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Source: Folia Zoologica, 68(1) : 43-47

Published By: Institute of Vertebrate Biology, Czech Academy of Sciences

URL: https://doi.org/10.25225/fozo.075.2019

Folia Zool. – 68 (1): 43–47 (2019) DOI: 10.25225/fozo.075.2019

Detection of earthworm *chaetae* in mammal faeces: methodological implications

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Received 2 December 2018; Accepted 22 February 2019

Abstract. Earthworms are an important food resource for a large number of mammals and one of the most largely used method to assess their contribution to mammals' diet is the search for *chaetae* in faecal samples. Nevertheless, the observation and the identification of these bristles often require properly trained operators and suitable instruments, since their detection can be difficult and time-consuming. We tested the "standard" method for *chaetae* detection by washing 1046 scats – belonging to four mammal species of the Western Italian Alps (*Vulpes vulpes*, *Martes* spp., *Lutra lutra*, *Sus scrofa*) – through three sieves differing in mesh size (1.00, 0.54 and 0.21 mm). Mesh size significantly affected the detection of earthworm *chaetae*, suggesting that the use of three sieves should be recommended in faecal analyses. Moreover, both earthworm size and mean number of *chaetae* per individual should be assessed in each study area prior to diet analysis.

Key words: scat analysis, laboratory procedures, mesh size, *Vulpes vulpes*

Introduction

Diet analysis is a major research issue in mammal ecology, especially for predators (Pineda-Munoz & Alroy 2014). This kind of research mainly relies on the examination of stomach contents and faecal samples, the second being a non-invasive technique which does not imply the death of target individuals (Balestrieri et al. 2011). Although widely used, faecal analysis is not free from methodological issues (reviewed by Reynolds & Aebischer 1991), which may lead to either incomplete or unreliable results. In particular, the detection and quantification of some food items can be particularly arduous, especially when undigested remains are very small (< 1 mm), transparent and dispersed within each faecal sample, as in the case of earthworm *chaetae*.

Earthworms represent a major proportion of the animal biomass of temperate ecosystems (Bouché 1982), and thus play a key role in the diet of several species, including many vertebrates (Granval & Muys 1995). On the basis of the frequency of occurrence (FOC) of earthworms in their diet, earthworm consumers have been classified as occasional (FOC < 10 %), regular (10 % < FOC < 50 %) or primary (FOC > 50 %) predators (Granval & Muys 1995). Following this classification, the European badger (*Meles meles*) is considered a primary earthworm consumer (Skoog 1970, Kruuk & De Kock 1981, Kruuk & Parish 1981, Lucherini & Crema 1995, Balestrieri et al. 2004), and one of the most important mammalian predators of these invertebrates (Granval & Muys 1995), together with shrews (Soricidae) and moles (*Talpa europea*) (Macdonald 1983). Among European carnivores, many authors have highlighted the consumption of earthworms also by the red fox (*Vulpes vulpes*) (Jefferies 1974, MacDonald 1980, Cavani 1991, Lucherini & Crema 1994, Balestrieri et al. 2011), and occasionally, least weasel (*Mustela nivalis*), pine marten (*Martes martes*) and raccoon (*Procyon lotor*) (Macdonald 1983, Granval & Muys 1995). In addition, earthworms are an important food resource for wild boars (*Sus scrofa*; Baubet et al. 2003, 2004).

Since earthworm tissues usually undergo a complete digestion, their detection in faecal samples is mainly related to the observation of gizzard rings (Bradbury 1977) and *chaetae* (Kruuk & Parish 1981, Wroot 1985). While gizzard rings are fragile, being therefore susceptible to damage during storing processes (e.g. drying or freezing; Wroot 1985), *chaetae* appear to be a more suitable indicator to assess earthworm contribution to mammal diets. Indeed, these bristles are composed by a protein-chitin complex, hardened

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by a quinine tanning (Dennel 1949, Morgan 2001), allowing a strong resistance to the digestion process and bacterial degradation. *Chaetae* (also called *setae*) are earthworms' locomotion structures and protrude from spots placed laterally and ventrally on every segment of the worm, except the first and the last ones (Sims & Gerard 1985, Edwards & Bohlen 1996, Morgan 2001). Typically, *chaetae* are *ƒ*-shaped, with a median nodule representing the insertion of retractor muscles (Sims & Gerard 1985, Edwards & Bohlen 1996) (Fig. 1a-c and 1e). In the Lumbricidae, typically there are eight sigmoid *chaetae* per segment (Edwards & Bohlen 1996). Similar structures are also involved in earthworm copulation process: genital *chaetae* are situated in the region of the genital pores (the 26th in *Lumbricus terrestris*; Hegner 1933), are longer and more slender than the ordinary ones and show a hook-like process in their distal end (Sims & Gerard 1985, Edwards & Bohlen 1996, Morgan 2001) (Fig. 1d).

Since the early 1980s, for assessing the occurrence and volumetric contribution of earthworms in the diets of mammal carnivores, most reports (e.g. Ciampalini & Lovari 1985, Pigozzi 1991, Fedriani et al. 1998, Goszczyński et al. 2000, Fischer et al. 2005, Mysłajek et al. 2016) have made reference to the pioneering study on badger food habits in Scotland by Kruuk & Parish (1981). Briefly, this method entails that each scat is washed through a sieve with a mesh size of 0.5 mm, catching the rinsing water in a beaker. Then the solid fraction is subsampled by pipette, after it has been allowed to settle, and examined using a stereoscopic microscope.

Although this method has subsequently been the object of some criticism (Reynolds & Aebischer 1991), to our knowledge laboratory procedures have no longer been tested. Therefore, in this paper we aimed to verify the effectiveness of sieves with different mesh sizes in retaining earthworm *chaetae*, and provide some methodological recommendations for improving the precise assessment of the contribution of earthworms to carnivore diets.

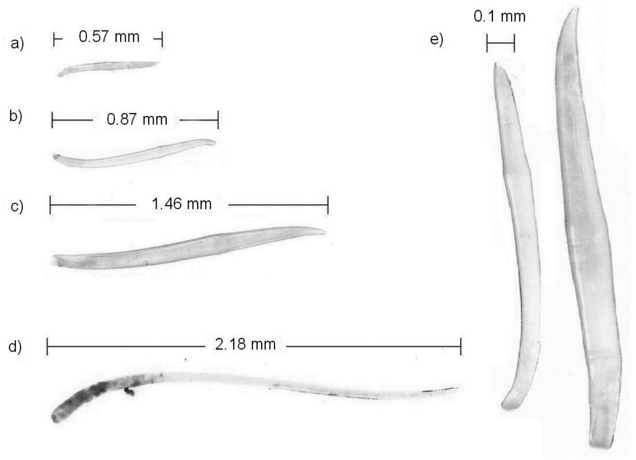

Fig. 1. General morphology of ordinary (a, b, c, e) and genital (d) earthworm *chaetae*.

Material and Methods

This study was performed using faecal samples belonging to mammal species from the northwestern Italian Alps: red fox, pine- and stone marten (*Martes* spp.), Eurasian otter (*Lutra lutra*) and wild boar (*Sus scrofa*). Scats were identified to species-level in the field, based on their morphology and size (Davison et al. 2002, Monterroso et al. 2013, Laguardia et al. 2015), and, eventually, the occurrence of footprints (Prugh & Ritland 2005). Uncertain samples were discarded.

The study area coincided with the Gran Paradiso National Park (710.4 km^2; NW Italy). From December 2013 to July 2015, scats were collected in a large range of Alpine habitats, between 900 and 2300 m above sea level. Eurasian otter spraints were collected while monitoring the movements of an individual escaped from an otter centre (Ferrari et al. 2017).

In laboratory, each faecal sample was washed using three sieves with progressively smaller mesh sizes: 1.00, 0.54 and 0.21 mm respectively. The resulting three fractions were analysed separately, recording earthworm *chaetae* presence/absence in each of them. Each fraction was partitioned, in as many Petri dishes as necessary to investigate each sub-sample accurately, and diluted in a thin water layer. *Chaetae* were sorted from other food remains by means of a stereo-microscope (Leica EZ4D) with magnification from 8× to 35× and multiple light sources (above, below and backwards). In addition, bristles were photographed using the software "Leica Application Suite 3.0.0 – LAS EZ", interfacing with the stereo-microscope.

Results were expressed as absolute percent frequency of occurrence [% OCC: (number of occurrence of earthworm *chaetae*/total number of faeces) × 100], a parameter largely used in literature to estimate the importance of food categories in mammals' diet. To test whether mesh size affects the detection of earthworm *chaetae* in faecal analysis, we ran a Generalised Linear Mixed Model (GLMM), with the presence/absence of *chaetae* in each fraction as dependent variable, and mesh size as fixed effect (categorical with three levels: 0.21, 0.54 and 1.00

mm). To incorporate the dependency among samples belonging to a same species, the univocal code of each sample and species were included in the model as nested random effects (Bolker et al. 2009). GLMM was performed using lme4 (Bates et al. 2015) package for R 3.2.2 (R Core Team 2015).

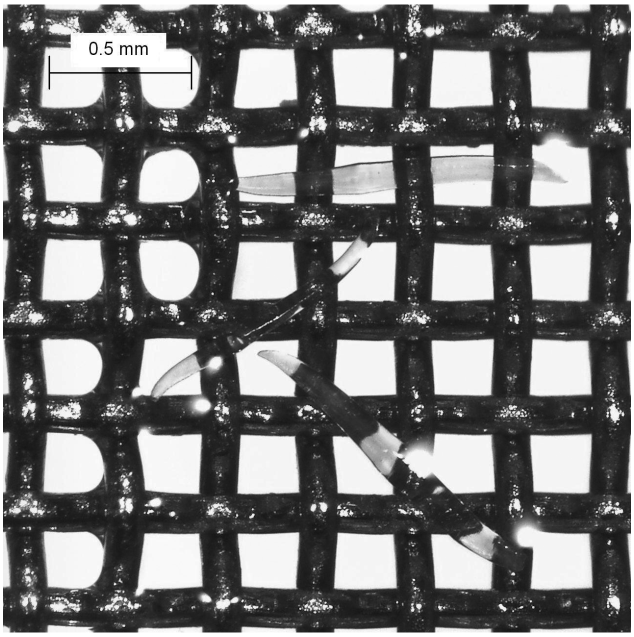

Fig. 2. Stereo-microscope magnification (35×) demonstrating the effectiveness of 0.21 mm sieves in holding earthworm *chaetae*.

Table 1. Percent frequency of occurrence (% OCC) of earthworm *chaetae* in the three fractions obtained by washing each faecal sample (N = number of samples analyzed) through three sieves with progressively smaller mesh sizes (1.00, 0.54, 0.21 mm).

Species	N	% OCC		
		1.00 mm	0.54 mm	0.21 mm
Vulpes vulpes	969	8.5 %	4.6 %	44.0 %
Martes sp.	31	0.0 %	0.0 %	12.9 %
Lutra lutra	23	0.0 %	0.0 %	4.3 %
Sus scrofa	23	4.3 %	8.7 %	43.5 %

Results

Overall, 1046 samples were collected and analyzed, 969 belonging to the red fox, 31 to *Martes* sp., 23 to the Eurasian otter and 23 to the wild boar. Earthworm *chaetae* occurred in the faeces of all investigated mammals: red fox (43.96 %), wild boar (43.48 %), *Martes* sp. (12.90 %) and Eurasian otter (4.35 %) (Table 1).

In our study area, *chaetae* ranged between 0.5 and 2.5 mm in length and from 0.05 to 0.1 mm in width (Figs. 1, 2). Small hair fragments and the detached bristles of *Rosa* sp. seeds, both appearing vitreous and sometimes amber-coloured, were identified as a potential source of mistakes in *chaetae* detection, being similar especially to the genital ones. However, hairs and rose bristles are softer than earthworm *setae*, thus they can easily be recognized by bending them with a tweezer. Moreover, little crystal splinters may appear similar to *chaetae*, but their occurrence in faecal samples is rare and their shape is usually irregular.

Mesh size significantly affected the recording of earthworm *chaetae* in faecal samples. In particular, the 0.54 and the 1.00 mm sieves had a negative effect on bristles detection with respect to the 0.21 mm one (binomial GLMM; 0.54 mm: $z = -18.69$, $P < 0.001$; 1.00 mm: $z = -18.27$, $P < 0.001$).

Discussion

Effective laboratory procedures can minimize the uncertainty of scat-based diet investigations by allowing the reliable assessment of the contribution of each prey type (Reynolds & Aebischer 1991), a goal which may be of outermost importance, especially when the target prey is a key resource, affecting predators' Darwinian fitness components (Avery et al. 1993) or macronutrient balance (Raubenheimer & Simpson 1997). Moreover, as properly stressed by Reynolds & Aebischer (1991), variation in laboratory procedures are likely to hinder the reliable comparison of results between dietary studies.

We demonstrated that the use of three sieves with different mesh sizes allows a proper sample filtration and the effective detection of earthworms *chaetae*. In our study area, the best instrument to avoid *chaetae* loss during the washing process is a sieve with meshes not larger than 0.21 mm. Most diagnostic undigested remains – namely hairs, seeds, feathers and fragments of insects' exoskeleton – are retained by the 1 mm sieve, while the use of a third sieve with an intermediate mesh-size (0.3-0.5 mm) allows to lower the volume of the *chaetae*-rich sediment of the smallest fraction. Our results suggest that the percentage of *chaetae* retained by the two upper sieves is negligible and probably proportional to the number of earthworms eaten. Nonetheless, by analysing earthworms from lowland areas of northern Italy, it has been reported that, on average, 31 % of *chaetae* is lost through washing and sieving (Rosso 1991). Such an error should be taken into account whenever volumes or biomasses have to be assessed.

The detection of earthworm *chaetae* in the smallest fraction is best performed by diluting the sediment in a known quantity of water. Rather than examining the whole mixture, it is less time consuming to place a sub-sample in a Petri dish (Kruuk & Parish 1981, Reynolds & Aebischer 1991). We suggest removal by a Beral pipette 3-5 0.5 ml sub-samples after homogenization of the mixture. To avoid double-counting, each sub-sample may be further separated in several (6-10) drops, which can be examined separately. The average number of *chaetae* must then be multiplied by the total volume of the mixture to estimate the total number of bristles within the faecal sample.

The conversion of the total number of *chaetae* to earthworm biomass is a delicate task. With reference to Kruuk (1989), most studies considered 1000 the mean number of *chaetae* per earthworm. Such a number is valid for as large earthworms as *Lumbricus terrestris*, which has, on average, 134.7 segments and 0.8 mm long *chaetae* (Wroot 1985), while the average wet weight is 2700-3000 mg (Carley 1978). However, smaller species are expected to have less segments and smaller *chaetae* than *L. terrestris* (Wroot 1985). As an example, in northern Italy the mean number of *chaetae* per earthworm has been assessed to be 400, corresponding to an average of ~ 50 segments (Pedrazzini 1990), while earthworm mean weight is 602 mg (Zenato 2010). This also entails that the use of a single sieve with a mesh size of 0.5 mm (Kruuk & Parish 1981) will increase the risk of *chaetae* loss, enhancing the probability of underestimating earthworm contribution to the diet of the target species wherever (as in NW Italy; Balestrieri et al. 2004, 2009) earthworms smaller than *L. terrestris* are usually preyed on. As an example, we suspect that the not-thought-out application of Kruuk and Parish's method may explain the recorded variation in earthworm importance in badger diet throughout the Alps (Balestrieri et al. 2009), where small epigeic species form the bulk of earthworm biomass (Omodeo 1952, 1962).

We argue that the assessment of the contribution of earthworms to the diet of mammals, especially those species, such as badger (Kowalczyk et al. 2003), for which worms represent a key resource, would greatly benefit by the prior estimation of both the mean size and number of *chaetae* of a sample of earthworms representative of the study area. This may be easily achieved by digesting earthworms using a concentrated hydrochloric acid solution (Pedrazzini 1990).

Acknowledgements

The research was funded by the Gran Paradiso National Park; we are especially grateful to Bruno Bassano for logistic and scientific support, while Caterina Ferrari gave us the opportunity of analysing Eurasian otter spraints. We also thank Carla Martínez Ucha and Yolanda Merchan Fernandez, who provided a fundamental help in fieldwork and laboratory analyses, and Elena Piano, for her help with statistical analyses.

Literature

Avery H.W., Spotila J.R., Congdon J.D. et al. 1993: Roles of diet protein and temperature in the growth and nutritional energetics of juvenile slider turtles, *Trachemys scripta*. *Physiol. Zool. 66: 902–925.*

Balestrieri A., Remonti L. & Prigioni C. 2004: Diet of the Eurasian badger (*Meles meles*) in an agricultural riverine habitat (NW Italy). *Hystrix 15: 3–12.*

Balestrieri A., Remonti L. & Prigioni C. 2009: Exploitation of food resources by the Eurasian badger (*Meles meles*) at the altitudinal limit of its Alpine range (NW Italy). *Zool. Sci. 26: 821–827.*

Balestrieri A., Remonti L. & Prigioni C. 2011: Assessing carnivore diet by faecal samples and stomach contents: a case study with alpine red foxes. *Cent. Eur. J. Biol. 6: 283–292.*

Bates D., Maechler M., Bolker B. & Walker S. 2015: Fitting linear mixed-effects models using lme4. *J. Stat. Softw. 67: 1–48.*

Baubet E., Bonenfant C. & Brandt S. 2004: Diet of wild boar in the French Alps. *Galemys 16: 101–113.*

Baubet E., Ropert-Coudert Y. & Brandt S. 2003: Seasonal and annual variations in earthworm consumption by wild boar (*Sus scrofa scrofa* L.). *Wildlife Res. 30: 179–186.*

Bolker B.M., Brooks M.E., Clark C.J. et al. 2009: Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol. Evol. 24: 127–135.*

Bouché M. 1982: Lombriciens de France, Ecologie et Systématique. *INRA, Paris*.

Bradbury K. 1977: Identification of earthworms in mammalian scats. *J. Zool. Lond. 183: 553–555.*

Carley W.W. 1978: Water economy of the earthworm *Lumbricus terrestris* L.: coping with the terrestrial environment. *J. Exp. Zool. 205: 71–78.*

Cavani C. 1991: Quality of the diet of foxes (*Vulpes vulpes)* in a Mediterranean coastal area (Central Italy). *Hystrix 3: 63.*

Ciampalini B. & 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 costal area. *Z. Säugetierkd. 50: 226–234.*

Davison A., Birks J.D.S., Brookes R.C. et al. 2002: On the origin of faeces: morphological *versus* molecular methods for surveying rare carnivores from their scats. *J. Zool. Lond. 257: 141–143.*

Dennell R. 1949: Earthworm *chaetae*. *Nature 164: 370.*

Edwards C.A. & Bohlen P.J. 1996: Biology and ecology of earthworms. *Chapman & Hall, London.*

Fedriani J.M., Ferreras P. & Delibes M. 1998: Dietary response of the Eurasian badger, *Meles meles*, to a decline of its main prey in the Donana National Park. *J. Zool. Lond. 245: 218–222.*

Ferrari C., D'Alfonso M., Moris V. et al. 2017: Diet in a free ranging individual of *Lutra lutra* in Valsavarenche. *J. Mt. Ecol. 10: 7–12.*

Fischer C., Ferrari N. & Weber J.M. 2005: Exploitation of food resources by badgers (*Meles meles*) in the Swiss Jura Mountains. *J. Zool. Lond. 266: 121–131.*

Goszczyński J., Jędrzejewska B. & Jędrzejewski W. 2000: Diet composition of badgers (*Meles meles*) in a pristine forest and rural habitats of Poland compared to other European populations. *J. Zool. Lond. 250: 495–505.*

Granval P. & Muys B. 1995: Predation on earthworms by terrestrial vertebrates. In: Golovatch S. & Penev L. (eds.), Proceedings of the International Union of Game Biologists XXII Congress. *Pensoft, Sofia: 480–491.*

Hegner R.W. 1933: Invertebrate zoology. *The Macmillan company, London*.

Jefferies D.J. 1974: Earthworms in the diet of the red fox (*Vulpes vulpes*). *J. Zool. Lond. 173: 251–252.*

Kowalczyk R., Zalewski A., Jędrzejewska B. & Jędrzejewski W. 2003: Spatial organization of badgers (*Meles meles*) in Białowieża Primeval Forest, Poland, and the influence of earthworms on badger densities in Europe. *Can. J. Zool. 81: 74–87.*

Kruuk H. 1989: The social badger. *Oxford University Press, Oxford.*

Kruuk H. & De Kock L. 1981: Food and habit of badgers (*Meles meles* L.) on Monte Baldo, northern Italy. *Z. Säugetierkd. 46: 295–301.*

Kruuk H. & Parish T. 1981: Feeding specialization of the European badger (*Meles meles*) in Scotland. *J. Anim. Ecol. 50: 773–788.*

Laguardia A., Wang J., Shi F.L. et al. 2015: Species identification refined by molecular scatology in a community of sympatric carnivores in Xinjiang, China. *Zool. Res. 36: 72–78.*

Lucherini M. & Crema G. 1994: Seasonal variation in diet and trophic niche of the red fox in an alpine habitat. *Z. Säugetierkd. 59: 1–8.*

Lucherini M. & Crema G. 1995: Seasonal variation in the food habits of badgers in an alpine valley. *Hystrix 7: 165–171.*

Macdonald D.W. 1980: The red fox, *Vulpes vulpes*, as a predator upon earthworms, *Lumbricus terrestris*. *Z. Tierpsychol. 52: 171–200.*

Macdonald D.W. 1983: Predation on earthworms by terrestrial vertebrates. In: Satchell J.E. (ed.), Earthworm ecology – from Darwin to vermiculture. *Chapman & Hall, London: 393–414.*

Monterroso P., Castro D., Silva T.L. et al. 2013: Factors affecting the (in)accuracy of mammalian mesocarnivore scat identification in south-western Europe. *J. Zool. Lond. 289: 243–250.*

Morgan M. 2001: The earthworm *chaetae*. *IOP Publishing PhysicsWeb, Accessed 17 October 2015. http://www.microscopy-uk.org.uk/mag/artfeb01/mmchaetae*

Mysłajek R.W., Nowak S., Rożen A. et al. 2016: Ecology of the European badger (*Meles meles*) in the Western Carpathian Mountains: a review. *Wildl. Biol. Pract. 12: 36–50.*

Omodeo P. 1952: The particularity zoogeography of earthworms. *Ital. J. Zool. 19: 349–369.*

Omodeo P. 1962: Oligochètes des Alpes. *Mem. Mus. Civ. Stor. Nat. Verona 10: 71–96.*

Pedrazzini O. 1990: Use of the biotope and food ecology of a badger (*Meles meles* L.) in the area of the cultivated plains of the province of Pavia. *Degree thesis, University of Pavia, Pavia, Italy.*

Pigozzi G. 1991: The diet of the European badger in a Mediterranean costal area. *Acta Theriol. 36: 293–306.*

Pineda-Munoz S. & Alroy J. 2014: Dietary characterization of terrestrial mammals. *Proc. R. Soc. Lond. B 281: 20141173.*

Prugh L.R. & Ritland C.E. 2005: Molecular testing of observer identification of carnivores faeces in the field. *Wildl. Soc. Bull. 33: 189–194.*

R Core Team 2015: R: a language and environment for statistical computing. *R foundation for statistical computing, Vienna, Austria. https://www.R-project.org*

Raubenheimer D. & Simpson S.J. 1997: Integrative models of nutrient balancing: application to insects and vertebrates. *Nutr. Res. Rev. 10: 151–179.*

Reynolds J.C. & Aebischer N.J. 1991: Comparison and quantification of carnivore diet by faecal analysis: a critique, with recommendations, based on a study of the red fox *Vulpes vulpes*. *Mammal Rev. 21: 97–122.*

Rosso C. 1991: Environmental preferences and trophic rate (*Meles meles* L.) in a cultivated area of the Po Valley. *Degree thesis, University of Milan, Milan, Italy.*

Sims R.W. & Gerard B.M. 1985: Earthworms: keys and notes for the identification and study of the species. *Synopses of the British Fauna (New Series) 31: 1–171.*

Skoog P. 1970: The food of the Swedish badger, *Meles meles* L. *Viltrevy 7: 1–120.*

Wroot A.J. 1985: A quantitative method for estimating the amount of earthworm (*Lumbricus terrestris*) in animal diets. *Oikos 44: 239–242.*

Zenato M. 2010: Methods of noninvasive genetic census applied to the study of a badger's social behaviour (*Meles meles*). *Degree thesis, University of Milan, Milan, Italy.*