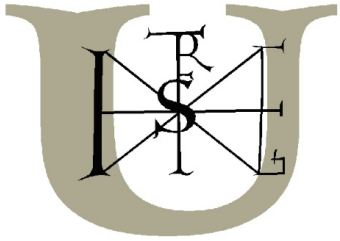

SZENT ISTVÁN UNIVERSITY FACULTY OF AGRICULTURAL AND ENVIRONMENTAL SCIENCES

Developing and testing non invasive survey methods on Carnivore species

Theses of Ph. D. dissertation

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1. BACKGROUND AND OBJECTIVES OF THE STUDY

Carnivores have been regarded as competitors for mankind since the ancient times. The diet of these species usually contains game animals and small mammals but they often prey on livestock as well. Problems resulting from predation have been solved by fences, trapping, hunting, poisoning and constant guarding with guard dogs in the historical times (Altai 1958). In the XXth century both scientist and the lay public believed that carnivores have relatively small ecological impact on the ecosystem compared to producers and primary consumers (Rosenzweig 1973, Terborgh & Estes 2010, Heltai & Lanszki 2013). For the first glance this seems to be true when we take a look at the trophic pyramid. Carnivores can be found in relatively small numbers on the upper levels, as secondary or tertiary consumers, as well as on the apex of the pyramid (Fryxell et al. 2014). Since the lower levels of the pyramid have a large number of plants and herbivores, they have an inherent effect on the levels above them, therefore the removal of some carnivores (upper regions) should not cause any particular concerns for the functioning of the ecosystem. Later, however, it became clear that these animals have important top-down ecological regulation effects (Estes & Palmisano 1974, Wilmers et al. 2003, Ripple et al. 2014).

The order of carnivores has 252 terrestrial species (IUCN Red Lista). There are 65 species in the IUCN Red List that fall into one of the threatened categories (*critically endangered, endangered, vulnerable*), which means 26% of the species on our planet are at risk and an additional 4% lacks the basic data for classification (*data deficient*). Based on this, on a global level, nearly one-third of the species condemned to receive human help to survive. Unfortunately, however, sometimes we do not have the most basic information about our wildlife resources and biodiversity (Joppa et al. 2016). Due to the fact that carnivores are elusive and often occure in small densities only indirect signs (e.g. hair, footprints, prey remains, faeces) can inform us about their presence. With the rapid development of molecular biological methods, these indirect or non invasive genetic samples (NGS) are becoming more accessible and can aid conservation efforts.

From the above, it might be clear that in the XIth century it is an important task to monitor carnivores both for conservationists and for wildlife managers. Thanks to rapidly developing technology methods become more sophisticated and delicate. However, investigating species is inherently multi-leveled. Animals can be monitored for their presence (i), or population changes (indices) (ii), as well as for their densities and population dynamics (iii). Each levels and new methodologies must be kept under constant supervision, due to the rapid development of field methods and laboratory techniques. With the recent growth in the

interest in NGS (Fig. 1) and by eliminating potential human disturbance reliable and unbiased data can be obtained on wild animals. Nonetheless, the effectiveness and reliability of field and lab methods have to be constantly verified if developing cost-effective and accurate methods for monitoring species is our primary goal.

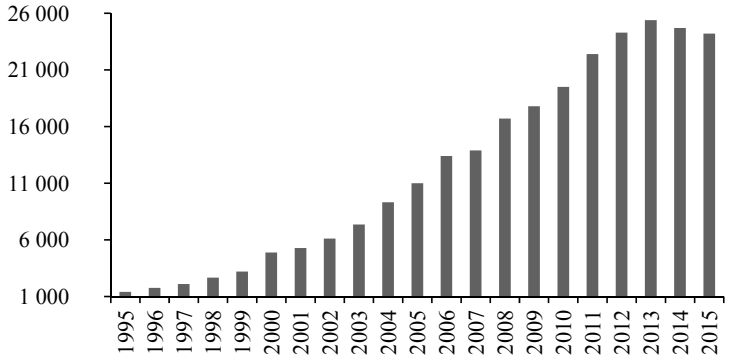

Fig. 1. Internet search results on „non invasive" + „carnivore" keywords in the last decade (Google Search).

For the reasons mentioned above my main aim was to develop and test monitoring methods to investigate the occurance of carnivore species in Hungary.

Hair collecting techniques vary widely and their effectiveness is presumably dependent on several factors so my goal was to renounce or strengthen the use of hair sampling methods for monitoring purposes in Hungary. I was interested in the following research questions:

1. Which hair sampling methods are the most suitable to study Hungarian carnivore species?
    A, Are there any typically used hair trap types and attractants for given species groups?
    B, How often are hair trapping methods used throughout Europe on these species?
2. Are there any differences in the morphology based identification succes among different species and body regions?
    A, Is there a difference in the amount and concetration of DNA collected from different body regions?
3. Are there any differences among the effectiveness of various hair traps and hair collecting surfaces?
4. Is there a difference between morphological and mtDNA based species identification?
    A, Can species be identified with the same success and accuracy?

5. Is it possible to develop and test hair traps that collect samples from Hungarian carnivore species in field conditions?

A, Do these NGS contain enough morphological and genetical information for species specific identification?

6. Is the special type of natural hair trap, the bird nest, suitable for describing the mammal fauna of urbanized and natural habitats?

7. What faunistical data can be gathered from different Natura 2000 sites by using hair collecting devices?

8. What are the best hair traps in field conditions for collecting carnivore hairs?

2. MATERIALS AND METHODS

2.1. Study areas

2.1.1. Bird nest collection

Bird nest analysis has been carried out in three different areas. Merzse swamp (2011) is a semi-natural habitat close at Budapest, Lower Park (2012) at Gödöllő is city park sorrunded by urban habitats, while Sár-hegy (2016) is covered by decidous forests and it is a Natura 2000 site at Mátra.

2.1.2. Enclosures for collecting reference hair samples and testing hair snagging devices

Reference hair samples were collected at Veresegyház Medveotthon, Budakeszi ZOO, Horkai Animal Training Center and Hungarian Natural History Museum. Hair trap prototypes were tested at Budakeszi ZOO during the winter and spring of 2013.

2.1.3. Field tests of hair traps and opportunistic hair collection

The hair trap monitoring tests have been carried out at two Special Protection Areas (Natura 2000, SPA) and ten smaller Special Areas of Conservation (Natura 2000, SAC). Field studies were done between February of 2014 and March of 2015. Beside the two main sites (Mátra SPA and Kiskunság SPA) several opportunistically gathered hair samples were sent for analysis from various locations of the North Hungarian Mountains (e.g. Zemplén, Aggtelek).

2.2. Study methods

2.2.1. Quantitative literature review

Scientific journal articles were collected from 2006 to 2015 with Google Scholar and Science Direct search engines. Artifical hair traps and species occuring in Europe were in the focus of the literature search. However, owing to the scarcity of European articles I also used literatures from the whole Holartic ecozone. Similar or vicariating species were grouped together and examined as one (e.g. Bobcat + Eurasian lynx + Canadian lynx = „Lynx").

2.2.1. Bird nest collection

The location of the nests was recorded on a GPS device (Gekko 201). The samples were placed in a separate paper bags and labeled with GPS point, date and collector's name. The nests were then dried in a well-ventilated room and placed in a freezer. Before the

laboratory tests were started I placed the samples under a UV sterilizer. The nest samples were disassembled for hair sub-samples on a white sheet. The hairs were then placed in snap-lock plastic bags and provided with a unique code (collector's name, field identifier, lab ID, date of collection, date of disassemble).

2.2.2. Hair trap tests in enclosures

During the trials in the enclosures I have tested three trap types (A – rub pad, B – modified live-catching box trap („cubbies"), C – two-end opened PVC trap („cubbies")). Traps were placed in the enclosures of the following species: ferret, wildcat, Eurasian lynx, stone marten, golden jackal and brown bear. Hairs (under hairs and guard hairs) were placed in plastic bags and stored in a freezer (-20°C) until further use. Samples were processed in 3-4 weeks after collection.

2.2.3. Hair collection at Natura 2000 sites

Field collecting of the hair samples started in the winter of 2013 and ended in the summer of 2015. I have placed hair traps on 100 locations in Mátra (85 A-type, 4 wire brush B-type, 9 adhesive B-type, 6 wire brush C-type and 8 adhesive C-type). At Kiskunság 38 traps were placed (6 A-type, 4 wire brush B-type, 8 adhesive B-type, 9 wire brush C-type and 11 adhesive C-type).

Baits and attractants were refreshed in every 2nd or 4th week when hair samples were also collected. During the sample collection, we had only minor contact with the trap in rubber gloves. This was important to avoid cross contamination and to minimalize human odoer around the trap.

2.3. Laboratory methods

2.3.1. Species identification based on hairs' morphological characters

Hair samples were prepared based on Tóth (2003), Tóth (2015), Teerink (1991) and Lanszki (pers. comm.). Contaminations like dust and mud were removed using alcohol (70%) and ethyl-ether.

Blind testing the anonymous samples of 11 species (red fox, Eurasian lynx, wildcat, golden jackal, stone marten, Eurasian badger, racoon, racoon dog, ferret, grey wolf and brown bear) and four body regions (dorsal, lateral, abdominal and snout) were carried out with three experts.

2.3.2. Species identification based on mtDNA

Hair samples for DNA concenteration (µg/µl), quality (A260/A230) and species identification were analysed at Nagy Gén Diagnosztikai és Kutatási Ltd., Biomi Ltd., CIBIO-InBIO (Vairão, Portugal) and National Agricultural Research and Innovation Centre, Agricultural Biotechnology Institute.

2.4. Data process and softwares

Descriptive statistics, β-diversity calculations, and graphs were produced using Excel (Microsoft Office, 2016). Differences among body regions were calculated with Chi^2-test. Wilcoxon-test, and the repeated measures ANOVA were used to determine the differences in DNA amounts. The statistical tests were performed using Prism 6 (InStat GraphPad 2016) software.

The differences in species diversity of different areas were determined by using the Jaccard correlation coefficient (Jaccard-index).

Maps were made by using the QGIS Geospatial Software (Quantum GIS Development Team (2016), Open Source Geospatial Foundation Project).

3. RESULTS

3.1. Quantitative literature review

Altogether 26 scientific articles were found that have matched my search criteria. Some of these articles dealt with more then one species, thus the final sample amount was larger (n=35). Approximately half of the studies (n=15, 53.85%) were carried out in the Palearctic fauna region, while the other were in the Nearctic (n=12, 46.15%). Between the two fauna regions species were splitted as follow: lynx (50-50%), small felids (80-20%), canids (40-60%), bears (60-40%), otter (50-50%), badger (100-0%) and other mustelids (42.86-57.14%).

Rub pads were primarly used by lynx (n=7, 87.5%), while small felids used mostly lure sticks/scent stations (n=5, 80%). Two studies were found that sampling canids with rub pads is possible. Natural rub objects (e.g. trees) were solely used by bears (n=5, 100%). Enhanced (e.g. with barbed wire) naturals rub object were also used mostly by bears (n=3, 60%). In case of the otter, one technique sampled the animals with modified leg-hold traps (50%) and another one with natural hair trap (song bird nest, 50%). Badger hairs could be found in bird nests (n=2, 66.66%) or on tracks with barbed wire (n=1, 33.33%). Based on one study mustelids cannot be sampled by rub pads (n=1, 12.5%), but they are effectively sampled by different cubbies (n=5, 62.5%).

From the 41 techniques found the rarest was the modified leg-hold trap (otter, n=1, 2.44%) and hair corral (bear, n=1, 2.44%). Rubbing devices (n=15, 36.59%) and birds nest (n=9, 21.95%) were frequently used to gather hair samples from different species. From the seven species groups only bear hair could not be found in field studies that used bird nest as sample device.

Most of the hair collection studies focused on core areas (n=32, 84.21%) where the target species were known to occure in larger densities. Studies focusing on the edge of target species area were less common (n=3, 7.89%). Three studies (7.89%) were carried out in enclosures.

3.2. Pilot studies

3.2.1. Morphological and genetical identification

Knowing which body region a hair belong to could influance species identification. Regarding the single body regions, dorsal and lateral hairs showed higher identification rates

(61%, SE=29.14 and 55%, SE=26.97, respectively) compared with abdominal and snout ha
(21%, SE=22.45 and 24%, SE=21.53; Fig. 2). No difference could be observed between t
identification success of dorsal and lateral hairs (Chi^2-test, χ^2=0.203, p=0.887). However
significant difference was shown between identification based on all body regions a
identification based on dorsal and lateral hairs (Chi^2-test, χ^2=5.506, p=0.019).

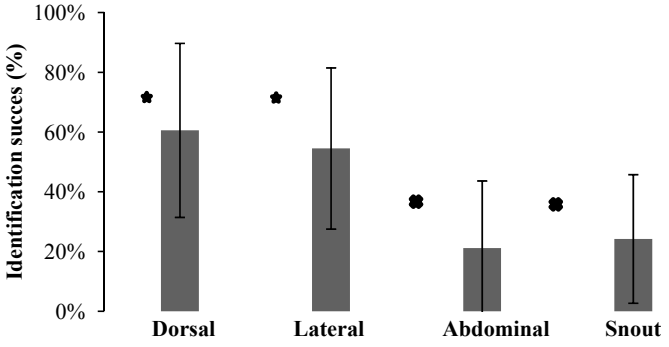

Fig. 2. Summarized results of the identification success based on body regions.

The mtDNA was extracted successfully from all 40 (100%) of the invasive samples and all
(100%) of the noninvasive samples. The mean DNA concentration for invasive samples w
18.23 µg/µl, (SE=2.6) and 9.18 µg/µl (SE=3.28) for noninvasive samples. Among invasi
samples, badger yielded the highest average DNA concentration (20.75 µg/µl) while red f
and grey wolf produced the lowest average values (15.5 and 15.75 µg/µl, respectively). Mo
hairs resulted in a significantly higher amount of DNA (Wilcoxon-test n=28, r=0.8
p<0.0001). I did not find any significant difference in DNA concentration among differe
body regions (Repeated ANOVA, ns, F=1.502, p=0.242; Fig. 3).

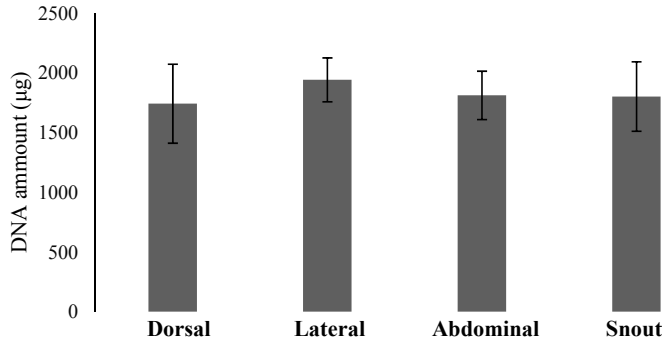

Fig. 3. Differences in DNA amounts in hairs collected from different body regions (Repeated ANOVA, ns, F=1.502, p=0.242).

3.2.2. Blind test: identification of the reference samples

The mean success rate of the three experts on species identification was 40% (SE=30.13). On average the species with the highest identification success rates were bear, badger, raccoon dog and lynx with respectively 75% (SE=30.94), 67% (SE=38.51), 58% (SE=16.65) and 58% (SE=31.93) succes, while raccoon and grey wolf showed the lowest values (both: 17%, SE=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%, Fig. 4).

From 36 investigated samples three (8.33%) could not be identified by morphological or molecular methods because of sample degradation. Six (16.67%) samples could be identified based on only their morphological features and nine (25%) were identified only by mtDNA based approach. Morphology based approach could identify on species level in 18 occasions (50%), whereas mtDNA approach did the same for 22 times (61.11%). Morphological identification did not show misidentification (0%), while genetical approach (16s rRNS BLAST) took four (17.39%) steppen polecat samples as if they belonged to black-footed ferret.

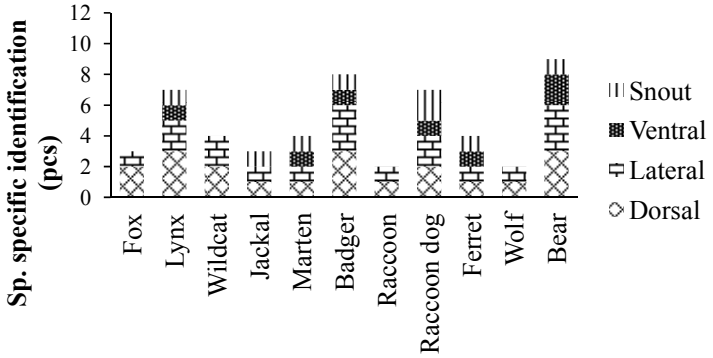

Fig. 4. Summarized results of species specific morphological identification (y-axis: one point is one expert's species specific identification based on one body region, in total 12 points can be gained).

3.2.3. Hair trap prototypes in enclosures

In total, the sampling resulted in 304 guard hairs from the six target species. Altogether, type A traps collected the most samples (n=125, x=20.83, SE=20.44) followed by type B (n=115, x=38.33, SE=33.38) and type C (n=64, x=32). From ferret, I could collect 110 hairs (x=10.1, SE=10). Stone marten produced the second largest hair sample with a total of 94 hairs (x=14.46, SE=10.73), followed by golden jackal (n=50, x=25) and lynx (n=38, x=9.5, SE=8.54). From wildcat and bear I could only gather a relatively small sample (n=8, x=1.14, SE=2.26; n=3, x=0.75, SE=0. 96, respectively). As hair collecting surface, the wire brush produced the most hair samples (n=131, x=13.1, SE=14. 87). Adhesive surface proved to be the second best hair collector (n=79, x=15.8, SE=13.53). I could only collect a relatively small sample with screws (n=16, x=1.46, SE=2.84). The efficiency of Velcro tape and barbed wire were similar (n=47, x=4.7, SE=7.44; n=31, x=7. 75, SE=12.23 respectively).

3.3. Field collected hair samples

3.3.1. Nests and artifical nest boxes: natural hair traps

At the urban sample area (Gödöllő) I have found 15 nests. From these nests 41 hair samples could be identified. Altogether seven categories have been established in Gödöllő. The six species categories were as follow: brown hare, dog, European mole, horse, hazel dormouse and human. One category remained for the unidentified hairs. The most common sample was human hair (81.81%).

At the semi-urbanized sample area (Merzse swamp) I could collect 13 nests and 34 mammal hairs were identified. From the eleven categories, five were species (brown rat,

brown hare, Eurasian otter, edible dormouse and human), two twin-species (hazel dormouse-forest dormouse and weasel-stoat). Other categories were higher taxa (e.g. Rodents) or unidentified. Human hair (n=7), weasel-stoat (n=6) and the twin dormice (n=6) were the most common samples. Otter and edible dormouse hairs were found in two different nest but only one occasion.

At the most natural sample area (Sár-hegy, Natura 2000 SAC) 12 nests or articial nest boxes were found with 55 hair samples. Occurance of nine species (wild boar, red fox, mouflon, fallow deer, brown hare, roe deer, badger, edible dormouse, Eurasian beaver, human) and two twin-species (brown rat-black rat, hazel dormouse-forest dormouse) were detected át Sár-hegy. Wild boar (n=12, 21.82%) and other ungulate (n=14, 25.45%) hairs were the most commonly found samples.

3.3.2. Artifically scented hair traps on Natura 2000 sites

The first year (2014) field testing of hair sampling devices yielded 51 hair sample at the Mátra sample area. From these 19 samples (37.25%) came from faunistically irrelevant sources (human (n=4) , unidentified (n=15)). The most common samples were ungulate hairs (n=16, 32%), followed by small carnivores (n=7, 14%). From the 51 samples altogether 13 (25.49%) belonged to carnivore species.

During the second year (2015) I have collected 23 samples at Mátra and from these 8 (36.36%) were irrelevant for the fauna (human (n=6), unidentified (n=2)). Small rodent hairs were found most often (n=7, 31.82%), which were followed by ungulates (n=6, 22.27%). From the 23 samples only one belonged (4.55%) to carnivore species.

At the Kiskunság samples site 51 hair samples were collected during the first year (2014). Ten samples (19.61%) were irrelevant to the faunistical list (human (n=3), unidentified (n=7)). Small carnivores (n=18, 35.29%) were most commonly found. The second most common species group were the small rodents (n=13, 25.49%). Almost half of the samples came from carnivore species (n=24, 49.02%).

During the second year 27 samples were collected at the Kiskunság and nine of them (33.33%) belonged to irrelevant sources (human (n=2), unidenified (n=7)). Common hair samples belonged to small mammals (n=7, 26.91%) and mesocarnivores (n=6, 23.08%). Altogether 27 samples (29.63%) were carnivore hairs.

Altogether 17 species were detected on the two samples sites but three of them (cat, dog, water buffalo) were introduced to the samples site by human activities. Most species

were detected at Mátra in 2014. The Jaccard-index that described the similarity between t mountaneus (Mátra) and lowland (Kiskunság) sample sites is 0.53.

3.3.3. Efficiency of scented hair traps

During the first trapping campaing at Mátra traps were checked for 16 times. One average day 2.63 sample (SE=2.73) could be collected and 23.13 traps (SE=7.23) could checked and rebaited. In 2015 altogether 31 field occasions have been carried out and 0. samples (SE=1.08) were collected. The amount of checked and rebaited traps on a da average was similar of the previous year (24.1, SE=9.57).

On the Kiskunság sample site 11 field days were spent and 47 samples were collec during altogether 275 checking and rebaiting. During one day of field work 4.27 samp (SE=1.68) were collected and 25 traps (SE=7.6) were checked. The second trapping campai resulted in 27 hair samples with altogether 153 checking and rebaited of the traps. On avera I could find 4.5 samples (SE=1.76) and 21 traps could be checked during one field day.

It seems that the most suitable period for hair sample collection is the first quarter the year. At the Mátra sample site I could collect 33 samples (x=8.25, SE=4.86) during t period, while the rest of the year yielded 29 samples (x=4.83, SE=5.31). Similar results w shown at Kiskunság (first quarter: n=50, x=12.5, SE=4.2; rest of the year: n=27, x=5 SE=7.6; Fig. 5).

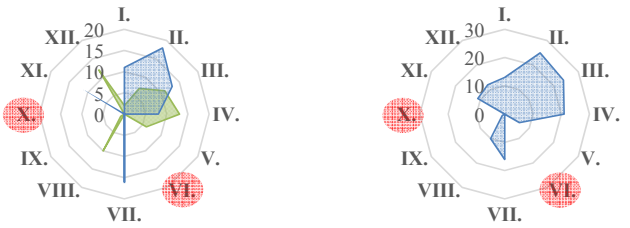

Fig. 5. Left: number of hair samples collected at Mátra (blue) and Kiskunság (green) (2014-2015). Right: th bulk number of collected hair samples in both study sites (Mátra, Kiskunság) (2014, 2015). Red dots: in Octol there was only one sampling occasion and in June sampling was not carried out.

3.3.4. Opportunistic sample collection

From the 27 samples that were collected troughout Hungary with different methods (40.74%) were identified as large carnivore samples. The rest of the samples were determir

3.4. NEW SCIENTIFIC RESULTS

1. Based on quantitative literature review and field studies I have proved that hair collecting is effective in the species core areas, and it is less effective in the case of occasional occurances at the edge areas. At the same time, I demonstrated the occasional presence of lynx in the Mátra on the basis of regular hair trap based monitoring method, as well as I confirmed the presence of some large carnivores (wolf, bear) from the North Hungarian Mountains.

2. I have successfully proved that the dorsal and lateral guard hairs are the most suitable for the species level identification. I have also shown that there is no significant difference among the different body regions regarding the quantity of extracted DNA amount. Based on morphological blind tests of hair samples, I have demonstrated that there are differences among the species in case of species specific identification accuracy. Eurasian badger and brown bear can be easily and accurately identified on a species level based on dorsal or lateral guardhairs.

3. After „blind testing" morphological identifications I have produced and tested the prototypes of various hair traps and successfully collected identifiable hair samples from Hungarian carnivore species. I have determined the most suitable hair-collecting surfaces (wire brush, double-sided adhesive tape) that can be used for regular hair trap based field monitoring, and I have proved that these prototypes can collect hairs with sufficient quantity and quality of DNA for species identification. Most hairs were collected by modified live-catching box traps and PVC cubbies.

4. For the first time in Hungary, I have performed regular species monitor with hair collecting rub pads. I have compiled a mammal species list based on the data collected by hair snagging devices on two Natura 2000 sites (Mátra SPA and Kiskunsági SPA). With the hair collecting devices I have successfully collected hair samples from several Hungarian carnivore species.

5. Simultaniously with the above mentioned I also successfully tested a natural hair collecting method, the bird nest analysis, in an urbanizied area (Gödöllő) and an area with less human disturbance (Merzse swamp). I have also shown that there are enough high quality guard hairs in urban bird nests to detect and identify elusive and rarely seen species based on hairs'

morphological characters. By this method, I was able to demonstrate the occasional occurrence of the strictly protected otter in the Merzse swamp (Budapest).

4. CONCLUSIONS AND SUGGESTIONS

Quantitative literature review

Among the natural hair collection methods bird nest analysis is a rarely tested one. Hairs, as lining materials of song bird nests, provide the occurance data of different mammal species. The methodology presented by Tóth (2008) seems promising, but in addition to my own research, there is only one scientific literature available on the subject (Ondrušová & Adamík 2013). Of the target groups only bear hairs were not found in the bird nests, all the other carnivores could be detected based on hair samples. Only a small sample of data collected by this method have been processed using genetical approach (Patkó, unpublished data). This analysis showed a positive picture, since all three examined hair samples were identified (dog and fox) based 12S rRNA despite the fact that the hairs were exposed to degradation in the nests for more than half a year. In bird nests and artifical nest boxes many hairs can be accumulated, so beyond the morphological identification mtDNA-based approaches can be suitable to determine an area's species composition. Such "simple" presence data may be of particular relevance, for example, in the case of detecting Natura 2000 species.

Most non invasive hair collection (n=32, 84.21%) were performed on core areas where the target species presence is proven, sometimes even in a high population density. However, some studies (n=3, 7.89%) were carried out at the target species edge area. There are probably many well-designed and executed hair collection studies that have not been published in international journals due to unsuccessful collection of the samples, however information about methodology would also be of crucial importance to practitioners. The results of unsuccessful studies (Comer et al. 2011, Anile et al. 2012), however, rarely appear in peer reviewed journals. It seems that non invasive hair traps can be used more effectively in areas where the target species is more common. Yet, the presence and occurance of carnivores remain difficult to investigate. An aid may be when methods (e.g. camera traps, hair traps) are combined with each other (Long et al. 2008, Meek et al. 2014) in a long field monitoring (several years).

Morphological and genetical identification

The accuracy of the morphological identification was significantly influenced by the body regions where hairs were orginated from. Scinetific literature generally refer to the dorsal guard hairs' suitability for identification (Tóth 2002, Shajpal et al. 2008), but the blind tests by

the three independent experts showed that the lateral guard hairs are just as good for identifying species as the dorsal ones (Chi^2-test, χ^2=0.203, p=0.019). However, hairs from the dorsal and lateral regions of the body can be better identified on a species level than the hairs from the snout and abdomen (Chi^2-test, χ^2=5.506, p=0.019).

The hair traps that I have tested can collect samples from the dorsal and lateral body regions and sometimes from the snout. For example, dogs like to rub on their backs (mostly on the ground) (Ausband et al. 2011), while felids tend to use their cheeks and sides to rub on the objects (Schmidt & Kowalczyk 2006). Based on this, the field collected samples have the best quality morphological characters for determinig species. I have also found that the DNA amount did not change among the body regions, so the tested devices could presumably provide suitable hair samples for determining mammal occurances at a given sample area.

Blind test: identification of the reference samples

During the blind test the three experts could identify roughly half of the hair samples on a species level. This does not necessarily mean that in the other cases identification was a failure. In most of the cases, experts identified a higher taxonomic category (e.g. felids, mustelids), because they were not sure about the exact species. This "self-restraint" is a crucial part of morphological based identification (Spaulding et al. 2000, Lobert et al. 2001), since it is better to have a „lower resolution" (e.g. genus or family level) but to have it as a definite identification than an imprecise one (Monterroso et al. 2013). The bear and badger were the most easily determined species, while raccoon and wolf could be identified on a species level with the least succes. Based on the blind test it has been confirmed that on the basis of the morphological characters, the determination of hair samples can vary greatly among the different species. This may be important, for example, in cases of human-wildlife conflicts (e.g. bear or wolf attacks). The hairs that can be found on the location of the conflict can be easily and cheaply determined if the damage is caused by a bear, but in the case of wolf it is probably only possible to identify the sample on a family level (canid).

The effectiveness of genetic identification is influenced by several factors. The amount of DNA may differ among species, also the DNA rapidly degrades in a warm and humid environment (Long et al. 2008), and tests based on costly STR markers are usually required for accurate species determination. The reliability of the morphological identification, on the other hand, is primarily influenced by subjective factors. It is hugely important for the investigator to have experience in the method and to use various hair identification atlases (Teerink 1991, Tóth 2015), as well as his or her own reference collection. The experience of

the reserachers should be controlled by "blind tests", therefore the identification can be more objective and reliable (De Marinis & Asprea 2006).

Hair trap prototypes in enclosures

I could collect 304 hair samples from six carnivore species with traps tested in the Budakeszi ZOO. I could get most of the hair samples (n = 125, x = 20.83, SE = 20.44) with the type A trap (rub pad). However, these traps were put in the enclosures of all six species, while type B and type C were not. It was not possible to collect samples from the stone marten with the rub pads, but based on martens behaviour (rarely rubbing) this result is not suprising. Similar concerns were stated in a North American study on fishers (Long et al. 2007). B-type traps (modified live-catching box traps) have collected many hair samples (n=115, x=38.33, SE=33.38) although they were placed only in three species (wildcat, European rabbit, ferret) enclosures. Type C traps (PVC cubbies) collected less hair samples (n=64, x=32) than the two other trap types, but they were placed only in two species (rabbit, ferret) enclosures. The low samples size might be the result of the fact that PVC tubes are hard to stabilize properly, due to this animals may not feel confortable in an unknown moving object. However, the material cost is really low (2-2.5 EUR/pcs) for making the PVC cubbies, so I recommend this trap type for further testing, especially in case of small mustelids. In addition, Tóth (2003) fixed the cubbies to the ground with large nails at the sides of the tubes in order to stabilize the traps.

As a hair collection surface the wire brush proved to be the best (n=131, x=13.1, SE=14.87). However, the two-sided sticky tape also produced a large number of samples (n=79, x=15.8, SE=13.53), but removing the hairs from the adhesive proved to be a challenging task. Moreover, the sticky tape does not always remain adhesive in cold and wet environment, the tape is difficult to change at field conditions, and the hair can be broken during the removal process (Mowat & Paetkau 2002, Patkó unpublished data).

Nests and artifical nest boxes: natural hair traps

In my most urbanized study area (Gödöllő Lower Park and University Park) I have found 15 nests and identified 41 hair samples. The following species inventory was made at Gödöllő: brown hare, European mole, hazel dormouse, horse, dog and human. Most samples were human hairs, in 81.81% of the nests human hair samples were found. I collected 13 nest in Merzse swamp and successfully identified 34 hair samples. Of these, I have identified five species (brown rat, brown hare, edible dormouse, Eurasian otter, human) and two twin-species (hazel and forest dormouse; weasel and stoat). In one nest otter hairs were found. From a faunistical point of view, the otter may be of particular interest. The presence of the otter has

not yet been proved from this area, but according to literature data otter has a stabile population and wide area of occurance in Hungary (Lanszki et al. 2008, Lanszki 2008), so it may well have appeared in the Merzse swamp also. I have collected 12 nests and next boxes from the most natural sample site, Sár-hegy SAC sample site of Mátra. A total of 55 hair samples (x=4.58, SE=6.69) were found. Altogether 11 species have been been described from this site. Wild boar and other ungulate species' hair samples were most commonly found. The occurance of the Eurasian beaver is surprising at Sár-hegy, but vagrant individuals in search for new locations may occur nearby, even though the lake (St. Anna) at Sár-hegy only considered as temporary water. In the vicinity of the sample site there are several larger water surfaces (e.g. Markaz water reservoir, Domoszló fishing lake), which can be considered as suitable habitats for beavers. In 2005, several individuals were released in a fishing lake near Sár-hegy, and beavers are now also considered to be a more widely spread species on a national level (Haarberg 2007).

I believe that the quantity of nests and artifical nest boxes, as well as the amount of hair samples found in them, shows that the method can be efficiently used at urban (Patkó et al. 2014) and natural areas (Láng 2016, Tóth 2008, Ondrušová & Adamík 2013). However, for determining species based on hair samples reference materials and practice are needed (Lobert et al. 2001, Spaulding et al. 2000).

Artifically scented hair traps on Natura 2000 sites

During the first field sampling at Mátra, I collected 51 hair samples of which 13 (25.49%) belonged to carnivore species. Most commonly (n=16, 32%) ungulate species occurance was shown. In addition to humans, I could detect 12 species or twin-species from the area. In one case I found Eurasian lynx hair on a rub pad. The lynx was previously unknown from this area. Detection of several species is fortunate when the aim is to compily a species list (faunistical data), but cross contamination can complicate DNA based approaches when more hair samples are found on one trap (Long et al. 2008). Contamination can be reduced by shortening the time interval between rebaiting periods, using species-specific attractants, or by the thorough morphological pre-selection of hair samples. During the sampling in 2015, less hair samples were collected (n=23). The lower sample size could have been accidental, but the sample size also could have been effected by field personnel whom were less experienced in collecting hair samples. Studies have demonstrated that the lack of practice in case of volunteers leads to less accuracy, for example, in case of density estimation

(Foster-Smith & Evans 2003). In a longer term study, experience of field personnel is likely to be increased, which can ultimately lead to minor inaccuracy.

During the first year of sampling at Kiskunság, I could collect 51 hair samples, just as in Mátra. Most commonly (n=18, 35.29%) small carnivores have been sampled. Apart from human, 12 other species or twin-species were shown from Kiskunság. Of the 51 hair samples, 24 (49.02%) belonged to carnivores. In the second sample collection campaign, I could collect less hairs (n=27). Eight (29.63%) of these samples belonged to carnivore species. Many of the samples, however, were not the hairs of native animals typical of the area, but belonged to stray or feral animals (e.g. dog, cat) of nearby farms. The golden jackal sample was not collected by the species specific sampling device (rub pad), but with a small modified live-catching box trap. This phenomenon is presumably not uncommon, and other sources also mention that traps occasionally sample non target species (e.g. because of sniffing around, rubbing, or scratching the traps) (Ausband et al. 2011).

Efficiency of scented hair traps

In the study area of Kiskunság the hair collection was more successful than at the Mátra. The first quarter of the year seems to be the most suitable time for hair collection. During the field visits I found 20-25 hairs per month from January to April. However, in October, I had not visited the traps and in June I have only visited the field once. At the same time, there were field visits on both sites when I could not find a single hair sample. In general, PVC cubbies and modified box traps have collected more samples. These tools have been repeatedly snagged hairs, so one trap in a campaign has often collected more than one sample. The number of hairs which can give a sample may vary among the international literature (Patkó et al. 2016b). One sample can be when a species specific identification is carried out, but this may be misleading. For some species (e.g. bear, badger, roe deer), based on a single well-developed dorsal guard hair species can easily be determined, so a piece of hair can be a sample. If authors do not clarify this issue (sample size, number of hairs) in the articles it could be deceiving for the readers. In this study, it was also the case that the samples usually contained less than five hairs, but this is not a unique feature of hair collection researches (Bullington, pers. comm.). Tom (2012) reported that none of his hair traps could collect samples repeatedly, and traps could snag an average of 1.7 hairs.

Opportunistic sample collection

Of the 27 opportunistically collected hair samples 11 (40.74%) belonged to large carnivore species. In other cases horse (n=2, 7.4%), red or roe deer (5%, 18.52%), wild boar (n=5, 18.52%), fox (n=1, 3.7%), felids (n=2, 7.4%) and human (n=3, 11.11%) hair samples were mistakenly identified at the field as large carnivore hairs.

Randomly collected samples in field can also help to prove the presence of protected Natura 2000 species. More than 40% of the samples turned out to be large carnivore hairs, which proves that random search for hair samples can result in a succes if field personnel know what to look for. With these random searches the presence of wolf and bear, which has already been known mostly from unreliable tabloid sources, has been proven securly and scientifically.

5. AUTHOR' S PUBLICATION RELATED TO THE SUBJECT OF THE DISSERTATION

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