



Noninvasive Genetics Knowledge from the Brown Bear Populations to Assist Biodiversity Conservation

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Abstract: Genetic monitoring has proven helpful in estimating species presence and abundance, and detecting trends in genetic diversity, to be incorporated in providing data and recommendations to management authorities for action and policy development. We reviewed 148 genetics research papers conducted on the bear species worldwide retrieved from Web of Science, SCOPUS, and Google Scholar. This review aims to reveal sampling methodology and data collection instructions, and to unveil innovative noninvasively genetic monitoring techniques that may be integrated into the genetic monitoring of a large bear population. In North American studies, hair samples were collected more often than faeces, whereas in Europe, both faeces and hair samples surveys are recommended, usually focusing on faeces. The use of the Isohelix sample collection method, previously tested locally and, if suitable, applied at the national level, could generate numerous advantages by reducing shortcomings. Additionally, dogs trained for faeces sampling could be used in parallel with hunting managers, foresters, and volunteers for sample collection organised during autumn and winter. It was stated that this is the best period in terms of cost-efficiency and high quality of the gathered samples. We conclude that large-scale noninvasive genetic monitoring of a large bear population represents a challenge; nevertheless, it provides valuable insights for biodiversity monitoring and actions to respond to climate change.

Keywords: noninvasive genetic sampling; brown bear; management plan

1. Introduction

Some anthropogenic activities harm the environment [1,2]; however, reducing these disturbances in human-dominated landscapes is a challenge for humanity [2]. The negative impact on the environment can be easily observed in the species' biodiversity, which is also negatively impacted [3]. Species loss due to human impact is documented in various studies and books [3–6]. Biodiversity is also correlated to climatic changes [7,8]. The challenge of predicting the complex action of climate evolution makes biodiversity conservation even more difficult [8]. However various models have been developed to predict changes in climate [8,9]; therefore, diminishing potential climate change impacts on species loss is possible [10–12].

Wildlife populations are disturbed in various ways by humanised landscapes, through poaching [13], habitats' alteration under different climatic [7] and vegetation type conditions [14], loss of habitats [3,15], and gene flow limitation, thus reducing landscape connectivity [16–18]. The consequences following these threats affect the survival of wildlife populations [19–21]. At the same time, there is a clear need for considering climate change adaptation and long-term sustainability that is well anchored in very effective policies [22,23]. Wildlife conservation is closely related to population management [24],



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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). and appropriate management of wildlife populations implies constant species monitoring [25] and conservation measures specific to targeted species, or, even more specifically, sex-biased conservation measures, if needed [26]. However, genetic monitoring has played a significant role in the conservation and management of species, and the understanding of their ecology [27,28].

Most of the worldwide genetic studies related to bear species are mainly targeted at the regional level. Previous studies, such as in North America, Scandinavia, and the Life DINALP BEAR project (across Croatia, Slovenia, Austria, and Italy), delivered valuable information from comprehensive genetic studies, including interconnected transboundary bears' populations. Therefore, the conservation actions should consider the particularities of species conservation statuses at each country level, with a high focus on the shared transboundary populations [29]. Moreover, the periodic monitoring of threatened large carnivores' populations reveals changes in population conservation status, documented in the European Habitats Directive Annexes 92/43/EEC [30,31]. Genetic methods used for population size estimation and population monitoring are essential for the effective long-term management of wildlife populations [28]. Consequently, each country should allocate resources for establishing permanent genetic monitoring programs [32]. Accurate monitoring efforts of the large carnivores usually require high costs [33–37]. These are also difficult to conduct for wide-ranging species [38], especially because the individuals have greater ecological importance than economic importance [39].

Ursids are of great importance worldwide; however, in addition to their charisma [40], the bear has an ecological and economic value in its habitats [39,41,42]. The brown bear (*Ursus arctos*) has the widest distribution worldwide; it ranges from North America to Eurasia [41]. The species is known for its opportunistic behaviour regarding diet [43]. However, the intraspecific competition for food can be high [44], even if their diet is omnivorous [43,45]. Some of the populations, depending on the living area, have a great preference for plants and a low interest in a carnivorous diet [45]. Interesting facts have been concluded following this behaviour, such as the bears becoming more aggressive towards humans due to higher intraspecific predation [44]. For some studied bears, the feeding locations during the hyperphagia period impacted the selection of their den locations during winter [46].

The brown bear is listed for protection and conservation by several international acts and regulations (Bern Convention, Washington Convention on International Trade in Endangered Species of Wild Fauna and Flora) [41]. Large carnivores, including brown bears, have high priority in conservation across the European continent [47]; the species was declared to have a community interest that needs high protection (it was included in Annex IV of the Habitats Directive), so that the bear population conservation required a declaration of SAC within the Natura 2000 network (Annex II of the Habitats Directive) [48]. Therefore, conservation efforts were considered when bear hunting was banned for some states [49], better-quality habitats were modelled [50], and reintroduction actions of brown bears in specific ecosystems were accomplished [51].

Twenty-two European countries share ten brown bear populations with a permanent species presence, mostly native [47]. The Carpathian population is shared between the Czech Republic, Slovakia, Eastern Serbia, Romania, Ukraine, and Poland [52]. The roaming of individuals between these countries is facilitated by favourable and untouched habitats, the lowest fragmentation rate in Europe, and primarily by the high rate of human acceptance [52].

In Romania, this apex predator population registers approximately 6000 individuals [53], and human-bear conflict across the country has continuously increased in recent years (according to the A2 action in the frame of the LIFE FOR BEAR). The National Action Plan (NAP) (http://www.forbear.icaswildlife.ro/wp-content/uploads/2018/05/plan.pdf last accessed on the 21 December 2021) was approved in 2018 and establishes the main direction for species management and preservation of the favourable conservation status as defined by the last report to the European Commission under Article 17 of the Habitat Directive (https://ec.europa.eu/environment/nature/knowledge/rep_habitats/index_en.htm last accessed on the 19 December 2021). Proper implementation of the NAP requires rigorous monitoring of the population in Romania, as part of the intensive population monitoring objective. However, by analysing the relative strengths and weaknesses of different monitoring field methods, noninvasive genetics could contribute in choosing the optimal strategy and the more efficient allocation of resources for monitoring the Romanian brown bear population [27]. In this way, the human errors when collecting genetic samples (faeces and hair samples) will be reduced to the minimum. At the same time, proper genetic methods will deliver useful biological information, such as that regarding genetic diversity, demography, population bottlenecks, inbreeding, gene flow, or isolated populations [27,53–55]. Moreover, a well-established noninvasive genetic monitoring programme will improve data and information about the bear population's evolutionary history, connectivity, and genetic health [56].

Consequently, this research review aims to provide: (1) relevant insights to develop and improve the methodology for organising the sampling in the Romanian brown bear population, which may lead to a successful research development, with promising results enhancing the institutional (National Institute for Research and Development in Forestry Marin Dracea) and national capacity (Ministry of Environment, Water and Forests); (2) to build upon clear instructions for rigorous training concerning the methodology and instructions for data collection; and (3) to unveil innovative noninvasive better-adapted genetic monitoring techniques. These objectives will contribute to establishing the noninvasive genetic monitoring method that best fits the Romanian brown bear population. Moreover, our approach will impact on future management actions taken for the bear population, and thus increase the degree of conservation of the brown bear population in Romania.

2. Materials and Methods

This research was conducted following the guidelines provided by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRSIMA) (Figure 1). Two of the most popular scientific databases—Web of Science and SCOPUS [57]—were used for identifying relevant scientific literature. Moreover, the web-based database Google Scholar was used, achieving good results in combination with the Web of Science database [58]. All three authors searched within the mentioned databases using the keyword searches "*noninvasive*" AND "genetic" AND "bear"/"brown bear". Sometimes, the country was specified as a keyword to receive the necessary results from the searched databases. The authors worked independently and shared their results periodically. The ending date for the search was December 2021.

The first assessment was performed to determine whether to include the records in this review. Therefore, before screening the full text, the studies considered appropriate were selected by (1) title, (2) abstract, and (3) keywords. As the first exclusion, documents from the results that were not on bear species or within the objective of this review were excluded. The grey literature was not an exclusion criterion, even if peer-reviewed literature was preferred; hence, reports following national bear projects, guidelines for noninvasive genetic methods, and national management action plans for bear populations were considered important for this literature review (Table 1).

Table 1. The categories of the reviewed literature related to noninvasive genetics on bear populations.

Category	Peer-Reviewed	Guidelines	Reports	Action Plans
Ν	140	3	3	2
Total		14	18	

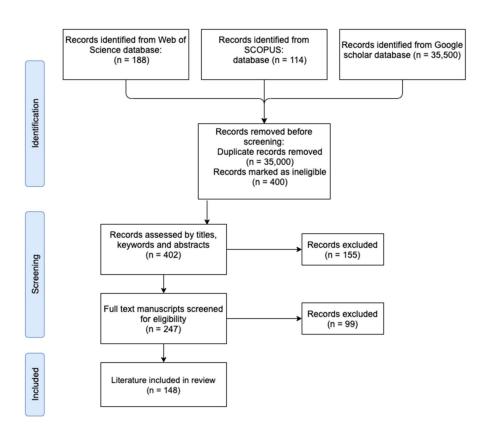


Figure 1. PRISMA literature search flow diagram. The number of works (n) screened, included and excluded in our review are identified at each step of the process.

In the second step, we applied the inclusion/exclusion criteria. The inclusion criteria were (a) studies focused on noninvasive genetic sampling methods, which include sampling detailing, samples' collection and storage, and quality assessment of the sample; (b) efficacy of noninvasive genetic sampling (cost and resources effectiveness); (c) research performed on bear populations across the world; (d) studies published in English (from Europe, North America, and Asia); (e) studies that had significant results/recommendations for developing a good workflow for future research. The exclusion criteria were: (A) studies in fields other than wildlife research; (B) studies that only contained recommendations regarding the laboratory work; (C) studies that used the same datasets and had no new information about the noninvasive genetic sampling; (D) studies that only analysed samples collected invasively.

Although documents from the first results that were not on bear species or within the objective of this review were excluded, 6 of the included studies addressed other species and obtained promising results that we assumed we could adapt to our species of interest. Moreover, a total of 9 studies included in this review used the same samples' database (partially or completely) from another research.

This review encompasses significant information about sampling and sample collection methods used by authors in their studies to monitor bear populations with noninvasive genetics. Additionally of interest were the studies that assessed the quality of the collected samples depending on the bears' diet, the location of the collected samples, the weather conditions when the samples were collected, human error, and other factors [59]. From all the studies identified as appropriate for the review, data were extracted as follows: (1) sampling scheme, including location and bear population type/size, (2) data collection and storage, and (3) significant results and recommendations. Thereby, most of the topic-specific studies should be covered by the chosen web-based databases.

3. Results

Using the three databases, 35,802 papers (peer-reviewed and grey literature) were retrieved, from which 247 were considered relevant for full text screening according to the first exclusion step (Figure 1). Table 1 shows the quantity of the scientific literature according to its classification, based on the inclusion/exclusion criteria after completing the second step, namely, the screening. Finally, the present review consisted of 148 relevant studies, of which the majority are peer-reviewed articles (n = 137), with the remainder being grey literature (three guidelines, three reviews, three reports, and two national action plans).

The final database on the noninvasive genetic sampling (nNGS) of different bear populations worldwide is represented in Figure 2. It includes Europe (Cantabrian, Pyrenees, Apennine, Dinaric—Pindos, East Balkan, Carpathian, and Scandinavian brown bear populations), North America (black bear and grizzly brown bear populations), and Asia (brown bear and Asiatic black bear populations, Malayan sun bear, and Gobi bear).

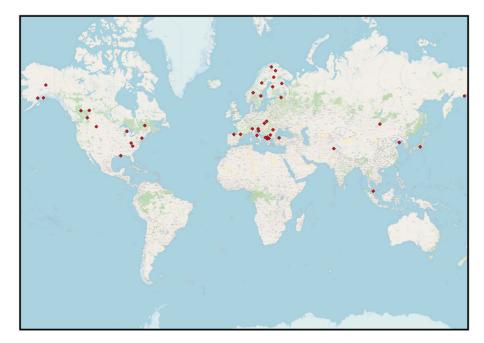


Figure 2. Locations referred to in the retrieved literature.

3.1. Sampling Scheme Including Location and Bear Population Size

The genetic sampling scheme is one of the preliminary stages when conducting genetic research and monitoring [60]. Pilot studies are recommended for establishing the proper sampling strategy (including sampling scheme, timespan between investigations, trap spacing, and subsampling, if necessary) [61]. Table 2 reveals essential information of the evaluated studies worldwide related to the nNGS of the bear population. It includes the sampling scheme used by the authors, the type and number of samples that were analysed, the bear population, and the location of the study.

Location	Population	Sampling Scheme *1	N Samples * ²	Temporal Extent	Study
Europe					
Northern Europe (E-W)	Scandinavian brown bear	M, SS	3365 F, H, T	2001, 2002, 2004, 2006	[62]
Northern Europe (Se, Norw, Fi, Karelia)	Scandinavian brown bear	Dataset obtai	ned from [63]	2005–2017	[64]
Northern Europe	Scandinavian brown bear	Dataset obtai	ned from [65]		[66]
Northern Europe	Scandinavian brown bear	Dataset obtained from reg	ional monitoring programs	2006–2013	[65]
Northern Europe	Scandinavian brown bear	Dataset obtained from reg	ional monitoring programs	2006–2012	[67]
Sweden	Scandinavian brown bear	Dataset obtai	ned from [55]	2001–2002	[68]
Sweden	Scandinavian brown bear	OS	1904 F	2001–2002	[63]
Sweden	Scandinavian brown bear	OS	5185 F		[69]
Slovenia (south)	Dinaric brown bear	Dataset obtained	from a pilot study	2004–2007	[59]
Slovenia	Dinaric brown bear	SS	1053 F	2007–2008	[70]
	M, SS, OS		4687	2015	[71]
Slovenia	Dinaric brown bear	Dataset obtained from regional and national studies		2007	[72]
Slovenia	Dinaric brown bear	CM, OS, SS		2007	[73]
Carpathian brown	bear	M, OS	339 T, B, F, H, bones	2004–2009	[74]
Slovakia	Carpathian brown bear		76 H, F	2005–2006	[75]
Slovakia	Carpathian brown bear		140 F, H	2007–2008, 2010	[76]
Romania	Carpathian brown bear	HT, OS, SS	1426 F, H	2017–2018	[77]
Poland	Carpathian brown bear	HT, SS	858 H	2010	[78]
Bulgaria	Eastern Balkan brown bear	HT, CM, OS, M	355 F, H	2004–2008, 2009–2012	[79]
Greece	Eastern Balkan brown bear	HT, TS, CM, M, SS	382 H, F, B	2006–2010	[80]
Greece	Eastern Balkan brown bear	HT, SS	860 H	2007–2010	[81]
Greece (Kastoria)	Eastern Balkan brown bear	HT, OS	232 H, F, B	2011	[82]
GR, FYROM, ALB	Eastern Balkan brown bear	HT, SS	191 H		[83]
FYR Macedonia	Eastern Balkan brown bear	HT, OC, SS	106 H	2008-2009	[84]

 Table 2. Sampling schemes retrieved from the reviewed literature.

Table 2. Cont.

Location	Population	Sampling Scheme *1	N Samples * ²	Temporal Extent	Study
Albania	Eastern Balkan brown bear	HT, M, TS, SS	12 H	2008–2009	[85]
	HT, OC, OS, SS		643 H	2011	[86]
Italy	Apennine Brown Bear	TS, M, OS	80 H, F, T	1991–2002	[87]
	HT, TS, OS		1164 F, H	2003-2004	[33]
Italy (Alps)	Alp Brown Bear	HT, TS, CM, M, OS, SS	2781 F, H	2002, 2003–2008	[51]
Spain		TS, SS	96 F	1990–1992	[88]
Spain		CM, M, OS, SS	133 F, H, B, T	2004–2006	[89]
Spain	Cantabrian Brown Bear	TS, SS	151 F, H	2017	[31]
France	Pyrenean Brown Bear	TS, OS, SS	153 F	2014–2019	[90]
North America					
Alberta, Canada	Grizzly	HT, TS, OS, SS	183 F, 958 H	2016	[91]
	TS, SS		880 F	1999, 2001	[92]
Alberta, Canada	Grizzly	HT, SS	3363 H	2004	[93]
BNP, Canada	Grizzly and American black bear	HT, CM	6236 H, T	2006–2008	[36]
BC, Canada	American black and brown bears	HT, SS	447 H	1995	[94]
Quebec, Canada	American black bear	HT, SS	411 H	2005	[95]
Alaska	Brown bear	HT, SS	2245 H	2014 - 2017	[96]
Alaska	Grizzly bear	HT, SS	466 H	2002–2003	[97]
	HT, SS		345 H	2003–2005	[98]
Alaska	Brown bear	TS, OS, SS	428 F, saliva	2014	[99]
Montana, USA	Grizzly bear	HT, SS	33741 H	2004	[100]
Northern New York, USA	American black bear	HT, SS		2006	[101]
Louisiana, USA	Louisiana black bear	HT, SS	922 H	1999	[102]

Table 2.	Cont.
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Location	Population	Sampling Scheme *1	N Samples * ²	Temporal Extent	Study
Louisiana, USA	Louisiana black bear	OS	448 H	1999	[103]
NLP, Michigan, USA	American black bear	HT, SS	1564 H, T	2003	[104]
Kentucky-Virginia, USA	American black bear	HT, SS	1503 H	2012–2013	[105]
New York, USA	American black bear	HT, SS	1985 H	2012	[106]
North Carolina, USA	American black bear	HT, SS	468 H	2001–2002	[61]
Asia					
Pakistan	Brown bear	TS, SS	136F	2004	[107]
	HT, OS		272 H	2008	[37]
Mongolia	Gobi bear	HT	200 H	1996–1998	[108]
GKM, Turkey	Brown bear	CM, M, OS	154 H, T	2008–2014	[109]
Malaysia	Malayan sun bear	HT	69 H	2017, 2019	[110]
Japan	Asiatic black bear	OC	99 corn-bite samples	2004	[111]

 *1 HT = hair trapping; TS = transect sampling; CM = capture for management; M = mortalities (including hunting and corpses); OS = opportunistic sampling; OC = occasional collection; SS = systematic sampling. *2 F = faeces sample; H = hair sample; T = tissue sample; U = urine sample; S = saliva sample; B = blood sample.

The samples used for the genetic analyses were mainly faeces (scat samples) and hair samples collected with different hair traps (further information is presented in Section 3.2). Sometimes, to improve the data collection, samples from accidentally killed bears were also included [72]. Moreover, in the regions where bears were hunted, most studies included tissue samples, typically in Northern Europe [38,62,67,112] and North America [104]. Tissue samples collected from GPS-collared bears and from legally killed individuals following a derogation have also been used in Bulgaria [79].

The sampling methods were correlated to the size of the bear population, the objectives of the study, and the expected results. Both opportunistic and systematic samplings were used in the literature listed in Table 2 (in 57 peer-reviewed research studies).

The studies covering large areas with large bear populations generally adopted the systematic sampling scheme in combination with other sample-collection methods to enlarge the capture probability, as was the case of the Northern Europe population [62,69], the Dinaric—Pindos population, including the transboundary population [71], the Carpathian population [113], and the North America bear populations [36,91,104].

Specific regions where bears usually live were opportunistically surveyed [62,66,77], but not as a single sampling scheme, mainly for the small bear populations. In this situation, the samples can be dispersed across the range, and sample collection may be challenging [86,88,114,115] The research from Italy on a sized population using various monitoring methods has proven that not a single scheme has managed to identify all the individuals. The sampling methods combined several techniques, namely, hair trapping, opportunistic collection of faeces and hair samples, and the transect method. In addition, this study (Table 2) suggested that opportunistic samples are usually helpful when gathering evidence following bear damage [33]. In addition, samples gathered through opportunistic methods usually had lower genotyping success than the samples from hair traps [51].

Sampling a large bear population is challenging; the number of the collected samples has a significant impact on the quality of the study [68]. The large population implies many samples need to be collected and many resources are required. Data collection is critical, and a highly intensive noninvasive genetic sampling can provide good results for large bear populations, as was previously successfully conducted in LIFE DINALP BEAR, in which the target of 3000 samples was exceeded by over 56%, and samples were collected with "good temporal and spatial coverage" [71]. In another study (sampling in November–December 2017), 128 bear faeces samples were collected from the eastern part of the small Cantabrian bear population using 25 km² plots. As a result of the sampling of 624.5 km of transects and 151 collected samples (faeces and hair), a minimum of 33 individual bears were identified [31].

Generally, known areas with permanent bear presence have been sampled using equal grids [31,36,86,91,100] or by having the entire range surveyed [65,71,88,104]. The grids can be geographically biased, e.g., Bulgaria, where they were unequally scattered in the targeted mountain regions with a bear presence [79]. Sometimes the sporadic presence of bears was also targeted [72]. Furthermore, areas where bears were known not to exist were included in the sampling scheme in Sweden [69].

When sampling, it is essential to consider the bear behaviour, particularly when hair collection is the goal of the sampling scheme, and the method includes natural rubs [78,81,83,85]. Usually, when sampling for hair collection, the area was divided into grids so that at least one hair trap was placed in an established grid [33,62,78,81,86,116]. The fixed points were specific for hair-collection methods, while hair samples were also opportunistically collected from areas with bear frequency, such as certain power poles used for rubbing [83–85,117], rub trees [78], or buckthorn patches during the berries ripening season [86].

In North America, hair samples were collected more often than faeces, whereas in Europe, the surveys generally included both faeces and hair samples, especially when monitoring rare and elusive species [118]. Some authors even recommended using both types of samples for accurate results following the monitoring of a bear population [113].

Choosing the best sampling scheme is essential; therefore, combining more types of sampling may lead to identifying more individuals or improving the capture mark recapture (CMR) results and resolution.

3.2. Samples' Collection and Storage

Samples' collection and storage significantly influence the genetic analyses' results, in addition to the sampling scheme. A suitable protocol requires a low human error; thereby, it may contribute to accurate DNA extraction and consequently to high qualitative study results [93].

The samples' collection can be conducted by people with different backgrounds as long as they follow a well-established set of instructions. Therefore, pre-season training for the personnel is needed, along with regular updates on results [33,59,71]. In Sweden, for instance, one monitoring technique collected samples opportunistically by moose hunters together with volunteers and personnel from the Scandinavian Brown Bear Research Project [69]. Data collection using volunteers in the framework of bear monitoring programmes has been successfully implemented in other studies [55,69,71,73]. A clear advantage is to monitor the sampling effort in real time by plotting samples on maps; in this way, the "blank areas" are avoided, and forces can be concentrated in particular areas [71].

The number of collected samples plays a significant role, and it is recommended to establish the number of samples to be collected (faeces and/or hair samples) from the beginning. However, 2.5–3 times more scat samples should be collected relative to the number of assumed bear individuals from the study area because it is supposed that 20–30% of the samples will not be genotyped [68].

Regarding the samples' labelling, most of the studies included the geographic location and additional data about the sample registered from the field, while several studies used georeferenced samples [31], including location and date [69,81]. Additionally, the name of the team/person who collected the sample can be recorded [69] to monitor the operators' work. Thereby, remarks can be made, if necessary, which will address the specific issue the operator is facing; thus, human errors may decrease, resulting in a more qualitative study with reduced costs.

As mentioned previously, the sample type (faeces or hair samples) determines the protocol for a noninvasive sample collection from the field. The following subsections present recommendations and relevant remarks for collecting bear faeces and hair samples retrieved from the reviewed articles.

3.2.1. Faeces Collection

First, the age of each faeces sample must be approximated according to the specific smell, visual appearance, and presence of mucous and insect larvae to decide if the sample should be gathered. According to other similar studies, subjective age estimation must be considered for the success of DNA genotyping [59,97,107,113]. Usually, it is suggested not to collect scat samples older than five days because the chances of being properly genotyped are small in a sample of this age [59]. Moreover, the sampling season increases the effectiveness of future noninvasive genetic studies on European bear populations if this is planned correctly [59]. In addition, laboratory costs may also be lower if only higher-quality DNA samples are genotyped, which usually means faeces collected during the autumn period [33].

Approximately 1 cm³ of scat sample [90,119] was collected with a wood stick [107] and stored in a collection tube/bag [69] in most of the reviewed studies. According to the instructions, the scat sample should be collected from the outer layer (no ground contact) and not from the scat top because the DNA from there could have been washed by rain [59].

In many cases, the sample was collected in solutions with different percentages of ethanol [89,120], from 95% [33,68,90,107] to 96% [31,59,79]. Similarly, it is recommended to use a scat-detection dog, as the mean number of bear scats collected per year may increase significantly; for instance, in the study from the French Pyrenees it was four times higher, and the costs were much lower, because of the validation of the bear scat. The dog also managed to detect the bear cub scats, which are the most difficult to collect by humans because they can be easily confused with faeces from other species. Moreover, a better understanding of the bear's diet is possible using this method. Human-only teams collected 337 scat samples, and the trained dog indicated 239 of them; however, using trained dogs is also cost-effective [90,121].

Another innovative method for faeces collection was revealed in elephant monitoring from Africa, whereby a swab to collect the sample was used [122]. This swab was initially used for collecting saliva samples [123]. The swab was used to scrub the entire surface of the dung pile without touching the ground and the sample was stored in a lysis buffer (Isohelix), in a 2 mL Eppendorf safe-lock tube. This nNGS collection method was efficiently used for elephant monitoring in Gabon, Central Africa, and may be feasible for European brown bear population monitoring. The swab method provides several advantages: the storage tube is smaller and safer than the classic 50 mL tube, the operator error decreases in this case, and the samples may be stored at ambient temperature for 1–4 weeks. A limitation of this practice is that the scat sample is preferably fresh in the field [124] (not older than 3 days) or at least in good condition. Moreover, it should be taken into account that this method does not fit every bear population, regardless of its size, because finding fresh faeces samples in a low-density bear population is difficult [125].

Similarly, in another study from Oman and the United Arab Emirates, a combination of the Isohelix DNA Isolation Kit (provided by Cell Projects Ltd., Harrietsham, Kent, UK) and the QIAamp DNA Stool Mini Kit (provided by Qiagen Ltd., Germantown, MD, USA) was used for faeces collection and DNA extraction from the endangered Arabian tahr [126]. During the research on phylogenetic evidence for the ancient Himalayan wolf, the wolf scat samples were swabbed and stored in Isohelix solution [124]. The swabbing technique provided a higher quality of DNA concentration following the extraction [122].

Following the evaluated studies, some recommendations for faeces collection can be made. The sampling period has a significant impact on the results [68]; thus, it should be chosen accordingly. Therefore, it is recommended to sample bears during autumn and winter because these seasons overlap with the hyperphagia behaviour and it does not interfere with the cubs' period (April–June) [31,127]. Hence, in this period, the number of samples may increase significantly.

In addition to the sampling month, the bear's diet can negatively influence genetic scat analyses. For instance, scats with beech nuts have a high genotyping success rate, but scat age estimates may bias this because faeces with beech nut content may look older than they are [59]. Several studies suggest that plants in the bear diet will affect DNA extraction and inhibit PCR reactions [63,128]. However, other studies obtained acceptable results from faeces samples composed predominantly of plants [107,125].

In addition, rainfall and sunlight exposure were considered other factors of DNA degradation [59,129,130]. However, if the scats are exposed to sunlight and rainfall, they may look older and be excluded by the sampler, as documented in recent research [59].

3.2.2. Hair Samples Collection

Hair sample collection methods from the reviewed research studies are presented in this section in terms of the sample characteristics, followed by the description of the collection and storage techniques. The types of hair traps that may be suitable for monitoring the Carpathian brown bear are mentioned, along with the recommended sampling periods for their installation, and the guidance regarding improving the DNA quality.

The hair traps can be baited, or passive (unbaited); further information regarding the baits and/or lures that can be used as attractants is presented in this chapter [131].

First, it is also necessary to mention the types of hair traps that are successfully used for noninvasive samples collection from ursids. Therefore, Table 3 presents the studies in which several types of hair traps have been used, with their specific characteristics, if they placed bait/lure, and where the research was conducted.

Table 3. Types of bear hair traps.

Hair Trap	Specifications	Bait and/or Lure	Location	Study
Hair corral	At least a single strand of barbed wire stretched around 4 or more trees at 50–55 cm above ground	yes	Italy, Poland, Malaysia, Turkey, California, Michigan, Montana, Alberta, BC, Quebec (Canada)	[33,35,36,78,86,94,109, 110,132–134]
Adhesive rub stations	Tree trunk or wooden blocks wrapped with duct tape	yes	Malaysia	[110]
Power poles	Covered with barbed wire	yes, not on purpose	Greece, Albania, FYR Macedonia, Turkey, Montana	[81–83,85,109,117,135]
Natural rubs (bear rub trees)	equipped hair snagging devices (e.g., barbed wire)	no	Italy, Greece, Bulgaria, Romania, Poland, Alberta, Montana, BC, California, Alaska, Mongolia, Japan, Russian Far East,	[37,77,78,108,132,133, 135,136]
Path traps	Barbed wire installed across known bear travel routes or at feeding routes	no	Italy, Poland, Alaska, Yellowstone Lake,	[37,77–79,108,133]
Modified hair snares	Barbed wire constructed in such way, that allows the bear to escape but keeps hair samples while doing it, and it disables after the process	no	Southeast Alaska	[97]

There are different types of hair traps, and it has been proved that this has a significant influence on trapping success [78]. The relevant studies are described in Table 3, which illustrates the type of bear hair traps and the operating procedures from different study locations. Some studies have used natural rubs, taking advantage of the bears' natural behaviour to rub on wooden and/or power poles [81,83–85,117]. The use of power poles as hair traps is not feasible everywhere, e.g., in Albania; although some of are made of wood, others are concrete, and bears do not rub on them [85]. In addition, in Turkey, most of the poles were exposed to sunlight, so that their genotyping success was very low in comparison to the rub trees [109].

Hair corrals were the most common hair traps, consisting of a single or even doublestrand (designed for cubs [33]) stretched around four or more trees at the height of 50–53 cm [36,37,94] above the ground, enclosing a pile of branches and woody debris in the centre, frequently with attractants [35,36,78,94,131]. The height of the strand usually depends on the field personnel who installs it.

These were followed by natural rubs. In Eurasia, it seems that hair traps based on the rubbing behaviour (the natural rubs) are more effective than corrals [37], in comparison to North America [78]. Habituation may be a severe issue; therefore, traps' movement is recommended (≥ 1 km) within each sampling grid [37,86] every session or at the middle of

the sampling session [33]. It is underlined that previously detected bears are likely to be captured again, lured by the baited station. Moving traps is recommended in other similar studies [93,101].

Other studies have used path traps. Barbed wire was installed with different methods in areas where bears were frequently seen (ungulated feeding sites, locations where salmon aggregates during spawning, known den locations, feeding points). Usually, the locations of the hair traps were chosen according to the experts' opinion [36,93] The subjective sampling increases the capture probability [137]. For instance, in a study in Banff National Park, these traps were placed 1 km apart in the vicinity of seasonal food sources or known wildlife paths, far from heavy anthropic areas and tourists' trails [36]. Being far from anthropic areas, the chances of the trap being vandalised were minimal [104]. A study from Poland concluded that the natural rubs had the highest efficiency, followed by smola tree traps. The path traps were considered to be the most ineffective in this situation [78] and were recommended to only be used in known important feeding sites, where bears aggregate [138]. Conversely, these have been successfully used across known spawning streams in Lake Aleknagik (Alaska). The traps have been placed across salmon spawning streams where bears tend to agglomerate. In addition, camera traps were installed nearby to evaluate the bears' behaviour regarding the passive path traps [116]. Some of the researchers have used camera traps to assess the bears' behaviour when reaching the hair trap to make future remarks and recommendations [78,116], an action that is helpful for future studies. Unbaited path traps were also installed in Kenai Fjords National Park, in south-central Alaska, in targeted locations near salmon spawning streams or dense berry patches. This forces the operator to deploy the traps during specific periods (late July-early August, to coincide with the berry productivity and salmon runs) [98]. The passive traps are preferred, especially in CMR studies, because they may increase the sample size without affecting the species' behaviour [98,118].

Bear's attitude regarding the barbed wire was studied, and it was concluded that female bears accompanied by cubs are shyer when contacting the barbed wire compared to the males [116,139]. Additionally, single individuals were more intimidated by the wire than in groups, and cubs were the most curious. However, the percentage of bears that contacted the wire was still high (80.9%), of which 28 were females and 90 males. In another study from Alaska, the bears were less reluctant to contact the wire at night (44.9%), followed by daylight (23.7%), dusk (17.1%), and, finally, dawn (14.3%) [116].

a. Hair clumps: qualitative collection and storage.

Only hair clumps that match the bear hair should be chosen following the sampling. The operator's experience is crucial because he must identify the species hair from the barbed wire. A hair clump is considered a sample worth collecting when it contains a minimum of five underfur hairs on a set of barbs [37,81]. The number of hair follicles from a sample is usually positively associated with the amplification success [63,69,109,110,117,134]. The bunch of hair from one wire is usually considered an individual [78] and is kept separate from others [89]. If the operator succeeds in identifying hair clumps from different individuals, it would be a significant advantage in terms of the costs and quality of the study. In addition, the contamination of the sample with other DNA (from other individuals or humans) should be avoided as much as possible [97].

Many studies collected the hair samples with sterilised forceps or latex gloves [33]. These are generally maintained in the dark in paper envelopes [90,97] and, in some situations, with silica gel packs [37,77,86,109,135,140,141]. In this way, they can be preserved at room temperature [31,79,109]. Prior to the laboratory analyses, some studies recommend freezing the hair samples [142]. In Macedonia, the envelopes were placed in Ziplock bags with silica gel [84], whereas in Slovakia and Italy, the hair material was stored either in paper envelopes or in 70% [108] or 95% ethanol [54]. In the studies on the Pasvik population and in Banff National Park, the hair material was stored dry in paper envelopes [36,62]; some were labelled with barcodes [36,100].

The trap checking interval is crucial for the quality of the collected sample. The hair traps were visited at least once per month [84], at 14-day intervals [36,37,78], or even as often as 7 days [61,104,110], to prevent contamination between individuals' DNA. It is recommended to have the trap checked once a week, or at least every 14 days [97]. After the samples' collection, the barbed wire must be flamed [36,86,100,102,105]. In addition, the functionality of the trap should also be checked, so that the trap's effectiveness is high [78].

Concerning the samples' analysis prioritisation, the hair samples must be examined as soon as possible due to quick DNA degradation [143]. It should be noted that the hair samples are sensitive to sunlight and moisture. When wet samples were collected because of the traps' location (path traps placed near streams; Table 3), they were dried near a heat source [97]. This fact was also observed in another study when hair samples from grizzly and black bears that were not in direct sunlight or exposed to moisture produced high quality genotypes [135,140].

The bear activity season did not substantially impact the hair-trapping success in the situation from Poland [78]. However, the laboratory costs may be lower if the hair sampling period is favourable, and only samples with high-quality DNA are processed, which usually means hair collected during spring [33].

b. Attractants.

The use of attractants is vital to create effective unnatural hair traps. When surveying wildlife, bait and lure have different meanings. There are two types of attractants: natural and unnatural. The first are objects from the habitat which are used by the bear species, such as the rub trees or snags. The unnatural attractants can be divided into rewarding attractants, such as bait (any type of food preferred by ursids), and non-rewarding attractants, such as a scent lure (attractive smells for bears) [131]. There are cases when the bears are involuntarily attracted to some places that are man-made, such as the wooden power poles (Table 3) from Greece and FYR Macedonia that were treated with creosote. This was enough to trigger the bears' rubbing behaviour [81–84,117]. Equivalent to creosote effects, the smola trees from Poland have been used due to their effectiveness as hair traps [78].

The sampling plots for the natural traps depend on the most visited sites where the bears rubbed in the past [81]. The rubbing behaviour is easily observed in the field through the presence of bites and claw signs [84]. Buckthorn was also used as natural rubs [86], even if further studies indicate that bears tend to prefer coniferous trees for rubbing, mainly fir and spruce [78,136,144] with large diameters [78,136,145,146].

For food bait, a mixture of peanut butter and oats or bacon was used [134]. For ursids, raw chicken, fish (also canned), meat (also rotten), carcasses, honey, fruit jam, maple syrup, livestock blood, fruits and vegetables, and pastries [105] are recommended to be used as baits [131].

As scent lures, cattle blood or/and rotten fish may be used. In Poland, ~300 mL of inedible liquid of cattle blood and rotten fish juice (3:1) was used as a lure [91]. In Italy, aged cattle blood (~5–6 L) and decomposed fish oil (2:1) were used as a lure placed on the pile of rotten wood from the centre of a barbed wire encasement (hair corral trap type from Table 3). The pile was covered with leaves, moss, and other forest debris [86,94,131]. Scents of milk, eggs, canned fish, and food scraps (one month old) were used in another study as non-rewarding liquid scent lures [37]. Scent lures and food bait were also used in Michigan's northern Lower Peninsula study. Cherry syrup and scat from black bear individuals at a zoo were used as attractants [134]. Another study from North America used bacon and anise extract to lure bears to the installed hair snares [104].

It is important to replace the bait and refresh lures after the hair sample collection, and especially after precipitation [37].

c. Effectiveness of hair sampling.

It has been revealed that hair sampling had a higher success (86%) in identifying individual grizzly bears; the hair samples' DNA identified nearly two times more unique bears than the DNA from the scat samples. In addition, gender identification also had

higher success from hair sampling than scat sampling [91]. Compared to other European studies (from Italy and Sweden), the scat sampling success was significantly lower in the present situation [51,91]. However, when studying large bear populations, competition can also affect the efficiency of hair traps, e.g., the study conducted in Banff National Park indicates that detection varies between the two studied bear populations (grizzly versus black bear), whereas differences between genders' behaviour due to the same variation in the hair trap type have also been observed [36].

Nevertheless, in the case of the small Apennine bear population, future recommendations include the hair snagging method as a primary sampling method, together with at least two different secondary types of hair traps [86]. Hair trapping and opportunistic sampling may provide vital information for the other small bear population from the Italian Alps, following a two-year pilot study where different methods have been tested [33]. These two methods are the most feasible and cost-effective for monitoring this bear population; moreover, it was observed that the hair trapping cost per bear sample may decrease if sampling occurs from the end of May to mid-August, when capture probability is maximised [33,93]. In North America, the standard monitoring method for the grizzly bear population is hair snag sampling, but faeces sampling is promising [91].

On the contrary, the pilot study conducted in the Southern Carpathians on the large Romanian bear population recommends that the sampling is focused on faeces collection in future studies, and that hair samples be considered an alternative [113]. The limitation of the hair traps in this situation is that they can be male-biased, especially during the mating season, and this drawback is also revealed in other research studies and guidelines [37,78,83,100,116,131,147,148].

Regardless of these factors, the capture probability is a limitation when using hair traps. Almost every reviewed study recommends improving the capture probability [34,61,98] or also including other sample-collection methods [33,61,77].

4. Noninvasive Genetics in Bear Conservation and Management

Following noninvasive genetic monitoring of the bear populations, valuable findings can improve management and conservation actions. It was proven that further research on the Gobi bear and implementation of conservation management actions are necessary, due to a noninvasive study conducted to determine its status [108].

Genetic analysis always facilitates an appropriately developed management plan [89,104]. It has been proven that a bear population's genetic structure at a large spatial and temporal scale may be investigated using noninvasively collected genetic data, and even the future shape of the population can be predicted based on the results following the genetic study [62,66,149–151]; however, if needed, the restocking demand can be assessed [87].

Particular attention has been drawn to the increasing bear population from Sweden and its consequences, particularly regarding the coexistence of humans and bears. The bear population has been monitored, including using noninvasive genetic methods, and potential upcoming management issues were identified. A solution was found by the specialists, namely, zoning the targeted areas' carrying capacity [152].

Following the Slovakian genetic study, the recommendations were that the continuous brown bear habitat must be kept to ensure the genetic diversity of West Carpathian bears [108]. However, genetic diversity is not the only condition for long-term population survival. This is also influenced by other factors, such as environment and life history factors [67].

The nNGS proves the need to study transboundary bear populations to provide interesting outcomes [64,71,79,84,153,154]. Three individuals' DNA from FYR Macedonia was also detected in Greece, and this demonstrates the presence of a single interconnected population [84]. The research on the Bulgarian bear population revealed that the apparent mitochondrial lineage separation shows a pattern of male-triggered gene flow. Sample material from two male bears was found following the noninvasive genetic method in both Bulgarian regions; hence, this is considered evidence that narrow corridors exist [79,155].

Female philopatry is demonstrated in this study, as observed in other studies [66,74,79,156]. Dispersal behaviour is mainly exhibited, regardless of sex, due to the increased population density [95].

However, it is suggested that the increased population size and individuals' dispersal do not necessarily imply an increase in the gene flow [65]. The results of an older study indicated some limitations of gene flow between the population from the eastern and western part of Northern Europe [62], even if in recent decades the Scandinavian brown bear population has recovered substantially [65].

Additionally, the results from noninvasive studies may be used as forensic samples. In Norway, DNA extracted in a research study matched with a bear from an illegal hunting case, and the outcome of the study was used as evidence in the trial [112].

Most of the genetic studies conducted on brown bear populations from Europe estimated the number of individuals, their density, and sex ratio to reveal the species' conservation status and distribution [69,81]. Some authors made assumptions and demonstrated the current need for connectivity between bear populations starting from these indicators [56,64,65,71,76,79,80,84,87,89,107] or the sex-biased philopatry [53,65,74,79,95,156–158].

A total of 10 European studies conducted in Northern Europe were retrieved following the results of their noninvasive genetic sampling. Sweden and Norway have developed national bear monitoring plans in which both noninvasive (scats and hairs) and invasive samples are collected permanently (tissue and/or blood from legally harvested bears) [159].

Usually, CMR estimations are difficult to obtain because of the large sample sizes needed, especially if there are extensive areas that must be covered [69,160]. The opportunistic monitoring and the national bear monitoring program provide the management authorities of Northern Europe with a significant amount of data regarding the Scandinavian bear population [159]. The genetic monitoring programs could include the Y chromosome, which is essential for male survival [161], e.g., a study from Northern Europe included the Y-SNP (UAY318.2C839) [67], and this has also been found among the East European bear population [85]. Other studies included additional samples to increase the recapture probability (e.g., tissues from legally harvested bears [104]) and build a diverse dataset. It is acknowledged that bear populations are dynamic, and the "ideal" population size will change depending on the landscape alteration and human dimension [73]. For this reason, a permanent, long-term genetic monitoring program is valuable for every state that hosts a bear population [81,86].

Sustainable genetic monitoring programmes that involve local volunteers are effective. Some of them were found to be rewarding [42], whereas others were not but were still successfully accomplished [55,69,71,73,91,117]. It is suggested that the monitoring effort may increase by having volunteers and standardised data-collection techniques [69,71]. Therefore, the developed database contains essential information that could be, and already is, used in other research studies [59,64–68,72,112], e.g., two different genetic studies concluded that, in addition to the small population effects and habitat quality, mortality thresholds are critical when recovering a large carnivore population [133].

The LIFE DINALP BEAR project provided important outcomes following an exhaustive effort replicated with the help of volunteers. It concluded that the size of the brown bear population from Slovenia increased 41.3% in the last eight years. A total of 1962 samples (including transboundary individuals) were successfully genotyped, resulting in 599 unique bears (545–655 individuals). A total of 552 participants were involved in this intensive sampling with a high recapture rate of 69.5% [71]. This study was challenging in terms of resources, but provided valuable results to be transferred into the species' management and conservation and into the noninvasive genetic knowledge.

For nuisance bear identification, saliva samples were collected by swabbing the surface of a corn-bite sample from a damaged corn field. However, from 99 corn-bite samples, only 30% contained sufficient DNA, and a minimum number of 21 individuals responsible for the damage were detected. Consequently, this study from Japan demonstrated that if agricultural damage samples are collected as quickly as possible, individual bears can be identified [111]. Additionally, saliva samples from partially consumed salmon carcasses were proven to be more cost-effective and have higher quality than the faeces samples in another study [99].

In addition to the genetic approaches, conventional monitoring techniques are also recommended to be used. When data are available from telemetry, direct observations, harvest records, and presence signs, they should be combined with the genetic data to achieve accurate maps of species distribution and relative local population densities, which can be further analysed [34,36,65,152]. Another study concluded that noninvasive genetic sampling and the CMR modelling approach are promising tools when monitoring large carnivore species at a regional scale [31]. Additionally, in another study it is mentioned that, currently, traditional monitoring of bear populations is frequently supplemented by the genetic identification of the species based on samples collected noninvasively [51,162]. Moreover, noninvasively genetic collected data were successfully complemented by the traditional bear population monitoring in the Cantabrian Mountains, namely, the direct observation method through counting females with cubs of the year [163].

Very often, it is suggested to use both noninvasive DNA sampling and the phototrapping method. If combined with traditional monitoring techniques, the genetic approach will improve the quality of the population study. Consequently, the noninvasive genetic approach does not exclusively assess the individual attributes (age, body condition, reproduction status, distinct signs) [38]. Moreover, it was concluded that the camera trap was more likely to detect grizzly bears than the hair trap in the sampling sites where they both were deployed. The same situation occurred for sun bears, when the camera detected more visits than the hair trap [110].

Another limitation in noninvasive population evaluation is individual heterogeneity (IH), which must be carefully considered. This problem can be solved by developing better laboratory and field protocols. It is even recommended to conduct a pilot study to assess genotyping error rates [164] and detect IH bias sources in the study area. Biological and ecological knowledge and information should be included to validate a model [165]. The use of two capture methods (e.g., hair traps and tissue from hunting individuals) contributes to minimising the individual capture heterogeneity [104]. Avoiding DNA contamination is also vital. Maintaining rigorous conduct is very important while manipulating the sample, beginning with collecting, transporting, storage, and laboratory analysis [70,97,104,106]. For this reason, guidance from similar studies should be considered. In addition to the quality, the low DNA quantity can negatively influence accurate genetic typing; hence, the laboratory personnel should be further instructed to be careful about the amount of DNA extracted from field samples [75].

Noninvasive genetic monitoring requires some resources in the field and the laboratory. From all the reviewed methods, opportunistic sampling showed lower costs and was less challenging for a small bear population. During this research, the opportunistic approach was considered to be the most affordable, at approximately 600 euros per individual [33], which is higher than the Romanian 2021 minimum wage of about 500 euros. Due to budgets being constrained in some situations, it is recommended that only high-quality samples are genotyped [61].

The interpretation of the results after a noninvasive genetic study is essential. The bear population estimation following the study conducted in Bulgaria was not considered to be reliable for the decision-making process when discussing management actions. It was deemed that cubs and individuals from neighbouring countries were included in the three-year sampling period (e.g., migrants from Greece or other mountainous regions). Establishing a wide area may achieve more accurate population size estimation and an improved dataset, including sampling of genetic material, sampling across state boundaries [71,84,153], and a standardised capture–recapture design [79]. The importance of sampling neighbouring countries was also proven in a recent study that underlined the need to closely monitor the Fennoscandian bear, including a regular sampling of bear populations from Finland, Sweden, and Norway. The researchers recommended the holis-

tic approach with a regular sampling of noninvasive genetic material to assess the bear populations [64].

5. Conclusions

A comprehensive noninvasive genetic study can be developed following this review, which evaluated former research studies related to the nNGS on bear populations across the world. The three databases (WoS, SCOPUS and Google Scholar) provided the needed literature; thus, the 148 screened papers allowed the collation of important information related to noninvasive sampling schemes, faeces and hair sample collection, and recommendations from the worldwide studies on bear populations. Depending on the size of the bear population, the location of the study, and the available resources, various suggestions were extracted to enhance the knowledge about noninvasive genetic sampling of the bear population.

The insights from the peer-reviewed and grey literature cited in this manuscript highlight that the minimum number of individuals in the Romanian brown bear population could be estimated through a rigorous genetic study, with considerable efforts and coordination during sampling, and will contribute to the proper implementation of the NAP. Following this review, the development and improvement of the methodology and instructions for genetic sampling are achievable; innovative data collection is vital for large bear population monitoring. The minimum number of individuals and species' genetic diversity and evolutionary potential could be effectively assessed through a large-scale noninvasive genetic monitoring study that embraces large bear habitats. However, this goal represents a significant challenge.

A number of main ideas to be applied at the national scale can be derived from this review:

- The Isohelix method to collect the samples needs to be tested in a local study and, if suitable, applied nationally, by considering the numerous advantages of this method and the high number of people involved in sample gathering, in addition to the shortcomings associated with the storage of such large quantities of samples.
- Trained dogs for faeces gathering should be used across the brown bear distribution, in parallel with hunting managers, foresters, and volunteers.
- Both faeces and hair samples should be collected using the systematic and opportunistic schemes, with a large focus on faeces.
- Samples following damage should be gathered without allowing much time to pass after the damaging event (these can be used for further forensic analysis).
- Sampling should be organised during autumn and winter because these seasons overlap with the hyperphagia behaviour, and it does not interfere with the cub's period.

Therefore, we advocate the noninvasive genetics approach for the establishment of a permanent monitoring program with multi-year coverage. This is recommended as the primary mechanism for better understanding of functional connectivity, gathering species' presence and abundance, and detecting trends in genetic diversity. These strategies provide rich information to be incorporated in recommendations to management authorities for action and policy development, which are crucial for the species survival.

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