NORTH-WESTERN JOURNAL OF ZOOLOGY 12 (1): 130-140
Article No.: e151706

http://biozoojournals.ro/nwjz/index.html

Even a hair casts its shadow: review and testing of noninvasive hair collecting methods of carnivore species

László PATKÓ[1,*], Nikolett UJHEGYI[1], László SZABÓ[1], Fehér PÉTER[1], Gergely SCHALLY[1], Mária TÓTH[2], József LANSZKI[3], Zsolt NAGY[4], László SZEMETHY[1] and Miklós HELTAI[1]

1. Institute for Wildlife Conservation, Szent István University, H-2100 Gödöllő, Páter Károly u. 1.
2. Department of Zoology, Hungarian Natural History Museum, H-1431 Budapest, PO Box 137.
3. Department of Nature Conservation, University of Kaposvár, H-7401 Kaposvár, PO Box 16
4. Nagy Gén Diagnostic and Research, H-1165 Budapest, VakBottyán u. 37.
*Corresponding author, L. Patkó, E-mail: patkolaszlo88@gmail.com

Received: 05. February 2015 / Accepted: 17. July 2015 / Available online: 29. May 2016 / Printed: June 2016

Abstract. Studying carnivores requires sophisticated methods by wildlife biologists. Hair collecting is one among many useful noninvasive techniques. In this study, we have compiled a quantitative literature review, tested three hair collecting devices, tested the reliability of morphological identification, and determined the amount of mtDNA in different hair samples. Rub pads, as hair collecting devices worked well on carnivores like golden jackal (*Canis aureus*) and Eurasian lynx (*Lynx lynx*). Modified box traps collected more samples than PVC tubes, and were generally better on small mustelids like ferret (*Mustela putorius furo*) and stone marten (*Martes foina*). We have found that some species like wolf (*Canis lupus*) and raccoon (*Procyon lotor*) are hard to identify, while others like brown bear (*Ursus arctos*) or badger (*Meles meles*) are rather easy to distinguish from other mammals based on guard hair morphology. The lateral and dorsal guard hairs proved to be the most valuable for identification, thus both of these regions of the body provide reliable samples. The applied hair collecting methods served enough hair samples from all eleven carnivores studied in this work for further mtDNA analysis. Amount of DNA did not show significant difference among species or body regions.

Key words: noninvasive, hair collecting, carnivore monitoring, PVC tube, cage trap, rub pad, Hungary, pilot study.

Introduction

For wildlife biologists, it has always been a challenge to examine rare and elusive animals, such as several carnivore species (Long et al. 2008). Although in Europe populations of large carnivores like grey wolf (*Canis lupus*) and brown bear (*Ursus arctos*) showed increasing tendency in the last decades (Deinet et al. 2013), many other species are experiencing massive population declines and fragmentation in their distribution range (Ripple et al. 2014). Sophisticated noninvasive monitoring techniques can serve as a reliable data source of these species (Boitani & Powell 2012), and the hair trapping is one of these.

Noninvasive hair trapping is a relatively cheap and easy monitoring technique. Controlled hair-trap pilot studies in animal enclosures usually have reported success (Heurich et al. 2012, Portella et al. 2013). However, when the monitoring technique is being tested in natural environments, it usually results less satisfactory (Comer et al. 2011, Portella et al. 2013). Hair collecting methods are generally used to detect only one or a few carnivore species (Triant et al. 2004, Weaver et al. 2005, Schmidt & Kowalczyk 2006, Comer et al. 2011) or families that are fairly easy to identify by specific hair characteristics (Long et al. 2008, Portella et al. 2013). However, noninvasive hair trapping can be used in long-term studies as well to collect occurrence data on a given mammal community (Castro-Arellano et al. 2008).

Hair collecting studies could aim to estimate population parameters such as genetic structure, home ranges, density and relatedness (Long et al. 2008). Owing to this, nowadays noninvasive genetic sampling became more widespread and morphological analysis became an overlooked and outdated method. Still, the identification of species based on dorsal guard hairs is an often used method (Tóth 2002a, 2002b, Sahajpal et al. 2008, Tóth 2008, Ondrušová & Adamík 2013). Teerink (1991) described that abdominal and lateral hairs are less distinctive but share similar characters with those of dorsal hairs of a carnivore. However, quantitative and comparative examination of hairs from different body parts has not been reported in the literature so far. Such examinations were based mostly on individual experts' empirical experiences. Matthew (2012) states that species identifi-

cation based exclusively on hair morphology could be subjective or inaccurate. In some cases species-specific identification cannot be done because of unusual colour variation (e.g. albinism, Nedyalkov et al. 2014) or overlapping morphological characters. Thus, DNA analyses are often required for a diagnosis confirmation. For this reason, noninvasive genetic sampling is becoming an essential tool widely used in many monitoring and population studies (Long et al. 2008). On the other hand, DNA may quickly degrade due to environmental conditions (Farrell et al. 2000) and genetic information can be flawed by contamination among individuals or species. Hair samples can fail to produce usable information if the sample is from more than one individual or species (Pauli et al. 2008). The amount of DNA can also vary widely among species (Long et al. 2008). In these cases, morphological identification can be a viable integration.

Our aims were to compile a literature review on different hair-traps (i), conduct a pilot study in an enclosure with various hair-trap types (ii), study the reliability of species identification based on morphological examination of hair samples from different body regions (iii), test the circumstances that might influence the reliability of morphological hair identification (iv), and determine the amount of DNA for species-specific identification (v).

Materials and methods

Literature review on hair traps

The research articles dealing with hair collecting were selected from the browsers of scholar. google. hu (GS), www. sciencedirect. com (SD) and www. researchgate. net (RG) searching for the following keywords: "noninvasive", "noninvasive monitoring", "carnivore", "hair collection", "lynx", "wildcat", "wolf" and "bear". In the case of noninvasive methods we focused on hair collection techniques. Faeces, blood, skin and saliva samples were excluded from the search. Species of interest were the carnivores occurring throughout Europe, such as brown bear, grey wolf, Eurasian lynx (*Lynx lynx*) and wildcat (*Felis silvestris*). Furthermore, we have focused on monitoring methods, thus publications discussing fragmentation, bottlenecks, barrier effects and other population genetics related topics were excluded from the search.

We filtered the obtained references using the following approach: (i) is the publication about hair-related noninvasive technique? (Y/N)→ (ii) is the publication about monitoring or abundance estimation? (Y/N) → (iii) is the publication about carnivore species of our interest (bear, lynx, wolf, wildcat, felids, canids, ursids)? (Y/N). If all answers were yes, the reference was selected for the literature review.

Pilot study of hair traps

The noninvasive hair sampling was carried out in February and March 2013, in the Budakeszi ZOO, Hungary. Samples were taken from the resident carnivore species: lynx, European wildcat, brown bear, golden jackal (*Canis aureus*), stone marten (*Martes foina*) and ferret (*Mustela putorius furo*).

Different hair collecting devices were used (Fig. 1.). Technical parameters of the traps were as follows:

T*ype A:* rub pad was constructed using 10x10 cm pieces of thick fabric with three stapled 210 cm velcro-strips (1), with approximately 2 cm distance from the edge of the pad. For alternative hair collecting surface wire brushes (2) or 8-9 screws (3) were used.

Type B: this hair trap was made from a cage trap (85x15x15 cm). Velcro tape (1), adhesive pads (2), wire brush (3) or barbed wire (4) were mounted on the upper-inside of the cage. The trap door was blocked, which prevented the trap from triggering.

Type C: PVC tubes with diameters of 10.5 cm ("ferret-sized") and 12.5 cm ("marten-sized"), and 45 cm length. The upper-inside of the tube was constructed as in the case of modified cage trap (type B, 1-4).

Type B and C traps were placed on the ground while type A was affixed on the cage walls with wires. We

Figure 1. Trap types used at Budakeszi ZOO pilot study
(from left to right: type A (1: Velcro, 2: wire brush, 3: screws), type B and type C).

placed B traps in the enclosures of wildcat, stone marten and ferret. Type C was tested only on stone marten and ferret. Type A was placed in all six species' enclosure. Food baits (chinchilla, chick, leveret and guinea pig) were refreshed in every 3-4 days in type B and C traps. All surfaces (1-3) of type A were tested in one week long periods at all enclosures. Type A traps (1-3) were placed in an enclosure at the same time. B and C traps were tested for 4 weeks in marten and ferret and 3 weeks (B) in wildcat enclosures. Only one surface type of B or C traps (1-4) was placed in enclosures at a time and after a week they were changed to another type.

Hair samples were stored in room-temperature in snap-lock plastic bags without any additional conservation technique and were analysed in 3-4 weeks after collection.

Identification of species based on hair morphology

Three individual experts carried out the blind identification. Experts were told to lead the protocol individually, and note any difficulties (e. g. insufficient sample size, trouble in species-specific identification) they experienced during their work. Hair samples were collected invasively with forceps from eleven carnivore species (lynx, European wildcat, golden jackal, stone marten, European badger, ferret, grey wolf, brown bear, red fox (*Vulpes vulpes*), raccoon (*Procyon lotor*) and raccoon dog (*Nyctereutes procyonoides*) in the Budakeszi ZOO, November, 2012. Samples were collected from four regions of the body (dorsal, lateral, abdominal and snout), and stored in room temperature in snap-lock bags without any additional conservation. In total, 44 independent hair samples (11 species, 4 body regions) were identified by all three experts.

The samples were cleaned in 70% alcohol then kept in ether for a few hours to remove grease and dust from the hairs' surface. Cuticular impressions were made in 20% gelatine with thymol preservative. Canada balm or immersion oil were used for the preparation of the medullar slides. The applied methods and the nomenclature were based mainly on Teerink (1991). 400x and 100x magnification were used for microscopic examination. We performed a Paired-samples t-test in InStat (GraphPad Software) to detect significant differences in species identification based on all body regions rather than only on dorsal and lateral hairs.

Determining DNA amount and purity

Additional 28 samples were collected for the analysis from the different hair-trap prototypes in Budakeszi ZOO. Genomic DNA was obtained from samples originated from both invasive and noninvasive samplings. Four or five hairs were weighed in a 1.5 ml Eppendorf tube. DNA was extracted from hair samples using Genomic DNA kit (Zymo Research). The extraction of gDNA was performed using NucleoSpin® Tissue DNA isolation system (Macherey-Nagel protocol). The gDNA concentration was assessed spectrophotometrically by NanoDrop (Rockland, DE, USA). DNA volume was 100 µl in all cases, where 50 µl Biotin-Elution buffer was used. The samples of purified DNA were stored in sterile tubes at -20 °C. Samples with an A260/A230 ratio greater than 1.8 were considered "high-quality DNA sample", if greater than 2.0 as "ideal DNA sample". We performed an ANOVA to detect any significant differences in DNA amount among samples from different body regions.

Results

Literature review on hair traps

Overall, Google scholar returned the highest number (GS=601261) of records among the three engines followed by Science Direct (SD=31525) and ResearchGate (RG=283). The keyword combination that produced the greatest amount of data was <Hair+Collection+Bear> (GS=295000) followed by <Hair+Collection+Wolf> (GS=98500) and <Noninvasive+Wolf> (GS=92700) (Table 1). In general, carnivore related keywords that produced the greatest number of hits were "bear" (GS=348900, SD=14393, RG=119) and "wolf" (GS=199950, SD=14888, RG=108). The "lynx" and "wildcat" related searches resulted in lowest numbers (GS=15605, SD=410, RG=22; GS=8446, SD=15, RG=5, respectively) (Table 1). After the content filtering we found 20 pertinent research articles 9 (45%) of which were related to European carnivore species of our interest (6 on bear, 2 on lynx, 1 on wolf and 1 on wildcat). In all cases, GS gave the less specific but the largest number of hits.

The amount of hairs collected in the analysed studies varied greatly, ranging from 1 (Comer et al. 2011) to 12564 (Stetz et al. 2010) (x=1226.1, SD=3005.98). The proportion of samples from which was possible to extract good quality DNA varied from 10.54% (García-Alaníz et al. 2010) to 87.5% (Ausband et al. 2011).

In some cases no reliable individual genotypes were found (Portella et al. 2013), while in others (Stetz et al. 2010) it was possible to identify up to 379 different individuals (x=47.5, SD=85.57). Trap nights also showed a huge variance (min=388, max=35296, x=5254.6, SD=9173.41). Maximum 4.9 individual could be caught in 100 trap nights (min=0, x=1.49, SD=1.63). Trap station numbers varied from 15 to 888 (x=133.0, SD=199.10). Trap density varied from 0. 01/km^2 up to 13/km^2 (x=1.34, SD=3.71) (Table 2).

Efficiency of hair-trap devices

In total, the samplings resulted in 304 guard hairs from the six target species. Altogether, type A

Table 1. Search results by different search engines.

Keywords+Species	Google Scholar	Science Direct	ResearchGate
Noninvasive+Monitoring +Carnivore	5 610	316	12
Noninvasive +			
Bear	41 800	6 210	50+
Lynx	2 720	114	14
Wolf	92 700	10 171	100+
Wildcat	525	27	3
Hair+Collection+			
Carnivore	20 100	1 249	9
Bear	295 000	7 474	50+
Lynx	11 900	251	2
Wolf	98 500	3 863	3
Wildcat	7 730	67	0
Noninvasive+Hair+			
Carnivore	2 650	163	8
Bear	12 100	709	19
Lynx	985	45	6
Wolf	8 750	854	5
Wildcat	191	11	2

traps collected the most samples (n=125, x=20.83, SD=20.44) followed by type B (n=115, x=38.33, SD=33.38) and type C (n=64, x=32).

From ferret, we could collect 110 hairs (x=10.1, SD=10). Stone marten produced the second largest hair sample with a total of 94 hairs (x=14.46, SD=10.73), followed by golden jackal (n=50, x=25) and lynx (n=38, x=9.5, SD=8.54). From wildcat and bear we could only gather a relatively small sample (n=8, x=1.14, SD=2.26; n=3, x=0.75, SD=0. 96, respectively).

In the case of type A traps, jackals produced the most hair samples (x=25), but in this case we could only sum up two sample occasions, thus no standard error was calculated. Brown bear and wildcat samples were very rare (x=3; 8, respectively). We could not collect hair samples from stone martens with type A (ferret), and lynx produced x=6.5, x=9.5 samples, respectively.

With type B traps stone marten produced almost twice as much (n=40, x=10, SD=14.14) hair samples as the ferret (n=24, x=6, SD=5.83).

Sampling with type C trap did not result in any samples in the case of wildcat (n=0), but collected slightly more samples from ferret (n=61, x=20.33, SD=13.43), than from stone marten (n=54, x=13.5, SD=9.54).

The wire brush as hair collecting surface produced the most hair samples (n=131, x=13.1, SD=14. 87). Adhesive surface proved to be the second best hair collector (n=79, x=15.8, SD=13.53). We could only collect a relatively small sample with screws (n=16, x=1.46, SD=2.84). The efficiency of Velcro tape and barbed wire were similar (n=47, x=4.7, SD=7.44; n=31, x=7. 75, SD=12.23 respectively, Fig. 2.).

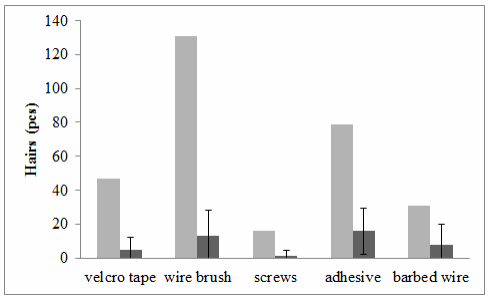

Figure 2. Collected hairs by different surfaces at Budakeszi ZOO (n=light grey, x=dark grey).

Identification of species based on hair morphology

The mean success rate of species identification of the three independent analyses was 40% (SD=30.13). On average the species with the highest identification success rates were bear, badger, raccoon dog and lynx with respectively 75% (SD=30.94), 67% (SD=38.51), 58% (SD=16.65) and 58% (SD=31.93), while raccoon and grey wolf showed the lowest values (both: 17%, SD=19.23)

All experts could identify the dorsal and lateral hairs of bear and badger (100%). Raccoon and grey wolf hairs identification success were doubled when only dorsal and lateral hairs were used for species identification (33%). Although, in the case of stone marten and ferret body regions did not seem to affect the efficiency of morphological

identification (33%-33%) (Fig. 3, 4). Wolf, racoon, golden jackal and fox were the four hardest to identify species specifically.

Regarding the single body regions, dorsal and lateral hairs showed higher identification rates (61%, SD=29.14 and 55%, SD=26.97) compared

Table 2. Results of DNA extraction from different carnivore hair samples (reference).

Number	Sample	Estimated hairs (pcs)	~ DNA concentracion (ng/µl)	DNA purity (A260/A230)
	Grey wolf			
1	dorsal	>10	13	pure (>2)
2	lateral	>10	16	
3	abdominal	>10	20	
4	snout	>10	14	
	Red fox			
5	dorsal	>10	12	pure (>2)
6	lateral	>10	18	
7	abdominal	>10	15	
8	snout	>10	17	
	Eurasian lynx			
9	dorsal	>10	19	pure (>2)
10	lateral	>10	20	
11	abdominal	>10	16	
12	snout	>10	17	
	European wildcat			
13	dorsal	>10	15	pure (>2)
14	lateral	>10	21	
15	abdominal	>10	16	
16	snout	>10	18	
	Golden jackal			
17	dorsal	>10	17	pure (>2)
18	lateral	>10	17	
19	abdominal	>10	21	
20	snout	8	14	
	Stone marten			
21	dorsal	>10	21	pure (>2)
22	lateral	>10	21	
23	abdominal	>10	18	
24	snout	>10	19	
	European badger			
25	dorsal	>10	21	pure (>2)
26	lateral	>10	20	
27	abdominal	>10	20	
28	snout	>10	22	
	Raccoon			
29	dorsal	>10	16	pure (>2)
30	lateral	>10	21	
31	abdominal	>10	19	
32	snout	>10	18	
	Raccoon dog			
33	dorsal	>10	20	pure (>2)
34	lateral	>10	19	
35	abdominal	>10	19	
36	snout	>10	23	
	Ferret			
37	dorsal	>10	20	pure (>2)
38	lateral	>10	21	
39	abdominal	>10	17	
40	snout	>10	18	

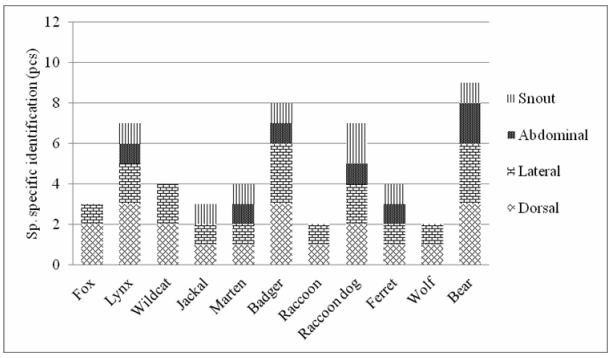

Figure 3. Summarized results of species-specific morphological identification (y-axis: one point is one expert's species-specific identification based on one body region, in total 12 points can be gained).

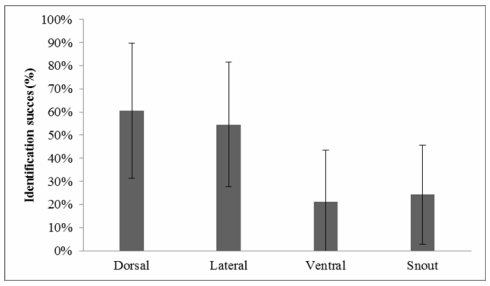

Figure 4. Summarized results of the identification success based on body regions.

with ventral ad snout hairs (21%, SD=22.45 and 24%, SD=21.53; Fig. 4). No difference could be observed between the identification success of dorsal and lateral hairs (Paired-samples t-test, t_{10}=1.49, p=0.1669). However, a significant difference was shown between identification based on all body regions and identification based on dorsal and lateral hairs (Paired-samples t-test, t_{10}=4.765, p=0.0008).

Determining DNA amount and purity

The DNA was extracted successfully from all 40 (100%) of the invasive samples and all 28 (100%) of the noninvasive samples (Table 2, 3). The mean DNA concentration for invasive samples was 18.23 ng/µl, (SD=2.6) and 9.18 ng/µl (SD=3.28) for noninvasive samples. Among invasive samples, badger yielded the highest average DNA concentration (20.75 ng/µl) while red fox and grey wolf produced the lowest average values (15.5 and 15.75 ng/µl, respectively, Table 2). More hairs resulted in a significantly higher amount of DNA (Wilcoxon signed-rank test n=28, r= 0.843, p < 0.0001). We did not find any significant difference in DNA concentration among different body regions (Repeated ANOVA, p= 0.237).

In the case of noninvasively collected hairs, samples from stone marten yielded the highest average DNA concentration (10.13 ng/µl) while samples from brown bear produced the lowest average values (5 ng/µl Table 3). More hairs resulted in a significantly higher amount of DNA (Wilcoxon signed-rank test n=28, r=0.843, p < 0.0001). A260/A230 quotient were higher than 2 in all samples (100%).

No significant difference could be shown in total DNA amount among body regions in the case of reference samples (Repeated ANOVA, p= 0.237).

Discussion

GS gave the most search results for each species. More hits were received on "bear" and "wolf" keywords than on "lynx" and "wildcat". This can be associated with the successfulness of bear related surveys (Woods et al. 1999, Mowat & Strobeck 2000, Triant et al. 2004). In these studies, bears reacted well on the two most commonly used sampling devices: hair corrals or rub objects, and a huge number of samples were collected. We could analyse only one wildcat and two lynx related papers, which were dealing with hair collection techniques. Felid monitoring can often result in contradictions. Wildcat monitoring is usually based on rub posts. In Germany for example, it was a huge success (Steyer et al. 2013), but with similar technique wildcats at Sicily refused to rub (Anile et al. 2012). Two canid-related surveys were found, one which dealt with wolf and coyote (*Canis latrans*) monitoring based on rub pads (Ausband et al. 2011), and one which tested tube traps on Saint Joaquin kit foxes (*Vulpes macrotis mutica*) (Clark et al. 2002). Most contradictions

Table 3. Results of DNA extraction from different carnivore hair samples (hair traps).

Sample	Estimated hairs (pcs)	~ DNA concentracion (ng/µl)	DNA purity (A260/A230)
Eurasian lynx	2	4	pure (>2)
	7	11	
	>10	14	
	2	5	
European wildcat	2	4	pure (>2)
	>10	9	
Golden jackal	>10	13	pure (>2)
Stone marten	5	10	pure (>2)
	>10	12	
	2	6	
	5	9	
	>10	11	
	5	10	
	>10	15	
	6	8	
Ferret	2	5	pure (>2)
	8	9	
	>10	10	
	>10	11	
	7	9	
	8	10	
	7	9	
	3	5	
	>10	15	
	5	11	
	>10	12	
Brown bear	2	5	pure (>2)
	2	5	

occurred in the case of felids, but it is clear that hair collection based on artificial traps depends on many factors (e.g.: trap nights, refreshing period, study interval, rarity and behaviour of the species). Noninvasive sample collecting is still a developing method. We found several papers that dealt with monitoring techniques, but their data were still unsatisfactory for a comprehensive statistical analysis. However, we can present the wide and variable nature of these techniques as guidance (Table 2). A huge proportion of these calculations is derived data, based on other values given in the articles. We believe that more detailed basic data would help experts to carry out fieldworks with higher efficiency. For further calculations, intervals given in trap-nights (e.g. Steyer et al. 2013) are the best. In some literature, it was not completely clear how long the study lasted, thus calculated trap night values might be inaccurate. We have encountered the same problems with refreshing periods. These were given in approximate values (app. 7-day intervals, Woods et al. 1999) or were not given at all (Portella et al. 2013).

The dense structure of wire brush combed the most hair from the animals at Budakeszi ZOO (Fig. 2.). Brushes collected almost twice as much hair samples as the second best surface (adhesive). We recommend using wire brushes because samples are harder to remove from the adhesive material. Several times lynx and jackals destroyed the rub pads (type A) and started to chew them. Screws, as hair collecting surfaces did not work well. We think this might be because we used too large (50 mm) screws and too soft fabric attached to, and the screws were too loose to collect hair samples. However, similar methodology was used successfully in other studies (nail: 30 mm, Castro-Arellano et al. 2008; nail: 32-38 mm, Kendall & McKelvey 2008; nail: 50 mm, McDaniel et al. 2000).

Most hair samples were collected from stone marten and ferret. We could not collect hair sample by type A traps from marten and only three samples were collected from bears. Sampling the hairs of bears proved to be more successful by rub trees (Stetz et al. 2010) or hair corrals (Triant et al. 2004). Stone martens and other mustelids are

Table 4. Quantitative literature review based on studies that aimed to monitor carnivore species by hair collecting.

Target species	Method	Interval	Hairs collected	DNA survey	Individuals	Area (km²)	Traps, trap stations	Trap nights	Hit/100 trap nights	Traps/Area (km²)	Literature
Black and brown bear	Hair corral (with experimental design)	28	293	153	25		20				Woods et al. 1999
	Hair corral	35	154	100	33	4900	22			0.004	
		43	1753	1496	54	4096	64	2653	2.052	0.016	
Grizzly and black bear		50	4245	1308	109	9866	76	3800[c]	2.834	0.008	Mowat & Strobeck 2000
		56	635	166	37	5030	73	4088[c]	0.888	0.015	
Louisiana black bear		30	922	571	89	1150	100	3000[c]	2.670	0.087	Triant et al. 2004
Apennine brown bear	Opportunistic		80		30	500					Lorenzini et al. 2004
Cantabrian brown bear	Opportunistic and systematic		134[g]	41	48	750					Pérez et al. 2009
Grizzly bear	Bear rub object (tree, post etc.)		12564	1891	379	31410					Stetz et al. 2010
Coyote	Rub pub (hanging or ground)	31	73	62	-*		120	4296			Ausband et al. 2011
Wolf		56	99	?	26[a]		30	388	4.902		
San Joaquin kitfox	Hair-sampling tube	20			8*		15	3140[c]			Clark et al. 2002
Margay, jaguarundi, ocelot	Rub pad	240	389	41	31[a]		888		1.46 (0.015)[b]		García-Alaníz et al. 2010
Mountain lion, jaguar, jaguarundi, margay, oncilla		62 & 122			0	80		1551	0		Portella et al. 2013
Eurasian lynx		~350[e]	229	186[c]		595	113	1656-10622[d]		0.190	Schmidt & Kowalczyk 2006
Margay		48	117	83	0	1445.3	30	1440[c]		0.021	Downey 2005
Canada lynx		~30									McDaniel et al. 2000
-		60[j]	3355 (pcs)			1445	138	8419		0.096	Castro-Arellano et al. 2008
Black bear, fisher, bobcat		2003-2004 summer	29	7[k]	13*	24963	74			0.003	Long et al. 2007
Bobcat, mountain lion, coyote, grey fox	Rub post		315	256	4	138	161	2254[c]	0.011		Ruell&Crooks 2007
Eurasian lynx			15864 (pcs)				148[f]				Heurich et al. 2012
European wildcat		35296	37	24	6	334.5	356	35296	0.07	1.064	Steyer et al. 2013
Ocelot		20			6	6	78[h]	1560[c]		13	Weaver et al. 2005
Bobcat		12 weeks	1		4[a,*]	13.18	20	1680	0.059	1.517	Comer et al. 2011

Legend continued on the next page

Table 4 (continued) legend:

Trap night and Catch/100 trap nights: calculated if it was not given in the literature
Hairs collected and Suitable for DNA survey: given in sample size (sample size can vary among studies)
Number of traps: can be given in pieces or in stations, depends on the study
*: individual identify was not carried out, shows the sample size
a: with nontarget species
a': no target species
b: all species (target + nontarget)
c: exact data was not given in literature
d: trap nights and trap numbers varied over sampling periods (min-max, calculated)
e: five sampling period, each varied between 46 and 94 days, calculated
f: a summary for different trap types
g: both hair and scat samples
h: number of traps varied between sampling periods (maximum value shown)
i: three sampling periods were summarized
j: for each trap, not for summarized interval
k: only 15 samples were sent to the DNA lab

usually sampled by cubbies or modified traps (Kendall & McKelvey 2008). With type B traps, we managed to get some samples from martens and ferrets too. Because of the small sample size significant difference cannot be shown between sample size collected by type B and type C traps, but type C collected more hairs. We argue that the structure of the trap can affect the results. A cylinder shaped tube is harder to stabilize than a rectangular trap and animals might avoid poorly stabilized traps. The wildcat did not visit type C trap, although these kinds of traps were used successfully to catch wild and domestic cats (Biró et al. 2004).

Identification success varied among body regions and species. Bear, badger, raccoon dog and lynx were easy to identify for all experts. Raccoon and grey wolf were hard to identify correctly (17% success).

According to international experience, the hair identification keys based on morphological characters of adult animals' dorsal guard hairs reveal the most clues (Tóth 2002a). This study has pointed out a significant difference between the identification success rates of the different body regions and there was no significant difference between identification success of dorsal and lateral hairs. Thus, guard hairs from both regions could be suitable for morphological identification because these two kinds of hairs have same macro- and micro-morphological features. This might be important for hair collecting because dogs like to rub with their back and neck (Ausband et al. 2011) and cats usually rub their cheeks and necks to surfaces (Schmidt & Kowalcyk 2006). If the traps can elicit "rub response", both canids and felids will probably leave hairs from the dorsal, lateral or snout regions of the body. If only dorsal and lateral identification are taken into account, the identification success usually increases. We have found that some taxa are easy to identify on a genus, or even on a species level. Badger and bear were identified species specifically by all the three experts (100%), but in the case of wolf and raccoon dog the identification success was lower (33%). All guard hairs of stone marten and ferret might look more or less the same, according to their macroscopic and microscopic features, so the average identification success was 33% on all body regions.

Meanwhile, among invasive samples red fox and grey wolf produced the lowest average values (Table 2). More hairs resulted in a significantly higher amount of DNA. We did not find any significant difference in DNA concentration among different body regions (Repeated ANOVA, $p= 0.237$).

Among noninvasive samples, stone marten yielded the highest average DNA concentration while brown bear produced the lowest average values (Table 3).

Badger (invasive sample) and stone marten (noninvasive sample) yielded the highest average DNA concentration. DNA concentration was satisfactory in all species in the case of reference hairs. All hair samples had an A260/A230 quotient higher than two, which means DNA purity was satisfying. We could not find a significant relationship among body regions or species in the case of DNA amount, DNA concentration or A260/A230 quotient. In conclusion, all of the examined species can contain sufficient amount and good quality DNA for further identifications.

The minimum number of guard hairs would be ideally 4-5 pieces per specimens and under hairs are not specific and cannot be used for proper morphological identification. For gathering presence or absence data, identification based on mtDNA would be a suitable and cheap method (e. g. Melton & Holland 2007). However, we must address that DNA degradation may be rapid in warm and wet environment (Kendall & McKelvey 2008), thus morphological identification in such circumstances is still a viable method, due to the enduring nature of cuticula and medulla patterns. Moreover, in the case of DNA approaches, genotyping errors can often occur (Taberlet & Luikart 1999, Pompanon et al. 2005). In some cases, presence data can be collected based solely on morphological features.

The hair-traps (rub pad (A), PVC tube (B), modified cage trap (C)) were placed to the enclosures of different carnivore species. Hairs were originated mainly from dorsal and lateral regions, because of the specific construction of hair collecting surfaces and/or the animal natural behaviour (see: Schmidt & Kowalcyk 2006, Ausband et al. 2011). All the three experts stated that dorsal guard hairs are the best for identifying species, but according to the blind test both dorsal and lateral guard hairs are suitable for species-specific identification. Furthermore, we have found that DNA amount and purity did not differ in species or in body regions in case of Hungarian predators, thus probably all field collected samples should be suitable for genotyping if samples are handled correctly (silica gel or -20°C deep freeze (Schwartz & Monfort 2008). We could also point out that a few rare and elusive Hungarian carnivore species, like the lynx and the bear could be readily identifiable using hair trapping, although some animals, like wolf usually require DNA genotyping for species-specific identification. Based on our results we believe hair collection can be a viable tool for researches with low budget. This method can be tested on several fields, like wildlife passage use to avoid vehicle collisions (Cserkész & Farkas 2015), compiling a mammal inventory (Patkó et al. 2013, Ondrušová & Adamík 2014) or gathering genetic information on elusive but abundant species (Woods et al. 1999). Due to these result, field testing of different hair collecting structures are being carried out in several Natura 2000 sites in Hungary.

Acknowledgements. The research was supported by the "Sustainable Conservation on Hungarian Natura 2000 Sites" (registration number SH/4/8) Swiss-Hungarian partnership program and by the Ministry of Human Capacities ("Research Centre of Excellence-9878-3/2015/FEKUT"). We would like to express our gratitude to Anett Weigl for her help in trap assembly. Special thanks must be addressed to Péter Szabó, director of Budakeszi ZOO for providing the opportunity for collecting reference material. All co-workers of the wildlife park should be thanked to, particularly Zsolt Szilágyi because of his tireless help in the process of collecting the reference material.

References

Anile, S., Arrabito, C., Mazzamuto, M.V., Scornavacca, D., Ragni, B. (2012): A non-invasive monitoring on European wildcat (*Felis silvestris silvestris* Schreber, 1777) in Sicily using hair trapping and camera trapping: does it work? Hystrix 23: 45-50.

Ausband, D.E., Young, J., Fannin, B., Mitchell, M.S., Stenglein, J.L., Waits, L.P., Shivik, J.A. (2011): Hair of the dog: Obtaining samples from coyotes and wolves noninvasively. Wildlife Society Bulletin 35: 105-111.

Biró, Zs., Szemethy, L., Heltai, M. (2004): Home range sizes of wildcats (*Felis silvestris*) and feral domestic cats (*Felis silvestris f. catus*) in a hilly region of Hungary. Mammalian Biology 69: 302-310.

Boitani, L., Powell, R.A. (2012): Carnivore Ecology and Conservation. Oxford University Press. Oxford. 528 pp.

Castro-Arellano, I., Madrid-Luna, C., Lacher, T.E., León-Paniagua, L. (2008): Hair-trap efficacy for detecting mammalian carnivores in the tropics. Journal of Wildlife Management 72: 1405-1412.

Clark, H.O., Cypher, B.L., Kelly, P.A., Williams, D.F., Clifton, S.D. (2002): Use of a hair-sampling tube to detect the San Joaquin Kit Fox. Transactions of the Western Section of the Wildlife Society 38: 29-30.

Comer, C.E., Symmank, M.E., Kroll, J.C. (2011): Bobcats exhibit low detection rates at hair collection stations in East Texas. Wildlife Biology in Practice 7: 116-122.

Cserkész, T., Farkas, J. (2015): Annual trends in the number of wildlife-vehicle collisions on the main linear transport corridors (highway and railway) of Hungary. North-Western Journal of Zoology 11(1): 41-50.

Deinet, S., Ieronymidou, C., McRae, L., Burfield, I.J., Foppen, R.P., Collen, B., Böhm, M. (2013): Wildlife comeback in Europe: The recovery of selected mammal and bird species. ZSL, BirdLife International and the European Bird Census Council. London. 307 pp.

Downey, P.J. (2005): Hair-snare survey to assess distribution of Margay (*Leopardus wiedii*) inhabiting El Cielo Biosphere Reserve, Tamaulipas, Mexico. Master Thesis. Oklahoma State University. Oklahoma. 40 pp.

Farrell, L.E., Roman, J., Sunquist, M.E. (2000): Dietary separation of sympatric carnivores identified by molecular analysis of scats. Molecular Ecology 9: 1583-1590.

Garcia-Alaniz, N., Naranjo, E., Mallory, F.F. (2010): Hair-snares: A non-invasive method for monitoring felid populations in the Selva Lacandona, Mexico. Tropical Conservation Science 3: 403-411.

Hanke, P.U., Dickman, C.R. (2013): Sniffing out the stakes: hair-snares for wild cats in arid environments. Wildlife Research 40: 45.

Heurich, M., Müller, J., Burg, M. (2012): Comparison of the effectivity of different snare types for collecting and retaining

hair from Eurasian Lynx (*Lynx lynx*). European Journal of Wildlife Research 58: 579-587.

Kendall, K.C., McKelvey, K.S. (2008): Hair collection. In: Long, A. R., MacKay, P., Ray, J., Zielinski, W. (ed.): Noninvasive Survey Methods for Carnivores. Island Press. Washington. 385 pp.

Long, R.A., Donovan, T.M., MacKay, P., Zielinski, W.J., Buzas, J.S. (2007): Comparing scat detection dogs, cameras, and hair snares for surveying carnivores. Journal of Wildlife Management 71: 2018-2025.

Long, R.A., MacKay, P., Ray, J., Zielinski, W.J. (2008): Noninvasive Survey Methods for Carnivores. Island Press. Washington. 385 pp.

Lorenzini, R., Posillico, M., Lovari, S., Petrella, A. (2004): Non-invasive genotyping of the endangered Apennine brown bear: a case study not to let one's hair down. Animal Conservation 7: 199-209.

Nedyalkov, N., Koshev, Y., Raykov, I., Bardarov, G. (2014): Color variation of small mammals's (Mammalia: Rodentia and Insectivora) coats from Bulgaria. North-Western Journal of Zoology 10(2): 314-317.

Matthew, B.T. (2012): A comparison of noninvasive survey methods for monitoring mesocarnivore populations in Kentucky. Thesis and Dissertation—Forestry. University of Kentucky. Kentucky. 151 pp.

McDaniel, G.W., McKelvey, K.S., Squires, J.R., Ruggiero, L.F. (2000): Efficacy of lures and hair snares to detect lynx. Wildlife Society Bulletin 28: 119-123.

Mowat, G., Strobeck, C. (2000): Estimating population size of grizzly bears using hair capture, DNA profiling, and mark-recapture analysis. Journal of Wildlife Management 64: 183-193.

Ondrušová, K., Adamík, P. (2013): Characterizing the mammalian hair present in Great Tit (*Parus major*) nests. Bird Study 60: 428-431.

Paine, R.T. (1980): Food Webs: Linkage, interaction strength and community infrastructure. Journal of Animal Ecology 49: 666-685.

Pauli, J.N., Hamilton, M.B., Crain, E.B., Buskirk, S.W. (2008): A single-sampling hair trap for mesocarnivores. Journal of Wildlife Management 72: 1650-1652.

Pérez, T., Vázquez, F., Naves, J., Fernández, A., Corao, A., Albornoz, J., Domínguez, A. (2009): Non-invasive genetic study of the endangered Cantabrian brown bear (*Ursusarctos*). Conservation Genetics 10: 291-301.

Pompanon, F., Bonin, A., Bellemain, E., Taberlet, P. (2005): Genotyping errors: causes, consequences and solutions. Nature Reviews Genetics 6: 847-846.

Portella, T.P., Bilski, D.R., Passos, F.C., Pie, M.R. (2013): Assessing the efficacy of hair snares as a method for noninvasive sampling of Neotropical felids. Zoologia Curitiba 30: 49-54.

Ripple, W.J., Estes, J.A., Beschta, R.L., Wilmers, C.C., Ritchie, E.G., Hebblewhite, M., Berger, J., Elmhagen, B., Letnic, M., Nelson, M.P., Schmitz, O.J., Smith, D.W., Wallach, A.D., Wirsing, A.J. (2014): Status and ecological effects of the world's largest carnivores. Science 343: 151-162.

Ruell, E.W., Crooks, K.R. (2007): Evaluation of noninvasive genetic sampling methods for felid and canid populations. Journal of Wildlife Management 71: 1690-1694.

Sahajpal, V., Goyal, S.P., Jayapal, R., Yoganand, K., Thakar, M.K. (2008): Hair characteristics of four Indian bear species. Science & Justice 48: 8-15.

Schmidt, K., Kowalcyk, R. (2006): Using scent-marking stations to collect hair samples to monitor Eurasian lynx populations. Wildlife Society Bulletin 34: 462-466.

Schwartz, M., Monfort, S. (2008): Genetic and endocrine tools for carnivore surveys. In: Long, L.A., MacKay, P., Zielinski, W.J., Ray, J.C. (ed.): Noninvasive Survey Methods for Carnivores. Island Press. Washington. 385 pp.

Stetz, J.B., Kendall, K.C., Servheen, C. (2010): Evaluation of bear rub surveys to monitor grizzly bear population trends. Journal of Wildlife Management 74: 860-870.

Steyer, K., Simon, O., Kraus, R.H.S., Haase, P., Nowak, C. (2013): Hair trapping with valerian-treated lure sticks as a tool for genetic wildcat monitoring in low-density habitats. European Journal of Wildlife Research 59: 39-46.

Taberlet, P., Waits, L.P., Luikart, G. (1999): Noninvasive genetic sampling: look before you leap. Trends in Ecology & Evolution 14: 323-327.

Teerink, B.J. (1991): Hair of West-European Mammals. Cambridge University Press. Cambridge. 224 pp.

Tóth, M. (2002a): Identification of Hungarian mustelidae and other small carnivores using guard hair analysis. Acta Zoologica Academiae Scientiarum Hungaricae 48: 237-250.

Tóth, M. (2002b): [*Haircapture: possibilities and restrictions of the method*]. Vadbiológia 9: 117-122. [in Hungarian with English Abstract]

Tóth, M. (2008): A new noninvasive method for detecting mammals from birds' nests. Journal of Wildlife Management 72: 1237-1240.

Triant, D.A., Pace, R.M., Stine, M. (2004): Abundance, genetic diversity and conservation of Louisiana black bears (*Ursus americanus luteolus*) as detected through noninvasive sampling. Conservation Genetics 5: 647-659.

Weaver, J.L., Wood, P., Paetkau, D., Laack, L.L. (2005): Use of scented hair snares to detect ocelots. Wildlife Society Bulletin 33: 1384-1391.

Woods, J.G., Paetkau, D., Lewis, D., McLellan, N.B., Proctor, M., Strobeck, C. (1999): Genetic tagging of free-ranging black and brown bears. Wildlife Society Bulletin 27: 616-62.