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**Mgr. Filip Scheiner**

*Geochemická data ze schránek foraminifer a jejich možnosti v rekonstrukci paleoprostředí:  
případová studie z miocénu Centrální Paratethydy*

*Geochemical markers from foraminiferal tests as a tool for reconstruction of  
paleoceanological environments: a case study from the Miocene of the Central Paratethys*

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Doc. RNDr. Katarína Holcová, CSc.

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Filip Scheiner

## Abstrakt

Tato práce se zabývá použitím různých geochemických proxy na foraminiferách v oblasti fosilního epikontinentálního moře - Centrální Paratethydy v období langhu a následnými na nich založenými paleoceanografickými, paleoekologickými a paleoenvironmentálními interpretacemi. Jsou zde dopodrobna popsány a diskutovány použité metodologické postupy jako například analýza izotopů uhlíku a kyslíku na jednotlivých schránkách foraminifer, které byly speciálně vybrány v závislosti na problematice studovaného prostředí. Další z řady použitých geochemických proxy jsou paleotermometrie založená na poměru Mg/Ca ve schránkách foraminifer a organické proxy z celkové horniny sestávající se z analýzy izotopů organického uhlíku, kalkulace základních n-alkanových indexů a analýzy celkového obsahu organického uhlíku. Tyto metody byly následně kombinovány spolu s paleoekologickými daty týkající se foraminifer, což umožnilo definici jednotlivých vodních mas ve studované oblasti spolu s identifikací cirkulačních schémat/režimů, které panovaly v Paratethydě v období langhu. Dále bylo také interpretováno množství lokálních paleoekologických a paleoenvironmentálních implikací. Ukázalo se, že Paratethyda měla totožnou hydrografii povrchových vod s oblastí Středozemního moře na rozdíl od odlišné hydrografie vod spodních. Paratethydní spodní vody byly zřejmě regionálního původu a procházely svým vlastním vývojem ve studovaném časovém období. Anti-estuáriový cirkulační režim byl základním cirkulačním schématem v oblasti Paratethydy ve studovaném časovém intervalu, což pravděpodobně souvisí s událostí uzavírání Indicko-Mediteránního průlivu, které jako takové ovlivnilo i vývoj cirkulačního režimu v Mediteránu samotném. Teploty mořské vody poukazují na podobu studované oblasti s dnešními subtropickými regiony a vývoj teplot vykazuje spíše stabilní trend navzdory očekávání mírného ochlazení v tomto časovém období po středně miocenním klimatickém optimu. Centrální Paratethyda představovala spíše širomořské prostředí s převahou marinní primární produkce. Fluktuace fyzikálněchemických podmínek na mořském dně působily jako hybatel paleoenvironmentálních změn, které značně ovlivnily populační dynamiku bentických foraminiferových společenstev.

## **Abstract**

This thesis deals with the use of geochemical proxies on foraminifera for paleoceanographical, paleoecological and paleoenvironmental interpretations in the fossil epicontinental sea - the Central Paratethys during the Langhian. It discusses the used methodologies and approaches that were specially chosen to fit the problematic of the studied area such as the single test analysis of carbon and oxygen stable isotopes on foraminifera. Other geochemical methods were represented by Mg/Ca based paleothermometry and by several organic geochemistry proxies on whole rock samples (n-alkane indices,  $\delta^{13}\text{C}_{\text{org}}$ , and carbon ratios – TOC/TIC/TC). These were further combined with foraminiferal paleoecological data, which allowed identification of particular water masses in the studied region as well as the prevailing circulation patterns/regimes during the studied interval in the Paratethyan marine realm. Additionally, there were interpreted various regional paleoenvironmental and paleoecological consequences. The Paratethys had similar hydrography of surficial waters with the Mediterranean, conversely to the bottom waters that were different, probably of a regional origin with their own evolution during the studied time interval. The anti-estuarine circulation regime, which was probably linked with the closure of the Indian-Mediterranean gateway that also affected the Mediterranean circulation patterns, was the basic pattern in the Paratethyan marine realm during this time interval. Moreover, the seawater temperature points to a similarity with modern subtropical regions and there is a minor warming trend present despite the expected cooling in the time interval after the Middle Miocene Climate Optimum. Lastly, the Central Paratethys represented a rather open marine environment with dominant marine algal productivity and with changes in paleoecological conditions at the seafloor caused by fluctuations in physicochemical conditions that affected the population dynamics of benthic foraminiferal communities.

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## 1. Introduction

The Central Paratethys was a part of a large system of epicontinental seas - the Paratethys - located from Western Europe up to the inner Asia. This system evolved during the Eocene-Oligocene and ceased to exist in the Late Miocene (Rögl, 1999; Popov et al., 2004). This marine realm was characterized by episodic activations and closings of gateways enabling the Paratethys, or to its individual parts, a communication with the adjacent Mediterranean Sea and with the Atlantic and Indian Ocean (Kouwenhoven and Van der Zwaan, 2006). The Paratethys was composed of the Western, Central and the Eastern part and during different geological time intervals the individual basins were either isolated or connected with the surrounding marine and oceanic realms based on the existing paleogeographical situation (Rögl, 1999). Moreover, this system experienced a broad variety of fluctuations regarding prevailing paleoenvironmental parameters such as temperature or salinity as well as variations in physicochemical composition of seawater (e.g. Karami et al., 2011; Vara et al., 2013; Kováč et al., 2017; Scheiner et al., 2018). This epicontinental marine system has no exact equivalent at present thus representing a unique fossil environment.

The Central Paratethys, which was chosen as our study area, represents a part of this system located in Central Europe (Rögl, 1999). Our studied time interval is ~ 14.5 - 13.8 Ma, which corresponds with the upper part of the Langhian period, which is a part of the Middle Miocene (Holcová et al., 2015; Ogg et al., 2016). During this time interval, the Central Paratethys was mainly influenced by the Mediterranean Sea thanks to an open connection between the Paratethys and the Mediterranean, which is interpreted for this time interval (Rögl, 1998; Kováč et al., 2017; Holcová et al., 2019a). Moreover, this interval corresponds to the closure of the Indian-Mediterranean gateway, which had a great impact on the Mediterranean-Paratethys marine system. It triggered the circulation regime change in the Mediterranean from an estuarine to an anti-estuarine pattern (Kouwenhoven and Van der Zwaan, 2006). In the Paratethys realm, this event is documented by a marine transgression (Rögl, 1999). Several mathematical modelling studies tested relationships between the Paratethys and the Mediterranean during the Langhian and they proposed the presence of an anti-estuarine circulation regime in the Paratethys during the first phase of the shallowing of the Indian-Mediterranean gateway, which further resulted in a decrease in water exchange between the Indian Ocean and the Mediterranean. According to those models, an inflow of the Paratethyan bottom waters back into the Mediterranean could have played a role in a formation of deep Mediterranean waters (Karami et al., 2011; Vara et al., 2013; Karami et al., 2009). Nevertheless,

the exact relationship between these two marine systems remains unclear. The climatic evolution of this area during the Langhian period is very interesting. The global climatic changes such as the Middle Miocene Climate Optimum (MMCO), Mi-2a (14.96 Ma) and Mi-3 (13.8 Ma) cooling events took place during this time interval (Zachos et al., 2001; Zachos et al., 2008) and the Paratethys was also greatly influenced by a variety of local factors such as the rise of the Carpathians that affected the mesoclimatic conditions in a broader regional scale (Kováč et al., 2017).

In the Paratethys area, the majority of paleoenvironmental studies was based on classical paleontological approaches combined together with sedimentology or with any other related disciplines (e.g. Holcová et al., 2015; Kopecká, 2012; Rupp and Hohenegger, 2008; Ozdínová and Soták, 2015). Those approaches mainly used the composition of foraminiferal assemblages as paleoenvironmental proxies that are based on various ecological preferences of different species. Lately, several studies using stable isotopes of carbon and oxygen on foraminifera emerged together with few individual studies using other geochemical proxies on foraminifera such as Mg/Ca (e.g. Šutovská and Kantor, 1992; Gonera et al., 2000; Báldi, 2006; Báldi and Hohenegger, 2008; Kováčová et al., 2009; Grunert et al., 2010; Holcová and Demeny, 2012 and Peryt, 2013). However, the studies often used the so-called “bulk” carbonate samples for carbon and oxygen isotope analyses, which now the latest studies show that it is not appropriate (Scheiner et al., 2018). It is due to the limitations, which are very abundant in this area - the microfossils preservation state and possible diagenetic alteration of foraminiferal calcium carbonate. The other important limitations are e.g. large local variations in physicochemical parameters of seawater that can bias results of geochemical analyses or signals of any isotopic trends (Scheiner et al., 2018). The species selection is another crucial factor, because many of the Paratethyan foraminiferal species are different from their modern relatives or they are even extinct (Báldi, 2006; Cicha et al., 1998). Therefore, a great attention had to be paid regarding the species selection for subsequent geochemical analyses, because geochemical proxies are often calibrated on recent foraminiferal species (e.g. Anand et al., 2003; Elderfield et al., 2006 among others). It is apparent that using geochemical proxies on foraminifera in the environment of the Central Paratethys could be tricky. The benefits of using geochemical proxies on foraminifera are at first their high precision in comparison with indirect approaches based on composition of assemblages and secondly the complexity of information they can provide. The other benefits are linked with the amount of material, which is needed for the analysis itself because if there are only limited samples available it could be an important factor.

The main aim of this Ph.D. study was to test several basic geochemical proxies on foraminifera in the environment of the Central Paratethys - stable isotopes of carbon and oxygen and Mg/Ca ratio - to discover possible limitations or any other problems that could be caused by the specificity of this environment. The secondary aims were to use the data based on the above-mentioned proxies to reconstruct the paleoenvironment of the Central Paratethys and compare these reconstructions based on the geochemical proxies on foraminifera with reconstructions based on classical paleoenvironmental and paleoecological approaches, which are dominant in the studied area. Moreover, basic organic geochemistry proxies on whole rock samples were tested as supportive paleoenvironmental proxies for determining the local paleoenvironment and its evolution.

### **1.1 List of studies:**

This Ph.D. thesis is based on the following studies, referred further as roman numbers in bold. All these studies can be found in their original form as an annex to this thesis.

**I. Scheiner, F., Holcová, K., Milovský, R., Kuhnert, H., 2018.** Temperature and isotopic composition of seawater in the epicontinental sea (Central Paratethys) during the Middle Miocene Climate Transition based on Mg/Ca,  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  from foraminiferal tests. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 495, 60-71.

**II. Holcová, K., Kopecká, J., Scheiner, F., 2019.** An imprint of the Mediterranean middle Miocene circulation pattern in a satellite sea during the Langhian: a case study from the Carpathian Foredeep (Central Paratethys). *Palaeogeography, Palaeoclimatology, Palaeoecology*, 514, 336-348.

**III. Scheiner, F., Holcová, K., Milovský, R., Doláková, N., Rigová, J., 2019.** Response of benthic foraminiferal communities to changes in productivity and water mass conditions in the epicontinental sea during the Middle Miocene. *Marine Micropaleontology*, in review.

**IV. Holcová, K., Dašková, J., Fordinál, K., Hrabovský, J., Milovský, R., Scheiner, F., Vacek, F., 2019.** A series of ecostratigraphic events across the Langhian/Serravallian boundary in an epicontinental setting: the northern Pannonian Basin. *Facies*, in press, DOI: 10.1007/s10347-019-0576-1.

## **The studies description:**

### **Study I. - the Central Paratethys, the LOM-1 locality**

This study deals with the application of the selected geochemical proxies on foraminifera in the studied area. It is the basic study dealing with new methodologies and presenting unique  $\delta^{18}\text{O}$ ,  $\delta^{13}\text{C}$  and Mg/Ca datasets. The methodological part is very important in this study. Further, the temperature conditions as well as the definition of particular Paratethyan water masses are presented in this study. The author was responsible for the entire preparation setup and for the application of the new methodologies as well as he participated in geochemical analyses itself. Further, the author made the processing of the raw data together with the subsequent paleoenvironmental interpretations and the final conclusions.

### **Study II. - the Paratethyan marine realm, the Central Paratethys, the LOM-1, OV-1 and RY-1 cores**

This study represents a review article dealing with an identification and comparison of particular Paratethyan water masses and circulation patterns based on the synthesis of isotopic and paleoecological data from the studied area during the Langhian. The main result of this study is the proposal of a model that characterizes the prevailing circulation patterns/regimes in the Paratethyan marine realm and their change during the studied interval. Further implications consisted of interpretations of local paleoenvironmental aspects. The author was responsible for the selection and comparison of the isotopic data from the Paratethyan area with the Mediterranean data that allowed identifying of the particular water masses. Moreover, the author is largely involved in the subsequent interpretations regarding the composition of the circulation model during the studied time interval.

### **Study III. - The Central Paratethys, the LOM-1 locality**

This study focuses on paleoenvironmental interpretations based on various organic geochemical proxies from whole rock samples combined together with the benthic foraminiferal stable isotopic data of the selected benthic species. This combination of methods allowed determining of the origin of the organic matter, productivity rate and the subsequent response of the benthic foraminiferal communities to these changes in paleoecological parameters. Moreover, this study deals with the interpretation of the studied environment in a regional paleogeographical perspective. The author was responsible for the preparation setup of the stable isotopes as well as for further data processing and their interpretations. Also, the interpretations of all organic geochemical proxies together with the final conclusions was made by the author.

#### **Study IV. - the Central Paratethys, the Pannonian Basin, the ŠO-1 core**

This study identifies important ecostratigraphic events during the Langhian/Serravalian boundary in the Pannonian Basin (Paratethyan area). The study describes paleoenvironmental turnovers that are recognized in a broader region based on the detailed multiproxy data (e.g. palynology, stable isotopes on foraminifera and molluscs, sedimentology, quantitative analysis of calcareous nannoplankton, molluscs, red algae and foraminiferal assemblages) from the studied sequence on the parastratotype locality ŠO-1. In the studied sequence, it is possible to recognize four units that describe the particular paleoenvironmental turnovers in the studied region. The author was responsible for the preparation of the foraminiferal stable isotopic samples as well as for the consecutive data processing and interpretations of the foraminiferal  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$ .

Overall, all the publications deal with important paleoenvironmental and paleoceanographical changes during the Langhian period in the Paratethyan marine realm. The Central Paratethys as a part of this marine realm was studied in detail in all studies, but several assumptions such as the changes in the prevailing circulation patterns/regimes are applied on the Paratethyan marine realm in a broader scale. The LOM-1 locality was studied in detail because this locality provided important geochemical datasets that are unique in the studied area. As the supplementary material, the OV-1 and ŠO-1 cores were selected mainly because of the preservation state of foraminiferal microfauna. All studies illustrate the potential of geochemical proxies on foraminifera in a paleoenvironmental research of a fossil epicontinental sea - the Paratethys in this case. Moreover, there are shown many paleoenvironmental interpretations such as the circulation patterns evolution, seawater temperature evolution, definition of the particular water masses and numerous paleoecological consequences, which otherwise would remain unclear. It clearly helped to clarify many scientific questions regarding the paleoenvironmental settings during the Langhian period in the Paratethyan marine realm.

## **2. The research problem definition**

As was described earlier, the use of geochemical proxies on foraminifera is not very common in the area of the Central Paratethys despite being a basic tool in a modern oceanographic research. The main reasons are linked with preservation state of microfossils, species selection and with using “bulk” samples for geochemical analyses. These limitations and their possible solutions were the basis for formulation of the main aims of this thesis.

The Ph.D. thesis was therefore build on the following aims:

- to test the use of geochemical proxies on foraminifera and to test their methodologies in the specific environment of the epicontinental sea
- to use innovative analytical procedures - high precision single test measurements of carbon and oxygen stable isotopes and to find out its benefits in the studied environment
- to compare paleoenvironmental reconstructions based on classical approaches with reconstructions based on geochemical proxies on foraminifera and identify their possible cons/pros
- to provide detailed, high resolution paleoenvironmental interpretations for the area of the Central Paratethys

### **2.1 Geological setting**

The primary material for this study came from the shallow core LOM-1 (GPS location: 49° 23.945' N and 16° 24.542' E, 382 m a.s.l.), which was drilled near the town Lomnice located in the Brno-venkov district in the Czech Republic, see Figure 1. This shallow core drills Middle Miocene sedimentary sequences of the Carpathian Foredeep. The total length of the core is 21.5 m. The Carpathian Foredeep is a peripheral foreland basin located on the eastern margin of the Bohemian Massif. The tectonic positioning of the Carpathian thrust wedge is responsible for its formation caused by the subsurface loading of the Alpine-Carpathian orogenic belt on the Bohemian Massif during Early to Middle Miocene (Nehyba and Šikula, 2007; Nehyba et al., 2008). Pelitic sediments that are called “Tegels” dominate the Middle Miocene sedimentary sequences and those sediments are classically interpreted as outer shelf deposits or hemipelagite deposits (Papp et al. 1978; Cicha 2001; 103 Nehyba et al. 2008). Several accompanying sedimentary members such as coarser-grained sandstones, conglomerate bodies, carbonate bioherms and thin volcanoclastics are appearing alongside the “Tegels” in the Middle Miocene

sedimentary sequences of the Carpathian Foredeep (Doláková et al., 2008; Nehyba et al., 1999). Calcareous clays, sandy clays, quartzose sands and calcareous sands are the main sedimentary representatives of the Middle Miocene deposits at the Lomnice locality (Nehyba et al., 1999; Holcová et al., 2015).

The supplementary material comes mainly from the OV-1 and ŠO-1 cores. The OV-1 core (GPS location: 49°06.820'N and 16°20.237'E) is located westwards near Brno and it represents the western margin of the Carpathian Foredeep basin. The ŠO-1 core (location: WGS 84: 47.8290 N and 18.8502 E) is located in the eastern part of the Danube Basin and it represents a parastratotype of the local Badenian stage (Papp et al., 1978). All the details regarding geological setting including description of the sedimentary sequences can be found in Holcová et al. (2019a, 2019b); (study II., IV.).

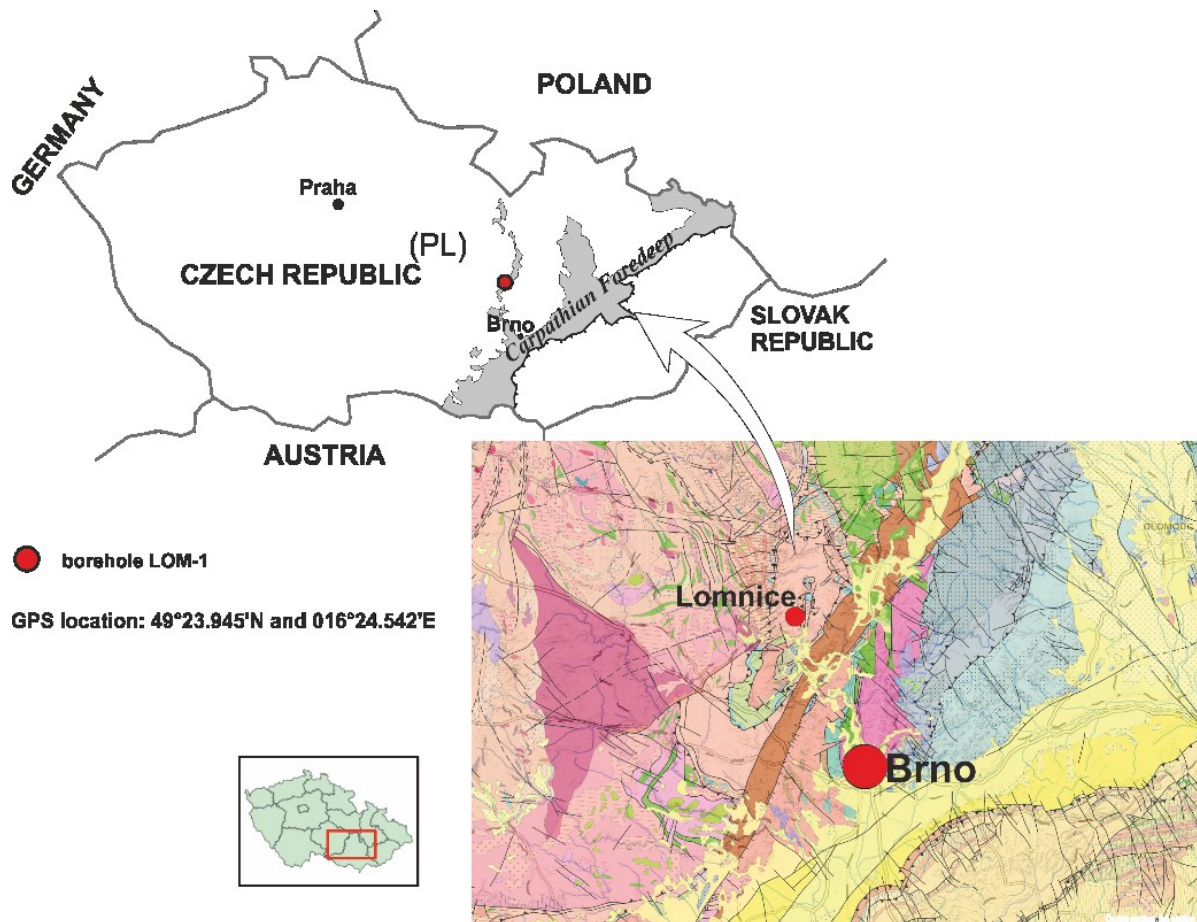


Figure 1: The LOM-1 core - GPS location: 49°23.945'N and 16°24.542'E (modified after Holcová et al. (2015) and Scheiner et al. (2018, 2019)).

The material from the LOM-1 core was chosen for this research because of the presumed exceptional preservation of microfossil assemblages, which was essential for the application of the selected geochemical proxies. Holcová et al. (2015) had already evaluated the LOM-1 core from the paleoenvironmental perspective, thus it represented an ideal material for comparison of results that were based on classical paleoenvironmental and paleoecological approaches with results based on geochemical proxies on foraminifera. Further, during the research itself it was discovered that the microfossil assemblages are truly well preserved and suitable for using as a “substrate” for geochemical studies. The age span was estimated using the biostratigraphy succession from the Mediterranean area based on the occurrences of the index planktonic foraminiferal and calcareous nannoplankton species (*Praeorbulina circularis*, *Orbulina suturalis* and *Sphenolithus heteromorphus*; Holcová et al., 2015). Holcová et al. (2015) correlated the LOM-1 core with the interval from 14.4 - 14.35 Ma, which is shortly after the Middle Miocene Climate Optimum (MMCO), but before the main onset of the Mi cooling events (Ogg et al., 2016; Miller et al., 1996).

## **2.2 Methods**

The foraminifera were picked up using a binocular microscope from the wet sieved fraction of ~ 63 - 2000 µm. Further, the preservation check based on the inner wall structure was applied. In the first step about forty foraminiferal specimens of ten different species was examined under the scanning electron microscope (SEM) and after that each of those specimens were crushed and analysed under the SEM again. The preservation of each sample was determined using this procedure. The samples that passed this first step was checked once again. In the second step, the check was carefully done on the multiple tests of the preferentially selected species to obtain the final list of well-preserved samples for subsequent geochemical analyses. This step was crucial to exclude any possible diagenetic alteration of foraminiferal tests, which otherwise can bias geochemical results. Although the preservation state of the selected samples was considered as suitable/well preserved, it was not the perfect case such as e.g. the exceptional preservation of pristine foraminifera in deep oceanic deposits. The following geochemical analysis showed that this check was well-designed and that no significant diagenetic alteration of foraminiferal calcite was present. Nevertheless, it should be noted that this preservation state is rare in the area of the Central Paratethys. The final step was to get foraminiferal tests from the selected samples into uplift using distilled water to be able to pick up only the floating ones with no or minor sedimentary or mineral infillings.

Another important part was the selection of the particular foraminiferal species for the subsequent geochemical analyses. We selected planktonic and benthic foraminiferal species that have different ecological preferences to document all parts of the water column as well as the seafloor. *Globigerinoides trilobus* and *Globigerina bulloides* were selected as representatives of planktonic foraminiferal species and *Cibicidoides* spp., *Melonis pompilioides* and *Gyroidinoides* spp. were selected as benthic foraminiferal representatives. *Globigerinoides trilobus* - shallow-dwelling, with an affinity to oligotrophic warm waters represents surficial waters in the uppermost water column (Reynolds and Thunell, 1985; Hemleben et al., 1989; Chapman, 2010; Schiebel and Hemleben, 2005) whereas *Globigerina bulloides* - shallow dwelling, with an affinity to high nutrient waters represents mixed surficial waters (Schiebel et al., 1997; Hemleben et al., 1989). *Cibicidoides* spp. represents an epifaunal to shallow infaunal species with an affinity to high oxic conditions (Kaiho, 1994; Murray, 2006) thus it represents oxic bottom waters. *Gyroidinoides* spp. is considered as a shallow infaunal species with low food demands (Schmiedl et al., 2004) and finally *Melonis pompilioides* representing intermediate, rather static infauna with a preference of higher nutrient levels (e.g. Sjoerdsma and van der Zwaan, 1992; Sen Gupta and Machain-Castillo, 1993; Miao and Thunell, 1993; Rathburn and Corliss, 1994; Schmiedl et al., 2004; Mackensen et al., 2000). The specimens for the geochemical analyses were selected of approximately the same size for each taxon to exclude any possible ontogenetic effects that can bias the results. Moreover, the species were also selected based on their occurrence overlap to the present foraminiferal microfauna or at least they were selected from close/related lineages that have their common occurrence at present.

### **2.2.1 Stable isotopic analysis of carbon and oxygen**

The stable isotopic analyses were carried out on a MAT253 gas isotope ratio mass spectrometer (Thermo Scientific) coupled to a Kiel IV (Thermo Scientific) automatic preparation line housed at the Earth Science Institute, Slovak Academy of Sciences, Banská Bystrica. The analyses were done using the single test approach, which means that from a single foraminiferal test it was obtained both, carbon and oxygen isotopic value. The internal precision was an average of 0.02‰ for carbon and an average of 0.03‰ for oxygen. The isotopic values are reported as  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  vs. VPDB. For further details regarding this methodology, please see Scheiner et al. (2018); (**study I.**). Despite the preference of using the single test approach, for some operations such as the  $\delta^{18}\text{O}_{\text{seawater}}$  calculation it was needed to obtain mean isotopic values. In those cases, the mean isotopic values were calculated using the weighted arithmetic

mean from multiple single-test measurements per sample. This was due to the large variability of isotopic values in particular samples. The weighted arithmetic mean was based on the determination of the main cluster of isotopic values. Each value within the main cluster was assigned the weight of 1 whereas the values lying outside the main cluster were assigned the weight of ½. This approach provided more precise mean isotopic values than using pure arithmetic mean (**study I**). The  $\delta^{18}\text{O}_{\text{seawater}}$  was calculated based on the formula by Shackleton (1974) - the formula is:

$$\delta^{18}\text{O}_{\text{seawater}} = \delta^{18}\text{O}_{\text{calcite}} + 0,27 - \frac{4,38 - \sqrt{4,38^2 - 4 * 0,1 * (16,9 - \text{Temp.})}}{2 * 0,1} \quad (1)$$

### 2.2.2 Mg/Ca ratio analysis

For Mg/Ca analysis, foraminifera of approximately the same size were picked up and for each sample and taxon a combined weight of ~ 300 µg of was processed (cf. 15 - 30 specimens). All samples were cleaned according to the procedure of Barker et al. (2003). The cleaned and dissolved samples were further analysed on a Thermo-Finnigan Element 2 sector field inductively coupled plasma mass spectrometer housed at the Department of Geosciences, University of Bremen, Germany. The analytical procedure followed that described by Kuhnert and Mulitza, (2011). For further details, please see Scheiner et al. (2018); (**study I**). Based on the analysed species, the following species-specific paleotemperature equations were used. The general exponential dependence of Mg/Ca on temperature can be described by the following formula:

$$\text{Mg/Ca} = B \exp(AT) \quad (2)$$

where T is  $\delta^{18}\text{O}$  calcification temperature and B and A are pre-exponential and exponential constants. The calibration errors are systematic and affect all samples the same way. The calibration for *Globigerinoides trilobus* follows that described in Anand et al. (2003). For *Globigerina bulloides* a mean from three different calibrations by Lea et al. (1999), Maschiotta et al. (1999) and Elderfield and Ganssen, (2000) were used because those studies used either culture, core tops or their combination and the results varied. For *Cibicidoides* spp. the calibration by Lear et al. (2002) was used and for *Melonis pompilioides* the calibration was based on the same study (Lear et al., 2002) as the calibration for *Cibicidoides* spp. The average errors were not greater than ±1.7 °C. *Gyroidinoides* spp. was not a subject of the Mg/Ca ratio analysis because two other benthic species were already analysed as indicators of bottom waters temperature.

There were two main potential sources of uncertainties. The first one was linked with salinity changes because Mg/Ca increases by ~ 10 % per salinity unit (e.g. Nürnberg et al., 1996; Lea, 2003; Kısakürek et al., 2008; Arbuszewski et al., 2010). However, no correction was applied because regarding the salinity there were no information available and based on the paleoecological composition of foraminiferal assemblages there seems to be no relevant fluctuations either. The second source of uncertainty was linked with variations of Mg/Ca concentration in seawater. Because of the long residence time of those elements in seawater, no specific corrections were applied although it resulted in a slight underestimation of the reconstructed paleotemperatures. For details, please see Scheiner et al. (2018); (**study I.**).

### **2.2.3 Organic geochemistry methods**

Whole rock samples from the LOM-1 core have been used for the organic geochemistry proxies analyses. All the samples were at first mechanically cleaned using tap water to remove any dust particles or any other impurities. After this step, samples were treated with HPLC acetone to remove any organic remains. All the following geochemical analyses were carried out at the Earth Science Institute, Slovak Academy of Sciences, Banská Bystrica.

#### **2.2.3.1 Carbon isotopes ( $\delta^{13}\text{C}_{\text{org}}$ )**

Carbon isotopes were analysed on an isotope ratio mass spectrometer (IRMS) MAT253 coupled to an elemental analyzer, Flash 2000 HT Plus (Thermo). The samples were combusted at 1000°C in a quartz tube packed with chromium oxide, electrolytic copper and silvered cobaltous/cobaltic oxide. Purified CO<sub>2</sub> gas was further separated from other gases on a capillary GC column (Poraplot Q, Agilent) and introduced into the IRMS in continuous flow mode with helium as a carrier gas. All values are reported in per mille PDB, and typical external precision measured on standards was 0.1 per mille. For further details see Scheiner et al. (2019); (**study III.**).

#### **2.2.3.2 TOC/TIC/TC analysis**

The carbon ratios (total organic carbon/total inorganic carbon/total carbon) were measured on an elemental analyzer Ströhlein C-MAT 5500. Samples (pulverized) were digested in HCl and ~ 50 mg were combusted in the temperature range 50-1000°C. The evolved CO<sub>2</sub> was measured using an infrared detector to calculate the carbon content. The carbon ratios concentrations were calibrated using pure CaCO<sub>3</sub>. The precision error in the weight range of samples was 0.08 wt %. For details see Scheiner et al. (2019); (**study III.**).

### 2.2.3.3 N-alkane and isoprenoid analysis

Cleaned and pulverized samples were Soxhlet-extracted using dichloromethane and methanol mixture (ratio 8:2) under a continuous reflux for several hours and after that, the total extracts were condensed and transported into vials. The extracts were analysed by a GC-MS (Trace GC Ultra and ITQ 900, Thermo Fisher Scientific) on a nonpolar capillary column Zebron ZB5-HT with helium as a carrier gas and the identification and quantification of chromatograms was performed with the Xcalibur software (ThermoFischer) using the ion fragment  $m/z$  85. The n-alkane and isoprenoid (pristine (Pr), phytane (Ph)) numerical values were used for the calculation of basic n-alkane ratios and indices such as pristane-phytane ratio (Pr/Ph), carbon preference index (CPI), average chain length index (ACL) and terrigenous-aquatic ratio ( $TAR_{HC}$ ). Pristane-phytane ratio describes redox conditions of a source-rock depositional environment (Didyk et al., 1978). If  $Pr/Ph < 1$  it indicates an anoxic source-rock depositional environment, while if  $Pr/Ph > 1$  it indicates an oxic source rock depositional environment (Didyk et al., 1978). Carbon preference index is a maturity indicator and it is based on a gradual change in the distribution of long-chained n-alkanes (eq. 3; Ratnayake et al., 2006). The main source of long-chained n-alkanes are waxes derived from vascular plants. If CPI values are  $> 1.5$  it points to relatively immature sediments; if CPI values are lower, then it indicates mature sediments or a limited input of long-chained n-alkanes from terrigenous environments. CPI is also used for hydrocarbon source tracing because terrestrial vascular plants have typical CPI values being  $> 5$  (e.g. Eglinton and Hamilton, 1967; Rielley et al., 1991; Bouloubassi et al., 1998). CPI values around 1 indicate petrogenic or marine hydrocarbon origin (Tareq et al., 2005; Jeng, 2006).

$$CPI = 0.5 \times \left( \frac{C_{23}+C_{25}+C_{27}+C_{29}+C_{31}+C_{33}}{C_{24}+C_{26}+C_{28}+C_{30}+C_{32}+C_{34}} + \frac{C_{23}+C_{25}+C_{27}+C_{29}+C_{31}+C_{33}}{C_{22}+C_{24}+C_{26}+C_{28}+C_{30}+C_{32}} \right) \quad (3)$$

Average chain length quantifies an average number of carbon atoms in n-alkane molecules present in a sample (eq. 4; Poynter and Eglinton, 1990). It is possible to distinguish terrigenous input that is represented by higher ACL values from an aquatic bio-productivity, which is conversely represented by lower ACL values (Eglinton and Hamilton, 1967; Rielley et al., 1991; Smith et al., 2007; Derrien et al., 2017).

$$ACL = \frac{25(nC_{25})+27(nC_{27})+29(nC_{29})+31(nC_{31})+33(nC_{33})}{nC_{25}+nC_{27}+nC_{31}+nC_{33}} \quad (4)$$

Terrigenous-aquatic ratio relates and quantifies these above-mentioned inputs (eq. 5; Bourbonniere and Meyers, 1996). If  $TAR_{HC} \gg 1$  it indicates a terrigenous origin of the organic matter and conversely if  $TAR_{HC} < 1$  it indicates a marine bio-productivity.

$$TAR_{HC} = \frac{C_{27}+C_{29}+C_{31}}{C_{15}+C_{17}+C_{19}} \quad (5)$$

For detailed description of these indices and other effects related to them, please see the study by Scheiner et al. (2019); (**study III.**).

#### 2.2.4 Paleoecological data

The previously published paleoecological data by Holcová et al. (2015) were used as the primary paleoecological data mainly the detailed composition of foraminiferal assemblages. Based on these data, the benthic foraminiferal oxygen index (BFOI; Kaiho, 1994, 1999) was calculated. This index provides an information about the amount of dissolved oxygen in bottom or pore waters based on the composition of foraminiferal benthic assemblages. BFOI is based on the classification of foraminiferal species into oxic/dysoxic and suboxic groups and on a relative prevalence of one of these groups in a sample. This index and classification of species was developed based on modern foraminifera and their recent habitats, which makes this index uneasy to use in the studied fossil environment, where there are many species with unknown habitat preferences or their preferences are only derived based on their modern relatives/equivalents. Therefore, Báldi (2006) reworked the Kaiho's classification for the area of the Central Paratethys and tried to assign exact microhabitats to the most common benthic species based on morphological principles. However, due to a certain disparity in both classifications there was made an own proposal of the classification of foraminifera to oxic/dysoxic and suboxic groups to fit the studied area. For further details regarding the particular classifications, please see the original studies by Kaiho (1994, 1999), Báldi (2006) and Scheiner et al. (2019); (**study III.**).

### 3. Results

All results are available in the original studies, mainly because of their quantity. The stable isotopic results from the LOM-1 core are published in Scheiner et al. (2018) and Scheiner et al. (2019); (**study I. and III.**). The datasets contain more than two hundred individual stable isotopic data points for each, carbon and oxygen isotopes. The datasets of mean isotopic values are also available in the mentioned studies. The Mg/Ca dataset is available in Scheiner et al.

(2018); (**study I.**) and it contains forty-four Mg/Ca measurements. The organic geochemistry with all datasets including  $\delta^{13}\text{C}_{\text{org}}$ , TIC/TOC/TC/CaCO<sub>3</sub>, n-alkane and isoprenoid indices are available in Scheiner et al. (2019); (**study III.**). The paleoecological data regarding the composition of foraminiferal assemblages are summarized in the review study by Holcová et al. (2019a); (**study II.**). The BFOI primary data and results are available in Scheiner et al. (2019); (**study III.**). The datasets regarding the stable isotopes of carbon and oxygen from the supplemental profiles OV-1 and ŠO-1 can be found in Holcová et al. (2019a, 2019b); (**study III., IV.**).

## 4. Discussion

### 4.1 Stable isotopes and possible diagenetic alteration - single test approach

Thanks to the unique methodology of the single test analysis of stable isotopes on foraminifera, it was possible to determine the isotopic variability and to identify potential outliers that can be caused by a diagenetic alteration of biogenetic calcium carbonate. Even by using the above-described preservation check during the preparation of the samples it is not possible to unequivocally exclude their diagenetic alteration. Thanks to the single test approach, the outliers that were most likely diagenetically altered were discarded before further data processing. In our datasets, only slightly more than 3% of samples reached outlying values. The anomalously high/low values that were considered as outliers have for example  $-5.4\text{‰ } \delta^{18}\text{O}$  (or values as high as  $-11.2\text{‰ } \delta^{18}\text{O}$ ) in a set of values, which have the calculated mean of  $-0.68\text{‰ } \delta^{18}\text{O}$ . If using this single outlying value the mean of this set would shift to  $\sim -1.18\text{‰ } \delta^{18}\text{O}$ , which is quite different from the real value, but when seeing only the mean value it does not seem as an effect of an outlier. Thanks to the single test approach, it was able to individually check all values as well as check them as a set of related values and therefore precisely identify the outliers. This is not possible when using the so-called “bulk” samples for stable isotopic analysis of carbon and oxygen. Isotopic variability is very important factor that is present in the studied area for all species. It points to a variation in isotopic composition of particular foraminiferal species within individual samples as well as a variation in isotopic composition within a whole set of samples. In a fossil material, this variability is very often present but the magnitude of this variability was not known for the Paratethys because using “bulk” samples do not allow its precise determination. Scheiner et al. (2018, 2019); (**study I., II.**) shows, that the isotopic variability for the Paratethyan foraminiferal species is quite broad thus it has to be taken into account in paleoenvironmental interpretations, please see Figure 2.

It is not frequently discussed phenomenon, but it is extremely important especially in environments where there are short-time fluctuations of paleoenvironmental parameters.

These along with the low material quantity requirements were the most important pros of the used methodology compared to common approaches using “bulk” foraminiferal/carbonate samples. It is apparent that for a precise interpretation of foraminiferal stable isotopic data in the studied area, the single test approach is the proper one and it should be commonly used. Details regarding the single test approach can be found in Scheiner et al. (2018); (study I).

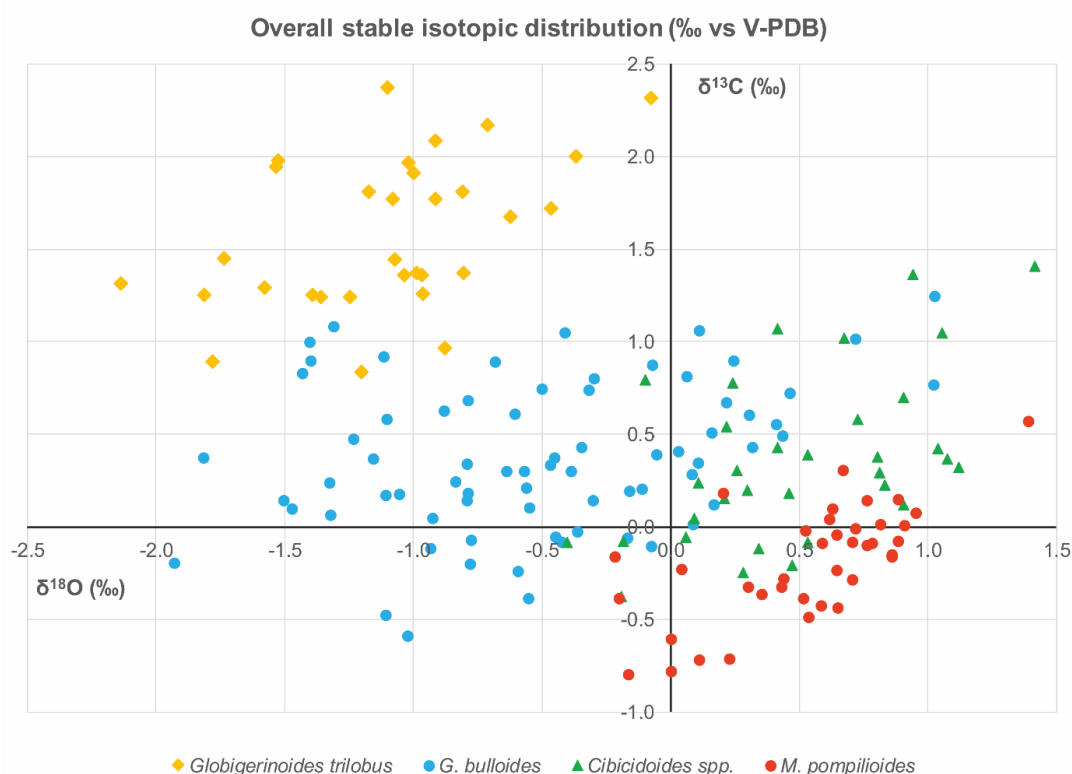


Figure 2: The species isotopic variability in the samples from the LOM-1 core. The picture is taken from Scheiner et al. (2018).

#### 4.2 Foraminiferal $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$

The foraminiferal stable isotopic data were interpreted using autecological principles. The global  $\delta^{18}\text{O}$  signal definitely contributed to the variability seen in the studied samples however, it is probably overprinted by local factors as well as with the species isotopic variability.  $\delta^{18}\text{O}$  is often related with changes in temperature and salinity in the studied area, especially for planktonic species. Nevertheless, temperature changes were only minor, which is shown by the Mg/Ca derived paleotemperatures thus most of the variations are attributed to

changes in salinity and to the species isotopic variability. *Globigerina bulloides* exhibits a broad variance, which is probably related with different populations of this species from various seasons and their mixing in the studied fossil material. It is because of the high nutrient preference of *Globigerina bulloides* (Hemleben et al., 1989; Schiebel et al., 1997). Concerning the benthic species (*Melonis pompilioides*, *Cibicidoides* spp. and *Gyroidinoides* spp.) the variations mainly reflect temperature and paleoenvironmental changes at the seafloor such as pH and  $[\text{CO}_3^{2-}]$  concentrations as well as the global  $\delta^{18}\text{O}$  signal. The  $\delta^{13}\text{C}$  in the studied samples is primarily related with differences in prevailing trophic conditions during different seasons. Surely, the global  $\delta^{13}\text{C}$  trend is also present however; the data resolution together with the species isotopic variability does not allow its clear identification in the studied samples. For benthic species, various vital and microhabitat effects are definitely present but it is not possible to distinguish their exact proportion. Moreover, the food preferences of each species are very important factors affecting their  $\delta^{13}\text{C}$  signal. Regarding the benthic species, their life position/life strategies and microhabitat preferences are closely linked with the above-mentioned food preferences. Overall, all the isotopic values reflect the presumed habitats of the studied species well. Another important factor is that there is no observable relationship between surficial and bottom waters which is implying to different hydrographies. All details regarding the stable isotopes and their paleoecological and paleoenvironmental interpretations are discussed in Scheiner et al. (2018, 2019) and Holcová et al. (2019a, 2019b); (study I-IV.).

### 4.3 Mg/Ca based paleotemperature time series

The calculated paleotemperatures are equivalent to modern subtropical regions. There are three warming periods in the studied interval, which are indicated by peaks in temperature calculations of all selected species. The temperature profile has clearly a cyclical character of an alternation of warmer and cooler periods. However, there are no signs of a gradual cooling in this time interval right after the Middle Miocene Climate Optimum and conversely there is a minor warming trend present. The temperature changed simultaneously in bottom and surficial waters, see Figure 3. The calculated temperatures reflect the calcification depth of the selected species well. There is an interesting offset as high as 6°C between benthic infaunal *Melonis pompilioides* and epifaunal *Cibicidoides* spp. in favour of *Cibicidoides* spp. It can be attributed to changes in the physicochemical composition of seawater - pH and  $[\text{CO}_3^{2-}]$ , which could have affected the incorporation of Mg/Ca in foraminiferal tests (Raitzsch et al., 2008; Yu and Elderfield, 2007; Russel et al., 2004) because it is very unlikely that pore and bottom waters had different temperatures. The temperature profile with discussed details is available in

Scheiner et al. (2018); (**study I.**). Furthermore, this presumed change of the physicochemical composition between pore and bottom waters is debated in Scheiner et al. (2019); (**study III.**). The Mg/Ca based temperatures clearly show a minor warming trend in the Central Paratethys despite the presumed cooling during this time interval right after the MMCO. Additionally, Mg/Ca drew an attention to the change of physicochemical parameters of bottom waters, which were further tested using the organic geochemistry proxies and stable isotopic data from selected benthic foraminiferal communities.

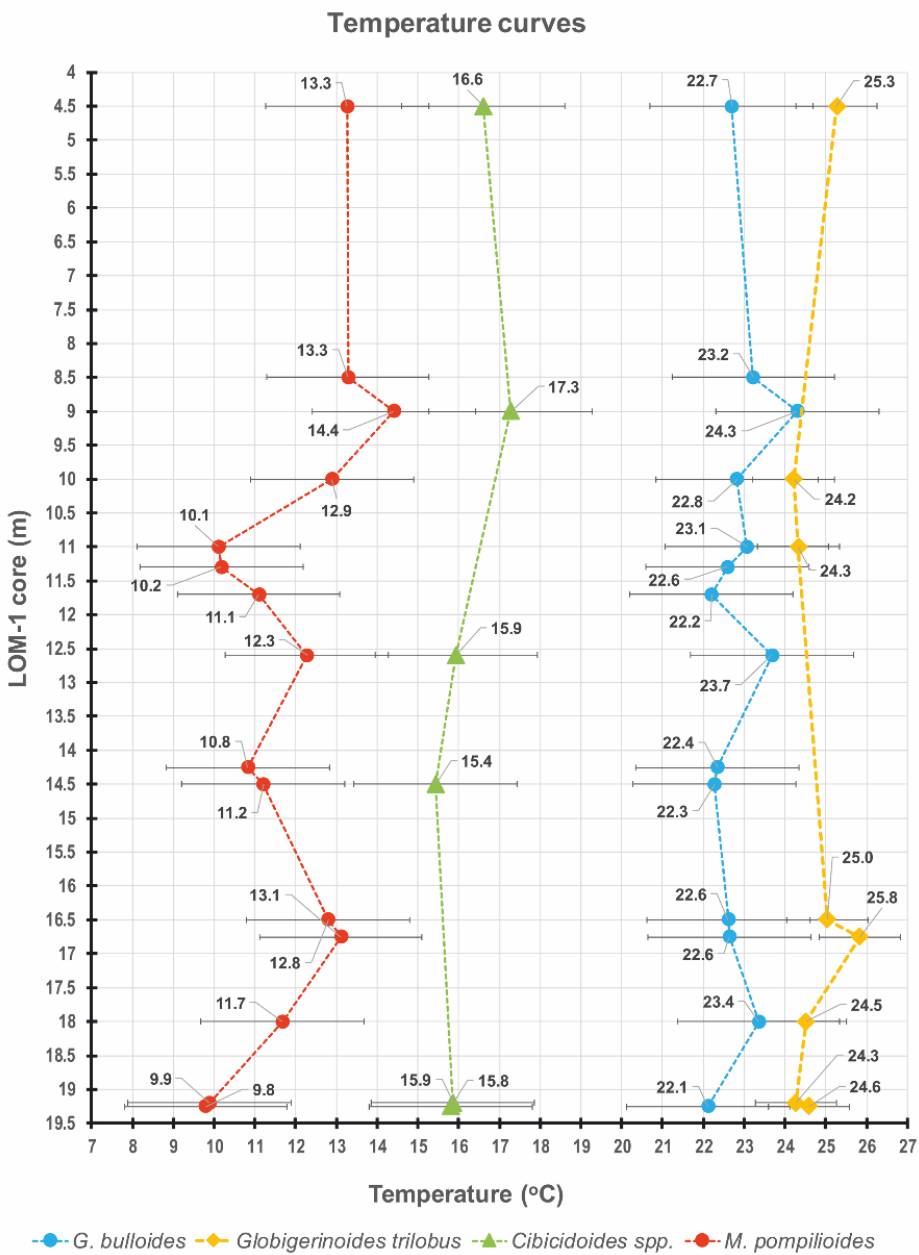


Figure 3: The calculated temperature time series for the LOM-1 core. The figure is taken from Scheiner et al. (2018).

#### 4.4 Comparison with the Mediterranean and identification of water masses

The stable isotopic datasets were further compared with isochronous data from related areas. Firstly, the datasets were compared with the Vienna Basin (Báldi and Hohenegger, 2008) as a representative of the same marine realm, the Central Paratethys but with slightly different paleoenvironmental conditions, which is exactly what the stable isotopic data are reflecting. The  $\delta^{18}\text{O}$  is similar in both basins and the only difference is the  $\delta^{13}\text{C}$  that probably points to a dissimilarity in trophic conditions/nutrient input between these localities. Furthermore, the datasets were compared with isochronous data from the Mediterranean area from localities with comparable bathymetrical settings (Mader et al., 2004; Mourik et al., 2011; Kocsis et al., 2008). It is because during this time interval the Central Paratethys had an unrestricted connection with the Mediterranean (e.g. Rögl, 1999) thus it makes sense to compare the data with the adjacent Mediterranean area. The comparison was mainly based on two foraminiferal species - planktonic *Globigerinoides trilobus* and benthic *Cibicidoides* spp. In all cases, the single test data had to be recalculated to mean  $\delta$  values to be able to compare them with the published data, which were obtained by different methodological approaches. The recalculation was done using the weighted arithmetic mean and it is described in the Methods section or it is discussed in detail in Scheiner et al. (2018); (**study I**). Based on this mean values it was able to calculate  $\delta^{18}\text{O}_{\text{seawater}}$  for each sample and each species to obtain the signal for the Paratethyan seawater. Thanks to the factors linked with the  $\delta^{18}\text{O}_{\text{seawater}}$  calculation such as the input data uncertainties it was apparent that it is not suitable for a detailed comparison with the Mediterranean, but it revealed the dissimilarity between surficial and bottom waters. The first step how to identify the particular Paratethyan water masses was the comparison of the foraminiferal  $\delta^{18}\text{O}$ . It showed that the data for planktonic foraminifera are in a very good agreement and conversely the  $\delta^{18}\text{O}$  data for benthic species were quite different. It indicates a similarity in the hydrography of surficial waters and a difference in the bottom waters hydrography. The surficial and bottom waters of the Central Paratethys were characterized in detail by their  $\delta^{18}\text{O}$  and temperature ranges, see Figure 4. This process led to the determination of the Central Paratethys Bottom Water. To elaborate these findings and to focus on a larger regional scale, it was logical to make a synthesis of the previously published data from several Paratethyan basins. It focused on a slightly broader time interval of  $\sim 14.6$  to  $13.9$  Ma and it used multiproxy data to deduce the evolution of circulation patterns/regimes in the Paratethys during the Langhian period. As the key tool, the foraminiferal oxygen stable isotopic data from five localities were used, which were further compared with the isochronous data from

the Vienna Basin (Báldi and Hohenegger, 2008) and from the Mediterranean area (Mourik et al., 2011) throughout the studied time interval. This comparison was based on a presumption that foraminiferal  $\delta^{18}\text{O}$  reflects general isotopic characteristics of particular water masses in which foraminifera had calcified although other effects, such as local variations (changes in temperature/salinity; species isotopic variability) and the global signal contribution are definitely present.  $\delta^{13}\text{C}$  was used to test the possible relationship between surficial and bottom waters at single localities. The interpretations were confronted based on the facts from recent paleogeographical studies despite the paleogeographical characteristics being considerably complicated (e.g. Kováč et al., 2017). The anti-estuarine circulation regime was the main circulation pattern during the studied time interval, which was suggested in several earlier studies (e.g. Báldi, 2006; Kováč et al., 2017). The Mediterranean waters entered the Paratethys as a surficial inflow, which is reflected in the similarity of the planktonic  $\delta^{18}\text{O}$  between the Paratethys and the Mediterranean. The similarity is being more pronounced in younger samples. The existence of the anti-estuarine circulation regime was probably a consequence of the closure of the Indian-Mediterranean gateway, which established this type of regime also in the Mediterranean (Kouwenhoven and Van der Zwaan, 2006). Conversely, the Paratethyan bottom waters are characterized by the benthic  $\delta^{18}\text{O}$ , which is different from the Mediterranean. The Paratethys shows notably greater  $\delta^{18}\text{O}$  values than those reported from the isochronous interval from the Mediterranean (Paratethys  $\delta^{18}\text{O} \sim +0.3$  to  $+1$  ‰ vs. Mediterranean  $\delta^{18}\text{O} \sim -1$  to  $0$  ‰). It agrees well with the previous definition of the Central Paratethys Bottom Water in Scheiner et al. (2018); (**study I**). It was suggested that the Paratethyan bottom waters were regionally formed and they were probably cooler or saltier compared to the Mediterranean waters at the same depth during this time interval. Moreover, there should be an outflow of the Paratethyan bottom waters back to the Mediterranean, but there is no evidence that could clearly support this presumption. However, there are no indications of an enclosed bottom water circulation, and Cornacchia et al. (2018) also suggested an open connection between the Paratethys and the Mediterranean based on Sr and Nd isotopes. During the time interval of  $\sim 14.4$  to  $13.9$  Ma the bottom waters  $\delta^{18}\text{O}$  experienced a change but the Paratethyan surficial waters  $\delta^{18}\text{O}$  values remained similar to those from the Mediterranean. During this time interval the bottom waters signal suddenly overturns and a certain similarity with the Mediterranean  $\delta^{18}\text{O}$  values appears. However, the most important fact is that the isotopic trend in the Paratethys was reversed compared to the trend from the Mediterranean and global oceans. It points to the Atlantic Ocean being the major influence in the Mediterranean propagating the global  $\delta^{18}\text{O}$  signal evolution. The Paratethyan bottom waters  $\delta^{18}\text{O}$  signal experienced its own evolution,

which was probably triggered by some regional aspects. The paleobiological data indicate a shallowing of the basin during this time interval, which could be one of the factors affecting the evolution of the Paratethyan bottom waters. Additionally, the Mg/Ca based paleotemperatures shows a minor warming during this time interval that could be a result of the shallowing as well as it could be linked with an enhanced inflow of the warm Mediterranean waters. Nevertheless, it is very unlikely that solely those factors are responsible for the change regarding the Paratethyan bottom waters evolution. The suggested hypothesis is that the change in the Paratethyan bottom waters could have been linked with a restriction during their formation. It is because the bottom waters were probably of a regional origin and an intensive tectonic activity together with the shallowing of the basin could lead to a restriction or isolation of the source area where those waters had formed. Whether the waters were isolated or they were unable to fully develop remains still unclear. Further, it would result in their substitution by a new water mass, which origin could be linked with the inflowing Mediterranean waters within the anti-estuarine circulation regime. There are also interpretations of many regional aspects concerning the studied localities. All the details regarding the definition of the Paratethyan water masses and the evolution of the circulation patterns during the studied interval can be found in Scheiner et al. (2018) and Holcová et al. (2019a); (study I., II.).

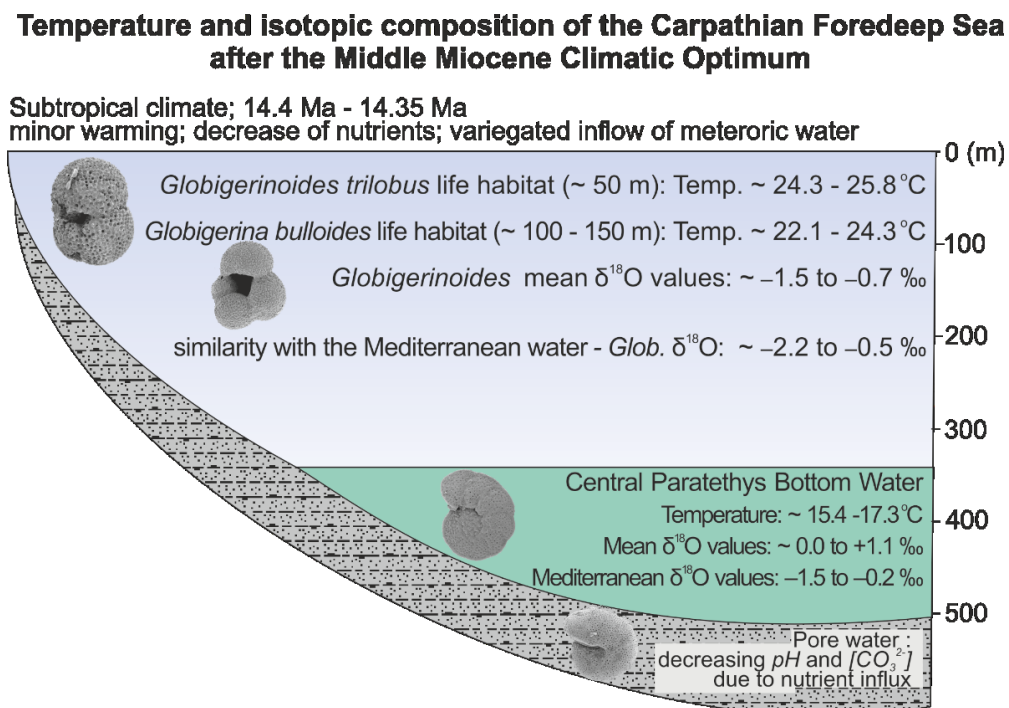


Figure 4: A simplified model showing a different hydrography of surface and bottom waters in the Central Paratethys during the time interval of ~ 14.4 - 14.35 Ma. The picture is modified after Scheiner et al. (2018).

#### 4.5 The main sources of the organic matter in the Central Paratethys

The  $\delta^{13}\text{C}_{\text{org}}$  values were compared with the typical  $\delta^{13}\text{C}_{\text{org}}$  values for various marine environments (e.g. estuaries/river deltas; open marine environment; carbonate platforms; gulfs). The mean value of the  $\delta^{13}\text{C}_{\text{org}}$  in the LOM-1 samples is  $-20.66\text{‰}$  whilst the lowest value is  $-22.96\text{‰}$ . The comparison revealed that the analysed values are mostly within the range that is typical for an open marine environment with dominant marine algal productivity, see Figure 5. All the analysed values are within the expected range of  $\delta^{13}\text{C}_{\text{org}}$  values for the studied environment thus, there were no indications pointing to any diagenetic alteration of samples although these effects are discussed in Scheiner et al. (2019); (**study III.**). Another factor is linked with the partial isotopic composition of organic matter that results in a mixing of various inputs. The typical values for the particular members are given in Scheiner et al. (2019) and in Figure 5; (**study III.**). Furthermore, there was a presumed exclusive dominance of  $\text{C}_3$  plants in terrestrial ecosystems during the studied time interval  $\sim 14.4\text{ Ma}$ , because according to the literature  $\text{C}_4$  plants started to be a significant part of terrestrial ecosystems much later in the Late Miocene (Cerling et al., 1997; Osborne and Beerling, 2006). It would result in more negative  $\delta^{13}\text{C}_{\text{org}}$  values of terrigenous members because of the  $\text{C}_3$  plants dominance, which have more negative  $\delta^{13}\text{C}_{\text{org}}$  signal. The TOC gradually decreases towards the top of the LOM-1 core with the highest value of 2.08 wt % and with the lowest value of 0.14 wt %. The higher values from the lower part of the core point to rather higher production rates that are decreasing towards the top of the core. In comparison with oceanic values, the values from the bottom part of the core are relatively high. Conversely, the upper part of the core represents a range that is typical for an open ocean (Meyers, 1994). The unexpectedly high TOC in the bottom part of the core could be linked with a high production rate but also with deposition-related processes. Burial speed and subsidence together with decreased oxygen conditions at the sediment could influence the deposition of organic matter (Katsouras et al., 2010). Moreover, an intensive volcanic activity accompanying the rise of the Carpathians (Kováč et al., 2017) could have led to fluctuations in the basin morphology and thus influencing the organic matter deposition. The carbonate content at the bottom part of the core is relatively stable around 40 wt %, whereas in the uppermost part there is a rapid rise to  $\sim 70\text{ wt \%}$  marking a rise in the carbonate production in the uppermost part of the LOM-1 core. The LOM-1 core is not a representative of a carbon-dominated environment thus it rather represents a deeper open marine environment but the rise in the carbonate production in the uppermost part of the core is probably linked with the minor warming trend and with the shallowing of the basin (Scheiner et al., 2018;

Holcová et al., 2019a; **study I, II**). This could lead to a transition to a more carbon-dominated environment with an enhanced production of carbonate, which is supported by other studies and paleobiological markers (e.g. Papp et al., 1978). The hydrocarbon indices also point to a predominant marine algal productivity in the studied area and they are in accordance with the other used proxies as well as with the foraminiferal paleobiological data. Overall, the studied environment represents an open marine environment with a dominant marine algal productivity that is gradually decreasing. There are no indications of any relevant freshwater influence pointing to a presence of large river deltas/estuaries. It is in agreement with the paleoceanographical studies by Scheiner et al. (2019) and Holcová et al. (2019a); (**study I, II**) and it supports the suitability of the LOM-1 locality for such paleoceanographical studies. Moreover, the interpretation of an open marine environment is interesting from a paleogeographical perspective, because traditionally this locality was situated “out” of the Carpathian Foredeep as its western margin. The presence of an open marine environment indicates that the coastline was probably situated more westwards on the Bohemian Massif.

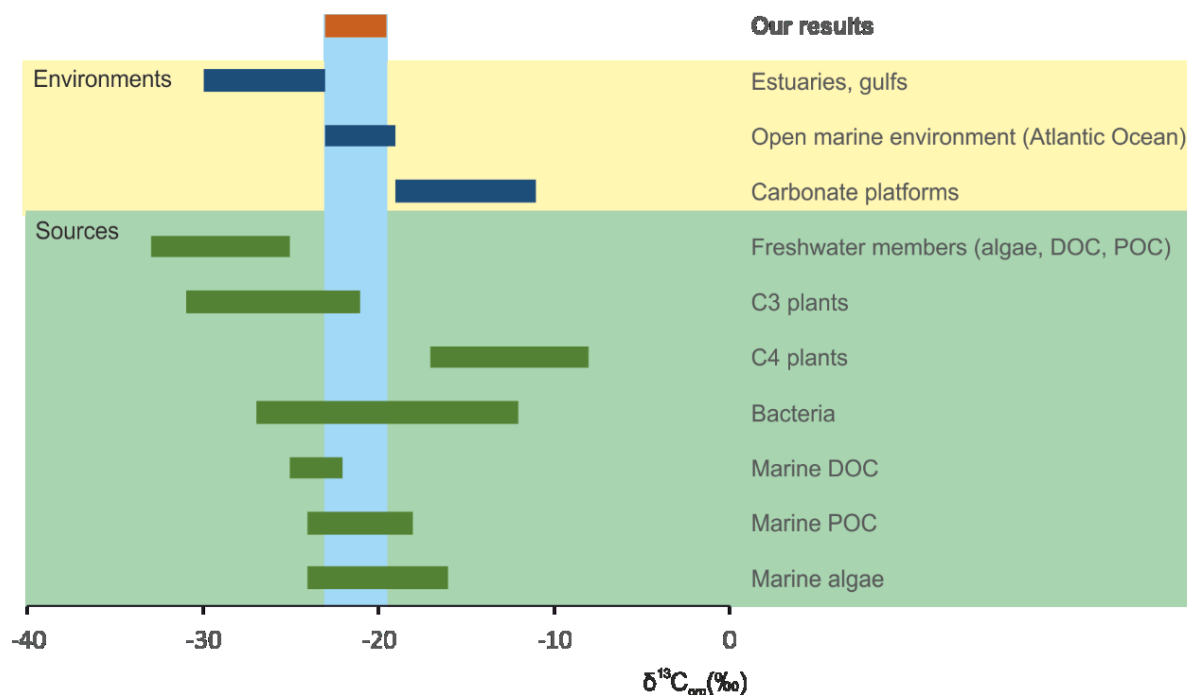


Figure 5: The typical  $\delta^{13}C_{org}$  ranges for various sources and environments in comparison with the LOM-1 data. The picture is taken from Scheiner et al. (2019).

#### 4.6 Paleoeological dynamics at the seafloor

The LOM-1 core sedimentary sequences were deposited under oxic conditions, which is indicated by the Pr/Ph ratio. It agrees with the presence of rich benthic fauna in the studied material because the faunal assemblages do not reflect any anoxic or dysoxic conditions. BFOI indicates mostly low oxic conditions but there is a large scatter in this index. During the preparation of the material, there were commonly observed pyrite infillings in foraminiferal tests. Those pyrite infillings are often formed under anaerobic conditions and the formation is related with the so-called reducing zone/layer in the sediment (McNeil, 1997). This zone/layer often starts in the first cm's of the sediment depth in normal marine environments (McNeil, 1997). These infillings are more common in the bottom part of the core that is associated with the higher TOC content and higher production rate. This leads to an assumption that high content of organics at the seafloor promotes bacterial activity that consumes oxygen and thus resulting in an expansion of the reducing zone/layer in the sediment. Nevertheless, the infillings could be just a selective phenomenon dependent on different types of foraminiferal tests that can have various predispositions to diagenesis. The benthic foraminiferal stable isotopic data reflect the life habitat and life strategy of the selected species well. There is a presence of various vital effects and the above-mentioned isotopic variability as well as the presence of the global isotopic signal. One of the important components of vital effects are the food preferences of particular species that are reflected in their  $\delta^{13}\text{C}$  signal (Schmiedl et al., 2004) thus the most important factors for interpreting the  $\delta^{13}\text{C}$  data are food preferences and life habitat of the selected foraminiferal species. The  $\delta^{13}\text{C}$  patterns and preferences of the selected species are discussed in detail in Scheiner et al. (2019); (**study III.**). The presence of *Gyroidinoides* spp. could be used for bathymetrical estimations because this species is considered as a bathymetric indicator in the Mediterranean region and its presence indicates depths of ~ 500 - 600 m (Barbieri, 1991). The BFOI calculation appears to be influenced mainly by the presence of deep and intermediate infauna, such as *Buliminas*, *Bolivinas* and *Uvigerinas*. When these species are commonly appearing in a sample, the calculated BFOI is often shifted to negative values indicating suboxic conditions at the seafloor. Nevertheless, several suboxic indicators such as *Melonis pompilioides* are appearing also in samples that are indicating high-oxic conditions but often in these samples, there is only a scarce occurrence of *Buliminas* and *Bolivinas*. This illustrates that BFOI reflects rather the deep infaunal composition of foraminiferal assemblages than the benthic assemblage as a whole, making it not reliable for using in the studied environment of the Central Paratethys. Based on the above-mentioned problematics and

foraminiferal  $\delta^{13}\text{C}$  data it was possible to describe the paleoenvironmental settings at the seafloor. Generally, a rather higher oxygen content together with an intensive nutrient influx promotes bacterial activity that results in a higher oxygen consumption in the sediment causing a drop in oxygen concentrations (McNeil, 1997). This allows a vertical rise of the less oxygenated zone to which many species could be very sensitive. Especially, species with rather static infaunal life strategy (e.g. *Melonis pompilioides*; Mackensen et al., 2000; Schmiedl et al., 2004) could thrive under slightly limited oxygen conditions utilizing degraded organic matter. However, when the oxygen levels and other factors associated with it, such as pH or carbonate ion concentration reach a certain threshold it could lead to a substitution of assemblages in favour of more adapted/tolerant ones or even to a disappearance of infaunal assemblages from the sediment at all. This process could be influenced by a seasonality, productivity rate and could alternate based on actual environmental settings. This scenario of vertical migration of the less oxygenated zone is in a good agreement with the situation reflected in our samples from the LOM-1 core. Moreover, the change in the circulation pattern proposed by Holcová et al. (2019a) is detectable in the foraminiferal  $\delta^{13}\text{C}$  data as well as increasing of the seasonality in the upper part of the core, which is probably linked with the shallowing of the basin together with the above-described paleoenvironmental changes. Generally, there are no indications of dysoxic or anoxic conditions at the seafloor but there were fluctuations of oxygen levels and pH within the sediment. The physicochemical conditions at the seafloor and in the sediment fluctuated based on the rate of primary productivity linked with an intensive bacterial activity causing a migration of the less oxygenated zone in the studied environment in seasonal or short-term variations. For more details regarding the paleoecological situation at the seafloor, please see Scheiner et al. (2019); (**study III.**).

#### **4.7 Paleoenvironmental turnovers during the Langhian/Serravalian boundary**

The paleoenvironmental turnovers are described based on the detailed analysis of the parastratotype sedimentary sequence from the ŠO-1 core in the Pannonian Basin. Using biostratigraphical markers, the studied locality was correlated with the time interval of ~ 14.4 - 13.5 Ma. This sequence is formed by four basic units characterizing the studied paleoenvironmental settings: 1) transgressive siliciclastic-carbonate complex - representing inner carbonate ramp settings where there are climatic oscillations such as events of increased rainfalls; 2) tuffaceous siltstones - representing the sea level high-stand with an introduction of cooler waters into this area, which is accompanied by cyclical events of an increased precipitation; 3) lowstand sandstones - indicating shallowing of the basin and an appearance of

seagrass meadows, which is correlated with the Ser 1 event representing the onset of the Wieliczkiian salinity crisis; 4) transgressive siltstones - representing a normal marine environment related with the Serravalian transgression event with marks of the presumed climate cooling. For further details, please see the study by Holcová et al. (2019b); (**study IV.**).

## **5. Summary**

This thesis illustrates a range of paleoenvironmental, paleoecological and paleoceanographical interpretations in the area of the Central Paratethys or in the Paratethys marine realm during the Lughian period. It illustrates the potential of using various geochemical proxies on foraminifera together with paleobiological data and with several other proxies, such as the organic geochemistry proxies, to obtain conclusions that would be impossible or very difficult to deduce using classical paleontological approaches. Moreover, this thesis discusses in detail the methodological aspects of the used proxies including new approaches that were specifically designed for the studied environment. The single test approach proved to be a reliable methodology for an analysis of carbon and oxygen stable isotopes on foraminifera because it helps with the identification of diagenetically altered material and it allows detecting of the species isotopic variability. Mg/Ca based paleothermometry appeared to be a robust tool to precisely estimate seawater paleotemperatures in the fossil epicontinental sea, when used properly. Additionally, the analysis of  $\delta^{13}\text{C}_{\text{org}}$  together with the TOC analysis and with the analysis of basic n-alkane indices represents an interesting tool, which together with foraminiferal paleobiological data can be used to interpret paleoenvironmental conditions at the seafloor. Generally besides the developing of effective methodologies the combination of the above-mentioned methods allowed an insight to a complex paleoceanographical evolution of the Paratethys-Mediterranean marine system. The determination of particular water masses as well as the exact identification of the prevailing circulation patterns are the most important results of this research. These form the basis on which the future research could be initiated. Lastly, the numerous local interpretations that were an integral part of this research are no less important and represents invaluable data regarding the local paleontological and paleoenvironmental studies. The concept presented by this thesis shows how to combine classical approaches together with the modern ones to form a viable state-of-the-art paleoceanographical research in a fossil epicontinental setting. Therefore, all the aims on which this thesis was built on were successfully fulfilled.

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## Annex

**I. Scheiner, F., Holcová, K., Milovský, R., Kuhnert, H., 2018.** Temperature and isotopic composition of seawater in the epicontinental sea (Central Paratethys) during the Middle Miocene Climate Transition based on Mg/Ca,  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  from foraminiferal tests. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 495, 60-71.

**II. Holcová, K., Kopecká, J., Scheiner, F., 2019.** An imprint of the Mediterranean middle Miocene circulation pattern in a satellite sea during the Langhian: a case study from the Carpathian Foredeep (Central Paratethys). *Palaeogeography, Palaeoclimatology, Palaeoecology*, 514, 336-348.

**III. Scheiner, F., Holcová, K., Milovský, R., Doláková, N., Rigová, J., 2019.** Response of benthic foraminiferal communities to changes in productivity and water mass conditions in the epicontinental sea during the Middle Miocene. *Marine Micropaleontology*, in review.

**IV. Holcová, K., Dašková, J., Fordinál, K., Hrabovský, J., Milovský, R., Scheiner, F., Vacek, F., 2019.** A series of ecostratigraphic events across the Langhian/Serravallian boundary in an epicontinental setting: the northern Pannonian Basin. *Facies*, in press, DOI: 10.1007/s10347-019-0576-1.

## Prohlášení školitele o podílu studenta na publikacích, které jsou součástí doktorské práce

*Jako školitel studenta Mgr. Filipa Scheinera prohlašuji, že student se podílel na pracích, které byly předloženy jako součást jeho disertační práce, následujícím podílem:*

**Scheiner, F., Holcová, K., Milovský, R., Kuhnert, H., 2018.** Temperature and isotopic composition of seawater in the epicontinental sea (Central Paratethys) during the Middle Miocene Climate Transition based on Mg/Ca,  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  from foraminiferal tests. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 495, 60-71.

*70% příprava materiálu, spolupráce při analýzách, kompilace a interpretace výsledků a příprava a finalizace rukopisu*

**Holcová, K., Kopecká, J., Scheiner, F., 2019.** An imprint of the Mediterranean middle Miocene circulation pattern in a satellite sea during the Langhian: a case study from the Carpathian Foredeep (Central Paratethys). *Palaeogeography, Palaeoclimatology, Palaeoecology*, 514, 336-348.

*50% příprava vzorků pro geochemickou analýzu, interpretace geochemických dat, výrazný podíl na tvorbě cirkulačního modelu, výrazný podíl na přípravě a finalizaci rukopisu*

**Scheiner, F., Holcová, K., Milovský, R., Doláková, N., Rigová, J., 2019.** Response of benthic foraminiferal communities to changes in productivity and water mass conditions in the epicontinental sea during the Middle Miocene. *Marine Micropaleontology*, in review.

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**Holcová, K., Dašková, J., Fordinál, K., Hrabovský, J., Milovský, R., Scheiner, F., Vacek, F., 2019.** A series of ecostratigraphic events across the Langhian/Serravallian boundary in an epicontinental setting: the northern Pannonian Basin. *Facies*, in press, DOI: 10.1007/s10347-019-0576-1.

*20% interpretace geochemických dat, podíl na přípravě rukopisu*

Doc. RNDr. Katarína Holcová, CSc.

školitelka

## The declaration of the supervisor

*As the supervisor of Mgr. Filip Scheiner I declare that Mgr. Filip Scheiner participated on the studies that were submitted as a part of his Ph.D. thesis by the following share:*

**Scheiner, F., Holcová, K., Milovský, R., Kuhnert, H., 2018.** Temperature and isotopic composition of seawater in the epicontinental sea (Central Paratethys) during the Middle Miocene Climate Transition based on Mg/Ca,  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  from foraminiferal tests. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 495, 60-71.

*70% - preparation of the material; collaboration during the geochemical analysis; compilation of the results and preparation and final editing of the manuscript*

**Holcová, K., Kopecká, J., Scheiner, F., 2019.** An imprint of the Mediterranean middle Miocene circulation pattern in a satellite sea during the Langhian: a case study from the Carpathian Foredeep (Central Paratethys). *Palaeogeography, Palaeoclimatology, Palaeoecology*, 514, 336-348.

*50% - preparation of the material for the geochemical analyses; interpretation of the geochemical data; a significant contribution to the proposed circulation model and a significant contribution to finalization of the manuscript*

**Scheiner, F., Holcová, K., Milovský, R., Doláková, N., Rigová, J., 2019.** Response of benthic foraminiferal communities to changes in productivity and water mass conditions in the epicontinental sea during the Middle Miocene. *Marine Micropaleontology*, in review.

*70% - partial preparation of the material; compilation and interpretation of the results; preparation and final editing of the manuscript*

**Holcová, K., Dašková, J., Fordinál, K., Hrabovský, J., Milovský, R., Scheiner, F., Vacek, F., 2019.** A series of ecostratigraphic events across the Langhian/Serravallian boundary in an epicontinental setting: the northern Pannonian Basin. *Facies*, in press, DOI: 10.1007/s10347-019-0576-1.

*20% - interpretation of the geochemical data; a contribution to the preparation of the manuscript*

Doc. RNDr. Katarína Holcová, CSc.

The Ph.D. supervisor