Traces of past reintroduction in genetic diversity: the case of the Balkan chamois

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Running head: Genetic diversity of reintroduced Balkan chamois

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Abstract
The translocation of wild animal species became a common practice worldwide to re-establish local populations threatened with extinction. Archaeological data confirm that chamois once lived in the Biokovo Mountain but, prior to their reintroduction in the 1960s, there was no written evidence of their recent existence in the area. The population was reintroduced in the period 1964–1969, when 48 individuals of Balkan chamois from the neighbouring mountains in Bosnia and Herzegovina were released. The main objective of this study was to determine the accuracy of the existing historical data on the origin of the Balkan chamois population from the Biokovo Mountain and to assess the genetic diversity and population structure of the source and translocated populations 56 years after the first reintroduction. We used 16 microsatellite loci to analyse the genetic structure of three source chamois populations from Prenj, Čvrsnica and Čabulja Mountains and from Mt. Biokovo. Both STRUCTURE and GENELAND analyses showed a clear separation of the reintroduced population on Biokovo from Prenj’s chamois and considerable genetic similarity between the Biokovo population and the Čvrsnica–Čabulja population. This suggests that the current genetic composition of the Biokovo populations does not derive exclusively from Prenj, as suggested by the available literature and personal interviews, but also from Čvrsnica and Čabulja. GENELAND analysis recognized the Balkan chamois from Prenj as a separate cluster, distinct from the populations of Čvrsnica and Čabulja. This suggests that the Neretva River and the state M17 road are geographic barriers for the species dispersal, as they form a genetic boundary.

Keywords: Balkan chamois, Biokovo, genetic structure, microsatellite, Prenj, translocation

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Introduction

The (re)introduction and translocation of wild species for various purposes became a common practice worldwide and was used as a conservation tool for rescuing and re-establishing extirpated populations (Cullingham and Moehrensclager 2013). Northern chamois (Rupicapra rupicapra L.) is one of the examples of successfully (re)introduced species (Apollonio et al. 2014) in many areas of Europe (Crestanello et al. 2009; Martínková 2012; Šprem and Buzan 2016), but also on other continents such as South America (Corlatti et al. 2011) and New Zealand (Christie 1964). Past translocations of chamois left a genetic signature in recent populations which can be now used for reconstructing undocumented events (Crestanello et al. 2009).

Today’s populations of the Northern chamois in northern Dinaric mountains in Croatia are descendants of successfully (re)introduced individuals captured on mountain areas in Bosnia and Herzegovina (Rupicapra rupicapra balcanica) and Slovenia (Rupicapra rupicapra rupicapra; Apollonio et al. 2014; Šprem and Buzan, 2016). Since different subspecies were involved in past reintroduction, a contact zone was formed on the northern Velebit Mountains where these subspecies hybridize (Šprem and Buzan 2016).

The Balkan chamois (Rupicapra rupicapra balcanica) is one of the seven recognized subspecies of the Northern chamois. It is found both in the mountainous regions of Croatia and in the mountain ranges of the eight other countries of the Balkan Peninsula, from north to south: Bosnia and Herzegovina, Serbia, Montenegro, Kosovo, North Macedonia, Albania, Bulgaria and Greece. The lack of continuity of these habitats and overhunting in the post-Neolithic period have severely fragmented subspecies present distribution (Corlatti et al. 2020). In addition to low colonization rates and reduced gene flow between isolated populations, which may lead to genetic differentiation due to the inbreeding effect and loss of allelic variants, the Balkan chamois is threatened by poaching (Papaioanou and Kati 2007), habitat change (Kavčić et al. 2019), unsustainable hunting (Šprem and Buzan 2016) and by the introduction of other subspecies of chamois, mainly Alpine chamois (Iacolina et al. 2019).

As a conservation measure, the Balkan chamois is listed in Annexes II and IV of the European Union Habitats Directive 92/43/EEC (OJ L 206, 22.7.1992) and in Appendix III of the Bern Convention (OJ L 38, 10.2.1982). The Balkan chamois is one of the most poorly studied subspecies of Northern chamois and knowledge on the genetic diversity and structure of the Balkan chamois population is limited and restricted to regional-local studies (Markov et al. 2016; Šprem and Buzan 2016; Papaioannou et al. 2019; Rezić et al. 2022).

The genetic structure of the Balkan chamois population on the Biokovo has been studied only by Šprem and Buzan (2016), and few other ecological studies have included this population in a population density estimation (Kavčić et al. 2019, 2021). The paleontological findings in the Baba cave, which are more than ten thousand years old, confirm that chamois once lived in Biokovo (Šabić 2011) but, before the reintroduction in the 1960s, there was no written evidence of the recent existence of chamois in this area. According to historical records, the chamois on Biokovo Mt. are descendants of individuals translocated from the "Prenj" hunting district in Bosnia and Herzegovina established in 1961 (Rapač and Kunovac 2020) which included both Prenj and Čvrsnica massifs. This hunting district had, in that time, stable and numerous chamois populations and was used for many reintroduction programmes in Balkan (Rapač and Kunovac 2020). The reintroduction of chamois on Biokovo Mt. was the result of the planned introduction on this area by the Union of Hunting Association, the municipality of Makarska and the Union of Hunting Association of Imotski, mainly with the aim of
increasing the population size for hunting purposes (Šabić 2011). Prior to the reintroduction from the "Prenj" hunting district, assessment of the suitability of the Biokovo habitat was made and, after a positive evaluation, a first release of 7 individuals (3 males and 4 females) took place on 1 November 1964 (Šabić 2014). A total of 48 chamois were successfully reintroduced in the period between 1 November 1964 and 23 October 1969 (Šabić 2014). The success of the reintroduction of the Balkan chamois in the Biokovo Mt. is reflected by the latest population size estimate (Kavčić et al. 2021), according to which this area is now inhabited by at least 600 individuals.

The main objective of this study was to determine the accuracy of historical data on the origin of chamois in Biokovo, and to assess and document the genetic status of both the source and translocated populations 56 years after reintroduction by using microsatellite markers.

**Methods**

*Ethical statement*

All samples used in this study were from hunted (regular hunting activities approved by the competent Ministry of Agriculture of the Republic of Croatia within the annual game management plans) and from remains of naturally dead animals (samples from Bosnia and Herzegovina).

*Population sampling*

We collected 20 samples from Biokovo and 29 samples from three areas which serve as source population for reintroduction and possible recent recolonization (Prenj, Čvrsnica and Čabulja mountains). Details of sampling locations are given in Fig. 1 and Table 1. The samples were collected between 2017 and 2020. After collection, the samples were preserved in 96% ethanol, delivered and stored at -80°C in the Laboratory of molecular ecology, University of Primorska.

*DNA extraction and microsatellite amplification*

We extracted DNA from tissue samples (N = 49) using the commercial peqGOLD Tissue DNA Mini Kit (PEQLAB Biotechnologie GmbH) following the manufacturer’s protocol in a volume of 150 µL. DNA concentrations were measured with Qubit® dsDNA BR Assay Kit (Invitrogen BR Assay Kit, Carlsbad, CA, USA) on a 3.0 Qubit Fluorimeter (Life Technologies, Carlsbad, CA, USA). Sixteen microsatellites were amplified using PCR multiplex sets previously investigated in studies with chamois (Zemanová et al., 2011; Buzan et al., 2013; Šprem and Buzan, 2016; see Supplementary file: Table S.1). The PCR protocol described in Rezić et al. (2022) was used for amplification of microsatellite regions. Genotyping errors were assessed by re-genotyping of 10 randomly chosen individuals from the final data set and comparing these genotypes with to the initial ones. Fragment analysis was performed on an ABI 3130 Genetic Analyzer (Applied Biosystems) using the GeneScan LIZ500 (-250) Size Standard (Applied Biosystems). Microsatellite genotypes were analyzed using Gene Mapper v. 4.0 software (Applied Biosystems).

*Microsatellite data analysis*

We used the Expectation-Maximization (EM) algorithm implemented in FREENA (Chapuis and Estoup, 2007) to estimate null allele frequencies for each microsatellite locus, as they can cause significant heterozygote deficit and population deviation from Hardy-Weinberg equilibrium (HWE). Values of null allele frequency greater than \( r \geq 0.20 \) were reported (see Supplementary file: Table S.2). FREENA software was also used to calculate global \( F_{ST} \)
values and \( F_{ST} \) values for each pair of analysed populations, both with and without the use of the excluding null alleles (ENA) correction method, as described in Chapuis and Estoup (2007). The Wilcoxon Two Sample test was used to compare the corrected \( F_{ST} \) values with the original \( F_{ST} \) values and to test the significance of null alleles in the analyses. The Wilcoxon Two Sample test was performed by R ver. 4.0.5 package stats (R Core Team, 2020).

We considered each sampling location as a separate population due to limited dispersal of subspecies between mountain ranges (see Table 1). The exact probability test for each locus and population was used to test the deviation of the observed genotype frequency from \( HWE \) using the Markov chain method with 10,000 dememorization steps, 500 batches and 10,000 subsequent iterations in GENEPOL ver. 4.7.2 (Rousset, 2008). The same test, based on a Markov chain method implemented in Genepop, was used to analyse pairwise linkage disequilibrium (\( LD \)) between all pairs of loci in all populations. A sequential Bonferroni procedure (Holm, 1979) was applied to correct for the effect of multiple comparison tests by using the adjust \( p \)-values function implemented in R ver. 4.0.5 package stats.

The Bayesian clustering program STRUCTURE ver. 2.3.4. (Pritchard et al., 2000) was used to estimate the most likely number of ancestral genotypes (\( K \)) within the entire sample, and to estimate the proportions of each ancestral genotype in Balkan chamois individuals. We run the analysis allowing for admixture and correlated allele frequency with ten independent runs for each \( K \) between 1 and 7 with a burn-in 500,000 steps followed by \( 10^5 \) Markov chain Monte Carlo (MCMC) iterations. The results of the repeated runs for each value of \( K \) were combined with the Greedy algorithm in CLUMPP ver. 1.1.2 (Jakobsson and Rosenberg 2007), and the summary outputs were combined with DISTRUCT ver. 1.1 (Rosenberg 2004). To estimate the most likely \( K \), we applied the ad hoc summary statistic \( \Delta K \) developed by Evanno et al. (2005). STRUCTURE HARVESTER (Earl and vonHoldt, 2012) was used to compare the average estimates of the likelihood of the data, \( \ln[\Pr(X|K)] \) for each value of \( K \). The same software was used to generate graphs for the mean log posterior probability of the data (mean±SD).

The modal proportions of ancestral genotypes for each individual in each sampled area from the run with the highest log-likelihoods was plotted on a map using QGIS ver. 2.18.21 (QGIS Development Team, 2018).

We estimated the effective population size (\( N_e \)) of populations using the linkage disequilibrium-based method (Hill 1981; Waples 2006; Waples and Do 2010) implemented in NeESTIMATOR V2 (Do et al. 2014). Rare alleles below an allele frequency of 0.02 were excluded (as recommended by Waples and Do 2010). The effective population size for Čabulja Mt. was not calculated due to small sample size.
The robustness of the results of STRUCTURE was estimated by analysing the same data with
the spatial Bayesian clustering model implemented in GENELAND software (Guillot et al.
2005a). Although there are several Bayesian clustering methods that perform spatial analysis
of genetic data, GENELAND was chosen because it provides the most accurate estimates of
ture genetic structure (Safner et al. 2011). We followed the recommendations of Guillot et al.
(2005a) to set up the analysis. The algorithm was run in two steps. In the first step, the
algorithm was run ten times to infer $K$ under the uncorrelated frequency model, with the
parameter indicating the degree of uncertainty of the spatial coordinates set to 10. The
MCMC iterations were set to $10^6$ with a thinning of 100. The number of populations was set
from $K=1$ to $K=5$. The maximum number of nuclei in the Poisson–Voronoi tessellation was
set to 300. After determining the number of population clusters in the first step, we ran the
algorithm setting $K$ to this number and leaving the other parameters as in the first step.

Results and discussion

The sixteen microsatellite loci yielded a total of 95 alleles, which varied between 2 (for locus
ETH10 and SR-CRSP-6) and 10 (for locus BM1258) with an average value of 5.937 alleles
per locus (see Supplementary file: Table S.1).

The values of null allele frequencies were low for most analysed loci, except for loci ETH10,
SY434, TGLA53, and SR-CRSP-6, whose frequencies were estimated to be $r \geq 0.20$ (see
Supplementary file: Table S.2). The presence of null alleles was found in all three
populations from Bosnia and Herzegovina. The various factors caused by natural population
mechanisms such as disassortative mating, bottleneck, fluctuations in population size can
cause heterozygote deficit that can be interpreted as false positive presence of null alleles
(Dąbrowski 2014), which led to the decision to retain all analysed loci.

The Prenj population deviated from Hardy–Weinberg equilibrium (HWE) but the deviation
was significant at the 0.05 level only for locus SY434 after applying sequential Bonferroni
adjustment (Table 1). This may be a consequence of the recent severe bottleneck in this
population, which was previously stable. Gafić and Džeko (2009) noted that the population of
Balkan chamois in the Prenj hunting district, which included both the Prenj and Čvrsnica
massifs, was about 4,000 individuals in 1966, but due to the war in the 1990s, the population
was greatly reduced by illegal hunting, with up to 95% of the population lost. Since no
deviation from HWE was observed at this locus in other populations, we retained it in all
subsequent analyses. After applying the sequential Bonferroni correction to the linkage
disequilibrium results, no significant value was observed.

The Prenj population had the highest values of observed (0.636) and expected (0.637)
heterozygosity, and allelic richness (2.517). A similar pattern was recorded in the study of
Šprem and Buzan (2016) where the Prenj population had the highest values of the allelic
richness, observed, and expected heterozygosity and significantly deviated from HWE. In
Šprem and Buzan (2016) study, the Biokovo population had the lowest allelic richness. The
observed number of alleles ($A$) varied from 3.375 in the Čabulja population to 4.625 in the
Biokovo population. All populations had private alleles ($N_{pr}$) and the highest number of
private alleles (11) was observed in Prenj population from Bosnia and Herzegovina (Table 1).
Effective population size was estimated for three sampled sites, excluding the Čabulja
population due to small sample size (Table 1). Čvrsnica had the lowest results for $N_e$,
although very similar to those estimated for Prenj. The higher estimates for the Biokovo
population should be taken with caution, considering our results suggest the presence of
multiple funding sources. Additionally, Do et al. (2014) showed that microsatellite loci could lead to a slight upward bias for the linkage disequilibrium method when the critical value is set to $p = 0.02$.

**Table 2.** Pairwise $F_{ST}$ values between four studied populations of Balkan chamois

<table>
<thead>
<tr>
<th>Populations</th>
<th>Čvrsnica</th>
<th>Čabulja</th>
<th>Biokovo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prenj</td>
<td>0.084*</td>
<td>0.047 ns</td>
<td>0.074*</td>
</tr>
<tr>
<td>Čvrsnica</td>
<td>0.024 ns</td>
<td>0.072*</td>
<td></td>
</tr>
<tr>
<td>Čabulja</td>
<td></td>
<td>0.027 ns</td>
<td></td>
</tr>
</tbody>
</table>

*p values: NS – non–significant value; * - significant at $p < 0.05$.

The lowest $F_{ST}$ value was found between Čvrsnica and Čabulja ($F_{ST} = 0.024$), while the highest and significant $F_{ST}$ value (0.084) was observed between two neighbouring populations from Bosnia and Herzegovina (Prenj and Čvrsnica; Table 2). The global $F_{ST}$ values were 0.067 ($CI = 0.031–0.108$) without using the correction method and 0.071 ($CI = 0.038–0.112$) with the ENA correction method for null alleles. The Wilcoxon Two Sample test showed no significant differences between the corrected and original $F_{ST}$ values ($p = 0.734$), indicating that the presence of putative null alleles did not affect the analysis (see Supplementary file: Fig. S.1).

The algorithm developed by Evanno et al. (2005) identified $K=3$ as the optimal number of ancestral genotypes detected by STRUCTURE analysis for four analysed populations and detected another peak at $K=5$ suggesting a possible further genetic structure subdivision within the populations (see Supplementary file: Fig. S.2). According to the STRUCTURE results, populations from Prenj, Čvrsnica and Biokovo had high proportions of genomes from a single ancestral genotype ($q \geq 0.88$ in all three populations), which for Prenj defer comparing to Čvrsnica and Biokovo, while in the population from Čabulja the highest proportion of same ancestral genotype as in Čvrsnica was lower 0.63 (Fig. 1a). Difference in genetic composition between Balkan chamois from Prenj and other populations is likely due to a barrier to gene flow between the studied populations, but also probably a consequence of recent bottleneck effect in populations, due to extirpation of chamois in Balkan civil war (Frković 2008) and population local adaptation. Čvrsnica, Čabulja and Prenj Mts. are restricted to habitat patches, particularly to steep river canyon of Neretva, which may suggest their small scale fragmentation. The Neretva River, one of the largest rivers in the eastern part of the Adriatic Basin, separates the Prenj Mt. from the Čvrsnica and Čabulja Mts. and forms the official border between the "Čvrsnica" and "Prenj" game reserves (Jurić 1998). Landscape features of the Neretva River valley, as well as the close by state road M17, built as a part of the European Route E73, can be effective barriers for the species natural spreading, as they hinder gene exchange between the studied populations. But also the population decline in the last Balkan civil war can contribute that small groups of chamois may stay isolated in restricted habitat patches where they are geographically very close to each other. Individual proportions of ancestral genotypes assigned by STRUCTURE support the higher levels of admixture in Čabulja when compared to the other populations (Fig. 1b). According to the $q$ values, only one individual from Čabulja had a $q$ value above the threshold of 0.75 sharing similar genotype as individuals from the Čvrsnica population, while
all others had admixed genotype \((q < 0.70)\). One individual from Čvrsnica stood out from the others with a \(q = 0.23\) proportion of ancestral genotype that was present mostly in Prenj population. As previously mentioned, natural migration between individuals from Prenj and Čvrsnica is currently unlikely, due to the presence of barriers, however these might not be a case before the construction of infrastructure. STRUCTURE indicated that the reintroduced Balkan chamois population in the Biokovo Mt. is genetically more similar the population from Čabulja, suggesting that the reintroduced individuals in the Biokovo Mt. may have originated from this area as well as from Čvrsnica. According to Jurić (1998), 992 individuals of Balkan chamois were counted in the Čvrsnica hunting ground which also includes the territories of the neighbouring Čabulja Mt. It is possible that populations Čvrsnica and Čabulja were connected in the past and formed a single population, which can be inferred from the results of STRUCTURE. Due to the civil war in the Balkans in the 1990s and continued illegal hunting and poaching, this single population has dwindled in numbers and become fragmented and isolated in the high mountain habitats (statements of local people).

The divergence between Prenj and Biokovo from Čvrsnica and Čabulja populations may be due to the historical founder effect and more recent genetic drift due to isolation, and local adaptation. Despite decades of long unsustainable hunting, predation, poaching and natural events (Šprem and Buzan 2016) the inbreeding levels in the analysed populations are still moderate. The results of genetic composition of Biokovo population can influence population viability through time and it is very important to monitor genetic parameters of the reintroduced population to prevent a loss of genetic diversity due to inbreeding and genetic drift (DeMay et al. 2017).

To improve the previous analyses, the spatial context of individuals was taken into consideration and tested with GENELAND. This analysis revealed a similar pattern of clustering of individuals as STRUCTURE, but suggested an additional fourth spatial cluster along the MCMC chain (see Supplementary file: Fig. S.3). GENELAND detected two spatial clusters among the three analysed populations in Bosnia and Herzegovina, while one spatial cluster was corresponded to the population in Biokovo (Fig. 1). The distinction between Prenj and Biokovo was also detected by Šprem and Buzan (2016), who used the BAPs algorithm for spatial clustering of groups and showed the separation of the Balkan chamois population of the Prenj Mt. from the Biokovo Mt. population. The fourth cluster revealed by GENELAND spatial model, was a so-called “ghost cluster” (Frantz et al. 2009), since no individual was assigned to it. Ghost clusters are not uncommon but are still a poorly understood phenomenon that can be caused by a heterogeneous distribution of samples (Aziz et al. 2018). It is possible that all the clusters identified by GENELAND represent a true genetic subdivision, but the degree of differentiation between them was too low for the clustering to be consistent (Frantz et al. 2009). Another possibility is that the GENELAND model might overestimate the number of genetic clusters when analysing populations which are affected by isolation by distance (Frantz et al. 2009).

Future studies will need to incorporate non-invasive genetic sampling, telemetry and behavioural patterns to confirm possible migration and gene flow between these two populations. In the available literature there is no indication of the exact location where the animals released on Biokovo were caught. It is only known that the individuals came from the Prenj hunting district, which included two game reserves called ”Čvrsnica” and ”Prenj” (established in 1893 by the Austro-Hungarian Empire) and which were declared protected areas (Rapaić and Kunovac 2020). Jurić (1998) stated in his master's thesis that the translocation of Balkan chamois from the game reserve ”Čvrsnica” started in 1962 and lasted
until 1970. During this period, a total of 101 Balkan chamois were translocated to different areas in Bosnia and Herzegovina and abroad, but the author did not write any additional information about the location where the animals were caught or the places where they were translocated. It is not yet known whether these data exist. Therefore, it is very important to establish standards for documenting and monitoring species translocation projects.

Conclusions
Non-invasive monitoring of genetic parameters of both reintroduced and source populations of endangered Balkan chamois, together with demographic monitoring, is crucial for sustainable management practices and improving conservation strategies to maximize the chances of population persistence. Our genetic diversity results show that the Balkan chamois population from Biokovo can serve as a potential source for future translocations, especially to the source habitats, Čvrsnica and Čabulja, that are currently threatened by loss of genetic diversity due to unsustainable hunting and poaching, leading to inbreeding and genetic drift.

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Figure legends

Figure 1. Results of the analysis of sixteen microsatellite loci in four Balkan chamois populations. (a) Geographical representation of results from STRUCTURE and GENELAND software. The pie charts show the results from STRUCTURE for K = 3. The different colours of the pie charts represent the proportions of each ancestral genotype per individual q (in %) in each of the four predefined Balkan chamois populations. The size of the pie charts indicates the number of samples collected at each location. The different shapes and colours of the chamois silhouettes represent the results of the spatial analysis under uncorrelated frequency model performed in GENELAND. The three spatial clusters are shown, while the assignment to the fourth ghost cluster was not shown because no individuals were assigned to it (see text for details). The dashed line indicates the national border, while the state road M17 in Bosnia and Herzegovina is marked with an orange line. The green lines represent connections with other main roads. The course of the river Neretva is marked by a blue line; (b) Genetic structure of the 49 Balkan chamois individuals analysed, shown as a bar plot from STRUCTURE at K = 3. Each vertical bar represents an individual, and the percentage of each colour corresponds to the percentage of the respective ancestral genotype. The studied populations are separated by a black line.
Table 1. Genetic diversity of four Balkan chamois populations assessed using sixteen microsatellite loci

<table>
<thead>
<tr>
<th>Population locality/country</th>
<th>N</th>
<th>$H_O$ (SD)</th>
<th>$H_E$ (SD)</th>
<th>$HWE$</th>
<th>$F_{IS}$ (IC 95%)</th>
<th>A</th>
<th>AR</th>
<th>$N_{pr}$</th>
<th>$N_e$ (IC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prenj</td>
<td>12</td>
<td>0.636 (0.274)</td>
<td>0.637 (0.150)</td>
<td>0.013*</td>
<td>0.046 (-0.147–0.116)</td>
<td>4.500</td>
<td>2.517</td>
<td>11</td>
<td>10.500 (6.500–18.600)</td>
</tr>
<tr>
<td>Čvrsnica</td>
<td>12</td>
<td>0.552 (0.287)</td>
<td>0.536 (0.206)</td>
<td>0.011NS</td>
<td>0.014 (-0.131–0.038)</td>
<td>3.937</td>
<td>2.223</td>
<td>3</td>
<td>7.700 (4.100–13.600)</td>
</tr>
<tr>
<td>Čabulja</td>
<td>5</td>
<td>0.575 (0.251)</td>
<td>0.535 (0.187)</td>
<td>0.913NS</td>
<td>0.059 (-0.415–0.089)</td>
<td>3.375</td>
<td>2.376</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Biokovo</td>
<td>20</td>
<td>0.584 (0.132)</td>
<td>0.597 (0.138)</td>
<td>0.389NS</td>
<td>0.048 (-0.050–0.084)</td>
<td>4.625</td>
<td>2.356</td>
<td>6</td>
<td>57.200 (28.600–371.800)</td>
</tr>
</tbody>
</table>

**Notes:**
- BIH – Bosnia and Herzegovina, HRV – Croatia; $N$ – number of samples; $H_O$ – observed heterozygosity; $H_E$ – expected heterozygosity; SD – standard deviation; $F_{IS}$ – inbreeding coefficient; IC 95% – 95% Confidence Interval; $HWE$ – Hardy-Weinberg equilibrium (after Bonferroni adjustment p values); NS – non-significant value; * – significant at $p < 0.05$; A – average number of alleles; AR – allelic richness; $N_{pr}$ – number of private alleles; $N_e$ – effective population size and its confidence interval estimated with the chi-square (i.e., parametric).