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Review

The Road ahead on Implementing Non-Invasive Genetic Monitoring of Multispecies in the Carpathians

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Abstract: Genetic monitoring represents a valuable tool for natural resource managers in managing and conserving wild populations of plants and animals. Even though there is a clear need to establish genetic monitoring programmes urgently, several barriers could occur depending on the region, such as lack of funding, gaps in national strategies, poor international collaboration, and transboundary issues. This review aims to analyze the genetic and non-genetic variables used in previous studies and projects to reveal the premises for conducting genetic studies on multispecies using existing knowledge. However, we found that sex marker, the number of samples per individual per year, and the rate of non-invasive samples were correlated with each other, as well as the number of sample types used and monitoring time. We show that developing nations are indeed challenged by the cost of large-scale and temporal coverage of the genetic studies. In contrast, high amounts of funds, complex methods, and national genetic monitoring programs are concentrated in well-developed countries, and the results are providing powerful tools for both species management and long-term conservation.

Keywords: non-invasive; genetic monitoring; data sharing; multispecies

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1. Introduction

Non-invasive DNA-based population monitoring is a powerful tool in wildlife management and conservation [1–4]. Most of the non-invasive genetic studies from Europe disclosed the conservation status and distribution of the species by estimating the number of individuals, population density, sex ratio, and phylogeography [5–9].

A non-invasive genetic monitoring program extended over a long period to assess species conservation is difficult to establish [10] as shortages may occur, like limited funding [11], and poor communication among scientists and practitioners, which is later translated to weak knowledge transfer between countries [11–13], and insufficient transborder studies [7,14,15]. Pilot studies and a review of the literature before the actual monitoring are necessary to establish the best genetic sampling possible [16,17]. Each region must implement particular approaches for monitoring forest stands or forest resources [18,19], especially when using non-invasive genetic monitoring [20]. The region's specifications and experience with genetic monitoring [15,21] may lead to challenges when comparing data gathered through different methods [22]. Generally, policies are crucial when conducting monitoring activities which strengthen sustainable management of forest lands and resources [23]. As an example, in Scandinavia, wildlife non-invasive genetic sampling has been implemented for a long time with the direct support of national policies [24], while other European countries do not share these benefits. Lack of funding to conduct genetic studies usually lead to limitations such as smaller areas of study, and low number of species and genetic markers [13,25].

Sometimes, the researcher is constrained to obtain satisfying results with limited resources [26], and, unfortunately, this is not always possible in non-invasive genetic sampling. However, if the appropriate population data to focus on are documented in similar studies [17], the investigator can start looking optimistically towards the expected outcomes. The mature database built on microsatellite genetic studies still weighs for future research [4,27]; thus, developing modern technology, like SNPs, should not make the existing data from STRs obsolete [4]. In addition, the Convention on Biological Diversity (CBD) proposes a holistic perspective of wildlife conservation efforts, suggesting that extensive datasets may lead to more knowledge worth sharing among working groups, thus reaching the best decisions concerning the safeguarding of biological diversity [13,28].

Comparative studies between different areas may provide relevant information on region/species requirements or the need to adapt research protocols [29,30]. Knowledge is improving and it demands adaptation to the environment [31], which is only possible when cooperation is encouraged [32]. Overall, improving information transfer between different bodies and researchers is essential for efficient and adaptive resource management of forest stand [33], wildlife species [34], and other forest resources [35].

This review aims to reveal the premises for conducting non-invasive genetic studies on multispecies using existing knowledge, thus exposing the influence of data gathered and analyzed during non-invasive genetic monitoring activities on the retrieved studies' results. For this, we have established the following objectives: (1) to evaluate the connection between genetic and non-genetic variables of the wildlife population; (2) to determine the country/region pattern regarding the non-invasive genetic method; and (3) to evaluate the knowledge transfer related to non-invasive genetic monitoring between countries.

2. Materials and Methods

2.1. Literature Search

This review includes peer-reviewed articles and grey literature (i.e., research reports obtained from European projects). We searched the following keywords: "non-invasive" AND "genetics" OR "genetic" AND "monitoring" AND "bear" OR "wolf" OR "Eurasian lynx" OR "wildcat" OR "mountain hare" OR "wolverine" AND "Carpathian" OR "Alps" OR "Dinaric-Alps" OR "Apennines", "Balkan" OR "Scandinavia" OR "Karelia" in Google scholar. Before screening, the title, the abstract, and the keywords were checked. Afterwards, the results were filtered out using the settled region. The process is shown in the following figure (Figure 1).

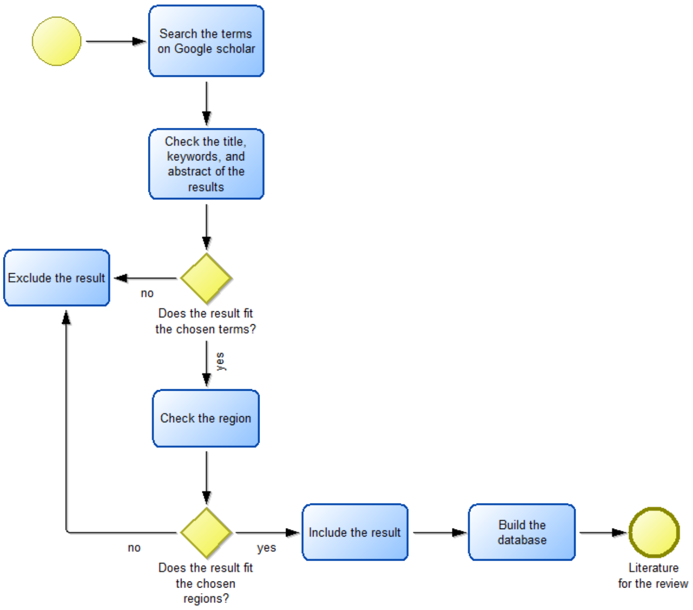

Figure 1. Flowchart of the literature search process.

Therefore, non-invasive genetic studies on bear, wolf, wildcat, mountain hare, and wolverine implemented in the Carpathian Mountains, Alps, Dinaric-Alps, Apennines, Balkan, Scandinavian, and Karelian regions were retrieved in this review paper, as shown in Figure 2.

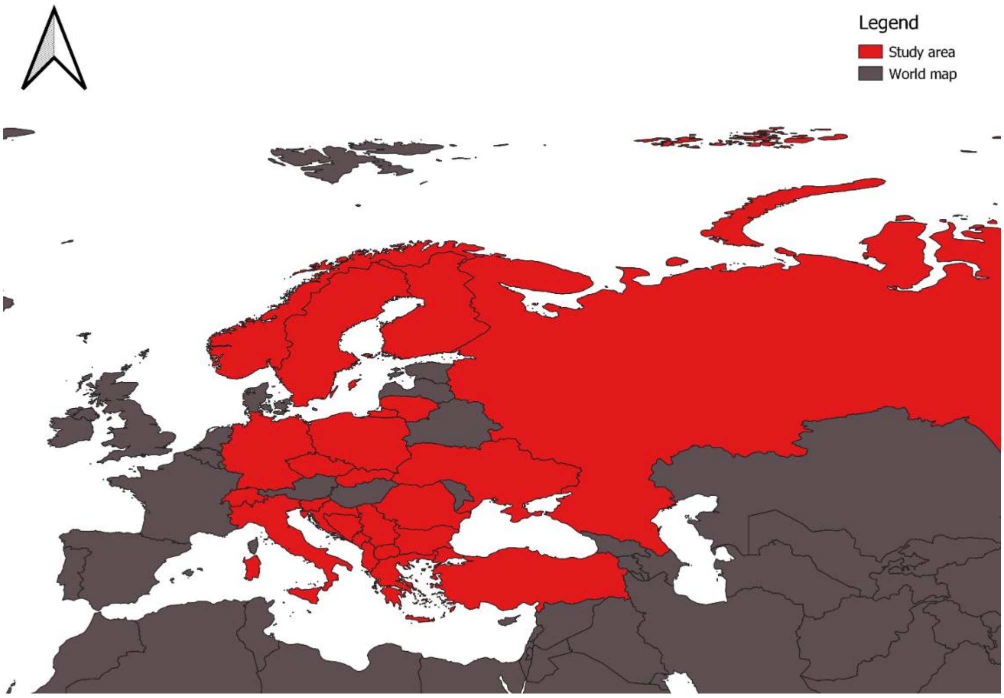

Figure 2. Location of the retrieved literature.

2.2. Results of Literature Search

Fifty-five studies have been considered relevant to the current review paper, based on the inclusion/exclusion criteria, of which 44 are peer-reviewed articles and 12 are grey literature on multispecies non-invasive genetic studies. Then, we extracted the wildlife species, the region, and the number and type of the collected samples. In addition, we retrieved information on the implemented methods (genetic/genomics), number of STRs, sampling data, timespan, number of collected samples, types of samples (faeces, hair, urine, blood, tissues, saliva, from hunter trophies, or museum specimen), and number of individuals. We also noted the type of results obtained for each study and divided them into two groups: population structuring and dynamics (density, structuring, mortality rate, immigration, hybridization, and bottleneck), and genetic diversity and other dynamics (expected and observed heterozygosity, Hard-Weinberg equilibrium, degree of inbreeding, genetic diversity, and the use of mtDNA).

2.3. Data Treatment

In addition to the study area and species, we used nine quantitative variables for our analysis: (a) length of the study (years); (b) total number of samples; (c) number of samples per individual per year (total number of samples divided by the number of individuals sampled divided by the length of the study); (d) rate of non-invasive samples (number of non-invasive samples divided by the total number of samples); (e) number of different sample types used; (f) number of STRs; (g) sex markers; (h) how many results have been obtained for population structuring or dynamics (PSD results); or (i) genetic diversity and other dynamics (GDD results) (for a detailed list of results taken into consideration, see paragraph 2.2). Concerning both PSD and GDD results, the aim is to establish two variables that allow the studies to be classified in a clear and understandable way according to their objectives, and therefore their type of results (concentration on genetics and dynamics applied either to the structure or the genetic pool of the population considered).

2.4. Principal Component Analysis (PCA)

One objective was to test correlations between the methods used and the types of results obtained. We considered the methodological variables mentioned in the previous paragraph and the number of calculations for the population structuring and dynamics (PSD) or the genetic diversity and other dynamics (GDD). We chose to carry out a Principal Component Analysis (PCA) to reduce our dataset's dimensionality [36]. It also allowed the study area and the studied species to be considered as auxiliary variables and to be included in the analysis. The statistical analysis was carried out using RStudio version 4.1.3 [37] with the following packages: "FactoMineR" (statistical analysis), "factoextra" and "corrplot" (visualization of results), and "missMDA" (input of missing values, which represented 11% of the quantitative dataset) [38–41].

2.5. Multiple Correspondence Analysis (MCA)

We tested correlations between methods and results more in-depth: we tried to understand if a given calculation on the PSD or the GDD could be linked to a specific pattern of methodological variables. For each study, we noted whether a given calculation (population structuring, number of alleles per locus, etc.) has been conducted (1) or not (0), and we assigned a level of intensity (class 1, 2 or 3) to all four methodological variables (Table 1). These classes divide each dataset into groups of homogeneous size.

Table 1. Methodological variables used in the MCA and their classes.

Class	Number of Samples Collected Per Individual and Per Year	Number of Sex Markers Used	Number of STRs Used	Number of Sample Types
1	[0;1.6]	0	[0;10]	[1;2]
2	[1.6;6.3]	[0;+]	[11;15]	[3;4]
3	[6.3;+]	n/a	[16;+]	[5;+]

MCA was the statistical analysis chosen for our goal and dataset, since it highlights correlations in a dataset composed of qualitative variables [42]. The MCA was implemented with the four methodological variables; one calculation (on the PSD or GDD) at a time was considered as a supplementary variable and visualized on the graphical display. Four additional MCAs were obtained by removing the methodological variables one by one to observe potential changes in supplementary variables. The statistical analysis was carried out using RStudio version 4.1.3 [37] with the following packages: "ade4" (statistical analysis), "factoextra" (visualization of results), and "missMDA" (input of missing values, which represented 11% of the quantitative dataset) [39,41,43].

2.6. International Cooperation and Exchange of Information

A study in at least two different countries was considered an international cooperation [44]. All cooperations were noted and grouped in a symmetrical matrix in which columns and rows were named according to countries. A similar matrix was built for gathering the exchanges of information between our set of studies (when results were compared with those from other studies) [45]. This matrix was non-symmetrical since the results of some studies were compared with those of regions that were not included in our analysis. The two matrices were then used to draw chord diagrams on RSudio version 4.1.3 [37] with the "circlize" package [46].

2.7. Statistical Analysis with RStudio

Analysis of the data was carried out using the following packages on RStudio version 4.1.3 [37]: "FactoMineR" v2.4 [38], "missMDA" v1.18 [41], "factoextra" v1.0.7 [39], "corrplot" v0.92 [40], "ade4" v1.7-19 [43], and "circlize" v0.4.15 [46].

3. Results

3.1. Primary Data Analysis

The number of papers by analyzed species that were retrieved in this review were: brown bear (31), wolf (14), Eurasian lynx (9), wildcat (2), mountain hare (2), and wolverine (1). The monitoring activities (number of papers) were in Scandinavia (17), the Carpathians (14), the Balkans (11), the Alps (6), the Dinaric Alps (4), the Apennines (3), both in the Alps and Apennines (2), and both in the Dinaric Alps and the Carpathians (2). Most studies used more than 80% non-invasive samples, focused on one sample type, and did a small number of calculations. Missing values represent 11% of these data. Indeed, not all the studies contained all the information we looked for, indicating that every region has its particular approach to implementing non-invasive genetic monitoring, which is also related to the research's desirable outcome.

3.2. PCA

Since both PCAs were conducted with the same methodological variables adding either the PSD or the GDD variable (number of results obtained concerning either the population structuring and dynamics or the genetic diversity and other dynamics), we will refer to them as PSD – PCA and GDD – PCA. We used the Kaiser criterion to determine the optimum number of Principal Components (PCs) to be included in our analysis [36]. For both PCAs, the four first PCs were analyzed and cumulatively represent 74.4% (PSD – PCA) and 72.5% (GDD – PCA) of total variance in our dataset. The PSD – PCA was chosen to link study areas and species to the other variables since its PCs explained more variances than GDD – PCA (1.9% more variance explained by the four first dimensions of the PSD – PCA). The results of GDD – PCA can, however, be found in the Appendix section (Figures A1–A3). All variables were well-represented (cosine squared close to 1) on at least one PC in both PSD – PCA and GDD – PCA (Figures 3 and A1). In both PCAs, two groups of correlated variables resulted: (i) the use of sex markers, number of samples collected per individual per year, and the rate of non-invasive samples are correlated (group 1); as well as (ii) the number of types of samples used and the time of monitoring (group 2). In the GDD-PCA, the amount of STRs is closer to group 1, and the GDD results are loosely correlated to group 2. In the PSD – PCA, the number of STRs are closely correlated to the PSD results, and both are more loosely correlated to group 2.

Concerning the study species, monitoring actions of wolves tend to gather more samples, use more STRs, and have more PSD results than other species (Figure 4); more calculations on the PSD were done. Monitoring actions of Eurasian lynx have more significant variance (the range of their coordinates on PC1 is 1 unit higher than the coordinates of other monitoring actions) and higher values in time and number of sample types used, but lowest total number of collected samples. Monitoring actions of brown bears seem to be homogeneously distributed along PC1 (great variance in rates of non-invasive samples, samples collected per individual and per year, number of sex markers, and time of study) and to use fewer STRs, and have poor PSD results. While monitoring actions of Eurasian lynx have a more specific pattern, monitoring actions of brown bears and wolves are quite alike, especially regarding the two first PCs: a high range of coordinates along PC1 and lower along PC2 (linked with the number of microsatellites, of PSD results, and number of sample types).

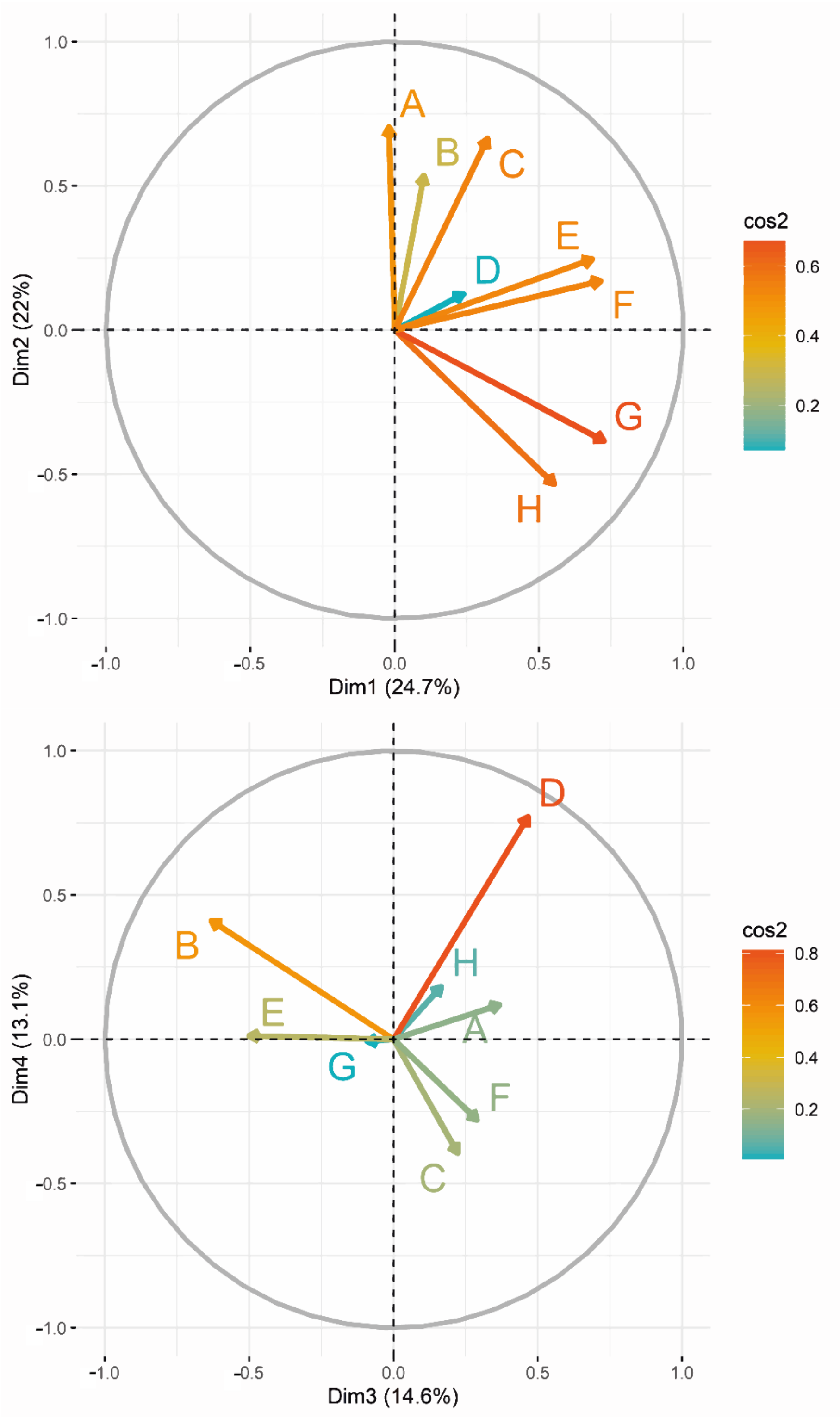

Figure 3. Variables factor maps on four dimensions (PSD – PCA). The factors are colored according to their quality of representation (A = rate of non-invasive samples; B = number of samples per individual per year; C = number of sex markers used; D = total number of samples gathered; E = number of STRs used; F = number of calculations done regarding the Population Structuring and Dynamics (PSD); G = number of types of samples used; H = time of study).

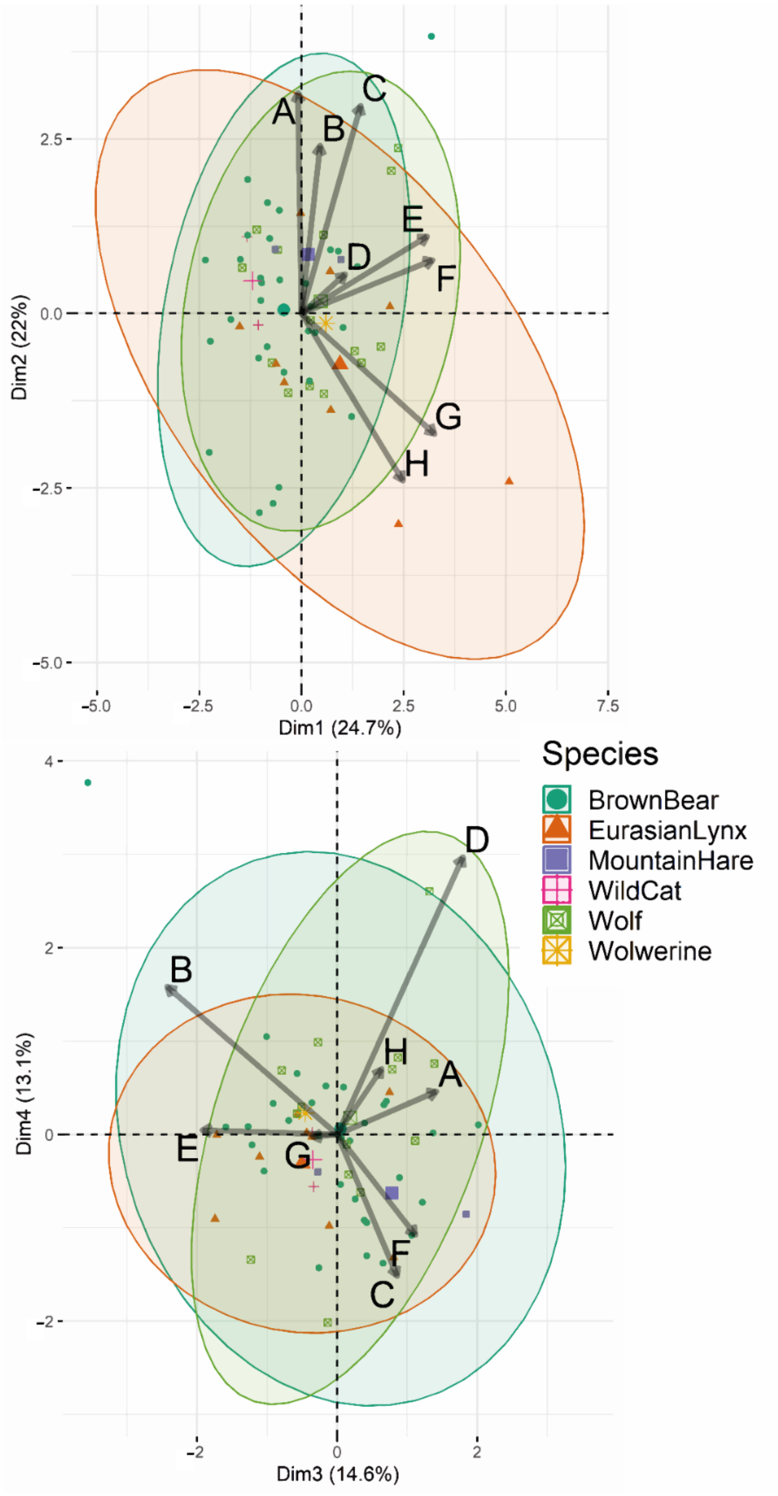

Figure 4. Biplot of variables and individuals classed by studied species (PSD – PCA).

Concerning the study areas, it appears that Balkan studies have a minor variance and smaller values in the rate of non-invasive samples, number of STRs, sex markers, and PSD results, as well as higher values of number of samples collected per individual and per year (Figure 5). Scandinavian studies show higher values in length of study and in the number of collected samples. Studies from the Alps have a short monitoring period and small numbers of sample types used, as opposed to DinaCarp studies (although there are only a few). Dinaric Alps studies have a specific pattern with high values of rate of non-invasive samples, samples per individuals per year, and sex markers, but little variance in time and no diversity of sample types. Carpathian studies have a short period of monitoring, a small amount of sample types and number of collected samples, as well as a significant amount of STRs and PSD results.

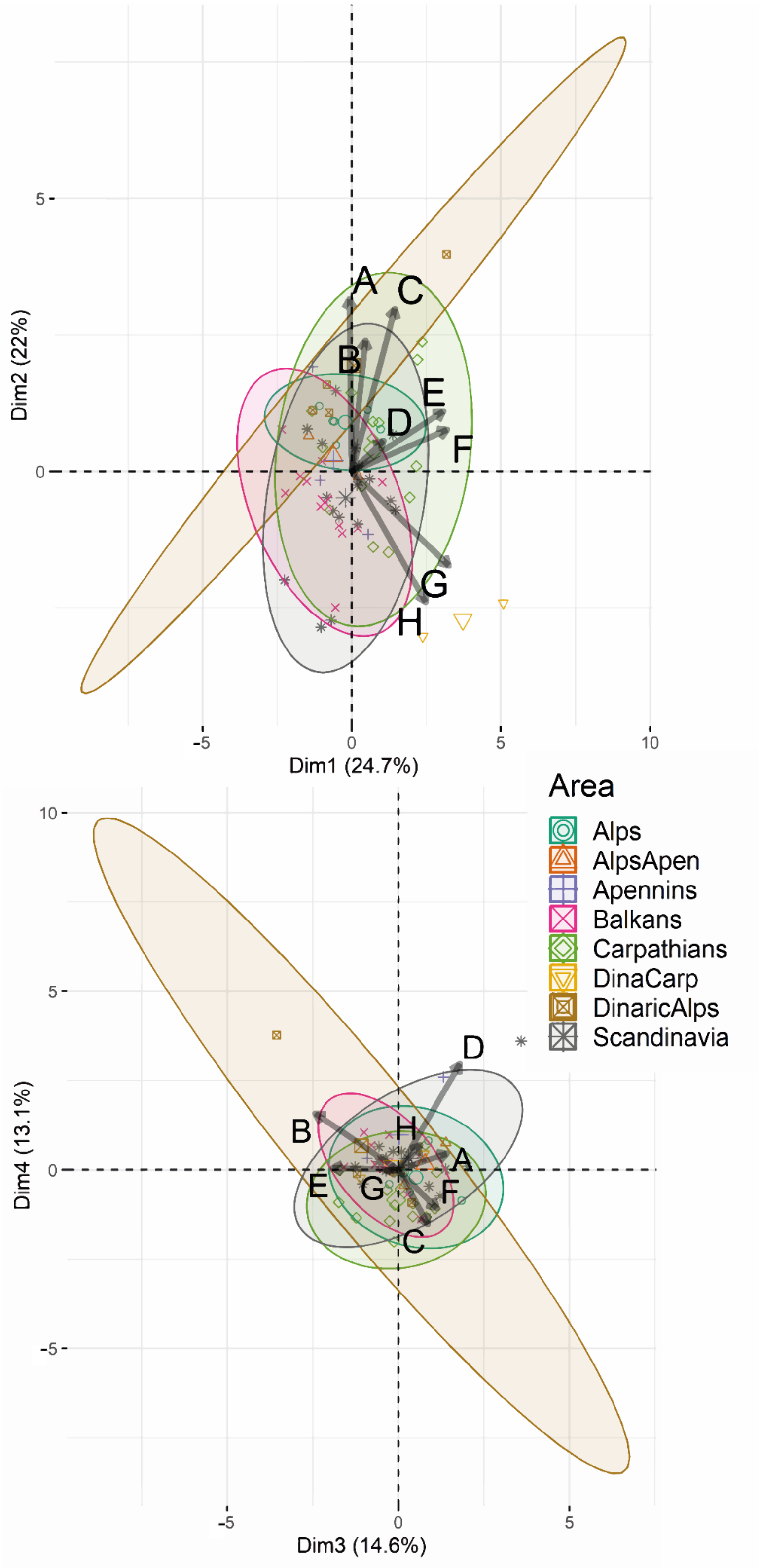

Figure 5. Biplot of variables and individuals classed by study areas (PSD – PCA).

3.3. MCA

The optimum number of dimensions was determined by analyzing the screen plot. For all five MCAs, the two first dimensions were included in the analysis and represent 45.7% of the variance in the main MCA (all four variables), and from 49.7 to 54.1% in the other four; all figures concerning the four additional MCA can be found in the Appendix section: Figures A4–A7. Previous studies [42] suggested that every dimension whose eigenvalue has a value higher than 0.2 could be selected; however, in our case, no clear results were found for the first two dimensions, and even less for the others. We considered it relevant to show and analyse only the results concerning the two first dimensions. Nevertheless, specific patterns of methodological variables were searched for. On Figure 6, it appears that the variables are well represented on dimensions 1 and 2 of the main MCA, except for the use of sex marker (both 0 and 1 values). Three groups of correlated variables can be observed. Class 3 of the number of samples collected per individual per year is correlated to class 3 of the number of STRs and class 2 of the number of sample types. Class 3 of the number of sample types, class 1 of samples collected per individual per year, and class 2 of number of STRs are correlated. Finally, class 1 of number of STRs, class 1 of sample types, and class 2 of samples collected per individual per year are correlated.

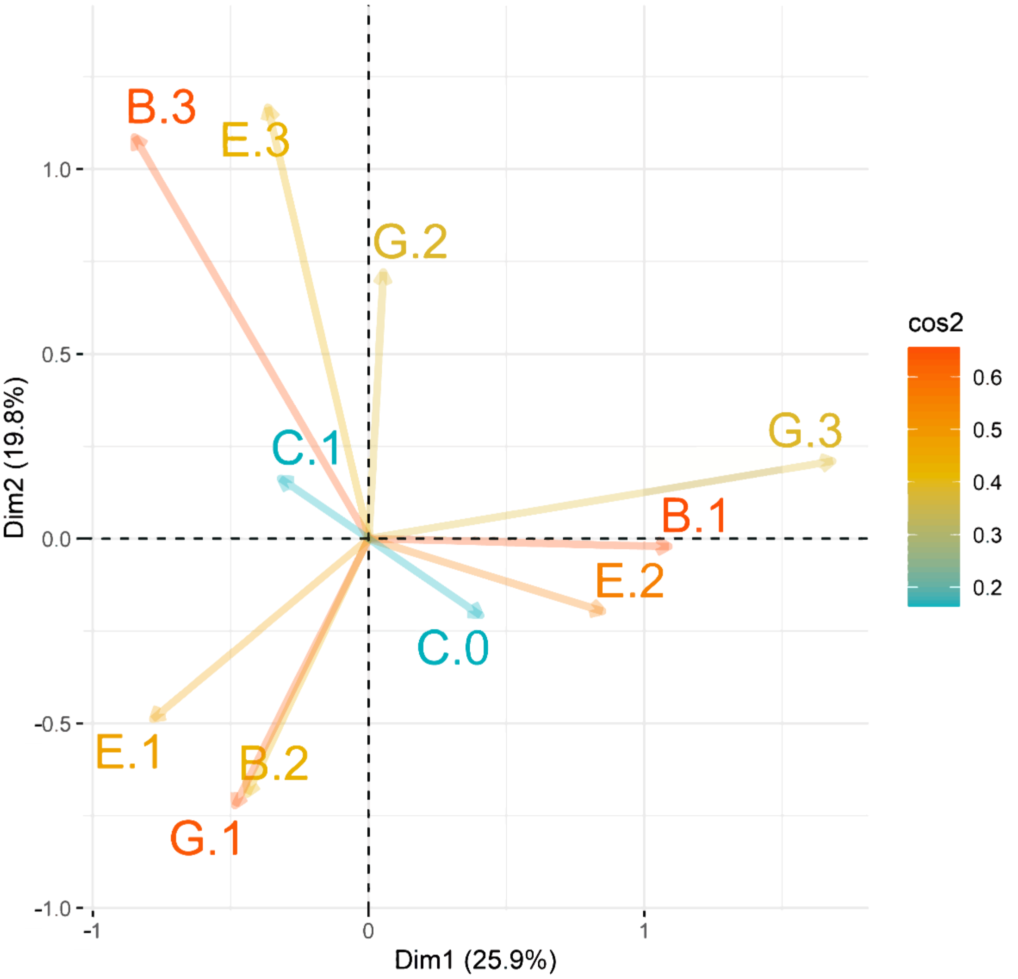

Figure 6. Graphic representation of the variables of the MCA colored according to their quality of representation (B = number of samples collected per year and per individual; C = number of sex markers used; E = number of STRs used; G = number of sample type). Figures refer to the classes from Table 1 (see 2.5).

Species and location seem to present the same tendency as in the PCA analysis (Appendix section: Figures A5 and A6). However, the number of samples collected per individual per year seems to have a more significant impact here than in the previous statistical analysis: from the four additional MCAs, the one in which this variable has been excluded differs the most from the main MCA (Appendix section: Figures A4–A7). While the number of sample types and STRs also have a great impact, no significant change was observed when the use of sex marker was excluded. We found no link between the methods used and the type of calculation performed, except a slight tendency in mortality estimation (Appendix section: Figure A7). To conclude, three patterns of methodological variables can be observed, but our analysis cannot link them to a type of result obtained.

3.4. International Collaboration and Exchange of Information

Figure 7 illustrates the knowledge transfer related to non-invasive genetic monitoring between countries from the retrieved literature. More explicit figures reflecting the cooperation for each region can be found in the Appendix section (Figures A8 and A9). For all chord diagrams, official country acronyms were used and summarized in the Appendix section, Table A1.

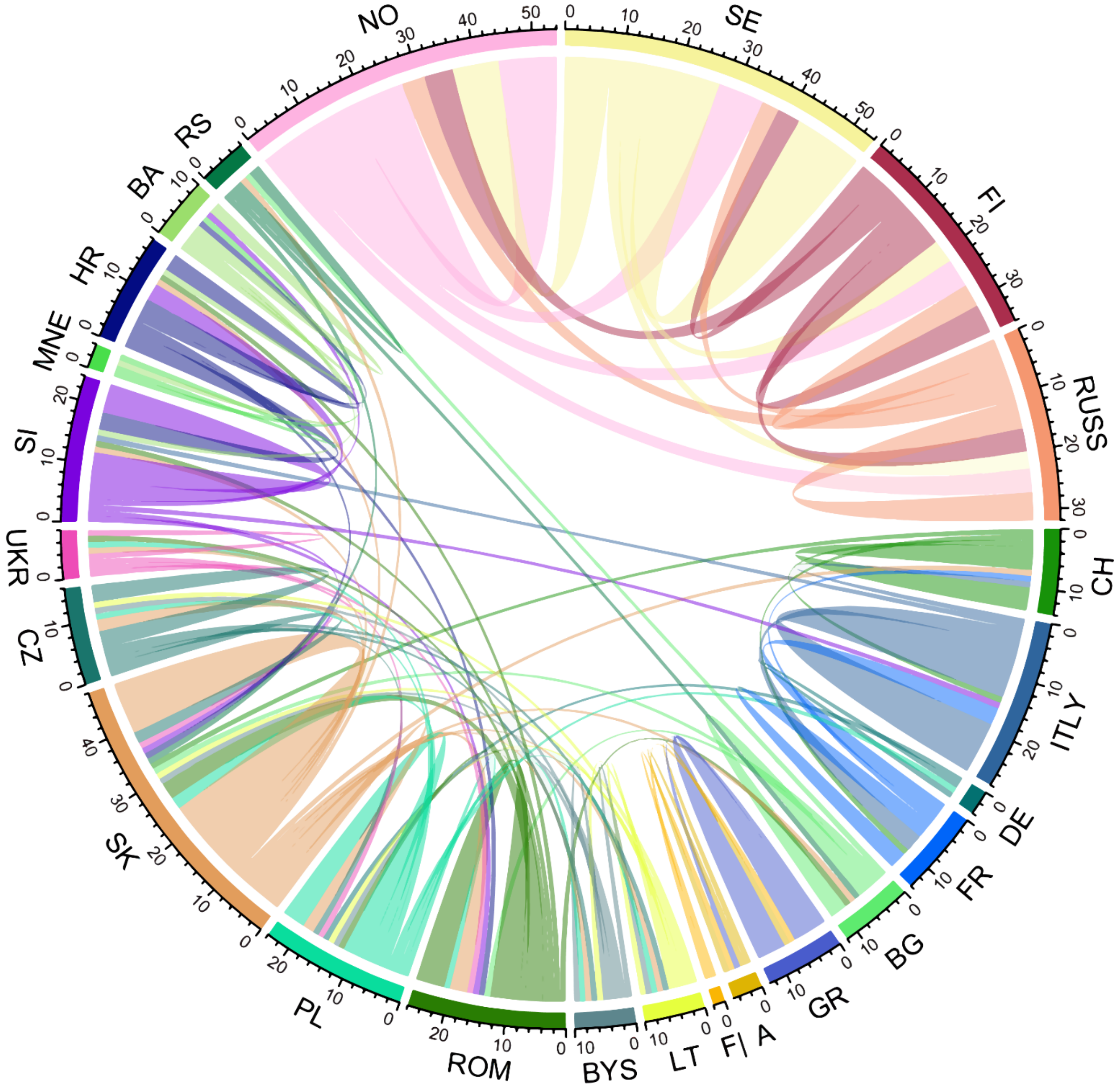

Figure 7. Graphic representation of the international cooperation found between our studies (see Table A1 for country acronyms. A = Albania, F = Former Yugoslav Republic of Macedonia).

Our results suggested that the outcomes of the studies implemented in the Carpathians are more likely to be shared with other regions, followed by those from the Dinaric Alps, the Balkans, and the Alps. Scandinavia tends to avoid collaboration with other regions. Nevertheless, an intense collaboration can be found within the Scandinavian region, but also when it comes to studies from the Carpathians and the Dinaric Alps: Czech Republic, Ukraine, Bosnia and Herzegovina, and Montenegro, which appear in more international studies than in those conducted by themselves. Moreover, Slovakia, Poland, Romania, and Croatia collaborated with more countries from their region. Lithuania shows great international collaboration considering the small number of studies. Cooperation in Scandinavia is the most homogeneous between its countries, in contrast with Balkan studies. Regarding international exchanges of information, studies from the Balkans gather data from other regions, followed by the Dinaric Alps, the Carpathians, Apennines, and Alps, while Scandinavia seems to be more isolated from this flux. However, we highlight the particularity of our results which are valid only for the retrieved studies.

4. Discussion

We searched for specific patterns of methodological variables, and for correlations between the methods used and the types of results obtained, through two statistical analyses (PCA and MCA). Our results indeed showed area- and species-specific patterns in methodology, as well as a positive correlation between the number of PSD results and that of STRs used. We also visualized the international cooperation and the exchanges of information between our set of studies in order to evaluate the knowledge transfer related to non-invasive genetic monitoring between countries. We might imagine that comparison among countries has some limitations. The results supported our assumption and disclaimed great disparities in international cooperation within studied areas, and highlighted the intense connectedness between Scandinavian countries.

4.1. Area- and Species-Specific Patterns Built by Correlations within Methodological Variables

Specific patterns linked with the study areas are visible. All the studies we have from the Dinaric Alps were conducted for less than one year: the values of sample per individual per year were consequently high; plus, almost only non-invasive samples were used. These two methodological variables might explain the specific pattern of this study area. Scandinavian studies may vary in time (from 0.25 to 12 years); only one of the retrieved studies lasted less than a year. Every dataset from a monitoring activity is added to a shared intraregional database, thus giving this region a global and long-term view over its species [47]. The areas with reduced time of study and number of sample types used (Dinaric Alps and Carpathians) might not have much funding support from the state and poor long-term view over their species. Our results indeed showed that the time for which a study is conducted and the number of sample types are correlated, and both can be linked to the funding resources; a high number of sample types used might cost more since different protocols (gathering, storage, DNA extraction kits, etc.) on the samples would be required [48]. Surprisingly, our results do not link the number of samples per year and per individual to these variables, and thus to the funding support. As we have retrieved studies with focus on non-invasive collected samples, the correlation between the number of samples per individual per year and the rate of non-invasive samples could be explained by low DNA quality. Low-quantity DNA is usually extracted from non-invasive samples [49–54]. More samples collected per individual could be needed in order to overcome this issue.

Developing nations are still challenged by the cost of precise and high-scale genetic data, while high-cost and complex methods, as well as national programmes for monitoring genetic diversity, are concentrated in countries which are allocating higher amounts of money [22]. The number of STRs used indeed depends on funding [55], and we found a correlation between this variable and the number of PSD results: studies highlight that the estimation of population structuring and its precision depends on the number of STRs used [5,55,56]. This suggests that some calculations on the PSD require many STRs and thus sufficient funding: the study area and its financial context have a great impact on the monitoring approach, limiting the comparisons among regions and countries [22]. However, large-scale projects could be implemented in nations with modest funding support: an extensive genetic monitoring programme for the brown bear based on non-invasive collected samples is currently ongoing at the national scale in Romania. A study of this dimension is the first of its kind across the world.

We also witnessed species-specific patterns. Monitoring actions of wolf population usually search for hybridization with dogs: more STRs are needed, and it adds one unit to the number of PSD results [5,57]. Monitoring actions of Eurasian lynx tend to use more types of samples: hair samples are more easily collected from Eurasian lynx than other species because of their marking behavior and their sensibility to certain olfactory attractants which could be placed on hair traps [58]. A less pronounced trend in the methods used in studies of lynx might be explained by a more recent interest in this species. There is not a pool of old studies on which new genetic monitoring activities could be based; therefore, no homogenization through time is yet to be observed, and those studies tend to be less diverse than those of the other species.

The results we obtained with the PCA cover 74.4% of the observed variance and the results are valid for this coverage, while the rest (around 25%) could explain other particularities not considered here. Our results would also have been more legitimate if we had the same amount of studies carried out in each study area and on each evaluated species. Finally, our sampling may be in part biased since some of our papers were written by the same author(s): these monitoring activities are not independent and may show some similarities. The results of the MCA did not extend beyond those of the PCA: no clear correlation was found between the methodological variables and the results obtained. Even though the need to use a larger number of STRs when estimating population structuring is well known according to the scientific literature [5,47–49,56,58,59], it was not reflected by our results, whether our sample coverage was not representative enough, or other factors like the study area have not been included. One hypothesis could be that the specific differences between the studied areas may prevent a universal trend (between the methodological variables and the results obtained) from being seen, in the same way as specificities limit comparisons among countries [22].

4.2. International Collaboration and Exchange of Information

The chord diagram which shows the cooperation of the countries from our dataset succeeds in indicating some trends of knowledge transfer among European genetic studies. Scandinavia has complex datasets and they are specialized on multispecies genetic studies which are considered the best in terms of species conservation management and cost-efficiency [60]. The knowledge transfer between Northern Europe and other regions is very weak in the studies we have evaluated. National programs of Scandinavian countries support genetic monitoring, to which designated funding and national policies are assigned [24]. Following a good management plan for bear species, the Dinaric-Alps region leads successful hunting management activities [61]. However, they do not benefit from national endorsement in comparison to Northern Europe. Lack of national regulations leads to not well-organized genetic studies, which makes the knowledge transfer in wildlife genetics and management difficult. The situation is similar in the Carpathian studies, which are grouped by regions with their own specificities. As in the previous case, the studies usually contain few details, and there are a lot of information gaps, i.e., no inbreeding studies, lack of genetic variables, and lack of policies and legal framework concerning wildlife genetic monitoring. The information gap generated by Ukraine has a bad influence on the wildlife conservation genetics from the Carpathian Mountains; therefore, ensuring connectivity in the Carpathian region needs to be enforced [62]. Moreover, the connectivity with the Balkan region and the Dinaric-Alps should be evaluated to get new perspectives on the gene flow. Balkan regions like Greece tend to concentrate on single species and adapt the monitoring methods as much as possible. Their proposed wildlife management actions are based on the scientific process [63].

Policies that facilitate diverse access to data are required to enable comparisons among regions and to overcome challenges in biodiversity conservation [22,64]. A good practice is successfully shared between nations and regions if these manage to keep similar non-invasive genetic approaches [22].

5. Conclusions

Our results reflect the need to gain knowledge from previous programs and suggest urgently starting genetic monitoring programs with a clear focus on multispecies. Regarding the Carpathian studies, we have identified few particularities such as a short period of monitoring, a small number of collected samples and sample types, as well as a significant amount of STRs used. The number of samples collected per individual per year seems to impact an existing pattern of methodological variables significantly but could not be linked to a result type. Area-specific patterns could limit comparisons, while economic context significantly impacts the monitoring approach and thus limits the comparisons among regions and countries. Our results confirmed that Carpathian studies results were shared with other studies in other regions and/or inside the region (such as Slovakia, Poland, Romania, and Croatia). The road towards adopting joint genetic monitoring on multispecies in the Carpathians implies using many STRs (and SNPs), and no matter the results and methods, we should secure a high number of samples collected per individual per year. Making such extensive genetic studies more accessible, a common friendly, up-to-date, graphical database could be in the hands of natural resources managers, managers, and practitioners. However, the standards of successful existing genetic monitoring programs like Scandinavia's should be, in principle, targeted, primarily due to their transfer into management practice. The best-case scenario would be having coordinated studies which use synchronized non-invasive genetic methods to enhance the knowledge transfer through transboundary monitoring. Nevertheless, this requires much effort, and several barriers could occur, such as lack of funding, gaps in national strategies for genetic monitoring, poor international collaboration between researchers, transboundary issues, etc.

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Data Availability Statement: Data available on request.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Appendix A

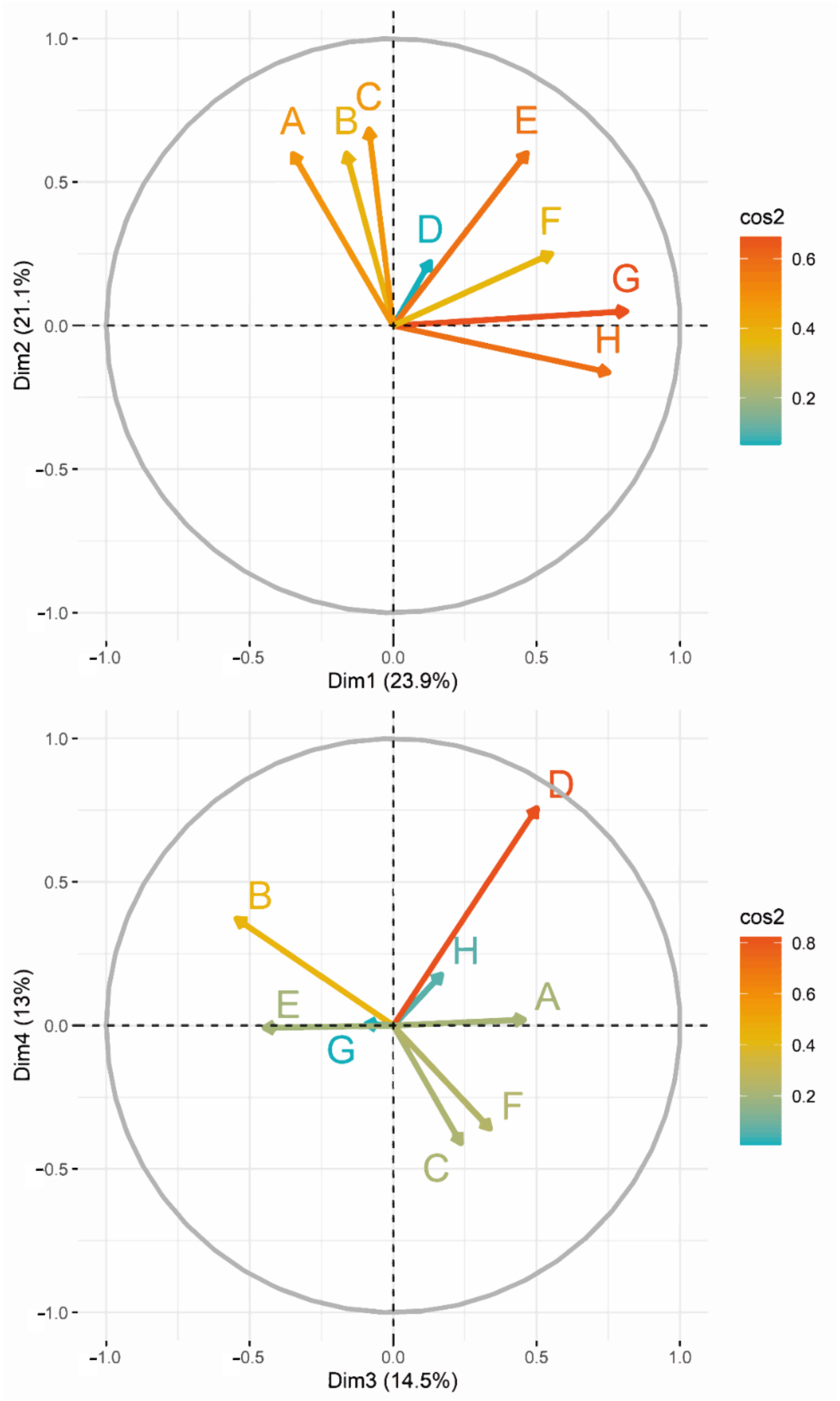

Figure A1. Variables factor maps on four dimensions (GDD – PCA). The factors are colored according to their quality of representation (A = rate of non-invasive samples; B = number of samples per individual per year; C = number of sex markers used; D = total number of samples gathered; E = number of STRs used; F = number of calculations done regarding the Genetic Diversity and other Dynamics (GDD); G = number of types of sample used; H = time of study).

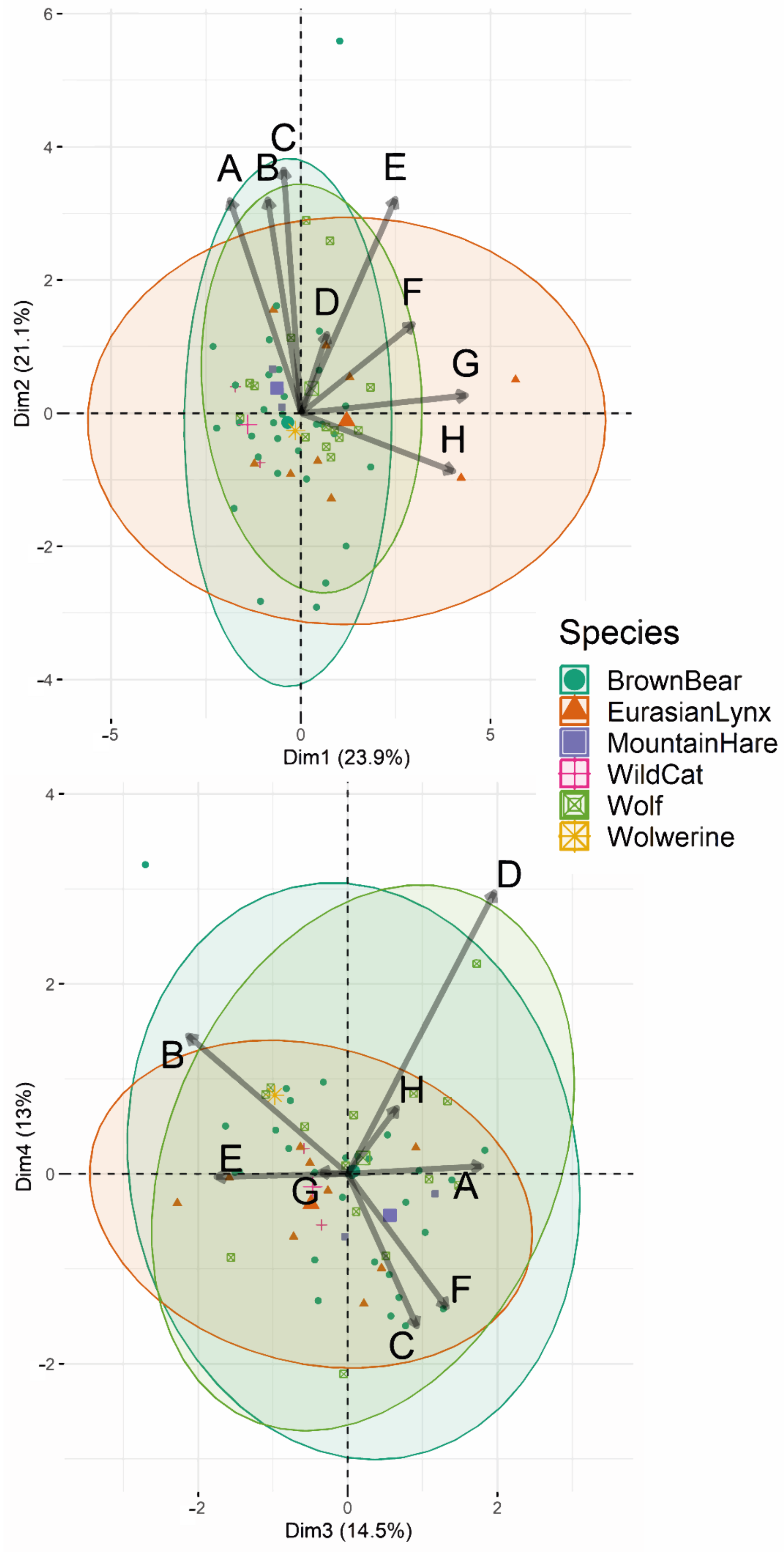

Figure A2. Biplot of variables and individuals classed by studied species (GDD – PCA).

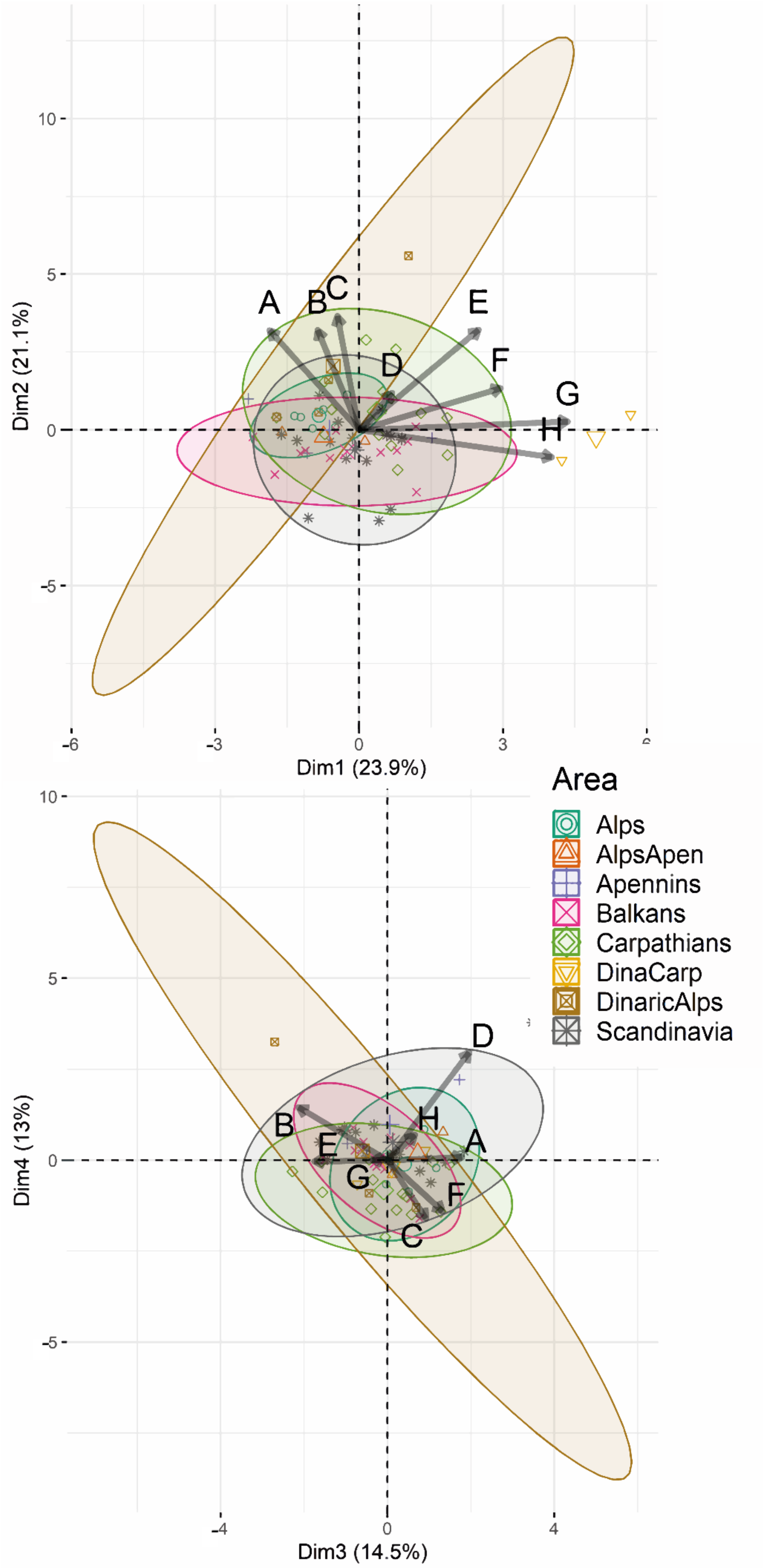

Figure A3. Biplot variables and individuals classed by study area (GDD – PCA).

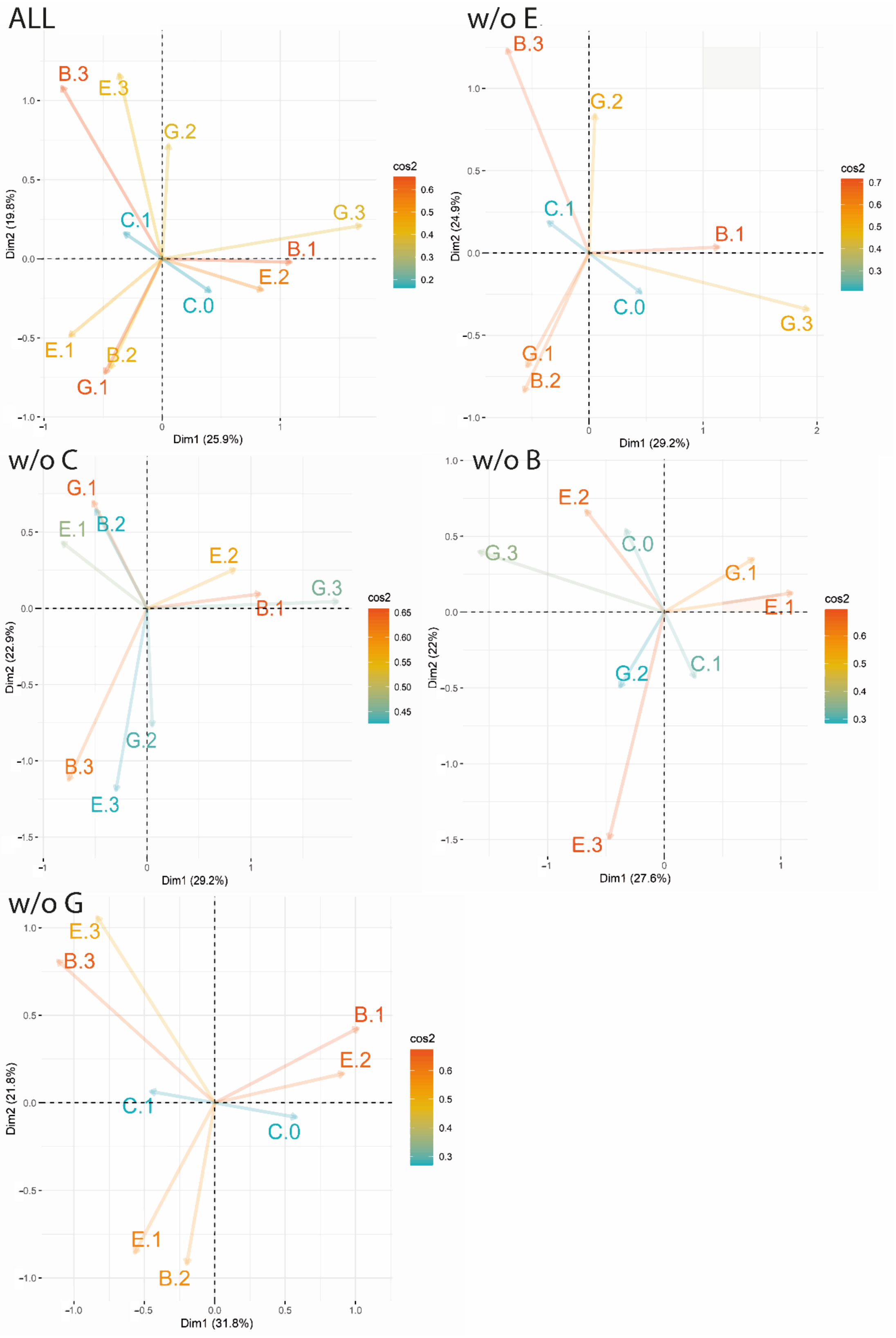

Figure A4. Graphic representation of the variables in the five MCAs coloured by their quality of representation: the main MCA with all variables (**ALL**), the one without "STRs" (**w/o E**), "Sex marker" (**w/o C**), "Samples per individual per year" (**w/o B**), and "Number of types of sample" (**w/o G**).

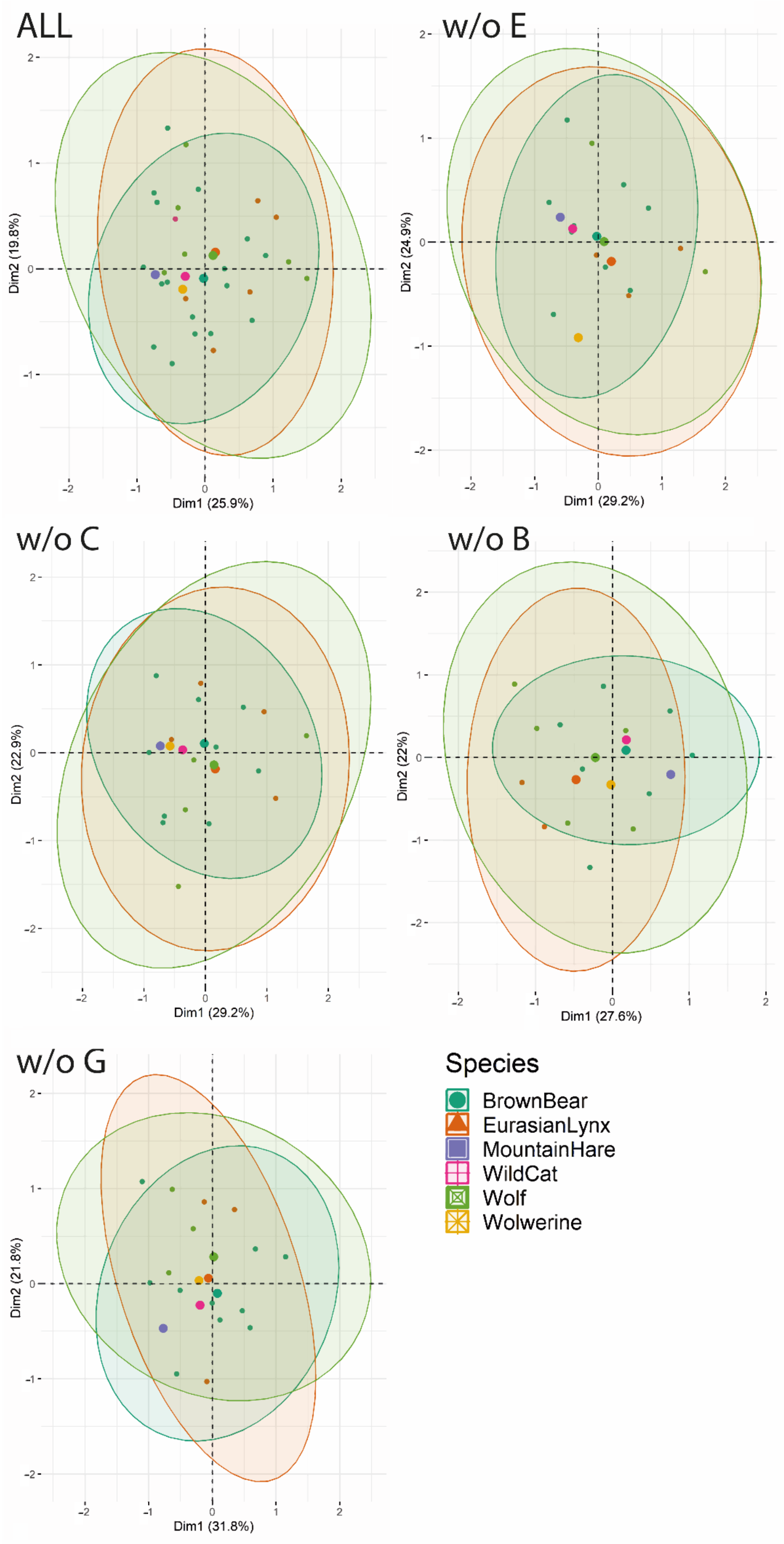

Figure A5. Graphic representation of individuals classed by species for all five MCAs.

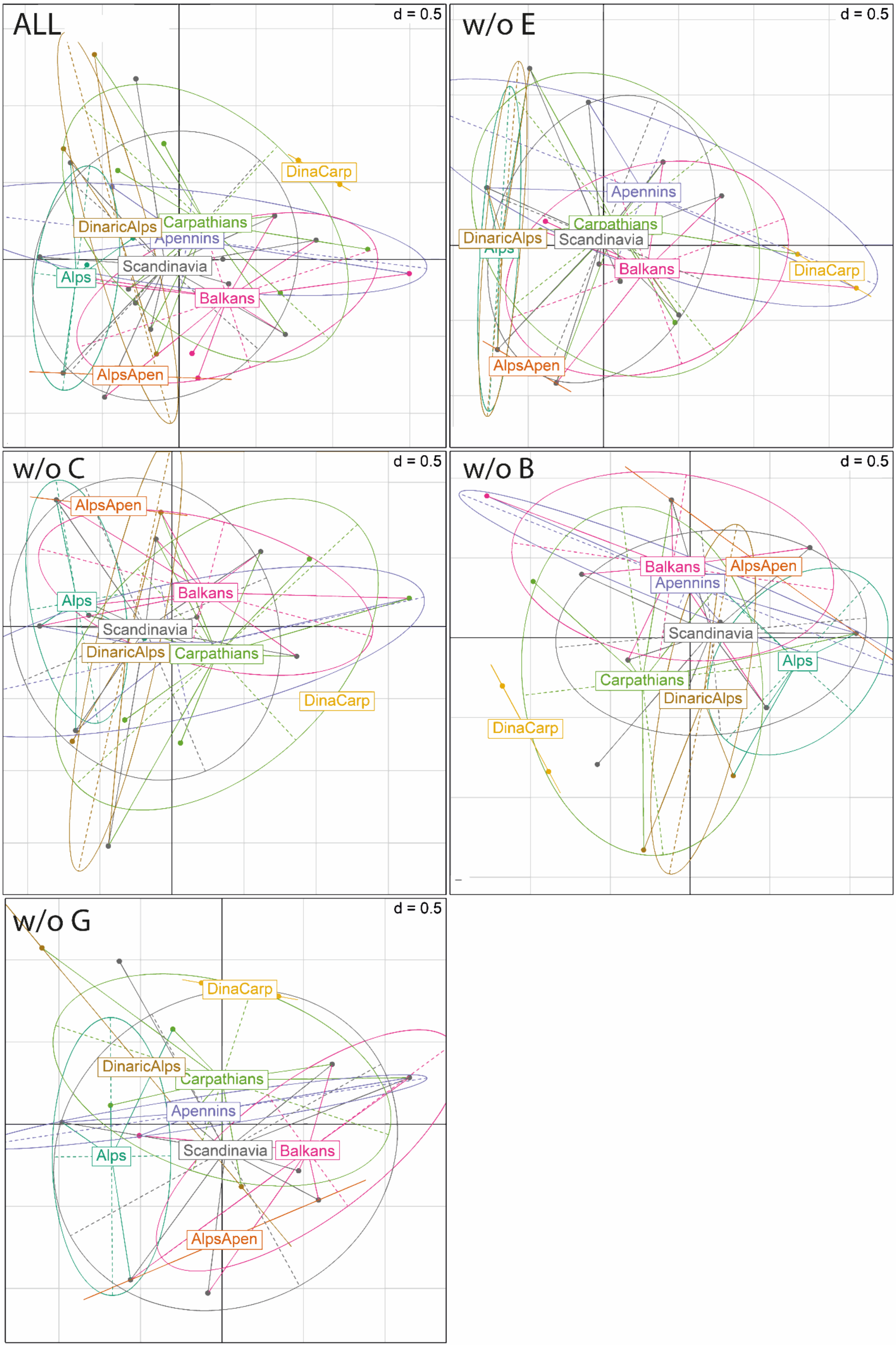

Figure A6. Graphic representation of individuals classed by study area for all five MCAs.

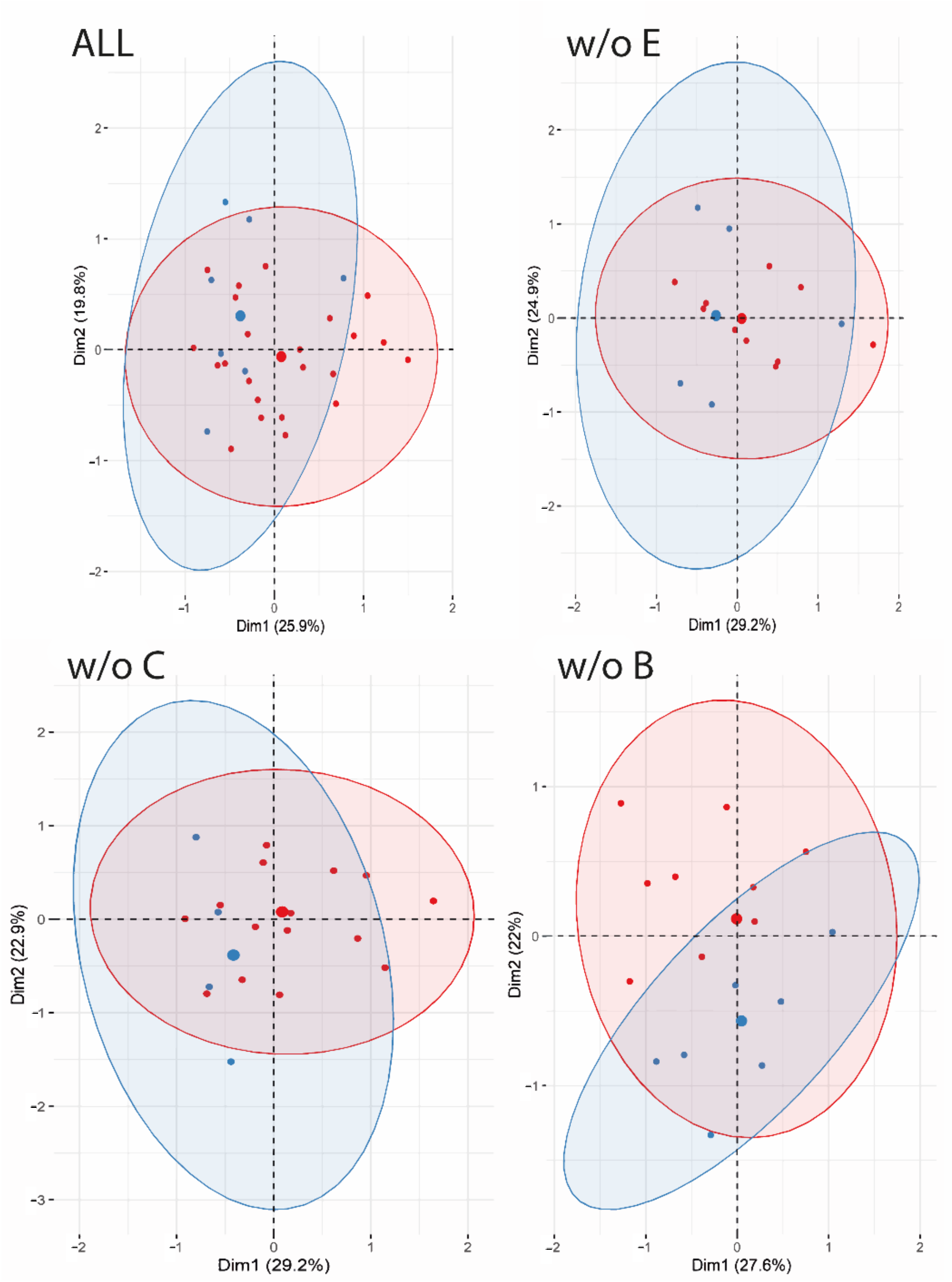

Figure A7. *Cont.*

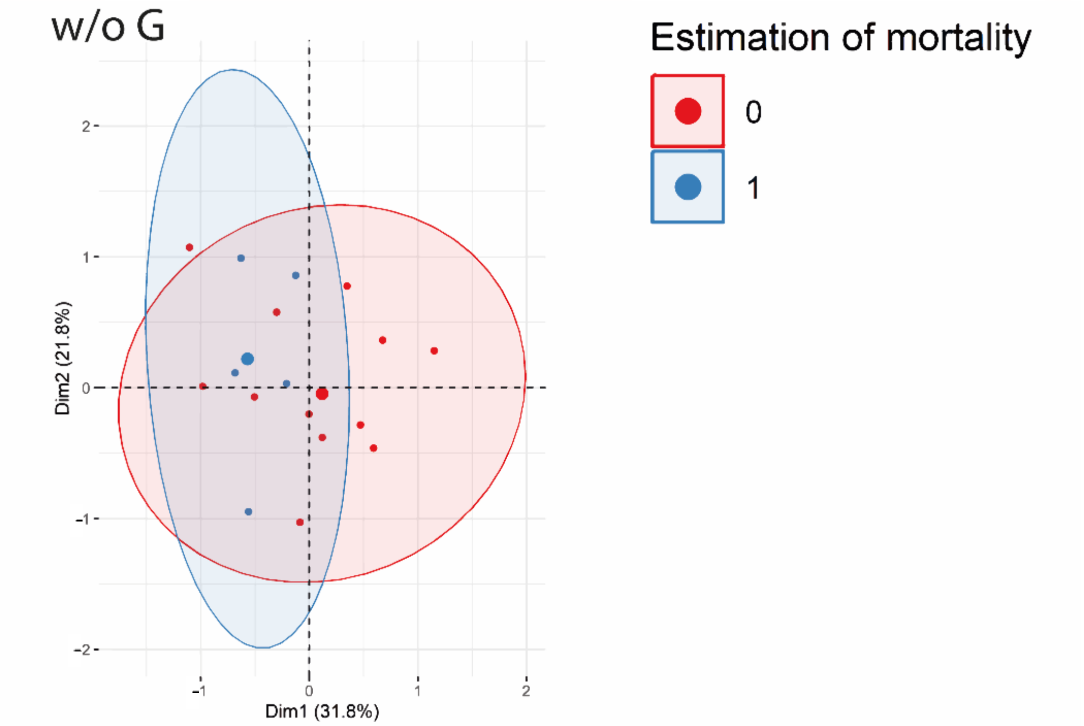

Figure A7. Graphic representation of estimation of mortality calculated (1) or not (0) for all five MCAs.

Table A1. Country acronyms used in Figures 7 and A8.

Country	Acronym
Norway	NO
Sweden	SE
Finland	FI
Russia	RUSS
Switzerland	CH
Italy	ITLY
Germany	DE
France	FR
Bulgaria	BG
Greece	GR
FYROM	FYROM
Albania	ALB
Lithuania	LT
Belarus	BYS
Romania	ROM
Poland	PL
Slovakia	SK
Czech Republic	CZ
Ukraine	UKR
Slovenia	SI
Montenegro	MNE
Croatia	HR
Bosnia Herzegovina	BA
Serbia	RS

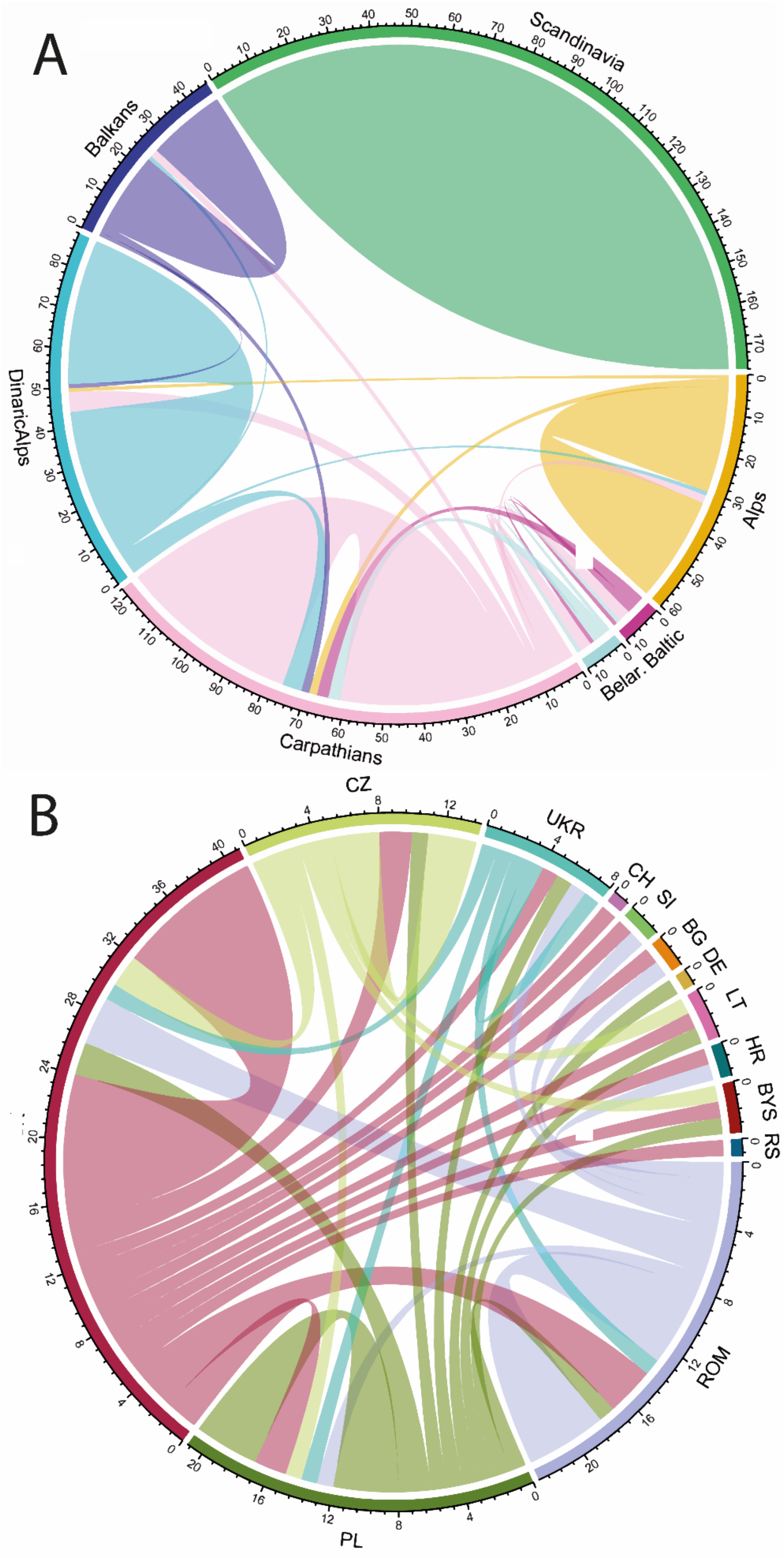

Figure A8. *Cont.*

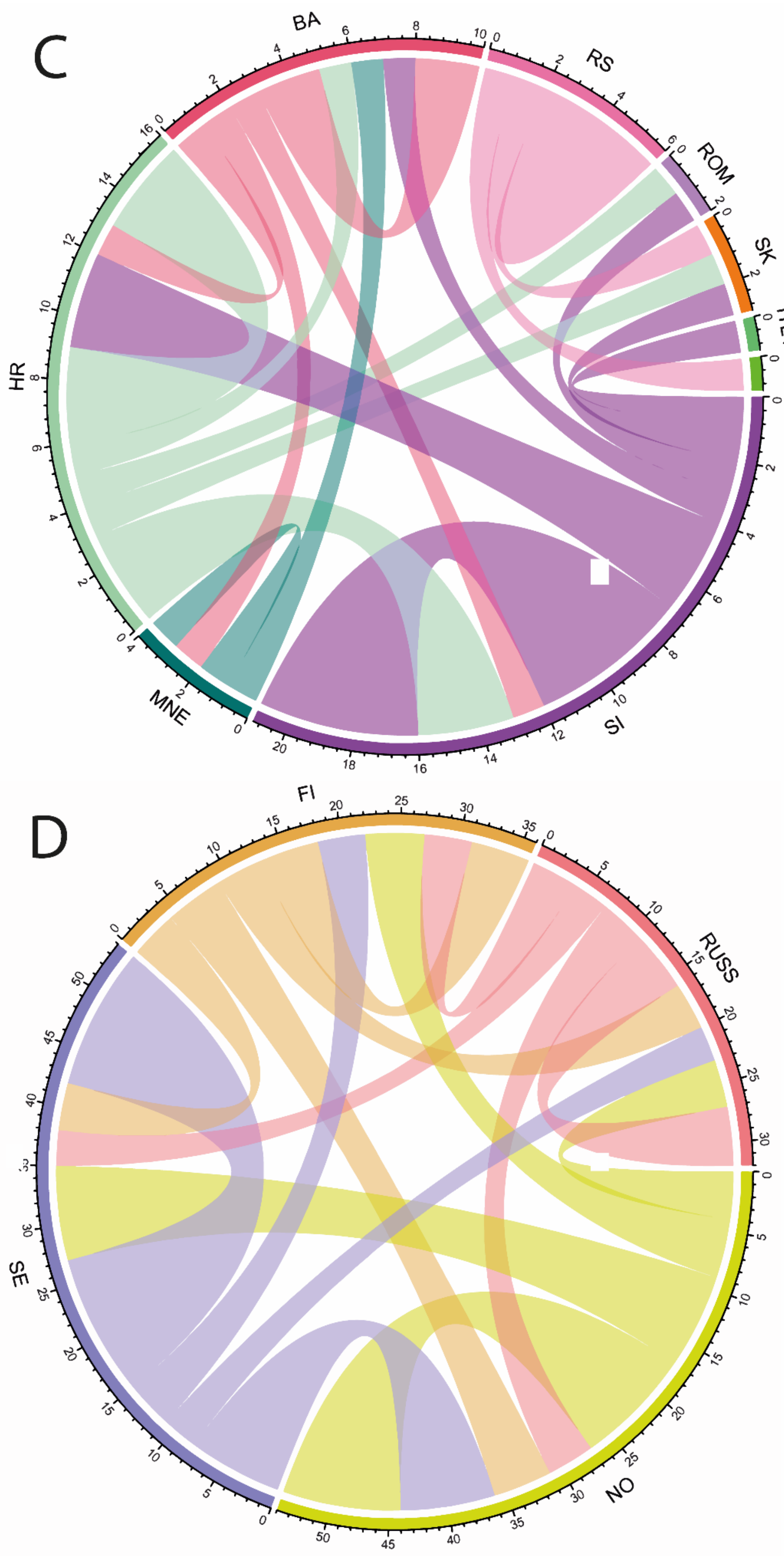

Figure A8. *Cont.*

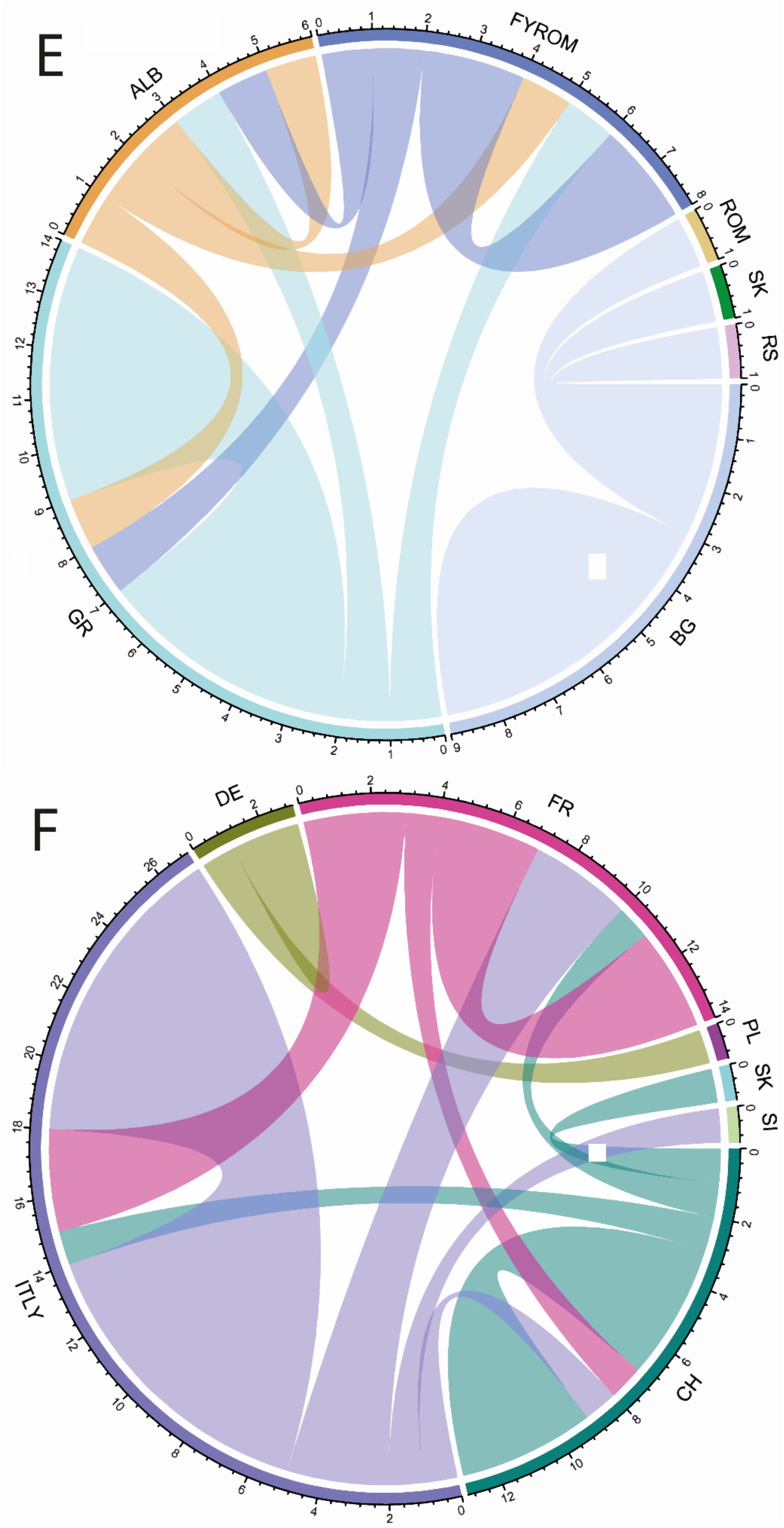

Figure A8. International collaboration between study areas: (**A**) Studies from the Apennines and the Alps are grouped in "Alps", Belar. = Belarus; and countries belonging to the Carpathians (**B**); International collaboration between countries belonging to the Dinaric Alps (**C**) and Scandinavia (**D**); International collaboration between countries belonging to the Balkans (**E**) and the Alps (**F**).

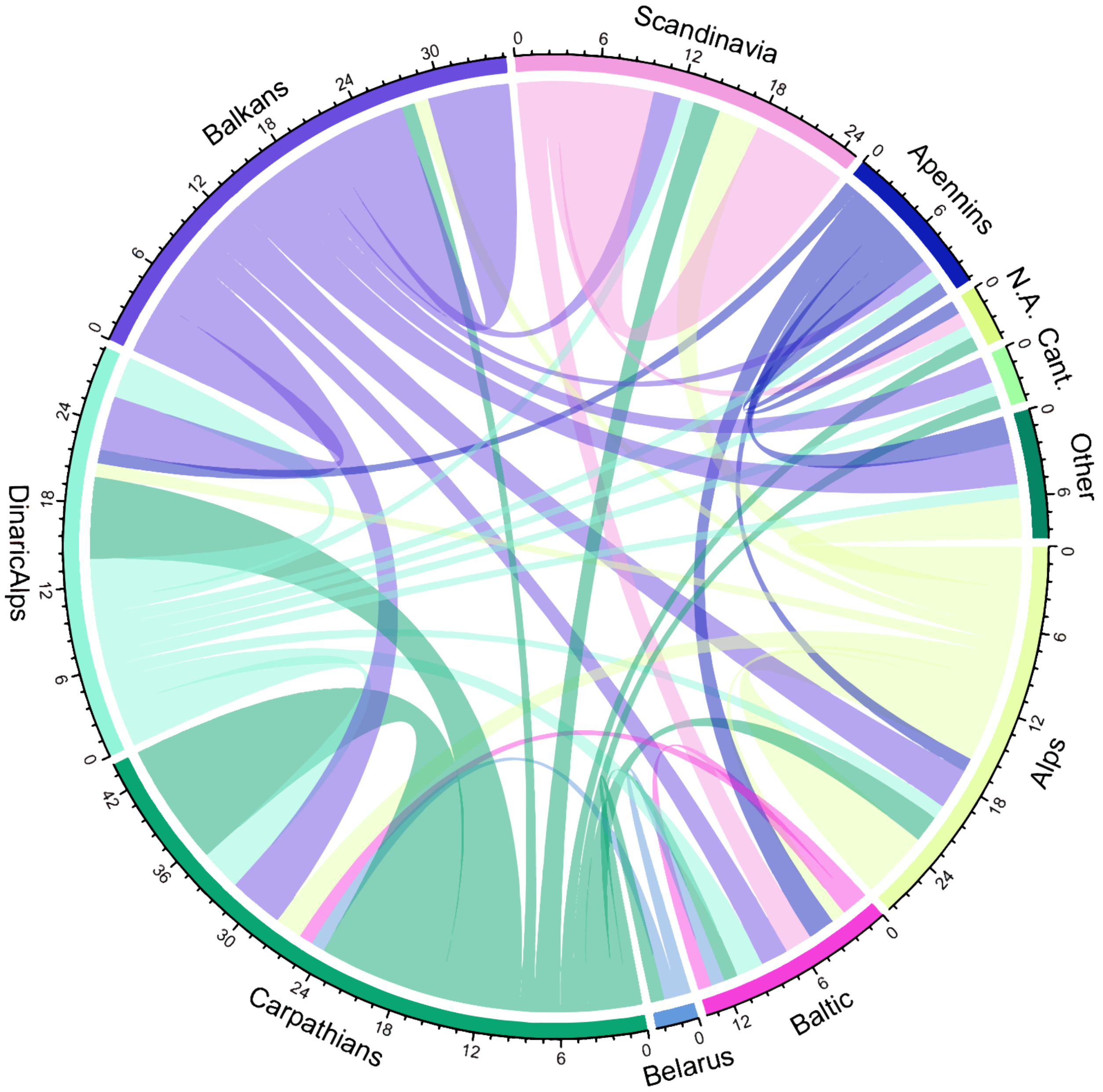

Figure A9. International sharing of information (N.A. = North America, Cant. = Cantabrians, Other = Alps-Dinaric-Pindos, North Africa, Middle East, Western Europe, Iberic Peninsula, Vosges-Palatinian, Bohemia-Bavara).

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