International Journal of Speleology | 53 (1) | 25-37 | Tampa, FL (USA) | January 2024

Available online at scholarcommons.usf.edu/ijs

International Journal of Speleology
Official Journal of Union Internationale de Spéléologie

Subaqueous carbonate speleothems as paleotemperature archives – clumped isotope thermometry and stable isotope compositions of inclusion-hosted water

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Abstract: Clumped isotope measurements of carbonates and stable isotope analyses of water trapped in fluid inclusions are both promising techniques to determine carbonate formation temperatures. Cave-hosted carbonate deposits (speleothems) are excellent targets for such studies, but kinetic fractionations and diagenetic influences frequently deteriorate the temperature data obtained from these methods. However, subaqueous carbonate deposits may provide reliable data, as kinetic fractionations are less significant in underwater environments. In this study, subaqueous speleothems, whose formation temperatures were directly measured in the water, were investigated. Additionally, temperatures calculated from the oxygen isotope fractionations between calcite and fluid inclusion-hosted water were compared with clumped isotope temperatures obtained for subaqueous carbonate formations in cave-hosted lakes. The clumped isotope temperatures fit the measured and calculated fluid inclusion temperatures within the analytical precisions. Carbonate deposits formed at elevated temperatures (~50°C or above) may undergo post-formational calcite-water oxygen isotope exchange, altering the composition of the inclusion-hosted water. In contrast, subaqueous speleothems formed at about 20-25°C appear to preserve the primary isotopic compositions. Our study shows that subaqueous carbonate speleothems are useful targets for clumped isotope and inclusion water analyses, making them valuable paleotemperature archives.

Keywords: subaqueous speleothem, clumped isotope, stable isotope, fluid inclusion, formation temperature

Received 11 September 2023; Revised 13 February 2024; Accepted 17 February 2024

Citation: Demény, A., Berentés, A., Rinyu, L., Kovács, I., Surányi, G., Virág, M., 2024. Subaqueous carbonate speleothems as paleotemperature archives – clumped isotope thermometry and stable isotope compositions of inclusion-hosted water. International Journal of Speleology, 53(1), 25-37. https://doi.org/10.5038/1827-806X.53.1.2480

INTRODUCTION

Carbonate speleothems (referred to as "speleothems" hereon for simplicity) are ideal subjects for paleoclimate research, as they can be dated with absolute dating methods, are formed in closed cave environments that protect them from significant late-stage alteration, and they provide various proxy data from which past climate conditions can be deciphered (see Fairchild & Baker, 2012). Temperature is one of the most important variables during speleothem formation, which is primarily investigated using stable oxygen isotope compositions (henceforth referred to as "oxygen isotope compositions", as no radioactive isotope is discussed here) of carbonates and waters, as well as clumped isotope measurements of carbonates. The oxygen isotope composition of carbonate is one of the most widely applied proxy datasets in speleothem studies, appearing in over a thousand international publications, whereas carbonate clumped isotope analysis has a shorter history that began with the seminal paper of Ghosh et al. (2006). As such, we first describe the background of oxygen isotope thermometry, then discuss clumped isotope thermometry. Stable hydrogen and oxygen isotope compositions of waters and carbonates

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are expressed as δ^2H and δ^{18}O values (relative to V-SMOW), respectively.

The oxygen isotope data of speleothems are used to determine changes in temperature, humidity, precipitation amount, seasonal variations, moisture transport routes, and meteorological teleconnections (e.g., Lachniet, 2009; Fairchild & Baker, 2012; Zhang et al., 2019; Demény et al., 2021a; Parker & Harrison, 2022; Treble et al., 2022). Oxygen isotope thermometry is based on the temperature dependence of carbonate-water oxygen isotope fractionation (McCrea, 1950). When the carbonate and water oxygen isotope compositions are measured, or estimated in the case of water (due to the absence of direct measurements of paleodripwaters), and the fractionation's temperature dependence is known (see later), the formation temperature can be calculated. However, calcite-water oxygen isotope thermometry suffers from several drawbacks. True equilibrium between the precipitating calcite and the solution is seldom reached (Daëron et al., 2019) due to kinetic fractionations. Numerous studies have investigated the temperature dependence of calcite-water oxygen isotope fractionation and reported different fractionation equations (laboratory experiments: O'Neil et al., 1969; Friedman & O'Neil, 1977; Kim & O'Neil, 1997; Watkins et al., 2014; Jautzy et al., 2020; theoretical calculations: Horita & Clayton, 2007; Chacko & Deines, 2008; empirical observations on natural occurrences: Coplen, 2007; Demény et al., 2010; Tremaine et al., 2011; Johnston et al., 2013; Daëron et al., 2019). The lowest temperature for a given calcite-water oxygen isotope fractionation value is yielded through the equation obtained with statistical mechanical calculations by Chacko & Deines (2008). The highest temperature is provided by the empirical equation obtained for extremely slowly depositing (and formed closest to equilibrium) speleothem calcites (Daëron et al., 2019). The temperature difference between the two equations for given calcite-water oxygen isotope fractionation values in the temperature range of about 0 to 60°C is about 10 to 13°C. Johnston et al. (2013) defined a calcite-water temperature calibration, which applies to most speleothems, that contained earlier published empirical data (e.g., Demény et al., 2010; Tremaine et al., 2011), as well. In addition to temperature, the local calcite-water oxygen isotope fractionation depends on several factors (e.g., pH and growth rate, Dietzel et al., 2009; Watkins et al., 2014), which are not known for subrecent or fossil speleothems. Degassing-related kinetic oxygen isotope fractionation may also affect calcite compositions (e.g., Mickler et al., 2006). As a result, an appropriate equation that best fits the local speleothem environment should be selected for reliable paleotemperature calculations.

Water oxygen isotope composition can be determined directly by extracting inclusion-hosted H_2O. Conventionally, the oxygen isotope composition was measured in the extracted H_2O by mass spectrometry that required a CO_2-H_2O equilibration in micro-sealed vessels (Socki et al., 1999) or a reaction of H_2O with fluorine compounds (O'Neil & Epstein, 1966). These methods are complicated (micro-equilibration) or dangerous (fluorination). As a result, newer techniques, such as using a high-temperature conversion elemental analyzer (TC/EA) attached to a mass spectrometer and the application of laser spectroscopy, induced a revolution in fluid inclusion stable isotope analyses (e.g., Vonhof et al., 2006; Dublyansky & Spötl, 2009; Arienzo et al., 2013; Affolter et al., 2014), as they have improved throughput relative to the micro-equilibration method and do not require the use of dangerous fluorine compounds. Laser spectroscopy is particularly promising, as it enables the direct and combined determination of ^{2}H/^{1}H and ^{18}O/^{16}O ratios in the H_2O vapor introduced into the spectroscope in a vacuum (Czuppon et al., 2014; Demény et al., 2016) or in a nitrogen carrier gas (de Graaf et al., 2020 and references therein). In contrast, TC/EA analyses determine the δ^2H and δ^{18}O values separately (Vonhof et al., 2006; Dublyansky & Spötl, 2009). Numerous studies have measured the δ^{18}O values of inclusion water in speleothems to determine past temperatures and/or hydrological changes (e.g., van Breukelen et al., 2008; Griffiths et al., 2010; Wainer et al., 2011; Rowe et al., 2012; Labuhn et al., 2015; Meckler et al., 2015; Allan et al., 2018; Affolter et al., 2019; Wortham et al., 2022; Weissbach et al., 2023). However, although some speleothems yielded reliable temperature data, others may have suffered diagenetic alterations that changed the original oxygen isotope compositions in the inclusion-hosted water (Demény et al., 2016; Affolter et al., 2019; Demény et al., 2021b).

With the development of clumped isotope analyses of carbonates and the discovery of the temperature dependence of the preferential bonding („clumping") of ^{13}C and ^{18}O (Ghosh et al., 2006), it became possible to directly determine the temperature of the carbonate formation. Clumped isotope compositions are expressed as Δ_{47} values (i.e., the deviation of the abundance of ^{13}C^{18}O^{16}O from the thermodynamically determined stochastic distribution, Eiler, 2007). Calibrations of the temperature dependence of the Δ_{47} values of carbonates were established using laboratory experiments (e.g., Jautzy et al., 2020), natural carbonate formations with known precipitation temperatures (e.g., Kele et al., 2015), and a combination of natural carbonate analyses and laboratory experiments (Anderson et al., 2021). Although many different carbonate formations yielded consistent Δ_{47}-temperature relationships (Anderson et al., 2021), speleothems rarely yielded reliable formation temperatures (e.g., Meckler et al., 2015; Duan et al., 2022), due to kinetic fractionations along the karstic water migration routes, on the stalagtite surfaces, and at the drip water arrival point on the tips of the stalagmites (Deininger et al., 2021). Nevertheless, speleothems that formed at a very low growth rate (i.e., reaching dynamic equilibrium between the carbonate and the solution) and underwater can potentially represent carbonate deposits that would provide reliable clumped isotope temperatures, as demonstrated for the Devils Hole and Laghetto Basso calcites by Daëron et al. (2019). The kinetic fractionation effects of $CO_{2(aq)}$ degassing on clumped isotope compositions is discussed in detail by Bajnai et al. (2021). Additionally, travertines that precipitate from strongly degassing solutions underwater appear to form in clumped isotope equilibrium (Kele et al., 2015), in contrast to stalagmite carbonate, which precipitates from a thin solution film that rapidly releases CO_2.

Subaqueous speleothem formations are promising for clumped isotope thermometry due to limited CO_2 degassing and evaporation during carbonate precipitation, which reduce the effects of kinetic fractionation. Clumped isotope analyses of subaqueous speleothems have been successfully used for Δ_{47}–temperature calibration (Breitenbach et al., 2018) or to decipher paleohydrological and paleotemperature changes in the aquifer (Kluge et al., 2014; Gázquez et al., 2018; Bajnai et al., 2021).

In a recent study, Koltai et al. (2024) compared temperature data obtained from clumped isotope analyses, nucleation-assisted fluid inclusion microthermometry (NA-FIM), and calcite-water oxygen isotope thermometry (OIT) using fluid inclusion $\delta^{18}O$ analyses of calcite spars collected in quarries and caves. The found that in a temperature range of 10 to 50°C the clumped isotope temperatures fit the NA-FIM data with an approximate uncertainty of ±7°C, whereas the OIT temperatures yielded mixed results (although it should be noted that only three samples were measured).

The aim of this study was to investigate subaqueous speleothem carbonates that were formed in cave-hosted lakes, as well as flowstone and travertine occurrences, both formed from flowing water. We determined their clumped isotope compositions and compared the Δ_{47}–based temperatures with temperature data either directly measured in the host water or yielded by stable isotope analyses of the inclusion-hosted water. As a result, the study showed that subaqueous carbonates can provide reliable clumped isotope paleotemperature archives in cave settings.

Cave locations and samples

Carbonate samples were collected from four locations (Molnár János Cave and Szent Lukács IV well in Budapest, Central Hungary; Berger Károly Cave, W Hungary; and Béke Cave in NE Hungary) and included subaqueous speleothems, flowstone, calcite rafts, travertine, and fresh calcite precipitates on glass slides, covering a temperature range of 9.8 to 51°C (Table 1).

Table 1. The studied samples, their locations and descriptions, as well as local water temperatures and pH values. Sources: Molnár János Cave and Szent Lukács Well IV - Virág (2018); Béke Cave - Czuppon et al. (2018); Berger Károly Cave - this study. FI: fluid inclusion analysis. CL: clumped isotope analysis.

Sample	Location	Material	Water temperature	pH	Analyses
M1 (“Vulkánok”)	Molnár János Cave	carbonate cone formed in a cave-hosted lake			FI, CL
M2 (Lukács IV)	Szent Lukács Well IV	carbonate precipitation at the orifice of a thermal well	51°C	7.0	CL
M3 (calcite rafts)	Molnár János Cave	calcite rafts formed in a cave-hosted lake	23 ± 1°C	7.0	CL
BNT-2 top	Béke Cave	flowstone surface	9.8 ± 0.1°C	7.0 ± 0.5	CL
Berger plate #17	Berger Károly Cave	calcite precipitation on glass slide placed into the water of Lángos Lake	24.3 ± 0.1°C	7.4 ± 0.1	CL
L1	Berger Károly Cave	drill core of calcite deposit in the Lángos Lake			FI, CL
L2	Berger Károly Cave	drill core of calcite deposit in the Lángos Lake			FI, CL
T1	Berger Károly Cave	drill core of calcite deposit in the Titkok Tava Lake			FI, CL
T2	Berger Károly Cave	drill core of calcite deposit in the Titkok Tava Lake			FI, CL
L-top	Berger Károly Cave	carbonate crust on the top of the Lángos Lake drill core	24.3 ± 0.1°C	7.4 ± 0.1	CL
T-top	Berger Károly Cave	carbonate crust on the top of the Titkok Tava Lake drill core	19.1 ± 0.1°C	7.4 ± 0.1	CL

Thermal karst environments are represented by the Molnár János Cave in Budapest, Central Hungary and by the Berger Cave in Western Hungary (Fig. 1A). The Molnár János Cave (N 47° 31’ 05”, E 19° 02’ 09”) contains a large underground lake, whose temperature is 23 ± 1°C, pH value is about 7.0, and the water’s $\delta^{18}O$ value is −10.8‰ (Virág, 2018). The water temperature and pH were measured during sample collection. A special carbonate formation, morphologically resembling a volcano (thus called „Vulkánok” or „volcanos” in Virág, 2018) was collected (sample M1 “Vulkánok”, Table 1). The cone-shaped carbonate deposits are approximately 20 cm in height (Fig. 1B) and have central chimneys, representing the orifices of thermal water emanations into an underground lake. A sample was taken from near one of the chimneys (Fig. 1B). The carbonate cones are composed of coarse-grained calcite with abundant fluid inclusions (Fig. 1C). The fluid inclusions are arranged in growth zones within isometric calcite crystals and can therefore be considered primary. The growth zones may represent an evolution during formation, but as the inclusions are extracted from gram-sized sample batches, the subsequently formed inclusions are measured together. A travertine calcite deposit (sample M2, Table 1) was collected at the orifice of Szent Lukács well IV, ~100 m away from the Molnár János Cave. The well water temperature was 51.0°C, its pH value is about 7.0 (both measured during calcite sample collection), and its δ^2H and δ^{18}O values are –85 and –11.7‰, respectively (Virág, 2018). As an example of recently forming carbonate in the Molnár János Cave, calcite rafts were also collected from the Szent Lukács (Saint Luke) shaft of the cave that were formed on the water surface (Fig. 1D) and deposited on the bottom. The calcite rafts usually contain micrite in their internal parts, covered by microsparite (Fig. 1E, Virág, 2018).

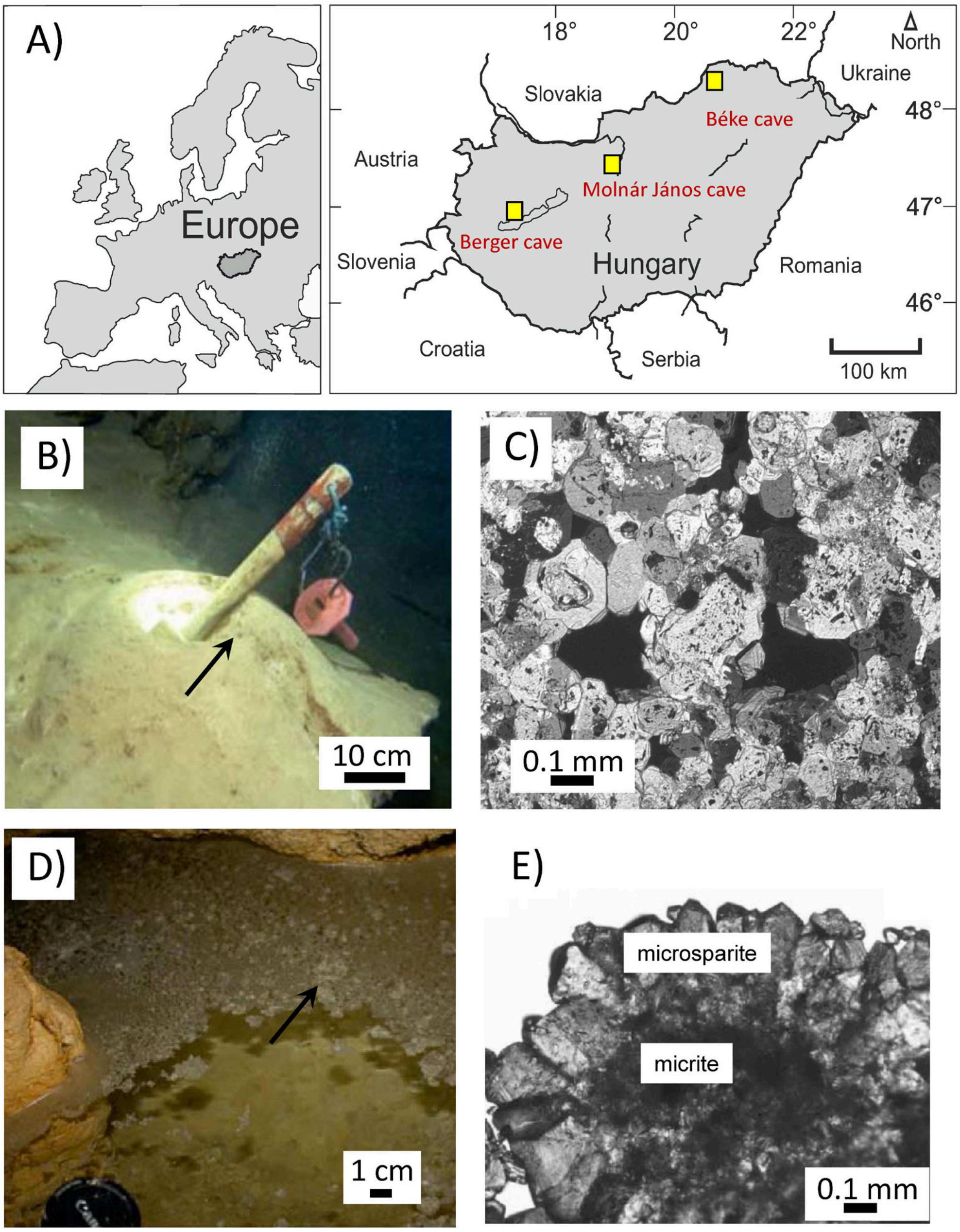

Fig. 1. A) Cave locations studied in this paper. B) The Vulkánok („volcanos") carbonate cones in the Molnár János Cave (photo by Dénes Szieberth). Black arrow shows the approximate sampling location. C) Optical microscopic picture with crossed nicols of the Vulkánok carbonate. D) Recently forming calcite rafts on the lake surface (shown by black arrow) of the Szent Lukács shaft of the Molnár János Cave (source: Virág, 2018). E) Optical microscopic image (with one nicol) of a calcite raft of the Molnár János Cave (source: Virág, 2018).

The Berger Károly Cave is situated beneath the town of Tapolca (N 46° 53' 14", E 17° 26' 30", Fig. 1A). It is strictly protected, and only expert cavers can enter with the permission of the Balaton Uplands National Park. The cave, discovered in 2002, stretches about 2 km long. It is a thermal karstic cave, with abundant carbonate deposits on the walls and several lakes, with thick calcite deposits (Figs. 2A, B). The calcite deposits and local physicochemical parameters (water temperature, pH, chemical composition) of two lakes (Lángos and Titkok tava lakes) were investigated from 2020 to 2022 but only on 6 occasions, due to restricted access to the lake chambers. Although monitoring activity was limited, the closed environment with reduced human influence, the extensive carbonate deposition in the lake pools, and the stable physicochemical conditions (see below) made the cave valuable for the study of subaqueous speleothem formation. The lakes are situated about 500 m away from the artificial entrance. The water temperatures of Lángos Lake and Titkok tava Lake were 24.3 ± 0.1 and 19.1 ± 0.1°C, respectively, in 2022 February and

July, and both lakes had pH values of 7.4 ± 0.1. In February 2022, 5x5 cm glass plates were placed just above and below the water levels (Fig. 2A) and were retrieved in July 2022. Carbonate deposition was observed on the glass plates placed in Lake Lángos, from which plate #17 (sample "Berger plate #17", Table 1) yielded enough carbonate to study. Drill cores were collected from the lake carbonate deposits at two locations in Lakes Titkok tava (Fig. 2B) and Lángos. Both cores were analyzed at two sampling points (samples L1, L2, T1, T2, Table 1, Fig. 2C), as well as at the top of the core, where a carbonate crust was detected (L-top and T-top for Lángos and the Titkok Tava, respectively) (Fig. 2C, Table 1). Fluid inclusions are usually primary, scattered within isometric calcite crystals, and are distributed in growth zones (Fig. 2D). The carbonate crusts that formed at the top of the drill cores are about 1 mm thick (Fig. 2E). The top crust of the Lángos core contains needle-like crystals at its base, covered by isometric crystals (Fig. 2F).

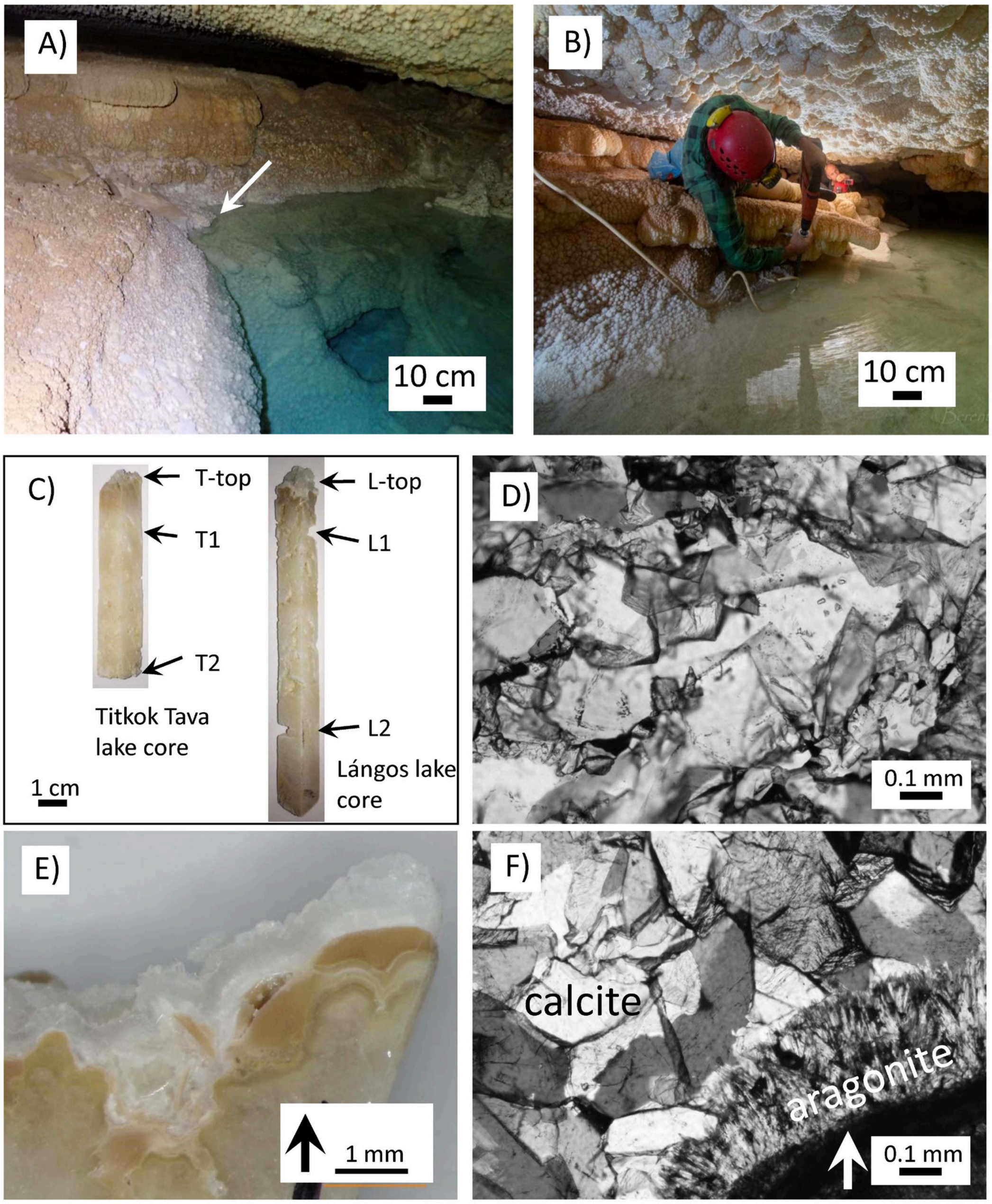

Fig. 2. A). Sample location of the Lángos Lake of the Berger Károly Cave. Glass plates were placed on the calcite deposit at the upper left corner of the lake shore (shown by the white arrow). B) Core drilling at the Titkok Tava Lake in the Berger Károly Cave. C) Drill cores (quarters of cores with 2 cm diameter) from the Titkok Tava Lake (samples „T") and from the Lángos Lake (samples „L"). D) Optical microscopic picture (one nicol) from the middle of the Lángos Lake drill core. E) The top of the Lángos Lake drill core with thin carbonate crust. F) Optical microscopic picture (one nicol) of the carbonate crust of the Lángos Lake drill core.

The Nagy-tufa flowstone deposit (Fig. 3A) is formed in Béke Cave, Northern Hungary (N 48° 27' 39", E 20° 32' 34", Fig. 1A). A detailed description of the cave site and flowstone formation is given in Demény et al. (2016). The cave temperature was 9.8 ± 0.1°C from 2013 to 2015 (Czuppon et al., 2018), and the BNT-2 drill core (Fig. 3B) was collected in April, 2014. Carbonate precipitation was active on the flowstone surface during the collection period. The water flowing on the flowstone was fed by an underground spring (Demény et al., 2019). Water discharge takes place mainly in early spring and carbonate is generally precipitated from a flowing water layer of several cm depth (Szabolcs Leél-Őssy, personal communication). Hence, the carbonate deposition on the flowstone surface is different from stalagmite formation where carbonate is usually precipitated from splashing water drips and a thin water film. The drill core covered the last 4200 years, with the topmost carbonate yielding a modern ^{14}C activity value (Demény et al., 2019). The BNT-2 top sample (Fig. 3C), comprising a dendritic calcite fabric (Fig. 3D), was scraped from the youngest part of the core and thus represents recently formed calcite at the flowstone location.

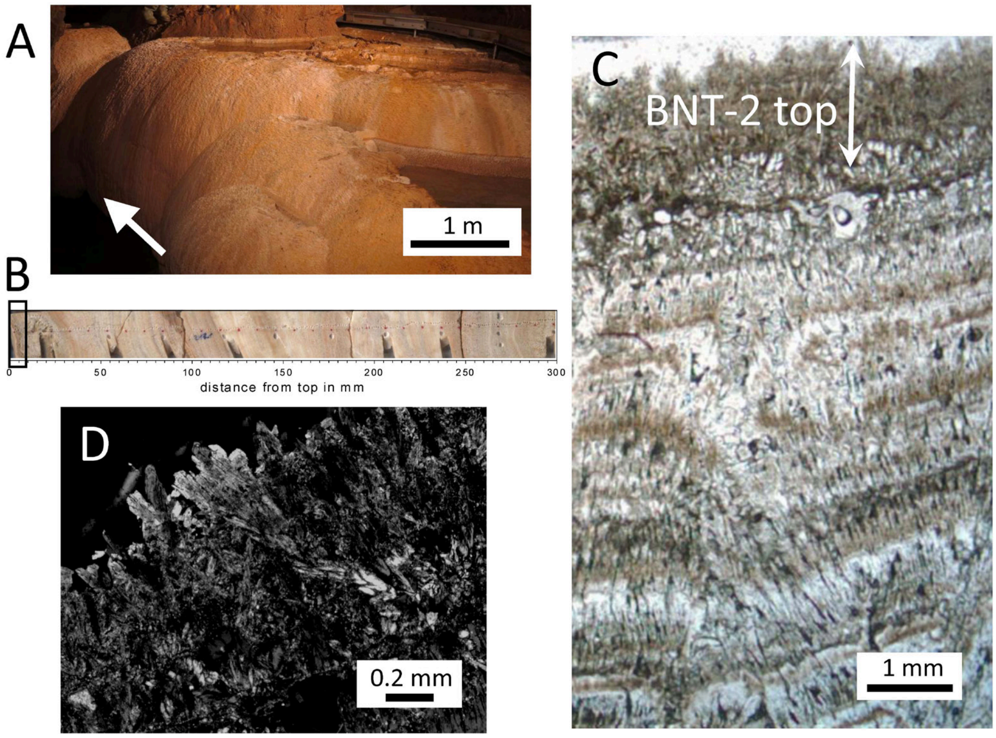

Fig. 3. A). The Nagy-tufa deposit of the Béke Cave, NE Hungary. White arrow shows the location of drill core sampling. B) The BNT-2 drill core. Box shows the location of C. C) Optical microscopic (taken with one nicol) fabric of the uppermost part of the BNT-2 drill core with the location of the BNT-2 top sample. D) Optical microscopic (taken with crossed nicols) fabric of the BNT-2 top sample layer.

METHODS

Petrographic analysis was conducted using a Nikon Eclipse E600 POL optical microscope on polished, thin (~100 µm) sections at the Institute for Geological and Geochemical Research in Budapest, as well as determination of the mineral compositions of the carbonate crusts, using a Rigaku D/Max Rapid II diffractometer, which was operated with CuKα radiation at 50 kV and 0.6 mA. In situ analyses on the surface of a polished thin section were conducted using a 100 µm collimator. A built-in CCD camera was used to select the measurement areas. The Imaging Plate (IP) was read by a laser scanning readout system in approximately 1 min. The acquisition time for each measurement was set to 5 minutes. 2DP RIGAKU software was used to record the diffraction image from the laser readout, allowing the operator to determine the area to integrate for a 2Θ versus intensity plot. This plot was read into the RIGAKU PDXL 1.8 software for data interpretation.

Clumped and oxygen isotope analyses of carbonates were carried out at the Isotope Climatology and Environmental Research Centre (ICER), HUN-REN Institute for Nuclear Research (ATOMKI) in Debrecen, Hungary. Carbonate sample analysis was performed on a Thermo ScientificTM 253 Plus 10 kV Isotope Ratio Mass Spectrometer (IRMS), after phosphoric acid digestion at 70°C, using a Thermo Scientific Kiel IV automatic carbonate device which is coupled by inert silica coated capillary to the IRMS. To eliminate the organic contamination from the extracted carbon-dioxide gas an additional Thermo Scientific™ PoraPak trap was installed between the two cold fingers of the Kiel device. The temperature of the trap was controlled by a Peltier module. The trap was filled with PoraPak™ Q 50-80 mesh porous polymer adsorbent, which was sealed with glass wool at both ends. The operation temperature of this trap was -30°C. Generally, an hour-long 120°C PoraPak trap cleaning and regeneration procedure is required before each measurement process. 100–120 µg aliquots of each carbonate sample measurement were replicated at least 8-12 times and measured alongside carbonate standard samples. Due to the relatively small sample size, clumped isotope analysis was achieved based on the long-integration dual inlet (LIDI) workflow (Müller et al., 2017), implemented in Thermo Scientific IsoDat NT 3.0.94.17 acquisition software. ETH1, ETH2, and ETH3 were used as normalization standards, and IAEA-C2 was used as a monitoring sample to determine the long-term reproducibility of the instrument. Simultaneously, the stable isotope composition of conventional carbonate was also determined for the same samples. Negative background, which is caused by secondary electrons on higher faraday-cup detectors, was corrected by applying the pressure-sensitive baseline (PBL) correction (Bernasconi et al., 2013) on all raw beam signals. Peak scans at 5 different intensities were used for the PBL correction algorithm, which is implemented in Easotope Software (Release 20190125, concept by Cédric John, programmed by Devon Bowen) (John & Bowen, 2016). The corrected data were exported into a csv file for further data processing. Data evaluation, standardization, and analytical error propagation of the Δ_{47} clumped-isotope measurements were carried out with the D47Crunch python software (Daëron, 2021), using the revised IUPAC parameters for ^{17}O correction (Baertschi, 1976; Gonfiantini et al., 1995; Meijer & Li, 1998; Assonov & Brenninkmeijer, 2003; Brand et al., 2010; Daëron et al., 2016; Schauer et al., 2016; Bernasconi et al., 2018). The samples were analyzed during different periods, so the reproducibility of the instruments changed over time. The repeatability of the Δ_{47} anchors (ETH1, ETH2 and ETH3), based on the D47Crunch calculation, varied between 0.026 and 0.037‰, while the repeatability of the IAEA-C2 monitoring sample varied between 0.011 and 0.040‰ (details in Supplementary information). A carbonate-based standardization scheme was used by applying ETH1, ETH2, and ETH3 InterCarb calcite standards (Bernasconi et al., 2021). As such, the Δ_{47} data were shown directly on the I-CDES90 scale, without having to apply a correction for acid fractionation. The Δ_{47} results are given in the I-CDES90 scale (Bernasconi, et al. 2021), and apparent temperatures in °C were calculated based on the Δ_{47}-temperature calibration from Anderson et al. (2021), with temperature uncertainties propagated from the 95% confidence interval of the Δ_{47} value, as well as from the uncertainty of the calibration equation. The oxygen isotope compositions of calcite are expressed as $\delta^{18}O$ values in ‰, relative to V-SMOW. Measurement data, averages, and uncertainties of the samples and standards are provided in the Supplementary information. The average $\delta^{18}O$ reproducibility for sample measurements is ± 0.09‰. The analytical accuracy is demonstrated by the IAEA-C2 analyses, which reference material was measured as unknown. As the Supplementary Information worksheet shows, the $\delta^{18}O$ (V-PDB) values of the IAEA-C2 calcite were –9.11 ± 0.09‰ (N=18), –9.00 ± 0.10‰ (N=27), –8.98 ± 0.11‰ (N=26), and –9.10 ± 0.14‰ (N=26) in four measurement runs, whereas Bernasconi et al. (2018) reported a $\delta^{18}O$ value of –9.0 ± 0.05 (N=11) for the IAEA-C2 calcite.

The stable hydrogen and oxygen isotope compositions of the inclusion-hosted water were determined at the Institute for Geological and Geochemical Research in Budapest, following Demény et al. (2016). Sample chips (2-5 mm pieces) of about 1-2 g were crushed under vacuum in 10 mm (outer diameter) stainless steel tubes, and the extracted H_2O was purified by vacuum distillation to remove non-condensable gases and CO_2, which was extracted at liquid nitrogen temperature, along with H_2O. The H_2O was introduced into a model LWIA-24d (Los Gatos Research Ltd.) liquid water isotope analyzer. The amount of H_2O was calibrated by injecting known water amounts. Corrections for measurement drifts, amount effects, and memory effects were conducted using three laboratory water standards, as described by Czuppon et al. (2014). The isotope compositions are expressed as δ^2H and $\delta^{18}O$ values in ‰, relative to V-SMOW. Based on measurements of carbonate-hosted inclusion waters with known isotopic compositions, the estimated analytical accuracies are about ± 0.5 and ± 2‰ for $\delta^{18}O_{fi}$ (where „fi" means „fluid inclusion") and δ^2H values, respectively (see Demény et al., 2021b). Formation temperatures were calculated using the $\delta^{18}O$ values of calcite ($\delta^{18}O_{cc}$) and inclusion-hosted water, as well as a set of calcite-water oxygen isotope fractionation equations (Friedmann & O'Neil, 1977; Chacko & Deines, 2008; Kim & O'Neil, 1997; Tremaine et al., 2011; Johnston et al., 2013; Daëron et al., 2019) to cover equations obtained by theoretical calculations, laboratory experiments, and measurements on natural carbonates with known formation temperatures.

RESULTS AND DISCUSSION

Clumped isotope temperatures

The most straightforward test of clumped isotope-based temperature determinations is to compare them with the measured formation temperatures of recently formed calcites (e.g., Kele et al., 2015). The results of this study are listed in Table 2.

The calcite rafts that formed at 23.2°C on the surface of the lake in Molnár János Cave (sample M3) yielded Δ_{47} = 0.586 ± 0.009‰ (I-CDES90°C, Crunch, ±1SE, as all Δ_{47} data henceforth), corresponding to 27.9 ± 6.4°C when using the Anderson et al. (2021) calibration (henceforth applied for all Δ_{47} temperatures; temperature uncertainties are within the 95% confidence interval). The travertine deposit that formed at the orifice of the Lukács IV thermal well at 51.0°C (sample M2) yielded a Δ_{47} value of 0.530 ± 0.008‰, corresponding to 49.5 ± 7.4°C. The glass plates that were placed underwater and on the uppermost surfaces of the drill cores in Berger Károly Cave are covered by an actively forming carbonate crust (Fig. 2E), so their compositions can be directly compared with the monitoring data. The calcite from plate #17 was collected at Lake Lángos in Berger Cave and yielded a Δ_{47} value of 0.605 ± 0.015‰ (21.4 ± 9.6°C), which is close to the measured water temperature (24.3°C). The carbonate crust of the Lake Titkok tava (sample T-top, Fig. 2C) sample is composed 100% of calcite, whereas the carbonate collected from the top of the Lángos drill core (sample L-top, Fig. 2C and E) for clumped isotope analysis contained calcite and aragonite (Fig. 2F). As Fig. 2E shows, the carbonate crust begins with a layer of needle-like crystals, and then is covered by isometric crystals, which are similar to the fabric of the internal part of the core (Fig. 2D). Micro-XRD analyses showed that the needle-like crystal layer is aragonite, and the carbonate covering is calcite. The L-top powder sample collected from the carbonate crust for clumped isotope analysis was a mixture of the two carbonates (approximately 35% calcite and 65% aragonite, as indicated by XRD analysis). However, the Δ_{47} temperature (20.2 ± 6.9°C) is close to the measured water temperature of the lake (24.3°C). The uncertainty of the calculated temperature (± 6.9°C) is larger than the difference between the calculated and the measured temperatures (4.1°C), so it is not possible to determine if the aragonite and the calcite covering crystallized at different temperatures, or if the clumped isotope composition was affected by differences in the aragonite and calcite clumped isotope calibrations. However, de Winter et al. (2022) found that aragonite and calcite behave similarly in terms of clumped isotope geochemistry. The carbonate crust of the Titkok tava drill core (T-top) is made of calcite, and its Δ_{47} temperature (24.5 ± 7.0°C) is also close to the measured water temperature (19.1°C).

The Nagy-tufa flowstone deposit (sample BNT-2 top) of Béke Cave yielded a Δ_{47} value of 0.638 ± 0.009‰, which corresponds to a formation temperature of 11.0 ± 5.8°C, as compared with the measured cave temperature of 9.8°C (Czuppon et al., 2018).

These clumped isotope temperature data were plotted in Fig. 4A jointly with the measured water temperatures. The good agreements between the clumped isotope temperatures and the measured water temperatures that plot along the 1:1 line in Figure 4A indicate that the subaqueous calcites are useful in clumped isotope studies if a temperature uncertainty of 5-10°C is acceptable. This conclusion is in agreement with the results of Koltai et al. (2024), who investigated calcite spar formation temperatures by means of clumped isotope analyses and nucleation-assisted fluid inclusion microthermometry.

Table 2. A) Clumped isotope compositions and (B) stable isotope compositions of calcites (“cc”) local waters and inclusion-hosted water ("w"), as well as measured or calculated temperatures (in °C). Anderson temperature: clumped isotope temperature calculated using the Anderson et al. (2021) calibration. CI: confidence interval. δ^{2}H and δ^{18}O values are in‰, relative to V-SMOW. Water temperatures in (B) were either directly measured in local water or were calculated using fluid inclusion (“fi”) data. Temperatures calculated using the equation of Tremaine et al. (2011) and oxygen isotope fractionations ($\Delta^{18}O_{cc-w}$) between calcites and local water or inclusion-hosted water are shown for comparison. In panel (B) * denotes fluid inclusion (fi) compositions and temperatures calculated from fi data. **: source Virág (2018).Temp.: temperature. Numbers of measurements (N) are the same for both clumped and oxygen isotope measurements. With the exception of the Vulkánok sample, the fluid inclusion analyses were obtained during single measurements due to sample amount requirements (about 2 g).

(A)	**D47Crunch Δ_{47} [I-CDES90°C,‰]**					**Anderson temperature**		
	N	**Value**	**±1SD**	**±1SE**	**±95% CI**	**Value**	**±1SE**	**±95% CI**
M1 (“Vulkánok”)	12	0.52	0.016	0.008	0.017	53.6	4.5	7.8
M2 (Lukács IV)	12	0.53	0.021	0.008	0.016	49.5	4.3	7.4
M3 (calcite rafts)	11	0.586	0.019	0.009	0.017	27.9	3.7	6.4
BNT-2 top	11	0.638	0.031	0.009	0.019	11.0	3.3	5.8
Berger plate #17	8	0.605	0.044	0.015	0.029	21.4	5.1	9.6
L1	11	0.587	0.033	0.014	0.027	27.5	5.2	9.5
L2	12	0.599	0.047	0.013	0.026	23.3	4.8	8.9
T1	12	0.593	0.042	0.013	0.026	25.2	4.9	9.1
T2	11	0.618	0.039	0.014	0.027	17.0	4.7	8.7
L-top	21	0.608	0.036	0.010	0.021	20.2	3.9	6.9
T-top	21	0.595	0.040	0.010	0.020	24.5	3.9	7.0

(B)	**$\delta^{18}O_{cc}$**	**±1σ**	**δ^{2}H (local water & fi*)**	**$\delta^{18}O_{w}$ (local water & fi*)**	**Temperatures (local water & fi*)**	**$\Delta^{18}O_{cc-w}$ temp. (Tremaine)**	**Clumped isotope temp.**
M1 (“Vulkánok”)	12.16	0.09	-86.2 ±2 *	-13.7 ±0.5 *	41.8 ±7.5*	45.8	53.6
M2 (Lukács IV)	14.55	0.08	-85.0 **	-11.7 **	51.0 ±0.5	43.7	49.5
M3 (calcite rafts)	18.84	0.03		-10.8 **	23.2 ±0.5	24.4	27.9
BNT-2 top	23.99	0.05	-64.6 ±1.5	-9.4 ±0.2	9.8 ±0.5	5.6	11.0
Berger plate #17	19.71	0.08	-70.9 ±0.1	-10.0 ±0.05	24.3 ±1	24.0	21.4
L1	18.02	0.12	-72.0*	-11.2*	24.4 ±6.8*	26.5	27.5
L2	18.67	0.13	-71.2*	-11.0*	22.2 ±6.8*	24.1	23.3
T1	17.87	0.11	-66.7*	-10.7*	27.8 ±6.9*	30.2	25.2
T2	19.78	0.11	-74.7*	-10.6*	18.9 ±6.6*	20.4	17.0
L-top	20.53	0.1	-70.9 ±0.1	-10.0 ±0.05	24.3 ±1	19.7	20.2
T-top	20.10	0.08	-71.0 ±0.15	-10.1 ±0.2	19.0 ±1	21.4	24.5

Clumped isotope vs. oxygen isotope thermometry

In addition to clumped isotope thermometry, speleothems may offer another tool with which to determine paleotemperature, the temperature dependence of the oxygen isotope fractionation between the speleothem’s host calcite and the water trapped in fluid inclusions. As described in the Introduction, both the calcite and the inclusion-hosted water can be analyzed for their oxygen isotope compositions, and when the temperature dependence of the oxygen isotope fractionation between these components is known, the formation temperature can be calculated. The oxygen isotope compositions of the calcites and inclusion-hosted waters were measured, and their temperatures were calculated as yielding the highest and lowest temperatures, respectively, using the fractionation equations of Daëron et al. (2019) and Chacko & Deines (2008). The averages of the two extreme ends and their deviations from the averages are listed in Table 2 and are plotted in Figure 4B against clumped isotope temperatures. The formation temperatures were also calculated using various calcite-water oxygen isotope fractionation equations (see Methods), which are intermediate between the Daëron et al. (2019) and the Chacko & Deines (2008) equations. As the Tremaine et al. (2011) equation yielded temperatures closest to the clumped isotope temperature values, these data are also listed in Table 2B.

The „Vulkánok" sample provides a fossil example of subaqueous carbonates. The inclusion-hosted water was analyzed along with the Δ_{47} measurements, and the calculated temperatures are plotted in Figure 4B. The average inclusion-based temperature is 41.8 ± 7.5°C, which is slightly lower than the Δ_{47} temperature (53.6 ± 7.8°C). One possible explanation is that the formation temperature and the present day ambient temperature (about 20°C in the Molnár János Cave) are so different that the host calcite and inclusion water have undergone post-formational oxygen isotope exchange. At decreasing temperatures, the calcite-water oxygen isotope fractionation becomes larger (McCrea, 1950). Because the host calcite dominated by its mass, the $\delta^{18}O$ value of the entrapped water is shifted in a negative direction to approach the larger calcite-water oxygen isotope fractionation. This explanation is supported by the observation that the hydrogen isotope compositions of the nearby thermal well (Lukács IV, –85.0‰, Virág, 2018) and the Vulkánok inclusion water (–86.2 ± 0.3‰) are very close to each other, whereas the $\delta^{18}O$ values of the well water (–11.7‰, Virág, 2018) and inclusion-hosted water (–13.7 ± 0.1‰) are significantly different, indicating that the latter has suffered a post-formational isotope shift. The $\delta^{2}H$ data indicate that the Vulkánok carbonate cones were deposited from thermal water similar to that of the Lukács IV well. Using the –12.0‰ $\delta^{18}O_{water}$ value that corresponds to the $\delta^{2}H$ value, as well as the measured $\delta^{18}O_{cc}$ value of 12.2‰, the Daëron et al., (2019) and Chacko & Deines (2008) equations yield an average of 51.8 ± 7.9°C. This is very close to the Lukács IV well (51.1°C) and the Δ_{47} temperatures (53.6 ± 7.8°C). Both temperatures are plotted in Fig. 4B, indicating the temperature shifted from the 1:1 line to a lower inclusion-based value. Thus, the paleotemperature calculation based on the inclusion-hosted water's $\delta^{18}O_{fi}$ value is roughly estimated to be at or above 40-50°C.

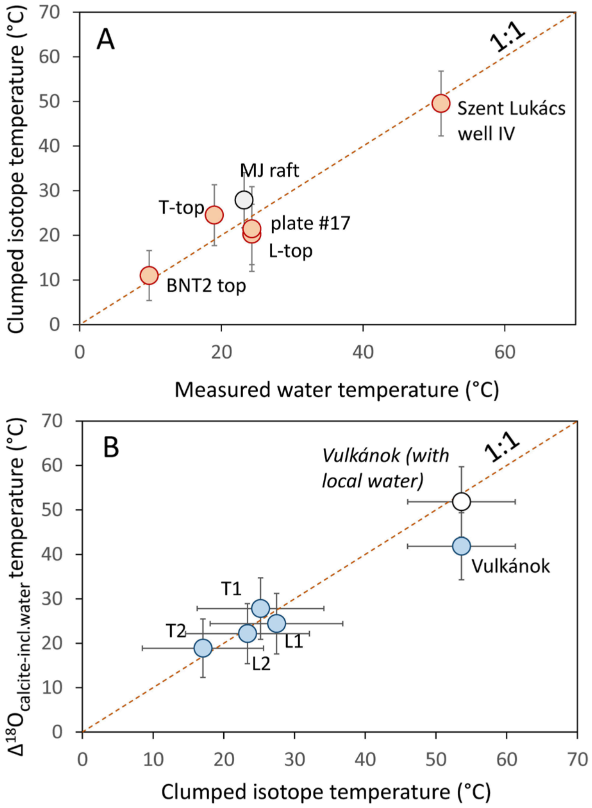

Fig. 4. A) Formation temperatures obtained by clumped isotope analyses of subaqueous speleothem deposits vs. measured water temperatures. BNT2 top: sample taken from the top of the BNT-2 core (Nagy-tufa flowstone deposit, Béke Cave, NE Hungary). T-top: the calcite crust formed at the top of the drill core taken at the Titkok tava Lake deposit of the Berger Cave, W Hungary. L-top: the calcite-aragonite crust formed at the top of the drill core taken at the Lángos Lake deposit of the Berger Cave, W Hungary. Plate #17: farmed carbonate collected from the glass plate #17 placed in the Lángos Lake, Berger Cave, W Hungary. MJ raft: calcite rafts collected at the lake bottom of the Molnár János Cave, Budapest. Szent Lukács Well IV: recently forming travertine deposit at the orifice of a thermal well, Budapest. Measured water temperature uncertainties are smaller than the sample dots. B) Temperatures obtained from measured oxygen isotope fractionations between calcite and inclusion-hosted water ($\Delta^{18}O_{calcite-incl.water}$) vs. clumped isotope temperature data. Samples L1, L2, T1, and T2 are shown in Fig. 2A. The data for the „Vulkánok" deposit are from direct measurement of inclusion-hosted water $\delta^{18}O$ (filled circle) and from fractionation calculation with local thermal well compositions (empty circle).

The Berger Károly Cave drill core samples represent another fossil speleothem formation, but in a lower temperature environment of around 20-25°C (see above). The L1, L2, T1, and T2 samples yielded clumped isotope temperatures between 17.0 and 27.5°C (with an average uncertainty of ±9.3°C), whereas the inclusion-based temperatures ranged from 18.9 to 27.8°C (with an average uncertainty of ±6.8°C) (Table 2), plotted on the 1:1 line in Figure 4B. The two types of temperature data can also be compared with local water temperatures. The Lángos Lake (L1 and L2) and the Titkok tava Lake (T1 and T2) samples yield average clumped isotope temperatures of 26 ± 10°C and 21 ± 13°C, respectively, which agree with local lake temperatures (24.3 and 19.1°C, respectively) within the uncertainties. Even the slightly lower temperature at Lake Titkok Tava appears in the clumped isotope data, although it should be noted that the difference is well within the clumped isotope temperature uncertainties. The inclusion-based temperatures of the two occurrences are indistinguishable (23 ± 8°C for the L1-L2 samples and 24 ± 11°C for the T1-T1 samples) and agree with local water temperatures within the uncertainties. These observations have twofold significance: (i) although the uncertainties of the obtained temperatures are large, the calcite deposits that precipitated in cave-hosted lakes appear to be appropriate for both clumped isotope and oxygen isotope thermometric analyses, and (ii) the local thermal karst environment is stable. The latter will be investigated in subsequent studies on the (paleo) hydrological evolution of Berger Károly Cave.

The good agreements of clumped isotope and inclusion-based oxygen isotope temperatures, as well as the good fit to the 1:1 line in Figure 4B for lower temperature formations all indicate that subaqueous calcites are useful in both clumped isotope and inclusion isotope studies, although with an expected temperature uncertainty reaching ±10°C.

CONCLUSIONS

Cave-hosted subaqueous carbonate deposits were investigated using clumped isotope analyses and measurements of the stable isotope compositions of inclusion-hosted waters and carbonates to determine if they yield reliable formation temperature data. The clumped isotope analyses of carbonate deposits with known formation temperatures (flowstone, travertine, calcite rafts, lake deposits) yielded close-to-measured temperatures, indicating that these deposits provide reliable temperature data (with an uncertainty of about ±5-10°C). The subaqueous carbonate deposits that formed in cave-hosted lakes were also investigated with the stable isotope analyses of inclusion-hosted waters. Together with the oxygen isotope composition of the host carbonate, they yielded realistic formation temperatures. However, post-formational oxygen isotope exchange can occur in hydrothermal carbonates formed at about 50°C, leading to inclusion-based temperatures that are below the original formation temperature. The results indicate that subaqueous carbonate speleothems are valuable clumped isotope paleotemperature archives, as kinetic isotope fractionations do not significantly affect them in an underwater environment. If the formation temperature is close to the post-formational conditions, then the primary oxygen isotope fractionations between the speleothem calcite and the water trapped in fluid inclusions can be preserved and also used in paleotemperature calculations.

ACKNOWLEDGEMENTS

The sampling conducted for this study in Berger Károly Cave was permitted by the directorate of the Balaton Uplands National Park (No. 102-2/2022). Rezső Szilaj and Dénes Szieberth provided essential help during sampling in Berger Károly and Molnár János caves, respectively. The authors are indebted to Ariana Gugora for polishing the English of the manuscript. Constructive reviews and helpful comments of three anonymous reviewers, and the editorial handling by Bogdan P. Onac are gratefully thanked. The study was financed by the Eötvös Loránd Research Network (projects SA-41/2021; ELKH KEP-1/2020). The clumped isotope facility of the Institute for Nuclear Research was supported by the European Union and the Hungarian State, co-financed by the European Regional Development Fund in the project of GINOP-2.3.2-15-2016-00009 'ICER'.

Authorship statement: AD designed the research and wrote the paper; ÁB, GS, and MV conducted field work; IK provided XRD analyses and interpretations; LR conducted the clumped isotope analyses.

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