CRYPTIC: CRYPtogams' Traits In the Carpathians

Final report

"Multifold meanings of „Cryptic"

There are several meanings for the adjective „cryptic". One meaning is mysterious, or puzzling, something we cannot quite grasp. Another meaning refers to a limited timeframe, like abrupt; terse; short, but we can also find here the expression „cipher". A cipher is an algorithm in cryptography, for performing encryption or decryption – a series of well-defined steps that can be followed as a procedure. The number of suggested synonyms for cryptic – enigmatic, mysterious, hard to understand, confusing, mystifying, perplexing, puzzling, obscure, abstruse, arcane, oracular, Delphic, ambiguous, elliptical, oblique — are also remarkable and clearly refer to the complexity of the meaning of „cryptic", the word that was chosen as a key word and core slogan of our proposal. In our context, I prefer the meaning „hard to understand". The name of the group of plants „Crytogams" also comes from the word cryptic. Algae, mosses and lichens belong here." These sentences come from our proposal written in 2016 that got support from NKFIH. In the last six years we worked on broke some "codes" written by Nature.

The main aim of the Cryptic project "CRYPtogams' Traits In the Carpathians" is to carry out a trait-based study of cryptogams in parallel with accurate species-level identification. The work focuses primarily on diatoms then Scots pine, but lichen and moss traits were also incorporated from the mountain lakes of the Carpathians. We collected sediment surface samples from the deepest points of altogether 53 lakes of the Carpathians, where cores were obtained for palaeoecological reconstruction. During the processing of 40 samples, 410 entities were distinguished, some of them could be new taxa. A database has been built in order for quantitative pH and total phosphorus reconstruction (buczko.eu). On another direction of our work is focusing the long-term changes of mountain and lowland lakes as demonstrated by diatom assemblages.

Retezat Mts. Lake Bucura and Lake Gales: First study of diatom traits was used for reconstruction of Holocene environmental changes in two deep lakes in Retezat Mts; in Lake Bucura and Lake Gales. Traits helped to understand the impact of erosion on aquatic assemblages (Hubay et al. 2018).

Balaton - Siófok: The first high-resolution diatom record was presented from the Lake Balaton, covering the last 7500 years (Buczkó et al., 2018). Lake level changes are reconstructed on the basis of changes in the ratio of different life forms of diatoms as diatom traits. Fragilarioid taxa were dominant through the core in the shallow Lake Balaton, but an episode of higher ratio of planktonic forms (*Lidavia balatonis*, *Aulacoseira granulata* and *Pantocsekiella ocellata*) referred to a lake level higher but fluctuating more than today between before ca. 2000 cal yr BP. The diatom-based reconstruction was in accordance with the geochemical and zoological records, proving the usability of siliceous remains in the paleolimnological reconstruction even a large and shallow lake as Lake Balaton, where the wind combined with shallowness disturb the preservation of biotic remains in the sediment.

Lake Ighiel In the case of Lake Ighiel, three cores have been studied and analyzed. Two of these cores are short cores (SC3 and SC4), covering ca one hundred year, while IGH1 covers 6200 years. Results of SC4 were published, that covers ca. 88 years. Clear and drastic changes were observed in the record, especially in the last two decades, when the planktonic diatoms practically replaced the benthic forms. Based on the associated diatom life forms and habitat preferring taxa (trait-based approach), we were able to

reconstruct the successive shifts of environment and find the climatic drivers of these changes. We concluded that the recent shifts in diatom assemblages may reflect the forcing of atmospheric nitrogen (N) deposition, as a key driver of recent diatom community turnover in mountain lakes. This project sheds light on the complex link between the drivers of catchment-scale impacts on one hand, and lake proxy responses on the other, highlighting the importance of an integrated historical and palaeolimnological approach to better assess lake system changes (Haliuc et al. 2020).

Lake Bâlea: Lake Bâlea is the largest glacial lake of Fagaras Mts, situated at 2034 m a.s.l. Altogether a short 32 cm long core was studied, where the upper 21 cm of the sediment covers approximately 200 years. In Lake Balea we also concluded that the recent shifts in diatom assemblages may reflect forcing from atmospheric nitrogen (N) deposition. This observation in our diatom record surprisingly similar to the species reorganisations found in North American alpine lakes. The nitrophilous diatom, *Asterionella formosa* became dominant after 1980, replacing centric diatoms and other fragilaroids within the planktonic diatom community. Even though other anthropogenic disturbance connected to tourism and recreation brought in new environmental stressors since 1992 e.g. more hotels were built on the lakeside with parking lots added and the number of tourists multiplied, it did not effect the planktonic diatom assemblages. However, these new stressors induced changes in other biotic assemblages and based at the rate of change (RoC) curves, we can conclude that both the aquatic and terrestrial environment experienced continuous high rate of change in the last nearly 30 years, which is unprecedented in the last 500 years (Szabó et al., 2020).

Lake Latorice: The high-resolution diatom analysis on a subalpine lake of the Pâreng Mountains, Lake Latorice was performed. The counting procedure, statistical analyses were completed, and the main results are under publication in a multi-proxy study. The thousand-year diatom-based history of Lake Latorica was found clearly climate-driven, where our data can contribute significantly to better understanding the Little Ice Age (submitted to Holocene, Szabó et al., after Rev. 1.).

Diatom traits in wetlands: The application of trait-based diatom analysis in studying the effects of environmental changes in wetlands was a major step forward in the project. The main goal of studying the side arms and oxbows of Szigetköz region was to detect changes in the epiphytic communities at structural (core species, changes in the relative abundance of common species) and functional (trait changes) levels (Ács et al. 2020).

Soda lakes: As another direction of the diatom study in the Cryptic project a trait-based diatom index was proposed and tested in the soda lakes. In a large data set covering the soda lakes of the Carpathian basin, a species- and a trait-based diatom ecological status index were developed. (Stenger-Kovács et al. 2018, 2019).

Our another study area in the Carpathian Mountains was the Ciomadul Mountains, where we focused on the paleoenvironmental study of the last glacial maximum. As a result of almost two decades long work, two comprehensive book chapters were published on the 26,200-years long history of **Lake St. Anne**, with diatom-based quantitative pH reconstruction, and trait-based analysis which yielded unexpected results. The crater lake has been strong acidic lakewater (low pH) during the Holocene – and still today – was previously more alkaline during the Last Glacial Maximum and began to turn to acidic status about 16,400 years ago. The book chapter describes the potential causes like permafrost melting, change in the degree of dust fall, change in the pH of precipitation water, general warming, vegetation change around the lake,

but the onset of volcanic after activity in the crater cannot be ruled out either (Buczkó et al., 2022, 2023, Magyari et al., 2022, 2023).

Close to completing the project we had possibility to write four review articles in 2022. Two of them focused on **the ecosystem services of diatoms and chrysophytes** (B-Béres et al., 2022, Lengyel et al., 2022.). The paleolimnological significance of Chrysophyte cysts and the role of the indicator in trait-based and taxon-level determination were summarized. It has been pointed out that the most important supporting services are related to primary production, nutrient- and biogeochemical cycling, as well as sediment formation (Lengyel et al., 2022). The **two other reviews were about the salinization**, one of the most important drivers that have had serious ecological consequences on the biota. Trait-based analyses of diatoms highlight the competitive advantages of motile guild and the extreme trait categories in these environments. Salinization induces the spread and invasion of marine and brackish species into inland freshwaters as well as freshwater species tolerating elevated conductivity and/or need specific ions. Diatom-inferred salinity reconstructions are widely applied to assess the past changes of salinity.

One of the consequences of salinization was detected in the rapid expansion of a small-celled (<15 µm) monoraphid **aquatic invasive species** (AIS) along the Central European lotic systems with the support of Cryptic project. Weighted average regression was employed to determine the *Achnanthidium delmontii's* ' optima and tolerances for 18 environmental variables. Invasion is an increasing problem in the diatom assemblages of mountain lakes (Buczkó et al., 2023).

Beyond trait-based analysis we also worked on **alfa-taxonomy**, the high-resolution taxonomical discovery. We have nice progress in the taxonomy of the genus *Gomphonema* in the Carpathians. Our long-term aim is to build a reliable database for quantitative reconstructions. In doing so, rare taxa are constantly being unearthed, they need special attention. As a step for it, we detected and documented the occurrence of *Gomphonema lacunicola* from the subalpine lake of the Pâreng Mountains from Lake Câlcescu as the first Romanian data on the species (Buczkó et al., 2022). We reported and documented the first Romanian occurrence of the diatom *Gomphonema angustivalva* E. Reichardt 1997 from a lake sediment core of Lake Balea, a proglacial lake of the Carpathian Mountains. The first Hungarian record of a rare *Gomphonema* (Bacillariophyta) species from temporary ponds (*Gomphonema jadwigae*) was also published (Ács et al. 2019). Valuable contributions were made to diatom biogeography of *Dorofeyukea rostellata* by Trábert et al. 2019.

Pine traits: Natural Pinus sylvestris populations of the Carpathians are considered as remnants of the past, descendants of the once existed large populations. Outcompeted by broadleaf species and spruce during the climate warming of the Holocene Scots pine could survive only in extreme ecological conditions like rocky sites or ombotrophic peat bogs. Due to the harsh local conditions in the peat bogs populations exhibit special aspect, trees are usually tiny because of the lack of nutrients. We studied microsatellite markers in stress responsive genes developed for *Pinus cembra* and their transferability to *Pinus sylvestris*. We successfully tested the marker usability in Scots pine (Köbölkuti et al 2018, 2020). We measured morpho-phenological traits of trees from different geological and ecological sites. Generative morphological traits (seeds) have revealed differentiation among populations in extreme habitat sites (Köbölkuti & Höhn 2018). Functional trait analysis including tree ring characteristics of Scots pine revealed that range-edge populations adjust their functional traits to the special local ecological conditions, leading to increased intraspecific variability in their morpho-anatomical structure (Palla et al., 2021). Peat bog populations adapted their wood anatomical traits to the generally hydric, cool and anaerobic conditions

of the peat bogs, Wood anatomical variability among tree rings and the corresponding short-term climate response of populations differed from the adaptive responses of the trees to the ecological characteristics of the habitat. In addition to the different phylogeographic origin evidenced in former studies phenotypic differentiation by the habitat type of the studied populations linked to the variance in morpho-anatomical traits have contributed to the survival of the peripheral Scots pine populations at the species' range margins (Palla et al., 2021a, b).

The most problematic part of the project is about the **lichens and mosses**. The drastic changes in the Hungarian Natural History Museum provided less possibilities for experts for focusing to these plants living around the mountain lakes. Fortunately, the sampled material is rich and large so hopefully these groups can significantly contribute to the of project.

Moss traits: As a first approach water chemical and physical features of five mires (Nyírjes lake at Sirok, Kis-Mohos and Nagy-Mohos at Kelemér, Bábtava and Nyíres lake in the Bereg region) were analysed in North Hungary. This work provided information for preparing our sampling campaign in the Cryptic project in order to compare the water chemical and physical properties of Transylvanian and Hungarian mires. The morphological variation of Sphagnum recurvum group was also investigated. We used traditional and new morphological traits also during the analysis. We also tried to change the prediction database. We demonstrated that LDA can be useful to determine the samples, but 10-12 % of samples are still unidentifiable. More than 250 moss samples were collected between 2017 and 2019, but they are only partly identified. We must admit, we have less progress regarding the mosses and use of moss traits.

Lichens: check list) of the lichen flora of Călimani Mts was completed: Having studied ca 300 specimens and 23 publications, 170 taxa of lichen-forming and lichenicolous fungi are reported from various sources, 140 from published literature records and old herbarium specimens, and 30 species from recently collected material (Lőkös et al. 2018). New floristic data were published from Romania (Papp et al. 2020). Altogether 620 lichen specimens were collected in the frame of CRYPTIC project and already 90 specimens are deposited in the Lichen Collection with accession number.

Outputs summary of Cryptic project

During the project we had to change our dissemination strategy concerning scientific publications, and modified the list of target journals, from the planned Q2-Q4 periodicals planned in the Proposal (Phytotaxa, Nova Hedwigia, Diatom Research, Fottea, Journal of Paleolimnology, Journal of Bryology) to better ranking Q1-D1 journals (Hydrobiologia, Science of Total Environment, Ecological Indicators, Ecology and Evolution, Scientific Reports and PLoS One).

The other concept concerning publication strategy was to publish our data base in local journals, especially in Studia Botanica Hungarica. This periodical is published by the Hungarian Natural History Museum aiming to publish "articles primarily discussing taxonomy, nomenclature, floristics and biogeography, and focussing on the Carpathian Basin and Balkan Peninsula, and the surrounding areas…". We believe that this journal can function as a perfect data repository to publish our data base documenting cryptogams.

In the frame of this OTKA project 33 research papers were published during the 6 years of the project and 4 manuscripts have been submitted that are currently under review. The number of publications in Q1 ranked journals is 14 and the total impact factor generated so far is 57.3.

Moreover 24 abstracts, 4 book chapters, 5 conference papers and 3 dissertations are also among the outputs of the project. There are 4 items without NKFIH number mentioned in the Acknowledgement, namely four book chapters written about the Lake Saint Anne. It was an editorial decision, that here no project numbers were allowed to mention in the volume, neither in the English, nor in the Hungarian versions.

As a part of dissemination, popular science contributions were written about the field trips, paleolimnology in climate research and global trends in freshwaters. The results about the history of Lake Ighiel seemed to capture the interest of the wider public, and upon the publication of our key findings and generated more the 40 other publications (second publishing appearance) in the Hungarian on-line platforms. We believe that the dissemination of our results was successfully carried out at all levels, also for the wider public, not just in scientific circles.

Conferences: Project members participated at least 18 international and 8 local conferences thanks to the financial support of the CRYPTIC project. Three MSc dissertations were partly supported by the project. An international workshop was organized in our topic, where diatomists joined in the efforts to reach our goals: https://riverine.hu/CenTrain500.

The very last scientific action of CRYPTIC project was to fulfill an invitation to Banska Bistrica to lecture about the global trends of siliceous organisms.

Overall, we think that despite covid pandemy and the personnel changes at the Botanical Department of the Hungarian Natural History Museum the CRYPTIC project was very successful. In the future we believe we will continue to enjoy CRYPTIC's aftereffects in scientific collaboration in the region. Thanks to the financial support of our CRYPTIC project, we had the chance that by studying the Carpathian's cryptogams we broke some "codes" written by Nature in traits and now we understand better the world around us.

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Winners or losers of the climate change?
Diatom (and siliceous algae) case studies from Cretaceous to Anthropocene

Ústav vied o Zemi, SAV, Ďumbierska 1, Banská Bystrica, štvrtok, 14:00

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