BABEȘ-BOLYAI UNIVERSITY

FACULTY OF BIOLOGY AND GEOLOGY

DOCTORAL SCHOOL OF INTEGRATIVE BIOLOGY

DOCTORAL THESIS

Ecology and conservation of fishes in Transylvanian river systems

SUMMARY

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Keywords: fish distribution, conservation, freshwater ichthyofauna, Natura 2000, non-native species, Transylvania, isolation, Gobio, barriers, fragmentation

SUMMARY

The present work is a cumulative thesis that compiles the scientific outcomes resulting from my doctoral studies. The scientific outcomes of this PhD thesis were collected and prepared in seven years (2016-2023) of research at the Hungarian Department of Biology and Ecology from the Faculty of Biology and Geology, Babeș-Bolyai University of Cluj-Napoca, Romania, where Prof. Dr. Péter László PAP genuinely supervised my work.

This PhD thesis contributes to the conservation of the ichthyofauna of Transylvania's (Romania) rivers by updating with quality data the knowledge about fish and lamprey species occurrence and distribution, discussing phylogenetic questions in case of a species complex, assessing the impact of barriers on ichthyofauna and proposing management measures. This thesis is a cumulative work of three chapters addressing different approaches and topics but comprehensive in their contribution to the real challenges faced by nature conservationists.

The overall objectives of the thesis are:

1. Update outdated knowledge and survey on the occurrence and distribution of fish and lamprey species in Transylvania (Romania) with data collected between 2007-2022 and provide recommendations for designation of new protected areas.
2. Address and understand the phylogenetic features, taxonomy, and distribution of Central European stream-dwelling gudgeons (*Gobio* spp.) by increasing the spatial resolution of sampling in the data-deficient middle Danubian (particularly Transylvanian) area.
3. Evaluate and discuss the effect of barriers on the fish fauna from the main rivers of Transylvania.

CHAPTER I

DISTRIBUTION AND DIVERSITY OF FISHES AND LAMPREYS IN TRANSYLVANIA (ROMANIA): A COMPLETE SURVEY AND SUGGESTIONS FOR NEW PROTECTED AREAS

Chapter I published: Nagy A.A., Erős N., Imecs I., Bóné G., Fülöp A., Pap P.L. (2023) Distribution and diversity of fishes and lampreys in Transylvania (Romania): a complete survey and suggestions for new protected areas. ZooKeys 1166: 351–373. https://doi.org/10.3897/zookeys.1166.102854

Our descriptive study addressed the most emergent challenges of freshwater fish and lamprey species conservation in Romania by revealing the status quo of all species in Transylvania's rivers. Over the past two decades, specialists have often faced a lack of updated

data about species distribution (e.g. when designating Natura 2000 sites, in impact assessment studies, compiling red lists). Consequently, many conservation measures or initiatives have been implemented based on outdated distribution data of fishes and lampreys. While several valuable studies addressing the ichthyofauna of Transylvania have emerged since the last national study conducted over 50 years ago (Bănărescu 1964, 1969), these have mostly been limited to case studies of individual rivers or hydrographic basins, lacking a comprehensive synthesis. We aimed to bridge this gap by providing up-to-date information on the distribution and abundance of fish and lamprey species. Therefore, our data serve as a foundation for future studies aiming to explore the causes of changes in Transylvania's ichthyofauna. In a larger sense, due to its comprehensive coverage (43% of Romania's territory), it can serve for monitoring, ecological or conservation studies including the rest of the territories of Romania. While our data complements existing knowledge on the major rivers of Transylvania and their main tributaries, it should be noted that standing water habitats were underrepresented, and reservoirs and artificial aquatic habitats were excluded from our assessments (Figure 1).

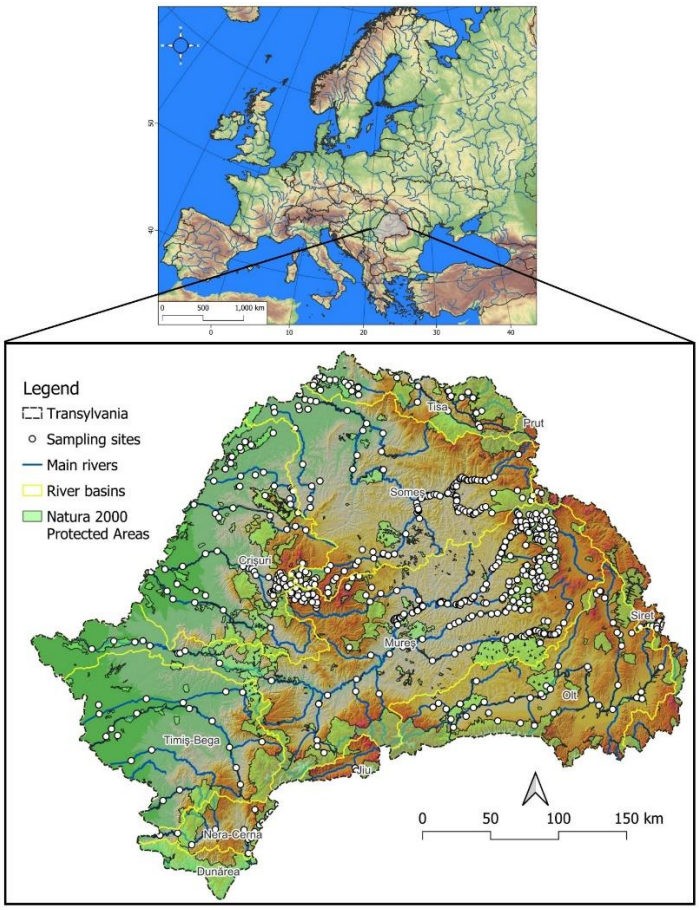

Figure 1. Position of Transylvania, Romania, in Europe, showcasing the main rivers, river basins, Natura 2000 sites (SCIs – Site of Community Interest), and the sampling sites surveyed in the study.

Compared with the historically recorded 77 species of fish and two lamprey species, our study identified 74 fish and three lamprey species (Table 1). The discovery of four new fish species (*Salvelinus alpinus*, *Neogobius melanostomus*, *Piaractus brachypomus*, *Pygocentrus nattereri*) and one lamprey species (*Eudontomyzon mariae*) in Transylvania, as well as new populations of several rare species (*Cobitis elongata*, *Sander volgensis*, *Umbra krameri*) highlights the need for further ichthyological research.

We also recorded apparently significant population declines in certain species (e.g. *Gymnocephalus schraetser, Umbra krameri, Carassius carassius, Tinca tinca, Lota lota*), while others have shown signs of recovery (*Leuciscus leuciscus, Zingel streber* – in Mureș and Someș rivers, *Zingel zingel* – in Someș river, *Hucho hucho* – in Mureș). One of the most remarkable returns we have noted is that of the *Leuciscus leuciscus*. This species was not observed for years in Transylvania's rivers, being recorded with certainty only in the basin of the Crișul Repede in the early 2000s. However, it has now been recorded in seven hydrographic basins (Tisa, Tur, Crasna, Barcău, Crișul Repede, Crișul Negru, Crișul Alb and Olt). It is worth noting that certain invasive species previously reported in Transylvania have expanded their distribution area in the past 50 years (e.g. *Lepomis gibbosus*). On the other hand, our assessment confirmed the presence of a few invasive species that had not been reported in this area 50 years ago but now populate large areas (e.g. *Pseudorasbora parva, Ameiurus melas*). Monitoring the distribution of recently recorded invasive species (e.g. *Perccottus glenii*) is essential in order to understand their effect on native fish populations. There is also a need for a similar systematic assessment of the ichthyofauna in standing waters and ponds.

Despite the negative impact of human activities on rivers in recent decades, such as river regulation, fragmentation, extensive loss of floodplains, and pollution (mainly from agriculture and human settlements), these water bodies still have a diverse fish community that deserves protection. Designation of new protected areas as part of the Natura 2000 network or other categories of protected areas can ensure the conservation of fish communities and therefore the management of protected areas remains essential for conserving protected species. However, urgent conservation measures are also needed to ensure the long-term survival of non-Natura 2000 fish species, particularly those that have suffered significant range reductions in the last decades (e.g. *Carassius carassius, Tinca tinca, Leucaspius delineatus, Lota lota, Thymallus thymallus*). Anthropogenic pressures on fish populations are increasing, making immediate conservation actions to safeguard Transylvania's diverse freshwater fish and lamprey populations.

Table 1. The complete checklist of freshwater fish and lamprey species of Transylvania (Romania). The taxonomy follows the FishBase online database (Froese and Pauly, 2023) with slight modifications.

No.	Scientific name	Recorded until 1969 (Bănărescu 1964, 1969)	New species recorded between 1964 and 2022	Present study	Origin	Natura 2000 protection	Observation
	Petromyzontidae						
1	*Eudontomyzon danfordi* Regan, 1911	x		X	native	yes	
2	*Eudontomyzon mariae* (Berg, 1931)			X	native	yes	
3	*Eudontomyzon vladykovi* Oliva & Zanandrea, 1959	x		X	native	yes	
	Acipenseridae						
4	*Acipenser gueldenstaedtii* Brandt & Ratzeburg, 1833	x			native	no	
5	*Acipenser ruthenus* Linnaeus, 1758	x		X	native	no	
	Anguillidae						
6	*Anguilla anguilla* (Linnaeus, 1758)	x			native	no	
	Cobitidae						
7	*Cobitis elongata* Heckel & Kner, 1858	x		X	native	yes	
8	*Cobitis elongatoides* Băcescu & Maier, 1969	x		x	native	yes	
9	*Misgurnus fossilis* (Linnaeus, 1758)	x		x	native	yes	
10	*Sabanejewia* sp. (incuding *S. balcanica* (Karaman, 1922) and *S. bulgarica* (Drensky, 1928))	x Bănărescu (1964) treated these two spp as ssp: *Sabanejewia aurata balcanica and S. a. bulgarica*.		x	native	yes	
11	*Sabanejewia romanica* (Băcescu, 1943)	x		x	native	no	
	Nemacheilidae						
12	*Barbatula barbatula* (Linnaeus, 1758)	x		x	native	no	
	Cyprinidae						
13	*Barbus barbus* (Linnaeus, 1758)	x		x	native	no	

14	*Barbus balcanicus* Kotlík, Tsigenopoulos, Ráb & Berrebi, 2002	x All species were treated together as *Barbus meridionalis petenyi* by Bănărescu (1964).	x (Kotlík et al., 2002)	x	native	yes	
15	*Barbus biharicus* Antal, László & Kotlík, 2016		x (Antal et al., 2016)	x	native	yes	
16	*Barbus carpathicus* Kotlík, Tsigenopoulos, Ráb & Berrebi, 2002		x (Kotlík et al., 2002)	x	native	yes	
17	*Barbus petenyi* Heckel, 1852			x	native	yes	
18	*Carassius carassius* (Linnaeus, 1758)	x		x	native	no	
19	*Carassius gibelio* (Bloch, 1782)	x		x	non-native	no	
20	*Cyprinus carpio* Linnaeus, 1758	x		x	native	no	
	Xenocyprididae						
21	*Ctenopharyngodon idella* (Valenciennes, 1844)		x (Bănărescu, 1981)	x	non-native	no	
22	*Hypophthalmichthys molitrix* (Valenciennes, 1844)		x (Bănărescu, 1981)	x	non-native	no	
23	*Hypophthalmichthys nobilis* (Richardson, 1845)		x (Bănărescu, 1981)	x	non-native	no	
	Tincidae						
24	*Tinca tinca* (Linnaeus, 1758)	x		x	native	no	
	Acheilognathidae						
25	*Rhodeus amarus* (Bloch, 1782)	x		x	native	yes	
	Gobionidae						
26	*Gobio gobio* sensu lato (Linnaeus, 1758)	x		x	native	no	The taxonomic position of stream dwelling gudgeons is still not clearly detailed (see Takács et al., 2021).

							Nowak et al., (2008) and Takács (2018) recommended the use of this taxonomic concept.
27	*Gobio obtusirostris* Valenciennes, 1842	The species was treated as a subspecies of *Gobio gobio* by Bănărescu (1964).	x (Takács et al.,2021)	x	native	no	
28	*Pseudorasbora parva* (Temminck & Schlegel, 1846)		x (Bănărescu, 1981)	x	non-native	no	
29	*Romanogobio kesslerii* (Dybowski, 1862)	x		x	native	yes	
30	*Romanogobio uranoscopus* (Agassiz, 1828)	x		x	native	yes	
31	*Romanogobio vladykovi* (Fang, 1943)	x		x	native	yes	
	Leuciscidae						
32	*Abramis brama* (Linnaeus, 1758)	x		x	native	no	
33	*Alburnoides bipunctatus* (Bloch, 1782)	x		x	native	no	
34	*Alburnus alburnus* (Linnaeus, 1758)	x		x	native	no	
35	*Ballerus ballerus* (Linnaeus, 1758)	x		x	native	no	
36	*Ballerus sapa* (Pallas, 1814)	x		x	native	no	
37	*Blicca bjoerkna* (Linnaeus, 1758)	x		x	native	no	
38	*Chondrostoma nasus* (Linnaeus, 1758)	x		x	native	no	
39	*Leucaspius delineatus* (Heckel, 1843)	x		x	native	no	
40	*Leuciscus aspius* (Linnaeus, 1758)	x		x	native	yes	
41	*Leuciscus idus* (Linnaeus, 1758)	x		x	native	no	
42	*Leuciscus leuciscus* (Linnaeus, 1758)	x		x	native	no	

43	*Pelecus cultratus* (Linnaeus, 1758)	x			native	yes	
44	*Phoxinus phoxinus* (Linnaeus, 1758)	x		x	native	no	
45	*Rutilus rutilus* (Linnaeus, 1758)	x		x	native	no	
46	*Rutilus virgo* (Heckel, 1852)	x			native	yes	
47	*Scardinius erythrophthalmus* (Linnaeus, 1758)	x		x	native	no	
48	*Squalius cephalus* (Linnaeus, 1758)	x		x	native	no	
49	*Telestes souffia* (Risso, 1827)	x		x	native	yes	
50	*Vimba vimba* (Linnaeus, 1758)	x		x	native	no	
	Serrasalmidae						
51	*Piaractus brachypomus* (Cuvier, 1818)			x	non-native	no	
52	*Pygocentrus nattereri* Kner, 1858			x	non-native	no	
	Siluridae						
53	*Silurus glanis* Linnaeus, 1758	x		x	native	no	
	Ictaluridae						
54	*Ameiurus melas* (Rafinesque, 1820)		x (Wilhelm, 1998)	x	non-native	no	
55	*Ameiurus nebulosus* (Leseur, 1819)	x		x	non-native	no	
	Esocidae						
56	*Esox lucius* Linnaeus, 1758	x		x	native	no	
	Umbridae						
57	*Umbra krameri* Walbaum, 1792		x (Bănărescu, 1981)	x	native	yes	
	Salmonidae						
58	*Coregonus albula* (Linnaeus, 1758)	x		x	non-native	no	
59	*Coregonus lavaretus* (Linnaeus, 1758)	x		*Coregonus. sp.*	non-native	no	
60	*Hucho hucho* (Linnaeus, 1758)	x		x	native	yes	
61	*Oncorhynchus mykiss* (Walbaum, 1792)	x		x	non-native	no	
62	*Salmo trutta* Linnaeus, 1758	x		x	native	no	
63	*Salvelinus alpinus* (Linnaeus, 1758)			x	non-native	no	
64	*Salvelinus fontinalis* (Mitchill, 1814)	x		x	non-native	no	
65	*Thymallus thymallus* (Linnaeus, 1758)	x		x	native	no	
	Lotidae						
66	*Lota lota* (Linnaeus, 1758)	x		x	native	no	

	Odontobutidae						
67	*Perccottus glenii* Dybowski, 1877		x (Covaciu-Marcov et al., 2011)	x	non-native	no	
	Gobiidae						
68	*Babka gymnotrachelus* (Kessler, 1857)		x (Cocan et al., 2016)	x	non-native	no	
69	*Neogobius fluviatilis* (Pallas, 1814)		x (Cocan et al., 2014)	x	non-native	no	
70	*Neogobius melanostomus* (Pallas, 1814)			x	non-native	no	
71	*Proterorhinus semilunaris* (Heckel, 1837)	x		x	non-native	no	
	Poeciliidae						
72	*Gambusia affinis* (Baird & Girard, 1853)	x			non-native	no	
73	*Poecilia reticulata* Peters, 1859		x (Bănărescu et al., 1997)		non-native	no	
	Centrarhidae						
74	*Lepomis gibbosus* (Linnaeus, 1758)	x		x	non-native	no	
	Percidae						
75	*Gymnocephalus baloni* Holčic & Hensel, 1974		x (Bănărescu, 1981)	x	native	yes	
76	*Gymnocephalus cernua* (Linnaeus, 1758)	x		x	native	no	
77	*Gymnocephalus schraetser* (Linnaeus, 1758)	x		x	native	yes	
78	*Perca fluviatilis* Linnaeus, 1758	x		x	native	no	
79	*Sander lucioperca* (Linnaeus, 1758)	x		x	native	no	
80	*Sander volgensis* (Gmelin, 1789)		x (Telcean and Bănărescu, 2002)	x	native	no	
81	*Zingel streber* (Siebold, 1863)	x		x	native	yes	
82	*Zingel zingel* (Linnaeus, 1766)	x		x	native	yes	
	Cottidae						
83	*Cottus gobio* Linnaeus, 1758	x		x	native	yes	
84	*Cottus poecilopus* Heckel, 1837	x		x	native	no	

Note: *Petroleuciscus borysthenicus* (Kessler, 1859) was reported from Mureș River basin by Nalbant (1995) but later the author admitted that it was a misidentification (pers. comm. Vasile Oțel, 27 February 2023).

Raw data of sampling sites, fish and lamprey species and their numbers
(Downloadable Excel File: https://zookeys.pensoft.net/article/102854/download/suppl/31/)
András Attila Nagy, Nándor Erős, István Imecs, Gábor Bóné, Attila Fülöp, Péter László Pap

Maps S1–S77 Distribution maps of fish and lamprey species in Transylvania, Romania.
(Downloadable PDF file: https://zookeys.pensoft.net/article/102854/download/suppl/32/**)**
András Attila Nagy, Nándor Erős, István Imecs, Gábor Bóné, Attila Fülöp, Péter László Pap

CHAPTER II

INCREASED SPATIAL RESOLUTION OF SAMPLING IN THE CARPATHIAN BASIN HELPS TO UNDERSTAND THE PHYLOGENY OF CENTRAL EUROPEAN STREAM-DWELLING GUDGEONS.

Chapter II published in: Takács P., Ferincz Á., Imecs I., Kovács B., **Nagy A.A.**, Ihász K., Vitál Z., Csoma E. (2021). Increased spatial resolution of sampling in the Carpathian basin helps to understand the phylogeny of central European stream-dwelling gudgeons. BMC Zool 6:3. https://doi.org/10.1186/s40850-021-00069-7

In the last decades, the populations of *Gobio gobio* in Europe have undergone taxonomic revision, resulting in describing several distinct new species (Figure 2). It has been suggested that distribution of the *Gobio obtusirostris* and *Gobio carpathicus* overlaps with territories from Transylvania (Kottelat and Freyhof 2007), although phylogenetic studies from this region missed. As a result, the reliability of the data regarding the distribution of these two *Gobio* species in our study area was doubtful. For this reason, it was essential to provide clarity and verified data regarding the distribution of these species in the rivers of Transylvania (completing the data and knowledge of the Carpathian basin beyond Romania), because Transylvania has served as Extra-Mediterranean refugia for many species, making it important to include this region in genetic studies. Additionally, the results of the phylogenetic studies on gudgeons collected in the Southwest region of the Carpathian basin may answer questions of whether separated, phylogenetically distinct *Gobio* species can be found in this area; or a „quasi genetic continuum" exists, formed by genetically less-distinct clusters living in the larger subdrainages of the Carpathian basin. Therefore, the aims of our study were: 1) to provide phylogenetic information about the characteristic fish species of a hitherto data-deficient area, 2) to clarify the phylogenetic relations of *Gobio* stocks inhabiting the inner area of the Carpathian basin. We also reviewed and compared the recently accepted distributions and the phylogeneticaly-verified *Gobio* distribution data in the Carpathian basin and its surrounding catchments.

A phylogenetic investigation using partial mtCR sequence data was conducted on 56 stream-dwelling freshwater fish *Gobio* spp.: individuals collected from 10 rivers from the data-deficient Transylvanian rivers, as well as one out this area, from the Argeș River (Southern Romania); and a review of the available data about Middle-Danubian stream-dwelling gudgeon lineages to delineate their distribution in the area. The results show that 7 of the 9 detected haplotypes are newly described, suggesting that the studied area hosts distinct and diverse *Gobio* stocks. Two valid species (*G. obtusirostris*, *G. gobio*), and a haplogroup with a doubtful phylogenetic position, "G. sp. 1" are detected in the area, showing a specific spatial distribution pattern. We refuted the assumption that a genetic continuum could characterize the Carpathian stream-dwelling gudgeons in light of the new results. In fact, in both the middle and lower part of the Tisa drainage system, a slightly separated but phylogenetically distinct and largely distributed, undescribed gudgeon species can be found (*Gobio* sp.1). Moreover, this group was detected in the surrounding watercourses (Olt and Timiș rivers) that have a current hydrologic connection to the Tisa drainage or had one in the recent past. Furthermore, our results demonstrate that the *Gobio gobio* cannot be excluded from the territory of Romania because it has been recorded in the basin of the Argeș River. Despite the relatively limited geographic range of the study, our results provide important information about the phylogenetic, taxonomic and distribution features of Central European gudgeons. In order to clarify the taxonomic position of the previously unknown groups, additional investigations are required and addressing the genetic status beyond the Carpathian populations (Southern and Eastern Romania) is needed to clarify the taxonomic position of the *Gobio* populations from this region.

Our findings emphasize the importance of surveys in the Carpathian aquatic system as they provide valuable additional genetic information. Therefore, we conclude that the increased spatial resolution of sampling in this area may also help to clarify the phylogenetic relationships of other fish taxa.

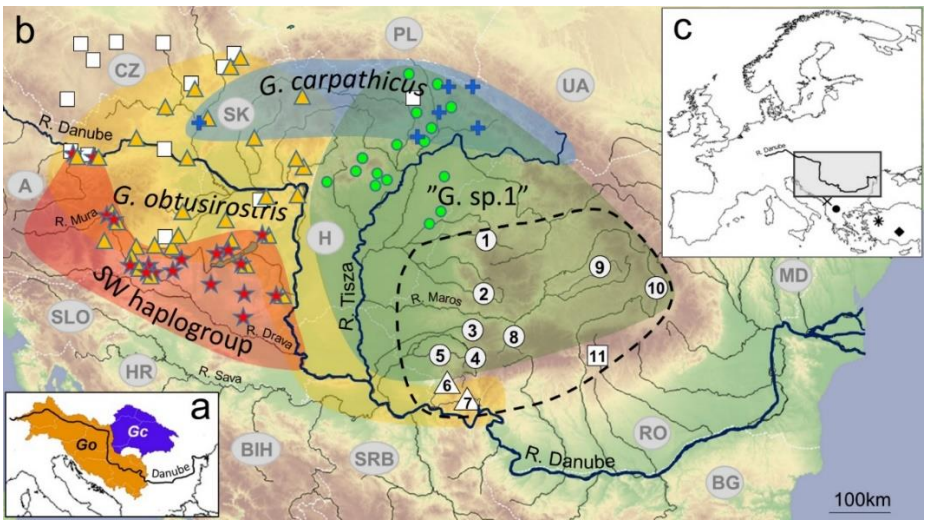

Figure 2 The distribution of the recently accepted Middle Danubian/Carpathian *Gobio* species (a) and the estimated distribution of *Gobio* lineages in the Carpathian basin and its surrounding areas derived from mtCR phylogenetic data (b). Location of the studied area in Europe is indicated on subfigure c. In this subfigure the collection sites of other close relative *Gobio* species also used for the phylogenetic analyses are indicated: *G. skadarensis*: ×, *G. ohridanus*: ●, G. sp.2: ✳, *G. insuyanus*: ◆.

CHAPTER III

EFFECT OF BARRIERS ON FRESHWATER FISH AND LAMPREY SPECIES RICHNESS AND DIVERSITY OF THE TRANSYLVANIAN (ROMANIA) RIVERS

Chapter III is a manuscript: Nagy A.A., Fülöp A., Erős N., Imecs I., Bóné G., Pap P.L. Effect of barriers on freshwater fish and lamprey species richness and diversity of the Transylvanian (Romania) rivers.

Recent studies show that the actual degree of river fragmentation in Europe is considerably higher than what is currently documented in existing databases (e.g. AMBER barrier atlas) and scientific records (Belletti et al., 2020). At a higher resolution, it is essential to address the issue of river fragmentation to plan conservation interventions and management strategies effectively. The objectives of this study were to evaluate fragmentation by surveying barriers (based on satellite images) along major Transylvanian rivers and their main tributaries (3835,6 km of river flow) and assess their effects on the number and diversity of fish species and propose conservation measures accordingly.

A total of 143 barriers (points of fragmentation) were identified (Figure 3), with an average density of 0.04 barriers per kilometer. Our results show that Transylvanian main rivers are less affected by fragmentation than other European rivers, and there are still few rivers and river sectors with few or no barriers in the studied sectors (Tisa, Vișeu, Hârtibaciu, Nera). Downstream barriers

were found to impact the number of fish and lamprey species but not their diversity, particularly reducing the number of native species while the number of non-native fishes remains unaffected. Although the diversity of fish and lamprey communities apparently was not affected by fragmentation, other measures of community functioning may reveal different responses of fishes to artificial barriers.

Additionally, we investigated the impact of Natura 2000 sites on the number and diversity of fishes. According to our resultswas no significant difference in species richness and species diversity between the Natura 2000 and non-Natura 2000 river sectors, leading to the following conclusions: i) many important sectors (i.e. with remarkable fish diversity) remained unprotected outside the Natura 2000 sites, which requires the designation of new protected areas; ii) the invested time and effort in the last decades into the management of the existing Natura 2000 areas and especially the conservation measures established explicitly for the conservation of the targeted species under the Habitats Directive have proven ineffective in practice; iii) the designation of new protected areas without the implementation of result-based conservation measures will not resolve the challenges posed by the increasing trend of river fragmentation in Romania. In order to use the available funds efficiently, it is desirable to prioritise barrier removal and connectivity restoration, especially by removal of barriers instead of constructing fish ladders, which typically provide connectivity for only a subset of species. In the cases when removing the barrier is not possible, the construction of fish passes can be a solution to reduce the negative impact of the fragmentation.

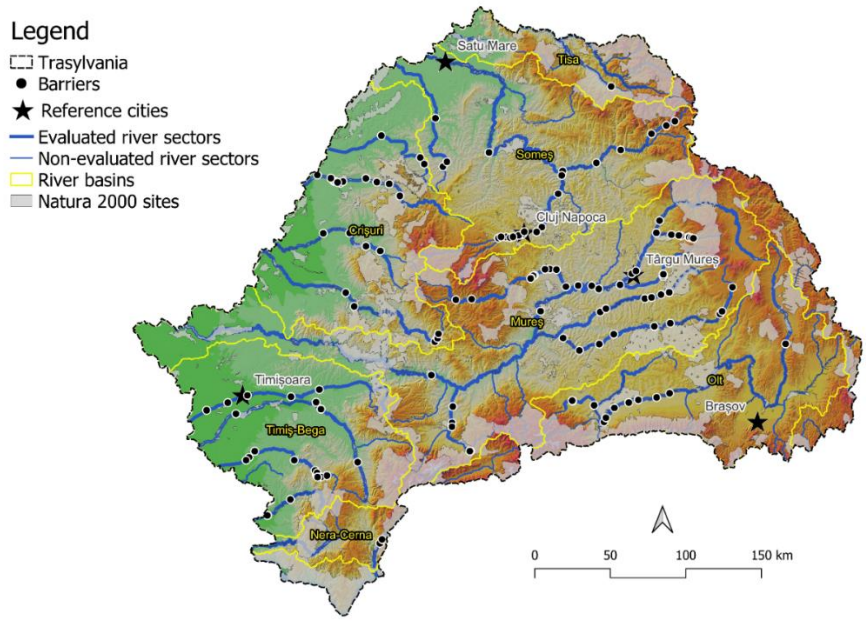

Figure 3. The map of Transylvania (Romania) showcasing the barriers surveyed in the study, the main rivers, river basins and Natura 2000 sites (SCIs – Site of Community Interest). River sectors evaluated for barriers are marked in bold.

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