European Commission

ENGAGING STAKEHOLDERS IN WILDLIFE MONITORING

A PILOT STUDY OF WOLVES IN SLOVAKIA USING NONINVASIVE GENETIC SAMPLING

Robin Rigg, Tomaž Skrbinšek & John Linnell

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The work described in this report constituted a pilot action on large carnivores developed within the project *Support to the European Commission's policy on large carnivores under the Habitats Directive – phase 2* (contract no. 07.0307/2013/654446/SER/B.3), financed by the European Commission and implemented by the Istituto di Ecologia Applicata, Rome, with guidance from the Large Carnivore Initiative for Europe (IUCN/SSC LCIE). The pilot action was implemented in November 2013 – November 2014 by the Slovak Wildlife Society in cooperation with the Slovak Hunting Union, Forests of the Slovak Republic state enterprise, organisations of the State Nature Conservancy of the Slovak Republic and the Animal Ecology Group at the Biotechnical Faculty of the University of Ljubljana.

TITLE: **Engaging hunters and other stakeholders in a pilot study of wolves in Slovakia using non-invasive genetic sampling**

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EXECUTIVE SUMMARY

One of the many controversies surrounding large carnivores relates to knowledge over their status (distribution, numbers and population trends). A major part of this conflict concerns whose knowledge is 'right' when different stakeholder groups proffer disparate information. Wolf numbers are intrinsically hard to census, with controversy arising from uncertainty in the interpretation of numbers of wolves from tracks and signs as well as the added complication of the same wolves being counted more than once as they move between hunting units. In an effort to address these issues, considerable effort has been invested in some parts of Europe to involve a diversity of stakeholders (hunters, foresters, livestock producers, naturalists) into the co-generation of knowledge through robust, objective approaches that maximise effort through 'citizen science'.

On the basis of these precepts we developed a project to address conflicts between stakeholders arising from differing perceptions of wolf abundance. Slovakia was a logical focus for activity due to its importance as a range state and ongoing controversy concerning wolf hunting management. The resulting one-year project, which began in November 2013, was one of the pilot actions on large carnivores at the population level developed within the project *Support to the European Commission's policy on large carnivores under the Habitats Directive – phase 2* (contract no. 07.0307/2013/654446/SER/B.3). As such it was financed by the European Commission and implemented by the Istituto di Ecologia Applicata, Rome, with support from the Large Carnivore Initiative for Europe (IUCN/SSC LCIE).

The action was implemented as a cooperative effort between the Slovak Wildlife Society, the Slovak Hunting Association, Forests of the Slovak Republic state enterprise and organizations of the State Nature Conservancy of the Slovak Republic as well as the University of Ljubljana's Animal Ecology Research Group.

Fieldwork was conducted from December 2013 to June 2014, but most intensively in January–February. Around 60 people participated, including trained conservation volunteers, hunters, foresters, national park staff, environmentalists, researchers and students. Opportunities for tracking were limited by an unusually mild winter with little snow. Nevertheless 112 non-invasive samples were collected for genetic analysis: 60 from urine, 50 scats and two hair samples.

Useable genotypes were obtained from 46 (41%) of the samples. We established that there were a minimum of 20 different wolves in the study area at the time of sampling. These animals were members of at least five and possibly up to nine separate family groups (packs). Genetic diversity was found to be relatively high, comparable to that reported in the large Dinaric wolf population. There was no evidence of hybridization between wolves and dogs. Based on the genetic data and assuming an average pack size of five individuals, the number of wolves in the study area was estimated at 20–45 individuals. However, there were insufficient data to permit a mark-recapture analysis so the upper figure is less robust and warrants further investigation.

This pilot action successfully served its purpose: testing procedures and identifying problems whilst obtaining sufficient results to demonstrate the potential utility of the approaches and provide opportunities to learn from the experience to produce a model for eventually up-scaling to the regional, national or transboundary levels. We believe it paves the way for further studies of wolves in Slovakia using molecular genetics that would form the basis for science-based management of this species at the population level.

INTRODUCTION

One of the many controversies surrounding large carnivores concerns a conflict of knowledge over their status (distribution, numbers and population trends). Part of this conflict concerns a simple lack of knowledge from areas where no field surveys have been conducted. However, a major part concerns a conflict over whose knowledge should count most, with local (lay) knowledge often being placed into opposition against more scientific knowledge or different stakeholder groups proffering disparate information. Wolf numbers are often hard to census, with controversy arising from uncertainty in the interpretation of numbers of wolves from tracks and signs as well as the added complication of the same wolves being counted more than once as they move between hunting units.

In an effort to address these issues, considerable effort has been invested in some parts of Europe (e.g. Nordic and Baltic countries) to involve a diversity of stakeholders (hunters, foresters, livestock producers, naturalists) into the co-generation of knowledge through robust, objective approaches that maximise effort through 'citizen science'. This combination takes advantage of the considerable voluntary manpower that stakeholder organisations can mobilise and the 'many-eyes' effect of widely distributed observers whilst maintaining and improving data reliability. It can thus help to reduce conflicts over the relative validity of different knowledge forms as the information is both dependable and generated in a cooperative manner.

On the basis of these precepts we therefore developed a project to address conflicts between stakeholders arising from differing perceptions of wolf abundance in Slovakia. This one-year project, which began in November 2013, constituted one of the pilot actions on large carnivores at the population level developed within the project *Support to the European Commission's policy on large carnivores under the Habitats Directive – phase 2* (contract no.

07.0307/2013/654446/SER/B.3), financed by the European Commission and implemented by the Istituto di Ecologia Applicata, Rome, with support from the Large Carnivore Initiative for Europe (IUCN/SSC LCIE).

Our aim was to work intensively with hunters and other stakeholders within a reference study area to obtain a census of the local wolf population. Building on traditional snow-tracking, supplemented by camera trapping, the action added the element of non-invasive genetic sampling to obtain an objective estimate of the true population size within the study area. Samples of wolf urine, scat and hair collected while snow-tracking or surveying forest roads can be used to verify the species (in cases where wolf and dog tracks may be confused) and identify individual wolves, their sex and kinship relations. Obtaining multiple samples from the same animals also permits the mapping of packs.

Slovakia is a logical country to be the main focus for this pilot action because of its importance as a range state (Kutal & Rigg 2008) and the background of ongoing controversy and conflicts between stakeholders concerning wolf hunting management (Rigg 2008). Conducting the study in proximity to state borders also provided the potential to organise sample collection in a neighbouring country in order to gain insight into the extent of transboundary wolf movements.

The action was implemented as a cooperative effort between the Slovak Wildlife Society, the Slovak Hunting Association, Forests of the Slovak Republic state enterprise and organizations of the State Nature Conservancy of the Slovak Republic as well as the University of Ljubljana's Animal Ecology Research Group, who were partners in the recently completed LIFE+ SloWolf project (LIFE08 NAT/SLO/000244) . By involving multiple stakeholders in a process of collaborative generation of data, the project aimed to demonstrate the potential utility of the approaches on a small scale and provide opportunities to learn from

the experience to produce a model for eventually up-scaling to the regional, national or transboundary levels. The work developed in this project covered four different tasks:

- Engaging key stakeholders and authorities in project activities;
- Conducting fieldwork with the involvement of local hunters, foresters and national park staff as well as conservation volunteers;
- Genotyping wolves in the reference area by non-invasive DNA sampling; and finally
- Running a workshop with stakeholder participation to present and interpret the results of genetic analysis and their implications for monitoring.

The goal of the pilot action was to obtain high quality data on the local wolf population in the reference study area with transparent and robust interpretation that is not contested by different stakeholders. The demonstration of a state-of-the-art approach to improving knowledge of large carnivore population status should help to foster a critical appraisal of traditional methods and will hopefully open the way for improved methods and collaborative data generation in the future.

STUDY AREA

We selected the Liptov region of northern Slovakia, bordering Poland, as the reference area for the study. It includes parts of three national parks (Tatras NP - SKUEV0307; Low Tatras NP - SKUEV0302 and SKUEV0310; and Veľká Fatra NP - SKUEV0238) as well as pastures where conflicts due to wolf predation on livestock are an issue during the grazing season (Rigg *et al.* 2011). Its proximity to the border with Poland provided the potential for a trans-boundary element to the study.

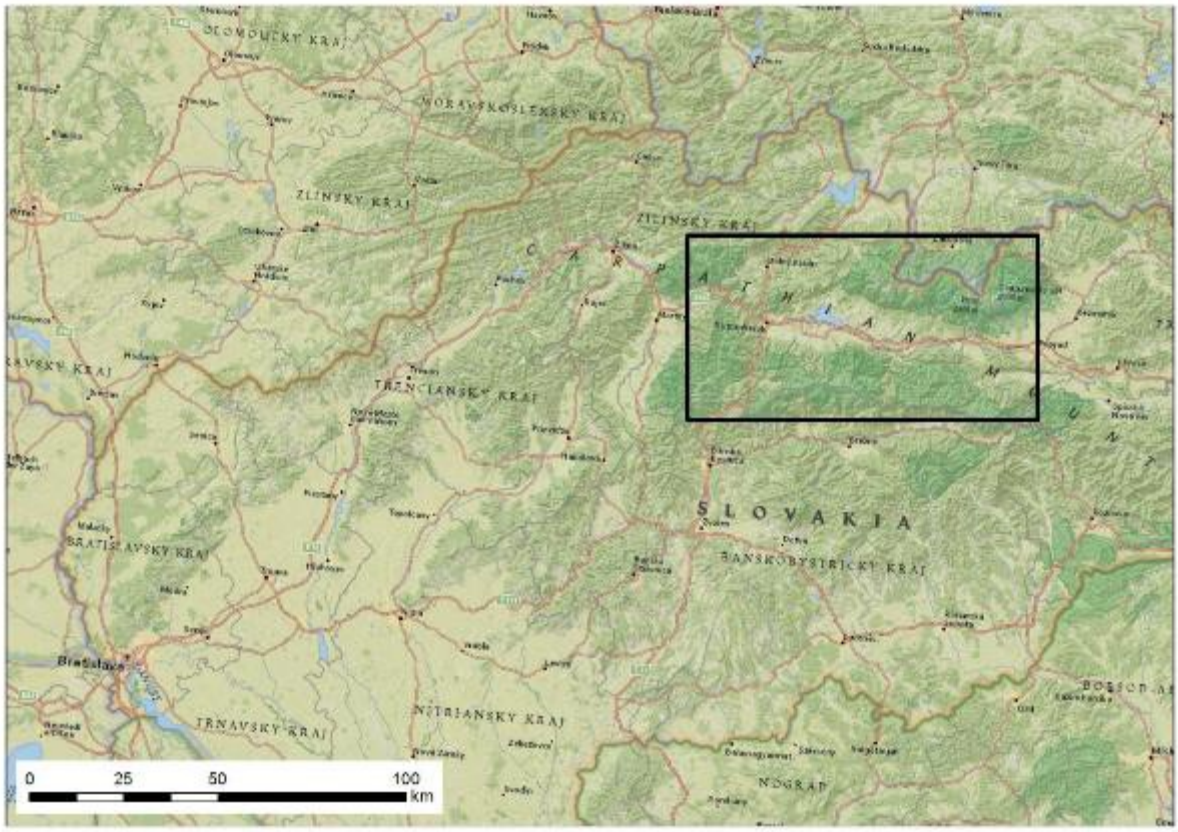

Figure 1. *The study area in the Tatra Mountains of northern Slovakia.*

ENGAGING STAKEHOLDERS

The concept of the project was discussed with the Director General of the Section for Nature Protection & Landscape Development at the Ministry of the Environment of the Slovak Republic on 13th November 2013 and, at his invitation, the planned activities were presented to the Ministry's multi-stakeholder large carnivore group, whose members represent hunters, foresters, protected area staff, environmentalists, researchers and others. The group and pertinent agencies were subsequently informed of the project's launch and invited to participate.

Discussions were held with the Executive Director of the Slovak Hunting Association at the stakeholder workshop held in Brussels on 5th December 2013 and collaboration was agreed. On 31st January we supplied the Association's representative with sample tubes and collection protocols. On 6th February the Association held an internal meeting of its members and representatives within the reference study area to discuss the project and disseminate sample collection kits. Sample collection materials and instructions were also provided to staff of the Tatras National Park, Veľká Fatra National Park and the Polish Tatras National Park as well as to staff of state enterprise Forests of the Slovak Republic.

After the completion and finalisation of genetic analyses, results were presented and discussed at a workshop held at the offices of the Slovak Hunting Association in Bratislava on 14th November 2014. In addition to representatives of the Association and the Slovak Hunting Chamber, the meeting was attended by officials from the Ministry of Agriculture & Rural Development and the Ministry of the Environment as well as representatives of hunters from three districts within the study area (Liptovský Mikuláš, Ružomberok and Martin). Feedback was prevailingly positive and willingness was expressed to explore future possibilities to strengthen hunter participation in monitoring, potentially including a national-level survey.

MATERIALS AND METHODS

Study design

We used simulations in program MARK (White & Burnham 1999) to estimate the required sampling effort. Assuming the presence of 5–10 packs of five wolves each in the study area and with an expected genotyping success rate of 50%, we simulated different sampling intensities to explore the expected confidence intervals. We performed 200 simulations for each combination of parameters, and used C^ = 2 extra-binomial variation. Results are presented in Table 1. We thus aimed to collect 130–150 samples, which would (with random sampling) provide a confidence interval of approximately 18–35%.

***Table** 1. Results of mark-recapture simulations done to plan for sampling effort.*

Study size (packs)	Sim. N animals	Sim. N samples	Samp. intervals	Nsamples/ interval	Mean est. N	Exp. s.e.(N)	Exp. 95%CI	Conf. interval	Gen. success	Plan. N samp.
15	**75**	225	6	38	76.10	3.07	**70 - 82**	**7.99%**	50%	**450**
15	**75**	150	6	25	76.85	4.61	**68 - 86**	**11.88%**	50%	**300**
15	**75**	112.5	6	19	78.32	10.61	**57 - 99**	**26.82%**	50%	**225**
10	**50**	150	6	25	50.14	2.80	**45 - 56**	**11.06%**	50%	**300**
10	**50**	100	6	17	51.45	5.47	**41 - 62**	**21.05%**	50%	**200**
10	**50**	75	6	13	51.42	8.45	**35 - 68**	**32.54%**	50%	**150**
5	**25**	80	6	13	26.13	1.87	**22 - 30**	**14.13%**	50%	**160**
5	**25**	65	6	11	26.11	2.39	**21 - 31**	**18.12%**	50%	**130**
5	**25**	40	6	7	27.11	6.49	**14 - 40**	**47.40%**	50%	**80**

A prioritization analysis was performed to help select which samples to include in cases when several samples were collected on the same day from the same locality.

Fieldwork and sample collection

Snow-tracking, camera trapping and collection of samples for genetic analysis began in late December 2013 and continued until June 2014, but were most intensive in January–February. A total of around 60 people participated, including trained conservation volunteers, hunters, foresters, national park staff, environmentalists, researchers and students (see Appendix 1). Efforts were somewhat hampered by an unusually mild winter with limited opportunities for snow-tracking. Nevertheless samples were collected throughout the study area and images of wolves were obtained using camera traps at several different localities (see Appendix 2).

Sample handling and quality control

Scat samples were collected in 1.5 ml flasks filled with DETs preservation buffer which preserves the target DNA and makes the samples simple to ship by post since it is non-toxic and non-combustible. Urine samples were collected in 50 ml flasks filled with EDTA-Ethanol-NaOH mixture designed to preserve the target DNA. Scat and urine samples were kept frozen prior to transport to the laboratory. Hair samples were collected in paper envelopes, air-dried and stored at room temperature.

Each 50 ml sample tube or re-sealable plastic bag containing a 1.5 ml tube was fitted with a label for field data entry to keep the data with the sample. Data for hair samples were written on each envelope. The estimated age of each scat was recorded, as previous studies have found the age of the scat to have a considerable effect on expected amplification success.

We used a dedicated laboratory for DNA extraction from non-invasive samples where we enforced strict rules regarding movement of personnel, equipment and material to prevent contamination, and used negative controls throughout. Pipette tips with aerosol barriers were used for all liquid transfers. Upon entry in the laboratory the data for each sample was

entered into a relational database. Barcodes were used to track samples through the genotyping process and eliminate manual data entry. Each critical step in analysis was photo-documented to 'catch' possible errors.

Genotyping and individual ID assignments

Samples were genotyped at 16 canine unlinked autosomal microsatellite loci in one PCR multiplex (AHT137, AHTh171, AHTh260, AHTk211, AHTk253, CXX279, FH2054, FH2848, INRA21, INU030, INU055, REN162C04, REN169D01, REN169O18, REN247M23, REN54P11) and the Amelogenin locus, which was used for sex determination. Preliminary testing showed that this marker set does not give specific PCR products for domestic or wild ungulate DNA (unpublished data). Loci AHTh260 and AHTk2011 and CXX279 were omitted from downstream analysis due to genotyping problems, yielding a total of 13 loci and sex ID locus useful for individual ID.

We observed very strict regimes for sample handling and analysis to avoid any possibility of contamination. In the first screening process, each sample was amplified with the full genotyping PCR protocol twice and analysed on an automatic sequencer (Applied Biosystem ABI 3130xl Genetic Analyzer). Samples that provided no specific PCR products at this stage were discarded; others were genotyped up to eight times, with reliability of the genotype being checked with Reliotype maximum-likelihood approach after each genotyping run.

Genotypes were then matched using MisBase software to see if they belonged to the same animal or to an animal with an already known genotype. If a genotype was reliably matched to another reliably genotyped sample, it was accepted even if the genotype reliability was still below 0.98 threshold (since the probability of a reliable match to a reliably genotyped sample in the presence of errors is marginal). If a sample was not matched to

another reliable sample, the analysis was repeated until reliability reached the 0.98 threshold, or discarded after eight replications if this threshold was not reached. If the quality index of a sample was below 0.4, unmatched samples were also discarded since the DNA quality was too low to provide a reliable genotype.

Since a large number of microsatellite markers relative to the expected number of animals in the study area was used, we allowed for some mismatch between samples. However, no direct incompatibilities (different alleles between samples) were allowed. To avoid the problem of incorrect individual assignment due to false alleles, we set a minimum threshold of two clear observations of an allele in separate PCRs / sequencer runs before the allele was considered 'true'.

Extended genotyping for 'reference' samples

The best-amplifying ('reference') sample of each individual animal was amplified using a panel of nine additional microsatellite loci (C09_250, C20_253, CPH12, CPH5, CPH7, CPH8, CPH9, Cxx_121, FH2010) for parentage and hybridization analysis, bringing the total number of useable microsatellite markers to 21. Another sex-ID locus (SRY) was used to double-check designation of sex. The same quality-assurance procedure that was applied for the individual-ID panel was also applied for the extended panel.

Genetic diversity

We used program Arlequin (Excoffier & Lischer 2010) to calculate genetic allelic diversity, expected and observed heterozygosity, and test for significant deviations from Hardy-Weinberg equilibrium. We compared the estimated genetic diversity with genetic diversity of the large wolf population in the Northern Dinaric Mountains (Skrbinšek, unpublished). We also calculated probability of identity and probability of identity for siblings

for each locus (Waits *et al.* 2001) to determine the power of our locus panel to distinguish individual animals.

Testing for hybridization with domestic dogs

Hybridization with dogs is an important threat to wolf conservation in many areas (Godinho *et al.* 2011). We used genotypes of 50 domestic dogs and 50 reference wolves from the Dinaric Mountains as known-sample clusters to determine if the canids detected in the study area in Slovakia were pure wolves. We used Bayesian clustering in program Structure (Pritchard *et al.* 2000).

Parentage and sibship assignments

Parentage and sibship assignments enable us to identify family groups and estimate the number of packs present in the study area even when data are too sparse to allow for a reliable mark–recapture estimate. We used program Colony (Jones & Wang 2010) to simultaneously assign parentage and sibship and determine family groups (packs) in the area.

RESULTS

Sample collection and genotyping success

A total of 112 non-invasive samples of wolves were collected: 60 urine samples, 50 scat samples and two hair samples. This was less than the study design called for so all were used for DNA extraction.

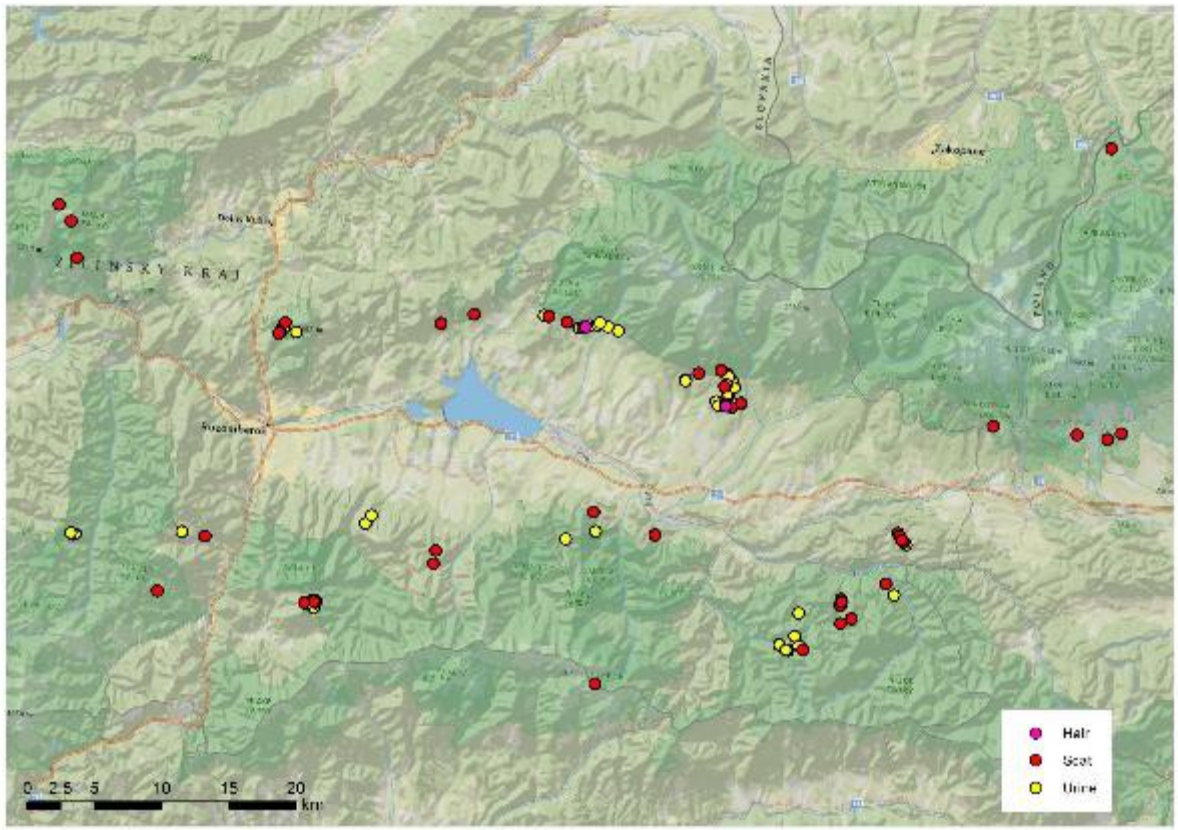

Figure 2. *Geographic distribution of collected samples.*

We successfully obtained genotypes from 54 samples (48%). Eight samples were problematic (mixed genotypes, unreliable genotype even after eight repetitions) and so were discarded, leaving a total of 46 useful samples (41%).

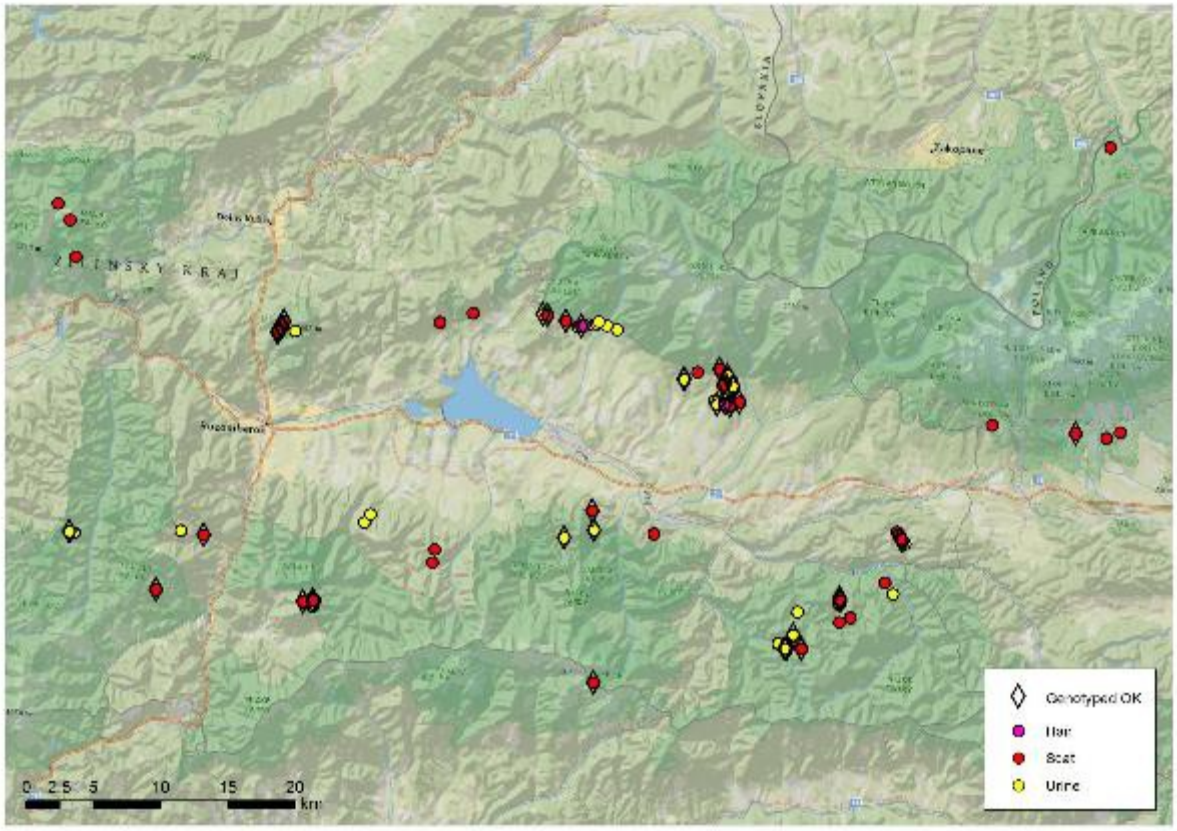

Figure 3. *Geographic distribution of successfully genotyped samples.*

Individual animals and recaptures

We identified 20 different animals, each 'captured' between one and nine times, among the collected samples. Eleven of these animals were recaptured, i.e. were identified from more than one sample. However, many recaptures occurred within a single day and originated from samples collected while following a single snow track. After eliminating such pseudo-recaptures, there were only four effective recaptures of four animals, which was not sufficient to allow any attempts at mark-recapture modelling.

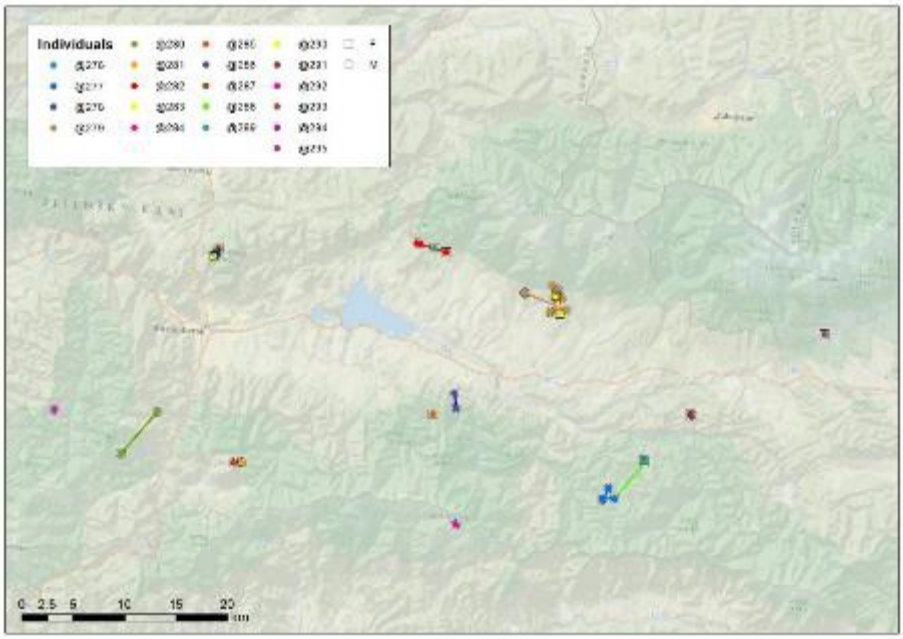

Figure 4. *Locations of samples of individual detected wolves. Lines connect samples from the same animal in chronological order.*

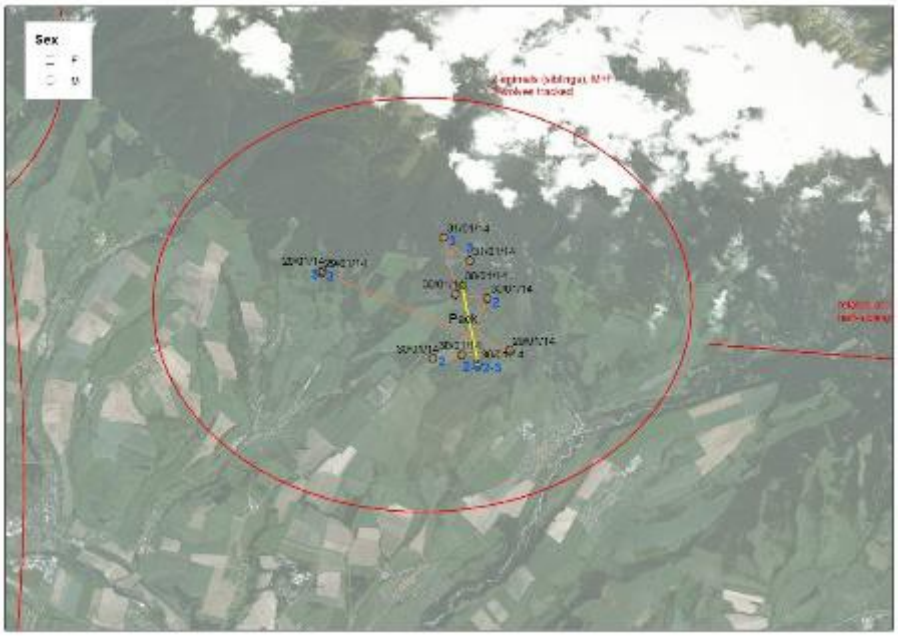

Figure 5. *Example of discrepancy between pseudo-recaptures and effective recaptures. Although the two wolves detected in the area shown in the figure appear to have been recaptured several times, these recaptures all originate from a single multi-day tracking session. There are actually no effective recaptures in these data.*

Genetic diversity

The genetic diversity data are presented in Table 2.

Table 2. *Genetic diversity indices, per-locus information content and results of Hardy-Weinberg Equilibrium tests. Ind/Par – locus was used for individual ID and parentage assignments (Ind) or just for parentage assignments (Par). N – number of individuals; A – allelic diversity; Ae – effective number of alleles; Ho – observed heterozygosity; He – expected heterozygosity; HW_p – p-value of Hardy-Weinberg equilibrium test; PI – probability of identity; Cm_PI – cumulative probability of identity; PIsib – probability of identity for siblings; Cm_PIsib – cumulative probability of identity for siblings.*

Locus	Ind/ Par	N	A	Ae	Ho	He	HW_p	PI	Cm_PI	PIsib	Cm_Pisib
AHTh171	Ind	20	6	4.57	0.85	0.80	0.99	0.07	0.06807	0.38	0.37951
REN169D01	Ind	19	5	4.46	0.95	0.80	0.60	0.07	0.00510	0.38	0.14574
AHT137	Ind	20	6	3.60	0.85	0.74	0.08	0.11	0.00055	0.42	0.06109
INRA21	Ind	20	6	3.45	0.70	0.73	0.15	0.11	0.00006	0.43	0.02610
REN162C04	Ind	20	4	3.28	0.80	0.71	0.31	0.13	7.90E-06	0.44	0.01143
INU030	Ind	19	4	3.27	0.47	0.71	0.06	0.13	1.01E-06	0.44	0.00502
AHTk253	Ind	16	4	2.91	0.69	0.68	0.71	0.15	1.50E-07	0.46	0.00233
REN54P11	Ind	20	5	2.88	0.75	0.67	0.56	0.14	2.16E-08	0.46	0.00108
FH2848	Ind	20	5	2.52	0.55	0.62	0.43	0.18	3.95E-09	0.50	0.00054
REN169O18	Ind	20	5	2.14	0.50	0.55	0.06	0.23	9.14E-10	0.55	0.00030
REN247M23	Ind	15	4	2.09	0.67	0.54	0.62	0.27	2.42E-10	0.56	0.00017
INU055	Ind	20	5	1.61	0.35	0.39	0.68	0.38	9.25E-11	0.66	0.00011
C20_253	Par	20	6	3.92	0.80	0.76	0.88	0.09	8.56E-12	0.40	0.00004
CPH8	Par	19	4	3.52	0.68	0.74	0.92	0.12	1.04E-12	0.43	0.00002
Cxx_121	Par	20	6	3.25	0.70	0.71	0.94	0.13	1.31E-13	0.44	8.28E-06
CPH7	Par	20	5	3.17	0.80	0.70	0.87	0.13	1.67E-14	0.44	3.67E-06
C09_250	Par	20	4	3.21	0.65	0.71	0.19	0.14	2.34E-15	0.44	1.63E-06
CPH9	Par	20	7	2.68	0.55	0.64	0.06	0.14	3.38E-16	0.48	7.80E-07
CPH12	Par	19	3	2.52	0.68	0.62	0.60	0.23	7.71E-17	0.51	3.97E-07
CPH5	Par	18	4	1.59	0.44	0.38	1.00	0.39	3.04E-17	0.67	2.66E-07
FH2010	Par	19	4	1.39	0.21	0.29	0.03	0.51	1.56E-17	0.75	1.98E-07
Average			4.86	2.95	0.65	0.64					

The panel of loci used to distinguish individual animals had considerable power: the probability of two unrelated animals sharing the same genotype was 9.25×10^{-11}, and the same probability for siblings was 1.1×10^{-4}, making incorrect assignments highly unlikely. We did not detect any deviations from Hardy-Weinberg equilibrium. Genetic diversity seemed high, and comparable to that observed in the large wolf population in the Northern Dinaric Mountains (Table 3; paired-samples t-test p=0.250).

***Table** 3. Comparison of expected heterozygosity between the Northern Dinaric Mountains (Slovenia and Croatia) and Slovakia. The difference is not statistically significant (paired-samples t-test p=0.250).*

Locus	Dinaric Mts.	Slovakia
AHT137	0.83	0.74
AHTh171	0.70	0.80
AHTk253	0.74	0.68
C09_250	0.77	0.71
C20_253	0.80	0.76
CPH12	0.70	0.62
CPH5	0.74	0.38
CPH7	0.52	0.70
CPH8	0.60	0.74
CPH9	0.46	0.64
Cxx_121	0.73	0.71
FH2010	0.70	0.29
FH2848	0.76	0.62
INRA21	0.67	0.73
INU030	0.72	0.71
INU055	0.72	0.39
REN162C04	0.55	0.71
REN169D01	0.73	0.80
REN169O18	0.52	0.55
REN247M23	0.81	0.54
REN54P11	0.67	0.67
Average	0.69	0.64
s.d.	0.10	0.14

Hybridization with dogs

Structure analysis showed no evidence of hybridization between wolves and dogs in the area. All detected canids clustered clearly as wolves (Figure 6).

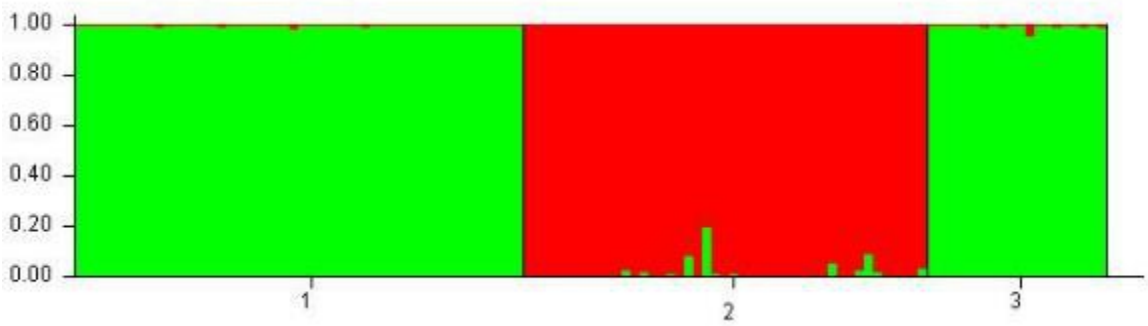

Figure 6: *Structure plot for the wolf-dog hybridization analysis, K=2. 1 – reference wolves, N Dinaric Mts.; 2 – reference dogs; 3 – canids detected in Slovakia in the present study. Each column is an individual, the proportion of green/red shows the estimated probability of assignment as a dog/wolf. The reference dog samples included several Czechoslovak wolf-dogs.*

Genetic distance between Dinaric and Carpathian wolves

Although both Dinaric and Carpathian (Slovak) wolves clustered clearly as wolves, there was considerable structure between Slovakia and the Dinaric Mountains (Figure 7, pairwise Fst = 0.145). Considering the large geographic distance between both populations and unfavourable habitat that lies between them, this result is expected.

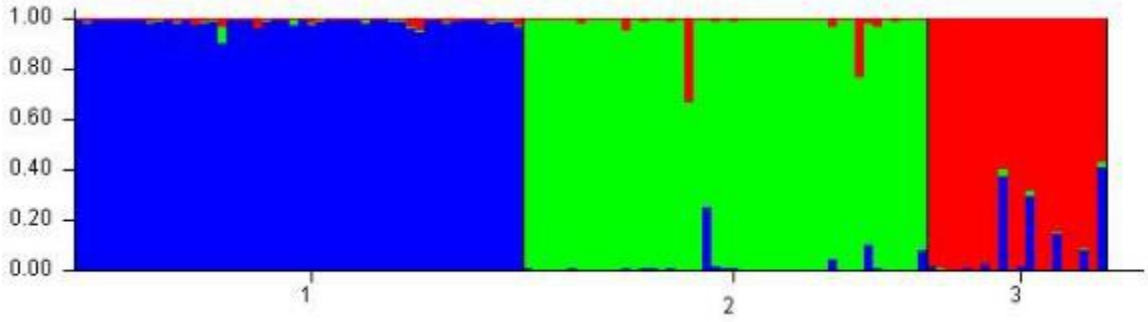

Figure 7. *Structure plot for the analysis of genetic structure between Slovakia and the Dinaric Mts., K=3. 1 – Dinaric wolves; 2 – reference dogs; 3 – Carpathian (Slovak) wolves. Each column is an individual, the proportion of blue/green/red shows the estimated probability of assignment to a Dinaric/dog/Slovak cluster.*

Parentage analysis and pack structure in the area

We identified nine groups of unrelated or low-relation animals among the samples (Figure 8). While there are five clear packs, there are also four additional, unrelated or low-relation animals that are either dispersers or members of poorly sampled residential packs. Since sampling intensity was relatively low and dispersers are usually more difficult to detect than resident animals, most of these animals probably belong to under-sampled packs.

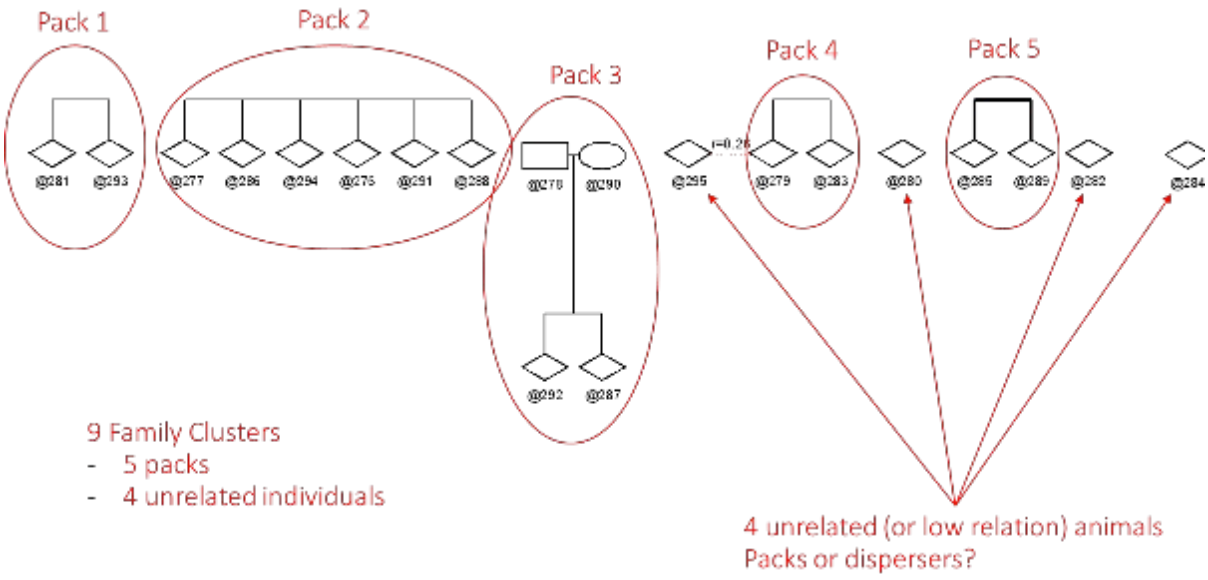

Figure 8. *Family structure detected by genetic sampling. There are five clear packs (siblings or parents/offspring pairs), and four additional, unrelated or low relation individuals that can be either members of poorly sampled packs or individual dispersers. Since sampling intensity was low and dispersers are usually more difficult to detect than resident animals, most of these animals probably belong to under-sampled packs.*

Spatial structure corresponds well with the estimated family structure, and shows that there are probably from five to nine residential packs in the area (Figure 9).

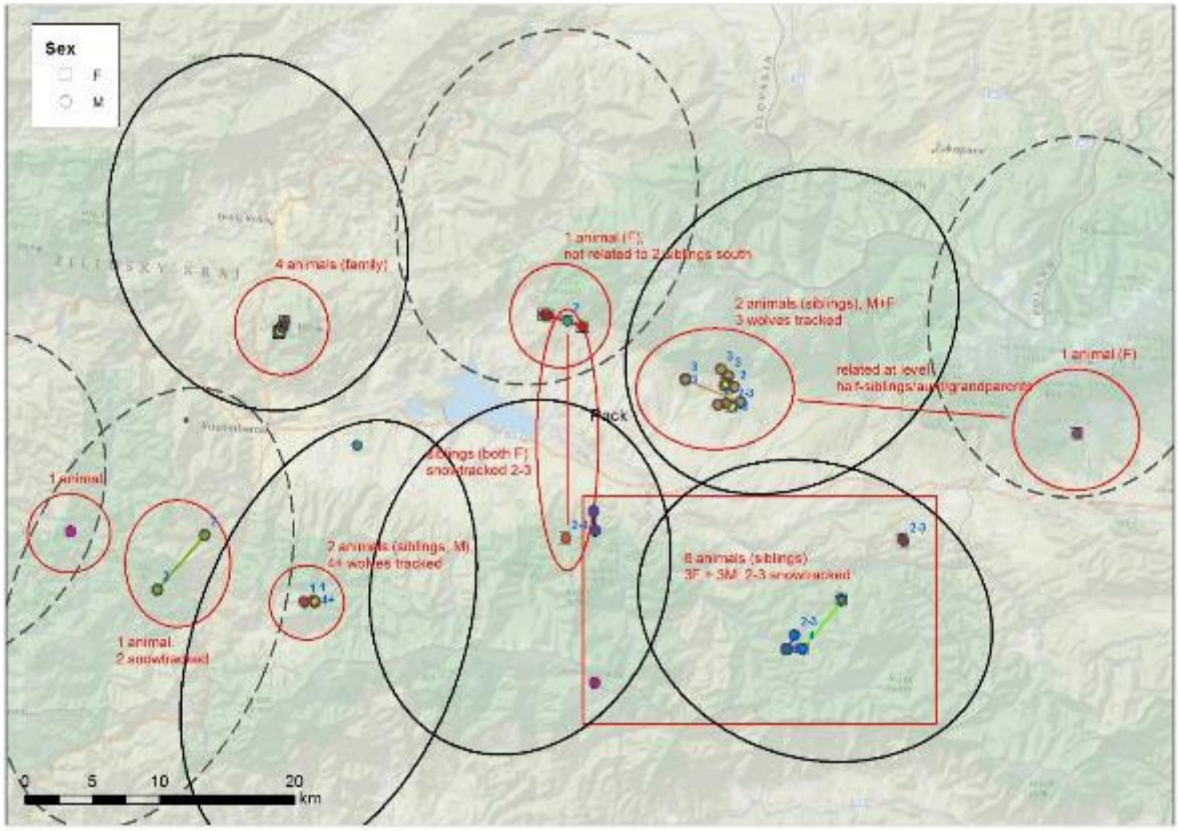

Figure 9. *Family groups and unrelated individuals (red lines), possible pack areas (black lines). Solid lines – detected family structure, dashed lines – detected unrelated individuals. The area of 'pack' ellipses is ~450 km^2, which corresponds to wolf home-range size in the Dinaric Mountains as determined by GPS telemetry.*

The map also show that the packs are distributed on both sides of the Liptov Valley that intersects the middle of the study area and seems to act as a partial ecological barrier (river, lake, agricultural areas, human settlements) separating wolf habitat.

DISCUSSION

The goal of this pilot project was to conduct a preliminary survey of the wolf population in a small study area in Slovakia using molecular genetics. While the aim of the study was to obtain hard data about the wolf population in Slovakia, it was mainly conceived as a 'proof of concept', aimed at demonstrating at the local level the applicability and usefulness of molecular genetics for obtaining robust, credible scientific data about wolves in the wild. The project also aimed to build trust between various stakeholders concerned with wolf management and to catalyze the formation of a larger network of interested people that could serve as a starting point for future, larger-scale genetic surveys of Slovak wolves.

Although the project was limited in both duration and financial resources, it nicely demonstrated the potential of molecular genetics tools for surveying wildlife populations. Despite the relatively low number of samples collected, we managed to obtain significant results. We established that there were at least 20 different wolves in the area at the time of sampling. We also established that these animals were members of at least five and possibly up to nine different family groups (packs). Based on genetic results and assuming an average pack size of five individuals (Rigg 2008), we can roughly estimate the number of wolves in the study area at 20–45 individuals. However, although the lower number is certain (being the actual number of genotyped individuals), the upper number is a preliminary estimate lacking the statistical robustness that a mark-recapture estimate would have provided.

An intrinsic difficulty of mark-recapture studies is that they critically depend on the number of recaptures to estimate total population size. This, however, was the least successful part of our study. The study design called for close collaboration with hunters but, despite high-level meetings and pledges, cooperation did not always materialise at the local level and we obtained only a small number of hunter-collected samples. The vast majority of samples

were collected by other volunteers, mostly during intensive snow-tracking. This often resulted in multiple samples being collected from the same animals within a small area and/or short time period, which is not sufficient to be treated as recaptures for the purposes of data analysis. The number of effective recaptures was therefore low, precluding a mark-recapture analysis. This deficiency highlights both the importance and the difficulty of engaging local hunters. It was also partly due to bad luck: nation-wide genetic sampling of brown bears was taking place at the same time, and hunters were less willing to participate in an additional study. This situation was aggravated by an unusually mild winter with little snow. Colleagues in Poland who were provided with sample tubes did not manage to collect any samples, citing a lack of wolf sign in areas where they would normally expect to find them.

A second issue for careful consideration in the design of future studies is the selection of sampling area. Due to the short time available for planning and implementation, we selected an area that we were confident could be surveyed adequately given the limited resources available. As this area was intersected by a human-populated river valley, which seems to present a partial barrier to wolf movement, we probably included more fragments of wolf pack territories than if a contiguous habitat patch was sampled in its entirety. This likely led to an underestimation of the required sampling effort. Although this had no bearing on our results, it highlights the edge effect that is an issue in relatively small-scale studies.

In conclusion, the pilot action successfully served its purpose: testing procedures and identifying problems whilst nevertheless obtaining sufficient results to demonstrate the value of the methodology. We believe it has paved the way for further, larger-scale studies of wolves in Slovakia using molecular genetics that would form the basis for science-based management of this species in the Western Carpathian Mountains.

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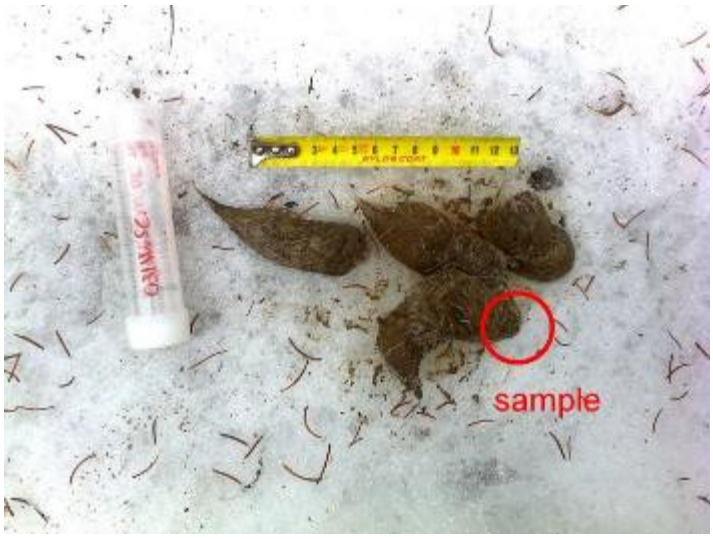
APPENDICES

Appendix 1: Photographs from meetings and fieldwork

The following images show planning and discussion meetings with hunters, foresters, volunteers and students as well as snow-tracking, sampling and camera trap installation.


sample

Appendix 2: Camera trap images of wolves

The following is a selection of images of wolves within the study area obtained by Slovak Wildlife Society staff and volunteers, hunters and national park staff using camera traps.


26.85 inHg - -3C 02/11/2014 05:13AM SWS CARC


Bushnell Camera Name0 28°F -2°C
03-19-2014 08:26:52


Ambush
3/2/2014 6:26 PM
Cuddleback


MOULTRIE
-6C
03/09/2014 02:15AM F16 N2


Ambush
3/31/2014 9:48 PM
Cuddleback


MOULTRIE
2C
05/13/2014 11:27AM F1 CERV GRUN


Bushnell
05-09-2014 04:26:21


8C
08/12/2014 06:48AM MYCAMERA