

## Report on the work carried out in the framework of the OTKA FK 140902 grant application

The Carpathian basin can be characterized by considerably diverse, but partly unexplored aquatic communities. The taxonomic position of some commonly distributed fish species (e.g. pike, gudgeons) has become questionable in the last period. Therefore the primary aim of our proposal to reveal the distribution and genetic patterns of these characteristic Carpathian fish taxa. Parallely, we wanted to test, intercalibrate and validate the usability of some well reproducible, accurate, fast, and relatively cheap „alternative” methodologies (scale and body geometric morphometry, and MALDI-TOF mass spectrometry) in the research fields of taxonomy, phylogeny and evolutionary ecology.

One of the main thrusts of our studies was to investigate the applicability of morphology and morphometrics as an additional method. By analysing literature data, we pointed out the main problem of morphometric studies, that authors tend to use the same few variables without specifying the most appropriate variables to be measured. Analyses of our own three datasets showed that the number of variables and the variable set compositions in most cases strongly influenced stock subdivision and the percentage of correctly classified individuals. It was also shown that the most useable variable sets for morphometric purposes can differ considerably depending on the taxon and goal of the survey. With the proposed methodological modifications, the efficiency of morphometric tests can be greatly increased. ([Bánó & Takács 2022](#))

During the project we provided additional distribution and phylogenetic data of the Carpathian gudgeons (genus: *Gobio*). We did not confirm our initial assumption that an unknown lineage would be present in the southeastern area of the region's water system. However, it has been proven that the *Gobio* sp1 cryptic species is the dominant taxon in the entire Tisza river system. ([Takács et al. 2021a](#))

We have demonstrated that in the case of the Carpathian cryptic *Gobio* lineages, the morphological differences are more likely attributed to varying environmental conditions rather than genetic differences. So it seems that morphometry is less suitable for detecting such small-scale phylogenetic differences. ([Takács et al. 2022a](#))

Proteomic analyses were also performed on the individuals collected for the morphological and phylogenetic studies. On one hand, we analysed whether this method could serve as an alternative to phylogenetic investigations, and on the other hand, we aimed to clarify methodological questions (e.g., how freezing preservation and the use of an environmentally friendly anesthetic (clove oil) affect the results). According to the study results, the method may be suitable for differentiating species, and even certain sub-species categories. At the methodological level, it was proven that refrigeration preservation significantly alters the protein profile, while the anesthetic method itself does not affect the results. ([Maász et al. 2020](#))

Since the sensitivity of the applied MALDI-TOF mass spectrometry has been proven to be capable of identifying even sub-species categories, the possibility has arisen that it could be suitable for the species and sex differentiation of hard-to-identify groups (e.g., sturgeon species) in aquaculture.

To test the usefulness of the method for the isolation of sturgeons, a preliminary experiment was designed to analyse the mucus peptid profiles of males and females of two sturgeon species

prepared for hormone induced reproduction before and after treatment. The results show that not only species and sexes can be distinguished by MALDI-TOF analysis. But also the effects of hormone treatment cause detectable changes in the mucus peptide profile. Based on these preliminary results of the above mentioned study, we submitted a HUNRENTech proposal (TECH24-41) in 2024. In this proposal we planned to build up a peptid profile database of mucus, caviar and meat of as many sturgeon species as available in Hungary. Our proposal was deemed worthy of support by the Hungarian Research Network. ([https://hun-ren.hu/szervezeti\\_hirek/10-projekt-reszesul-tamogatasban-a-hun-ren-kozpont-altal-meghirdetett-hunrentech-program-kereteben-106980](https://hun-ren.hu/szervezeti_hirek/10-projekt-reszesul-tamogatasban-a-hun-ren-kozpont-altal-meghirdetett-hunrentech-program-kereteben-106980)) But because of the grant, this work is still in manuscript, has not been published yet.

In addition to species of conservation importance, we conducted essential studies on two species of ecological and economic significance, the pike and the pikeperch, in the Carpathian Basin. According to the results of the population genetic study conducted in the Lake Balaton's pikeperch stocks, the lake hosts a genetically unique and diverse pikeperch population. The population's genetic characteristics were not significantly affected by the mass die-offs or increased fishing exploitation that occurred in the second half of the last century. The results of microsatellite analyses suggest that there is a considerable east-west gene flow in the lake, which can be explained by the trophic gradient observed along the lake's axis. Our findings are crucial for the future management planning of the pikeperch population in Lake Balaton ([Bánó et al. 2024](#)).

Northern pike populations of the Carpathian Basin are characterized by significant phenotypic variability. This was long attributed to the high level of habitat diversity. But as two new pike species have recently been described from European freshwaters, the possibility has arisen that the diversity of local populations may be better explained by genetic factors. According to the results of the phylogenetic studies, the area is primarily inhabited by the northern pike (*Esox lucius*), with no evidence of the presence of other pike species or unknown clades. However, all three known lineages of *E. lucius* were found in the study area. The most diverse group was the southern/Danubian clade, which led us to hypothesize that this group is native to the study area. The Atlantic clade was found sporadically, only in the western part of the Carpathian Basin, suggesting recent introductions. Pike individuals from the circumpolar clade were found in large numbers in nearly every region of the basin, although this group showed minimal allele variation, suggesting that they may have been introduced to the area or escaped from fish pond of the neighbouring countries and reached the inner area of the basin through the Centripetal river network of the basin.. The observed differences in the coloration and patterns cannot be explained by phylogenetic differences. It is more likely that the original striped pattern breaks down during the ontogeny, with the increasing body length, the typical spotted pattern of adult northern pike emerges. ([Takács et al. 2022b](#))

During the OTKA project, we also tested the usability of scale morphology and morphometry for taxonomic and ecological purposes. Scales have long been used in biology, (e.g. for back calculation of growth), but no comprehensive work has yet been done to analyse the taxonomic and ecological applications of scale size and shape. The results of our studies analysing thousands of scale images from a total of 192 freshwater fish species show that scale shape can be used to separate higher taxonomic categories (Ordo, family) and in some cases can even be used for species identification. The relative size of the scales can also be used to

distinguish closely related species. But it can also provide important information about the environmental requirements of a species. In freshwater fishes, we first showed that reophilic and pelagic species tend to have smaller scales than metaphytic ones. This pattern can be used to distinguish closely related species and to specify the environmental requirements of species with hitherto unknown habitat requirements ([Bánó et al. 2024a](#)). The database of scale photographs created, which can be further expanded in the later years, has been made available to the public ([Bánó et al. 2024b](#))

Knowledge of morphometric and morphological information not only facilitates the taxonomic classification of a species, but can also greatly contribute to understanding the role/function of a species in a given community. In the framework of this project, a morphofunctional database has been developed, taking into account recommendations from the literature, which currently contains data on about 60 fish species can be found in Hungarian natural waters. The database analyses show, we sought to answer the question of what makes invasive species, which are becoming increasingly common in natural waters in Hungary, successful. The results of our analyses show that invasive species (e.g. Ponto-Caspic gobies) are functionally unique, significantly different from all native species, which gives them an advantage in exploiting the available resources. ([Takács et al. 2021b](#)) In the future, we would like to expand the database to include data on other species that could potentially occur in the Carpathian Basin waters in the near future. By analysing the data, we can show the extent of functional similarity of these species (to define the potential competition), and thus predict the future competitive impact of species that may only appear in the future, or those thermophilic ones that are already here but may only spread in the future due to climate warming.

For several taxonomically unclear/questionable groups, such as the minnows (*Phoxinus* sp.), the stream dwelling Barbels (*Barbus* sp.) and the Danubian whitefin gudgeons (*Romanogobio* sp.), and perch (*Perca fluviatilis*) studies are being carried out with the involvement of foreign (Czech, Austrian, Serbian, Romanian, Slovakian, Swedish) colleagues, and partly analysed in foreign molecular labs. Unfortunately, these studies, especially the sample collections, have been significantly delayed by the Covid pandemic. The results of the phylogenetic and population genetics studies are already fully or partially available, with a number of very promising results. For example, in the case of minnows and whitefin gudgeons, it is not excluded that new species may be described from the area. These works will be published in the following period.

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