Final closing report of the NKFIH project K129265

„Phylogenetical, biogeochemical and paleoecological investigations on the endemic gastropod fauna of Lake Petea near Oradea"

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1.Introduction, aims

Ecological speciation is a prominent mechanism of diversification but in many evolutionary radiations, particularly in invertebrates, it remains unclear whether supposedly critical ecological traits drove or facilitated diversification. As a result, we lack accurate knowledge on the drivers of diversification. Numerous gastropod taxa represent celebrated model systems in the study of ecophenotypic plasticity. Understanding the underlying reasons for phenotypic plasticity and the resulting morphological disparity is one of the key topics of evolutionary biological and paleobiological research (Dewitt & Schneier 2004; Fusco & Minelli 2010; Gilbert & Eppel 2009; Schlichting & Pigliucci 1998; West-Eberhard 2003, 2005).

The thermal spring-fed Lake Pețea located in NW Romania southeast of the city of Oradea harbors a unique endemic warm water biota. It is the only location in Europe where thermal water endemic melanopsid *Microcolpia parreyssii* (Philippi, 1847) lived (Kormos 1903, 1904, 1905 a,b; Müller et al. 2014; Neubauer et al. 2014; Paucă 1937; Sîrbu et al. 2013; Sîrbu & Sárkány-Kiss 2002; Sümegi et al. 2012 a,b, 2018; Tóth 1891). Lake Pețea drained by the Pța creek is a very shallow lake with a maximum depth of 2-3 m and an area of a couple of tens of square meters, with average water temperatures of 30°C (Telcean & Cupșa 2013) (Fig.1) (Covaicu-Marcov et al. 2003; Müller et al. 2014, 2018; Sîrbu & Sárkány-Kiss 2002; Sîrbu et al. 2013; Telcean & Cupșa 2012, 2013). The bicarbonate, calcium, and sulfate-rich waters of the lake are maintained by underwater thermal springs deriving from the Lower Cretaceous aquifer system (Telcean & Cupșa 2013, Tenu et al. 1981, Gheorghe & Graciun 1993), so fluctuations in spring activity is the major driver of lake level control. As a result, it is very prone to natural and artificial environmental changes. Even minor fluctuations in depth, the surface area along with water temperature, chemistry, nutrient supply, and substrate conditions leading to habitat and food source loss and concomitant increased predation have a significant impact on the biota leading to extinction as well (Müller et al. 2014, 2018; Sîrbu & Benedek 2016; Telcean & Cupșa 2012, 2013).

The potential influences of the mentioned processes controlling population size and diversity in the past are well reflected in the diverse morphologies of subfossil melanopsids recovered from the lacustrine deposits. In contrast to the extant phenotypes of *Microcolpia parreyssii* (Philippi, 1847) with relatively clear and well-defined morphological traits of pronounced shouldering and the overall presence of axial ribs (Fig.1), Holocene and Pleistocene subfossil melanopsids display extreme morphological variability. Phenotypes range from smooth, slender elongate, keeled to bulkier smooth, ribbed specimens with varying degrees of shouldering, ornamentation, callus thickness, aperture shapes, and sizes, which captured the attention of paleontologists and biologists very early and led to the description of over 40 species and types (Brusina 1903; Kormos 1903, 1904, 1905 a,b; Neubauer et al. 2014; Pauča 1937; Sümegi et al. 2012a,b,2018; Tóth 1891). Up till the works of Sümegi et al. in 1999 and 2012, only a single center placed 11 m deep geological profile existed (Kormos 1903, 1904, 1905 a,b) exposing a continuous sequence of oligotrophic marls topped by eutrophic lacustrine clays and peat. Using the retrieved melanopsids Kormos (1903) presented a clear succession of smooth shelled forms to bulkier, globular shouldered and ribbed forms with the extant *Me. parreyssii* as an end member. Later studies attempted to revise the described taxa. Based on the most recent genetic data the melanopsids were assigned into the genus *Microcolpia*. Neubauer et al. (2014) implemented extensive morphometric and isotopic analyses on museum specimens of unknown stratigraphic location and found no clear separation of the fossil, subfossil and extant *Mi. parreyssii* specimens in the created morphospace and isotope space. No significant variation was observed in oxygen and carbon isotope values of the shells either (Neubauer et al. 2014). Using the profile data of Kormos (1903), where shouldered morphotypes closely resembling the traits of extant *Mi. parreyssii* were restricted to the upper eutrophic deposits, a major bottleneck with presumed cause (marginalization of gill-bearing melanopsids in oxygen poor environments) was proposed (Neubauer et al. 2014) where according to the interpretation a shrink in habitat reduced morphological variability of elongated forms and led to shifted morphologies to shouldered bulky specimens. So far however, a detailed high-resolution paleohydrological reconstruction of the lake using multiple sedimentological, geochemical proxies and solid chronology was missing. The reconstruction of the three major stages of lake evolution was based on stratigraphic observations made on profile of Sümegi et al. (2012) and using paleoecological data. Specimens of melanopsids and neritids of known stratigraphic position have not yet been exposed to extensive morphometric and biogeochemical analyses so a reliable understanding of factors driving morphological evolution is missing. So, the major aims of our project were 1.) establish a solid chronology and high-resolution paleohydrological reconstruction of the lake via multiproxy analysis of the sediments and gastropod shells of 2012 profile deepened in the central part of the lake 2.) quantify and analyze the overall morphological diversity of melanopsids and neritids 3.) reconstruct the temporal variation of morphological disparity and reveal potential underlying causes relying on the newly established paleohydrological, biogeochemical data. 4.) compare morphometric and isotope geochemical data with rescued specimens from Lake Petea presented by Müller et al. (2014) to understand if and how the morphology of *Mi. parreyssii parreyssii* changed in the modern lake leading to the preservation of a single taxon from the former wide array of morphotypes.

2. Reconstructed chronology and paleohydrology of the lake

The entire material derives from the 8.4 m deep geological profile of the 2012 campaign deepened in the central part of the lake (Sümegi et al. 2012a,b, 2018) (Fig. 1). The bedrock of the sequence consists of limestone pebbles and clayey silt overlain by ca. 2 m thick silt and organic-rich sediments (peaty, clayey silt). This is overlain by ca. 4 m of calcareous marl up to a depth of 2.5 m with thin pebbly, sandy intercalations in its top part. The next unit is a 1 m organic-rich lacustrine sediment with thin silty, sandy marl intercalations overlain by 1 m of clay-rich silty marls.

As part of our work a formerly lacking high-resolution chronostratigraphy was established (Gulyás & Sümegi 2023) as it is essential to interpret the depositional successions in the lacustrine system, decipher millennial-scale fluctuations in the lake level, and analyze the morphospace changes of endemic *Microcolpia* and *Th. prevostianus* gastropods. Macro-botanical remains provided accurate chronology, while aquatic gastropod shells are extremely ^{14}C-depleted with only negligible difference rendering them unsuitable for chronological applications (Gulyás & Sümegi 2023). Low pMC values of similar range reported from shells of spring waters of Nevada (Riggs 1984; McConnaughey et al. 2008; Brennan and Quaide 1997) imply a constant supply of ^{14}C-deficient carbon incorporated into the shells by spring waters feeding the lake from the Lower Cretaceous aquifers. The observed slight upward decrease in pMC values must be attributed to potential aeration which may hint at the gradual shallowing of the lake through time (Gulyás & Sümegi 2023) in line with our other palaeoecological, paleoenvironmental data.

Formerly the Pleistocene/Holocene boundary was placed to a 2 m depth of the profile, where a change from oligotrophic lacustrine marls to eutrophic lake sediments was notable. According to the new chronology, the initial phase of lake evolution started at 17.5 ky cal BP and the uppermost sample at 0.6 m yielded an age of 546 y cal BP. The new results put the mentioned environmental transition to 5.4 ky cal BP (Gulyás & Sümegi 2023) (Fig.1). The newly established chronology provided us with a resolution of ca. 300 years per sample.

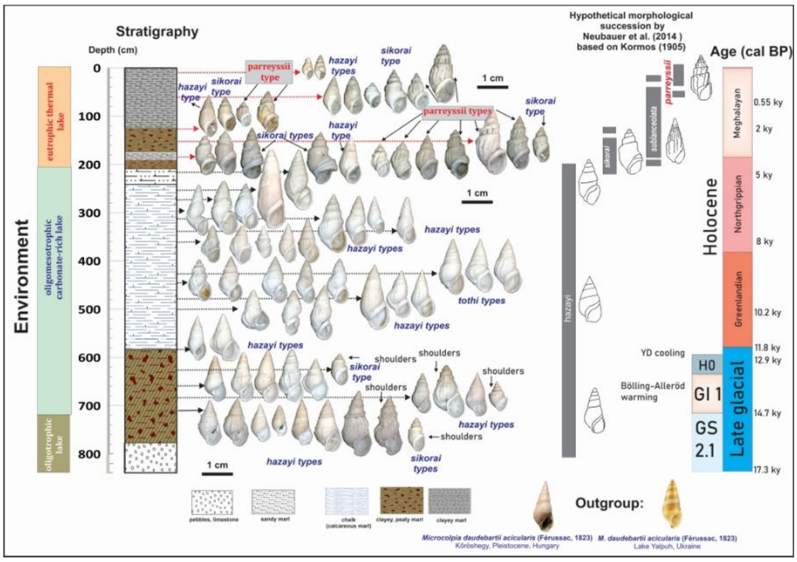

Fig.1. Litho-, chronostratigraphy of the studied Lake Petea sequence with the temporal occurrence of the most prominent endemic *Microcolpia* types and the formerly proposed succession of taxa leading to the evolution of *Mi. parreyssii parreyssii*

Detailed sedimentological and geochemical analysis of sediment samples (grain-size, organic and carbonate content, environmental magnetism marking input of magnetic rich minerals, concentration of small carbonate concretions > 0.5 mm related to underground spring water activity, selected elements marking detrital material input (Al, Ti, Si), temperature (Mg, Ca) or productivity (P)), mineralogical components), as well as selected elements recorded in shells reflecting water temperature changes (Mg/Ca ratio, Sr), increased thermal water input into the lake (As, Pb, Sr), paludification due to rotting of organic matter (S), were used to assess the paleo-hydrological characteristics of the pond. Abundances of shallow water gastropods with a preference for organic-rich, alkaline waters or inhabiting nearshore areas (Sümegi et al. 2012a,b) have been used to mark potential lowered lake levels confirmed by other sedimentological, geochemical proxies as well. One must bear in mind that the profile was deepened in the central part of the lake, so the abundant presence and high values of these shallow water markers in our sequence must truly mark major drops in the lake level. Abundance variations of the thermophilic endemic gastropod *Theodoxus prevostianus* (C. Pfeiffer 1828) hint at water temperatures reaching and or exceeding 20-23°C (Sîrbu & Benedek 2005, 2009; Sümegi *et al*. 2012a, 2018; Vásárhelyi 1956) signaling thermal water input into the lake and rising water temperatures.

Use of stable isotopes recorded in sediments and lacustrine organisms' shells is an essential part of paleolimnological investigations (Leng, 2005). In our work shells of modern (2014) and fossil *Microcolpia* and fossil *Th. prevostianus* were studied ($\delta^{18}O_{shell}$,$\delta^{13}C_{shell}$) in addition to lacustrine calcite and organic matter ($\delta^{18}O_{carbonate}$,$\delta^{13}C_{carbonate}$,$\delta^{13}C_{org}$, $\delta^{15}N_{org}$) (Figs.2,3). Changes in oxygen isotopes of shells and lacustrine carbonate are important proxies for paleoclimate as they reflect changes in the source of water to the lake and/or temperature, precipitation/evaporation ratio. Due to the extremely small size of the lake and its dominant source of thermal groundwater, the isotopic composition must reflect changes related to spring activity as well as water input from precipitation. So, for the evaluation of past oxygen isotope changes recorded in our *Microcolpia* and *Theodoxus* shells as well as lacustrine carbonates we looked specifically for data on modern-day precipitation which is highly depleted in oxygen (-9.9 ‰) (Nicula et al 2021), also isotope data of thermal waters feeding the lake in the area, likewise highly depleted (-10.9, ‰ on average with a range from -12.3 to -10.30 ‰ (Tenu et al. 1981, Nicula et al. 2021). So periodic inputs of thermal water and/or precipitation must result in a negative shift in both lacustrine carbonate and shell carbonate oxygen isotopic values concomitantly with a positive shift $\delta^{13}C_{shell}$ signaling reduced bioproduction. Modern rescued *Microcolpia parreyssii* shells measured as part of the project had significantly higher $\delta^{18}O_{shell}$ values (-10.2‰) than those of the fossil ones and plotted closer to the modern values of thermal waters and precipitation recorded in the region

(Fig.2). Highly negative $\delta^{13}C_{shell}$ values indicated that bioproduction in the modern lake is significantly higher than in prehistoric times. $\delta^{18}O_{shell}$ values of *Theodoxus* and *Microcolpia* clearly overlap and show a similar upward increasing trend to those of $\delta^{18}O_{carbonate}$ and regional speleothem records (Onac 2001, Tamas et al. 2005). Late glacial, early and mid-Holocene samples are heavily depleted compared to younger ones (Fig.2). Likewise, $\delta^{13}C_{shell}$ values are less depleted in the earlier oligomesotrophic part of the lake than in the eutrophic thermal water phase.

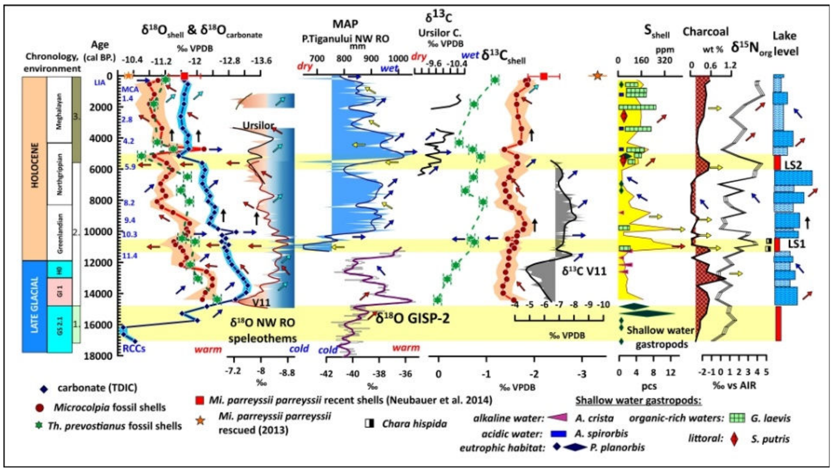

Fig.2. Comparison of geochemical data of shells, lacustrine carbonates, organic matter, charcoal concentrations, abundance of shallow water gastropods with regional speleothem, precipitation proxies, and isotope data for GISP-2

Based on the newly established chronology the temporal evolution of the lake can be divided into three intervals: Zone 1 represents the period from 17 to 14.3 ka cal BP (oligotrophic lake and rivulet), Zone 2 covers the period from 14.3 to ca. 5.5 ka cal BP (oligomesotrophic carbonate-rich lake), and Zone 3 (eutrophic thermal lake) represents the Late Holocene till 546 y cal BP (Gulyás & Sümegi 2023)

The initial phase of the lake's evolution corresponds to the period from 17 ka cal BP to 14.3 ka cal BP.; i.e. the Oldest Dryas. The age of thermal waters in the Cretaceous aquifer is around 20.3 ky cal BP (Tenu et al. 1981), so an initial charge-up of the system must have started after the LGM and should have reached the surface at a later stage. In this part, a shallow lake was formed with a considerable silt input and minimal clay and carbonate content in its sediments. The presence of limestone pebbles at the base of the sequence hints at the activity of a rivulet in creating the lake basin. Shells were highly fragmented or lacking in this interval. Initial $\delta^{18}O_{carbonate}$ values are high (-10.3‰) and are close to the modern precipitation (-9.9 ‰) (Nicula et al 2021). $\delta^{13}C_{carbonate}$ values are low (-6 to -8 ‰) close to characteristic values of river waters recorded in W. Europe (-6 to -10 ‰) (Leng, 2005, Jones 2008) implying that at this stage the only source of water was reduced precipitation and riverine water input. in line with the observed presence of carbonate pebbles. Based on our results it is the only zone where clay minerals of illite and kaolinite appear besides the dominant minerals of calcite, quartz, and muscovite present alone in the other parts of the sequence, marking increased physical and chemical weathering around the lake basin. Elements of detrital origin (Al, Ti, Si) have the highest values, in addition to magnetic susceptibility and clay confirming the input of significant amounts of magnetic mineral-rich sediment into the basin at times of higher rivulet discharge. Highly negative $\delta^{13}C_{org}$ values as well as positive $\delta^{15}N$ values confirm increased bioproduction and lower water levels in relatively dry conditions as organic matter produced by algae thriving in deeper waters is depleted in $\delta^{15}N$. The lack of carbonate nodules and the minimal carbonate content indicates a complete choking of the conduit of the thermal water spring feeding the lake. Sedimentation times are low indicating slow accumulation (22-24 years/cm).

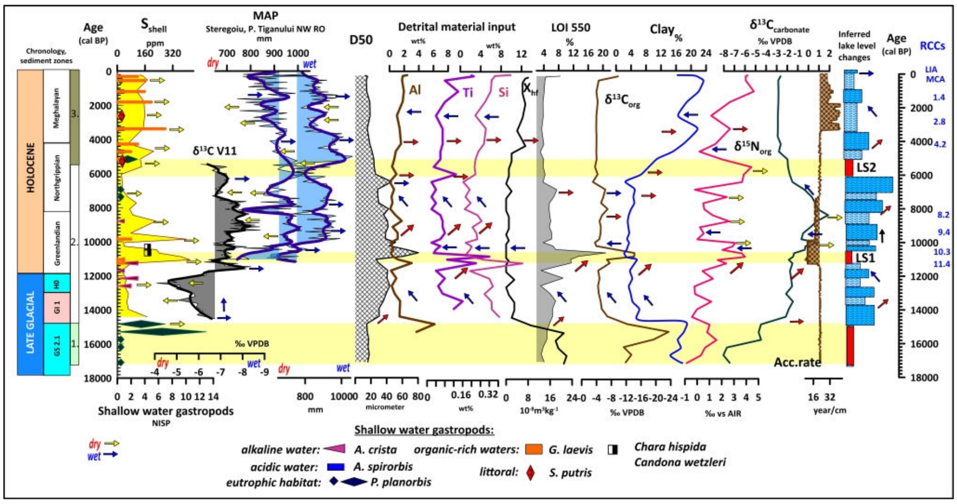

Fig.3. Comparison of variations in geochemical data of shells, organic matter, grain-size and sedimentation rates, detrital input, abundance of shallow water gastropods with regional speleothem, precipitation proxies, inferred lake level changes

There is a stepwise change in all parameters around 14.3 ky cal BP. The stepwise increase in carbonate concentration, average grain size, and Ca, Mg with a parallel significant reduction of the clay content, and increase in the organic content marks the next phase of lacustrine evolution into an oligomesotrophic lake. Wohlfarth et al. (2001) from Preluca Tiganului, NW Romania, obtained a similar age for the beginning of lake sedimentation. $\delta^{13}C_{carbonate}$ values are positively shifted to values ranging between -3 and +2 in the remaining part whole part of the sequence. These are characteristic of karstic water systems enriched in $\delta^{13}C$ within the mentioned range because of carbonate dissolution (Leng, 2005; Jones 2008) marking the first major input of underground thermal waters. The marked negative shift in oxygen isotope values of both shell and sediment carbonate to all-time lows close to marginal values of the Triassic thermal waters (-12.3‰) (Tenu et al. 1981 Nicula et al. 2021) clearly signals the increased input of deep-sourced thermal waters depleted in oxygen due to meltwaters from glaciers. A parallel positive shift of $\delta^{13}C_{shell}$ is also notable owing to the dilution of the water. It is also the period when the first representatives of the thermophilous melanopsids in the sequence appear marking the development of relatively warmer waters with temperatures around 16°C (Sümegi et al. 2012a,b, 2018)] most likely because of the significant warm water input. This transition is coeval with the Bölling/Alleröd interstadial (GI-1). The beginning of GI-1 in the Greenland Ice Core shows a rapid and considerable temperature amplitude increase of 10 °C (Björk et al. 1998, Walker et al. 1999) (Fig.2). The $\delta^{18}O$ values of speleothems from NW Romania (Tamas et al. 2005) and the growth intervals of speleothem in Scărişoara Ice Cave (Onac 2001) also indicate a rapid warming phase initiating at ∼14.8 ka cal BP in the area. This warming trend is notable throughout Central Eastern Europe (Feurdean et al 2014). Coevally increasing annual and summer temperatures are also seen in other pollen records from the region of NW Romania (Feurdean et al. 2008, 2014) and chironomid-based reconstruction of summer temperatures in the South Romanian Carpathians indicating an increase of ∼2.8 °C in summer air temperature during the same transition (Tóth et al. 2012). The emergence of warmer humid conditions must have resulted in the melting of local icefields, increased precipitation, and an elevated recharge of the groundwater system resulting in higher discharge of the thermal springs in the area supplying warm waters to the lake. The sudden increase in the concentration of carbonate concretions and steady input of coarser grain fractions signals the reopening of the conduit of the underlying spring. It is also the time when the first peaks of thermal water sourced As, Sr, Pb appear in shells. The concentration of elements marking detrital material input decreases, a strong positive shift of $\delta^{13}C_{org}$ values and negative shift of $\delta^{15}N$ in addition to the disappearance of shallow water gastropods signals a deepening of the lake.

In the interval dated to the Younger Dryas (HO) an increase in charcoal concentrations in our lake marks dry conditions corroborated by positive $\delta^{13}C$ values of nearby speleothems leading to lowered lake levels signaled by the appearance of shallow water gastropod taxa. The $\delta^{18}O$ values of speleothems from NW Romania (Tamas et al. 2005) are negatively shifted signaling cooling, while our shells and lake carbonate show a positive shift along with a decrease in thermal water sourced As, Pb and Sr in the shells and the abundance of the thermophilous gastropod *Th. prevostianus*. $\delta^{13}C_{carbonate}$ turns negative also signaling a reduced spring activity and the supply of $\delta^{18}O$ depleted thermal waters due to a drop in the groundwater levels.

The next marked change is dated between 11.7 and 11.4 ky cal BP with a slight negative shift in our oxygen isotope values, a positive shift in carbon isotope values. $\delta^{13}C_{carbonate}$ turns back, a second peak of thermal water sourced As, Pb and Sr is notable after the one at GI-1, with an increase in small carbonate concretions and the abundance of the thermophilous *Th. prevostianus* marking renewed spring activity. This must have resulted in a slight increase in the lake level. This period coincides with a rapid increase in precipitation connected to the 11.4 ka event also recorded in the negative shift of carbon isotope values of speleothems from NW Romania (Tamas et al. 2005). This rapid precipitation increases not only resupplied the underground springs with water but also increased nearby fluvial activity and erosional material into the already shallow lake as marked by the highest peaks of detrital elements (Al, Si, Ti) towards the end of the period. There is a stepwise change in sedimentation to higher accumulation rates marking increased sediment input resulting in a rapid shallowing of the lake leading to the emergence of a marked low stand, one of the largest in the lake's history (LS1) between 11.4 and 10.4 ka cal BP corroborated by the peak of organic-rich water preferring shallow gastropods, the largest peak in shell sulfur indicating intensive paludification at a time when according to regional paleoclimatic and paleohydrological data marked dry conditions develop. Early Holocene records for the lowlands of NW Romania show high summer temperatures (by ca. 4°C) and ca. 33% lower precipitation rates (Feurdean et al. 2008, 2014). High fire activity and a lowering of the lake level at Ştiucii Lake, and lower MAP inferred from the pollen records of the crater lakes of Steregoiu and P. Tiganului in NW Romania (Feurdean et al. 2012,2013) also indicate decreasing moisture availability in this interval. Speleothem records both in the southern and northern parts of the region (Tamas et al. 2005, Onac et al. 2002) indicate a gradual warming trend from ~ 11.5 ka into the Holocene (Figs. 2,3). Regional records also show a decrease in peat surface moisture as well as lake levels in NW and E Romania (Feurdean et al. 2008, 2014). A shift in river activity characteristic of lower discharge and related lower amount of precipitation again indicates the emergence of warmer and drier conditions (Persoiu 2010, Howard et al. 2004). The highest peaks in organic matter, negative $\delta^{13}C_{org}$ values as well and a peak in clay further underlie the scenario of a marked low stand. A positive shift in $\delta^{18}O$ of shells, and a reduction in As, Pb, Sr marks the periodic cessation of the thermal water supply. In this stratigraphic interval, a marked accumulation of macrocharcoal is noted. In addition, it is the only interval when shallow water *Chara hispida* remains appear (Benyó-K et al. 2020, 2023 a,b). These remains are completely lacking from the rest of the sequence indicating that strikingly shallow conditions during the oligomesotrophic phase appeared only during this low stand and higher water levels were not suitable onwards for the living. The presence of the ostracod *Candona wetzleri* also confirms this scenario (Bóni 2022a,b).

After this short interval there is a very pronounced stepwise shift in all parameters ($\delta^{18}O$ shell to negative, $\delta^{13}C_{org}$ and $\delta^{13}C$ of shells to positive, $\delta^{15}N_{org}$ to negative, As, Pb, Sr increase, organic matter rapidly decreases as well as concentration of detrital elements) from ca 10.4 ky cal BP marking increased precipitation and thermal water input into the system most likely connected to the humid conditions of the 10.3 ka event and a rise in the water.

In the remaining oligomesotrophic lake phase up to 5.4 ky cal BP a gradual decrease in clay content, the concentration of detrital elements, reduced charcoal and shell sulfur concentrations, low organic matter content, the disappearance of shallow water gastropods and characea marks a slow gradual increase in the water table in this interval and the emergence of a deeper water carbonate-rich oligomesotrophic lake as a result of increased precipitation. The carbonate content of the lake increases reaching peak values around 6 ky cal. BP. Iterative increases in the carbonate content, shell Ca, and sediment Ca values as well as concentrations of minor carbonate nodules in the sediment are accompanied by iterative abundance increases of the thermophilous gastropod *Theodoxus prevostianus* indicate that these periods of increased carbonate input must represent increased warm water discharge into the lake, creating ideal conditions for the thriving of the mentioned warm water gastropod taxa. Elements of strictly natural origin enriched in thermal waters (As, Sr, Pb) also show a strong correlation with the mentioned sedimentological parameters and periodic negative shifts of oxygen isotopes indicating the supply of depleted waters into the lake.

Oxygen isotopes increase up to about 8 ky cal BP, $\delta^{13}C_{shell}$ also, Mg/ Ca ratio and thermal water sourced Pb, As, Sr remains low and constant. The abundance of the thermophylous *Th. prevostianus* reaches its all-time minima indicating relatively stable water levels and lower temperatures. A change is notable after 7 ky cal BP with oxygen isotopes gradually turning negative again. Thermal water sourced Pb, As and Sr in the shells as well as Mg/Ca ratios, and carbonate content show a stepwise increase from here marking intense thermal water input into the lake and rising water levels and temperatures (Figs 2,3 .). Reduced charcoal accumulation rates in Lake Stiucii and T. Muced (Feurdean et al. 2012,2013, 2014) marked an overall decrease in fire activity. The gradual decrease in oxygen and carbon isotope values of nearby speleothem records in the Apuseni Mts (Tamas et al. 2005, Onac et al. 2002) well as the high MAP values reconstructed for the nearby peatlands Feurdean et al. 2012,2013, 2014) (Figs. 2,3) mark the emergence of cooler and wetter conditions offering a continuous supply of water into the underground karst system and ensuring constant water input into the lake and the maintenance of relatively higher water levels (Figs.2,3). At 6.3-6 ka cal BP an increase in concentrations of carbonate concretions, abundance of the thermophilous *Th. prevostianus* along with a negative shift in $\delta^{18}O_{shell}$ and positive shift in $\delta^{13}C_{shell}$ which may indicate renewed warm water input into the lake. Our record also shows an increase in detrital material input. The coeval increase in MAP at Steregoiu (Feurdean et al. 2012,2013) and decreases in oxygen and carbonate isotope values of Apuseni Mts speleothems (Tamas et al. 2005, Onac et al. 2002) hint at increased availability of moisture in this interval.

Another charcoal peak turns up in our records between 5.5-5 ka cal BP marking renewed fire activity (Fig.5) The abundance peaks of the eutrophic shallow water gastropod taxa *A. spirorbis* and the littoral habitat preferring *S. putris* , a marked positive peak in $\delta^{13}N_{org}$ values and a rapid negative shift in $\delta^{13}C_{shell}$ and positive shift in $\delta^{18}O_{shell}$ as well as increasing shell S values mark the development of shallow water conditions, increased paludification under drier conditions. (Figs.2,3). This major low stand (LS2) is the second after the first one between 11.4 and 10.3 ka cal BP. Low lake levels and reduced river activity were documented between 5.5 and 5.3 ka cal BP in the Eastern Carpathians and the Transylvanian Plain too (Persoiu 2010, Howard et al. 2004). A general increase in fire activity and dry peat surface conditions was identified between 5.5 and 4.8 ka cal BP in the Romanian Carpathians (Feurdean et al. 2012,2013). Warmer/drier climatic conditions were also reconstructed around 5.5 ka cal BP based on speleothem isotopes from the Apuseni Mountains (Tamas et al. 2005, Onac et al. 2002) and on pollen-based quantitative climate reconstructions in the Gutâiului Mountains, NW Romania (Feurdean et al. 2012,2013) (Figs. 2,3).

After ca. 4.5 ka cal BP there is a marked decrease in the D50 values, nitrogen isotope values a sharp increase in the concentration of small carbonate concretions and the abundance of *Th. prevostianus* (Figs. 2,3). accompanied by the complete disappearance of the eutrophic shallow water gastropod taxa. One of the most prominent negative $\delta^{18}O_{shell}$ peaks also hallmarks the strong input of thermal water and a rapid lake level rise giving birth to the eutrophic thermal lake. The sharp decrease in charcoal concentrations in our deposits here is also notable. There is an abrupt rise in the lake levels of the Eastern Carpathians from 5.3 ka cal BP (Magyari et al. 2009) and the Transylvanian Plain from 5 ka cal BP too (Feurdean et al. 2012,2013). A marked negative shift in oxygen and carbon isotopes of the Ursilor cave speleothem here with all-time low values around 4 ka cal BP marks the emergence of cooler and wetter conditions (Figs 2,3). Cooler and wetter conditions associated with the 4.2 kyr event (Mayewski et al. 2004) have been widely documented in the region by various records of fluvial activity, oxygen isotopes (Persoiu 2010, Onac et al. 2005), chironomid-based summer temperature (Tóth et al. 2012) and mire surface wetness reconstructions (Galka et al. 2016)

From ca. 3 ka cal BP there is a marked increase in the sedimentation times to all-time high in the entire profile (44 cm/y) (Figs.2,3). This is accompanied by a shift to a dominance of finer grain-size classes with a marked increase in the clay content. The concentration of small carbonate concretions also decreases, $\delta^{18}O_{shell}$ increases with a concurrent positive shift in carbon isotope values marking increasing temperatures, and eutrophication is also signaled by gradually increasing S values in the shell and the abundance of shallow water gastropods. It refers to the emergence of a shallow (1-2 m deep), eutrophic lake (Figs. 2,3). This drop in the lake level might be partially attributed to the emergence of drier conditions also seen in the increase in charcoal concentrations in the sediment marking intensifying fire activity in the neighboring areas. A high peat decomposition and elevated charcoal accumulation rates at 2.7 ka cal BP at the Tăul Muced peatbog NE Romania suggest also dry climate conditions. High fire activity and a lowering of the lake level at Ştiucii Lake, higher MAT and lower MAP inferred from the pollen records of the crater lakes of Steregoiu and P. Tiganului in NW Romania as well as high carbon and isotope values in Ursilor Cave speleothems at the Apuseni Mts, NW Romania also indicated decreasing moisture availability around 2.8 ka cal BP (Tamas et al. 2005, Onac et al. 2005, Feurdean et al. 2012,2013) (Figs. 2,3). This change is linked to reduced solar activity inferred at 2.8 ka from many peatlands in NW Europe (Galka et al. 2013). Palaeohydrological data from a wider regional scale in Europe (Poland, Germany) show dry climate conditions during this time (Swindles et al. 2007).

After 2 ka cal BP speleothem data from Ursilor Cave (Onac et al. 2005) indicate a cooling of the climate with the development of wetter conditions similar to wet surface mire conditions at Tăul Muced peatbog NE Romania (Feurdean et al. 2012,2013). This increase in moisture availability must have contributed to a slight rise in the lake level at Lake Pețea as seen in a stepwise decrease in the accumulation times to 26 year/cm (Figure 5). A parallel increase in the concentration of small carbonate concretions, a slight sand input and rise in detrital elements, and a minor rise in the abundance of the thermophilous gastropod *Th. prevostianus* here may hint at the increased water discharge of the underground hot springs feeding the lake coevally with the 1.4 ka event (Figs. 2,3). The last peak of shallow water gastropods and shell S and other isotopes marking dry conditions and a lowered lake level are dated to the period of the MCA. After that, all proxies point to the development of humid conditions and slightly increased water levels (Gulyás &Sümegi 2023).

3. Delineation of morphotypes of *Microcolpia*

Morphological variation of the *Microcolpia* types without reference to any previously described taxa was assessed using shell outline analysis (EFA) as well as traditional morphometric techniques relying on a mass database of 100 specimens per sample totaling around 3600 studied and quantified specimens. Besides the determination of major shape traits (PCs), quantified parameters included proportional density of the shells and shell thickness derived from micro-CT analyses. Also, shell size, whorl expansion rate and traditional length and angle-based morphometric parameters have been recorded enabling comparison with recent specimens, where only such parameters were available (Müller 2014, 2018).

Three major components (PCA) have been identified to accurately capture over 80 % of shape variation (Figs.4,7). The most important shape component (PC 1) capturing 68.6 % of the shape variation is related to the globularity of the shells (Figs 3,4.). Shapes with negative PC 1 eigenvalues are characterized by low apical angles and slight, equal inward displacement of the outline points of the spire on both sides compared to the consensus shape resulting in more slender shells. A slight widening of the middle part of the body whorl is notable, while the original curvature is preserved. A counterclockwise rotation, as well as the narrowing of the sides of the aperture, is also visible here (Fig.4).

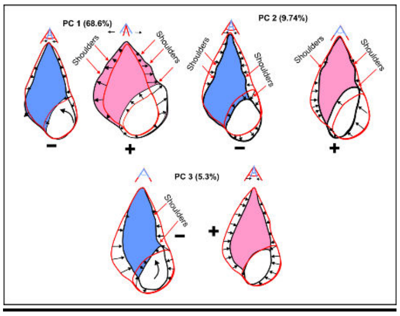

Fig.4.Shape deformations compared to the average shape (red) along the main axes (PCs) of shape variations

Shapes with positive PC 1 eigenvalue have wider apical angles and are characterized by a concomitant widening of the spire and the body whorls with a relatively equal outward displacement of the outline points compared to the consensus shape creating more globular and bulky shells (Fig.4). The body whorl remains arched while the sides of the lower part of the spire are somewhat flattened resulting in the appearance of shoulders. Parallelly an equal upward and sideward stretching of the aperture is also notable. PC 2 mainly describes shape differences of the body whorl (Fig.4). Here shapes with negative eigenvalues display a larger reduction of the apical angle compared to the consensus shape resulting in more compressed spires. A sidewise widening of the aperture and downward displacement of the lower half of the outer lip is also notable (Fig.4). In shapes with high positive PC 2 eigenvalues, the apical angle of the consensus shape is preserved while a clear flattening of the flanks of the body whorl and the penultimate whorl is visible marking the development of a strong shoulder just above the aperture and on top of the body whorl. Parallelly with the flattening of the flanks of the body whorl a sideward compression of the aperture is notable resulting in more elongated apertural areas (Fig. 4).

Finally, the third shape component (PC 3) giving 5.3 % of shape variation captures minor variations in both the body whorl and the spire (Fig.3). Shapes with negative eigenvalues preserve the apical angle of the consensus shape and there is an equal flattening of the sides of the body whorl as well as the lower part of the spire. This brings about the emergence of a strong shoulder just above the apertural area. The aperture is more compressed and slightly rotated counterclockwise. Shapes with positive PC 3 eigenvalues are characterized by a uniform inward displacement of all outline points compared to the consensus shape creating slenderer spindle-like shapes (Fig. 4). As seen on the dendrogram and scatterplots (Fig.5) two well-separated clusters with a minor overlap are present corresponding to the bulky shouldered forms with varying degrees of shouldering (Group1), and the slender, elongate spindle-shaped forms (Group 2). The more bulky, low-spired forms of both groups are generally smaller than the elongated high-spired forms implying that this type of shape variation is allometry-controlled. The observed minimal morphospace overlap between the two major subfossil groups (Group 1, 2) is attributed to such shapes in Group 1, where the spire is generally higher but shouldering is less pronounced yielding spindle-shaped outlines. These ribbed or smooth forms closely resemble the taxa described under the names of *Me. hungarica* and *Me. sublanceolata* by Kormos (1903, 1905) (Fig. 1). Subgroups represent various morphotypes distributed relatively linearly along the axis of the most important shape parameter expressing the globularity of the shells.

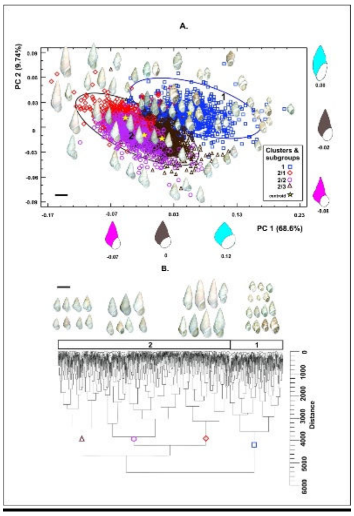

Fig.5.Identification of major morphotypes via cluster analysis and their distribution in the morphospace

Variations in shell globosity appear to be allometry-related, with smaller specimens being bulkier and lower-spired while larger specimens being elongated and relatively higher-spired. This allometric trajectory is applicable to both major subfossil groups. Axial ribs and keels, originally considered to have emerged in the second step of evolution by Neubauer et al. (2014), appear quite randomly in morphotypes of both Groups. Ratio of morphotypes with spiral striae and keels and smooth ones for Group 2 is the same. The appearance of similar keels was also recorded on thermal water melanopsid *Microcolpia daudebartii doboi* (Schréter, 1975) with elongated, high-spired, spindle-like shapes from Early Pleistocene deposits of Eger, Hungary (Schréter 1975; Sümegi et al. 2012 a,b, 2018) pointing to the presence of potentially similar factors influencing the development of these structures.

Group 1 unites all slightly or extensively shouldered high and low-spired mostly ribbed, subordinately smooth-shelled morphologies with spiral striae present on some specimens. So the two distinct major morphotypes in our view may be regarded as separate taxa in line with the observations of Tóth (1891) and Kormos (1904, 1905a,b). But separation of the subgroups as individual taxa must be refuted.

The co-occurrence of the mentioned morphotypes in the same stratigraphic horizons and random distribution lacking any major trend along the profile (Fig.1) imply that their development is not due to true phyletic evolution but rather direct modifications of the phenotype by environmental stimuli.

Based on the large-scale similarity of specimens of Group 2 to the morphotype of *Me. hazayi* Brusina, 1903 illustrated by Kormos (1905) relying on Brusina (1903)'s description and the marginal morphospace overlap with the specimens of *Mi. daudebartii daudebartii* (Prevost 1821) but isolation from *Mi. daudebartii acicularis* (Férussac 1823), the introduction of the taxonomic name *Mi. daudebartii hazayi* (Brusina 1903) is proposed. Members of Group 1 described as *Mi. parreyssii parreyssii* (Philippi 1847), *Me. sikorai. Me. hungarica, Me. sublanceolata* must be regarded as varying phenotypes of extant Mi. parreyssii parreyssii (Philippi 1847). The shouldered ecophenotypes of *Mi. parreyssii parreyssii* (Philippi 1847) are most abundant in the upper 2-2.5 m of the geological profile corresponding to the late Holocene(Fig.1). Ribbed, smooth as well as smooth-keeled specimens regarded originally as *Me. sikorai* Brusina (sikorai type in Fig.1) and *Me. sublanceolata* appear collectively with typical forms of extant Mi. parreyssii parreyssii (Philippi 1847) in the entire upper 2 meters corresponding to the eutrophic thermal lake phase (Fig.1). Smooth, spindle-shaped, high-spired *Mi. daudebartii hazayi* (Brusina, 1903) is also present, though highly subordinately (Fig.1).

The multiple presence of shouldered smooth-shelled morphotypes of Mi. parreyssii parreyssii (Philippi 1847), though minimally (2-3 %), in the lowermost samples dated to the Late Glacial is striking (Fig.11) as it questions the idea of gradual continuous two-step evolution postulated by Neubauer et al. (2014) from entirely smooth and elongated shapes to shouldered and keeled shells around the Pleistocene-Holocene boundary followed by the second step of transition of keeled and shouldered specimens into more compressed heavily shouldered forms with strong sigmoidal keels. The clear collective presence of more compressed heavily shouldered shells with dominantly smooth and elongated ones from the very start of lake evolution dated to the Late Glacial may hint at the prevalence of special conditions enabling the coeval development of these morphotypes. Melanopsids appear in Late Glacial horizons with *Theodoxus prevostianus* preferring temperatures above 23°C that may indicate the subordinate presence of hot water microhabitats in cooler waters.

A comparison of our morphometric data with recent specimens from Lake Petea presented by Müller et al. (2014) helped to understand how the morphology of *M. parreyssii parreyssii* changed in the modern lake. As we have seen the smooth, elongated, spindle-shaped M. daudebartii hazayi, though subordinately, is collectively present in the topmost part of the profile with the dominant shouldered smooth, ribbed morphotypes of *Mi. parreyssii parreyssii* after which it disappeared (Fig.1).

Based on our results modern *Mi. parreyssii parreyssii* living in the lake is significantly larger with wider apertures, a smaller aperture area than its subfossil representatives. There are also numerous subfossil specimens displaying extreme morphologies (downward displacement of the aperture, lip thickening, distortion of the last whorl and aperture) (Fig.1). All in all, this tends to show that the morphology of recent *Mi. parreyssii parreyssii* must have stabilized with a general increase in size.

4. Assessment of temporal morphological variation of *Microcolpia* and *Theodoxus prevostianus* and its potential causes in light of inferred paleohydrological changes

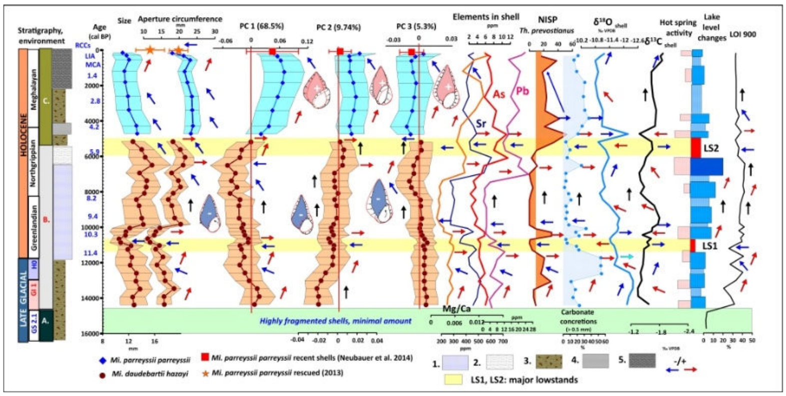

Fig.6.Temporal variation in shape and size parameters in light of selected paleohydrological proxy data

Globose shells (+ PC1) are restricted to GI-1 and the upper part of the last ca. 5 kys. Stepwise changes are notable after LS1 in all shape and size parameters but transition in terms of globularity seems continuous with minor shifts observable only at PC2, PC3, and aperture circumference. In the oligotrophic lake there is an upward decrease in globosity along an increase in size leading to the development of larger shells (Figs.1,6). Globosity (PC1) seems to show a similar trend recorded by parameters marking increased warm water input ($\delta^{18}O_{shell}$ and As, Sr, Pb in shells, abundances of *Th.prevostianus*, carbonate concretion concentrations) and Ca availability (Mg/Ca in the shells) mainly driven by provision of thermal water being the major source of these elements. As seen on PC2 apart from the GI-1 horizon, where globose shells are present shapes stay elongated with more globular sides of the body whorl close to the consensus shape. A shift to flattened flanks is notable in the upper part. PC3 also stays relatively constant close to the consensus shape during the oligotrophic phase, a marked negative shift to more elongated body whorl types is noted again in the upper part.

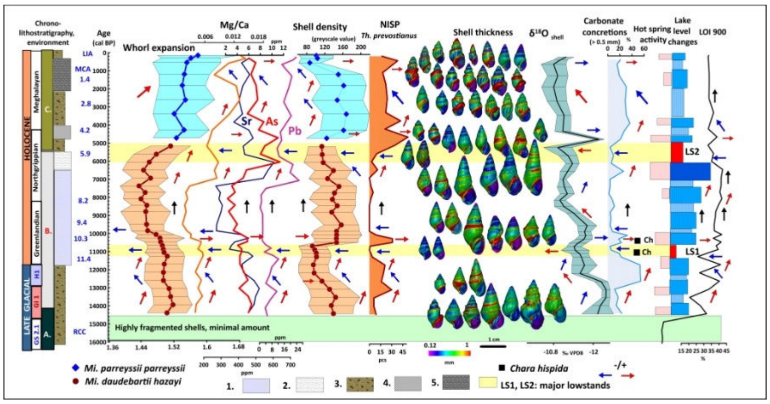

Fig.7. Temporal variation in shell density, whorl expansion and shell thickness of the two major *Microcolpia* taxa in light of paleoenvironmental data

While there is a considerable shift in whorl expansion rates from *Mi. daudebartii hazayi to Mi. parreysi*, variation of shell densities remains in the same range. A stepwise change in densities is notable at LS1 but no such major shifts are recorded in Wlog values. Variation across the LS2 boundary marking the transition to the eutrophic lake phase also appears to be gradual. Whorl expansion rates tend to show a strong positive correlation with proxies signaling increased thermal water input ($\delta^{18}O_{shell}$ and As, Sr, Pb in shells, abundances of *Th.prevostianus*, carbonate concretion concentrations) and Ca availability (Mg/Ca in the shells). Shells of both taxa are generally loosely coiled when warm water input increases and Ca decreases with an increase in Mg and vice versa. The coiling ratio is negatively correlated with shell. An expansion of the whorl rate allows for the conservation of calcium carbonate (Raup 1961) via changing the surface-to-volume ratio of the shell also seen in the decreased densities of our shells. Changes in whorl expansion also follow the same trend as the most important shape trait of globosity (PC1). Namely slender, elongated loosely coiled shells must have formed in relatively Ca-deficient environments, while the construction of thick shells and ribs, and keels is possible in Ca and Mg-rich waters. So, this seems to be the main driving factor of the shell morphological evolution and shell density as well as the shell thickness. Plastic shell responses are not limited to shell thickening via increased carbonate but altering shell shapes too like the aspect ratio (DeWitt 1998; DeWitt el al. 1998; Hollander et al. 2006; Bourdeau 2009; Bourdeau et al. 2015) as this may not require additional investment in shell deposition just a reallocation of shell materials allowing for the development of adaptive shell forms. This may hint at the development of bulkier forms at times of increased Ca and Mg input.

The same pattern in visible for the thermophilous *Th. prevostianus* with shell parameters showing a close correlation with Mg/Ca values of the shell and other parameters indicating increased warm water input. Size, lip thickness is positively correlated, while both have contrasting trends with shell elongation. A stepwise increase in size and the development of loosely coiled compressed, less elongated forms during the phase of the eutrophic thermal lake follows similar patterning to Microcolpia attributable to the formerly mentioned plastic shell responses to environmental change.

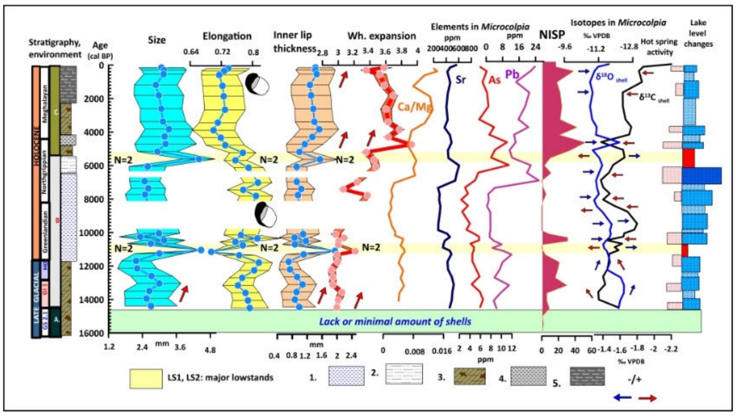

Fig.8. Morphological variance of *Th. prevostianus* in light of main paleohydrological proxies

Morphological variance of *Microcolpia* (Fig.9) is likewise connected to the above-mentioned processes with variances increasing at times of increased warm water input. Two major intervals of the low stands are clearly marked by extremely reduced variation besides many other small ones signaling the presence of two major bottlenecks.

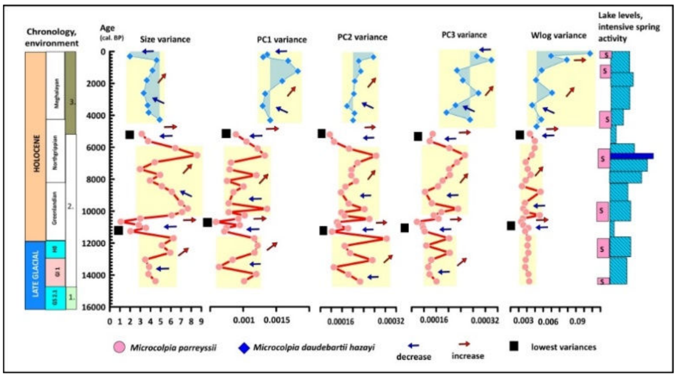

Fig.9 Changes in morphological disparity with lowest variances marked at times of major lowstands and decreased Ca and Mg availability

5. Concluding remarks, use of results in other habitat conservation strategies

Based on our results, the lake evolution was mainly controlled by major climate-driven hydrological changes also seen in regional paleoclimatic and paleoecological records (Figs 2-3) (Gulyás & Sümegi 2023). Our results clearly testify how sensitively our small-size lake system responded to minor and major rapid climate change events (RCCs) of the Holocene (Mayewski et al. 2004). The hydrological changes were mainly controlled by varying input of thermal water into the lake due to recurring increased/decreased recharge of the underground shallow karst water system. Major precipitation events mainly controlled nearby erosion and detrital material input into the lake, which was especially prominent when waters were relatively shallow and succeeding extreme dry events lead to the preservation of major low stands, extreme rise in eutrophical conditions and paludification at 10.5 ky cal BP and 5-5.5 ky cal BP after the 11.4 and 5.9 ka RCCs. Similar major transformations in the carbonate lakes of Hungary have been recorded around 10 ka and 9.7 ka. as part of our work too (Vári et al. 2023; Alzoubi et al. 2023). The driving factor of underground thermal water discharge was different though during the Late Glacial than the Holocene. During the Late Glacial, it was the warming of the climate at 14.5 ka cal BP connected to the Bölling/Alleröd interstadial that dominantly created increased recharge of the system via melting of regional ice sheets leading to activation of thermal water sources from the deeper Triassic aquifers providing oxygen-depleted waters as seen on initial extreme negative oxygen isotope values of the shells. Conversely, during the Holocene increasing/decreasing moisture availability was in control and lake level changes mainly reflected the effects of the rapid climate change events (RCCs) of the Holocene (Gulyás and Sümegi 2023). Warm water input through the provision of Mg and decreasing Ca rates had a major influence in creating morphological variability, as morphological variability was largest at times of major warm water input and higher lake levels. In other times diversity was relatively reduced. The largest reductions occurred at the time of the mentioned major low stands, which were quickly followed by thermal water pulses because of rapid recharge of the spring system in successive RCC-related humid periods (e.g. 10.3 ka, 5.9, 4.2 ka events). Low stands truly created major bottlenecks reducing genetic variability seen also in the appearance of extreme morphologies (Fig.1- @ 2 and 5 m depths) during the first stages of rapid climate meliorations (humid periods). Based on our findings *Microcolpia* and *Theodoxus prevostianus* responded mostly similarly and morphology is clearly environment controlled mainly through the availability of Ca and Mg in shell construction owing to varying thermal water input as well as lake level changes. Accordingly, all major and minor morphotypes must correspond to various ecophenotypes of the ancestor form. If we wish to separate the two major morphotypes this should be done at the subspecies level degrading *Mi. parreyssii parreyssii* to *Mi. daudebartii parreysii*.

The results provide important data for the conservation of the only remaining domestic (Kács) and 3 European habitats (Bad Vöslau, Bad Fischenau, Pesnica) of the endemic highly endangered domestic and European thermal water snails (*Microcolpia*, *Theodoxus prevostianus*), showing how these species have responded to paleohydrological changes caused by past rapid climate changes. Similar complex studies have not yet been carried out in these habitats, but our results suggest that they are of paramount importance. The coherent methodology and paleoecological and paleobiological research developed and applied here can thus serve as a basis for future essential research of this kind. In an environment with a warming climate and a hectic rainfall distribution, which will have a fundamental impact on thermal water supply, in addition to the human impact of diminishing supplies caused by water exploitation, artificial habitat conversion will be a particularly important aspect. Our results show that these species, which have existed since the Ice Age, have reacted and adapted sensitively to century-scale and millennial changes in the lake, producing extreme morphological variation that has allowed only the preservation of a single species (*Mi.daudebartii parreyssii*). The current rapid changes on a large scale may affect their future even more, especially if their habitat is reduced, eliminated or modified by human impact, as the recent disappearance of our endemic species due to the drying up of Lake Petea caused by the dwindling recharge from excessive water extraction or the disappearance of our endemic gastropod species due to habitat modification caused by canalization in the nearby Rabagan area (2016, 2017) is a good example to testify to present and future generations.

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