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Content

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ANALYSIS OF ICHTHYOFAUNA COMPOSITION, SPECIES DISTRIBUTION AND WATER QUALITY OF THE UPPER OLT RIVER BASIN

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Abstract: *The study aimed to assess the ichthyofaunal composition of the upper Olt basin, and to determine the conservation status of the existing fish species. At the same time, water quality was determined and possible sources of pollution were identified. Estimates were also made of the biogenic capacity of the water and, consequently, its productivity. Thus, for the ichthyofauna inventory, sampling stations were established on tributaries of the Olt River (23 tributaries with a total of 51 sampling stations). Sampling of macro invertebrates at the same stations identified 10 genera. Water sampling for the qualitative study was carried out for the fauna inventory stations resulting in 41 samples. The field studies carried out in the rivers of the upper Olt basin identified the presence of seven fish species, of which only the trout has an almost continuous distribution in the entire hydrographic network. Among the fish species of community interest, the presence of the Romanian barbel (Barbus meridionalis), the European bullhead (Cottus gobio), and the golden spinead loach (Sabanejewia aurata) was identified, with fragmented distribution in the streams with permanent flow, in short river segments, in the hilly and foothill areas for the common eel, and in the hilly and mountain areas for the common scoter. The analysis of the main water quality parameters shows an adequate water quality, characteristic of mountain rivers, with temperatures generally below 18 degrees Celsius, absence of pollutants, low concentrations of nitrates, nitrites, pH in the biological optimum for fish species, with the presence of phosphates in some of the sampling stations and ammonium ion. Investigations of benthic macro invertebrate communities revealed the presence of the main groups of invertebrates in mountain waters (Plecoptera, Trichoptera, Ephemeroptera, Diptera, Amphipoda, Coleoptera, Oligocheta, Platelmintes, Nematoda, Odonata), biological indicators of surface water quality, with continuous distribution in the river sectors but with different abundance over time, depending on the stages of larval development of insects and the frequency of floods.*

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Key words: *ichthyofauna, macroinvertebrate, Olt basin, quality parameters, biogenic capacity.*

1. Introduction

Mountain aquatic ecosystems are favourable habitats for a restricted number of fish species, characterized by increased demands in relation to environmental characteristics [4]. It is well known that individuals of different species and age classes [8, 18, 24] show specific requirements with respect to certain aquatic parameters: water temperature [17, 20], flow velocity [18, 28, 35], water depth [29], and concentrations of some dissolved chemicals in the water [31]. There is also a selection of favourable habitats according to the characteristics of the substrate [6, 29, 35], the abundance and distribution in the water mass of some shelter elements (rocks, roots and trunks of trees, hollows dug in the banks) [33]. Last but not least, the presence and, especially the abundance of individuals in certain sections of the river is conditioned by the abundance of and accessibility of food [5, 8, 11, 17, 22, 26, 30].

Dispersal is a determinant characteristic that influences the distribution and dynamics of animals and presents essential information for management planning [1, 27].

This paper presents the results of research carried out in 2019 on some aquatic ecosystems in the mountain area of trout distribution (*Salmo trutta* L., 1758), belonging to the upper course of the Olt River, upstream of the city of Brasov. The study aimed at analysing some hydrobiological aspects of the investigated rivers, as well as the ichthyofauna composition, and benthic macroinvertebrate communities, and at assessing the conservation status of the species and natural habitats. The characteristics of the runoff [13] were examined, the values of the main physico-chemical water quality parameters were determined, studies were carried out on the benthic macroinvertebrate communities [12, 15], and fish species [14] were identified in the analysed river sectors. The mentioned aspects facilitated the obtaining of the necessary information for the production of the distribution map of fish species in the rivers of the upper basin of the Olt River, the estimation of the water quality in the investigated river sectors, the characterization of the benthic macroinvertebrate communities and the main trophic resource of trout.

2.Material and Methods

2.1. Study area

The upper Olt basin, between the source and the confluence with the Homorod river, has a catchment area of 6,340 km^2, and crosses the Ciuc and Bârsei Depressions with an average altitude of the basin between 600-750 m [34].

The studies on the inventory of ichthyofauna species, the characterization of benthic macroinvertebrate communities, and the estimation of the physico-chemical water quality included a total of 23 tributaries of the main course, where 51 stations for biological sampling and water parameters measurements were located. The sampling stations were located on the following rivers: Zizin, Black River, Lemnia, Copolna, Zagon, Ghelinta,

Ghelinta Mica, Casin, Casinul Nou, Cașinul Mic, Turia, Bârsa Ursului, Bârsa Groseț, Cheile Râșnoavei, Cheișoara, Pârâul Rece, Țigănești, Pârăul Diham, Glăjarie, Cașin, Cașinul Mic, Cașinul Nou, and Turia. In order to analyse the ichthyofauna composition, classical capture methods were used, i.e. nets, carrying out qualitative studies, the captured fish being released alive.

Sampling for the benthic macroinvertebrates research was carried out in the same sampling stations used for the ichthyofaunal study, totalling 61 samples. The location of the sampling stations is shown in Figure 1.

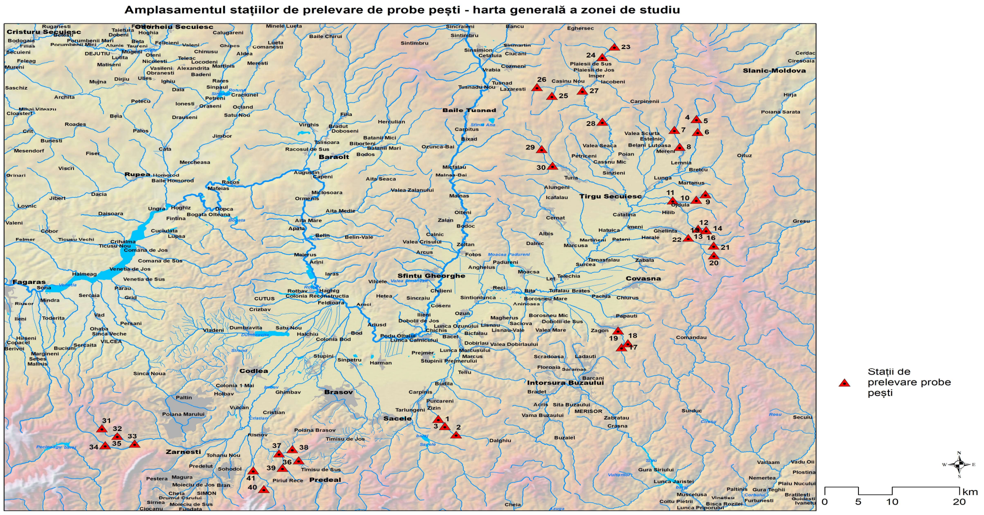

Fig. 1. *Location of sampling stations - General map of the study area*

2.2. Methods

The inventory activities for the ichthyofaunal study were conducted based on the established methodology developed for the study of fishes in mountain rivers [25]. The selected sampling stations were representative for the ecosystems investigated and included different types of microhabitats [33]. The sampling stations were established according to the characteristics of the aquatic parameters relevant for the habitat selection of salmonids at different stages of ontogenetic development (juveniles, fry, adults), i.e. water current velocity, water column depth, substrate granularity, presence of natural shelters. In order to identify taxa and estimate population size, 51 sampling stations were installed and 61 samples were taken for laboratory analysis. Water and benthic macroinvertebrate sampling were carried out in the vicinity of the sampling stations for the aquatic fauna samples, during the same period.

Water quality determination was performed in the laboratory with the PF12 PLUS photocolorimeter, the "VISOCOLOR ECO" kit for drinking water analysis, and the "PONSEL ODEON X" portable field kit.

Both the FP12 PLUS photocolorimeter and the “Ponsel Odeon X” kit are used to perform a precise qualitative analysis of the aquatic physico-chemical parameters mentioned in the article, in field and laboratory studies and frequently in aquaculture. The technical characteristics of the FP 12 PLUS photocolorimeter can be downloaded from office@adis-international.ro.

The analysis of the physico-chemical quality of water was done by determinations for a total of 41 water samples, taken during the month of July, from a total of 22 rivers delimited in the upper Olt River for each sample, 11 laboratory analyses were performed with the PF 12 Plus Photocolorimeter, and three field terminations were performed with the PONSEL ODEON X Handheld Water Quality meter (oxygen concentration, Ph, electrical conductivity). Water temperature was recorded with the aquaculture alcohol thermometer.

In order to establish the physico-chemical quality of the water, data on dissolved oxygen concentration (mg/l), nitrite concentration (mg/l $\mathrm{NO_2^-}$), nitrate concentration (mg/l $\mathrm{NO_3^-}$), ammonium concentration (mg/l $\mathrm{NH_4^+}$), phosphate (mg/l $\mathrm{PO_4^{-P}}$), sulphate (mg/l $\mathrm{SO_4^{2-}}$), sulphite (mg/l $\mathrm{S^{2-}}$), manganese (mg/l Mn), iron (mg/l Fe), zinc (mg/l $\mathrm{Zn^{2+}}$), chloride (mg/l $\mathrm{Cl_2}$), water pH, conductivity of water (µS/cm), and water temperature (degrees Celsius) were analysed.

The width of the river bed, the water depth expressed in meters and the current velocity expressed in m/s were chosen as hydrograph variables.

The sampling areas for fish were 50 m^2, where fish species were identified specifying the presence and absence at the sampling stations; the sampling areas for benthic macroinvertebrates were 0.5 m^2, biomass was reported at 1 m^2. The determinations were carried out at the invertebrate order level and aimed at identifying suprataxa and estimating the trophic potential of rivers for fish. Macroinvertebrate sampling targeted taxa characteristic of the fish fauna. The samples were fixed in 4% formalin for transportation, sorted and identified with a magnifying glass and binoculars [7, 23, 31, 32], and then weighed with electronic scales.

The macroinvertebrates collected from the sampling stations belong to genera and species common to mountain rivers and have no special protection status established by legislation.

Some of the fish species identified in the investigated river streams have protection status conferred by their inclusion in Annex II of the Habitats Directive, 92/43/EC, updated in April 2025. In order to protect the captured fish, including those with protected status, the method of capturing them by electrofishing was abandoned. Nets with mesh sizes adapted to the size of the fish were used. All captured fish were released live in the habitats from which they were caught, immediately after identification, without somatometric measurements or biological sampling for laboratory analysis. Only qualitative determinations (presence of the species in the rivers investigated) were performed.

In order to estimate the anthropogenic impact, the effects of human activities on the physico-chemical parameters of water and the quality of aquatic habitats for fish were analysed, identifying the forms of pollution and potential sources of pollution, namely damming/capturing of water for the purpose of taking flows for human communities, changes in the character of runoff by building dykes to protect settlements, the construction of concrete sills in the bed of the riverbeds to regularize the flow of water, wastewater discharge downstream of the settlements, mineral aggregate extraction, and logging works on the slopes of major riverbeds.

2.3. Statistical Data Analysis

A number of three hydrographic variables were taken into account, 14 variables expressing the physico-chemical characteristics of water, and seven variables related to fish fauna (seven species of fish).

From the total of 24 variables, five variables were eliminated, whose values did not exceed the permissible values namely anions SO^{3-} (sulphates), SO^{2-} (sulphites), cations H^{4+} (ammonium), Fe^{+} (iron), and Zn^{+} (zinc).

The analysis addressed concerns statistical indices of variability, normality and determining the form of the relationships between variables by applying specific tests. Statistical analysis took into account the indices of experimental distributions, dispersion indices (variance, standard deviation, coefficient of variation), normality of distribution, and correlation analysis (simple correlation).

3.Results

3.1. Qualitative and Quantitative Composition of the Ichthyofauna

The aquatic habitats investigated in the upper sector of the Olt River are specific to the mountain and submontane areas, characterized by the presence of the common trout (*Salmo trutta* L.), alongside the European bullhead (*Cottus gobio* L.), the common minnow (*Phoxinus Phoxinus* L.), the Romanian barbel (*Barbus meridionalis* Heckel), more rarely the stone loach (*Barbatulla barbatulla* L.), and the golden spined loach (*Sabanejewia aurata* De Filippi). Towards the lower part of the rivers, when they flow into the Olt River, the European barbel (*B. meridionalis* Risso) and the European chubs (*Petroleuciscus cephalus* L.) are more frequent, but also other species common to the aquatic habitats of the hilly area, namely the common sandpiper (*Alburnoides bipunctatus* Bloch), the common walleye (*Alburnus alburnus* L.*)*, species of the genus *Gobio* (e.g. *Cottus gobio* L.), and other species of the genus *Cobitis*. During the study period, seven fish species were identified, the dominant one being the common trout, whose presence was indicated in most of the investigated rivers, in the sampling stations in the mountainous area, with stable populations, with pre-reproductive and reproductive (adult) age specimens.

Among the fish species of conservation interest, the presence of the Romanian barbel (*Barbus meridionalis* Risso), the European bullhead (*Cottus gobio* L.) and the golden spined loach (*Sabanejezia aurata* De Filippi) was recorded in the investigated area. The common trout (*Salmo trutta* L.) was identified in 22 sampling stations (11% of the total) in the rivers Zizin, Black River, Copolna, Ojdula, Zagon, Ghelința, Ghelința Mică, Cașin, Bârsa Ursului, Cheile Râșnoavei, Cheișoara, Pârăul Rece, Pârăul Diham, and Glăjărie. The Romanian barbel (*Barbus meridionalis* Risso) was identified in 12 sampling stations (6% of the total) installed on the rivers Zizin, Copolna, Ojdula, Ghelința Mică, Cașin, Cașinul Mic, and Turia. The European bullhead (*Cottus gobio* L.) was identified in four sampling stations (2% of the total) on the rivers Zizin and Turia. The golden spined loach (*Sabanejewia aurata* De Filippi) was identified in six stations (3%

of the total) on the rivers Copolna, Ojdula, Cașin, and Turia. The stone loach (*Barbatula barbatula* L.) was identified in six stations (3% of the total) installed on the rivers Lemnia, Casin, Cașinul Nou, Cașinul Mic, and Turia.

The European chubs (*Petroleuciscus cephalus* L.) was identified in five stations (2.5% of the total) on the rivers Ghelința Mică, Cașin, and Turia. Summarized data are presented in Table 1. The distribution of the fish species identified is presented in **Appendix 1.**

Fish species identified Table 1

Species	No. of station
common trout (*Salmo trutta* L.)	22
European bullhead (*Cottus gobio* L.)*	4
common minnow (*Phoxinus Phoxinus* L.)	9
Romanian barbel (*Barbus meridionalis* Risso)*	12
stone loach (*Barbatula barbatula* L.)	6
golden spined loach (*Sabanejewia aurata* De Filippi)*	6
European chubs (*Petroleuciscus cephalus* L.)	5

Note: *Species of conservation concern

The species *Cottus gobio* (L.) and *Sabanejewia aurata* (De Filippi) are listed in Annex II of the Habitats Directive. The species *Barbus meridionalis* (Risso) has recently been reclassified as *Barbus petenyi* (Heckel) in Romania.

All mentioned species are common species for the ichthyofauna of Romania, being present in most mountain rivers.

3.2. Qualitative and Quantitative Composition of Benthic Macroinvertebrate Communities

The study of benthic macroinvertebrates in the rivers belonging to the upper Olt River basin allowed quantitative and qualitative estimates of invertebrate communities.

In the fieldwork, significant differences were observed between the quality, abundance, and composition of invertebrate communities sampled from different rivers, which can be explained by the frequent spring and early summer floods which affected the abundance and diversity of benthic macroinvertebrate communities differently.

The field surveys indicated the presence of 10 orders of invertebrates, some being recorded with high frequency (76% Ephemeroptera, 78% Trihoptera, 68% Plecoptera, 60% Amphipoda) in almost all monitoring stations, with numerical and gravimetric abundance corresponding to the harvesting period, and others with rare presence, such as odonates, which were identified in only one sample from the Ojdula River, and nematodes which also occur in only one station from the Bârsa Ursului River. Oligochaetes (with nine present) and platyhelminthes (with 10 present) are also mentioned with rarer presence.

From the numerical point of view, ephemeropterans are dominant, followed by amphipods, trihopterans, plecopterans, dipterans, and coleopterans. The presence of a higher number of invertebrate orders in some of the investigated rivers explains a better water quality, but also a better stability (uniformity) of the hydrological characteristics.

High biological diversity was found in the rivers:

- Zizin (six invertebrate orders present);
- Black River (seven invertebrate orders);
- Lemnia (eight invertebrate orders);
- Copolna (eight invertebrate orders);
- Ojdula (six- eight invertebrate orders);
- Ghelința (seven invertebrate orders);
- Cașinul Nou (seven invertebrate orders);
- Cheișoara (six invertebrate orders).

The lowest diversity was found in the Cașin River, station four (three orders of macro invertebrates), Bârsa Ursului, station S2 (two orders of invertebrates), Bârsa Groșet (three orders of invertebrates), Cheile Râșnoavei (three orders). A high biological diversity of macroinvertebrates can be observed especially in the upper sections of the rivers (upstream areas), less affected by floods, with more constant water temperatures. Summarized data are presented in Table 2.

Cyclograms of macro invertebrate communities in the analysed stations are presented in **Appendix 2**.

3.3. Physico-Chemical Water Quality of Investigated Rivers

The analysis of the values of the main physico-chemical parameters of the water, which condition the survival of aquatic organisms, shows that they are located between the limit's characteristic of mountain waters, specific for waters populated by salmonids, and the lower part of the same, the area of the aubergine moray, with some different values of the parameters compared to the trout area.

For the investigated rivers, the water temperature values ranged from 7 degrees Celsius at the stations on the Tiganesti and Paraul Doamnei streams, to 21 degrees Celsius, measured at the downstream stations of the Casin (stations three and four) and Turia rivers. It is mentioned that the characteristic values of the aquatic habitats favourable to trout remain below 16-17 degrees Celsius in summer, with fluctuations of only 2-3 degrees Celsius during the day. For monitoring stations where higher characteristic temperature values were recorded, the fish species of the ecological zone of the moraine (*Barbus meridionalis* Risso, *Gobio gobio* L., *Alburnus alburnus* L., *Petroleuciscus cephalus* L., *Sabanejewia aurata* De Filippi, *Sabanejewia romanica* Băcescu) were identified. Temperatures up to 17 degrees Celsius were recorded at 17 sampling stations. Temperatures above 18 degrees Celsius were measured at eight sampling stations. The dominance of the common trout (*Salmo trutta* L.) and its distribution in the investigated rivers is thus explained by water temperature values.

Table 2

Benthic macroinvertebrate communities

Order	No. of station
Ephemeroptera	39
Plecoptera	35
Trihoptera	40
Amphipoda	31
Diptera	18
Coleoptera	23
Oligocheta	9
Platelmintes	11
Odonata	1
Nematoda	1

Values between 6.7 and 8.03 were determined the pH (Table 3). These values fall within the optimum pH range for trout, which is considered to be between 6.5 and

7.5. The lowest values of this parameter (pH 6.5) were found at station S2 on Bârsa Ursului, and the highest at the stations on Cașinul Nou and Cașinul Mic. In the range 6.5-7.5 were samples from 13 sampling stations on Black River, Ojdula, Cașin (station one), Bârsa Ursului, and Bârsa Groset. For all stations pH values corresponding to the survival of trout and other identified fish species were recorded.

The physical and chemical parameters of water Table 3

Parameter	Values
Water temperature	7-21°C
pH	6,7-8,03
Oxygen concentration	8,3 mg/l
Nitrites concentration	< 1.2mg/l
Nitrates concentration	0.12mg/l
Sulphates	0
Sulphites	0
Ammonium cation	0
Phosphates	0.3-1.1 mg/l* (Casin station)
Fe^+	0
Zn^+	0
Chlorine	0

The dissolved oxygen concentration was high, above 8.3 mg/l. For trout, the optimum oxygen concentration values necessary for normal development are considered to be above 7.5 mg/l. In river courses, there is in general a tendency for dissolved oxygen concentration to decrease from upstream to downstream, the coefficient of oxygen diffusion in the water being correlated with temperature values.

Nitrate concentration was in most of the sampling stations below the detection threshold of the reagents used, which characterizes clean mountain waters. Values slightly above the detection limit were measured in stations S1 on the Cașin river, S1 on the Turia river, S2 on the Bârsa Ursului and Cheișoara rivers, the values found being less than 1.2 mg/l, without having a negative impact on fish populations or aquatic macroinvertebrates.

Values higher than the detection limit were also recorded for nitrites on the Black River in stations S2 and S3, Lemnia in S2, Copolna in S1, Cașin in all stations, Cașinul Mic, Bârsa Groșet, Cheișoara, and Glăjărie. The highest nitrite value was found in station S1 on the Cașin river (0.12 mg/l). These concentrations do not affect aquatic fauna. The presence of sulphates, and sulphites and ammonium was not detected. Phosphates were recorded with concentrations higher than the threshold of detection of reagents at Station S1 on Ghelința (0.3 mg/l), S2 on Ghelința, and S1 on Ghelința Mică. A higher concentration of phosphates was found at Station S1 on the Casin River (1.1 mg/l). Values higher than the limit of detection were also recorded on the streams Cașinul Mic, Turia, Bârsa Ursului (in both stations), Cheile Râșnoavei, Cheisoara, and Glăjarie. It is estimated that values higher than 0.5 mg/l phosphate cause water quality alterations. The presence of Fe and Zn was not found in any of the stations, and chlorine (Cl2) was measured in the S2 stations on Lemnia and Pârăul Diham with low concentrations.

The values of the analysed parameters certify an adequate surface water quality for aquatic organisms, which excludes the possibility of chemical or domestic pollution of the investigated waters.

However, it is possible that some of the watercourses flowing through human settlements may be potentially affected temporary, by runoff water carrying domestic waste, fuels, etc.

3.4. Statistical Data Analysis

For the statistical analysis of the data, a number of three hydrographic variables were taken into account, namely the width of the riverbed, the water depth expressed in meters, and the current velocity expressed in m/s. The variables expressing the physico-chemical characteristics of the water, 14 in number, refer to (Table 4): temperature (°C), pH (-lg[H_3O]) and the amount of oxygen dissolved in the water (mg/l), the anions SO_3- (sulfates) , SO_2- (sulfites), PO_4- (phosphate), NO_3- (nitrates), NO_2-(nitrites), Cl- (chlorine), the cations NH_4+ (ammonium), Fe+ (iron), Zn+ (zinc), Mn+ (manganese), also expressed in mg/l, and water conductivity (µS/cm). Variables related to the fish fauna, seven in number (seven fish species) were expressed by presence/absence indices 1 and 0, respectively.

The experimental distributions of these variables are presented in Table 4. Analysing the statistical indices of variability and in particular the values of the coefficient of variation, extremely high values are found for almost all variables, except for pH and dissolved oxygen in water with values below 10%. The high values of this index for the other variables suggest an extremely high variability and thus the heterogeneity of the sample [19]. Moreover, by applying the Shapiro-Wilk normality test (N = 41) for all the variables taken in the study, it was found that they deviate significantly from the normal distribution.

In this context, the realization of correlations between the analysed variables, respectively the establishment of the form of the links between them, was carried out by applying a non-parametric test, namely Spearman's test (rank correlation), for transgression probabilities $\alpha = 0.05$, $\alpha = 0.01$, $\alpha = 0.001$ (Table 5).

4. Discussion

Analysing the data of the correlation matrix concerning the correlations established between the physico-chemical and hydrographical characteristics of the water and the fish species present, the following aspects emerge:

- The first two hydrographic variables, i.e. bed width and water depth, do not correlate with any fish species;
- Water current velocity is significantly negatively correlated with the *Bb* (*Barbatulla barbatulla* L.) and *Pc* (*Petroleuciscus cephalus* L.) species. This can be attributed to the fact that these two species usually prefer still waters with low current velocities and high benthos, respectively, being omnivorous species.

Water conductivity correlates significantly negatively with *St* (*Salmo trutta* L.), distinctly significantly positively with *Bm* (*Barbus meridionalis* Risso), significantly positively with *Bb* (*Barbatula barbatula* L.), and significantly positively with *Sa* (*Sabanejewia romanica* Băcescu), *Pc* (*Petroleuciscus cephalus* L.) and *Pp* (*Phoxinus phoxinus* L.). The first negatively significant correlation practically corresponds to the theory that the amount of dissolved oxygen in the water is affected by the amount of salts in the water, *St* (*Salmo trutta* L.) being a sensitive species to this parameter [2, 3, 16]. The two most distinctly significant correlations are realized by the *Bm* (*Barbus meridionalis* Risso) and *Bb* (*Barbatulla barbatulla* L.) benthic omnivorous species, the former also being a moderately photophobic species. The other positively significant correlations for the *Sa* (*Sabanejewia romanica* Băcescu), *Pc* (*Petroleuciscus cephalus* L.), and *Pp* (*Phoxinus phoxinus* L.) species follow the same reasoning, with a somewhat lower weight on food specificity implicitly driven by this water mineralization.

Indices of experimental distributions for the variables studied Table 4

Variables	Mean	Median	Variance	Standard deviation	Coef. of variation	St. error of mean
Bed width	4,1005	4,0000	2,4930	1,5789	38,5058	0,24659
Depth	0,1743	0,1600	0,0047	0,0684	39,2598	0,01069
Speed	0,4000	0,3300	0,0439	0,2094	52,3599	0,03271
Conductivity	267,38	237,0000	23512,08	153,33	57,3459	23,94714
T water	15,268	15,0000	9,1012	3,0168	19,7587	0,47115
pH	7,5766	7,5900	0,1054	0,3246	4,2840	0,05069
O_2	10,9100	10,6100	1,1094	1,0533	9,6542	0,16449
NO_3^-	0,0829	0,0000	0,0895	0,2991	360,6601	0,04671
NO_2^-	0,0095	0,0000	0,0005	0,0217	227,8534	0,00338
PO_4^{3-}	0,1073	0,0000	0,0452	0,2126	198,0966	0,03320
Mn	0,0024	0,0000	0,0002	0,0156	640,3124	0,00244
Cl_2	0,0056	0,0000	0,0003	0,0173	308,8824	0,00271
Salmo trutta (L.)	0,5366	1,0000	0,2549	0,5049	94,0865	0,07885
Barbus meridionalis (Risso)	0,2927	0,0000	0,2122	0,4606	157,3875	0,07194
Cottus gobio (L.)	0,0976	0,0000	0,0902	0,3004	307,9164	0,04692
Sabanejewia aurata (De Filippi)	0,1463	0,0000	0,1280	0,3578	244,5233	0,05589
Barbatula barbatula (L.)	0,1463	0,0000	0,1280	0,3578	244,5233	0,05589
Petroleuciscus cephalus (L.)	0,1220	0,0000	0,1098	0,3313	271,6616	0,05174
Phoxinus phoxinus (L.)	0,2195	0,0000	0,1756	0,4191	190,9043	0,06545

Correlation matrix of Spearman's coefficients (ranks) for the studied variables Table 5

Variables	**Width**	**Depth**	**Speed**	**Cond.**	**T water**	**pH**	**O_2**	**NO_3**	**NO_2**	**PO_4**	**Mn**	**Cl_2**	**St**	**Bm**	**Cg**	**Sa**	**Bb**	**Pc**	**Pp**
Width	-	0,30	0,03	-0,04	0,26	-0,11	-0,09	0,02	**0,50****	0,16	0,09	-0,10	0,23	0,17	-0,06	-0,01	-0,13	0,16	**-0,11**
Depth	-	-	**0,43****	-0,03	-0,10	-0,15	-0,15	-0,07	0,26	0,11	-0,21	-0,26	0,07	-0,02	-0,08	0,02	-0,20	0,16	**-0,25**
Speed	-	-	-	**-0,32^{o}**	**-0,52^{ooo}**	**-0,39^{o}**	0,14	-0,09	0,15	-0,02	0,11	-0,20	0,08	-0,20	-0,05	-0,20	**-0,35^{o}**	**-0,37^{o}**	**-0,10**
Cond.	-	-	-	-	**0,48****	**0,71*****	-0,02	0,27	0,12	0,11	0,13	0,06	**-0,31^{o}**	**0,44****	0,29	**0,31***	0,41**	**0,38***	0,37*
T water	-	-	-	-	-	0,29	**-0,52^{ooo}**	0,10	0,16	0,12	0,13	-0,02	**-0,46^{oo}**	**0,72*****	0,26	**0,51*****	0,43**	**0,49****	0,47**
pH	-	-	-	-	-	-	0,13	0,02	0,06	0,07	0,25	-0,07	-0,30	**0,38***	**0,35***	0,02	0,40*	**0,32***	0,32*
O_2	-	-	-	-	-	-	-	-0,24	-0,07	-0,23	0,19	-0,02	**0,36***	-0,28	-0,26	-0,23	-0,28	-0,26	**-0,14**
NO_3^-	-	-	-	-	-	-	-	-	**0,34***	**0,48***	-0,04	0,21	0,08	0,02	0,22	0,14	0,14	0,17	**0,07**
NO_2^-	-	-	-	-	-	-	-	-	-	**0,32***	0,21	0,01	-0,08	0,19	-0,20	0,04	0,04	0,12	**0,04**
PO_4^-	-	-	-	-	-	-	-	-	-	-	0,29	-0,01	0,11	0,13	-0,00	0,03	0,08	**0,35***	**-0,05**
Mn	-	-	-	-	-	-	-	-	-	-	-	-0,05	-0,17	0,25	-0,05	-0,07	**0,38***	-0,06	**0,30**
Cl_2	-	-	-	-	-	-	-	-	-	-	-	-	-0,03	-0,21	-0,11	-0,14	**0,34***	-0,12	**0,03**
St	-	-	-	-	-	-	-	-	-	-	-	-	-	**-0,58^{ooo}**	-0,19	**-0,45^{oo}**	**-0,45^{00}**	-0,25	**-0,57^{ooo}**
Bm	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0,33***	**0,64*****	**0,34***	**0,58*****	0,69***
Cg	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0,10	0,10	0,13	0,42**
Sa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0,22	**0,48****	0,45**
Bb	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0,27	0,45**
Pc	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-0,02**
Pp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Note: * $p<0.05$*, $p< 0.01$**, $p<0.001$***, ^{o} $p<0.05^{o}$, $p < 0.01^{oo}$, $p <0.001^{ooo}$; t – *Salmo trutta* L., Bm – *Barbus meridionalis* Risso, Cg – *Cotus gobio* L., Sa – *Sabanejewia aurata* De Filippi, Bb – *Barbatulla barbatula* L., Pc – *Petroleuciscus cephalus* L., Pp – *Phoxinus phoxinus* L..

Water temperature is distinctly significantly negatively correlated with *St* (*Salmo trutta* L.), which are sensitive to large temperature fluctuations. Distinctly significant positive correlations for this variable are achieved with the *Bb* (*Barbatulla barbatulla* L.), *Pc* (*Petroleuciscus cephalus* L.), and *Pp* (*Phoxinus phoxinus* L.) species. This significance suggests an increased amplitude of these species relative to the mean water temperature. The highly significant correlations realized by this variable with the benthic species *Bm* (*Barbus meridionalis* Risso) and *Bb* (*Barbatulla barbatulla* L.) also suggest a temperature affinity.

The pH, expressed as the inverse of the log of the decimal logarithm of the concentration of hydronium ions (H_3O), correlates significantly with the species *Bm* (*Barbus meridionalis* Risso), *Cg* (*Cottus gobio* L.), *Bb* (*Barbatulla barbatulla* L.), *Pc* (*Petroleuciscus cephalus* L.), and *Pp* (*Phoxinus Phoxinus* L.). This correlation actually defines the species' tolerance of water acidity.

The amount of dissolved oxygen in the water achieves a single significant positive correlation with the *St* (*Salmo trutta* L.) species, suggesting that this parameter is vital for the species.

Nitrate and nitrite concentrations do not correlate significantly with fish species, but can be considered significant in relation to other parameters.

The phosphate anion shows significant positive correlations with the *Pc* (*Petroleuciscus cephalus* L.) species, taking into account the fact that, being relatively stable, it provides an important food source under low activity conditions.

The manganese cation and the chlorine anion show significant positive correlations with the *Bb* (*Barbatulla barbatulla* L.) species, the explanation for this correlation being speculative, given the miscibility and different electrical charges.

The significant negative and positive correlations realized between fish species are interesting, but speculative at least in the absence of a complete set of qualitative and quantitative information.

The cited species are distributed in two ecological zones of distribution, i.e. the *trout* zone, located in the upstream part of the rivers, characterized by high water flow velocity, lower temperatures (usually below 16 degrees Celsius), higher dissolved oxygen concentrations, peat type of runoff, with boulder or stony substrate and banks covered with forest vegetation [4]. In this area, trout occurs with high frequency together with *Cottus gobio* (L.), but also with *Barbatulla barbatulla* (L.) and *Phoxinus phoxinus* (L.), and sometimes with *Barbus meridionalis* (Risso). The second ecological zone described for the investigated rivers is that of the *Romanian barbel,* characterized by higher water temperatures, sometimes 18-20 degrees Celsius, laminar flow, stony or sandy substrate, sometimes the stones are covered by bioderma, lower water flow velocity, and higher turbidity. In these habitats, along with the *Romanian barbel* (*Barbus meridionalis* Risso) individuals of the species *spined loach* (genus *Sabanejewia*), *Petroleuciscus cephalus* (L.), *Alburnoides bipunctatus* (Bloch), *Alburnus alburnus* (L.), but sometimes, towards the upper limit of the area, *trout* and its accompanying species also occur. The distribution of fish species in the investigated river stretches is continuous (no artificial constructions or natural structures were identified that prevent or limit fish migration/dispersal), except for the upper river segments, where the steep slopes and rugged relief favoured the formation of natural waterfalls, representing natural barriers to the migration of trout to spawning areas. The distribution of species in the river sectors is not uniform, each species having specific requirements for habitat conditions (habitat selection). It can be appreciated that although few individuals of the described species were evidenced, the aquatic habitats offer favourable conditions for the development of ichthyofauna, except for the autumn and winter period when the river flows are considerably reduced, significantly decreasing the habitat area and its quality for fish species, and significantly exposing the populations to the risk of predation.

During the field monitoring period, pre-reproductive (less than one year for most fish species, and less than two years for trout) and reproductive individuals were identified for all fish species. However, the number of trout older than 2-3 years was low and can be explained by low river flows in the fall season, lack of suitable habitats and possibly fishing.

The invertebrate orders occurring in the majority of the sampling stations are *Ephemeroptera* (identified in 39 sampling stations), *Plecoptera* (identified in 35 stations), and *Trihoptera* (identified in 40 stations), followed by *Amphipods* (identified in 31 stations), *Diptera* (in 18 stations) and *Coleoptera* (in 23 stations). Oligochaetes were identified at nine sampling stations and flatworms at 11 sampling stations.

An estimate of the anthropogenic impact was made for each of the investigated rivers. First of all it should be mentioned that all the rivers investigated during the field activities showed values of aquatic parameters corresponding to the quality of unpolluted waters. The quality of surface water is also affected by the transportation of wood material through the bed of the weirs, the crossing of the weirs through the minor bed, and the temporary storage of wood material in inappropriate conditions. Although such situations have not been identified in the field, they represent potential sources of impact on the quality of aquatic ecosystems. The possibility of using surface water for drinking water supply may affect river flows in drought conditions. The regularization of watercourses is a factor impacting the quality of aquatic ecosystems and biological diversity [9].

Another source of impact on aquatic ecosystems is the discharge of wastewater.

An overall analysis of the monitoring points revealed that the majority of the data collected characterizes the aquatic habitat as little or not at all affected by anthropogenic factors.

Anthropogenic factors with medium or major potential impacts include: the presence of inhabited areas (settlements, industrial zones – [10]), uncontrolled clearing of riparian vegetation, bank siltation, and exploitation of mineral aggregates (gravel, sand, etc.).

5. Conclusions

The field studies carried out in the rivers of the upper Olt basin identified the presence of seven fish species, of which only the trout has an almost continuous distribution in the entire hydrographic network. Among the fish species of community interest, the presence of

Barbus meridionalis (Risso), *Cottus gobio* (L.), and *Sabanejewia aurata* (De Filippi) was identified with fragmented distribution in the streams with permanent flow, in short river segments, in the hilly and foothill areas for *Barbus meridionalis* (Risso) and *Sabanejewia aurata* (De Filippi), and mountain areas for *Cottus gobio* (L.). In the upper segments of the streams the dominant species is *Salmo trutta* L., with the common trout (*Cottus gobio* L.), *Phoxinus phoxinus* (L.), and rarely *Barbatulla barbatulla* (L.). The river stretches in the premontane zone are dominated by *Barbus meridionalis* (Risso) with which *Petroleuciscus cephalus* (L.) occurs.

The analysis of the main water quality parameters shows an adequate water quality, characteristic of mountain rivers, with temperatures generally below 18 degrees Celsius, absence of pollutants, low concentrations of nitrates, nitrites, pH in the biological optimum of fish species, with the presence of phosphates in some of the sampling stations and ammonium ion.

The investigations carried out on the benthic macroinvertebrate communities revealed the presence of the main invertebrate groups in mountain waters (*Plecoptera*, *Trichoptera*, *Ephemeroptera*, *Diptera*, *Amphipoda*, *Coleoptera*, *Oligocheta*, *Platelmintes*, *Nematoda*, *Odonata*), biological indicators of surface water quality, with a continuous distribution in the river sectors of the site, but with different abundance over time, depending on the stages of insect larval development and the frequency of floods. In terms of gravimetric abundance, reduced biomass was recorded in some of the sampling stations, explained by the unstable substrate, the lack of bioderma and the low number of fish species. The low gravimetric abundance is explained by high fluctuations of water flow during the year, by the decrease of flows to about 1/3 of the mean annual flow in the summer, fall, and winter seasons with strong exposure of fish populations to stress conditions (decrease of favourable habitat surfaces, appearance of physical barriers that prevent dispersal of individuals, predation, overfishing). These habitat conditions are unfavourable to fish species and alter the age class structure, with most individuals caught being up to three, rarely four years old in the case of trout.

The anthropogenic impact is low on fish populations and consists in the modification of the natural flow characteristics by the regularization and canalization of streams in inhabited areas, the installation of bottom sills to reduce water energy, the abstraction of drinking water, and the development of logging activities in the watersheds. In addition to these unfavourable aspects on the ichthyofauna, the presence of natural waterfalls that prevent the dispersion of individuals towards the upper segments of the rivers, frequent floods of different amplitudes, instability of the water bed, and poor trophic supply in conditions of frequent floods are also mentioned.

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A1. Distribution maps of identified fish species

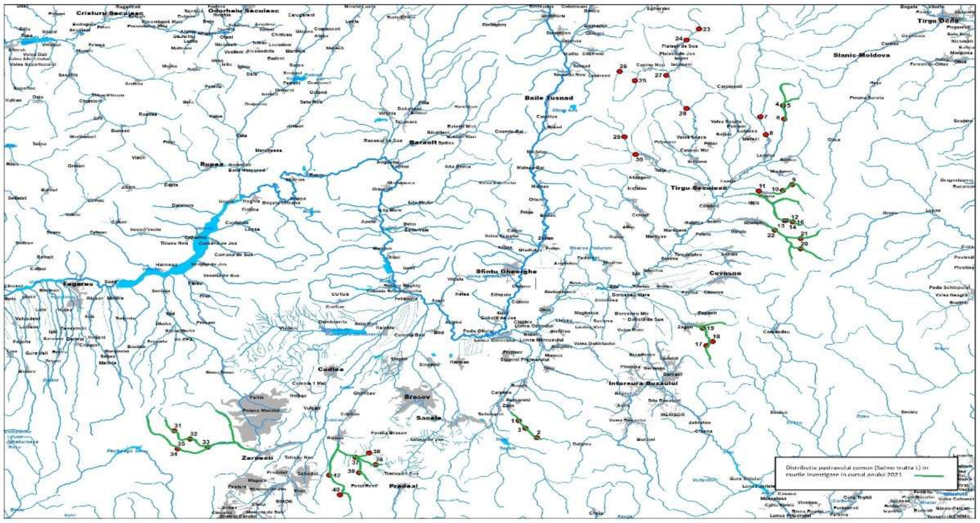


Fig. A1.1. *Distribution of Salmo trutta (L.)*

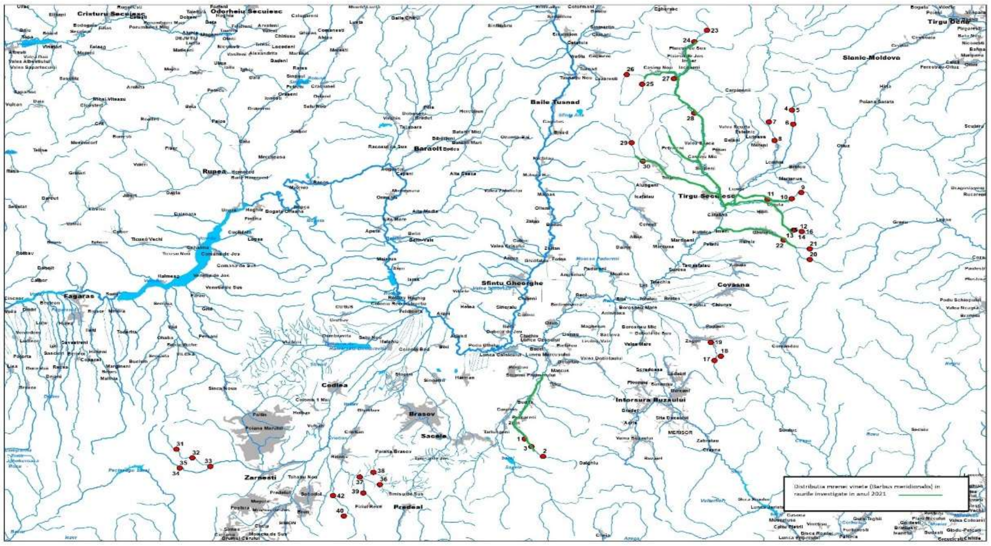


Fig. A1.2. *Distribution of Barbus meridionalis (Risso)*

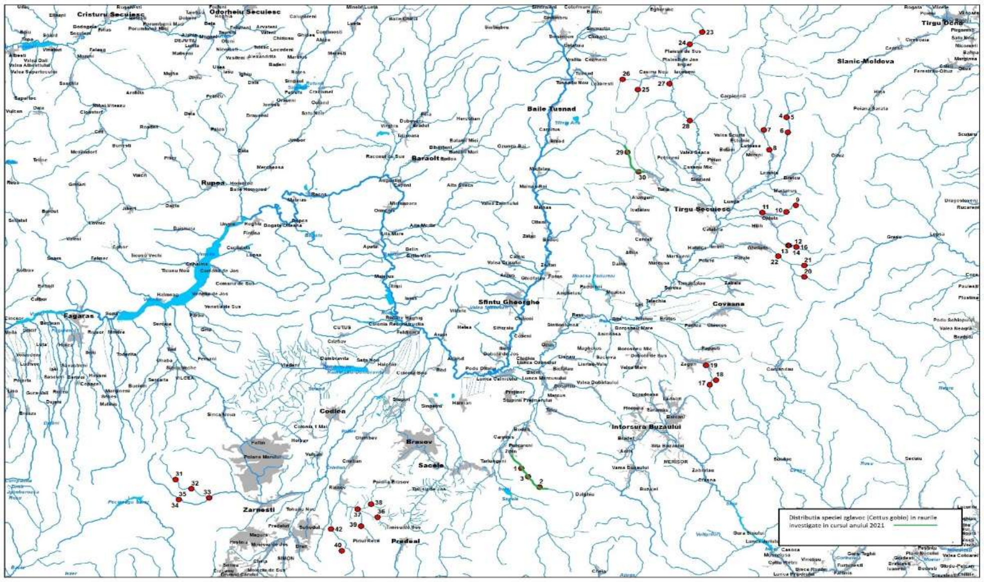


Fig. A1.3. *Distribution of Cottus gobio (L.)*

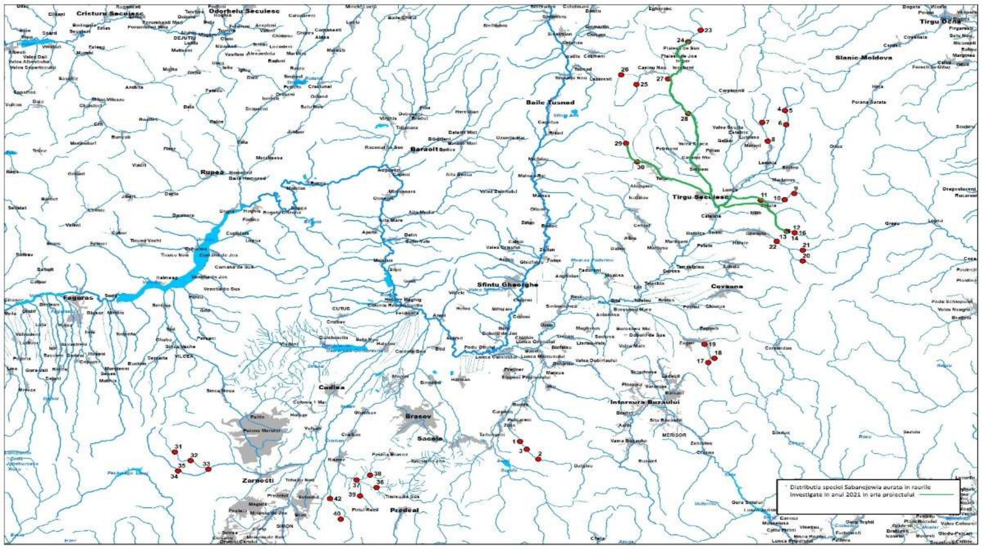


Fig. A1.4. *Distribution of Sabanejewia aurata (De Filippi)*

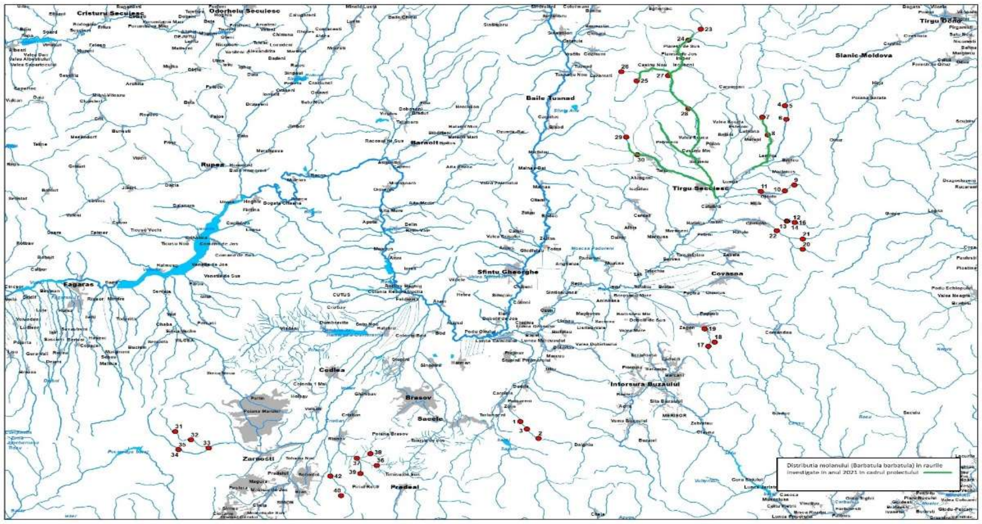


Fig. A1.5. *Distribution of Barbatulla barbatulla (L.)*

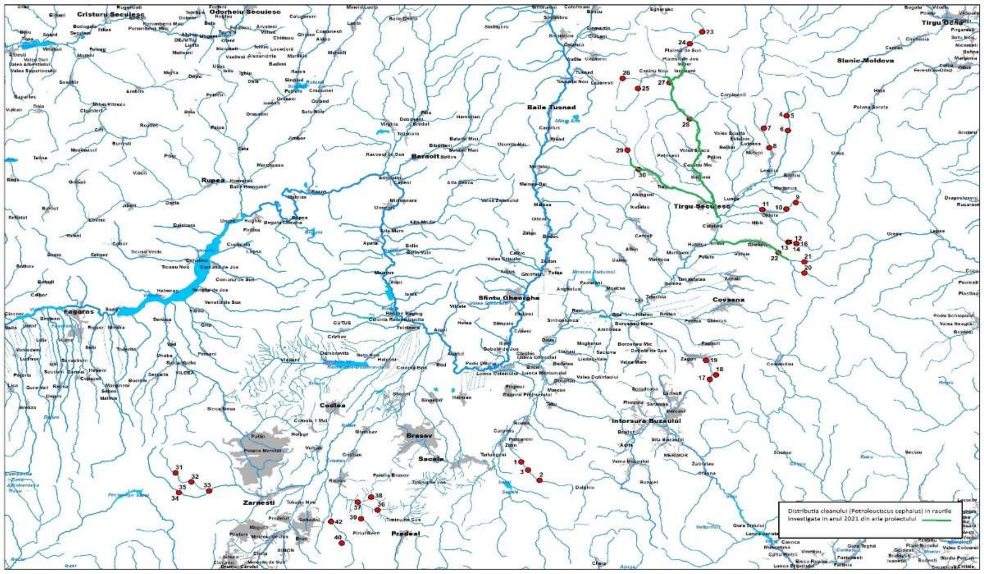


Fig. A1.6. *Distribution of Petroleuscius cephalus (L.)*

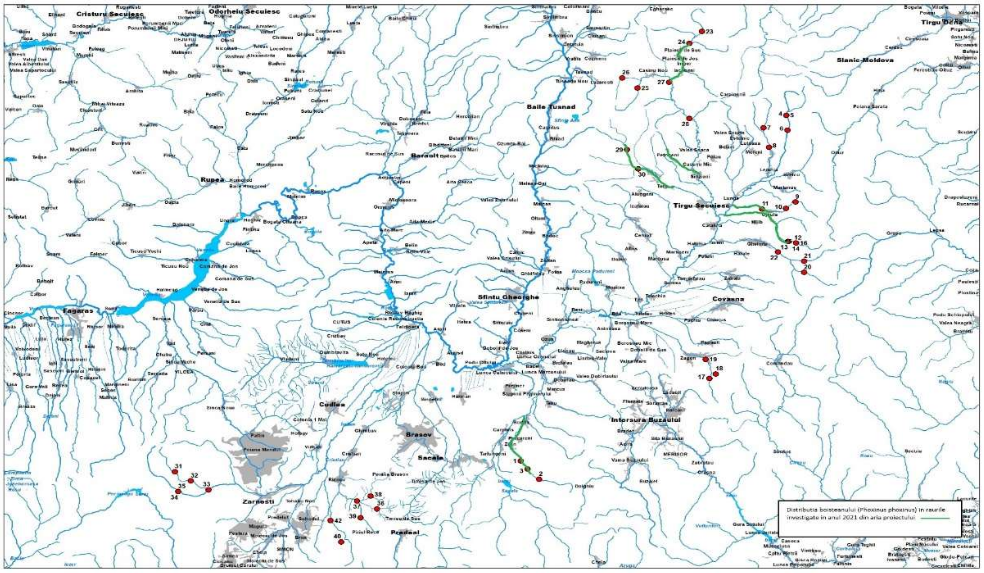
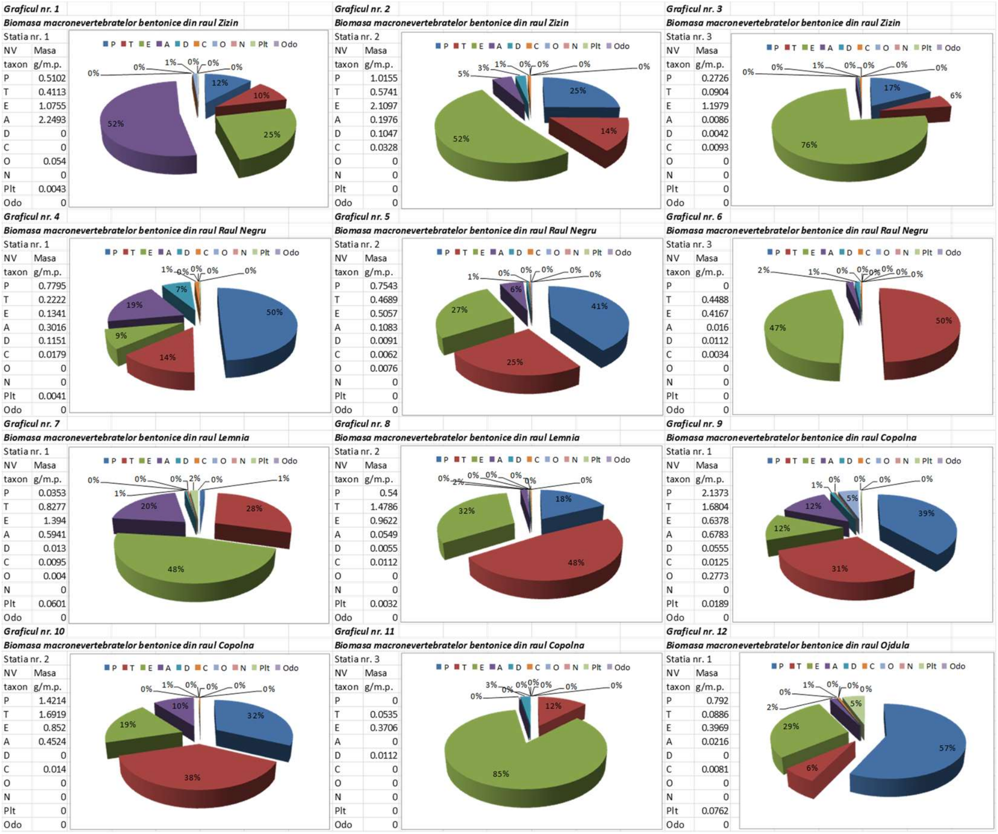


Fig. A1.7. *Distribution of Phoxinus phoxinus (L.)*

Appendix 2. Percentage shares of aquatic macro invertebrate orders in the trophic base of rivers



Graficul nr. 1

Biomasa macronevertebratelor bentonice din raul Zizin

Statia nr. 1

NV taxon	Masa g/m.p.
P	0.5102
T	0.4113
E	1.0755
A	2.2493
D	0
C	0
O	0.054
N	0
Plt	0.0043
Odo	0

Graficul nr. 2

Biomasa macronevertebratelor bentonice din raul Zizin

Statia nr. 2

NV taxon	Masa g/m.p.
P	1.0155
T	0.5741
E	2.1097
A	0.1976
D	0.1047
C	0.0328
O	0
N	0
Plt	0
Odo	0

Graficul nr. 3

Biomasa macronevertebratelor bentonice din raul Zizin

Statia nr. 3

NV taxon	Masa g/m.p.
P	0.2726
T	0.0904
E	1.1979
A	0.0086
D	0.0042
C	0.0093
O	0
N	0
Plt	0
Odo	0

Graficul nr. 4

Biomasa macronevertebratelor bentonice din raul Raul Negru

Statia nr. 1

NV taxon	Masa g/m.p.
P	0.7795
T	0.2222
E	0.1341
A	0.3016
D	0.1151
C	0.0179
O	0
N	0
Plt	0.0041
Odo	0

Graficul nr. 5

Biomasa macronevertebratelor bentonice din raul Raul Negru

Statia nr. 2

NV taxon	Masa g/m.p.
P	0.7543
T	0.4689
E	0.5057
A	0.1083
D	0.0091
C	0.0062
O	0.0076
N	0
Plt	0
Odo	0

Graficul nr. 6

Biomasa macronevertebratelor bentonice din raul Raul Negru

Statia nr. 3

NV taxon	Masa g/m.p.
P	0
T	0.4488
E	0.4167
A	0.016
D	0.0112
C	0.0034
O	0
N	0
Plt	0
Odo	0

Graficul nr. 7

Biomasa macronevertebratelor bentonice din raul Lemnia

Statia nr. 1

NV taxon	Masa g/m.p.
P	0.0353
T	0.8277
E	1.394
A	0.5941
D	0.013
C	0.0095
O	0.004
N	0
Plt	0.0601
Odo	0

Graficul nr. 8

Biomasa macronevertebratelor bentonice din raul Lemnia

Statia nr. 2

NV taxon	Masa g/m.p.
P	0.54
T	1.4786
E	0.9622
A	0.0549
D	0.0055
C	0.0112
O	0
N	0
Plt	0.0032
Odo	0

Graficul nr. 9

Biomasa macronevertebratelor bentonice din raul Copolna

Statia nr. 1

NV taxon	Masa g/m.p.
P	2.1373
T	1.6804
E	0.6378
A	0.6783
D	0.0555
C	0.0125
O	0.2773
N	0
Plt	0.0189
Odo	0

Graficul nr. 10

Biomasa macronevertebratelor bentonice din raul Copolna

Statia nr. 2

NV taxon	Masa g/m.p.
P	1.4214
T	1.6919
E	0.852
A	0.4524
D	0
C	0.014
O	0
N	0
Plt	0
Odo	0

Graficul nr. 11

Biomasa macronevertebratelor bentonice din raul Copolna

Statia nr. 3

NV taxon	Masa g/m.p.
P	0
T	0.0535
E	0.3706
A	0
D	0.0112
C	0
O	0
N	0
Plt	0
Odo	0

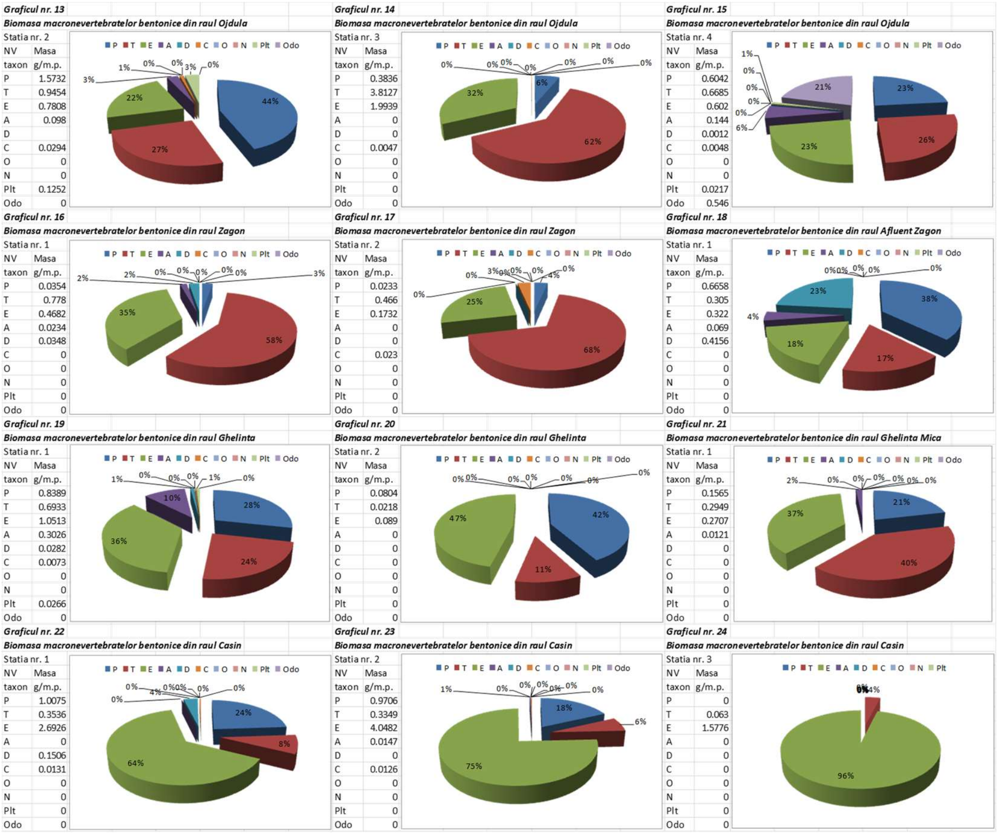
Graficul nr. 12

Biomasa macronevertebratelor bentonice din raul Ojdula

Statia nr. 1

NV taxon	Masa g/m.p.
P	0.792
T	0.0886
E	0.3969
A	0.0216
D	0
C	0.0081
O	0
N	0
Plt	0.0762
Odo	0

Fig. A2.1. *Macroinvertebrate order in the stream of Zizin, Râul Negru, Lemnia, Copolna*



Graficul nr. 13

Biomasa macronevertebratelor bentonice din raul Ojdula

Statia nr. 2

NV taxon	Masa g/m.p.
P	1.5732
T	0.9454
E	0.7808
A	0.098
D	
C	0.0294
O	0
N	0
Plt	0.1252
Odo	0

Graficul nr. 14

Biomasa macronevertebratelor bentonice din raul Ojdula

Statia nr. 3

NV taxon	Masa g/m.p.
P	0.3836
T	3.8127
E	1.9939
A	0
D	0
C	0.0047
O	0
N	0
Plt	0
Odo	0

Graficul nr. 15

Biomasa macronevertebratelor bentonice din raul Ojdula

Statia nr. 4

NV taxon	Masa g/m.p.
P	0.6042
T	0.6685
E	0.602
A	0.144
D	0.0012
C	0.0048
O	0
N	0
Plt	0.0217
Odo	0.546

Graficul nr. 16

Biomasa macronevertebratelor bentonice din raul Zagon

Statia nr. 1

NV taxon	Masa g/m.p.
P	0.0354
T	0.778
E	0.4682
A	0.0234
D	0.0348
C	0
O	0
N	0
Plt	0
Odo	0

Graficul nr. 17

Biomasa macronevertebratelor bentonice din raul Zagon

Statia nr. 2

NV taxon	Masa g/m.p.
P	0.0233
T	0.466
E	0.1732
A	0
D	0
C	0.023
O	0
N	0
Plt	0
Odo	0

Graficul nr. 18

Biomasa macronevertebratelor bentonice din raul Afluent Zagon

Statia nr. 1

NV taxon	Masa g/m.p.
P	0.6658
T	0.305
E	0.322
A	0.069
D	0.4156
C	0
O	0
N	0
Plt	0
Odo	0

Graficul nr. 19

Biomasa macronevertebratelor bentonice din raul Ghelinta

Statia nr. 1

NV taxon	Masa g/m.p.
P	0.8389
T	0.6933
E	1.0513
A	0.3026
D	0.0282
C	0.0073
O	0
N	0
Plt	0.0266
Odo	0

Graficul nr. 20

Biomasa macronevertebratelor bentonice din raul Ghelinta

Statia nr. 2

NV taxon	Masa g/m.p.
P	0.0804
T	0.0218
E	0.089
A	0
D	0
C	0
O	0
N	0
Plt	0
Odo	0

Graficul nr. 21

Biomasa macronevertebratelor bentonice din raul Ghelinta Mica

Statia nr. 1

NV taxon	Masa g/m.p.
P	0.1565
T	0.2949
E	0.2707
A	0.0121
D	0
C	0
O	0
N	0
Plt	0
Odo	0

Graficul nr. 22

Biomasa macronevertebratelor bentonice din raul Casin

Statia nr. 1

NV taxon	Masa g/m.p.
P	1.0075
T	0.3536
E	2.6926
A	0
D	0.1506
C	0.0131
O	0
N	0
Plt	0
Odo	0

Graficul nr. 23

Biomasa macronevertebratelor bentonice din raul Casin

Statia nr. 2

NV taxon	Masa g/m.p.
P	0.9706
T	0.3349
E	4.0482
A	0.0147
D	0
C	0.0126
O	0
N	0
Plt	0
Odo	0

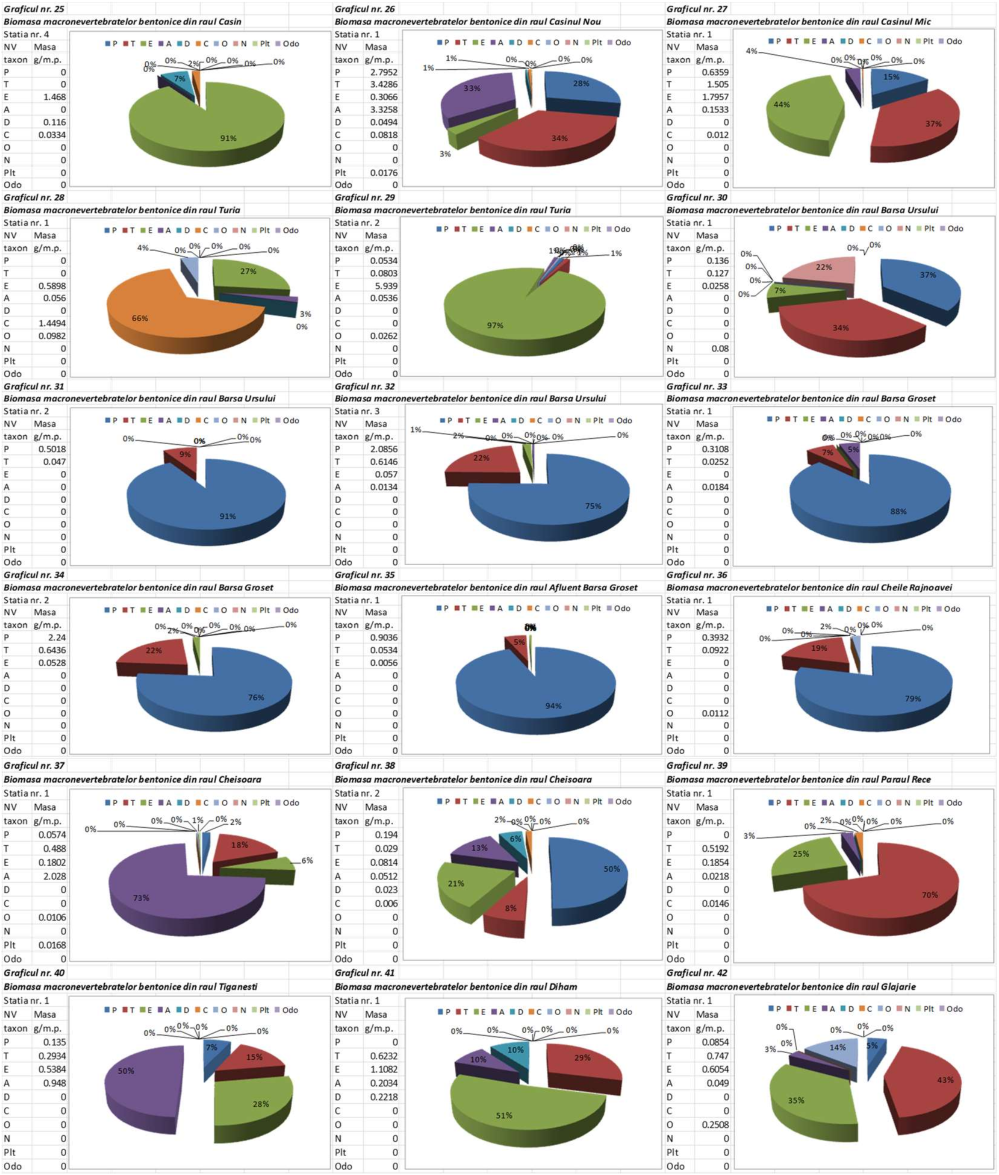
Graficul nr. 24

Biomasa macronevertebratelor bentonice din raul Casin

Statia nr. 3

NV taxon	Masa g/m.p.
P	0
T	0.063
E	1.5776
A	0
D	0
C	0
O	0
N	0
Plt	0
Odo	0

Fig. A2.2. *Macroinvertebrate order in the stream of Ojdula, Zagon, Ghelința, Cașin, Cașinul Mic*



Graficul nr. 25

Biomasa macronevertebratelor bentonice din raul Casin

Statia nr. 4

NV taxon	Masa g/m.p.
P	0
T	0
E	1.468
A	0
D	0.116
C	0.0334
O	0
N	0
Plt	0
Odo	0

Graficul nr. 26

Biomasa macronevertebratelor bentonice din raul Casinul Nou

Statia nr. 1

NV taxon	Masa g/m.p.
P	2.7952
T	3.4286
E	0.3066
A	3.3258
D	0.0494
C	0.0818
O	0
N	0
Plt	0.0176
Odo	0

Graficul nr. 27

Biomasa macronevertebratelor bentonice din raul Casinul Mic

Statia nr. 1

NV taxon	Masa g/m.p.
P	0.6359
T	1.505
E	1.7957
A	0.1533
D	0
C	0.012
O	0
N	0
Plt	0
Odo	0

Graficul nr. 28

Biomasa macronevertebratelor bentonice din raul Turia

Statia nr. 1

NV taxon	Masa g/m.p.
P	0
T	0
E	0.5898
A	0.056
D	0
C	1.4494
O	0.0982
N	0
Plt	0
Odo	0

Graficul nr. 29

Biomasa macronevertebratelor bentonice din raul Turia

Statia nr. 2

NV taxon	Masa g/m.p.
P	0.0534
T	0.0803
E	5.939
A	0.0536
D	0
C	0
O	0.0262
N	0
Plt	0
Odo	0

Graficul nr. 30

Biomasa macronevertebratelor bentonice din raul Barsa Ursului

Statia nr. 1

NV taxon	Masa g/m.p.
P	0.136
T	0.127
E	0.0258
A	0
D	0
C	0
O	0
N	0.08
Plt	0
Odo	0

Graficul nr. 31

Biomasa macronevertebratelor bentonice din raul Barsa Ursului

Statia nr. 2

NV taxon	Masa g/m.p.
P	0.5018
T	0.047
E	0
A	0
D	0
C	0
O	0
N	0
Plt	0
Odo	0

Graficul nr. 32

Biomasa macronevertebratelor bentonice din raul Barsa Ursului

Statia nr. 3

NV taxon	Masa g/m.p.
P	2.0856
T	0.6146
E	0.057
A	0.0134
D	0
C	0
O	0
N	0
Plt	0
Odo	0

Graficul nr. 33

Biomasa macronevertebratelor bentonice din raul Barsa Groset

Statia nr. 1

NV taxon	Masa g/m.p.
P	0.3108
T	0.0252
E	0
A	0.0184
D	0
C	0
O	0
N	0
Plt	0
Odo	0

Graficul nr. 34

Biomasa macronevertebratelor bentonice din raul Barsa Groset

Statia nr. 2

NV taxon	Masa g/m.p.
P	2.24
T	0.6436
E	0.0528
A	0
D	0
C	0
O	0
N	0
Plt	0
Odo	0

Graficul nr. 35

Biomasa macronevertebratelor bentonice din raul Afluent Barsa Groset

Statia nr. 1

NV taxon	Masa g/m.p.
P	0.9036
T	0.0534
E	0.0056
A	0
D	0
C	0
O	0
N	0
Plt	0
Odo	0

Graficul nr. 36

Biomasa macronevertebratelor bentonice din raul Cheile Rajnoavei

Statia nr. 1

NV taxon	Masa g/m.p.
P	0.3932
T	0.0922
E	0
A	0
D	0
C	0
O	0.0112
N	0
Plt	0
Odo	0

Graficul nr. 37

Biomasa macronevertebratelor bentonice din raul Cheisoara

Statia nr. 1

NV taxon	Masa g/m.p.
P	0.0574
T	0.488
E	0.1802
A	2.028
D	0
C	0
O	0.0106
N	0
Plt	0.0168
Odo	0

Graficul nr. 38

Biomasa macronevertebratelor bentonice din raul Cheisoara

Statia nr. 2

NV taxon	Masa g/m.p.
P	0.194
T	0.029
E	0.0814
A	0.0512
D	0.023
C	0.006
O	0
N	0
Plt	0
Odo	0

Graficul nr. 39

Biomasa macronevertebratelor bentonice din raul Paraul Rece

Statia nr. 1

NV taxon	Masa g/m.p.
P	0
T	0.5192
E	0.1854
A	0.0218
D	0
C	0.0146
O	0
N	0
Plt	0
Odo	0

Graficul nr. 40

Biomasa macronevertebratelor bentonice din raul Tiganesti

Statia nr. 1

NV taxon	Masa g/m.p.
P	0.135
T	0.2934
E	0.5384
A	0.948
D	0
C	0
O	0
N	0
Plt	0
Odo	0

Graficul nr. 41

Biomasa macronevertebratelor bentonice din raul Diham

Statia nr. 1

NV taxon	Masa g/m.p.
P	0
T	0.6232
E	1.1082
A	0.2034
D	0.2218
C	0
O	0
N	0
Plt	0
Odo	0

Graficul nr. 42

Biomasa macronevertebratelor bentonice din raul Glajarie

Statia nr. 1

NV taxon	Masa g/m.p.
P	0.0854
T	0.747
E	0.6054
A	0.049
D	0
C	0
O	0.2508
N	0
Plt	0
Odo	0

Fig. A3.3. *Macroinvertebrate order in the stream of Turia, Bârsa Ursului, Bârsa Groșeț, Râșnoava, Pârâul Rece, Țigănești, Glăjărie, Diham*

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GENETIC PATTERNS OF NATIVE HUNGARIAN OAK (*QUERCUS FRAINETTO* TEN.) STANDS ON THE BALKAN PENINSULA BASED ON CHLOROPLAST MICROSATELLITE MARKERS

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László NAGY[1] **Attila BENKE**[1]

Abstract: *The Hungarian oak (Quercus frainetto Ten.) is a meso-xerophilous species that belongs to the European white oaks, native to the Balkan Peninsula. Still, its distribution range also reaches South Italy and North-West Turkey. The northernmost autochthonous population can be found in North-West Romania. Despite the name, it is non-native in Hungary; the sporadic stands and old individuals found throughout the country are presumably of planted origin and were formerly introduced. Based on the climate envelope models, this oak species will expand northward and probably be one of the winners of the changing climate in the European temperate broadleaf forests. In this study, twelve native populations from the Balkans were analysed by applying chloroplast microsatellites to describe the genetic pattern of the different geographic regions throughout the main native distribution range. Another goal was to test the chloroplast markers' resolution and usefulness for provenance identification. For this reason, the chloroplast haplotype pattern of five non-native old stands from Hungary was also checked and analysed in parallel with the Balkans. Two main lineages were detected in this study, with two clearly separated northern and southeastern clusters and a transition zone between them at the central part of the distribution range. The north edge of the natural area presented a lower genetic diversity. The five Hungarian artificial stands showed a high genetic variability, and most have a completely different origin.*

Key words: *Quercus frainetto, haplotype, genetic structure, climate change.*

1. Introduction

The Hungarian oak (*Quercus frainetto* Ten., syn. *Quercus conferta* Kit.) is a close relative of the European white oak species in section *Quercus* within the subgenus *Quercus* [12]. However, its classification is still dubious. According to Camus [5], this oak species belongs to a separate section, *sec. Mesobalanus*, together with the

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southwestern European *Quercus pyrenaica* Willd., *Quercus macranthera* Fisch. & C.A.Mey. in the Caucasus, and the Asian *Quercus dentata* Thunb. Parallelly, Schwarz [35] established the series *Confertae* (in section *Dascia*) with the four taxa *Q. frainetto* (Ten.), *Q. vulcanica* (Boiss. ex Kotschy), *Q. pyrenaica* (Willd.), and *Q. kotschyana* (Schwarz). The centre of the distribution range lies on the Balkan Peninsula, but fragmented populations can also be found on the Apennines. The area reaches its easternmost edge in Northwest Turkey, in the Peloponnese in the South, and along the Hungarian border in the North [6]. *Quercus frainetto* (Ten.) is a thermophilous oak species finding its optimum between the continental and Mediterranean climate zones [23]. It forms mainly mixed stands with *Quercus cerris* (L.), *Fraxinus ornus* (L.), *Carpinus orientalis* (Mill.), and *Ostrya carpinifolia* (Scop.) in the lower elevations of mountains. It occupies a narrower ecological niche than *Quercus cerris* (L.) and *Fraxinus ornus* (L.). However, its drought tolerance is better than the Turkey oak's [29]. The growth rate of the species is relatively slow; the whole life cycle is shorter than the other white oaks, so more than 200-year-old individuals can rarely be found in stands [23]. Due to human impact, namely frequent coppicing, the trunk quality is usually poor. However, the species would be capable of forming good-shaped stems as well. Moreover, the former distribution range of *Q. frainetto* (Ten.) frequently overlaps with most historical agricultural areas. Hence, considerable vegetation loss is probable due to long-term human impact [3].

Based on various climate envelope models [21, 26], a potential northwestern shift can be predicted in the species' distribution range throughout the whole European continent, reaching even the Scandinavian region. On the other hand, considering the current distribution range (native and suitable), an apparent decline and habitat loss are also predicted based on the mentioned models. From this aspect, especially in contrast with the expanding Turkey oak and pubescent oak, Hungarian oak is not considered a real 'winner' of climate change in Hungary [21]. Therefore, careful and accurate planning is needed before setting up future afforestation to find suitable and stable sites for new stands.

In Hungary, *Q. frainetto* (Ten.) is considered non-native, but several smaller stands, in some cases a few individual trees, can be found mixed in the local broadleaf sessile oak stands. These are mainly artificial plantations; most are considered more than 100 years old based on the official forest inventories. However, the history of these old trees is unclear, and the exact planting records are missing or lost. Some of the stands are already mature, producing a considerable amount of acorns and, in some cases, regenerating naturally. For this reason, this oak species is at the centre of attention of local forest management. On the other hand, the information about the genetic composition and patterns in its natural range is scarce and limited. Chloroplast DNA markers with the PCR-RFLP method contributed to the knowledge of the white oaks' genetic pattern in different geographic regions, providing details about glacial refugia and the postglacial recolonisation routes [28]. Bordács et al. [2] investigated a few *Q. frainetto* (Ten.) stands in the Northern Balkans (eight in Romania and two in Croatia) along with the other white oak species, applying the same PCR-RFLP technique. According to their results, an overlapping haplotype pattern could be observed with the different oak species, supposing a broad and frequent outcrossing occurred between species during the postglacial migration. Today, the marker technique has slightly changed, and chloroplast microsatellites (cpSSR, chloroplast simple sequence repeats) have become more frequently used. These markers are more suitable for providing a detailed, fine-scale haplotype pattern of a region due to a higher mutation range than the traditional plastid marker regions [32]. Comparative studies also confirmed that an equivalent haplotype pattern can be obtained with both techniques [11, 18]. Today, more and more detailed genealogical reconstruction maps are available for local stands of several oak species throughout Europe, from Western and Central Europe [20, 25], in the Mediterranean and Balkan regions [13, 29], in Northeast Europe [7] to the East in the Crimea [37]. However, these studies focus on the main stand-forming oak species, *Q. robur* L. and *Q. petraea* (Matt.) Liebl.. The occurrence of *Q. frainetto* Ten. in the genetic studies is still sporadic, concentrating primarily on one location [8, 17] or a particular region of Europe (e.g., Italy - Di Pietro et al. [13]; Romania – [9]. Nevertheless, interest in this species is growing [1, 22].

This study aimed to test 10 cpSSR markers available in the literature for the genetic identification of different geographic locations for natural and possibly planted *Q. frainetto* (Ten.) stands. Twelve populations from the natural distribution zone were sampled and tested, where 13 different haplotypes were observed altogether. To check the method's accuracy for identification purposes, five additional locations were tested in Hungary with artificial (planted) origin. With the analysis of this extended data set, four additional haplotypes could be detected with unknown origins. In this article, we discuss the observed genetic pattern of natural *Q. frainetto* (Ten.) stands and the method’s accuracy for tracing genetic material.

2. Material and Methods

Leaf samples were collected during the summers of 2019, 2023, and 2024 for DNA extraction from 12 putatively native stands and five planted locations in Hungary. For the genetic analysis, 103 individuals from the native distribution range were used, and four individuals were used from each population in Hungary. The location data of the autochthonous populations included in the study are presented in Table 1 and on the distribution map in Figure 1.

Total genomic DNA was isolated from fresh leaves following the modified CTAB procedure of Dumolin et al. 1995 [14] (see also Bruegmann et al. [4]). DNA concentration was measured by Qubit 4 Fluorometer (Thermo Fisher Scientific) and standardized to 10 ng/µl. Ten chloroplast SSR markers were selected and arranged in two multiplex mixes for the test of amplification in *Q. frainetto* (Ten.) from the cmcs-series of Sebastiani et al. [36], the ccmp-series of Weising and Gardner [41], and the µdt/µkk-series of Deguilloux et al. [10]: Mix1) cmcs9 (VIC), cmcs1 (6FAM), ccmp6 (PET), cmcs8 (NED), cmcs6 (VIC), cmcs7 (6FAM), Mix2) µdt4 (PET), µdt3 (6FAM), µdt1 (VIC), µkk4 (NED). Primer pairs were synthesized with a universal M13(-21) tail on the 5’ end of the Forward primer following the description of Schulke [34] by IDT (Integrated DNA Technologies Inc., Coralville, Iowa, USA). Fluorescent dyes compatible with the Applied Biosystems G5 Matrix were used on the universal M13 (-21) primer 5' end. For the PCR, the GoTaq G2 Flexi (Promega, Madison, Wisconsin, USA) polymerase kit was used, and the protocol described by Schulke [34] was applied. PCR fragments were separated on a 1.75% agarose gel in 1× TAE buffer, stained with GelRed (Biotium, Fremont, CA, USA). Then, fragments were diluted (up to 20-fold) for capillary electrophoresis and multiplexed by dye and size in formamide (Hi-Di, Applied Biosystems, Waltham, Massachusetts, USA) using GeneScan LIZ 500 (Applied Biosystems) internal size standard. SSR genotyping was performed on an ABI 3730 DNA Analyzer (Applied Biosystems) by BIOMI Ltd. (Gödöllő, Hungary).

Table 1

Description of the sampled Hungarian oak (Quercus frainetto Ten.) populations of the natural distribution range and Hungary

Population ID	Sample number	Country	Location	Latitude	Longitude	Altitude (m a.s.l.)
RO_ARAD	7	Romania	Șiștarovăț	46.01	21.70	294
RO_COM	12	Romania	Comana	44.14	26.15	99
SE_FRUS	6	Serbia	Cserög (Fruska Gora Mt.)	45.18	19.66	186
SE_BELG	6	Serbia	Lipovica	44.63	20.40	278
SE_KRAL	11	Serbia	Kraljevo	43.77	20.68	249
CR_PAPUK	4	Croatia	Stara Ljeskovica (Krndija Mt.)	45.39	18.02	195
KOS_PRIST	10	Kosovo	Pristina	42.51	20.97	787
ALB_BAJRAM	4	Albania	Bajram Curri (Albanian Alps)	42.37	20.13	290
BU_BALK	10	Bulgaria	Botevgrad (Balkan Mt.)	42.97	23.83	600
BU_ROD	10	Bulgaria	Zlatograd (Rodope Mt.)	41.38	25.14	462
BU_STRAN	12	Bulgaria	Balgari (Strandzha Mt.)	42.11	27.77	210
TUR_STRAN	11	Turkey	Demirköy (Strandzha Mt.)	41.95	27.68	312
HU_IHAROS	4	Hungary	Iharosberény	46.37	17.05	242
HU_HEGYESD	4	Hungary	Hegyesd	46.93	17.53	172
HU_IBAFA	4	Hungary	Ibafa	46.15	17.98	254
HU_GYULAJ	4	Hungary	Gyulaj	46.56	18.25	218
HU_DIÓSJ	4	Hungary	Diósjenő	47.96	19.03	341

Following the fragment analysis, allele variants for the data analysis, the software HAPLOTYPE ANALYSIS by Eliades and Eliades [16] and GenAlEx 6.5 [27] were used. The different fragments and their frequencies for each marker region were detected, followed by the description of the unique haplotypes for each population. A genetic distance matrix was calculated based on the stepwise mutation model for each haplotype by GenAlEx, and a principal coordinate analysis was conducted based on this matrix to represent the genetic relatedness between the detected haplotypes. The following main population genetic indices were calculated by HAPLOTYPE ANALYSIS: the number of different haplotypes (A), the number of private haplotypes (P_h), the effective number of haplotypes (N_e), the haplotypic richness (H_R) was estimated using the rarefaction method [15], Nei's index of genetic diversity (H_E) was estimated without bias [24], and the mean genetic distance between individuals (D_{sh}^2) [19]. Finally, a Bayesian clustering method was applied using the software STRUCTURE 2.3.4. [31] for inferring genetic structure among the detected haplotypes with the following parameter settings: 200,000 repetitions in the burnin period, followed by 500,000 MCMC repetitions, with K set 1-6, and 5 iterations in each K, using the admixture model and no prior population information. Genetic clustering of the analysed haplotypes considering the most probable K was visualized with the "seaborn" library [40] in python environment.

QGIS (http://qgis.osgeo.org) was used for map construction [33].

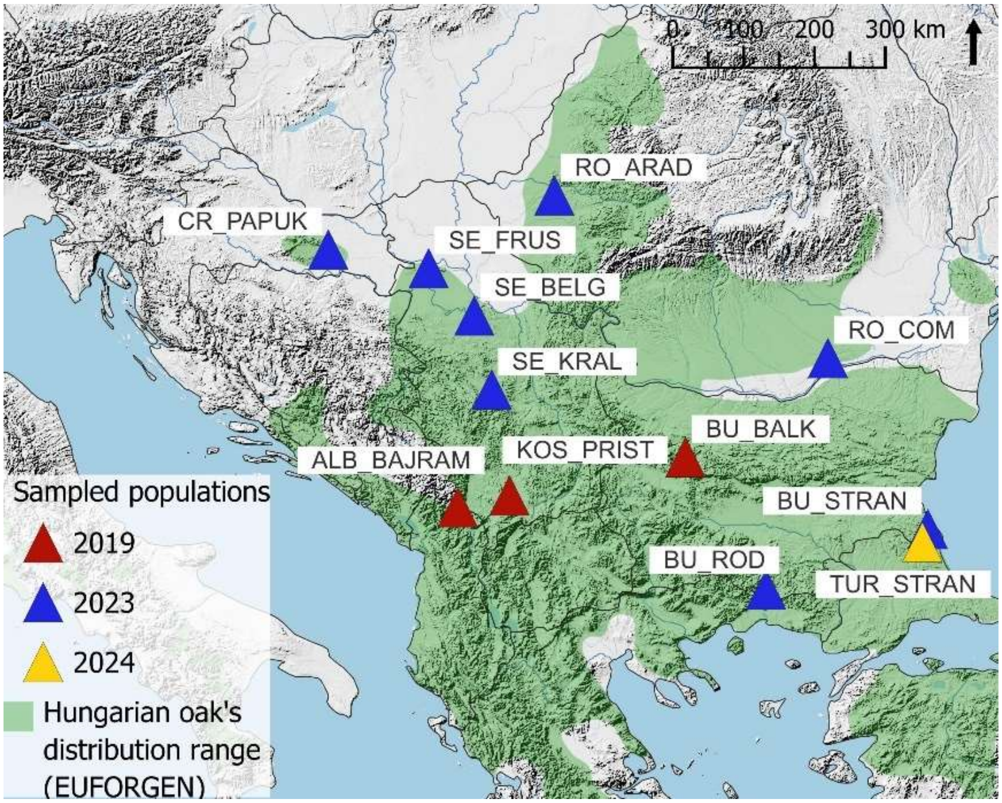

Fig. 1. *Sampled Hungarian oak (Quercus frainetto Ten.) populations in the natural distribution range*

3. Results

Out of the applied 10 cpSSR markers two (cmcs1 and cmcs7) were monomorphic, so these were excluded from further analysis. The allele composition of each of the remaining eight markers in the Balkan populations is represented in Figure 2. The most polymorphic markers are cmcs6 with five allele variants and µdt3 with four allele variants. The markers ccmp6, cmcs8, µdt4, and µkk4 contribute only two alleles.

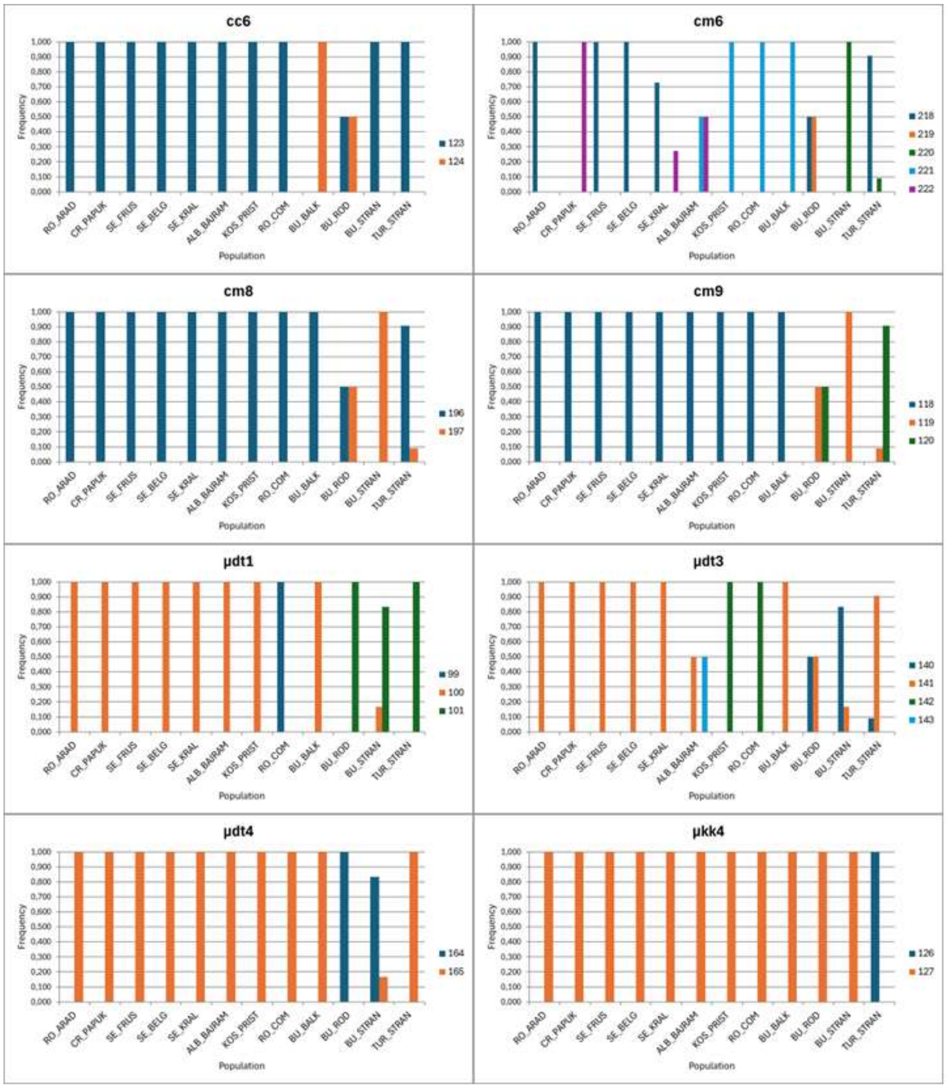

Fig. 2. *Allele composition of the applied polymorphic cpSSR markers in the Balkan populations*

In the 12 studied Balkan populations, 13 different haplotypes were detected (Figure 3). The main population genetic indices are presented in Table 2. Five populations (SE_KRAL, ALB_BAJRAM, BU_ROD, BU_STRAN, TUR_STRAN) hold two haplotypes. Most of the haplotypes are unique for one population, while the three populations in Serbia (SE_FRUS, SE_BELG, SE_KRAL) share the same haplotype with RO_ARAD, and SE_KRAL partly with CR_PAPUK. The highest diversity indices (R_h haplotypic richness, H_e genetic diversity, and D_{sh}^2 mean genetic distance between individuals) can be found in the population ALB_BAJRAM; however, this is represented only by four samples. The following two highest values are in the populations BU_ROD and SE_KRAL. The calculated mean genetic distance between individuals (D_{sh}^2) is the highest in the population BU_ROD with a value of 1.736 (Table 1).

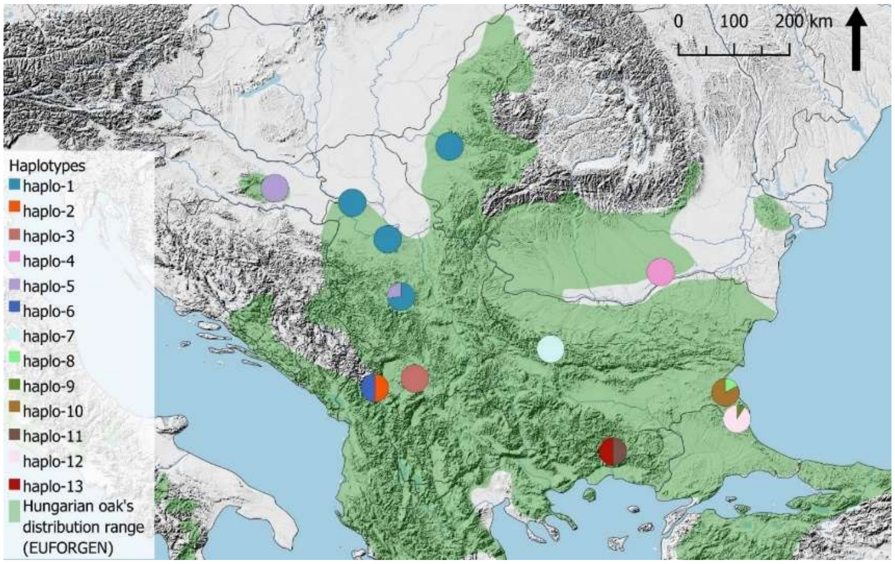

Fig. 3. *The 13 detected haplotypes in the 12 analysed Balkan populations along the natural distribution range of Quercus frainetto*

The genetic relatedness of the detected haplotypes can be represented by a principal coordinate analysis (*PCoA*) derived from the genetic distances (Figure 4). Haplotypes from the Southeastern Balkan region (in populations BU_ROD, BU_STRAN, TUR_STRAN) are well-separated from the other haplotypes and genetically loosely linked. The other haplotypes form a genetically uniform and close group. This latter group comprises the haplotype of the BU_BALK and RO_COM populations, which are also localized in a transition geographic zone.

Based on the STRUCTURE analysis, two main genetic clusters can be identified in the haplotype data set. The genetic partitioning of the two clusters within the recognized haplotypes is presented in Figure 5. Cluster 1 can be identified as a northwestern genetic type, while Cluster 2 as a southeastern type. Populations being geographically in a transition zone, namely ALB_BAJRAM, KOS_PRIST, RO_COM, and BU_BALK, hold shared haplotypes with the genetic pattern of the two clusters, showing a transition also in the genetic pattern.

Table 2

Main haplotype diversity indices of the analysed Hungarian oak (Quercus frainetto Ten.) populations in the Balkan region

Population	N	A	P	N_e	R_h	H_e	D_{sh}^2
RO_ARAD	7	1	0	1.000	0.000	0.000	0.000
CR_PAPUK	4	1	0	1.000	0.000	0.000	0.000
SE_FRUS	6	1	0	1.000	0.000	0.000	0.000
SE_BELG	6	1	0	1.000	0.000	0.000	0.000
SE_KRAL	11	2	0	1.658	0.788	0.436	0.873
ALB_BAJRAM	4	2	2	2.000	1.000	0.667	0.750
KOS_PRIST	10	1	1	1.000	0.000	0.000	0.000
RO_COM	12	1	1	1.000	0.000	0.000	0.000
BU_BALK	10	1	1	1.000	0.000	0.000	0.000
BU_ROD	10	2	2	2.000	0.952	0.556	1.736
BU_STRAN	12	2	2	1.385	0.576	0.303	0.341
TUR_STRAN	11	2	2	1.198	0.364	0.182	0.568
Mean	8.583	1.417	0.917	1.270	0.307	0.179	0.356

Note: *N*: sample size, *A*: number of haplotypes, *P*: number of private haplotypes, N_e: effective number of haplotypes, R_h: haplotypic richness, H_e: genetic diversity, D_{sh}^2: mean genetic distance between individuals.

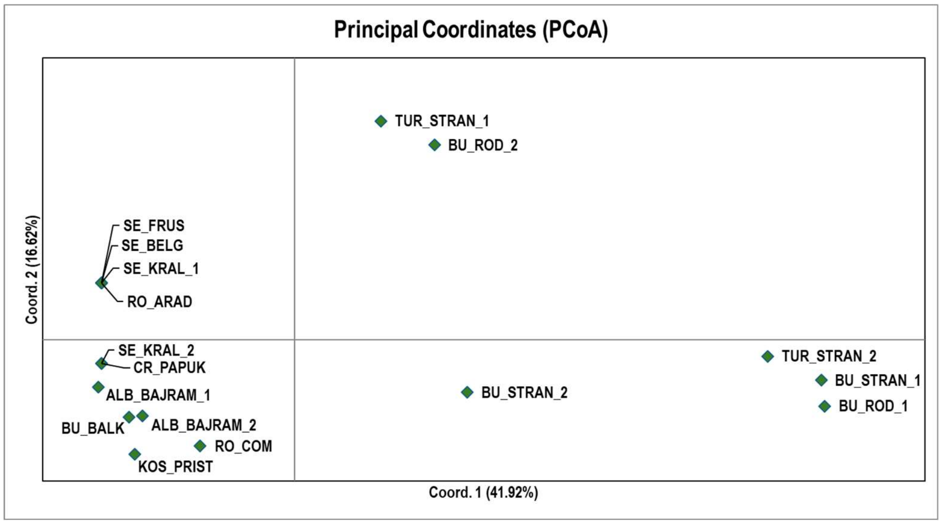

Fig. 4. *The genetic relatedness of the 13 detected haplotypes in the 12 studied populations in the Balkan region represented by a principal coordinate analysis (PCoA) of the genetic distances*

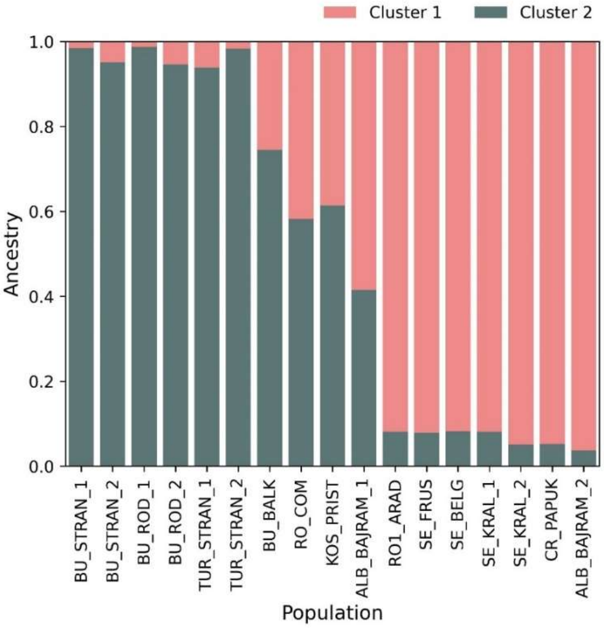

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Fig. 5. *The genetic partitioning of the two genetic clusters within the recognized haplotypes by STRUCTURE analysis*

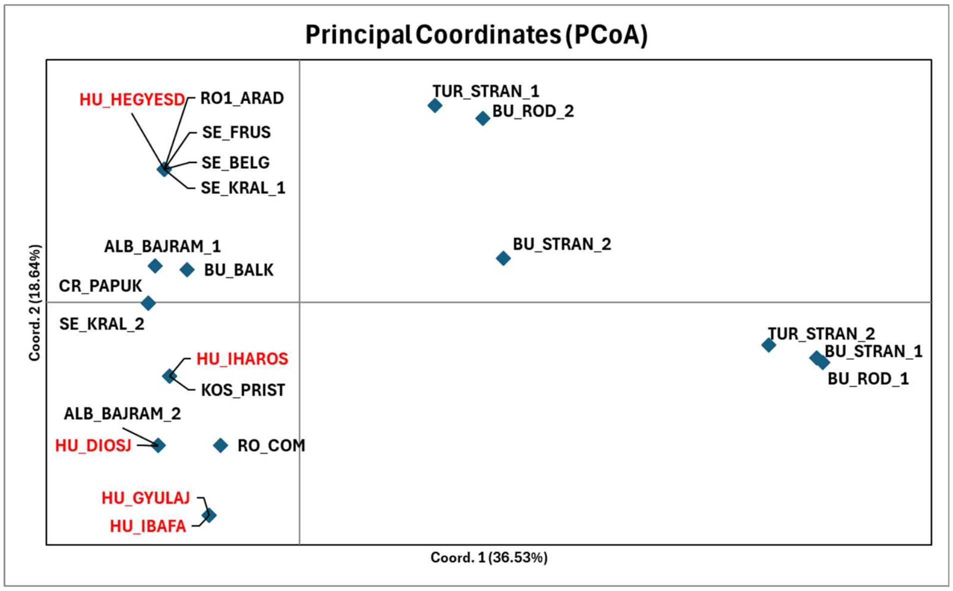

Fig. 6. *The genetic relatedness of the detected haplotypes of the 12 studied native populations in the Balkan region and the five Hungarian plantations (red) represented by a principal coordinate analysis (PCoA) of the genetic distances*

The haplotype composition of the five planted Hungarian populations is partly congruent with the analysed natural populations' genetic pattern. One new haplotype was detected, shared by the stands HU_IBAFA and HU_GYULAJ. The three other stands have different haplotypes that match one of the analyzed natural populations' haplotypes. The HU_HEGYESD haplotype is identical to the northern common haplotype (RO_ARAD and SE populations), the HU_IHAROS is identical to the KOS_PRIST haplotype, and the HU_DIOSJ matches the ALB_BAJRAM_2 haplotype. Based on the PCoA plot calculated from the genetic distances (Figure 6), the unique haplotype of the two Hungarian sites stands closer to the southern haplotypes. The unique haplotype of the two Hungarian sites stands closer to the southern haplotypes.

4. Discussion

Based on the eight applied cpSSR markers, a high genetic diversity could be observed, as almost all 12 Balkan populations on the natural distribution range were represented by a unique haplotype, and in some stands, we were able to detect two haplotypes despite the relatively low sample sizes. Therefore, the microsatellite method seems to give as high resolution as the former chloroplast marker technique applied by Petit et al. [28]. As a lab technical aspect, we should highlight that the eight cpSSR markers that were used can be analysed in one multiplex set to reduce costs.

In the case of Hungarian oak, we only have a few references with which to compare. The study of Bordács et al. [2] using the PCR-RFLP method is still the most comprehensive so far, as the study of Slade et al. [38] from the Central Balkan region contains only a few *Q. frainetto* Ten. samples. In the mentioned study, 11 populations were sampled mainly from the northern and central distribution range, including two stands from Croatia, eight from Romania (from the middle part and the southern plain), and two from Hungary. This study resulted in seven haplotypes belonging mainly to lineage A (western types) and partly to lineage E (eastern types). In our research, we could also distinguish these two main lineages with two clearly separated northern and southeastern clusters and a transition zone between them at the central part of the distribution range. We presume that haplo-1 in our study corresponds to the most common 'haplotype 5' from the former PCR-RFLP studies. On the other hand, a cross-check is needed to match the other, less frequent haplotypes with the previously published sets.

Genetic studies with cpSSR markers are more limited for Hungarian oak, and the applied marker sets in the different studies are not fully overlapping. The survey of Di Pietro et al. [13] is one of these, giving a detailed overview of the genetic patterns of five oak species comprising the whole Italian Peninsula, Sicily, and Sardinia. The study used five cpSSR markers and evaluated 60 natural Italian populations of *Q. robur* (L.), *Q. petraea* ((Matt.) Liebl.), *Q. pubescens* (Wild.), and *Q. frainetto* (Ten.). The analysis resulted in a complex haplotype structure along the geographic regions, where the central and southern part of the Italian Peninsula represented the highest haplotype variability. In contrast, the northern part and the Sicily and Sardinia regions showed lower variability, together with the marked isolation of Sardinia. Out of the detected 28 haplotypes, six were shared by 10-30 individuals per population, while 22 were restricted to a smaller number of individuals. In the frame of the study, 15 *Q. frainetto* (Ten.) individuals were investigated from four populations of the Central and South Italian Peninsula, and the analysis revealed five haplotypes within this sample set, with one exclusive (private) haplotype for this oak species. In general, both *Q. robur* (L.) and *Q. frainetto* (Ten.) displayed fewer haplotypes (six and five, respectively) compared to the *Q. petraea* ((Matt.) Liebl.) and *Q. pubescens* (Wild.) species complexes. The ratio between the number of haplotypes and the number of *Q. frainetto* (Ten.) individuals was between the values of *Q. petraea* (the highest) and *Q. pubescens* Wild. (the lowest) and was higher than for *Q. robur* (L.). Even if the Italian study used a partly different marker set with a lower marker number, it highlights considerable genetic variability for the Apennine region. These are possibly derived from a distinct lineage and are different from the ones observed in our analysis in the Balkan region.

From the genetic pattern obtained in our study, we can conclude that the northern edge of the natural area is represented by a lower genetic diversity, at least at the chloroplast level. On the other hand, the high number of detected haplotypes highlights the importance of high-resolution genetic sampling, especially for species with fragmented distribution patterns, like *Q. frainetto* (Ten.). The northeastern edge and the central part of the natural area were scarcely covered with sampling sites, so investigating new populations in the future may reveal new haplotypes in parallel. Suppose the method is applied for tracking reproduction materials to give a certificate of origin. In that case, an accurate and fully elaborated haplotype map is essential for the whole distribution range.

The second part of the study dealt with the history of some old stands in Hungary. As written documentation about the origin of seed lots and the year of afforestation is not available anymore, we decided to use the cpSSR marker technique to unveil at least the putative origin of these old stands. Besides this aspect, using acorns from these stands for new plantations can also be a fundamental question, as the different provenances may react differently in the future [30]. Surprisingly, the five analysed Hungarian stands also present high variability, and most of them have a completely different origin. Two stands (Gyulaj and Ibafa) share one haplotype that could not be identified among the sampled natural populations. This unique haplotype stands genetically close to the central pattern of the area, but based on the Structure clustering, it belongs rather to the northern cluster (lineage A). These stands may have derived from acorns, possibly originating from the Transylvanian territory of the Monarchy. In the case of one stand (Hegyesd), this hypothesis can be proved, as the most frequent haplotype dominating the northern part could be detected here. Two other old stands, in Iharosberény and Diósjenő, are represented with one-on-one haplotypes from the central regions near Kosovo and North Albania, respectively. The occurrence of these haplotypes in the former territory of the Monarchy (the inner Carpathian areas) is less probable; however, it is not entirely impossible. However, further studies are needed to unveil the exact routes of the original propagation material. The considerable variability detected among the five Hungarian stands highlights the need to check all the artificial stands with unknown origins. This is especially important, since the utilization of these resources for seed production stands is planned in Hungary.

Finally, considering gene conservation purposes of native (or even artificial) *Q. frainetto* (Ten.) stands, the study of Neouphytou et al. [25] provides valuable hints for future research. The study evaluated relict and managed oak stands of pedunculate oak, sessile oak, and pubescent oak in Central Europe. It demonstrated that the autochthony was preserved throughout historical times based on the chloroplast haplotype pattern. The study concluded that both relict and managed stands, species-pure or mixed, can be considered suitable for conserving autochthonous gene pools. Indeed, coppice-with-standard management could contribute to preserving autochthony in this region. However, the study also highlights that *Q. pubescens* (Wild.) exhibited a pronounced genetic structure based on the nuclear markers compared to *Q. petraea* ((Matt.) Liebl.) and *Q. robur* (L.) due to its smaller and isolated population pattern. Therefore, a finer resolution of gene conservation units is proposed for pubescent oak. We can also predict more or less similar patterns for the even more fragmented *Q. frainetto* (Ten.) stands. Furthermore, the results highlight the importance of evaluating nuclear genetic markers and the other occurring oak species in parallel. This can be important later from the viewpoint of possible hybridization between oaks, which can be probable to some extent, and which may also form the genetic patterns of Hungarian oak populations [9, 17].

5. Conclusions

The applied cpSSR method is adequate for tracking reproduction materials of autochthonous *Quercus frainetto* (Ten.) stands, as a considerable genetic variability was detected in the 12 studied populations in the Balkan area. Two main lineages with two clearly separated northern and southeastern clusters were detected, as well as a transition zone between them at the central part of the distribution range. The north edge of the natural area is represented by a lower genetic diversity, at least at the chloroplast level. The five analysed Hungarian artificial stands also presented high variability, and most of them have a completely different origin. However, a more detailed map is needed to fill the gaps and identify all the putative haplotypes of the other fragmented populations along the natural distribution range. The method can also serve as a tool for unveiling and reconstructing the history of old planted stands. This will be particularly important if the reproductive material is used for afforestation in the future.

Acknowledgments

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UNGULATE BROWSING ON OAK FOREST REGENERATION IN ROMANIA’S WESTERN PLAINS

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Abstract: *The increasing populations of ungulates in Europe have raised significant concerns regarding their impact on forest ecosystems, particularly in Romania. This study examines the development of both artificial and natural regeneration and the effects of ungulate browsing on forest regeneration in oak-dominated stands located in Romania’s Western Plains, at altitudes below 300 meters. Regeneration from three groups was measured over two consecutive years, in 2023 and 2024. For each sapling, several attributes were recorded, with species and browsing occurrence being the most critical. Estimates of game numbers, hunting bags, and other characteristics were collected from five hunting grounds, focusing on the main game species of interest: roe deer (Capreolus capreolus L.), fallow deer (Dama dama L.), and red deer (Cervus elaphus L.). The findings indicate a decrease in sapling density alongside an increase in browsing occurrence. Although protective measures such as fencing are employed, they often fall short of providing long-term solutions. This study advocates for the implementation of proper hunting quotas and the integration of ecological factors into wildlife management strategies to sustainably control ungulate populations. Ultimately, this research underscores the need for a comprehensive approach to mitigate the adverse effects of ungulates on forest regeneration and maintain ecological balance.*

Key words: *ungulate browsing, wildlife damage, forest regeneration, oak-dominated stands.*

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1. Introduction

The population of ungulates in Europe has seen a drastic increase over the past few decades, leading to their classification as invasive species due to the escalating damage they cause across various sectors collisions [1]. Central European countries, such as Austria and Germany, have been grappling with the challenge of controlling ungulate populations which have reached overabundance levels, resulting in damages amounting to hundreds of millions of euros each year [31]. In addition to posing risks to human safety through vehicle and the associated compensation costs [35], ungulates inflict significant harm on both the agricultural and forestry sectors. In forestry, the primary damages caused by ungulates are represented by browsing, fraying, and bark stripping. In recent years, browsing has emerged as one of the most prevalent forms of damage, hindering the proper development of young tree regeneration and causing both financial and labor-related challenges for forest managers [30]. These challenges often necessitate the implementation of protective measures, such as fencing and the use of deterrents, as well as replanting efforts. However, these solutions are costly and time-consuming, and they do not offer a long-term fix, as ungulate populations continue to prosper without effective population control and the presence of large carnivores to help maintain ecological balance.

The first scientific mention of problems related to ungulate browsing in Romania may have been made by Onofrei [26], who reported high levels of browsing in young pine plantations at O.S. Cerna and experimented with the use of polyethylene bags to prevent further damage. Between 1970 and 1978, Cioflec [9] reported in his study that up to 90% of Scots pine and Norway spruce saplings were browsed by roe deer during winter. The study suggested the use of a repellent, “Cervacol”, as it was found to be more effective than polyethylene bags in preventing browsing damage. The monocultures established over large areas in the 1960s and 1970s, predominantly replanted with Norway spruce, combined with the drastic reduction in gray wolf populations, led to an exponential increase in the population of red and roe deer, resulting in significant damage to young tree stands [17, 18]. In 1978, Popescu [29] started to raise awareness about the issues caused by ungulates in young coniferous stands. The study recommended treatments that emphasize natural regeneration, along with the implementation of fencing as a preventive measure. At the beginning of the 2010s, a more scientific approach to addressing browsing pressure on Norway spruce stands was discussed, and prevention techniques were analyzed [37, 38]. Following these initial studies, several additional investigations were conducted on ungulate browsing, primarily focusing on the northeastern region of Romania and exclusively examining coniferous stands in mountainous areas. In the past two decades, the populations of ungulate species in Romania, including red deer (*Cervus elaphus* L.), roe deer (*Capreolus capreolus* L.), and fallow deer (*Dama dama* L.) have significantly increased in both numbers and territorial range. In this matter, the focus should be focused not only on forest managers, but also on wildlife managers, as population control is done through hunting.

This study aims to analyze browsing occurrences in a forest stand type and region that have not been previously investigated in Romania, specifically focusing on the oak-dominated stands of Romania's Western Plains. The study will compare data from two consecutive years and try to identify the effects of browsing on sapling development and survival rates. Additionally, in this study, the evolution of game numbers and a possible correlation with a probable cause will be tested.

2. Material and Methods

2.1. Study Area

The study was conducted in the Western Plains, in the northwestern part of Romania, in Bihor County, specifically in the forests around the municipality of Tinca, located at approximately 46° 46' northern latitude and 21° 55' eastern longitude. All of the forest stands included in this study are administered by O.S. Tinca, which is subordinated to D.S. Bihor. Simultaneously, the study area overlaps with five hunting grounds (HG) in Bihor County: HG 25 Boboștea, HG 26 Păușa, HG 30 Peri, HG 31 Goroniște, and HG 35 Oșand. This area was chosen for the study because the ungulate density is relatively high and it has a history of significant damage caused by ungulates, which has, in certain cases, led to the complete reforestation of some already planted areas.

All of the forest units included in this study are oak-dominated, and the following oak species are present: Turkey oak (*Quercus cerris* L.), pedunculate oak (*Quercus robur* L.), Hungarian oak (*Quercus frainetto* Ten.), sessile oak (*Quercus petraea* (Matt.) Liebl.) and northern red oak (*Quercus rubra* L.). All of the forest stands were located below 200 m.a.s.l. and were included in 3 types of Ecological Groups (GE): GE55 "*Hilly area of hornbeam-oak forests (s-m), luvisols, medium edaphic soil volume*", GE73 "*Tabular plains of oak forests (m), pseudogley soils, medium to large edaphic soil volume*", GE75 "High plains of hornbeam-oak forests (s), brown and brown-red soils, luvisols-vertisols, medium to large edaphic soil volume".

For this study, 15 forest units with abundant regeneration and a total area of 84.29 ha were selected: 9 units where the predominant type of regeneration was artificial (15.71 ha), 4 units with predominant natural regeneration (62.86 ha), and 2 control units (5.72 ha). The control units were characterized by the exclusion of ungulates through the use of properly installed electric fencing. Among the artificial regeneration units, four had electric fences installed; however, the energizer, height, insulators, and wire used were not properly selected, which allowed easy access for all ungulate species. The main ungulates found in this study area are: roe deer (*Capreolus capreolus* L.), fallow deer *(Dama dama* L.), and red deer (*Cervus elaphus* L.). The hunting grounds are specific to the Western Plain region, with forests surrounded by agricultural fields. These agricultural fields comprise an overall higher proportion (68.4%) and act as feeding and resting zones for the majority of the year. However, during late winter and early spring, when the fields are cleared, the ungulates take refuge in the forests, placing higher pressure on the forest food resources.

2.2. Data Collection

A permanent sample grid was established, where data were collected for two consecutive years, 2023 and 2024. For the measurement of regeneration, two types of sampling grids were used. For artificial regeneration, a sampling grid of randomized square plots was employed, while for natural regeneration, a sampling grid of randomized circular plots was utilized. The square plots had an area of 100 m², with each side measuring 10 m, and the circular plots had an area of 100 m² with a radius of 5.64 m. A permanent sample grid with a total of 48 sample plots has been established: 20 sample plots for the artificial regeneration, 16 sample plots for the natural regeneration, and 8 sample plots for the control group. A mixed use of the two sampling plot shapes was implemented to facilitate the measurements of regeneration. This approach does not compromise the accuracy of the estimations, as the errors are minimal [27].

The data collection was based on a similar collection protocol conducted by Ruprecht et al. [33], where the following parameters were collected: the species, the saplings' height below 1.30 m, the diameter at root collar, the vigourity of the saplings, and the occurrence of browsing. Additionally, stand characteristics for each forest unit from the management plans were also recorded.

The wildlife data, all game estimates, hunting bag statistics, and characteristics of the hunting grounds were gathered from official reports from the Ministry of Environment, Waters, and Forests during the period 2013-2023.

2.3. Statistical Analysis

The analysis in this study can be divided into two sections: descriptive statistics of the regeneration and browsing occurrence and statistics on game population evolution. Firstly, synthetic description regarding the sapling density per hectare for each of the studied regeneration types was shown, followed by the species composition share, and a distribution of browsing occurrence.

To understand the growth of the game numbers in the past decade, a relative growth of ungulate species has been calculated, with the reference year being 2013. In order to test if the increase in both game numbers and density is caused by a deficient harvest sex ratio, Pearson correlation between the sex ratio of the harvested game and the annual growth of the game number for the following year was tested for each of the species. As both variables were independent of each other, this test aimed to assess whether the hunting bag had any effect on the annual growth.

3. Results

3.1. Description of the Regeneration and Browsing Occurrence

For the investigation cycles of 2023 and 2024, the density of saplings/ha (Figure 1) and the tree species composition were calculated. In 2023 investigation cycle, a total of 3,234 saplings from 7 species were measured. In the category of artificial regeneration, the sapling density was approximately 4,965 saplings/ha; in the category of natural regeneration, the sapling density was approximately 12,830 saplings/ha; and in the control group, the density was approximately 4,950 saplings/ha. For this investigation cycle, the share of tree species in the regeneration composition was: Turkey oak (*Quercus cerris* L.) with 52.59%, pedunculate oak with 31.37% (*Quercus robur* L.), European ash (*Fraxinus excelsior* L.) with 5.81%, Hungarian oak (*Quercus frainetto* Ten.) with 4.4%, red oak (*Quercus rubra* L.) with 2.53%, sessile oak (*Quercus petraea* L.) with 2.2%, and hornbeam (*Carpinus betulus* L.) with 1.1%.

In 2024 investigation cycle, a total of 3624 saplings from 7 species were measured. In the category of artificial regeneration, the sapling density was approximately 3,850 saplings/ha; in the category of natural regeneration, the sapling density approximated 13,600 saplings/ha; and in the control. group, the density was approximately 4,600 saplings/ha. For this investigation cycle, the share of the species in the regeneration composition was the following: Turkey oak with 55.6%, pedunculate oak with 29.27%, Hungarian oak with 4.72%, European ash with 4.42%, red oak with 2.9%, sessile oak with 2.1%, and hornbeam with 1.1%.

Between the two investigation cycles, the survival rate for the saplings from the artificial regeneration was 77.54%, and the survival rate of the control group was 92.2%. In the natural regeneration group, the survival rate was not calculated because the number increased as seedlings evolved into saplings and the numbers of saplings increased.

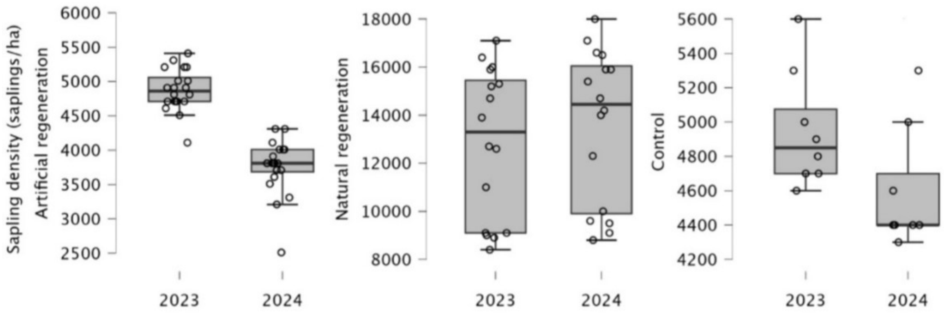

Fig. 1. *Sapling density for the 2023 and 2024 investigation cycles*

Regarding the browsing occurrence (Figure 2), in 2023, 51.2% of the measured saplings from the artificial regeneration were browsed, 10.5% of the measured saplings from the natural regeneration were browsed, and 12.4% saplings from the control group were browsed. The following browsing percentages were found for the following species from the artificial regeneration group: 59.2% of Turkey oak, 52.4% of pedunculate oak, 39.4 of European ash and 43.5% of red oak. In parallel, the following browsing percentages were found for the species from the natural regeneration group: 16.3% of Hungarian oak, 12.5% of hornbeam, 10.2% of sessile oak, and 9.3% of pedunculate oak. Simultaneously, the browsing percentages that were recorded in the control group are: 37.5% of hornbeam, 18.0% of Turkey oak, 15.0% of sessile oak, 12.9% of European ash, and 8.53% of pedunculate oak. In 2024, 47.3% of the measured saplings from the artificial regeneration were browsed, 12.3% of the measured saplings from the natural regeneration were browsed, and 11.6% from the control group were browsed. The following browsing percentages were found for the following species from the artificial regeneration group: 58.7% of Turkey oak, 53.1% of European ash, 45.5% of pedunculate oak, and 36.2% of red oak. The following browsing percentages were found for the following species from the natural regeneration group: 15.8% of Hungarian oak, 12.3% of pedunculate oak, 12.07 of Turkey oak, and 12.0% of hornbeam. The following browsing percentages were found for the following species from the control group: 40.0% of hornbeam, 16.7% of Turkey oak, 14.5% of sessile oak, 10.0% of European ash, and 8.0% of pedunculate oak.

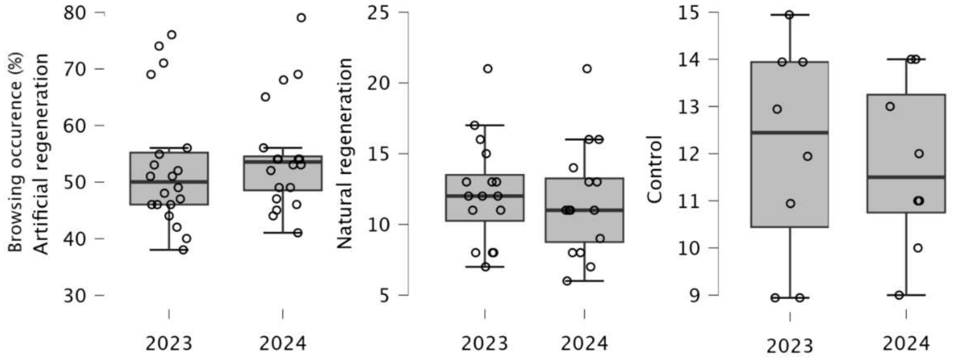

Fig. 2. *Browsing occurrence in the 2023 and 2024 investigation cycle*

3.2. Dynamics of Game Population Numbers

Based on the relative growth of game numbers in the 2013-2024 period, a trend of increase was identified for all three studied species (Figure 3). Based on the reference year, the growth for roe deer was 22.9%; for fallow deer - 46.2%; and for red deer - 42.3%. The estimated number of roe deers increased in 11 years (2013 – 2024) from 1,123 individuals to 1,457.

For the same period, the fallow deer population increased from 217 to 403 individuals, and the red deer population maintained the trend and almost doubled the number of individuals, from 459 to 795. Based on the previous numbers, the densities of ungulates across all five hunting grounds in 2024 are as follows: 3.39 ind./km² for roe deer, 0.93 ind./km² for fallow deer, and 1.84 ind./km² for red deer. Theoretically, during winter and early spring, when agricultural fields are often vacant and forests provide the majority of shelter and food, the densities within the forest during these periods can reach 10.8 ind./km² for roe deer, 2.98 ind./km² for fallow deer, and 5.8 ind./km² for red deer. Even though the majority of the hunting quotas were filled, the increase in ungulate populations remains ascending, indicating that the hunting quotas are not being adapted to the current trends in wildlife growth ecology.

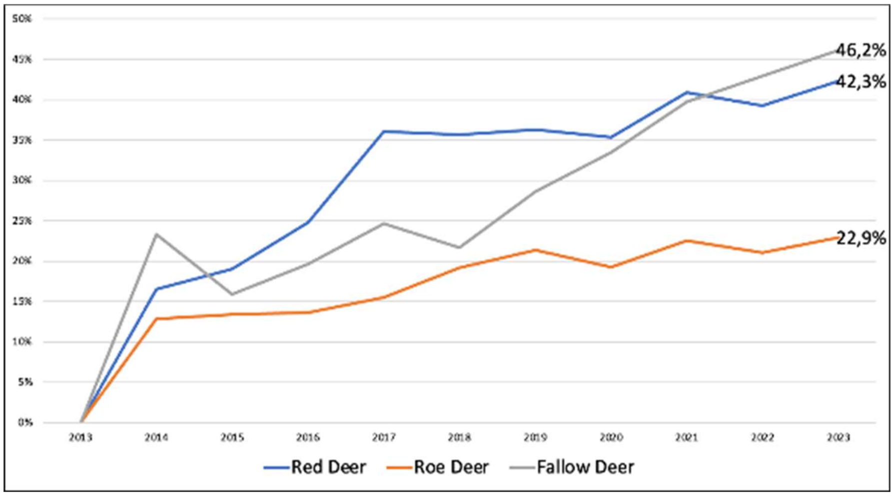

Fig. 3. *Relative growth of the studied ungulates*

Regarding the correlation between the annual growth of game population and the sex ratio of the hunting bag, the Pearson correlation test did not have any significance for any of the three studied species. It can be admitted that the annual growth of game numbers is not influenced significantly by the sex ratio of the harvested wildlife. Similar situation was observed in red deer population from Spain [7].

4. Discussion

Between the two investigation cycles in 2023 and 2024, a noticeable decrease in sapling density was observed in both artificial regeneration group, and in control group. It is important to note that this declining trend cannot be attributed solely to browsing; several factors contribute to the reduction in sapling numbers. Drought, underdeveloped root systems, and natural causes of sapling mortality are significant contributors [34, 39]. While browsing can directly result in sapling losses, particularly when severity is high, it also indirectly increases plant stress levels, further weakening them and exacerbating the decline in density [10]. In the case of natural regeneration, the number of saplings increased, primarily due to the transition of seedlings into the sapling class. The occurrence of browsing did not significantly change between the investigation cycles. This phenomenon can be attributed to two factors: first, the saplings that were browsed in the previous cycle may have perished, resulting in theoretically consistent levels of browsing; second, the same saplings may have been subjected to repeated browsing.

Even though this study is the first to document browsing occurrences and their effects in Romania, this phenomenon has been extensively studied in various regions of Europe. In England, significant damage caused by fallow deer in mixed oak plantations of pedunculate oak (*Quercus robur* L.) has been reported by Moore et al. [23, 24]. Additionally, both roe and red deer have shown a high preference for oak species in various high forest and coppice systems [14, 15, 19]. A similar study from

Southern Sweden indicated that pedunculate oak was one of the most browsed species by red deer [20]. In Central Italy, browsing by roe and red deer resulted in a loss of approximately 25% in volume in turkey oak (*Quercus cerris* L.) and chestnut coppices (*Castanea sativa* Mill.) [12, 28]. In Lower Austria, browsing of oak species significantly impacted restoration efforts in the area, favoring the development of Norway spruce [28]. A study in France done by Chevrier et al. [8] found that excluding roe deer allowed the canopy stage to be reached 10 years earlier. In French coppice systems, oak stumps can maintain a sprouting capacity of up to 80 years without browsing [21]. Overall, not only broadleaved species but especially oak species are highly preferred over coniferous species in terms of browsing preferences across most studies focused on multiple species. While browsing may not be the primary cause of mortality, it significantly influences plant health and regeneration. Furthermore, the main issue caused by browsing is the depreciation of timber quality in future stands [2, 41], which is a critical concern for the oak stands examined in this study, as their primary goal is to produce superior timber assortments.

The primary cause of ungulate browsing is considered to be the high densities of ungulates within forests during critical periods of the year [11]. Despite achieving the hunting quotas set for wildlife managers from the study area, the growth of ungulate populations remains uncontrolled. The sex ratio of the hunting bag did not show any significances based on the Pearson test, meaning that the structure of the hunting bag had no influence on the continuous growth of ungulate numbers. Additionally, the study area exhibits a low density of large carnivores, which further exacerbates the issue. This situation is mirrored at the European level, where ungulate populations have increased, even in regions with sufficient predator densities [4] and intense culling [16]. Although the hunting bag has increased across Europe, the number of ungulates has not decreased [3, 5, 6, 22, 32], highlighting a significant discrepancy that calls for a vital reassessment of wildlife management practices and hunting regulations, especially for the case of Romania, in particular because these regulations generally may have a delayed effect [13, 25]. Effective management strategies must take into account ecological factors and population trends to ensure sustainable wildlife populations and mitigate their impact on forest regeneration, because some ungulate species, particularly roe and red deer, can be considered invasive species in some regions of Romania. In the current context, even a stable and permanent density of large predators has proven insufficient to maintain a favorable ungulate population density. Another study reached similar conclusions indicating that human activities exceeded the presence of a large carnivore when it comes to browsing prevention [36].

While fencing has been successful in preventing ungulate browsing, this method is only effective when electric fences adhere to regulatory standards. However, it incurs additional costs for forest managers and does not provide a long-term solution. Promoting hunting to reduce browsing pressure is a more efficient prevention measure, as supported by similar research studies [40]. Therefore, it is crucial for authorities to offer proper hunting quotas to wildlife managers to better control ungulate numbers and address the challenges posed by browsing.

5. Conclusion

This study highlights the critical impact of ungulate browsing on forest regeneration in oak-dominated stands in Romania's Western Plains. Despite the implementation of protective measures, such as fencing and hunting, the findings reveal a trend of decreasing sapling density and persistent browsing occurrence over the investigation cycles. The lack of significant change in browsing levels indicates that saplings are subjected to similar pressures as their predecessors (intact saplings from the previous investigation cycle), indicating a cycle of vulnerability that undermines regeneration efforts.

To address these challenges, it is essential to adopt a management approach that includes the establishment of appropriate hunting quotas and the consideration of ecological dynamics in wildlife management strategies, including a more intensive harvesting quota. By integrating these practices, forest and wildlife managers could better control ungulate populations and prevent damages to the forest stands. Ultimately, fostering a balanced relationship between ungulates and forest regeneration is crucial for maintaining ecological integrity and ensuring the sustainability of forest resources in the region.

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PHYLOGENETIC STUDY OF *QUERCUS* SECTION *CERRIS* – INVESTIGATING EVOLUTION TO HELP UNDERSTAND ADAPTATION TO CLIMATE CHANGE

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Abstract: *Over the last 60 million years, the adaptability of oaks has enabled them to survive a series of climatic and geological transitions. These transitions have shaped the taxonomic groups within the genus. Understanding these phylogenetic structures can now contribute to a better understanding of the genetic basis of adaptation to current climate change conditions. In this study, we investigated the phylogenetic structures within the Old-World oak clade (Quercus subgenus Cerris), with a particular focus on section Cerris and its Western Eurasian taxa. To obtain a large amount of genomic data, we applied double-digest restriction site-associated DNA sequencing (ddRAD-seq) for genotyping. For the phylogenetic analyses, 29 taxa were sampled from three different arboreta. This dataset included all but one taxon from section Cerris, along with subspecies and natural hybrids. Additionally, for the analyses at the subgenus level, 10 species from sections Ilex and Cyclobalanopsis were included, with Notholithocarpus densiflorus serving as the outgroup. Our phylogenetic analyses clearly separated the three oak sections in every approach applied. Furthermore, at a finer scale, we confirmed the presence of subgroups within section Cerris, which could be considered subsections. Within section Cerris, the initial divergence was observed between Asian and Western Eurasian species, likely driven by past geological and climatic transformations. The Western Eurasian taxa of section Cerris were further divided into four monophyletic groups, corresponding to subsections Suber, Aegilops, Libani, and Cerris.*

Key words: *ddRAD-seq, reference mapping, cork oaks, sect. Ilex, sect. Cyclobalanopsis, Notholithocarpus.*

1. Introduction

The oak genus (*Quercus* L.) currently has the largest species diversity within the beech family (Fagaceae). The more than 400 oak species are distributed across all continents of the Northern Hemisphere. Within their distribution range, they are

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characterized by remarkable phenotypic and genetic diversity, which provides them with great adaptability. Oaks play a key role in temperate forest ecosystems almost everywhere in the Northern Hemisphere, often being among the dominant forest-forming elements (e.g., sessile oak and Turkey oak in Europe) [21].

Over the last 60 million years since their emergence, they have successfully adapted to drastic climatic and geological transformations while maintaining their significant ecological role [3].

However, these challenges have left imprints on the genomes of modern oaks, which are reflected in the formation of their various taxonomic units [8, 28]. Although the taxonomic classification of oaks has long posed challenges for botanical research, the application of molecular genetic methods has begun to stabilize the taxonomic system of oaks [9, 12]. This system divides oaks into two subgenera (*Quercus* and *Cerris*), which are further subdivided into eight sections. The evolutionary history, current distribution, species diversity, and number of sections of the two subgenera have characteristic differences, which may be expressed in differing neutral, as well as adaptive traits.

Section *Cerris* is the smallest group in the subgenus *Cerris* comprising only 15 species mainly in Western Eurasia, North Africa, and Southeast Asia [8, 21]. Although this small oak group has been rather underappreciated even in recent times, some species (such as Turkey oak (*Quercus cerris* L.)) may have great potential in forestry climate adaptation in Europe, mainly thanks to their drought tolerance and large genetic and phenotypic variability [14, 30]. However, there is only limited knowledge available in the literature on these oaks’ genetic attributes [8, 27], although it would be required for a complex assessment of their adaptability under the current climate change conditions. For this reason, in the present study, we conducted phylogenetic analyses primarily within section *Cerris* and, on a broader scale, within the subgenus *Cerris*. Our objectives were, on the one hand to investigate the phylogenetic relations of sections within the subgenus *Cerris*, and on the other hand to survey the fine-scale clustering of species within section *Cerris*. To allow for the detailed analyses of fine-scale genetic structures, we used a high-resolution method, namely double digest restriction site-associated DNA sequencing (ddRAD-seq) for genotyping. In addition, all species (excluding *Q. crenata* Lam.), subspecies and some natural hybrids of section *Cerris* were included in our analyses, which enabled the representative assessment of the subgroups within this section.

2. Materials and Methods

2.1. Plant Material

The plant material required for the phylogenetic analyses was collected from the Arboretum des Barres and Arboretum des Pouyouleix in France, as well as from the Kámon Arboretum of the University of Sopron, Forest Research Institute, Hungary. This collection consists of dried leaf samples from a total of 29 taxa (Table 1). The sampled individuals of the French arboreta mainly originate from natural habitats, which likely eliminates the uncertainty regarding species identity that may have arisen from previous seed exchange.

Sampled taxa and their distribution Table 1

Sampled taxa	Sample ID	Section	Distribution
Quercus acutissima Carruth.	Q3856	*Cerris*	East and Southeast Asia
Quercus acutissima subsp. *kingii*	Q3857	*Cerris*	Nepal - Laos
Quercus variabilis Blume	Q3866	*Cerris*	East and Southeast Asia, Japan
Quercus chenii Nakai	Q3861	*Cerris*	East Asia
Quercus afares Pomel	Q3858	*Cerris*	North Africa (Algeria, Tunisia)
Quercus suber L.	Q3864	*Cerris*	Central and Western Mediterranean
Quercus × numidica Trabut *(Quercus afares × Q. suber.)*	Q3855	*Cerris*	Algeria, Tunisia
Quercus cerris L.	Q3770	*Cerris*	Eastern and Central Mediterranean, Balkan Peninsula, Carpathian Basin
Quercus × hispanica Lam. *(Quercus cerris × Q. suber)*	Q3607	*Cerris*	Southwestern Europe
Quercus castaneifolia C.A. Mey	Q3860	*Cerris*	Hyrcanian region
Quercus brantii Lindl.	Q3859	*Cerris*	Southeastern Anatolia, Iran, Lebanon
Quercus libani Oliv.	Q3867	*Cerris*	From Southeastern Anatolia to Iran
Quercus look Kotschy	Q3863	*Cerris*	Lebanon
Quercus trojana Webb	Q3865	*Cerris*	Anatolia, Aegean region, Southeastern Italy
Quercus ithaburensis Decne.	Q3862	*Cerris*	Eastern Mediterranean, from Southeastern Italy to Palestine
Quercus ithaburensis subsp. *macrolepis*	Q3868	*Cerris*	Eastern Mediterranean, from Southeastern Italy to Syria (excluding Palestine)
Quercus ilex L.	Q3869	*Ilex*	Central and Western Mediterranean
Quercus baloot Griff. (two individuals)	Q3873, Q4302	*Ilex*	Kashmir, Pakistan, Afghanistan
Quercus engleriana Seemen	Q4301	*Ilex*	From Tibet to Southern China
Quercus phillyreoides A.Gray	Q3870	*Ilex*	Southern China, Japan
Quercus leucotrichophora A.Camus	Q4300	*Ilex*	Northern India, Nepal, Myanmar, Pakistan, Sri Lanka
Quercus glauca Thunb.	Q4305	*Cyclobalanopsis*	East and Southeast Asia, from Afghanistan to China
Quercus augustinii Skan	Q4307	*Cyclobalanopsis*	Vietnam, Southern China
Quercus myrsinifolia Blume	Q4304	*Cyclobalanopsis*	Central and Southern Japan, Korea, from Central and Southern China to Indochina
Quercus oxyodon Miq.	Q4303	*Cyclobalanopsis*	Thailand, India, Nepal, Bhutan, Burma, Southern China
Quercus acuta Thunb.	Q4308	*Cyclobalanopsis*	Japan, South Korea, Taiwan
Quercus gilva Blume	Q4306	*Cyclobalanopsis*	Central and Southern Japan, South Korea, Taiwan, Central China
Notholithocarpus densiflorus (Hook. & Arn.) Manos, Cannon & S.H.Oh	Q3872	*Notholithocarpus*	Southeastern Oregon, California

Only one taxon, *Quercus* x *hispanica* (Lam.), was sampled from the Kámon Arboretum of the University of Sopron Forest Research Institute, which may have been introduced to the arboretum through seed exchange, thus its hybrid origin may be uncertain. Since the aim of our study was both to investigate the relations between the sections of the subgenus *Cerris* and to conduct a detailed analysis of the species structures within section *Cerris*, six species from section *Cyclobalanopsis*, four species from section *Ilex*, and all species, subspecies, and some natural hybrids from section *Cerris*, except for *Quercus crenata* Lam., were collected during the sampling. In addition, we used *Notholithocarpus densiflorus* as an outgroup, which is commonly used in oak phylogenetic analyses as it is one of the closest living relatives of oaks.

2.2. Genotyping

To obtain a high amount of genomic data evenly covering the nuclear genome, the sampled taxa were genotyped by implementing the double digest restriction site-associated DNA sequencing (ddRAD-seq) method. This genotyping approach belongs to the family of reduced-representation methods based on next-generation sequencing [5]. Its major advantage over traditional marker types is that it enables the detection of hundreds of thousands of single nucleotide polymorphism (SNP) markers through even sampling across the entire genome [5, 23]. Moreover, this method samples not only non-coding but also functional regions, providing insights into genomic regions involved in various adaptive processes. In addition, it is applicable also for non-model organisms without a well-annotated reference genome, such as forest trees [22, 30].

For DNA extraction we used five leaf disks (with 5 mm diameter) per sample, following the protocol used in Dummolin et al. [10]. After DNA extraction, library preparation was performed the same way as in our recently published dataset [16], in which double digestion was carried out by the combination of the *Pst*I and *Msp*I enzymes. In the next step, the samples were sequenced on a NovaSeq 6000 platform in two separate runs.

2.3. SNP Calling

Bioinformatic processing of the raw sequences was carried out on the HPC server of the University of Sopron, Forest Research Institute, Sárvár, Hungary. The first step of processing involved quality control of the raw data by using the FastQC [1] and MultiQC [11] programs. During this process, we determined that the first five bases of the forward reads (essentially the restriction enzyme binding site) were of poor quality for almost all samples due to sequencing errors. Additionally, both forward and reverse reads contained poly-guanine and poly-adenine sequences, characteristic sequencing artefacts of the sequencing platform [2, 6, 29]. To address these issues, we performed quality filtering in the next step using the Cutadapt [19] program. This involved trimming the first five low-quality bases from the forward reads and removing the poly-guanine and poly-adenine content from the sequences. After the trimming process, only reads with at least 200 bp length were kept.

Next, we used the Stacks v2.62 software [4, 26] for SNP calling. During this process, we followed the reference mapping pipeline, as reference mapping also provides information on the exact location of the SNP loci in the genome. In addition, this approach helped us validate the SNPs by reducing noise in the reads through the soft clipping of sequences that did not match the reference genome.

Before conducting the reference mapping, we performed another quality filtering with the "process_radtags" program. During this process, the quality of the reads was checked by a sliding window algorithm (window size was set to 15% of the read length), and low-quality reads were removed (mean Phred score < 10 in at least one window). In addition, reads containing adapter sequences were also discarded.

In the following step, filtered reads were mapped to the Turkey oak genome (NCBI BioProject: PRJEB69237) by using the BWA MEM program [17], and then SAM files were sorted and converted to BAM files with the SAMTOOLS software [18]. In the next step, SNP loci were assembled in each individual with the "gstacks" program. After that, we used the "populations" function to generate the final SNP datasets. To allow detailed analyses on multiple levels of the investigated oak groups, three datasets were generated comprising different groups of taxa within the subgenus *Cerris*. The first dataset comprised all the sampled species, the second only the taxa within section *Cerris*, and the third only the Western Eurasian and North African members of this section. All three datasets were generated with the same filtering thresholds in the "populations" program: --min-populations 1, --min-maf 5.

Finally, we used the PLINK program [24] to carry out final filtering on the datasets by removing duplicated SNPs and loci with more than 30% missing data. The main attributes of the datasets are summarized in Table 2.

Main attributes of the generated datasets (where N is the sample size per dataset) Table 2

Dataset	N	No. of SNP loci	Genotyping rate [%]
subg. *Cerris*	29	992 942	58.07
sect. *Cerris*	16	580 854	66.97
sect. *Cerris* W-Eurasia	12	417 781	68.26

2.4. Phylogenetic Analyses

During our phylogenetic analyses, we applied multiple clustering approaches to investigate the genetic structure of the sampled taxa. Initially, principal component analysis (PCA) was performed with the PLINK software. First, we analyzed the subgenus level, and then repeated it for the Western Eurasian members of the section. Since the third principal component also explained a significant portion of the total variance in both analyses, results were visualized on 3D figures, representing the first three principal components. For 3D visualization, we used the Python libraries "matplotlib" [13] and "seaborn" [32].

Subsequently, as another approach, we investigated the species structures using the fastStructure program [25] at the level of the subgenus *Cerris*, section *Cerris* and its Western Eurasian taxa. In contrast to PCA, this method also allows for the estimation of the most probable number of genetic groups that can be built from the sampled individuals. The obtained results were visualized in Python using the "pandas" [20] and “matplotlib” [13] libraries.

Finally, a maximum likelihood phylogenetic tree was constructed using the subgenus level dataset by applying the IQ-TREE 2 software [22], which also allows for the selection of the appropriate substitution model. In the analysis, we applied the GTR+Γ model based on the AIC values of the automatic testing. The resulting maximum likelihood tree was also supported by bootstrap values calculated from 1000 iterations. The consensus tree, generated from these 1000 iterations, was visualized in R using the "ggtree" package [33].

3. Results

At the subgenus level, the *PCA* analysis efficiently separated the three sections under study (Figure 1). Among these groups, section *Cerris* exhibited the largest variance among individuals but mainly because of the split between the Western Eurasian and East Asian species. In section *Cyclobalanopsis*, *Q. gilva* (Blume) was markedly separated from the other species within the section. As for section *Ilex*, two distinct groups were identified: one comprising *Q. ilex* (L.) and *Q. baloot* (Griff.), and the other including *Q. engleriana* (Seemen), *Q. phillyreoides* (A. Gray), and *Q. leucotrichophora* (A. Camus). The outgroup species, *N. densiflorus* (Hook. and Arn.), was clearly distinguishable from the oak species forming a distinct group from the tree oak sections.

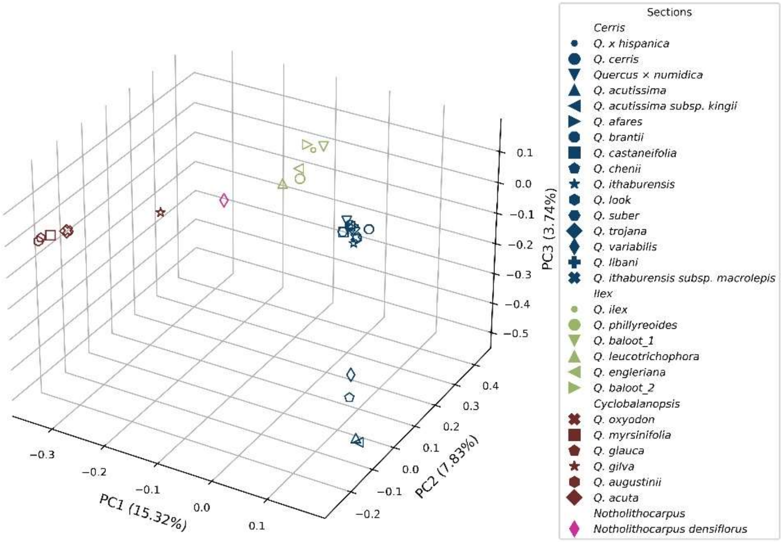

Fig. 1. *Results of the principal component analysis at the subgenus level*

At a closer view of the Western Eurasian group of section *Cerris*, three additional subgroups were identified in the PCA analyses (Figure 2). First, along the first principal component, *Q. suber* (L.) and its hybrid *Q.* x *numidica* (Trab.) were separated from the other species. After that, the remaining species were divided into two additional groups, where *Q. ithaburensis* (Decne.) and *Q. ithaburensis* subsp. *macrolepis* ((Kotschy) Hedge and Yalt.) were separated into their own group, primarily along the second principal component.

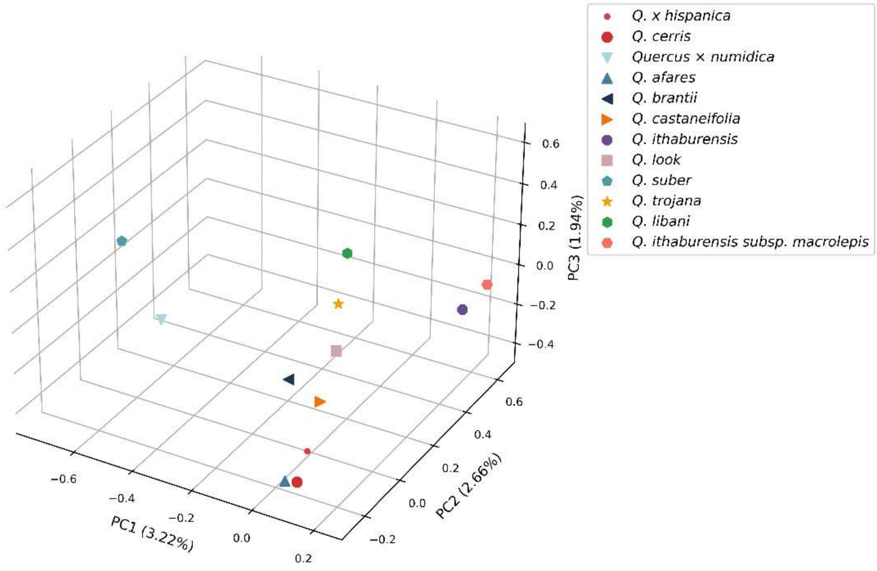

Fig. 2. *Results of the principal component analysis for the Western Eurasian species of section Cerris*

Further investigation of species structures was conducted using the fastStructure software at the level of subgenus and section *Cerris*, and its Western Eurasian members. At the subgenus level, considering all the examined taxa, the most likely number of groups to be established was four (Figure 3). In this case, the three sections and *N. densiflorus* (Hook. and Arn.) formed distinct genetic groups. Although this method was effective in the separation of the different oak sections, at the subgenus level it was unable to find further subgroups within the sections.

To investigate deeper structures, the analysis was then conducted for section *Cerris* and its Western Eurasian members. At the section level (Figure 4a), the most probable number of clusters was found to be two. Similarly to the previous results of the PCA, the section was divided into a group containing the Western Eurasian taxa and another group containing the Asian taxa.

Narrowing the scope to only the Western Eurasian group, the individuals were further divided into three genetic clusters (Figure 4b). One cluster included *Q. suber* (L.) and its hybrid *Q.* x *numidica* (Trab.), another included *Q. ithaburensis* (Decne.) and its subspecies *Q. ithaburensis* subsp. *macrolepis* ((Kotschy) Hedge and Yalt.), and the third group comprised *Q. cerris* (L.), *Q.* x *hispanica* (Lam.), *Q. afares* (Pomel), *Q. brantii* (Lindl.), *Q. castaneifolia* (C.A. Mey.), *Q. libani* (Oliv.), *Q. look* (Kotschy), and the *Q. trojana* (Webb) species and hybrids. These are essentially the same groups identified in the PCA.

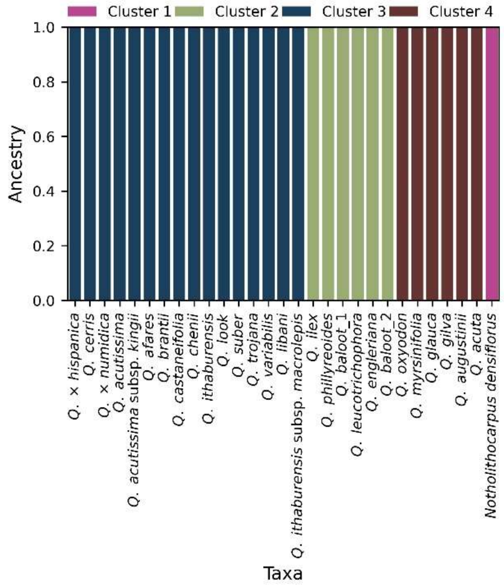

Fig. 3. *Genetic structures within the subgenus Cerris based on fastStructure analysis*

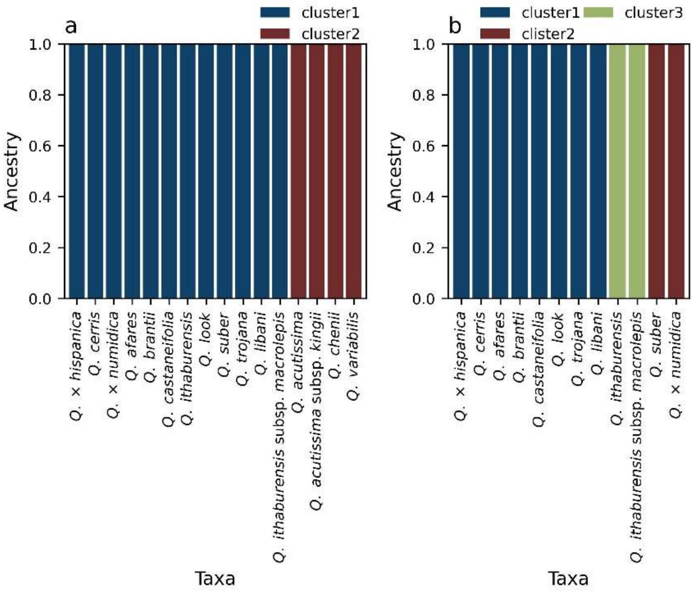

Fig. 4. *Genetic structures within section Cerris (a.) and its Western Eurasian group (b.) based on fastStructure analysis*

To validate the previous results and obtain an even more detailed picture of the species structures within subgenus and section *Cerris*, we also built a maximum likelihood tree using the IQ-TREE 2 software (Figure 5). As a result, the investigated sections were successfully divided into monophyletic groups, which could be considered sections.

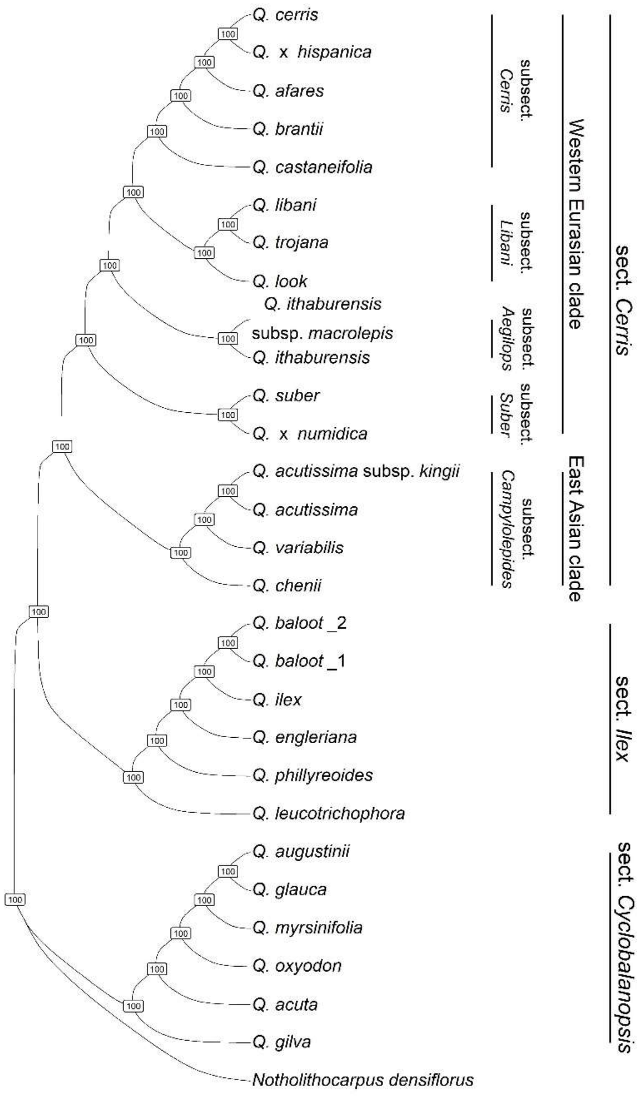

Fig. 5. *Maximum likelihood tree constructed for the subgenus Cerris by using the IQ-TREE 2 software (node support values were calculated from 1000 bootstrap iterations, we used the system published in Denk et al. [8] to annotate the tree)*

At the base of the tree, section *Cyclobalanopsis* takes the basal position, followed by the two sister sections, *Ilex* and *Cerris*. Within section *Cyclobalanopsis*, the species *Q. glauca* (Thunb.) and *Q. augustinii* (Skan) are the closest related, while *Q. gilva* (Blume) forms the outgroup of the investigated species. In the case of section *Ilex*, *Q. baloot* (Griff.), which is otherwise a Western Asian species, is more closely related to the Western Eurasian *Q. ilex* (L.) than to the other Asian species. In this case, *Q. leucotrichophora* (A. Camus) forms the outgroup of the species under study.

Since almost all the taxa belonging to section *Cerris* were included in the analysis, the clades formed on the tree can be considered relevant for the species structure within the section. According to this, there is a primary differentiation within the section, which divides the species into an Asian and a Western Eurasian clade. This validates the previous results of the PCA and fastStructure analyses. Within the Asian clade, *Q. chenii* (Nakai) is the most distantly related to the other species, similarly to the results of the principal component analysis. Within the Western Eurasian clade, there is also a finer clustering of species. This results in four additional monophyletic groups, one more than those identified in the fastStructure analysis. In this system, first, a separate clade is formed by the Western Mediterranean distributed *Q. suber* (L.) and *Q.* x *numidica* Trab. (*Q. afares* x *Q. suber*). This is followed by a monotypic clade that includes *Q. ithaburensis* (Decne) and its subspecies *Q. ithaburensis* subsp. *macrolepis* ((Kotschy) Hedge and Yalt.). In addition, there is a clade mainly consisting of species distributed in Asia Minor, which includes *Q. libani* (Oliv.), *Q. look* (Kotschy), and *Q. trojana* (Webb). Finally, a clade comprising species with more varied distributions can also be distinguished. This last group includes the Pontic-submediterranean *Q. cerris* (L.) and its hybrid *Q.* x *hispanica* Lam. (*Q. cerris* x *Q. suber*), the Hyrcanian *Q. castaneifolia* (C.A. Mey), the North African *Q. afares* (Pomel), and the Irano-Turanian *Q. brantii* (Lindl.).

4. Discussion

As a result of ddRAD-seq genotyping, a large number of genome-wide SNP data (nearly 1 million SNP loci) was obtained allowing also for fine-scale analyses of phylogenetic structures within subgenus and section *Cerris*. This high number of SNP loci included in our analyses contributed to minimizing the uncertainty of the results. For example, each taxon was assigned to specific genetic groups with 100% probability in each run of the fastStructure analysis. Similarly, in the maximum likelihood tree, all bootstrap values reached the maximum support value of 100%.

However, the use of such a large number of markers also resulted in the total variance being distributed more evenly across the loci. This means that the occurrence of a high number of loci with a lower differentiation might mask finer species structures. To address this issue, three separate datasets were created, representing different taxonomic levels within subgenus and section *Cerris*. According to the results, this strategy proved effective: compared to the larger dataset generated for the subgenus, the narrowed datasets allowed us to identify finer subsection-level structures within section *Cerris* and its Western Eurasian group.

In our principal component analyses, first, the structure of the entire subgenus was investigated, which revealed a clear separation among the three sections under study. These groups were also validated by subsequent fastStructure analyses and maximum likelihood phylogenetic tree construction. In addition, the phylogenetic tree further supported the monophyletic origin of each section.

Within section *Cyclobalanopsis*, *Q. gilva* (Blume) showed a higher degree of differentiation from the other species in our analyses. This separation was also confirmed by a previous study using a different marker system, which divided this species into a distinct lineage [7].

Investigating the finer structures within section *Cerris*, all three analyses consistently divided the taxa initially into two major groups, regarding their geographic distribution. This division into an East Asian and a Western Eurasian genetic group is in line with the latest results on this section's phylogeny [8]. In addition, a similar split has also been observed in other oak sections (e.g., sect. *Ilex* and sect. *Quercus*) and is often referred to as the Tethyan disjunction [15]. Namely, during the Cenozoic, the gradual transformation of the climate and the fragmentation of the former Tethys region accompanied by the orogeny of mountain chains could segment species ranges, limiting migration and gene flow. This probably caused the initial isolation, followed by differentiation among oak groups.

To obtain a more detailed insight into the Western Eurasian group of section *Cerris*, a dataset focusing exclusively on the species within this group was also generated. This allowed us to divide these species into three further genetic groups using both *PCA* and the fastStructure analysis. According to the PCA results, the separation of *Q. suber* (L.) and its hybrid *Q.* x *numidica* (Trab.) from the other species was the initial split in this group. This separation was also reflected in the structure of the phylogenetic tree.

Along the second principal component, *Q. ithaburensis* (Decne.) and its subspecies differentiated from the remaining taxa, a pattern which is also supported by the phylogenetic tree. For the remaining species and hybrids, no additional groups were identified in the PCA or fastStructure analyses. However, in the more detailed maximum likelihood phylogenetic tree, two additional monophyletic groups were divided.

One smaller group included *Q. libani* (Oliv.), *Q. look* (Kotschy), and *Q. trojana* (Webb) species with overlapping distributions in the eastern Mediterranean and Asia Minor. The second, larger group consisted of *Q. cerris* (L.), *Q.* x *hispanica* (Lam.), *Q. afares* (Pomel), *Q. castaneifolia* (C.A. Mey.), and *Q. brantii* (Lindl.), comprising species with quite diverse current distributions. For instance, *Q. cerris* has a Pontic-Submediterranean distribution, *Q. castaneifolia Hyrcanian*, and *Q. afares* have a *North African* distribution, and *Q. brantii* has a Irano-Turanian distribution. Despite these varied ranges, the genetic clustering of these taxa may suggest a formerly shared ancestral range that could be fragmented over time. This could be further supported by the endemism of certain species, such as *Q. afares* (Pomel) and *Q. castaneifolia* (C.A. Mey.), which may indicate that their current distributions arose from the breakup of a previously continuous range.

The geographic attributes of these species' ranges may suggest that they (or their ancestors) have been pushed back to the current restricted areas, likely due to climatic and geological transformations, and are now 'trapped', surrounded by regions unsuitable for colonization.

The formation of species groups within section *Cerris* has been supported by several studies [8, 27]. In addition, in the study carried out by Denk et al. [8] these groups were also considered subsections, which have similar structures to the groups identified in our study, with minor differences.

Among the four subsections, subsection *Campylolepides* defined in Denk et al. [8] is identical to the East Asian clade identified in our study. Subsection *Suber* includes *Q. suber* L. and *Q.* x *crenata* Lam. (which were not analyzed in our study), as well as *Q.* x *numidica* (Trab.) in our system (this species was not included in the study of Denk et al. [8]). Subsection *Aegilops* differs slightly from our results, as the mentioned study included *Q. brantii* (Lindl.) in addition to *Q. ithaburensis* (Decne.) and *Q. ithaburensis* subsp. *macrolepis* ((Kotschy) Hedge and Yalt.) in this group. Subsection *Libani* defined in Denk et al. [8] includes *Q. libani* (Oliv.), *Q. trojana* (Webb), and *Q. afares* (Pomel). In contrast, our results suggest *Q. look* (Kotschy) should be included instead of *Q. afares* (Pomel). Finally, subsection *Cerris*, as proposed in Denk et al. [8], comprises *Q. cerris* (L.), *Q. look* (Kotschy), and *Q. castaneifolia* (C.A. Mey.). However, our findings indicate a slightly different composition, excluding *Q. look* (Kotschy), while including *Q. afares* (Pomel) and *Q. brantii* (Lindl.).

Despite the differences in species composition, the presence of four subsections is also supported by our results. These differences probably arise from variations in the marker systems and sampling strategies used. Gene flow among species at different locations within their ranges may also affect results. For this reason, future studies with larger sample sizes from various parts of each species' range could help minimize these biases.

Since our sampling also included two natural hybrids, the results allow us to infer their true hybrid origins. From this perspective, the principal component analysis conducted with the Western Eurasian species of section *Cerris* proved informative. Observing the placement of the two hybrids on the PCA plot reveals a significant difference between them. While *Q.* x *numidica* Trab. (*Q. suber* × *Q. afares*) is situated between its parental species, *Q.* x *hispanica* Lam. (*Q. cerris* × *Q. suber*) does not display a similar pattern, instead clustering closely with *Q. cerris* (L.).

Although PCA alone is insufficient to directly assess the hybrid origins of the taxa, these findings may suggest that a true hybrid origin is plausible only for *Q.* x *numidica* (Trab.). It should also be noted that *Q.* x *hispanica* (Lam.) was the only taxon collected from the Kámon Arboretum of the University of Sopron, Forest Research Institute, where it likely arrived through seed exchange. This may raise questions about the taxon's identity. According to the PCA results, the investigated individual appears to be genetically closer to *Q. cerris* (L.) than to the other parent species *Q. suber* (L.). This might also be explained by the possibility that the sampled individual originated from a backcross involving a true hybrid ancestor. However, determining the true hybrid origin of the sampled *Q.* x *hispanica* (Lam.) would require further investigations, including additional samples collected from individuals in natural populations.

5. Conclusions

During our phylogenetic analyses, almost one million SNP markers were used to investigate the genetic structure of subgenus and section *Cerris*. As a result, the three investigated sections were clearly separated in all analyses. Since our study included almost all species, subspecies, and some natural hybrids of section *Cerris* (excluding *Q.* x *crenata* Lam.), we also obtained relevant insights into the species' genetic structures within the section.

Using our high-resolution datasets generated by the ddRAD-seq method, we confirmed the subsectional structures observed in similar studies. However, we noted some differences in the species composition of subgroups, which are likely the consequence of variations in the sampling design and the different marker systems used. Nevertheless, consistent with earlier studies, our results support the differentiation of section *Cerris* into five subsections. Among these, the differentiation between East Asian and Western Eurasian taxa was the most pronounced. In addition, Western Eurasian species further split into four sub-sections.

These findings on the phylogenetic structure of oaks belonging to section *Cerris* may contribute to future studies investigating the genetic background of the long-term adaptability of these species. Future studies may focus on specific aspects of section *Cerris* or its subgroups, such as the comparative analysis of *Q. cerris* L. (Turkey oak), and related species with forestry potential (e.g., *Q. afares* Pomel, *Q. castaneifolia* C.A. Mey.) would be required for the complex assessment of the species' potential in forestry climate adaptation. These studies could also provide valuable information about the species' adaptive strategies for limiting environmental factors (e.g., drought tolerance, oxidative stress tolerance etc.) and their adaptive responses under the current climate change conditions.

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MODELLING PHOTOGRAMMETRIC DATASETS IN ORDER TO ENHANCE URBAN PLANNING

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Abstract: *This paper explores the application of photogrammetric datasets to enhance urban planning through the development of detailed 3D city models. Using high-precision photogrammetric data, a Digital Terrain Model (DTM) was derived from dense image matching point cloud, in order to accurately extract the ground of the urban landscape. A Digital Surface Model (DSM) was also generated, capturing all features, including buildings and vegetation. The subtraction of the DTM from the DSM produces a normalized Digital Surface Model (nDSM), isolating the height of above ground features such as buildings. The nDSM is important to extract building heights. Using building footprints and the extracted height values, a LoD2 building model was developed. These models provide accurate representations of buildings, including roof shapes and heights, crucial for urban planning tasks such as infrastructure design and environmental analysis. This approach will try to demonstrate how integrating photogrammetry with advanced modelling techniques can significantly improve the accuracy and utility of urban planning tools.*

Keywords: *DSM, DTM, 3D building models, photogrammetry.*

1. Introduction

Urban planning has become increasingly reliant on advanced technologies to manage the complexities of modern cities. In particular, the integration of photogrammetry — the science of transforming 2D images into 3D models — has proven to be a powerful tool for collecting spatial data essential for urban development [24]. Photogrammetric datasets, generated using high-resolution aerial imagery or terrestrial data offer an efficient means of mapping urban environments with high precision. However, the raw data from photogrammetric surveys often require advanced modelling techniques to be fully utilized in practical urban planning scenarios. The application of modelling techniques to photogrammetric datasets enhances the ability to accurately represent three-dimensional structures of urban landscapes. Recent advances in

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computational algorithms, such as machine learning and artificial intelligence, have further improved the processing and analysis of these datasets, providing planners with more detailed and actionable insights. This is critical in areas such as infrastructure planning and environmental impact assessments, where the ability to predict and visualize changes to urban spaces is vital for effective decision-making [16]. By integrating time-series data from photogrammetric surveys, urban planners can track long-term trends and assess the effectiveness of planning strategies. For instance, the use of 3D city models in combination with geographic information systems (GIS) allows planners to simulate various scenarios, optimize land use, and enhance the design of urban spaces [25]. Despite the significant potential, challenges remain regarding the modelling of photogrammetric datasets effectively. Issues such as data noise, the need for high computational power, and the integration of heterogeneous data sources must also be addressed. Furthermore, the complexity of modelling urban environments accurately requires interdisciplinary collaboration between fields such as geospatial science, architecture, and urban planning. This article explores the current methodologies for modelling photogrammetric datasets and examines their applications in urban planning, with a focus on the potential for enhancing urban sustainability, mobility, and resilience when facing rapid urbanization [22].

2. 3D Building Models and Urban Planning

The modern city is often regarded as a connecting point between administrative-governmental functions with its citizens in domains such as economy, transportation, communication, technology, and services. Consequently, there is an increasing need for carefully planning the development of urban areas, which extends beyond the existing city boundaries to encompass the continuous growth and development of adjacent areas [18]. Another significant challenge for modern cities is climate change, which has long posed threats to urban environments. Urban centres have substantial energy demands for spatial conditioning and population mobility [13], contributing to greenhouse gas emissions. Government policies, administrative initiatives, and external factors, such as climate change and urban planning, directly influence the development of modern cities. Climate change exerts significant pressure on urban areas, requiring city leaders to address its impacts while ensuring that climate resilience becomes a cornerstone of municipal governance.

This was the starting point for the project Climate Resilient Development Pathways in Metropolitan Regions of Europe – CARMINE [9]. The project's goal is to assist metropolitan communities in Europe in becoming more resilient to climate change through the co-production of knowledge-based tools, strategies, and plans for enhanced adaptation and mitigation actions, aligned with the EU Mission Charter on Climate Adaptation by 2030. To achieve this objective, focusing on the 2030–2035 timeframe and with longer-term perspectives extending to 2050, CARMINE proposes an interdisciplinary approach that aims to:

- Co-create and co-develop decision-support services and guidelines to enhance resilience and adaptive capacity, including early warning

systems and disaster risk management frameworks;
- Cooperate closely with local and regional communities (stakeholders and users), policymakers, and decision-makers (local authorities) to co-develop cross-sectoral frameworks for adaptation and mitigation actions;
- Deliver science-based research and innovation solutions for multi-level climate governance, supporting local adaptation assessments and planning efforts.

CARMINE carries out its approach in eight Case Study Areas (CSA): Prague (CZ), Leipzig (DE), Funen-Odense (DK), Athens (GR), Barcelona (ES), Bologna (IT), Brasov (RO), and Birmingham (UK) [9]. In every CSA, a Living Lab will be developed in order to analyse the climate risks and socio-economic vulnerabilities. The final goal is to co-design decision-support tools and propose climate-resilient development pathways through the use of Digital Twins.

3D city models consist of 3D models of buildings, vegetation, street elements, urban infrastructure (e.g. hydrographic constructions, high voltage lines), and urban objects (e.g. traffic signs, monuments, statues and fountains). High resolution Digital Surface Models (*DSMs*) include all these elements, but do not distinguish between individual objects. Therefore, generating 3D models from point cloud datasets is the best option, as it preserves accuracy and facilitates analysis at an individual level. There are three methods of generating 3D building models: model-based, data-based, and hybrid. For the model-based generation of 3D building models, a building is considered to be made up of simple primitives, stored in a library of predefined models. In the case of complex buildings, the reconstruction approach is to decompose the building into multiple shapes and to match the models to these shapes. For the data-based generation of 3D building models, no assumptions are made about the shape of the buildings. These methods use point cloud segmentation, followed by individual segment assembly and model reconstruction. The methods used for segmentation include algorithms such as region growing, RANSAC [5].

The geometric and semantic detailing of a 3D model is known as the level of detail (*LoD*). Standardization defined by the Open Geospatial Consortium (*OGC*) is known as the CityGML standard [19]. In the CityGML version 2.0 five standard classes with different *LoDs* are defined (Figure 1). Higher levels have a superior degree of detail, are more precise, and have a structure of greater complexity.

Fig. 1. *LoDs in CityGML 2.0 [1]*

By combining 3D modelling with meteorological data in order to create a digital twin (*DT*) of the area, urban planners can foster sustainable development, enhance resilience, and improve the quality of life for urban residents. Digital twins were initially developed in manufacturing, representing high-fidelity digital plans of products to be made [12]. The defining feature of a *DT* is that the entire planning process occurs digitally, without further adjustments once the *DT* model is finalized and production begins. In construction, *DTs* align closely with Building Information Modelling (*BIM*), which consists of high-fidelity three-dimensional construction plans. These models may require adjustments for landscape integration, such as in the case of single form-work concrete bridges [7]. For urban environments, DTs aim to replicate essential physical and functional properties, including buildings, infrastructure, vegetation, terrain, and other elements. They also enable the integration of information from various processes. Creating *DTs* for urban environments involves 3D models derived from point clouds and/or images, which are considered data derivatives [15].

Based on the integration of photogrammetric data with advanced modelling techniques, a 3D model of the buildings is generated that could be used by decision-makers for various analyses and applications [2], also taking into account the city's climate data, thus improving the urban planning of the area. One application is the urban microclimate analysis, where detailed 3D building models enhanced with meteorological data are used to simulate airflow, temperature distribution, and solar radiation. This enables planners to mitigate urban heat island effects and optimize building orientation and material selection for better thermal performance [20]. Another key application is disaster risk management. 3D models integrated with real-time weather data allow for accurate simulations of flood risks and wind impacts in urban areas, facilitating the design of adaptive infrastructure and early warning systems [14]. The integration also supports green infrastructure planning. 3D models enriched with meteorological insights help identify optimal locations for parks, green roofs, and urban forests, improving air quality and reducing surface runoff during heavy rain events [6].

3. Case Study

3.1. Research area

We aim to demonstrate how the integration of photogrammetry with advanced modelling techniques can significantly improve the accuracy and utility of urban planning tools. The study area consists of 2 sq. km in the town Măgurele, in Ilfov County (Figure 2).

3.2. National Project for High Resolution Image Acquisition in Romania

The data used for the case study are derived from the ongoing national project for high resolution image acquisition in Romania [4]. The project's primary activities include acquiring high-resolution aerial imagery, conducting bundle block adjustment and dense image matching, and generating digital surface models (*DSMs*) and true orthophotos. Figure 3 shows the project areas at city level. The aerial data acquisition is done using advanced photogrammetric sensors. The images are composed of four spectral bands (red, green, blue, and near-infrared) and the spatial resolution is of 4, 9 or 15 cm, depending on the city type.

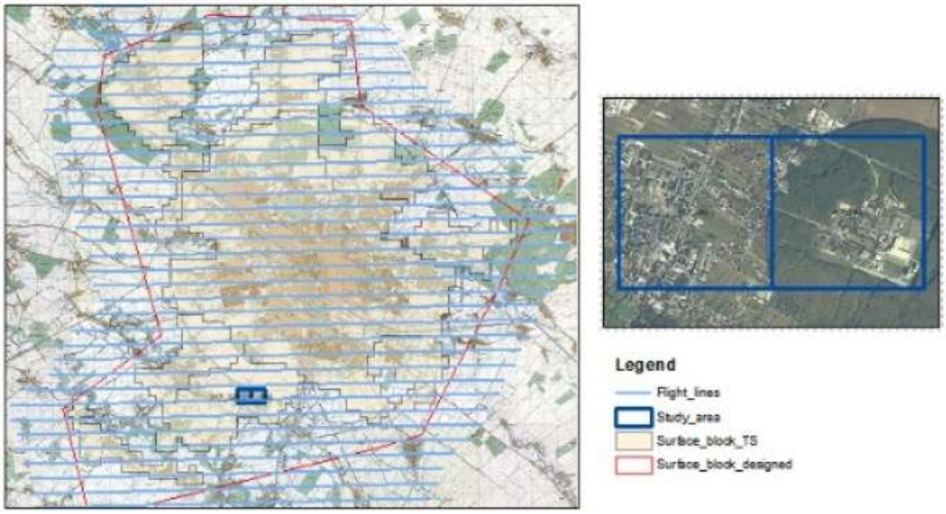


Fig. 2. *Study area*

Fig. 3. *True orthophoto generation in Romania*

The main outputs of the project are photogrammetric images, photogrammetric-derived point clouds via Structure-from-Motion (*SfM*), DSMs and true orthophotos. All products are delivered in the national Romanian reference system (Krasovski ellipsoid 1940, Stereographic projection 1970, and Black Sea normal altitude system 1975).

3.3. Data Acquisition

The aerial flight was carried out in April 2022, using an oblique ULTRACAM Osprey 4.1 camera produced by Vexcel. The main technical specifications of the photogrammetric flight are displayed in Table 1.

Dataset parameters Table 1

CAMERA PARAMETERS	Digital camera	UltraCam Osprey 4.1
	Focal length of the nadir sensor	79.6 mm
	Image Format (long track and cross track)	52.700 mm 77.245 mm
	Pixel size	3.760 μm x 3.760 μm
	Coordinates of the principal point	0.000 mm 0.000 mm
FLIGHT SPECS	Spatial image resolution	15 - 16.5 cm
	Spectral resolution	4 bands (R,G,B,NIR)
	Frontal overlap	78% - 82%
	Side overlap	58% - 62%
	Average flight height	3,365 m
	Flight days	24.04.2022 26.04.2022
	Number of images	2892
	Number of flight lines	41
	Block surface	1,518 sq. km
	Flight direction	East-West
	Number of GCPs	58 points
	Number of CHKs	57 points

The flight was performed in good meteorological conditions and the dataset obtained (raw images, GNSS and IMU measurements) went through a rigorous photogrammetric workflow, consisting of:

1. *Image Processing:* includes enhancing the geometric and radiometric image quality;
2. *Tie points extraction:* represents extracting the corresponding points in two or more overlapping images. Tie point identification can be done by brute force, which involves comparing each descriptor of image A with each descriptor of image B. This approach is simple, but quite inefficient. Examples of alternative strategies are: FLANN [17, 23], which uses a quick search structure, and MatchMe [21], which uses similarity measurements between descriptors;
3. *Bundle adjustment:* the 3D coordinates of the points and the exterior orientation parameters are determined based on the internal orientation parameters, the coordinates and the rotation angles measured for each projection centre and the previously identified tie points, and the GCPs coordinates. Both tie point extraction and bundle adjustment can be done with specialized software, such as Inpho

Match-AT (Trimble) or UltraMap (Vexcel);

4. Dense image matching: To obtain a dense point cloud, a corresponding point is needed for all the pixels in the image. SGM (semi-global matching) is a computer vision algorithm for the estimation of a dense disparity map from a rectified stereo image pair [8]. The method is used nowadays, also in improved versions.

The quality assessment of the aerial triangulation was performed using the check points (*CHKs*) that were precisely determined in the field using static GNSS observations and geometric levelling measurements. GNSS data were acquired with a minimum observation time of two hours per point and a sampling interval of 10 seconds. A total of 11 simultaneous measurements were performed to ensure the establishment of baseline vectors between the determined points. The GNSS measurements were made using Spectra Precision SP80 (240-channel) and E-Survey E300 PRO (800-channel) receivers, which support signals from GPS, GLONASS, Galileo, and BeiDou with subsequent data processing carried out using Spectra Survey Office software to guarantee accurate adjustments and coordinate transformations. The normal height of the GCPs and CHKs were determined through precise geometric levelling, and referenced to the National Levelling Network. For this purpose, digital levels, FOCUS DL-15, with an accuracy of 1.5 mm per double kilometre were used. The planimetric Root Mean Square Error ($RMSE_{XY}$ - Equations (1) to (3))) obtained was 9.14 cm and the altimetric RMSE ($RMSE_{H}$ – Equation (4)) was 17.61 cm, based on the 57 CHK. The densified photogrammetric-derived point cloud is comprised of 128 million points, with a density of approximately 60 points/sq m (Figure 4).

Fig. 4. *Input datasets: photogrammetric point cloud and building footprints*

$$RMSE_X = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(X_i - X_i^{ref}\right)^2} \quad (1)$$

$$RMSE_Y = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(Y_i - Y_i^{ref}\right)^2} \quad (2)$$

$$RMSE_{XY} = \sqrt{RMSE_X^2 + RMSE_Y^2} \quad (3)$$

$$RMSE_H = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(H_i - H_i^{ref}\right)^2} \quad (4)$$

where:

X_i, Y_i, H_i represent the measured coordinates in stereomodels for the CHKs [m];

X_i^{ref}, Y_i^{ref}, H_i^{ref} – the field coordinates of the CHKs [m];

n – the number of CHKs.

The national topographic database TopRo5 (at a scale of 1:5.000) was used to extract the building footprints [10]. All geospatial features were digitized using the INSPIRE Directive based on the infrastructures for spatial information established and operated by the Member States of the European Union [11].

3.4. Proposed Workflow

The CityEngine software, developed by Esri, uses a 3D generation procedure based on a series of rule files, written in a programming language called Computer Generated Architecture (*CGA*). These rules underlie the extrusion of polygons and the generation of 3D building models (Figure 5). Textures for buildings can also be added to the rules file.

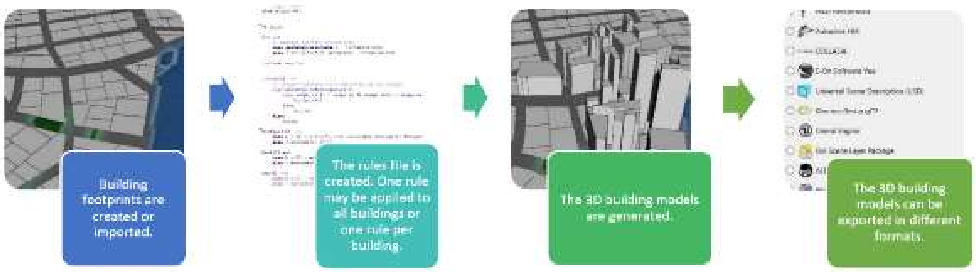


Fig. 4. *3D building models using CityEngine*

This approach requires information about the building footprints and the height of each individual building. These heights can be derived from data stored by public entities or from processed photogrammetric datasets and were introduced as an attribute.

In our approach, heights were extracted from the normalized Digital Surface Model (*nDSM*) and were assigned to the building footprints as attributes. To model the buildings in a 3D environment, the workflow in Figure 6 was conducted.

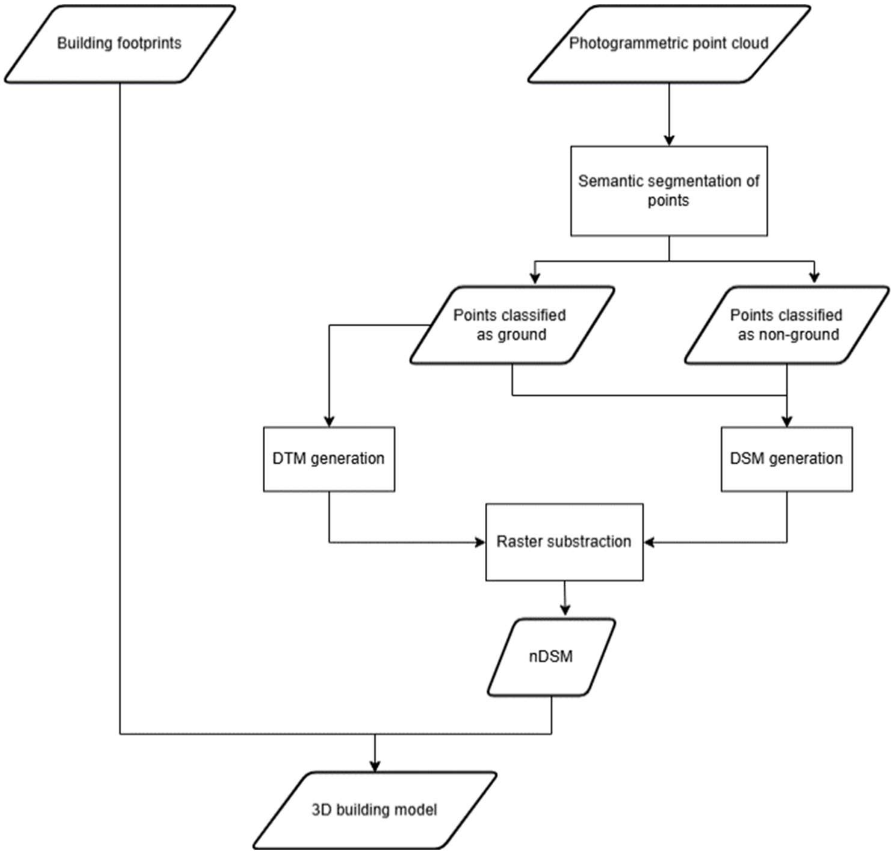


Fig. 6. *Proposed workflow for 3D building modelling*

The point cloud classification was performed in TerraScan using routines defined in a macro. The study area was analyzed and parameters used in the classification process, like building surface area, building heights, and maximum height for vegetation, were extracted from the data and included in the macro. Digital models were generated in Global Mapper using the *TIN* interpolation method, in raster format at a spatial resolution of 20 cm, as follows: digital terrain model (*DTM* - from ground points), digital surface model (*DSM* – from ground and non-ground points), and normalized digital surface model (*nDSM* – based on the arithmetic difference between *DSM* and *DTM* – Figure 7).

In order to assign the height values to the building footprints, geoprocessing tools from ArcGIS were used. The approach was based on the research performed by Bină [3]:

- Using the "Feature to Points" tool, the centroid points of the buildings were generated;
- Using “Extract Values to Points" tool, the pixel height value corresponding to the centroid point was extracted from the *nDSM*;
- By applying a spatial join between the centroid points and the footprints, the building height was transferred to the polygons.

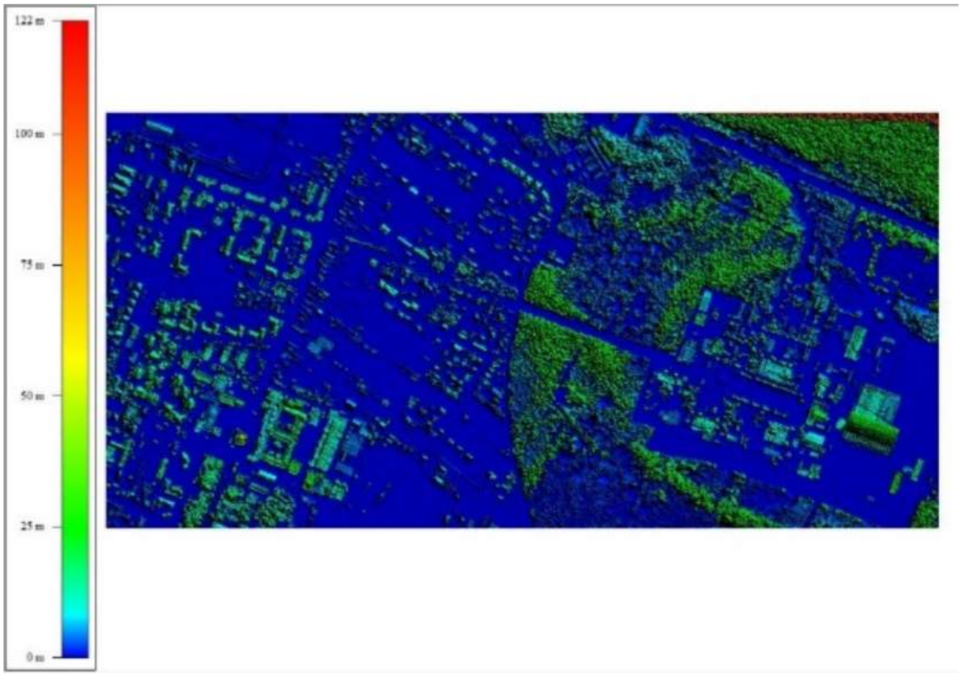


Fig. 7. *Normalized digital surface model*

The software used for 3D building models, CityEngine, uses a 3D generation procedure based on a series of CGA rule files. The principle is based on polygon extrusion, followed by generation of 3D building models.

For the quality assessment, the root mean square error of the building heights was computed (Equation (5)).

$$RMSE_H = \sqrt{\frac{\left(H_{ground_{truth}} - H_{3D_{model}}\right)^2}{n}} \qquad (5)$$

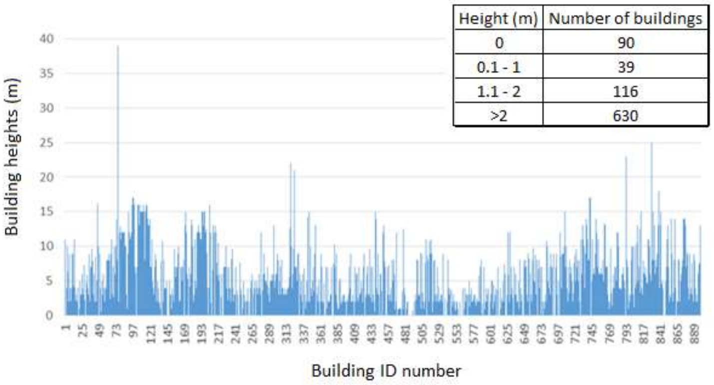
4. Results

The study area consists of 2 sq km in the town Măgurele, in Ilfov County (as shown previously). After performing the steps described above, the heights of the buildings were extracted from the *nDSM* (Figure 8).

By analysing the values for the buildings heights, it was concluded that 28% of the buildings have heights smaller than 2 meters (Figure 9). This situation can be due to: (1) the time difference between the collection of vector data (2012) and the time of the aerial flight (2022) during which some buildings were demolished, or (2) errors related to the process of digitization. So, our approach can also help in updating the national database, in order to identify and remove demolished buildings.

Figure 10 shows a demolished block of flats.

Fig. 8. *Building footprints highlighted by the height value*



Height (m)	Number of buildings
0	90
0.1 - 1	39
1.1 - 2	116
>2	630

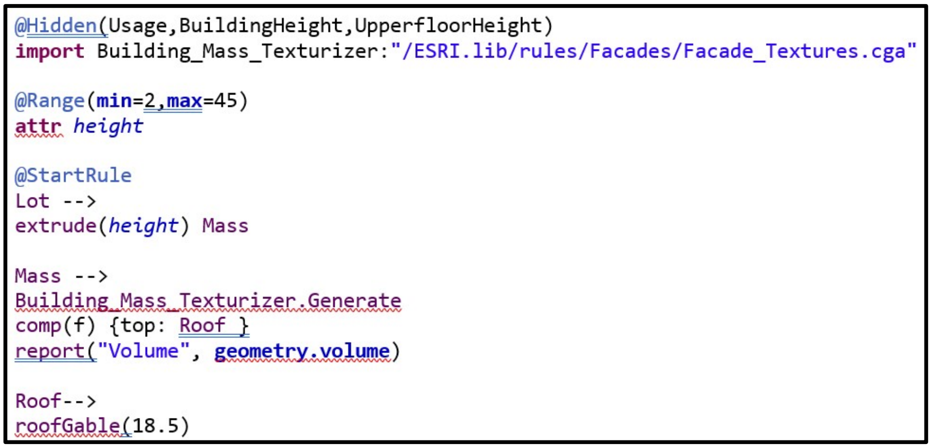
Fig. 9. *Statistical distribution of the building heights extracted from the nDSM*

a. b.

Fig. 10. *Identifying demolished buildings: a. orthophoto 2012; b. orthophoto 2022*

After performing an analysis of these situations, the building feature class was filtered and the updated database was composed of 716 features.

The software used for 3D building models, CityEngine, uses a 3D generation procedure based on a series of CGA rule files. The principle is based on polygons extrusion, followed by generation of 3D building models. The defined CGA rule (Figure 11) involves setting up some parameters, like the type of roof (gable roof), the inclination angle (18.5°), and the texture used for building facades.



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@Hidden(Usage,BuildingHeight,UpperfloorHeight)
import Building_Mass_Texturizer:"/ESRI.lib/rules/Facades/Facade_Textures.cga"

@Range(min=2,max=45)
attr height

@StartRule
Lot -->
extrude(height) Mass

Mass -->
Building_Mass_Texturizer.Generate
comp(f) {top: Roof }
report("Volume", geometry.volume)

Roof-->
roofGable(18.5)
```

Fig. 11. *CGA rule used to generate 3D building models*

Using the updated building footprints and the CGA rule defined previously, the 3D building models were generated (Figure 12).

To evaluate the quality of the performed modelling, 16 randomly distributed points were chosen. The points were located on the rooftops, in one of the corners of the buildings (Figure 13).

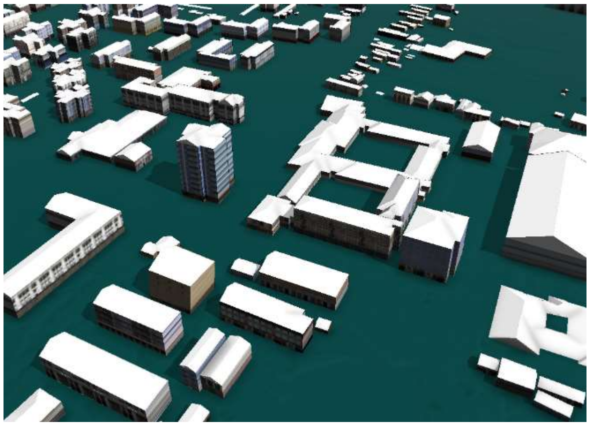


Fig. 12. *3D building models*

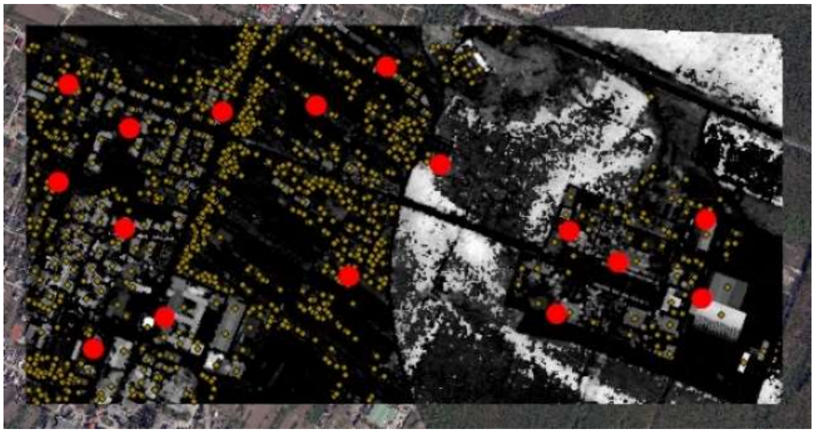
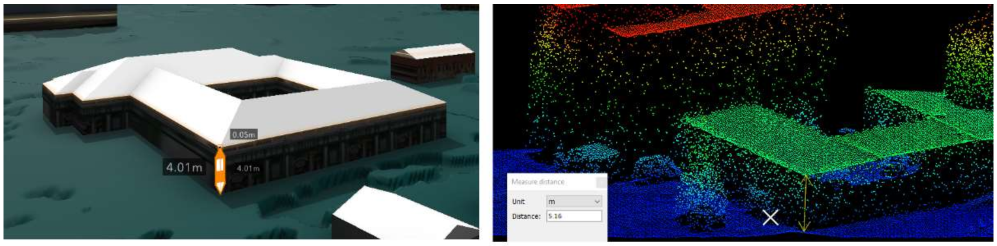


Fig. 13. *Points used to evaluate the quality of the 3D building model*

The building heights used as ground truth were extracted from a precise LiDAR point cloud. This dataset was collected in 2024 using a RIEGL VQ-1560i airborne LiDAR system, with a point density of 5 points/sq m. This flight provided high-resolution, highly accurate data that served as a reference for model validation. This precise ground truth was crucial for assessing the altimetric precision of the 3D building models, as it allowed for direct comparison between the height of the building in the models and the actual height of the buildings in the LiDAR point cloud (Figure 14). By using this ground truth, the study ensured the reliability of the results used for urban planning and analysis.



Measure distance
Unit: m
Distance: 5.16

Building height in 3D model

Building height in LiDAR point cloud

Fig. 14. *Example of a building height used in quality assessment*

By using the values extracted from the data and displayed in Figure 15, the root mean square error of 1.65 meters was computed.

It should be noted that all roofs were generated with the same angle of inclination (18.5°). Therefore, block-type roofs or those inclined in different directions can generate significant errors in the calculations. Some errors are due to the way in which the height of the buildings was extracted from the *nDSM*, namely using the building centroids, which in most cases, are situated on the ridge of the roofs, at the highest points.

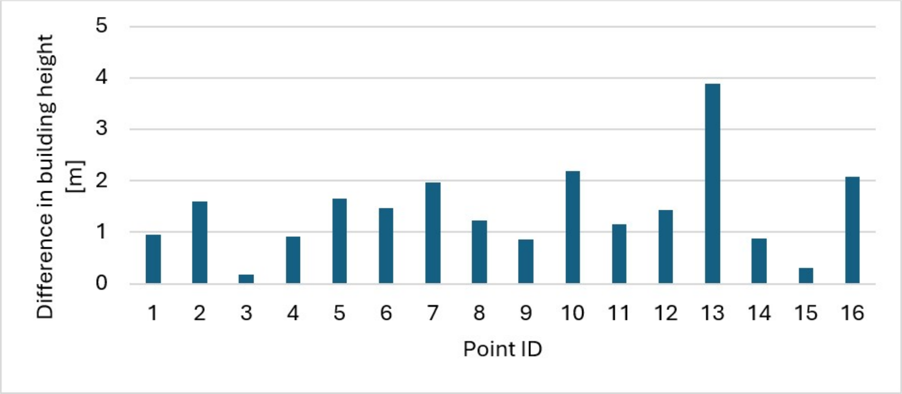


Fig. 15. *Differences in building height for RMSE evaluation*

To present the benefits of the 3D building models obtained, we propose a series of applications (Figure 16):

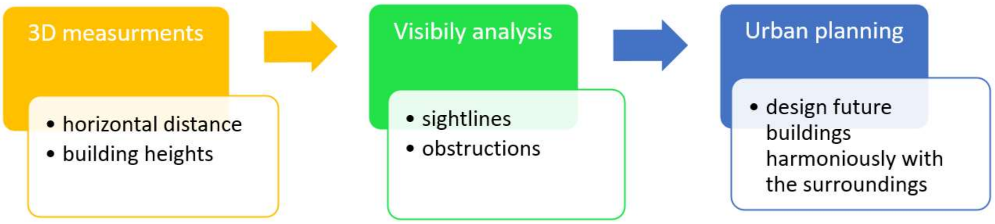


Fig. 16. *Applications of the 3D building models*

- *Precise Measurements of Buildings in a 3D environment* (Figure 17) - These models allow for detailed measurement of structures, including dimensions, angles, and volumes, which are critical for urban planning, historical preservation, and construction management [26].

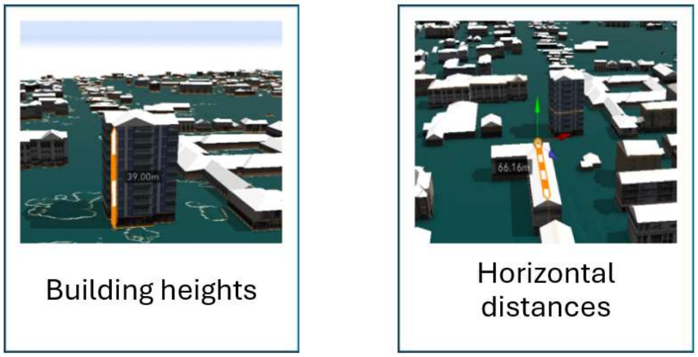
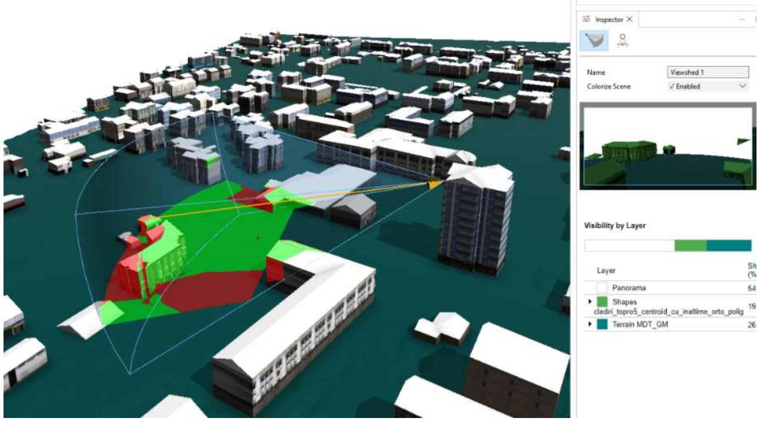


Fig. 17. *3D measurements*

- *Visibility analysis in different spot points* (Figure 18) – by determining sightlines and obstructions, urban planners can evaluate the visual impact of buildings, optimize placement for surveillance cameras, and ensure compliance with architectural guidelines. Visibility tools can also be used in emergency response planning, ensuring clear sightlines for navigation and evacuation. These analyses contribute to sustainable urban development by balancing aesthetics and functionality.

- *Planning future buildings* (Figure 19) - The integration of 3D data and simulations allows architects and urban planners to design future buildings in harmony with their surroundings. This includes factors such as natural lighting, airflow optimization, and minimizing the visual and ecological impact of new constructions. Research highlights the importance of considering the surrounding landscape to maintain cityscape coherence and reduce urban heat island effects.



Name: Viewshed 1
Colorize Scene: ✓ Enabled

Visibility by Layer

Layer	Sh (%
Panorama	54
🟩 Shapes cladiri_topro5_centroid_cu_inaltime_orto_polig	19
🟦 Terrain MDT_GM	26

Fig. 18. *Visibility analysis*

Fig. 19. *Planning a new building*

5. Conclusion

This study highlights the significant potential of 3D building models in advancing urban planning practices. The integration of high-resolution photogrammetric datasets provides a robust foundation for creating detailed, accurate 3D models of buildings, which are critical for informed decision-making in urban development. The workflow proposed can be applied at national level, by using the building footprints from the national database TopRo5 and the building heights from different datasets based on data availability in different areas. Through the application of advanced computational techniques, such as machine learning and artificial intelligence, urban planners can more effectively analyze and utilize these datasets to simulate various urban scenarios and optimize land use strategies.

Moving forward, it is essential to continue improving algorithms and technologies that can efficiently process large-scale photogrammetric data while reducing error margins. Based on the 3D models and meteorological data, decision-makers can generate different applications. In this sense, by combining a 3D building model with climate and weather data, a digital twin of a city can be developed and maintained in order to be prepared for many possible scenarios. The approach will be applied for the CSA Brasov in the CARMINE project, in order to assess the effects of drought.

While there are obstacles to overcome, the advancements in photogrammetric modelling offer substantial benefits for urban planning. As these techniques continue to evolve, they will likely play an important role in shaping the cities of the future, ensuring that urban growth is managed in an efficient, sustainable, and responsive manner.

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INVENTORY OF BAT SPECIES AND PROPOSED CONSERVATION MEASURES IN ROSCI0207 POSTĂVARUL AND ROSCI0195 PIATRA MARE

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George E. SÎRBU[1,2] **Flaviu VODĂ**[1,2]

Abstract: *This study presents an updated inventory of bat species and outlines specific conservation measures for the Natura 2000 sites ROSCI0207 Postăvarul and ROSCI0195 Piatra Mare. Conservation measures are key actions and mechanisms that need to be implemented for a Natura 2000 site to achieve its conservation objectives. It is mandatory to establish these necessary measures, whether they are applied within individual sites or, in some cases, even outside the site boundaries or across multiple sites. In the initial phase, using the inventory method and monitoring stations, species distribution and presence were assessed in the field based on the specific standard form for each site. From this perspective, the aim was also to estimate the population at the site level. Bat species distribution and presence were assessed using the acoustic and mist-netting methods. In addition to the species listed in the Natura 2000 Standard Data Form (SDF), several new species were recorded. These include Myotis bechsteinii, Myotis mystacinus, Myotis brandtii, Myotis nattereri, Pipistrellus pipistrellus, Nyctalus noctule, Eptesicus serotinus, Vespertilio murinus, and Plecotus auratus. The conservation status of the bat populations was evaluated based on population size, habitat quality, and future prospects. Management measures addressing current and future pressures are proposed, focusing on habitat maintenance and threat mitigation.*

Key words: *Natura 2000, conservative management, Chiroptera.*

1. Introduction

Conservation measures refer to specific actions and mechanisms implemented at the level of a Natura 2000 site, aimed at achieving its conservation objectives. These measures are essential tools for safeguarding the natural habitat listed in Annex I and the species listed in Annex II of the Habitats Directive that are present within sites, which must be tailored to their ecological requirements [7].

The implementation of these measures may occur exclusively within the

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boundaries of a site or, when ecologically justified, beyond its limits or across multiple sites simultaneously [7]. While conservation actions are typically developed at the local or site-specific level, they can also be formulated and coordinated at regional, national, transboundary, biogeographical, or European Union levels [6]. In certain cases, conservation efforts may extend beyond the Natura 2000 network itself, through horizontal measures targeting national ecological networks, landscape-level habitat connectivity or similar objectives [13].

The overarching aim of the management framework developed for the sites ROSCI0207 Postăvarul and ROSCI0195 Piatra Mare is to safeguard biodiversity through the implementation of evidence-based conservation measures. These include the designation, maintenance, and effective management of Sites of Community Importance (*SCIs*) and Special Areas of Conservation (*SACs*), ensuring the long-term viability of threatened habitats and species of European interest.

The sites Postăvarul (ROSCI0207) and Piatra Mare (ROSCI0195) serve as key ecological corridors and face pressures from urbanization and tourism. This study aims to assess the current bat populations and to propose management strategies based on site-specific ecological conditions.

2. Material and Methods

2.1. Study Area

2.1.1. Bat Species in the Study Area

Both sites are located in Brasov County, within the Alpine biogeographical region. Postăvarul (1,288.2 ha) and Piatra Mare (4,281.4 ha) support numerous bat species and serve as migration and dispersal corridors.

Bat species listed in the Natura 2000 SDF:

- *Barbastella barbastellus* Schreber (1308);
- *Myotis myotis* Borkhausen (1324);
- *Rhinolophus ferrumequinum* Schreber (1304; only in Postăvarul);
- *Rhinolophus hipposideros* Bechstein (1303).

2.1.2. ROSCI 0207 Postăvarul

The ROSCI0207 Postăvarul site, with an alpine area of 1.288 ha holds significant importance at regional, national, and European levels due to its role as a vital ecological corridor, forming part of the regional connection between the Bucegi Mountains and the Piatra Mare Massif. Maintaining the functionality of this corridor represents one of the main challenges for the entire European Carpathian chain. Through its substantial contribution to the efficiency and coherence of the Natura 2000 network, the ROSCI0207 Postăvarul site plays a crucial role in biodiversity conservation. The site encompasses segments of the two major migration and dispersal routes: the primary west-east route and the east-west route, which traverse the main ridges of the Bucegi Mountains and the Piatra Mare Massif [6].

In addition to its function as an ecological corridor, the site hosts habitats and species of conservation interest, further enhancing its ecological significance [6].

2.1.3. ROSCI 0195 Piatra Mare

The importance of the ROSCI0195 Piatra Mare site at regional, national, and European levels lies in its critical role as an ecological corridor, forming part of the regional corridor connecting the Bucegi Mountains to the Postăvarul Massif [13]. The ROSCI0195 Piatra Mare site, with an alpine area of 4.281 ha, plays a key role in maintaining ecological connectivity within the Carpathian region, addressing one of the primary challenges for biodiversity conservation across the European Carpathians. It contributes substantially to the efficiency and coherence of the Natura 2000 network by encompassing segments of two major migration and dispersal routes – west-east and east-west – that traverse the Bucegi Mountains and the Postăvarul Massif. Beyond its corridor function, the site also supports habitats and species of high conservation value [1, 3].

2.2. Data Collection

To achieve a comprehensive assessment, additional data was collected regarding the habitat, age, and composition of the forest vegetation, the presence of flowing or standing water, and potential shelters. In parallel, the assessment and mapping of pressures and threats to the species were also conducted [2, 4].

2.3. The acoustic method

The acoustic method [17] involved recording ultrasounds emitted by bats, which were subsequently analysed using specialized software (e.g., BatSound) to identify the species. This method can be applied in any type of habitat, including urban areas, and enables the collection of data on the distribution of bat species. A major advantage of this method is the ability to rapidly cover various types of habitats without causing disturbance to the bats. For recordings, high-performance ultrasound detectors were used, such as the D240X model [15], widely utilized in Europe for bat inventory and monitoring.

Bat activity was monitored by following predefined transects and stopping at stationary points, with the main objective of adequately covering the characteristic habitats of the Piatra Mare SCI. To ensure sufficient representativeness, samples were selected to thoroughly cover the SCI Piatra Mare and Postăvarul. Based on available maps, four routes were established, ranging from 2 to 8 km in length for Piatra Mare and 2 km for Postăvarul. During the transect surveys, GPS coordinates were recorded, allowing the collected data to be georeferenced (Figure 1).

The recordings – sonograms – were later analysed using specialized software (BatSound). The analysis of sonograms, based on features such as minimum frequency, maximum frequency, duration, and signal shape, allowed for the identification of recorded species and the determination of the type of activity bats were performing at the time of recording.

By examining the sonograms in terms of minimum frequency, maximum frequency, duration, and shape, we obtained information about the identity of the recorded species and the type of activity the bats were engaged in at the time of the recording [16].

To complement the acoustic method during the bat mating season, bats were captured using mist nets set up near underground shelters utilized by multiple species as swarming sites, particularly at cave entrances. This was conducted during mating season near known swarming sites, using protocols from Furmankiewicz and Górska-Kłęk [8]. Capture activities commenced immediately after sunset, under clear skies and calm weather conditions, and lasted for a minimum of four hours. The number and length of nets used depended on the size of the targeted cave entrances. The data recorded included species, forearm length, age, sex, and parasite presence. The bats were released within 30 minutes of capture. For each captured bat, specific data was recorded, including the time of capture, species, forearm length, age, sex, and presence of parasites. No mist netting was conducted near maternity colonies to avoid causing unnecessary stress [8].

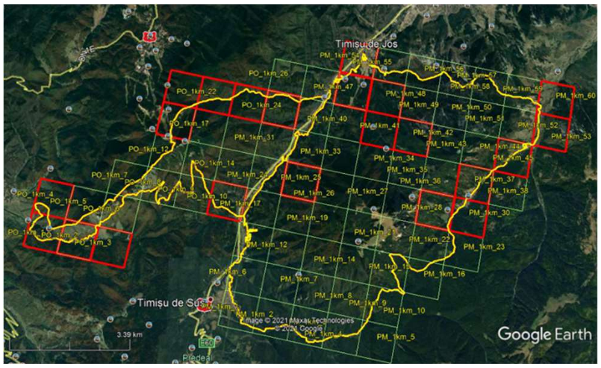

Fig. 1. *Distribution of bat sampling areas in ROSCI 0195 Piatra Mare and ROSCI 0207 Postăvarul*

3. Results and Discussions

The sonograms (Figure 2) from the recordings were subsequently analysed using specialized software (Pettersson Elektronik AB, 2004). By evaluating the sonograms based on minimum frequency, maximum frequency, duration, and shape, we were able to identify the species recorded and determine the type of activity the bats were performing at the time of the recording (Figure 3).

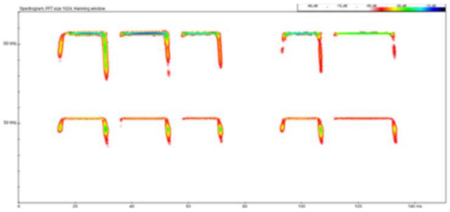

Fig. 2. *Sonogram map example for Rhinolophus hipposideros (Bechstein)*

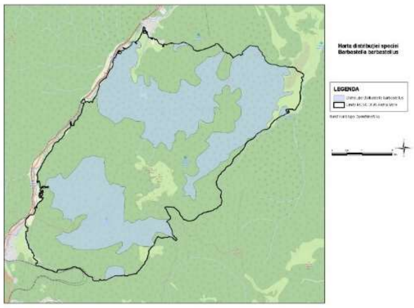

Fig. 3. *Distribution map example for Barbastella barbastellus (Schreber)*

In addition to aligning with the ecological requirements of target species and habitats, the proposed measures are formulated in accordance with the specific management objectives established for the category of protected natural area. They are consistent with national legislation, the guidelines of the International Union for

Conservation of Nature (IUCN) for the management of protected areas, and relevant European directives [9, 10].

The approach also considers national conservation priorities, the socio-economic and cultural context of local communities, and the specific ecological and geographical characteristics at regional and local levels [12]. The measures include preventive, corrective, and monitoring actions, applicable both within the site boundaries and, where ecologically justified, in adjacent or functionally connected areas. Emphasis is placed on forest habitat conservation, protection of natural and anthropogenic underground roosts, and on ensuring ecological connectivity – particularly via riparian corridors and other key movement pathways for bat species.

Several newly recorded bat species within the site, not previously listed in the Natura 2000 Standard Data Form, have been proposed for future inclusion in the form [11, 14]. Their presence has been duly considered in the development of conservation and management measures, which aim to ensure adequate protection and monitoring of both listed and non-listed species of conservation interest. Several additional species were recorded beyond the SDF-listed bats, including *Myotis bechsteinii* (Kuhl), *Pipistrellus pipistrellus* (Schreber), and *Nyctalus noctule* (Schreber). Detailed species presence and population estimates are presented in Tables 1 and 2.

Table 1

Standard Data Form bat species results in
ROSCI 0195 Piatra Mare and ROSCI 0207 Postăvarul

No.	Bat species (Standard Data Form)	ROSCI0207 Postăvarul	ROSCI0195 Piatra Mare
1.	1308 *Barbastella barbastellus* (Schreber)	Population type – permanent Population size (individuals): 25-50	Population type – permanent Population size (individuals): 50-80
2.	1324 *Myotis myotis* (Borkhausen)	Population type – permanent Population size (individuals): 50-100	Population type – permanent Population size (individuals): 50-100
3.	1303 *Rhinolophus hipposideros* (Bechstein)	Population type – permanent Population size (individuals): 25-50	Population type – permanent Population size (individuals): 50-100
4.	1304 *Rhinolophus ferrumequinum* (Schreber)	Population type – permanent Population size (individuals): 10-20	–

Table 2

Additional bat species identified in ROSCI 0195 Piatra Mare and ROSCI 0207 Postăvarul

No.	Additional bat species identified	ROSCI0207 Postăvarul	ROSCI0195 Piatra Mare
1.	1323 *Myotis bechsteinii* (Kuhl)	-	+
2.	1330 *Myotis mystacinus* (Kuhl)	+	+
3.	1320 *Myotis brandtii* (Eversmann)	+	+
4.	1322 *Myotis nattereri* (Kuhl)	+	+
5.	1309 *Pipistrellus pipistrellus* (Schreber)	+	+
6.	1312 *Nyctalus noctule* (Schreber)	+	+
7.	1327 *Eptesicus serotinus* (Schreber)	+	+
8.	1332 *Vespertilio murinus* (Linnaeus)	+	+
9.	1326 *Plecotus auratus* (Linnaeus)	+	+
10.	1313 *Eptesicus nilssonii* (Keyserling and Blasius)	-	+
11.	1321 *Myotis emarginatus* (E. Geoffroy Saint-Hilaire)	+	-
12.	1314 *Myotis daubentoni* (Kuhl)	+	-
13.	5003 *Myotis alcathoe* (O. von Helversen and K.-G. Heller)	+	-

Conservation measures and activity description Table 3

No.	Conservation measures	Activity description
1.	Periodic inspections of the natural/anthropogenic underground shelters within the site area will be conducted to inventory the colonies during different relevant periods of the year and to document any changes in the shelter conditions.	Clear-cutting in deciduous (for substitution purposes to natural fundamental type) and mixed forests must be halted, and ongoing management plans should be reviewed to eliminate any such planned interventions. It is also important to maintain or increase the percentage of mature forests, ensuring that the stands are managed under a forest regime. Riparian vegetation and meadow forests should remain undisturbed. Location of the measure: Deciduous and mixed forests within the site, particularly in valleys with streams that serve as travel routes and guidance for the species. Priority: High
2.	Monitoring of logging activities	
3.	Participation in the development of forestry plans aimed at increasing the proportion of mature deciduous forests (over 80 years old), which are the primary feeding habitats for the species.	
4.	Identification of additional cellars, mine galleries or caves as potential underground shelters. These may not have been discovered yet due to insufficient information, lack of timely exploration or various other reasons, and could be found in the future.	
5.	Regulation of speleological activities.	Combating acts of vandalism and the destruction of cave entrances.

The key pressures and threats to the species identified at the site level:

- B07 – Decrease in the percentage of mature forests in favour of younger age categories; this results in loss of shelters and changes in the spectrum of available food;
- B07 – Forestry activities other than those listed: intensification of forest exploitation; the intensification of exploitations affects the species in all possible ways: the number of suitable shelters and feeding habitats is reduced, habitats are fragmented;
- B02.04 – Removal of dry or drying trees;
- B02.02 – Deforestation;
- J03.02 – Reduction of habitat connectivity due to anthropogenic causes.

The management measures presented in Table 3 represent standard conservation practices widely supported by the scientific literature and by official guidance for the implementation of the Natura 2000 network [5, 7]. These measures are designed to maintain or restore the favourable conservation status of Annex I habitats and Annex II species, in accordance with the Habitats Directive (92/43/EEC).

4. Conclusions

In the socio-economic context of the site, management measures have been developed to address both the current and future pressures and threats faced by the bat species.

Conservation measures implemented within specific areas, and even outside the Natura 2000 network, contribute to the national and regional conservation status. Additionally, socio-economic factors must be considered within each site, as they can significantly influence or limit the conservation planning process.

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THE USE OF GEOGRAPHIC INFORMATION SYSTEMS IN THE PLANNING OF BICYCLE ROUTES IN THE ZĂNOAGA – BRAN AREA

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Abstract: *Everyday life is increasingly challenging and so the need to relax takes on new coordinates. Due to the fact that recent climate changes have made winters increasingly scarce in precipitation and snow periods shorter, there has been a growing concern to increase the offer of summer (relaxation) sports. In the Zănoaga area of Bran locality, an extensive sports complex is being developed. Appropriate IT facilities have been used for the development of bicycle routes, some of which are highlighted in the present work. Long trails are being developed, which are used by a certain segment of cyclists, as well as shorter trails with varying degrees of difficulty. These have (mainly) used the means provided by geographic information systems through VBA programming, which have been used to design trails of certain degrees of difficulty. A program has also been created using such sequences, which guides athletes to a particular route according to their experience, age, courage, and the type of bike they use.*

Key words: *sports complex, GIS, VBA.*

1. Introduction

The implementation of a project to create a bicycle path in the mountainous area, part of which is forested, is an investment that has both an economic impact, given the high costs of realization, and an environmental one. The positive impact is given by the fact that it stimulates ecotourism, leading to a healthy lifestyle, but also protects the environment by using non-polluting means. Based on the promotion of a healthy lifestyle and the fact that tourism overwhelmingly leads to a higher quality of life [6], most tourist resorts (and beyond) try to win their customers with a wide range of leisure offers [7]. It is clear that this offer is different both in terms of opportunities and quality of services [12], as Romania is recognized for its generous leisure options [11]. A great advantage of the tourist resorts in Romania is the different natural settings [23], each one beneficial and exploiting what nature offers in the most favourable way. All this, coupled with the

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fact that tourism in Romania has a considerable history [13], with ancient roots [10], demands high quality and standards from those who open new opportunities for tourists. This is also because it is not only the natural potential of the area that counts [16], but also a qualitative approach to the problem. Unfortunately, many sites only consider the quantitative aspect and lose sight of sustainability. On the other hand, as is well known, tourism not only has an economic dimension, but also social or even cultural aspects [19], providing an opportunity to promote certain local products or services [5], or even specific customs and traditions. Of course, time also influences the way of relating to tourism [15], each stage being influenced by the salient social aspects. Bran is par excellence a location that benefits from many assets and should make the most of them, with many places in the country and abroad focusing on such opportunities [4]. Moreover, this location should be a source of inspiration for other resorts [9]. Lately, there has been a return to the rural values of yesteryear, and there is a particular demand for rural tourism [22].

The forested mountain area under study, with its impressive natural landscapes and rare biodiversity [1], provides an ideal setting for the development of a bicycle trail that is accessible and attractive to outdoor sports enthusiasts. However, the creation of such a trail requires careful planning, responsible implementation and efficient natural resource management [17]. All this leads to the conclusion that the study area is an excellent location to develop a suitable sports centre [21]. Even though there is already some tradition in practicing winter sports in Bran, both the average segment of cyclists as experienced [2, 24] and those who are more experienced in these sports [3] were considered.

Tourism is one of the fastest growing economic sectors globally, and outdoor recreational activities such as mountain biking are increasingly popular. Creating a bike path in a forested mountainous area can attract tourists from all over the world who are looking for authentic experiences in the middle of nature [20]. There are many possibilities of automating topographic calculations [27, 28] to help in organizing the appropriate space for bicycle path design. Instead of depending on costly and environmentally damaging infrastructures, a well-designed bike trail can attract adventure sports and ecotourism enthusiasts, offering them an environmentally friendly and sustainable alternative to explore the mountain region [18].

A dedicated mountain bike route can help diversify the tourist offer in a given mountain region, attracting not only cycling enthusiasts, but also families, groups of friends or those looking to improve their health. Once built, the trail can become a year-round tourist attraction, having a positive impact on the local economy by increasing the number of visitors and supporting local businesses such as hostels, restaurants, local guides, and bike rental shops [8, 14].

In this paper, we explore the process of creating a bike path in a forested mountainous area, considering the benefits, challenges, and possible solutions for a successful project. Furthermore, we analyse how GIS can help in the implementation and development of such a project.

2. Materials and Methods

The present study has exhaustively considered the study of the terrain - its topography. For this purpose, the ComNav T300 GNSS equipment was used, which provides an accuracy of 8mm + 1 ppm RMS in the horizontal plane and 15 mm + 1 ppm RMS in the vertical plane, with which the coordinates of more than 3300 points were determined. Cadastral plans (including those equipped with forest boundaries) and area-specific orthophotos were used for various verifications. A Dell Latitude 5411 laptop for data processing, the software: ArcMap, AutoCAD Civil 3D, and Microsoft Office were also used. Last but not least, the coordinates of more than 3300 points determined with GNSS equipment were used.

The research methods were: the direct measurements method, with which the coordinates of the points were taken in the field; statistical methods with which the data taken with the GNSS equipment were filtered, and specific GIS methods: georeferencing of topographic plans, vectorization, and use of VBA programming sequences for various solutions.

3. Results and Discussion

After checking the coordinates of the points determined with the GNSS equipment stored in the *.rw5 file and eliminating those points for which the accuracies were not adequate, the 3D model of the studied area was created. The program AutoCAD Civil 3D was used to generate this TIN model on the basis of known elevations (Figure 1). Based on this model, contours were also created in the same program (Figure 2).

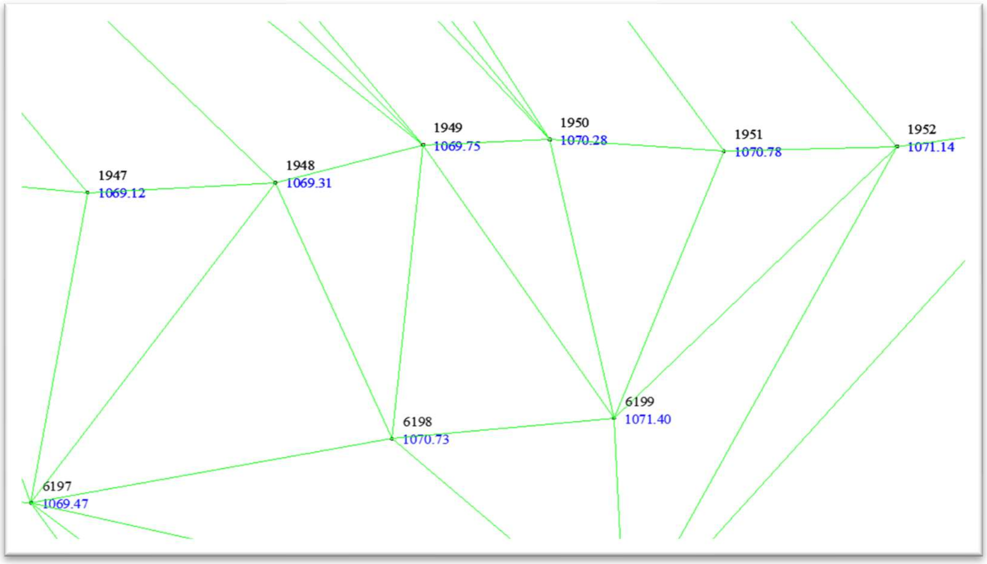


Fig. 1. *Making a 3D model in AutoCAD Civil 3D*

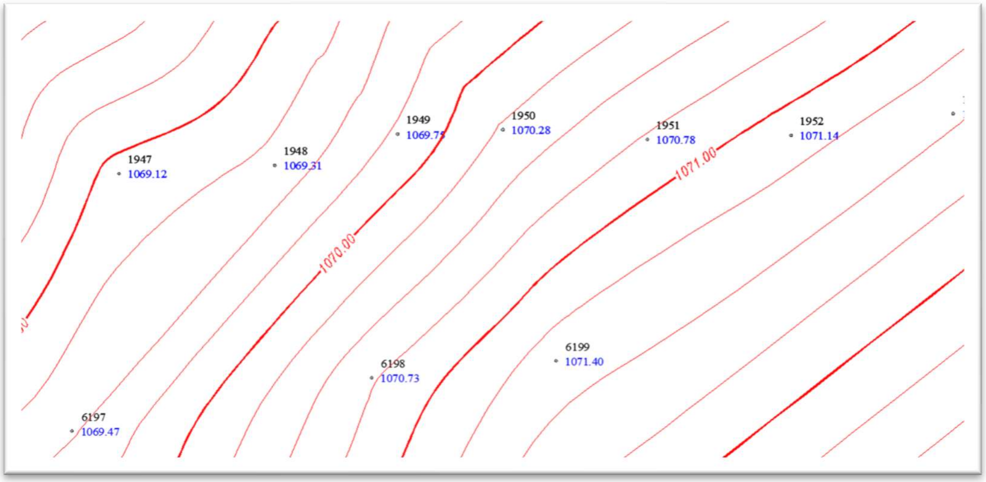


Fig. 2. *Drawing contour lines*

This step is mandatory for this project as the development of the various bicycle routes has to take into account certain orographic and other criteria. Also, the corresponding topographic plans were georeferenced and the configurations in the two situations were compared. Further on, the data was passed into the ArcGIS program and the studied area was modelled in 3D space. It went through the ArcCatalog and then through the ArcMap step to import the points determined on the ground and create the grid and 3D surface (Figure 3).

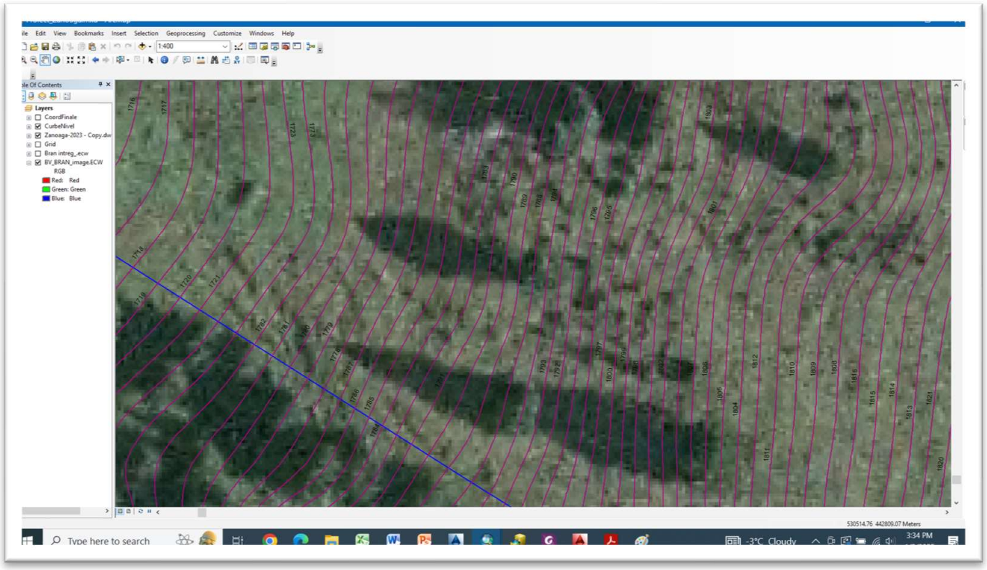
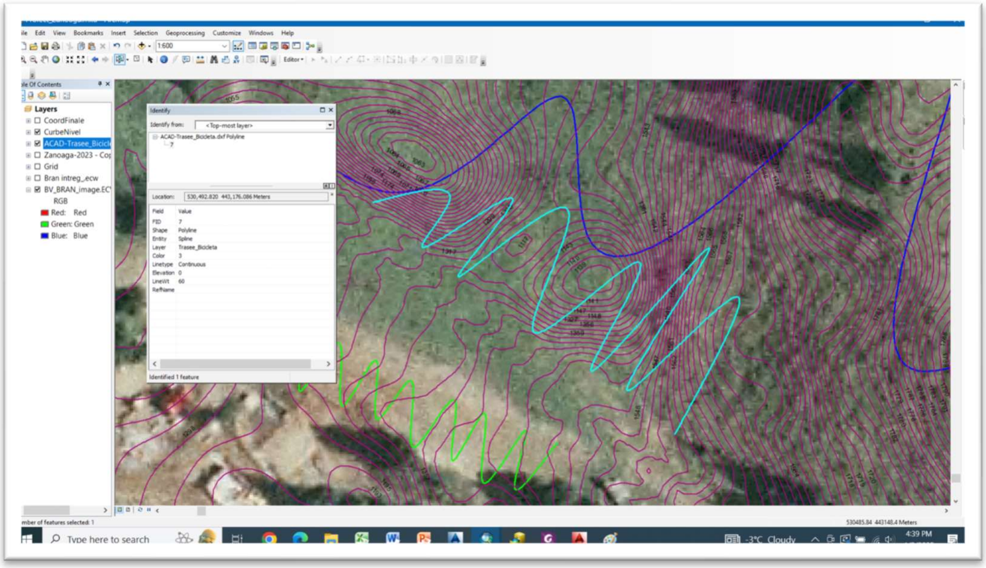


Fig. 3. *Visualizing the 3D model in ArcMap*

Next, data from the designer were used and the characteristic data of each route were extracted from the project: downstream end; upstream end; intermediate points (Figure 4).

These data were retrieved and fed into the GNSS equipment, the terrain was traversed and these points were staked out. It was found that all the projected routes required modifications, some minor, some major. "Workspaces" were mapped out where the route could be modified according to the projected maximum speed. For each designed route, determinations were made for the beginner route 1, to assess certain parameters (downstream elevation, upstream elevation, total length of the route, major slope break points, number of curves, main curves, etc.):

- Downstream point elevation - 945.25 m; this value was read directly from the 3D model;
- Elevation of the upstream point - 961.04 m; this value was read directly from the 3D model;
- Total length of the route - 395.8 m^2; this value was determined by a VBA sequence [25].



Identify from: <Top-most layer>

ACAD-Trasee_Bicicleta.dxf Polyline
7

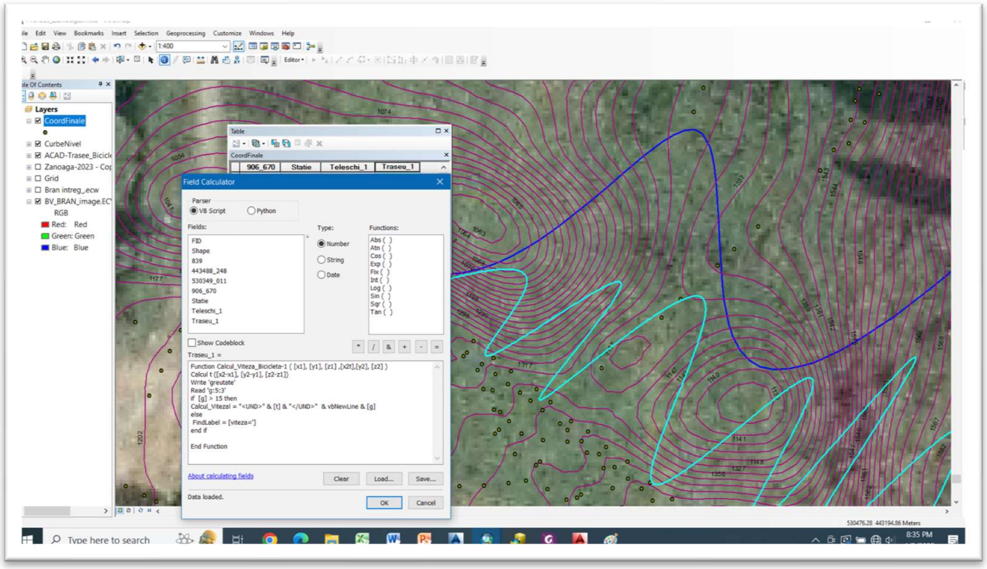
Location: 530,492.820 443,176.086 Meters

Field	Value
FID	7
Shape	Polyline
Entity	Spline
Layer	Trasee_Bicicleta
Color	3
Linetype	Continuous
Elevation	0
LineWt	60
RefName	

Identified 1 feature

Fig. 4. *Determining the main points of a route in ArcMap*

The operation was necessary since the route was determined by a spline curve. Within the VBA sequence, settings were made on the spline curve to determine the portions that could be associated with a line and those that could be associated with an arc, then the sequence was designed that summed the values of the lengths of all the line segments and all the arcs involved within that spline curve (Figure 5). Moreover, also by means of VBA sequences, the final path was determined, simulating the path in the area established in the field between certain points.



Traseu_1 =

```
Function Calcul_Viteza_Bicicleta-1 ( [x1], [y1], [z1] ,[x2t],[y2], [z2] )
Calcul t ([x2-x1], [y2-y1], [z2-z1])
Write 'greutate'
Read 'g:5:3'
if  [g] > 15 then
Calcul_Vitezal = "<UND>" & [t] & "</UND>"  & vbNewLine & [g]
else
 FindLabel = [viteza=']
end if

End Function
```

Data loaded.

Fig. 5. *VBA sequence for speed and route contour calculation for bike lane 1*

Similar, determinations were made on all other routes. Six cycle routes were proposed with varying degrees of difficulty and varying lengths from 395.8 to 1850.5 m and a difference in level from 15.79 to 140.5 m. The longest route has some sections that pass through forested areas, which is why steps have been taken to obtain approval from the Forestry Guard. It should be noted that there is no need to remove trees, as the route fits in perfectly with the spruce trees in the management unit in question. Finally, a program was also developed to help the participants to choose the most suitable route according to their experience [25]. A VBA sequence was created in the ArcMap program that took into account the following: the age of the participant, using appropriate coefficients with high values at young and old ages (over 70 years - including the health aspect of the individual), weight - this is very important as it is a factor that greatly influences the speed of travel: for this parameter a scale of 5 in 5 kg was created; experience and courage of the cyclist - here a scale of 20 steps was created. The program was installed on a tablet and can be accessed at the sports equipment rental centre. It involves going through a few steps where the participant is asked to enter the data specified above and the end is that the cyclist is recommended the most suitable track for them. There are situations in which a participant is suggested the possibility to ride more than one track.

4. Conclusions

Starting from the particular facilities offered by GIS in environmental problem solving [26], the present work continues these concerns and improves them. For the Zănoaga area of the touristic locality Bran, a sports complex for both winter and summer sports is in full development. This paper presented concrete ways in which geographic information systems can be successfully used in the design and development of mountain bike trails. Complex measurements of the study area were carried out and a 3D profile of the study area was produced. On this profile, the six bicycle paths were designed, which were subsequently adjusted by modifications in the main curves, and various GIS simulations were carried out with a certain degree of difficulty and a certain projected speed. VBA program sequences were created with which the final configuration of each track was determined. It was estimated that the use of GIS facilities resulted in a considerable reduction in the design time and, consequently, in the execution time of the trails by up to 35%. It is also considered that this investment, even if relatively low-cost, will be a major tourist attraction and a landmark of leisure enjoyment for a long time to come. In addition, the fact that this investment is particularly environmentally friendly, both in terms of the totally non-polluting cycling proposal and the fact that the routes developed do not have any impact on the forest, could recommend this type of investment in many other nearby localities.

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