by the previous studies (Carbone *et al.*, 2001; Kelly & Holub, 2008) and consisted of the mean number of independent detections per 100 trap days, over all camera stations.

Genetic analysis and identification

DNA extractions were performed with the Qiagen QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions in a separate and autonomous facility, under sterile conditions. Species assignment was performed using two diagnostic methods described by Oliveira *et al.* [2010; interphotoreceptor retinoid-binding protein (IRBP) fragment] and Palomares *et al.* [2002; domain 1 of the control region (CR)]. Amplifications were performed in a final volume of 10 μL using 5 μL of Qiagen PCR MasterMix, 0.2 μM of each primer and 2 μL of DNA extraction (*c.* 10 ng of genomic DNA). Thermocycling conditions for both fragments were as follows: 95°C for 15 min, followed by 40 cycles at 95°C for 30 s, 60°C (IRBP) or 58°C (CR) for 20 s and 72°C for 20 s, with a final extension step at 72°C for 5 min (IRBP) or 60°C for 10 min (CR). Polymerase chain reaction (PCR) amplifications were carried out in a thermocycler MyCycler (Bio-Rad, Hercules, CA, USA). Successful amplifications were purified using the enzymes exonuclease I and shrimp alkaline phosphatise, and sequenced for both strands with

BigDye chemistry (Applied Biosystems, Carlsbad, CA, USA). Sequencing products were separated in a 3130 XL Genetic Analyzer (Applied Biosystems). Pre- and post-PCR manipulations were conducted in physically separated rooms.

Sequence alignment was performed using Clustal W (Thompson, Higgins & Gibson, 1994) implemented in BioEdit software (Hall, 1999) and was manually checked and reassessed for any discrepancy. Species identification using IRBP followed the variants in Iberian wild carnivores reported by Oliveira *et al.* (2010). Aligned CR sequences were compared with the corresponding region of the mitochondrial genome from target species in the GenBank. Both markers were consistently used to increase identification confidence. Each marker has its own advantage: the IRBP nuclear marker is highly discriminative for Southern European carnivores (Oliveira *et al.*, 2010); however, mtDNA is usually available in higher quantity in non-invasive samples, increasing the species identification success. All molecular identifications were blind, that is, information from morphologic identifications was not available to the laboratory staff.

Data analysis

For the sake of reliance, the molecular species assignment of each sample was considered the correct one. The success of the genetic procedure was assessed as the proportion of samples with species identification over the total number of samples analysed. Samples were grouped in each study area and season on the basis of their morphological identifications. The accuracy of morphological identifications was expressed as the proportion of correct identifications over the total number of samples with molecular identification. Several factors were considered to potentially influence the accuracy of morphological identifications: study area, season and mammalian community composition. These relations were tested using a binary response variable, identification accuracy, where '1' corresponds to correctly identified samples and '0' for cases where morphological and molecular identification differed. Basic variables consisted of season (summer/autumn vs. winter/spring), study area (CNP vs. GVNP) and putative species ID (i.e. morphological identification: red fox vs. stone marten vs. European wildcat). Biological variables were estimated for a buffer area of 1 km surrounding each scat-searching transects. This buffer size roughly corresponds to the radius of a hypothetical circular home range of the target species in Europe and was a criterion previously used by other authors (Barea-Azcón *et al.*, 2006; Pita *et al.*, 2009). Data obtained from all camera traps included in the buffer area of a particular transect were pooled to estimate biological parameters potentially related to the morphological identification accuracy. Derived variables consisted of red fox, stone marten and European wildcat camera-trap successes, as well as the interactions between these variables, and carnivore species evenness (as defined by Heip, 2009). As available prey may be related to their consumption by predator species, the camera-trap success of three prey items – European rabbit, Iberian hare *Lepus granatensis* and small mammals (Rodentia) – was considered. Generalized linear models were used to model the identification accuracy, assuming a binomial error distribution and logit link function (Crawley, 2007). As the ratio for global model was ≈20, the corrected AIC values for small sample sizes (AIC_c) was used (Burnham & Anderson, 2002). The ΔAIC_c and model weights were used to compare and rank all tested models, which included all variable combinations and a null (intercept-only) model (Burnham & Anderson, 2002). We only considered the top models whose summed weights accounted for 95% of the total. Individual variable weights were estimated by summing the weights of all the selected models in which they were included. All statistical analyses were performed using R software (R Development Core Team, 2008). Models and model parameters were developed with the AICcmodavg package, version 1.21 (Mazerolle, 2011).

Results

A total of 320 putative scats from red fox, stone marten and European wildcat were submitted to genetic analysis. Approximately half of the samples were collected at each study area (44.7%, $n = 143$, at CNP and 55.3%, $n = 177$, at the GVNP). According to the season, 134 samples were collected in summer/autumn, while 186 were acquired in winter/spring. The majority of the collected scats (88.5%) was identified by morphological characteristics as belonging to either red fox (49.1%, $n = 157$) or stone marten (39.4%, $n = 126$), while potential European wildcat scats consisted only of 11.5% ($n = 37$) of the total sample. Species assignment based on molecular methods was achieved in 251 samples, resulting in an overall genetic identification success of 78.4%. The genetic identification success varied slightly across seasons, areas and putative species [species G = 4.501, 2 degrees of freedom (d.f.), $P = 0.105$; season G = 1.049, 1 d.f., $P = 0.306$; area G = 0.484, 1 d.f., $P = 0.487$], ranging from 64.0% (CNP at winter/spring) to 94.7% (GVNP at summer/autumn; Table 1). The IRBP nuclear fragment provided a lower identification success (34.3%) than the CR mitochondrial marker (97.6%). The identification success for both markers simultaneously was 31.1%.

Over a total of 251 genetically identified scats, 244 belonged to one of the target species (red fox, stone marten or wildcat), even though not always matching the morphological identification. The remaining seven samples were genetically assigned to polecat *Mustela putorius* ($n = 2$) and dog *Canis lupus familiaris* ($n = 5$). The morphological identification of putative red fox scats had an accuracy rate of over 82% (101 out of 117; 86.3% as average) across all seasons and study areas. Red fox misidentified scats belonged to stone marten ($n = 9$, 7.7%), dog ($n = 5$, 4.3%) and European wildcat ($n = 2$, 1.7%; Table 1). These genetically identified stone marten scats were mostly collected at CNP ($n = 8$), while most dog scats were collected at GNVP ($n = 4$). European wildcat scats morphologically assigned to red fox were collected both at CNP and at GVNP.

Putative stone marten scats were accurately identified by morphological characteristics in 77.8% (84 out of 108) of the occasions. Misidentified stone marten scats were genetically assigned mostly to red fox ($n = 22$, 20.4%) and, to a lesser extent, to polecat ($n = 2$, 1.8%; Table 1). Misidentification of red fox scats as stone martens occurred across all seasons and

Table 1 Red fox *Vulpes vulpes*, stone marten *Martes foina* and European wildcat *Felis silvestris* relative abundances and genetic results for the scats morphologically identified, collected at Cabañeros National Park (CNP) and Guadiana Valley Natural Park (GVNP), during the summer 2009 and winter 2010

						Proportion (%) of samples genetically identified as:				
Putative species	Season	Study area	TS	*n*	SGI (%)	Red fox	Stone marten	European wildcat	Polecat	Dog
Red fox	Summer/autumn	CNP	22.08 ± 22.04	26	64.00	**82.35**	17.65	0.00	0.00	0.00
		GVNP	4.16 ± 6.46	39	79.49	**93.55**	0.00	0.00	0.00	6.45
	Winter/spring	CNP	34.19 ± 34.68	54	77.78	**83.33**	11.90	2.38	0.00	2.38
		GVNP	2.27 ± 4.96	38	71.05	**85.19**	3.70	3.70	0.00	7.41
	Overall		16.78 ± 25.28	157	75.52	**86.32**	7.69	1.71	0.00	4.27
Stone marten	Summer/autumn	CNP	3.53 ± 5.72	30	90.00	7.41	**92.59**	0.00	0.00	0.00
		GVNP	1.63 ± 3.58	19	94.74	16.67	**72.22**	0.00	11.11	0.00
	Winter/spring	CNP	2.14 ± 3.83	32	75.00	45.83	**54.17**	0.00	0.00	0.00
		GVNP	6.26 ± 7.96	45	86.67	15.38	**84.62**	0.00	0.00	0.00
	Overall		3.34 ± 5.71	126	85.71	20.37	**77.78**	0.00	1.85	0.00
European wildcat	Summer/autumn	CNP	0.33 ± 0.99	1	100.00	100.00	0.00	**0.00**	0.00	0.00
		GVNP	2.56 ± 3.50	19	84.21	80.00	6.67	**13.33**	0.00	0.00
	Winter/spring	CNP	0.74 ± 1.95	0	–	–	–	**–**	–	–
		GVNP	1.89 ± 3.71	17	69.23	90.00	0.00	**10.00**	0.00	0.00
	Overall		1.29 ± 2.80	37	78.78%	84.62	3.85	**11.54**	0.00	0.00

Proportion of samples genetically identified: red fox, stone marten, European wildcat, polecat *Mustela putorius* and dog scats *Canis lupus familiaris*. Correct morphological assignments are marked in bold.

n, total number of putative red fox, stone marten and European wildcat scats sent for genetic analysis; SGI, proportion of scats identified through genetic analyses; TS, trap success, that is, the number of independent red fox detection per 100 camera-trap days (mean ± standard deviation).

Table 2 Models for accuracy of mammalian mesocarnivore scats morphologic identification

Model	*k*	ΔAIC_c	D^2	w_i	Cum.w	ER	Rank
Ssn, SA, MID, Wldc, Fox	7	0	24.70	0.31	0.31	1.00	1
Ssn, SA, MID, Wldc, Fox, Mrtn	8	1.17	25.05	0.17	0.48	1.80	2
Ssn, SA, MID, Fox:Mrtn	6	2.44	23.06	0.09	0.57	3.39	3
Ssn, SA, MID, Wldc	6	2.51	23.04	0.09	0.65	3.51	4
Ssn, SA, MID, Fox, Mrtn	7	2.81	23.69	0.07	0.73	4.07	5
Ssn, SA, MID, Wldc:Fox	6	3.59	22.65	0.05	0.78	6.03	6
Ssn, SA, MID, Wldc, Mrtn	7	3.93	23.28	0.04	0.82	7.13	7
Ssn, SA, MID, Fox	6	4.19	22.43	0.04	0.86	8.13	8
Ssn, MID, Mrtn	5	4.36	21.62	0.03	0.89	8.86	9
Ssn, SA, MID, Mrtn	6	5.66	21.91	0.02	0.91	16.13	10
SA, MID, Wldc	5	6.13	20.98	0.01	0.92	21.43	11
SA, MID, Fox	5	6.66	20.79	0.01	0.94	27.91	12
SA, MID, Wldc	5	6.89	20.71	0.01	0.95	31.41	13

ΔAIC_c, variation in Aikake's information criteria in relation to the highest ranked model; Cum.w, cumulative weight; D^2, squared deviance; ER, evidence ratio; Fox, red fox *Vulpes vulpes* trap success (detections per 100 trap days); Fox:mrtn, interaction between red fox and stone marten trap successes (detections per 100 trap days); *k*, number of model parameters; MID, morphologic identification; Mrtn, stone marten *Martes foina* trap success (detections per 100 trap days); SA, study area; Ssn, season; w_i, model weight; Wldc, European wildcat *Felis silvestris* trap success (detections per 100 trap days); Wldc:mrtn, interaction between European wildcat and stone marten trap successes (detections per 100 trap days).

study sites, while misidentification of polecat scats as stone martens only occurred in two samples collected in GVNP during summer/autumn.

The lowest overall accuracy rate corresponded to putative European wildcat scats (11.5%) and most misidentified samples were genetically assigned to red fox (84.6%; Table 1).

Data obtained from camera trapping revealed that while the three target mammalian mesocarnivores (red fox, stone marten and European wildcat) produced trap success within the same range of values in both seasons at GVNP, in the CNP study area the guild is highly biased towards the red fox (Table 1).

The models developed for scat identification accuracy hardly explained 25% of the observed variability (Table 2). The top 95% confidence model sets systematically included the season, study area and morphological species assignment, and these variables' individual weights were always higher than 0.90 (Supporting Information Appendix S1). The European wildcat and red fox trap successes were the fourth and fifth ranked variables, with weights of 0.63 and 0.60. The stone

marten trap success ranked next, while the remaining variables (interactions between target species trap successes) revealed a very limited influence in explaining the observed data structure (Supporting Information Appendix S1).

Model parameter estimates reveal that higher morphological identification accuracy was obtained in summer/autumn season and a positive effect of the GVNP study area (Supporting Information Appendix S2). Furthermore, morphological identifications had a significantly higher probability of being accurate for samples originally classified as belonging to red fox, while samples classified as European wildcat scats had the less chance of being accurately identified. Additionally, identification accuracy was significantly higher where wildcat trap success was lower and red fox trap success was higher (Supporting Information Appendix S2). Carnivore evenness and prey availability variables had very limited influence in explaining the observed data variability.

Discussion

Our results indicate that errors are common in the identification of mammalian mesocarnivore scats, and that its accuracy is influenced by biological, environmental and human-related factors. Morphological identification efficiency is generally assessed by comparison with alternative procedures (Barea-Azcón *et al*., 2006; Long *et al*., 2007). We used genetic identification to evaluate the accuracy of morphology-based scat identification. The technical difficulties inherent to the analysis of low quantity and quality DNA limit the efficiency of this approach (Broquet *et al*., 2007). However, our genetic identification success (78.4%) was in agreement with other studies: 72% in Fernandes *et al*. (2007), 81.1% in Oliveira *et al*. (2010) and 60% in Harrington *et al*. (2010). Mitochondrial assays are often more efficient than nuclear ones for non-invasive samples (Broquet *et al*., 2007). Nevertheless, both markers provided identification data simultaneously in nearly one-third of the samples, which proves that confirming species identification using two different markers is feasible and fruitful (Beja-Pereira *et al*., 2009). Our study areas reach high temperatures during summer season, which leads to a fast degradation of scat DNA (Santini *et al*., 2007), but in both areas, the overall amplification success was high (≈80%). On the other hand, the cold weather, low atmospheric moisture and reduced precipitation during winter should help preserve DNA. Therefore, a higher extraction success would be expected during winter. However, no evident seasonal differences occur in genetic identification success, neither among putative species identification in this work.

The morphological classification errors ranged between ≈14% for putative red fox and ≈88% for putative European wildcat scats. Most observed identification errors consisted of scats belonging to one of the three target species. Only seven samples (≈3%) actually belonged to other carnivores (polecat and dog). Our results are consistent with those of other authors who reported that substantial misidentifications have been perpetuated in scat-based studies on mammalian mesocarnivores in Europe. For instance, the scats of pine marten *Martes martes* were consistently misidentified in the UK, mostly with red fox (Davison *et al*., 2002). In another study, on American mink (*Neovison vison*), none of the genetically analysed scats belonged to the target species, rather being of pine marten or fox origin (Harrington *et al*., 2010).

The low variability explained by our models (25%) suggests that some important factors affecting the accuracy of morphological identification of scats were most likely not considered. Nevertheless, the accuracy of scat identification seems to be affected by the species assignment by morphological characteristics, the season and the study area. The relative abundance of target species also influenced accuracy, although to a lesser extent. Scats morphologically classified as red fox had the highest probability of being correctly identified, whereas those classified as belonging to European wildcat had the least chance of being correctly identified. The high abundances and marking behaviour may be responsible for the high detection rates of red fox scats (Cavallini, 1994; Monclús *et al*., 2008; Monterroso *et al*., 2011) and, hence, a higher probability of a given scat being from red fox.

Season also revealed a significant effect on the accuracy of scat morphological identification. Scats were more accurately identified when collected in summer/autumn than in winter/spring. The Mediterranean area is characterized by marked seasonal climatic variations (Blondel & Aronson, 1999), causing fluctuation in the availability of food resources throughout the year. Summer and autumn are characterized by a high diversity of food items, enabling segregation in the exploitation of key resources (Barrientos & Virgós, 2006). The reduced diversity of available food resources during winter most likely leads to a high dietary niche overlap (Carvalho & Gomes, 2004) and, as a consequence, higher similarities among scats should be expected. These seasonal fluctuations in species feeding behaviours may be responsible for the varying rates of scat identification accuracy.

Overall, scats collected in the GVNP had the greatest probability of being correctly identified compared with scats collected in CNP. Feeding resources show remarkable differences in their availability among the two study areas. While the European rabbit is very abundant in the GVNP (Monterroso *et al*., 2009; Sarmento *et al*., 2009), it is nearly absent in CNP (Guzmán, 1997). Moreover, fruits are more widely available in CNP than in GVNP.

Both the European wildcat and the red fox are considered as facultative specialists in European rabbit (Lozano, Moleón & Virgós, 2006; Delibes-Mateos *et al*., 2008), meaning that they preferably prey on it when it is available. However, when rabbits are not available, the European wildcat switches prey, mainly towards rodents (Lozano *et al*., 2006), while the red fox feeds on a wider variety of alternative foods (Díaz-Ruiz *et al*., in press). On the other hand, the stone marten diet in Mediterranean areas is highly variable (Serafini & Lovari, 1993; Genovesi, Secchi & Boitani, 1996; Rosalino & Santos-Reis, 2009). Different availabilities of feeding resources could lead to locally adapted strategies within the carnivore community, which likely led to varying scat morphological characteristics. However, as data on the local feeding ecology of target species are not available, an adequate evaluation of how diet composition affects scat identification accuracy is not possible.

Moreover, another potential uncontrolled factor could have some influence on the observed accuracy differences among study areas, which is the human factor. The prior knowledge that field technicians have on the carnivore community structure in each area might subconsciously bias their judgement. Varying error rates were also found in other studies across different study areas surveyed (Davison *et al*., 2002).

The red fox and stone marten abundances showed a positive relation to classification accuracy. The same pattern has been identified by Davison *et al*. (2002) with pine martens and red foxes. The more abundant the target species, the more likely it is to find its scats in a given area (Sadlier *et al*., 2004; Webbon, Baker & Harris, 2004). Moreover, several authors have referred that scats' misidentification rates tend to increase when targeting species are rare or when scats are difficult to detect (Bulinski & McArthur, 2000; Prugh & Ritland, 2005). Thus, when target species are common, accuracy rates increase, as supported by our models. However, wildcat abundance negatively influenced identification accuracy. The European wildcat distribution in southern Iberian Peninsula is strongly influenced by the availability of the European rabbit (Monterroso *et al*., 2009). As a consequence, where rabbit abundances are high, so is the abundance of European wildcat, and higher dietary overlap with the red fox is expected. Scats with similar contents, combined with the increased abundance of wildcat faeces, probably lead to a decrease in the accuracy of scat classification.

The use of scats to study mammalian carnivores is common in Europe, but the use of molecular methods to assess the reliability of the identification of the collected samples is scarce. For instance, among 35 studies on ecology of mammalian mesocarnivores using scats published in the last 10 years, and performed in 13 European countries, only 8.5% assessed the reliability of the identification of the collected samples based on molecular methods (Supporting Information Appendix S3). Our results suggest that error rates in carnivore scat identifications vary between species and target species abundance, becoming more severe for scarce species or when species with similar scats occur in equivalent abundances. We suggest that some cautionary measures can be implemented to minimize potential biases, such as restricting scat collection to specific well-known sites, used exclusively by the target species. Regardless, only one-third of the reviewed literature took such cautions (Supporting Information Appendix S3). In light of our results, as well as other recent studies (Davison *et al*., 2002; Janecka *et al*., 2008; Harrington *et al*., 2010), mammalian mesocarnivore studies undertaken using morphology of scats should be carefully reviewed for potential biases. As bias severity is associated with species rarity, serious consequences for the management of threatened species when data led unrealistic estimates of status and distribution are prone to occur (Birks *et al*., 2005; Miller *et al*., 2011). While this study focused on a three-species complex, the applicability of our conclusions can be extended to other carnivore species complexes, where similar problems are known to occur (e.g. Hansen & Jacobsen, 1999; Pilot *et al*., 2007).

Our results suggest that scat identification accuracy rates are circumstance-specific, and for that reason, should not be transferred or extrapolated. We recommend that future scat-based studies should implement measures (molecular or other) that allow researchers to determine their error rates in scat identification. If financial constraints prevent all samples to be analysed, at least a subsample should be subjected to a confirmation method, and error rates should be considered for subsequent analysis, ensuring adequate results and consequent ecological inferences.

Aknowledgements

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Supporting information

Additional Supporting Information may be found in the online version of this paper:

Appendix S1. Relative contribution of each variable for the models for accuracy of morphological identification of carnivore scats.
Appendix S2. Model averaged coefficients of factors for accuracy of morphological identification of carnivore scats.
Appendix S3. Review of published literature on scat-based studies on native mammalian mesocarnivores in Europe since 2003, obtained in the Web Of Knowledge search engine using the keywords 'scat', 'carnivore', 'Europe' and 'ecology'.
Appendix S4. List of references used in literature review presented in Supporting Information Appendix S3.