DIATOM-BASED PALEOENVIRONMENTAL RECONSTRUCTION IN LAKE BALATON, HUNGARY (TÓ34F)

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Abstract: A high-resolution diatom assemblage's database was built on a 65 cm long sediment core (Tó34f), obtained from the Siófok Basin of Lake Balaton in 2017. Light microscope photo-documentation was developed out of 15,797 diatom valves. The pictures were arranged according to taxonomy from centric diatoms via araphids, monoraphids to biraphid forms. 30 categories were established to group the diatom taxa instead of species level identification requiring high level of expertise. Some (1) characteristic, and easily identifiable taxa (Karayevia clevei, Cavinula scutelloides) were distinguished on species level, (2) taxa were grouped at genus level (like Amphora, Aulacoseira, Neidium), while (3) were joined based on their morphological features. Two large groups were created based on this approach, the "light fragilarioids" and the "heavy fragilarioids". Both groups contain diatoms with taxonomic problems, e.g. Staurosira, Staurosirella genera and other fragilarioids. This approach allowed us to make paleo-ecological reconstruction on (1) rough taxonomic data and (2) apply trait-based methods. Diatom assemblage's zones were defined by CONISS clustering method, validated by the broken stick model. Though the planktonic trait (referring the high lake level) is insignificant (< 5%) in most of the studied record, but some peaks (>35%) point to high water level. The most recent trend of increasing number of planktonic diatoms is hypothesized to be due to the recent global warming. We demonstrated that traits are a good alternative to time and expertise demanding species-level determination of diatoms, thus a quick method is found for paleo-ecological reconstructions, while the photo-base good for detailed taxonomic work at a future time.

Key words: Cryptic project, diatoms, Hungary, Lake Balaton, traits

INTRODUCTION

Due to the increasing human impact freshwater ecosystems have faced unprecedented and maybe irreversible changes (BATTARBEE et al. 2014, BENNION et al. 2015, CATALAN et al. 2013, MOSER et al. 2019, REAVIE 2020, RÜHLAND et al. 2015, SMOL 2019, WANG et al. 2012). Paleorecords from lacustrine sediments have great importance to evaluate the degree of environmental degradation from a longer perspective (KUEFNER et al. 2020). Recent global warming is affecting lake ecosystems by a series of direct and indirect effects, altering the dynamics of these sensitive habitats. According to our diatom-based preliminary results (BUCZKÓ et al. 2021), there were significant and synchronous rearrangements in lakes in the Carpathian region at the beginning of the industrial revolution (ca 1850) and around 1950, related to the use of nitrogen fertilizers. Since the early 2000s, rising temperatures have probably been one of the main drivers (SZABÓ et al. 2020). However, the high-resolution diatom analysis is extremely time-consuming and requires well-trained diatom experts exceeding the level of expertise of technicians and undergraduate students, which often impedes lake research projects and keeps the number of studied lakes lower than desired. On the other hand, it is hard to find adequate tasks for highly motivated undergraduate students in environmental studies. To meet the necessity and the possibility, to increase the number of studied lakes and to involve students in the diatom analysis of lacustrine sediments, we worked out a simplified investigating (counting) method for studying changes in the diatom assemblages.

Below we present the diatom results of a 65 cm long sediment core (Tó34f), obtained from the Siófok Basin of Lake Balaton in 2017 within the frame of GINOP-2.3.2-15-2016-00009 project. Izzati Hanun Binti Mahadi worked on this core and wrote her MSc theses, supervised by Katalin Báldi and Krisztina Buczkó. The permanent diatom slides deposited, and they are available in the Algological Collection of Hungarian Natural History Museum. The main aim of the thesis was to test, if there were any significant changes in the diatom composition in this short core.

Izzati Hanun Binti Mahadi's theses

Paleoclimatology is the study of past climates, however in the absence of instrumental data going back in time, the only way to learn about ancient climate is from climate archives, where the imprints of environmental change can be expected to be preserved. Lake sediments are excellent archives recording all sorts of climate related changes, however the key to reveal this information is by the mathematic formula of proxies. A proxy can be defined as variables in paleoenvironmental reconstruction where the measurable descriptors can stand in for desired variables such as temperature, salinity, nutrient content, wind speed and carbon dioxide concentration. These desired parameters are also known as target parameters (FISCHER and WEFER 1999). Useful climate proxies can be many sorts based on any organisms, like diatoms, ostracods, chironomids living in a lake, or pollen transported there mostly by wind, or in case of marine sediments corals, foraminifera and many more. Besides organisms, ice cores, tree rings, speleothems, or charcoal can be used as proxy.

For the here presented study, diatoms are used to study the past climate and human impact preserved in sediments of Lake Balaton, Hungary. Diatoms are single-celled silicified alga that usually has the size ranging from 2 to 200 μ m in diameter or length, but sometimes they can reach the size of 1 mm, or more (ROUND *et al.* 1990). Diatoms occur either as solitary cells or in colonies, which can take the shape of ribbons, fans, zigzags, or stars (RIMET and BOUCHEZ 2012). Some species can live as mucilage tubes. Regarding the habitats, they occupy the water column (planktonic life form) or attached to the different kinds of substratum (epiphytic) or freely move on the soft or solid surfaces (SABATER 2010).

Diatoms are highly responsive organisms to numerous factors, either environmental or biological. The factors could be light intensity, water temperature, substratum type, water velocity, mineral composition, and content and the availability of nutrients in the habitat. Their individual growth and community composition depending on the species present in the community will change in direct response to all factors controlling their growth (SABATER 2010). Diatoms are thus an important source of information about the past environment and ecological changes like climatic and lake-level changes, water pollution, eutrophication, and acidification (SMOL and STOERMER 2010). Diatoms are ubiquitous in aquatic environments, especially in lakes, as they can settle in various types of habitats within the lake by forming stable populations in either pelagic, epiphytic or benthic habitats based on the taxa. All taxa have unique compositional differences, and these differences can affect diatom valve preservation which highly depends on their life form. For example, planktonic diatoms mostly have their remnants floating around causing these taxa to be easily transferred and distributed through the sub-environments of the lake, whereas benthic and epiphytic taxa are more predominant in the sediment near their habitats as they sink down to the sediment more easily (FREY 1988, HASSAN 2015, HEGGEN et al. 2012). At the surface level, the identification of diatom taxa that dominates in certain habitats will allow us to make an inference about the status of water-level and also the level of nutrient availability in that particular habitat, as certain taxa

can survive in limited resource, but some requires ample resources to survive. Therefore, based on their strong species-environment relationship, we can make new investigations to reconstruct the status of the environment based on diatom assemblages/populations.

Studying the past environment of waterbodies of small, closed basins on land has become a common practice all over the world, as the lacustrine sediments of deep lakes turned out to preserve undisturbed evidence of changes in the past. Thus, this kind of lakes can be an ideal object of paleo studies such as taxonomic, evolutional and paleoenvironmental reconstruction. Information studying deep lakes is desirable for us to reconstruct climate models, whereas information on shallow lakes, though less ideal for climate models, but might have a great deal of economic and social importance (PADISÁK and REYNOLDS 2003). The sediment of shallow lakes has greater mixing due the effects of wind or due to other benthic organisms, making these processes much more important in a shallow lake than in a deep lake (KEARNS 1996). Therefore, shallow lakes, such as Lake Balaton, where a small-scale water level fluctuation can affect the lake fundamentally is more sensitive towards climate and human impact (KENNEY *et al.* 2002, MOSS *et al.* 2003, VÄLIRANTA *et al.* 2005).

Lake Balaton

Lake Balaton is the largest shallow lake in Central Europe. The basin of the modern Lake Balaton can be divided into four sub-basins with open water, known as Keszthely, Szigliget, Szemes and Siófok Basin besides the large reed lands of "Kis-Balaton" (meaning "Small Balaton") attached at the main water mass of the lake. Lake Balaton today is an elongated shallow lake that is about 77.9 km long, average width of 7.2 km, surface area of 593 km² and a mean depth of 3.14 m (HERODEK *et al.* 1988, ZLINSZKY *et al.* 2010). The shoreline variation of Lake Balaton could be affected by erosional processes surrounding the catchment area as well as eutrophication event that occurred in the lake, though in modern times rather artificially controlled. However, sedimentation process like silting up can be quite intense among the reeds at the northern shore (SZESZTAY *et al.* 1966). The accumulation of silt in the lake is varying due to the majority of sediment load that arrives in Keszthely Bay by the Zala River and deposited. Generally, an average rate of sedimentation rate in Lake Balaton is 0.43 mm/yr (TULLNER and CSERNY 2003).

The paleolimnological exploration of Lake Balaton is still scarce and uneven, in spite of the intensive and long ongoing neolimnological studies (HATVANI *et*

al. 2011, ISTVÁNOVICS and HONTI 2018) and the recent efforts (CSERNY and NAGY-BODOR 2000, MAGYARI *et al.* 2022, SZABÓ *et al.* 2021). There is still not enough information about its algal flora, especially in terms of diatom assemblages (BUCZKÓ *et al.* 2009) The objectives of this study are to (1) acquire the database about diatom assemblages in Lake Balaton and (2) compare the diatom record based on their traits with known climatic, hydrological and land use changes to find out what caused the ecological change.

Algological background

Being a young glacial formation, Lake Balaton is one of the best studied water bodies worldwide and there are many detailed ecological studies, but in terms of algal flora of the lake, it is still poorly studied. The history of palaeolimnological diatom studies in Lake Balaton were summarized in BUCZKÓ et al. (2005, 2019). Recently diatom-based reconstruction in Lake Balaton were conducted by BUCZKÓ et al. (2009, 2019), where the first study was carried out at the southwest part of the lake (Zalavár Pond), while the second one was in the northeast part (Siófok Basin). Both studies were a multiproxy involving investigation of trace elements, Cladocera subfossils and diatom remains from sediment cores taken at respective site location to reconstruct the trophic status and hydrological alterations of Lake Balaton. Moreover, the timescale for both was before present ranging from 7000 cal year BP to end of the 20th century. There is a study presenting the first high-resolution diatom record from Siófok basin offering new taxonomic finding and an updated record (ВUCZKÓ et al. 2019). Current study (Tó34f) was located between Zalavár Pond and Siófok basin thus the findings of this study will complement the previous data in understanding better the possible local variation of paleoclimate of Lake Balaton area. In addition to that, our current study's timescale was ranging from 1459-2017 AD (550 years interval from present) (Magyari, E., pers. com.), thus, the expected changes will be recent and possibly influenced by anthropogenic activity.

This study is partly focussing on diatom traits in determining the trophic status and hydrological alteration. This method is less time consuming, and the interpretation is direct to our objectives. Moreover, in present situation diatoms are mostly used for monitoring and assessment of water quality in running waters (KELLY 2013), but scarcely available for lakes. The information obtained from this study will serve as a good start to learn more about the status of the lake in the past or in present days. In addition, this will be the first diatom-based paleoenvironmental reconstruction using traits in Lake Balaton.

MATERIALS AND METHODS

Sediment coring

A sediment coring in order to get samples was carried out in Lake Balaton, Hungary during the coldest days of the winter of 2017, when massive ice developed on the lake allowing the possibility to drag the drilling rig to position without a boat. The drilling was carried out at the coordinates of N 46° 54' 57.80", E 17° 48' 55.38" on the surface of the lake (Fig. 1). The exact location of Tó-34f core is 2 km away from the shore, far from inflowing water from Zala river. A core of 65 cm in length was chosen to study in detail, that was taken at a water depth of 4.20 m, where the depth was determined using an echo-sounder at sampling time. The core was sectioned at 2 cm intervals and samples were stored in a refrigerator at 4 °C for further analysis. The core was obtained by Mihály Braun, Enikő Magyari, Mónika Tóth with several others helping.

Diatom analysis

All samples were treated with 10% hydrochloric acids (HCl) to remove carbonates and 30% hydrogen peroxide (H_2O_2) to remove organic matter



Fig. 1. Sampling location of Tó-34f. See the massive ice covering the lake making possible to take samples without boat. (Pictures courtesy from Magyari Enikő Katalin)

(BATTARBEE 1986). The samples were then left in water bath at 90 °C until all organic matter had been removed. The cleaned samples were mounted permanently onto microscope slides into Naphrax resin (refractive index =1.7). After the samples was completely dried, the diatoms were then observed under the light microscope (Olympus BX51 Light Microscope) at the Department of Physical Geology, Faculty of Science ELTE. Diatom counting was supervised using a Leica DM LB2 light microscope (equipped with 100 HCX PLAN APO objective and Olympus SC180 digital camera) at ×1,000 magnification under oil immersion and phase contrast in the Algological Collection of the Hungarian Natural History Museum. The counting process was conducted using oil emersion objectives and magnifications of ×1,000. Approximately, about 300 of diatom valves were counted per sample and identified to different taxonomic level, mainly in genus level. The first 50 diatom valves for all samples were photographed using digital camera (High definition HD1080p CNOS colour camera with HDMI and USB-2 interface) and arranged in photo plates (Photoshop CS4, 2008) accordingly). The purpose of these photo plates serves as important documentation for future research when higher taxa at species level is attempted, while for the current study the plates serve as useful aid in counting and identifying diatom valves according to its taxa.

High level, accurate identification of diatoms requires long expertise and clarifying the taxonomic position of some rare taxa is very time consuming. Moreover, in the here presented study only the taxa having > 2% of relative abundance were taken into consideration, so here the analysis of abundant and frequent taxa can give us information about the temporal changes along the sequence.

In diatom studies, the valves were categorized from genus to species based on their functional ability to adapt under particular environmental conditions and this functional trait can be inferred as life strategies (TAPOLCZAI *et al.* 2016) Traits are the basic units of any kind of ecological classification of organism and according to (VIOLLE *et al.* 2007), it can be defined as any morphological, physiological or phenological measurable feature at the individual levels. The categorization was made with reference mainly from the website of *https://diatoms.org/*. Within the araphid diatoms, light fragilarioids and heavy fragilarioids were separated, based on the degree of silification of valves.

Centric spp. (within Centric diatoms, *Aulacoseira* taxa were distinguished and excluded), *Aulacoseira* spp., light fragilarioids, heavy fragilarioids, *Ulnaria* spp., *Gomphonema* spp., *Eunotia* spp., *Amphora* spp., *Amphora* thumensis, Cymbella spp., *Cocconeis* spp., *Diploneis* spp., *Karayevia* spp., *Surirella* spp., *Nitzschia* spp., *Navicula* spp., *Caloneis* spp., *Pinnularia* spp., *Epithemia* spp., *Gyrosigma* spp., *Cymatopleura* spp., *Cavinula* spp., *Staurouneis* spp., *Neideomorpha* spp., *Sellaphora* spp., *Placoneis* spp., *Cymatopleura eliptica*, *Cymbopleura* spp., *Tryblionella* spp.

The diatom diagram presented in this study are the percentage of total valves counted. Diatom zonation can be used for qualitative analysis and for quantitative analysis as the determined zone representing significant changes in the assemblages (EGAN *et al.* 2019). Stratigraphic zone boundaries were defined using CONISS on square-root transformed data with Psimpoll version 4.26 program (BENNET 2005). The significant number of diatom assemblage's zones (DAZ) was obtained from a broken-stick model implemented in "Rioja" package (JUGGINS 2015). There are two sets of diatom diagrams created, where the first one is using the 50 count of diatom valves per depth, while the second one is using the 300 counts per depth. Each of these datasets are the results of diatom assemblages plotted along depth.

Diatom slides are deposited in the Algological Collection of the Hungarian Natural History Museum marked from HNHM-ALG-D 2020/53 to HNHM-ALG-D 2020/103.

RESULTS

Light microscope pictures were taken on the first 50 diatom valves for each sample and documented. Altogether 52 photo plates were prepared and used for environmental reconstruction of Lake Balaton. After the completion of photo plates, with aid of these photo-documentation, extra 250 valves were counted, and only pictures about those taxa that have not recorded before was taken. Thus, for the here presented study two datasets were created. For data analysis, we used the data from the second set (approximately 300 valves were counted for every sample) and a total of 30 diatom categories were created and used. The most abundant group taxa were the light fragilarioid (60%) heavy fragilarioid (50%), *Aulacoseira* spp. (25%), Centric spp. (25%) and *Diploneis* spp. (25%).

Altogether 30 genera or taxa were identified on variety of form at genus level and only taxa that had an abundance > 2% are illustrated in the diatom diagram. Cluster analysis using increment sum of squares method (CONISS) was applied to define intervals containing similar species assemblages and to identify zonation in the taxonomic profile. Based on the Broken-Stick model, four significant diatom assemblage zones and two subzones spanning the past of 550 years was identified (Figs 2–3).









Diatom assemblages' zones defined by the first 50 diatom valves

Figure 4 presents the diatom diagram from Siófok basin of Lake Balaton, showing the percentages of the 30 categories (minimum relative abundance of 2%) along the depth of the core. The diagram based on the counting of first 50 diatom valves. The significant four zones are divided at 60, 40 and 9 cm.

Tó34f-DAZ1⁵⁰: 65–60 cm

In this zone, the *Diploneis* spp. started with a moderate abundance (> 20%) reaching its peak in the core in this interval, then gradually decreasing never to become dominant in later zones. The diatoms belonging to Centric spp., shows a small peak here of 5% in abundance while *Aulacoseira* spp. shows a decreasing trend from 20% to 5% in relative abundancy. The light fragilarioid taxa can be seen a sharp increase from less than 5% to 20% in this zone while, for heavy fragilarioid taxa, a significant increase can be seen from 0% to 15% in this zone. *Amphora* spp., *Cocconeis* spp. and *Navicula* spp. has the abundancy roughly about 10% (Fig. 5).

Tó34f_DAZ2⁵⁰: 60-40 cm

In this zone, within the planktonic group, the *Aulacoseira* spp. has the highest abundancy by reaching 40%, while within the benthic group the highest peak can be seen in light fragilarioid taxa with 60%. Heavy fragilarioid taxa in the early part of the zone just 10% and reaching the peak of 40% followed by fluctuating values and then stabilize around 20%. Other species such as *Amphora* spp., *Diploneis* spp., *Nitzschia* spp., *Navicula* spp., cymbelloid spp., *Cavinula* spp. and *Karayevia* spp. are present in low abundance (< 5%).

Tó34f_DAZ3⁵⁰: 40-9 cm

The fragilarioid taxa (light and heavy) is still the dominant group in this zone, but we can see great fluctuations in their abundance especially for light fragilarioid. Relatively they dominate the zone, but there are other group of taxa that present in low abundance such as *Amphora* spp. (20%), *Cocconeis* spp., *Diploneis* spp., *Epithemia* spp. and *Karayevia* spp. However, the planktonic group – Centric spp. and *Aulacoseira* spp. – is rarely present in the zone (< 5%) (Fig. 6).

Tó34f_DAZ4⁵⁰: 9–1 cm

This topmost zone is characterized by the dominance of light fragilarioid in high abundance (60%) for most of the time in this zone. Contrary to the heavy fragilarioid group, that shows a definite decreasing trend reaching lowest abun-







Fig. 5. Photodocumentation of the first 50 diatom valves from the sample Tó34f, obtained in the 4–5 cm depth. *Aulacoseira* and other centric taxa are in the first row; the second and third row show the light fragilarioids. The first 4 valves in the 4th row are counted as heavy fragilarioids, and *Amphora* spp. are also in the 4th row. *Caloneis, Navicula* s. l., *Amphora* spp. and *Diploneis* valves are in the last row.



Fig. 6. Selected pictures from the Tó34f core, from 30-31 cm sample. There are light fragilarioids in the first row, with a Centric diatom at the end of the row. The second row show two pictures of light fragilarioids followed by *Cocconeis* spp. The second and third rows present the heavy fragilarioids. *Amphora* spp. are positioned in the last row, and the last picture belongs to *Epithemia* spp.

dance of less than 10%. Interestingly for the planktonic group, we can see a rise of Centric spp. at the topmost layer (45%), whereas the *Aulacoseira* spp. shows a lower abundancy of about < 20%. In this zone there are some other taxa present at low abundance, such as *Navicula* spp. (> 10%) and *Amphora* spp. (< 20%) (Fig. 7).

Diatom assemblages' zones defined by 300 diatom valves

Figure 8 presents the diatom diagram from Siófok basin of Lake Balaton, showing the percentages of the 30 categories (minimum relative abundance of 2%) along the depth of the core. The diagram based on the counting of 300 diatom valves. The significant four zones are divided at 54, 42 and 7 cm, and it looks reasonable to distinguished two subzones ate 26 cm within the Tó34f-DAZ3.

Tó34f-DAZ1³⁰⁰: 65-54 cm

This zone is characterized by variety of diatom taxa observable in the sediment at this level. The diversity of diatom in this zone is the highest compared to other zones as numbers of taxa is observed to be more than 2%. In the beginning of the zone, we can see an expansion of *Diploneis* spp. as it presents at moderate abundance from < 5% to 20% but as it moves toward to DAZ2 the abundancy decreases significantly (< 2%). We can also see at the deepest layer (65 cm) the planktonic taxa and the fragilarioid are relatively in moderate abundance (10–20%) but as the sediment goes deeper we can see a trend of sharp decrease for *Aulacoseira* spp., light fragilarioid and heavy fragilarioid. However, the trends to do not stay low in long period and gradually the abundances increase for these three groups. Centric spp. is already low in abundance from the beginning and as it reached the top layer. Other than these taxa, *Amphora* spp., *Surirella* spp., *Navicula* spp. and *Gyrosigma* spp. are present at low abundances.

Tó34f-DAZ2³⁰⁰: 54-42 cm

This zone is dominated by light fragilarioid for more than 60% of abundance while, heavy fragilarioid are present at roughly of 40%. However, we can see that light fragilarioid is much more stable in this zone whereas, heavy fragilarioid taxa show some fluctuations at the beginning of the zone but it remained constant as it moving toward to DAZ3. The species richness in this zone is not as high as the previous zone and *Aulacoseira* spp. and *Amphora* spp. are in low abundance (< 10%).



Fig. 7. Selected pictures from the Tó34f core, from 64–65 cm sample. There are 7 pictures about light fragilarioids in the first row, that follow 3 pictures of *Aulacoseira* spp. Some problematic taxa (small naviculoids, *Cocconeis* spp, a heavily dissolved centric taxon (*Pantocsekiella* cf. ocellata)) are in the second row. In the third row some monoraphid taxa are presented, *Hippodonta* sp., and *Navicula* s. l. diatoms. The last in the this row is a Centric taxon. There are *Diploneis* spp. in the 4th and 5th row.





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Tó34f-DAZ3³⁰⁰: 42-7 cm

This zone is divided into two subzones:

Subzone 1a (42–26 cm) is dominated by benthic group; fragilarioid taxa (heavy fragilarioid: 60% and light fragilarioid: ~30%) but, noted that the abundances for light fragilarioid is lower compared to the abundances in subzone 1b and DAZ2. As the depth moving upward to subzone 1b (20% to 40%) and downward to DAZ2 (30% to 40%) the percentage increases. Other than these two taxa, several other taxa such as *Amphora* spp., *Cocconeis* spp. and Epithemiod spp. are present at low abundances (< 10%). However, the planktonic group (Centric spp. and *Aulacoseira* spp.) is significantly depleted throughout both subzone 1a and 1b.

Subzone 1b (26–7 cm) is still dominated by fragilarioid taxa but we can see a slight opposite trend as the taxa shifted from subzone 1a to subzone 1b. For light fragilarioid, the percentage increases by 20% at the border of subzone 1a and 1b and it reached 50% of abundance at the end of the subzones, while for heavy fragilarioid the abundance decreases from 60% to 40% at the beginning of subzone 1b and continues to decrease till the percentage is at 30%. Other taxa like *Amphora* spp., *Diploneis* spp. and Epithemiod are present at low abundances (<10%).

Tó34f-DAZ4³⁰⁰: 7–1 cm

At the beginning of this zone, light fragilarioid remained constant at 50% before it increases to 60% and from there it slowly decreases to 40% as the sediment reached the surface layer, whereas heavy fragilarioid is present at low abundance (< 10%). *Amphora* spp. and *Navicula* spp. are also present at low percentage but much higher compared to the previous zone. In this zone, the percentage of planktonic group increases more than 2%, especially as they moving to the topmost layer (5–0 cm). *Aulacoseira* spp. present in low abundance (~10%) but for Centric spp., the highest abundance is at 30% on the topmost layer.

Diatom assemblage's zones based on diatom trait distribution

All 30 diatom group were recorded and distinguished into four ecological guilds; 1) planktonic guild, 2) high-profile guild, 3) low-profile guild and 4) motile guild:

Planktonic guild: Centric spp., Aulacoseira spp.

High-profile guild: light fragilarioids, heavy fragilarioids, *Ulnaria* spp., *Gomphonema* spp., *Eunotia* spp.

Low-profile guild: *Amphora* spp., *Amphora thumensis*, *Cymbella* spp., *Cocco-neis* spp., *Diploneis* spp., *Karayevia* spp.

Motile guild: Surirella spp., Nitzschia spp., Navicula spp., Caloneis spp., Pinnularia spp., Epithemia spp., Gyrosigma spp., Cymatopleura spp., Cavinula spp., Staurouneis spp., Neideomorpha spp., Sellaphora spp., Placoneis spp., Cymatopleura eliptica, Cymbopleura spp., Tryblionella spp.

Figure 9 presents the diatom diagram from Siófok basin of Lake Balaton, showing the percentages of the four diatom guilds along the depth of the core.



Fig. 9. Percentage of diatom traits plotted against depth as defined PASSY 2007 and modified RIMET and BOUCHEZ 2012).

Tó34F-DAZ1^{Traits}: 65–54 cm

In this zone, the planktonic guild was seen at moderate abundance for about 35% at the lowermost part of the sediment core (65 cm). Immediately at 64 cm, the relative abundance dropped to 20% and they remained around that until the sediment reached 60 cm. Gradual increase of their abundance is observed and reached a peak at 57 cm (> 30%). However, soon after that, an abrupt decrease can be seen at 54 cm where the planktonic guild present at low abundance (10%) for some quite period.

High-profile guild started at moderate abundance (40%) at the lowermost part of the core and having a significant decline in their abundance from 40% to 10% at 62 cm. This abundance of this high-profile guild is the lowest throughout the whole core. Not long after the decline, the guild is able to regain their abundance and an increasing trend can be seen for this, where they manage to reach high relative abundance at 50%.

Low-profile guild on the other hand, started at low abundance (15%) and doubled to > 30% which also seen as the highest abundance ever present throughout the whole core. A decreasing trend of the abundance can be seen starting from 62 cm and never to reach peak more than 30% again. However, in comparison between high-profile and low-profile, we can see complementary trends for both of this guild in the zone. Whenever there is any drop of relative abundance for high-profile guild, the low-profile guild is observed to have an increase in their abundance to complement the loss of high-profile in the ecosystem.

Motile guild started at low abundance (10%) and reached their peak for more than 30% at 62 cm for only a short period of time. At this point, it was the highest abundance ever reached by this guild and remained in low abundance starting at the end of this zone and throughout the whole core.

Interestingly, at 62 cm all three guilds (high-profile, low-profile and motile guild) had a significant change occurred.

Tó34F-DAZ2 Traits: 54-42 cm

In this zone a high-profile guild is dominating or present at high abundance (80%) for the whole zone. The planktonic guild is scarcely present in the zone, while the motile guild present in low abundance (< 10%). At the beginning of the zone, the low-profile guild is present at low abundance and approaching the end of the zone with a slight increase in its abundance (15%).

Tó34F-DAZ3 Traits: 42-7 cm

As this zone contains two subzones, thus we are going to interpret the result according to its subzones as well. The first Subzone 1a (42-26 cm) has the plank-

tonic guild remaining scarce for the whole zone, while the high-profile guild is the dominant. The relative abundance for low-profile guild is higher than in the previous zone where the peak is more than 20%. Interestingly, at 40 cm, 36 cm, 29 cm and 26 cm, we can see how low-profile and motile guild rise in abundance whenever there a decline occurs in the high-profile guild. Subzone 1b (26–7cm) has the planktonic guild remaining scarcely present at the beginning of the zone, but starting to show an increasing trend of their abundance from 9 cm. The highprofile guild is the dominant one in this zone, while low-profile and motile guilds are present at low abundance.

Tó34F-DAZ4 Traits: 7-1 cm

The last zone is characterized by the sudden increase of planktonic guild where they reach about 40% of their relative abundance at the topmost layer of the core (1 cm). From 3–1 cm, as we can see increasing trend of planktonic, a decreasing trend of high-profile can be observed. Low-profile guild is present at low abundance in this zone. Like motile guild, they present at low abundance (< 10%) at the beginning of the zone, however, at the top three layers we can see a slight increase of motile guild to reach more than 10%. This zone presents a significant change of trends for planktonic and high-profile guild.

DISCUSSION

Based on the diatom analysis 30 taxa were distinguished for Tó34f sediment core. Some of the most frequent taxa present through the core were Centric spp., *Aulacoseira* spp., fragilarioid spp., *Amphora* spp., and *Diploneis* spp. varying in abundance. In comparison to the study conducted by (BUCZKÓ *et al.* 2019), altogether 140 taxa were distinguished from the Siófok basin and about 150 taxa were distinguished from the core of Zalavár pond (KORPONAI *et al.* 2010). Fragilarioid taxa were present and dominant throughout most of the sediment core for current study, also true for the core from the Siófok basin and only certain zones in the Zalavár pond.

Zonation

There are some observable differences using the 50 and 300 datasets. Generally, both datasets show a quite similar trend or presence in its relative abundance throughout the sediment core with the same numbers of 30 taxa recorded. Although the trends are almost similar between these two datasets, we can see less important taxa having a higher curve and easily detected on the diagram for 50 compared to the second dataset that counted 300 per sample. This is due to 2% cut off level, meaning that in the 50 datasets more taxa reached abundance exceeding 2%, whereas for the second dataset of 300 valves less taxa reached the 2% and contributed to the higher number of taxa recorded. The most obvious difference that we could find is the way how e.g. fragilarioid taxa were presented. The percentage curve in the 300 datasets was much smoother and less fluctuating, compared to the much rougher and heavily fluctuating curves of the first dataset. This might be true for all curves, but especially obvious for fragilarioid. Noteworthy remarks, the result of zonation also depends on the counting accuracy. The boundaries of the diatom assemblage's zones can be seen to change significantly (Figs 4–5).

According to BATTARBEE (1986), based on the number of valves counted, there would be changes in the frequency of the dominant taxa and altogether in the percentage distribution of taxa in the assemblage, becoming more accurate with increasing sample size. Studies show that the counting process is varying from region to region. Thus, it is important to determine the correct numbers of count to get accurate data about the ecological condition of the past. ROUND (1993) suggested that 100 valves were sufficient for dominant species, but according to SOEPROBOWATI *et al.* (2016), the fixed count at 100 valves were less efficient and less effective in a region that has higher species richness, and there we need to count 100 valves of the dominant species. Moreover, there were significant differences between the counts of 100, 200 and 300, whereas 500 valves do not give any significant difference from 400 and 600 (SOEPROBOWATI *et al.* 2016). Therefore, it is recommended to count the valves at fixed count of 300 to 600 valves for each sample and as mentioned in earlier chapters, the here presented study will use the dataset from 300 counts per sample.

The here presented study aims to provide new information and a database based of the relative abundance of taxa that correlates to environmental condition. Several studies indicate how diatom traits can give information on nutrient availability, organic pollution and levels of disturbance (e.g. grazing and flow velocity) (B-BÉRES *et al.* 2016, BERTHON *et al.* 2011, PASSY 2007). Moreover, this trait-based approach helps to develop a fast method to assess the ecological pattern in a particular water system (MCGILL *et al.* 2006). Therefore, by studying diatoms, their abundancy and habitat preference inferred from their traits can give important information for the present state of the ecosystem and its future state, helping to sustain the biodiversity and ecosystem services of Lake Balaton.

The functional traits of this study is following description (PASSY 2007), that refers to the ecological guild of diatoms based on their ability to utilize available nutrients and their resistance to physical disturbance. In simpler categorization the traits are simplified into; low-profile, high profile, motile guilds and expanding planktonic guild. We going to see how the change of diatom abundancy

is based on trophic preference, but we must keep in mind, that Passy's system was developed for running waters, so its application in lentic waterbodies must be handled with caution.

However, the fragilarioid taxa for both light and heavy traits show high abundancy for the past 550 years. Although there are some fluctuations in certain years, but generally we can state, that, these taxa are the dominant one. This could be due to the high growth rate of fragilarioid taxa allowing them to maintain their biomass despite the environmental changes. Small-sized diatoms like *Fragilaria* spp. can compensate the dilution of water (influx of water) as these species has high growth rate making them able to maintain their biomass even during flooding.

Based on the percentage diatom diagram we can see fragilarioid taxa (both light and heavy) to strive and survive at any water levels. Due the LIA reigning already at the start of our record until the 17th century the water level in Lake Balaton was higher than present but the relative abundancy of fragilarioid taxa is still higher than other taxa. Moreover, as a general observation for a shallow lake, the fragilarioid taxa are often dominant and their presence corresponds in other lakes to periods of shallower deposition with higher organic matter and the presence of littoral fossils (PADISÁK and DOKULIL 1994), thus it is recognized as a shallow water living taxa. Similar study by (HAWES and SMITH 1994) indicates that some species like *Aulacoseira granulata*, the dominant *Aulacoseira* species in Lake Balaton can have high abundance in similar depth conditions as *Fragilaria* spp., which is in accordance with our result.

In the oldest sample the planktonic taxa rise in abundancy compared to other zones, and some studies says that moderate abundance of planktonic fresh water *Aulacoseira* spp. may indicate wet and eutrophic environment (LAING and SMOL 2003, ZALAT 2015) but some says it also may indicate greater depth and open lake conditions (HAMDAN *et al.* 2016). Moreover, high abundance of *Aulacoseira* taxa can be indicative for turbulent water during windy periods (BUCZKÓ *et al.* 2018).

Relative increase of non-planktonic taxa such as *Navicula* spp. and *Amphora* spp. may indicate the shallowing of the lake with low flooding period in arid and hyper-arid conditions (HAMDAN *et al.* 2016). Presence of *Navicula* spp. could indicate weak mixing at the bottom of lake which will manifest clear water and oligotrophic conditions. But since the presence of *Navicula* taxa in current study is low, it is not enough to indicate oligotrophic conditions at the bottom of Lake Balaton. *Navicula* taxa are motile diatoms thus, they can freely move on the surface, so even in a windy, changing periods of time, they can survive.

Back in 1860, there is a significant decline of water level in Lake Balaton as the Sió Canal was opened (BíRó 1984) and based on our diatom record not much changes occurred except for a gradually decrease of light fragilarioid and a gradual increase of heavy fragilarioid from 1850–1910. This scenario coincides with the end of LIA in 1850. During this time, the fragilarioid taxa is dominating, but the species variability between the light and heavy fragilarioid has changed. Light fragilarioid started to be more stable at high abundance (40–50%), whereas heavy fragilarioid having a decreasing trend significantly till the end of the core.

Trait distribution

The considered functional traits in the here presented study are low-profile, high profile and motile guild based on (PASSY 2007) descriptions. In addition to these three guilds, the planktonic guild was also accounted as a guild by RIMET and BOUCHEZ (2012). Low-profile guild consisted of diatom taxa that favoured low nutrient environment (BERTHON et al. 2011). These taxa regarded in lowprofile guild can resist high grazing pressure (high disturbance) allowing them to strive in oligotrophic rivers (RIMET et al. 2005). Simply, low-profile guild has advantage in low nutrient condition and has high resistance towards physical disturbance. On the other hand, the taxa of the high-profile guild have the highest abundancy in intermediate to high nutrient environments, but they cannot withstand high disturbance in the water system. The most common low-profile diatoms are usually from the Achnanthidium spp., Amphora spp. and Cocconeis spp. Other functional traits that considered in the current study is the motile guild. Several studies give an impression of motile guild possessing the ability to re-arrange their position freely, making them competitive even under limiting conditions (LENGYEL et al. 2015, PASSY 2007). Under extreme environmental conditions creating stress for the organism like loads of organic matter, high levels of turbidity or limited amount of light gives this motile taxon an adaptive advantage (STENGER-KOVÁCS et al. 2014). They can survive the extreme condition by crawling away or moving to another place where they can get the source of energy to sustain their survival (Table 1).

	· / ·	1	· · ·
		Physical disturbance	Nutrient enrichment
Low profile		+	-
High profile		-	+
Motile		_	+

 Table 1. Ecological guild's resistance to physical disturbance and nutrient enrichment based on PASSY (2007). (+ resist the perturbation; – do not resist the perturbation)

Tó34f-DAZ1 Traits

In the lowest diatom zone, the low abundance of low-profile guild such as the *Amphora* spp. and *Cocconeis* spp. suggest that the trophic condition in this

zone was never in extreme depletion of nutrients. Low-profile guild species can survive in limiting environment but for high-profile guild species need sufficient nutrient level to survive and based on the information that we have; high-profile guild is generally present in moderate abundance in the current zone. This indicate that to some extent the level of nutrient available during this period was sufficient for the high-profile guild to propagate moderately. It was also proposed by STENGER-KOVÁCS *et al.* (2013), that the relationship of low-profile guild and nutrient availability is inversely proportional as low-profile guild abundance will increase as the nutrient level decrease. This is because in limited nutrient environment, high-profile guild cannot survive, thus this guild will not produce any algae canopy preventing further (shading) nutrient limitation for low-profile guild species.

Planktonic diatom *Aulacoseira* spp. was the highest in its abundancy in the current zone. *Aulacoseira* spp. is a meroplanktonic organism where it can be suspended in the water column (KILHAM 1990) and during the resting stages they also can survive in the dark when they sink to the sediment surface of the lake bottom (SCHELSKE *et al.* 1995) by increasing their light harvesting capacity in order to adapt with low-light condition (TALLING 1957). *Aulacoseira* taxa appears to require a rather high nutrient environment and is a characteristic planktonic diatom in mesotrophic or eutrophic lakes and slow running rivers (KILHAM and KILHAM, 1975, KISS *et al.* 2012). Therefore, this group is likely to thrive in abundance during periods of strong winds where the thermal lake stratification breaks down causing the concentration of nutrients to increase (RÜHLAND *et al.* 2015, WANG *et al.* 2012). Low abundance of high-profile guild in this zone with the rise of *Aulacoseira* spp., we suggest that there was a great event of high turbulence during this time where nutrient availability is not the main focus for the rise of *Aulacoseira* spp.

The motile guild (*Nitzschia* spp., *Navicula* spp. and *Surirella* spp.) is also observed to be present in the current zone at low abundance. It is possible for the motile guild to replace the low-profile guild, as the motile guild is characterizing habitats enriched in nutrients (PASSY 2007, RIMET *et al.* 2015). Motile guild species are commonly threatened with the increase in flood velocity; thus we suggest that in this period the levels of flood intensity might have been higher than in the rest of the zone. However, expansion of *Diploneis* spp. in this zone may also indicate low water levels with high volume of macrophyte vegetation (MARCHETTO *et al.* 2008). The cumulative of planktonic guild for this zone presents at moderate abundance which may be indicative to moderate water level. The high-profile guild was in low abundance a possible indication of oligotrophic status and at the same time with moderate abundance of low-profile guild and motile guild.

Tó34f-DAZ2 Traits

In this stage corresponding to the second zone high-profile guild is present in high numbers, whereas the low number of low-profile guilds may indicate a high nutrient level with low physical disturbance. The characteristic of fragilarioid taxa of having high surface-volume ratio allows them better access to light and nutrients (STENGER-KOVÁCS *et al.* 2014) and with the expansion of the fragilarioid, the low-profile guild has limited substrate surface availability, and this affect their chances of survival as they cannot attach themselves to substrate to prevent from being carried away (STENGER-KOVÁCS *et al.* 2013). The lack of planktonic guild refers to shallow water level. In addition, with high abundance of high-profile guild and low abundance of low-profile guild and motile guild be an indicative of eutrophic status.

Tó34f-DAZ3^{Traits} [Subzones: 1a, 1b]

In the third zone, there are two subzones. For Subzone 1a, high-profile guild is the dominant and we can see there is a difference in abundance between light fragilarioid (30%) and heavy fragilarioid (60%) taxa. Interestingly, light fragilarioid and heavy fragilarioid cannot coexist in high abundance in the same system. Based on our results we can see the trend of heavy fragilarioid has the highest abundance, then the light fragilarioid will be in moderate or slightly lower and vice versa. This observable inverse proportional relationship between light and heavy fragilarioid is probably due to their weight that could play role during high water level which favours the heavy diatoms more than the light ones. The low-profile guild on the other hand is present in subzone 1a in low abundance but far from being important taxa, *Cocconeis* spp. is only prominent here, and far from dominant.

In the subzone 1b the fragilarioid taxa remained the dominant taxa, but it is interesting in the current study to observe an abrupt decrease of heavy fragilarioid (60% to 40%), whereas there is also an abrupt increase for light fragilarioid (20% to 50%). They seem to be inversely proportional. These changes occurred during the transitional period from subzone 1a to 1b (AD 1982–1916). In the current subzone light fragilarioid are important, while heavy fragilarioid started to decrease in abundancy.

Therefore, with the domination of high-profile guild in both subzones, it can be concluded, that the water conditions during this period were eutrophic with lack of deep water, also with a possible fluctuation of nutrient availability as we can see low-profile guild rise and occasionally shifts similarly in subzone 1b. The high-profile guild peaks with 80% with fluctuations followed by a significant decrease, an important trend to observe. This trend was probably caused by the authorities in the 19th and 20th century trying to lower the water-level of Lake Balaton by widening the Sió Canal resonsible for the lake's only outflow to protect the newly built Budapest–Rijeka railway from flooding (Kovács *et al.* 2010). The regulation of water level during this period has caused degradation of Kis-Balaton wetlands, functioning for centuries as the natural filter of Lake Balaton. Furthermore, the agriculture practice of the time extensively using phosphorousrich artificial fertilizers and the increased amount of sewer from rapidly growing settlements, as well as the untreated manure released from animal husbandry combined with the degradation of Kis-Balaton wetlands all contributed to the intense eutrophication of Lake Balaton in the 1930s and 1940s (DÖMÖTÖRFY *et al.* 2003, NGUYEN *et al.* 2005).

Tó34f-DAZ4 Traits

As for the last zone, high-profile guild is the dominant but mainly referring to the light fragilarioid. Again, the interaction between light and heavy fragilarioid has been vice versa, where either one species has higher frequency when the other has lower, though altogether both remained showing high abundance. The exception is when the abundance of heavy fragilarioids have been decreasing gradually, while the other started to decrease at the end of the zone. During this period with the presence of high-profile guild, the nutrient should be just as high as in the previous zone, but we can see sudden rise of the planktonic taxa (Centric spp.) and motile taxa (*Navicula* spp.).

The planktonic group such as Centric spp. has rather low abundancy in the last 500 years from 1459–1500 (10%) till recent times in 2000–2017 (25%). The presence of Centric spp. was only obvious during these two periods (1460–1500 and 2000–2017) and only little were observed in between them. Highest abundance of Centric spp. at 25% was seen during the early 21st century is probably an indication of temperature rise due to global warming where the elevated nitrate availability may play a crucial role in stimulating growth of Centric diatoms (Qu *et al.* 2018). Acceleration of global warming may increase the replacement of *Aulacoseira* spp. by Centric spp. observable at the beginning of 21st century (LI *et al.* 2021). Moreover, high portion of planktonic taxa indicate increasing water lever.

Approaching the end of this zone the nutrient concentration seems to be the limiting factor of algal growth. Probably, during this period the concentration of nutrients in the lake decreases from high levels to low levels reaching oligotrophic status due to steps that were taken to counteract the trend of eutrophication by the authorities to avoid algae blooms in Lake Balaton in the framework of the Kis-Balaton Water Protection System. As part of these efforts Kis-Balaton was re-flood again to become wetlands to filter out the harmful loads of nutrients and sediments arriving into the lake (DÖMÖTÖRFY *et al.* 2003). Latest studies confirmed that the Kis-Balaton wetlands do have a significant protective effect and can safeguard the lake from potential blooms, especially from toxic cyanobacterial blooms (MARINOVIĆ *et al.* 2021).

Recommendation

The here presented study has provided a good database for further research especially for multi-proxy analysis. The photo plates are available by request. Moreover, it proved that using trait-based diatom analysis in determining the trophic status of the lake, the method is useful in present-day biomonitoring, while much more convenient and less time consuming without requiring high level of expertise to conduct the assessment. When the age-depth model will be available (it is in progress), the reinterpretation of data is required.

Summary: Diatom based limnological changes in the mountain and lowland lakes of the Carpathian Region have been studied within the framework of the Cryptic project (supported by the NKFIH) mainly using paleolimological methods. In order to understand better the general trends, its drivers, and stressors in these lakes, it is necessary to increase the number of studied lakes. However, the analysis of a cores obtained from lacustrine sediments is time consuming and requires highly qualified specialists, making hard to involve early career researchers and undergraduate students in diatom analyses, even though processing a core can serve as an ideal thesis topic. In this paper we present the results of a core obtained in the Siófok basin of Lake Balaton in 2017 based on an MSc thesis. The 65 cm long core - marked Tó34f - was sampled in every centimeter, light microscopic images were taken of the first 50 diatom valves from each sample, and these were arranged in photo plates per sample. A total of 15 797 diatom images were taken. Later the documented diatoms were divided into 30 categories. Some characteristics, easily definable taxa have been distinguished at species level (e.g. Karayevia clevei, Cavinula scutelloides); most taxa have been grouped at genus level (e.g. Amphora, Aulacoseira, Neidium). Finally, we divided the weakly and highly silicified fragilarioid taxa, so characteristic of the Lake Balaton sediments, into two broad umbrella categories. For Centrales taxa – the species of Aulacoseira genus – a group was also erected. The counted diatoms were grouped according to diatom traits (PASSY 2007). This process resulted in a low taxonomic resolution trait-based record for the Siófok basin of Lake Balaton based on 65 samples. Four significant diatom zones were distinguished, with a depth of 54, 42 and 7 cm zone boundaries. The increase in the ratio of Centric diatoms in the upper section of the core coincides with the trend observed in lakes in the Northern Hemisphere. This could be due to increased trophic level and/or rising water temperatures, ultimately due to climate change. Analysis based on diatom traits clearly shows the trends observed in lakes of the Northern Hemisphere, a clear increase in the proportion of planktonic guilds. We demonstrated that trait-based diatom analysis is a simple and useful method for studying general trends as a pilot study, to be followed by highresolution taxonomic analysis. The here built database can provide a good basis for species-level analysis and quantitative reconstructions in the future.

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Összefoglaló: Az NKFIH által támogatott Cryptic projekt keretében paleolimnológiai módszerekkel vizsgáljuk a Kárpáti Régió hegyi és sík vidéki tavaiban zajló változásokat, elsősorban a kovaalgákra fókuszálva. Ahhoz, hogy jobban felismerhessük az általános trendeket, azt, hogy milyen változások történtek, meghatározzuk a ható tényezőket, minél több víztestet vizsgálatára van szükség. Egy-egy furat feldolgozása azonban erőforrásigényes, hosszú időt vesz igénybe, és magasan képzett szakemberekre van szükség. Ezért nehéz a munkamegosztás, pedig egyetemi hallgatók számára ideális szakdolgozati téma lehet egy-egy furat feldolgozása. Az itt bemutatott munka a Balaton siófoki medencéjében, 2017-ben mélyített fúrás eredményeit mutatja be egy MSc szakdolgozat alapján. A 65 cm hosszú, Tó34f jelzésű furat centiméterenként került feldolgozásra, minden mintából az első 50 kovaalga vázról fénymikroszkópos kép készült és ezeket a képeket mintánként fotótáblákba rendeztük. Összesen 15 797 diatóma kép készült, amit 30 kategóriába soroltuk. Néhány jellegzetes, könnyen határozható taxont faji szinten különítettünk el (Karayevia clevei, Cavinula scutelloides); a taxonok jelentős részét nemzetség szinten választottuk szét (úgymint Amphora spp., Aulacoseira spp., Neidium spp.). Végül két nagy gyűjtőkategóriába osztottuk a gyengén és erősen kovásodott fragilarioid fajokat (amelyek a balatoni üledék jellegzetes kovaalgái), illetve a sugaras szimmetriájú (Centrales az Aulacoseira fajok nélkül) taxonokat különböztettük meg. A 65 minta elemzése után egy kis taxonómiai felbontású diatóma rekord és egy, kovaalga guildek alapján készült (PASSY 2007), jelleg-alapú rekord készült el a Balaton siófoki medencéjére vonatkozóan. Négy szignifikáns diatóma zónát különítettünk el, 54, 42 és 7 cm-es mélység határokkal. A furat felső szakaszán a sugaras szimmetriájú diatómák arányának növekedése egybeesik az északi félteke tavaiban tapasztalt trenddel. Ennek oka lehet trofitásnövekedés és/vagy a víz hőmérsékletének emelkedésének – így végső soron lehet a klímaváltozás – következménye. A diatóma jellegek alapján végzett elemzések is egyértelműen mutatják az északi félteke tavaiban tapasztalt trendeket (a planktonikus guild arányának növekedését). Adataink alapján egyértelmű, hogy a trait alapú diatóma elemzés egyszerű és jól használható módszer az általános trendek vizsgálatához – amit aztán követhet nagyfelbontású taxonómiai elemzés. A jelen munka keretein belül felállított adatbázis jó alapot biztosít a faj szintű elemzéshez és az azokon alapuló kvantitatív rekonstrukciókhoz.

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