The ostracod clumped-isotope thermometer: A novel tool to reconstruct quantitative continental climate changes.

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STATEMENT

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ABSTRACT

This study presents the ostracod clumped-isotope (Δ_{47}) thermometer, a new tool that provides quantitative temperature and hydrological reconstruction from lacustrine systems, which are among the best archives to reconstruct continental paleotemperature variations. The relationship between Δ_{47} and the temperature at which ostracod shell crystallized, is determined by measuring Δ_{47} on different species grown under controlled temperatures in both natural environments (4 ± 2°C; 12 ± 1°C) and lab cultures (23 ± 0.5°C). No consistent offset between the two species originating from the same environment and precipitated at the same temperature is reported, suggesting the absence of a vital effect in ostracod Δ_{47}. In addition, the excellent agreement between the presented ostracod data and the carbonate clumped-isotope unified calibration (Anderson et al., 2021) suggests that the use of the latter can be extent to continental biogenic carbonates that do not present a kinetic effect. The ostracod-Δ_{47} thermometer constitutes

a reliable tool for continental palaeoclimate reconstructions that can be widely used in freshwater systems in all climatic belts.

INTRODUCTION

The projected future global temperature increase (Stocker et al., 2013 and IPCC, 2021) will affect continental areas differently depending on their geographic position. Lakes are distributed worldwide, on different climate belts, and from boreal to tropical regions. Lacustrine sedimentary archives constitute one of the most useful recorders of past climate changes (Cohen, 2003). Hence, their study provides the means to better constrain future climate variability and foresee mitigation or adaptation measures to preserve the ecosystem on the continents where human societies thrive (Gornitz, 2009). Because of their smaller size compared to oceans, lakes are more sensitive to environmental variations and thus record even rapid climatic events. In paleoclimatology the reconstruction of past temperatures and hydrology in lake-waters, which reflects atmospheric changes, is of particular interest.

Since the work of McCrea (1950) and Urey et al. (1951), stable oxygen isotopic analyses in carbonates constitute an essential component of paleoclimatology. Variations in carbonate $\delta^{18}O$ in lacustrine settings are commonly attributed to changes in lake water temperature and/or in the isotope composition of the lake. Today, well-constrained lacustrine temperature reconstructions remain challenging as the isotopic composition of water depends on the lake biological productivity and on the hydrological balance of the lake, including the source area of the evaporated water and the precipitation/evaporation ratio (Leng and Marshall, 2004). Therefore, a major limitation of the conventional stable isotope $\delta^{18}O$ technique for paleotemperature determination is the difficulty to discriminate between the temperature and isotopic composition of the water.

Carbonate clumped-isotope (Δ_{47}) paleothermometry (Eiler, 2007) opens the way to more precisely determined lacustrine paleotemperatures. The Δ_{47} technique is based on the temperature-dependent abundance of ^{13}C-^{18}O bonds in CO_2. The increased abundance of these bonds in carbonate is associated with decreasing water temperatures, providing a direct insight in carbonate temperature precipitation with an accuracy of ~ ± 2 °C. It is expressed using the Δ_{47} notation, which corresponds to the small overabundance of ^{13}C-^{18}O bonds relative to a stochastic isotopic distribution.

The Δ_{47} presents three main advantages over conventional stable isotope $\delta^{18}O$ technique:

- Δ_{47} is only temperature-dependent, and not influenced by the water isotopic composition (Schauble et al., 2006).
- Δ_{47} combined with the $\delta^{18}O$ of carbonate, reconstructs the isotopic composition of the water from which the carbonate precipitated ($\delta^{18}O_w$) and thereby changes in the precipitation over evaporation relationship.
- Δ_{47} values are not affected by a so-called "vital effect" (i.e., disequilibrium between the water isotopic signal and the one recorded by the organisms) for several organisms such as foraminifera and molluscs (Tripati et al., 2010; Grauel et al., 2013; Peral et al., 2018; Meinicke et al., 2020; Huyghe et al., 2022; de Winter et al., 2022).

The clumped paleothermometer is not commonly applied to lacustrine carbonates because the traditional micro- and macrofossils used for marine paleoclimate Δ_{47} studies (e.g., foraminifera, gastropods, and bivalves) are rare or absent in lakes. Δ_{47} technique has been performed on fossil ostracods, common in lacustrine sediments, by Song et al., 2022 and Yue et al., 2022 to reconstruct paleotemperatures and paleoelevation. Yet these authors did not explicitly test the robustness of this paleothermometry technique and the absence of a vital effect prior to its application. In this study, we fill this scientific need by carrying out Δ_{47} analyses on living ostracods that precipitated their shells under known temperatures.

Ostracods are small aquatic crustaceans (mostly 0.3-5 mm) with a stable low-Mg calcite shell mineralogy, which makes them ideally suited for targeted geochemical analyses (Holmes and De Deckker, 2012). They secrete their shell relatively rapidly (Chivas et al., 1983) starting from ions present in the host water at the time of valve calcification (Turpen and Angell, 1971). Ostracods grow by moulting and undergo eight juvenile stages (designated in descending order from A-1 with A-8 being the first instar) in their development before reaching adulthood (A). They occur in almost all aquatic environments and have an excellent and abundant fossil record. Because of their high sensitivity to the variation of several ecological variables (i.e., pH, salinity, temperature, water level, and oxygen saturation), ostracods are valuable proxies to reconstruct past environmental and climatic changes (Ruiz et al., 2013; Marchegiano et al., 2018, 2019, 2020, Marchegiano and John, 2022).

The unified Δ_{47} calibration recently published by Anderson et al., (2021) may be applied across a wide range of natural carbonate material precipitated at temperatures between 0.5 to 1100°C. It includes exclusively authigenic continental carbonates (i.e., tufa, travertines, speleothems) and synthetic carbonates. However, the good correspondence with previous published calibrations on foraminifera (e.g., Meinicke et al., 2020; Peral et al., 2018) and marine bivalves data (Huyghe et al., 2022; de Winter et al., 2022) confirm its validity also in biogenic carbonate that do not present any isotopic disequilibrium. Yet, temperatures data from continental biogenic carbonates remain missing to date.

This study improves the current understanding of the application of Δ_{47} to lacustrine environments with a particular focus on ostracod shells and it provides a new tool to reconstruct continental climate changes using lake sediments. This article aims to:

- Determine, for the first time, the relationship between Δ_{47} and precipitated temperature of freshwater ostracod shells by using ostracods grown under known temperatures (from lab cultures and natural environments)
- Investigate the potential so-called "vital-effects". This test relies on measuring of different ostracod species growing in the same environment and at the same temperatures. The selected ostracod species are among the most widespread and easily recovered in both recent and fossil records.

MATERIALS AND METHODS

Ostracod shells

The ostracod species *Heterocypris incongruens* (Ramdohr, 1808) was cultured at the Royal Belgian Institute of Natural Science (Brussels-Belgium) from September to December 2021 under controlled temperature at 23°C (± 0.5°C) (Fig.1). Living ostracods were collected in their natural environments during two field campaigns in January (Ghent-Belgium) and February (Granada-Spain) 2022 (Fig.1). The selected species have one generation per year, and they live only in the months during which they were collected. *Eucypris virens* (Jurine, 1820) and *Bradleystrandesia fuscata* (Urine, 1820) were sampled in a monitored temporary pond (Ghent-Belgium) and precipitated their shells between end of December and end of January, at a temperature of 4°C (± 2°C). *Herpetocypris brevicaudata* (Kaufmann, 1900) specimens were collected in a small river fed by groundwater in the Padul lake area (Granada-Spain). The collected adult precipitated their shells between November and beginning of February a period during which the water temperature is constant at 12°C (± 1°C). Water depths at both sites were lower than 10 cm. Adult specimens were selected to prevent a potential size effect in the samples since water isotopic composition and temperature could have changed from the juvenile stage, which can commonly last several weeks, to the adult stage. All the collected ostracods were euthanized and preserved in ethanol (see in Supplementary material for more details)

Clumped isotope analyses

The four samples, representing four different ostracod species that precipitated their shells at three different temperatures (see Tab1), are used for clumped isotope (Δ_{47}) analyses. To avoid contamination, each ostracod is cleaned to remove the organic matter. The two ostracod valves are gently opened to remove the body using two needles and each shell accurately cleaned with a thin brush and ethanol under the microscope. A total of 40 to 60 valves per replicate is needed to assure enough material (0.500 - 0.600 mg per replicate), whereby the exact number depends on the species (i.e., size and thickness of the shells). Each sample is replicated 5 to 11 times (300 - 700 valves per samples in total). The Δ_{47} analyses of ostracod shells are carried out at the Analytical, Environmental & Geo-Chemistry (AMGC) clumped isotope lab of the Vrije Universiteit Brussel (VUB), using a Nu Instruments Perspective-IS stable isotope ratio mass spectrometer (SIRMS) in conjunction with a Nu-Carb carbonate sample preparation system, as described in detail in De Vleeschouwer et al. (2022). The analyses are performed between October 2021 and November 2022. The carbonate standard ETH-2 is systematically measured and compared to InterCarb values (Bernasconi et al., 2021) to ensure the measurements quality control. The raw measured Δ_{47} values are processed using the IUPAC Brand's isotopic parameters (Daëron et al., 2016) and converted to the ICDES 90°C scale, using the most recent values for the ETH-1, ETH-3, and ETH-4 carbonate reference materials (Bernasconi et al., 2021) within the ClumpyCrunch software. Nu

Instruments does not require a background check. Both analytical and calibration uncertainties are propagated to calculate the final uncertainties on temperatures.

RESULTS

The Δ_{47} values of the ostracod samples range from 0.603 to 0.6648 with a SE from 0.0104 to 0.0148 ‰ (Fig.2 and Tab.1). The availability of sample material directly determined the number of replicated measurements carried out per sample. The large number of replicates per specimen ensures the robustness of the results. The repeatability along the 9 sessions of the standards used to standardize the results and the ostracod samples is of 31.7 ppm and 30.0 ppm respectively. The ETH2 standard, used to ensure and control the quality of the analyses, presents a Δ_{47} value of 0.2117 and a standard error (SE) of 0.0057 for 97 replicates. The difference between our measured value of ETH2 and the official one from Bernasconi et al., (2021) (ETH2- Δ_{47} value of 0.2086 ± 0.0015) is negligible (Δ_{47} of 0.0031) and falls within the calculated SE. This confirms both the accuracy and precision of the Δ_{47} analyses presented in this study. The homogeneity in the $\delta^{18}O$ and $\delta^{13}C$ values (supplementary material S1) indicate that the non-linear mixing effect (Deflies and Lohmann, 2015) did not affected our measurements.

DISCUSSION

Relationship between ostracod-Δ_{47} and temperature

The Δ_{47} – T relationship for ostracod shells is provided by measuring clumped isotopes on four species that precipitated their shells at three different temperatures. Mean measured Δ_{47} values follow the principle of clumped-isotope technique (Eiler et al., 2017) by decreasing with an increase in temperatures (Fig.2 and Tab.1). Indeed, they strongly correlate with the known calcification temperature. The use of a large number of valves (between 300 and 700) per sample ensures the consistency of the analyses. Fig.2, shows the ostracod data compared with the unified calibration and those from recent studies on both biogenic carbonates and inorganic speleothems (see references in Fig.2). All the measured ostracod samples fall within the 95% confidence interval of the regression line, suggesting that clumped-isotope thermometry can be applied to ostracods shells.

Vital effect in ostracods-Δ_{47}

The potential ostracod vital effect was assessed by measuring Δ_{47} on two different species that lived at the same time and environment and precipitated their shells at the same temperature. The long-lasting problem of the species-dependent vital effect, which currently limits unambiguous paleotemperature reconstructions based on $\delta^{18}O$, Mg/Ca and other commonly applied proxies, remains an active field of research. A vital effect exists on the $\delta^{18}O$ signal of several ostracod species (e.g., Grafenstein et al., 1999; Holmes & Chivas, 2002) but so far no study has investigated whether it also affects their clumped-isotope composition. The absence of a consistent offset (Δ_{47} 0.002 ± 0.01‰, Tab.1), within the analytical uncertainty, between the species *E. virens* and *B. fuscata* suggests the absence of a vital effect at the genus and species level. This is reinforced by the observation that all four species, coming from lab culture and different natural environments (i.e., river and temporary pond) record the corresponding temperatures.

These findings show that the ostracod-Δ_{47} thermometer is fully applicable throughout geological time, independently of the ostracod species, and in all kinds of freshwater environments where ostracods live, irrespective of the geographical area.

Implication for palaeotemperature reconstructions

Ostracods show the same T-Δ_{47} relationship as all other carbonates, supporting a common temperature relationship between biogenic and authigenic carbonates from both marine and continental environments (Fig.2). The strong agreement between all the datapoints from Fig.2 and the unified calibration (Anderson et al., 2021) supports that ostracods are not affected by isotopic disequilibrium effects. Consequently, the unified calibration is applied here to convert ostracod-Δ_{47} values in temperatures (Tab.1). The difference between the calculated temperatures and the temperature measured in situ is between 0.5 and 1 °C, which falls within the uncertainties of the measurements (SE from 0.8 to 1.7 °C). De Winter et al., (2022), suggests that for lower earth surface T, the combined foraminifera-based calibration from Meinicke et al., 2020 is more suitable. However, our data (Tab.1) appear to be in better agreement with the unified calibration. This difference may result from a more robust constraint on temperatures of calcification rather than using isotopically estimated temperatures as done in Meinicke et al. (2021).

CONCLUSIONS

Ostracod-Δ_{47} is a new freshwater paleothermometer to reconstruct temperature with a precision of around ± 2°C. The absence of a vital effect enables the use of the paleothermometer with all ostracod species in different lacustrine systems and throughout geological time. This makes the ostracod-Δ_{47} thermometer a powerful tool to reconstruct past continental climate change. Because of the good agreement between the ostracod data and the unified calibration (Anderson et al., 2021), the latter can be used for future application of clumped isotope in ostracods. The establishment of this new lacustrine proxy opens the door to new high-resolution continental paleoclimate and paleoenvironmental reconstructions and therefore has the potential to become a key tool in future lacustrine research. The ostracod-Δ_{47} thermometer is of particular importance in the upcoming global warming as the understanding of its effect on specific continental areas remains limited and its reconstruction challenging because of the large environmental variability on the continents.

Furthermore, to reconstruct reliable paleotemperature is important to know the life history (i.e., calcification season) of the used ostracod species. It is, thus, preferable to use only adult valves to avoid erroneous species identification and to reduce the inter sample temperature variability that could be caused by the different timing of shell calcification among the 8 moulting.

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REFERENCES CITED

Anderson, N. et al., 2021, A unified clumped isotope thermometer calibration (0.5-1100C) using carbonate-based standardization: Geochemistry preprint, doi:10.1002/essoar.10505702.1.

Bernasconi, S.M. et al., 2021, InterCarb: A Community Effort to Improve Interlaboratory Standardization of the Carbonate Clumped Isotope Thermometer Using Carbonate Standards: Geochemistry, Geophysics, Geosystems, v. 22, doi:10.1029/2020GC009588.

Chivas, A.R., De Deckker, P., and Shelley, J.M.G., 1983, Magnesium, strontium and barium partitioning in non marine ostracod shells and their use in paleoenvironment reconstructions – a preliminary study., *in* Applications of Ostracoda, University of Houston, TX, p. 238–249.

Cohen, A.S., 2003, Paleolimnology: the history and evolution of lake systems: Oxford, New York : Oxford University Press, 500 p.

Daëron, M., Blamart, D., Peral, M., and Affek, H.P., 2016, Absolute isotopic abundance ratios and the accuracy of Δ47 measurements: Chemical Geology, v. 442, p. 83–96, doi:10.1016/j.chemgeo.2016.08.014.

De Vleeschouwer, D. et al., 2022, Plio-Pleistocene Perth Basin water temperatures and Leeuwin Current dynamics (Indian Ocean) derived from oxygen and clumped-isotope paleothermometry: Climate of the Past, v. 18, p. 1231–1253, doi:10.5194/cp-18-1231-2022.

Defliese, W.F., and Lohmann, K.C., 2015, Non-linear mixing effects on mass-47 CO_2 clumped isotope thermometry: Patterns and implications: Non-linear mixing effects on mass-47 clumped isotopes: Rapid Communications in Mass Spectrometry, v. 29, p. 901–909, doi:10.1002/rcm.7175.

Eiler, J.M., 2007, "Clumped-isotope" geochemistry—The study of naturally-occurring, multiply-substituted isotopologues: Earth and Planetary Science Letters, v. 262, p. 309–327, doi:10.1016/j.epsl.2007.08.020.

Fiebig, J., Daëron, M., Bernecker, M., Guo, W., Schneider, G., Boch, R., Bernasconi, S.M., Jautzy, J., and Dietzel, M., 2021, Calibration of the dual clumped isotope thermometer for carbonates: Geochimica et Cosmochimica Acta, v. 312, p. 235–256, doi:10.1016/j.gca.2021.07.012.

Gornitz, V., 2009, Encyclopedia of paleoclimatology and ancient environments, Encyclopedia of earth sciences series: Netherlands, Springer.

Grafenstein, U. von, Erlenkeuser, H., Brauer, A., Jouzel, J., and Johnsen, S.J., 1999, A Mid-European Decadal Isotope-Climate Record from 15,500 to 5000 Years B.P.: Science, v. 284, p. 1654–1657, doi:10.1126/science.284.5420.1654.

Grauel, A.-L., Schmid, T.W., Hu, B., Bergami, C., Capotondi, L., Zhou, L., and Bernasconi, S.M., 2013, Calibration and application of the 'clumped isotope' thermometer to foraminifera for high-resolution climate reconstructions: Geochimica et Cosmochimica Acta, v. 108, p. 125–140, doi:10.1016/j.gca.2012.12.049.

Heip, C., 1976, The life-cycle of Cyprideis torosa (Crustacea, Ostracoda): Oecologia, v. 24, p. 229–245.

Holmes, J.A., and Chivas, A.R., 2002, Ostracod shell chemistry — overview, *in* Holmes, J.A. and Chivas, A.R. eds., Geophysical Monograph Series, Washington, D. C., American Geophysical Union, v. 131, p. 185–204, doi:10.1029/131GM10.

Holmes, J.A., and De Deckker, P., 2012, The Chemical Composition of Ostracod Shells, *in* Developments in Quaternary Sciences, Elsevier, v. 17, p. 131–143, doi:10.1016/B978-0-444-53636-5.00008-1.

Huyghe, D., Daëron, M., de Rafelis, M., Blamart, D., Sébilo, M., Paulet, Y.-M., and Lartaud, F., 2022, Clumped isotopes in modern marine bivalves: Geochimica et Cosmochimica Acta, v. 316, p. 41–58, doi:10.1016/j.gca.2021.09.019.

Leng, M.J., and Marshall, J.D., 2004, Palaeoclimate interpretation of stable isotope data from lake sediment archives: Quaternary Science Reviews, p. 21.

Marchegiano, M., Francke, A., Gliozzi, E., and Ariztegui, D., 2018, Arid and humid phases in central Italy during the Late Pleistocene revealed by the Lake Trasimeno ostracod record: Palaeogeography, Palaeoclimatology, Palaeoecology, v. 490, p. 55–69, doi:10.1016/j.palaeo.2017.09.033.

Marchegiano, M., Francke, A., Gliozzi, E., Wagner, B., and Ariztegui, D., 2019, High-resolution palaeohydrological reconstruction of central Italy during the Holocene: The Holocene, v. 29, p. 481–492, doi:10.1177/0959683618816465.

Marchegiano, M., Horne, D.J., Gliozzi, E., Francke, A., Wagner, B., and Ariztegui, D., 2020, Rapid Late Pleistocene climate change reconstructed from a lacustrine ostracod record in central Italy (Lake Trasimeno, Umbria): Boreas, v. 49, p. 739–750, doi:10.1111/bor.12450.

McCrea, J.M., 1950, On the isotopic chemistry of carbonates and a paleotemperature scale: v. 18, p. 849–857.

Meinicke, N., Ho, S.L., Hannisdal, B., Nürnberg, D., Tripati, A., Schiebel, R., and Meckler, A.N., 2020, A robust calibration of the clumped isotopes to temperature relationship for foraminifers: Geochimica et Cosmochimica Acta, v. 270, p. 160–183, doi:10.1016/j.gca.2019.11.022.

Peral, M. et al., 2018, Updated calibration of the clumped isotope thermometer in planktonic and benthic foraminifera: Geochimica et Cosmochimica Acta, v. 239, p. 1–16, doi:10.1016/j.gca.2018.07.016.

Ruiz, F., Abad, M., Bodergat, A.M., Carbonel, P., Rodríguez-Lázaro, J., González-Regalado, M.L., Toscano, A., García, E.X., and Prenda, J., 2013, Freshwater ostracods as environmental tracers: International Journal of Environmental Science and Technology, v. 10, p. 1115–1128, doi:10.1007/s13762-013-0249-5.

Schauble, E.A., Ghosh, P., and Eiler, J.M., 2006, Preferential formation of 13C–18O bonds in carbonate minerals, estimated using first-principles lattice dynamics: Geochimica et Cosmochimica Acta, v. 70, p. 2510–2529, doi:10.1016/j.gca.2006.02.011.

Song, B., Zhang, K., Farnsworth, A., Ji, J., Algeo, T.J., Li, X., Xu, Y., and Yang, Y., 2022, Application of ostracod-based carbonate clumped-isotope thermometry to paleo-elevation reconstruction in a hydrologically complex setting: A case study from the northern Tibetan Plateau: Gondwana Research, v. 107, p. 73–83, doi:10.1016/j.gr.2022.02.014.

Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M.M.B., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., and Midgley, P.M., 2013, Working Group I Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change: , p. 14.

Tripati, A.K., Eagle, R.A., Thiagarajan, N., Gagnon, A.C., Bauch, H., Halloran, P.R., and Eiler, J.M., 2010, 13C–18O isotope signatures and ‘clumped isotope’ thermometry in foraminifera and coccoliths: Geochimica et Cosmochimica Acta, v. 74, p. 5697–5717, doi:10.1016/j.gca.2010.07.006.

Urey, H.C., Lowenstam, H.A., Epstein, S., and McKinney, C.R., 1951, Measurement of paleotemperatures and temperatures of the Upper Cretaceous of England, Denmark, and the southeastern United States: v. 62, p. 399–416.

de Winter, N.J., Witbaard, R., Kocken, I.J., Müller, I.A., Guo, J., Goudsmit, B., and Ziegler, M., 2022, Temperature Dependence of Clumped Isotopes (Δ_{47}) in Aragonite: Geophysical Research Letters, v. 49, doi:10.1029/2022GL099479.

Yue, J., Xiao, J., Wang, X., Meckler, A.N., Modestou, S.E., and Fan, J., 2022, “Cold and wet” and “warm and dry” climate transitions at the East Asian summer monsoon boundary during the last deglaciation: Quaternary Science Reviews, v. 295, p. 107767, doi:10.1016/j.quascirev.2022.107767.

FIGURES AND TABLES

Samples name	Species	Environmental T (°C)	N	Δ_{47} (‰) I-CDES	1SE	T(C°) Anderson et al., 2021	T(C°) Meinicke et al., 2020
OSTRA-1	*E. virens*	4 (± 2)	11	0.6629	0.0104	4 (± 1.7)	5.5 (± 1.7)
OSTRA-2	*H. brevicaudata*	12 (± 1)	10	0.6364	0.0112	11.5 (± 2.1)	13 (± 2.1)
OSTRA-3	*B. fuscata*	4 (± 2)	9	0.6648	0.0109	3.5 (± 1.8)	5 (± 1.8)
OSTRA-4	*H. incongruens*	23 (± 0.5)	5	0.603	0.0148	22 (± 3.4)	23 (± 3.4)

Table 1: Ostracod clumped results. N = number of replicates per each sample.

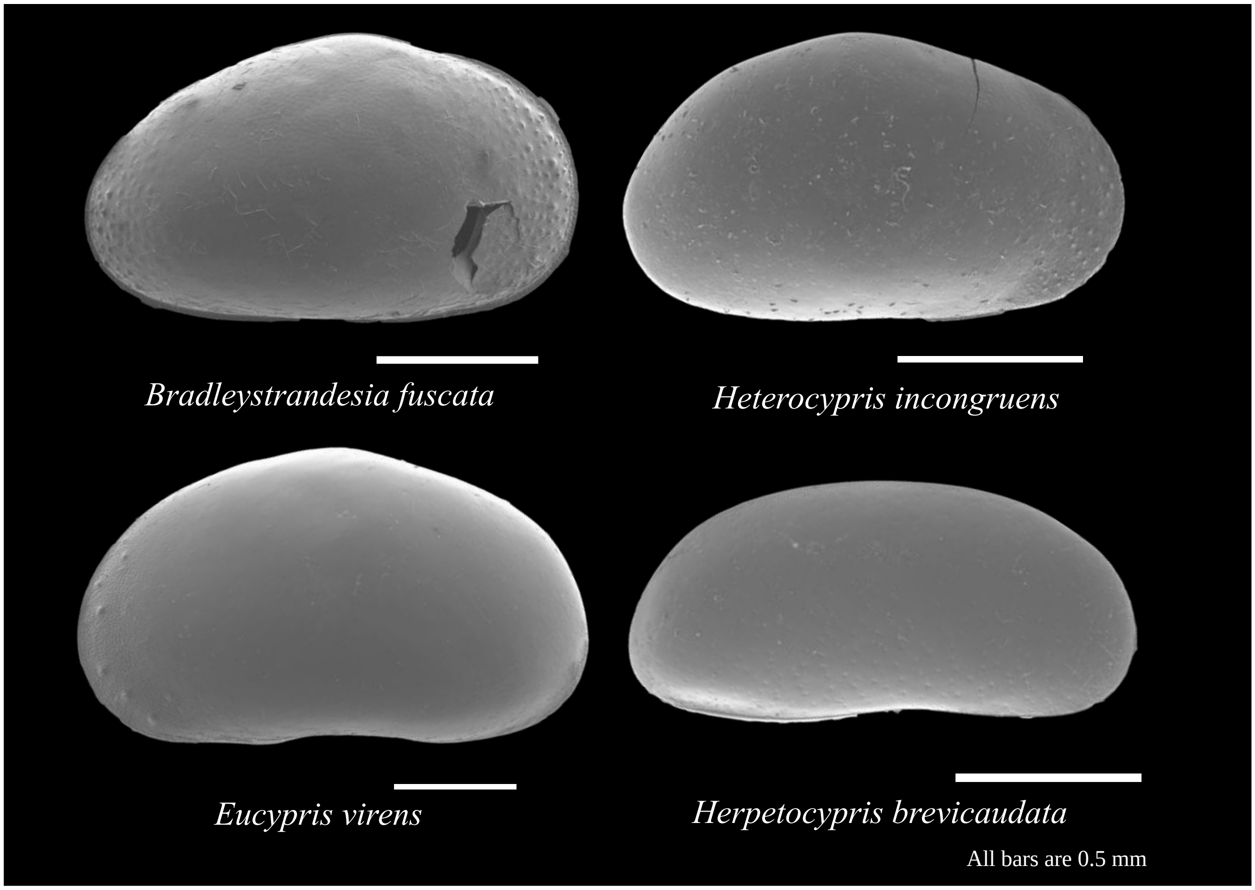
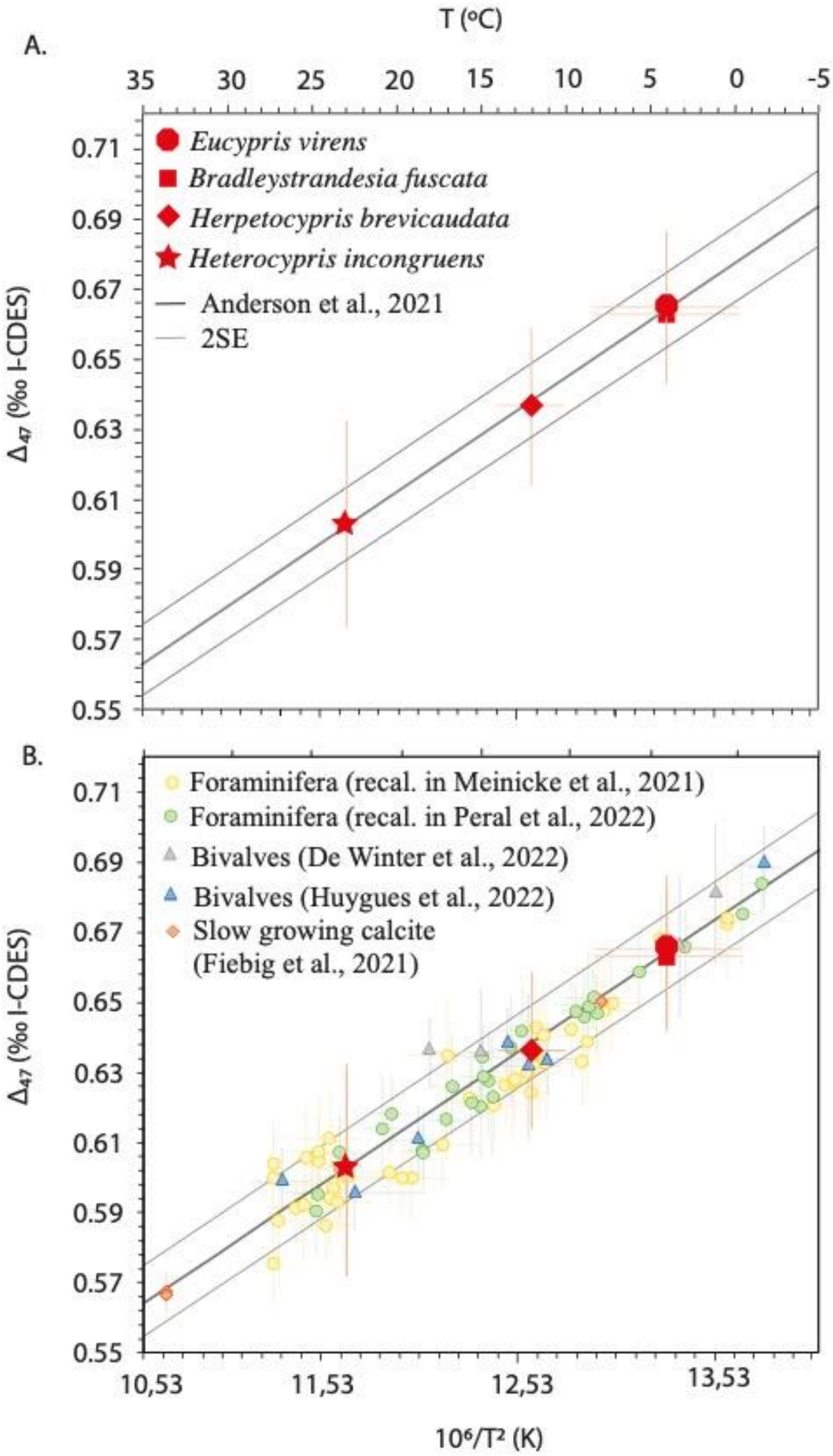


Figure 1: SEM pictures of ostracod shells


T (ºC)
A.
35
30
25
20
15
10
5
0
-5
Eucypris virens
Bradleystrandesia fuscata
Herpetocypris brevicaudata
Heterocypris incongruens
Anderson et al., 2021
2SE
Δ47 (‰ I-CDES)
0.71
0.69
0.67
0.65
0.63
0.61
0.59
0.57
0.55
B.
Foraminifera (recal. in Meinicke et al., 2021)
Foraminifera (recal. in Peral et al., 2022)
Bivalves (De Winter et al., 2022)
Bivalves (Huygues et al., 2022)
Slow growing calcite
(Fiebig et al., 2021)
10,53
11,53
12,53
13,53
10⁶/T² (K)

342

Figure 2: A. Clumped isotope (Δ_{47}) measured on living ostracods shells are presented against the known precipitated temperatures. The ostracod-Δ_{47} values are compared to the unified calibration of Anderson et al., 2021 (black line; grey lines indicate the 2SE confidence interval). B. Ostracod clumped-isotope data against precipitated temperatures compared with clumped-isotope data from marine biogenic carbonate and continental slow grown calcite speleothems. All Δ_{47} errors are expressed at 2SE. The horizontal lines indicate the variations of the temperature measured in the field per each sample.

SUPPLEMENTARY MATERIAL

LIVING OSTRACODS FROM LAB AND NATURAL ENVIRONMENTS

Lab Grown *Heterocypris incongruens*

The ostracod species *Heterocypris incongruens* (Ramdohr, 1808) was cultured at the Royal Belgian Institute of Natural Science (Brussels-Belgium) from September to December 2021.
This species is cosmopolitan and very common in shallow seasonal ponds and small permanent water bodies (Meisch, 2000). It tolerates a wide range of air temperatures, in the summer from 5 to 28 °C and in winter from -15 to 18°C (Horne and Mezquita, 2008). The length of its valves can vary from 1.2 to 1.9 mm for female and from 1.2 to 1.3 for male specimens.
Living specimens of *H. incongruens* were sampled using a rectangular hand-net (120 μm mesh size) in a temporary pond in a nature area called “Drongengoed” (Ursel, Belgium - 51.151N, 3.474E). The main population was kept alive in an aquarium filled with fine sand sediments (ca.

2cm thickness at the bottom) and water coming from their natural environment. Every three days they were fed with *Spirulina* algae. For this study, juvenile specimens were selected from the last three stages (A-1, A-2, A-3 valves). These specimens were isolated to make satellite populations in a (self-made) incubator system under controlled temperature at 23°C (± 0.5°C) and 50-50 Light-Dark diurnal circle. Deceased adult *H. incongruens* specimens were removed from the isolated temperature-controlled aquarium and their valves were used for Δ_{47} analysis.

Ostracod collected in natural environments

Living ostracods were collected in their natural environments using a hand-net (120 μm mesh size) during two field campaigns in January (Ghent-Belgium) and February (Granada-Spain) 2022. *Eucypris virens* (Jurine, 1820) and *Bradleystrandesia fuscata* (Jurine, 1820) come from a monitored temporary pond (Ghent-Belgium) only a few cm deep. *Eucypris virens* is a winter-early spring species (December/April) widely distributed (Europe, Greenland, Azores, North Africa, Central Asia, China, North America and Middle East) that prefers to live in temporary habitats, mostly ponds, which dry up before summer, as well as in streams, rivers, and rice fields (Meisch 2000). It tolerates a wide range of air temperatures (summer from 13 to 28 °C (July) and winter from -8 to 14°C (January), (Horne and Mezquita 2008)). The length of its valves varies from 1.4 to 2.4 mm in females and from 1.4 to 2.1 in males. Adults have a life spam of about 2 months and die shortly after egg production (Meisch 2000). The collected adult specimens precipitated their shells between December and beginning of January 2022 under temperature of 4°C (± 2°C).

Bradleystrandesia fuscata is a species characteristic of seasonal temporary ponds, mainly from autumn to spring and very rare during summer. In winter ponds larvae develop faster than in those in other seasons and they live only for a few weeks (Meisch 2000). The species tolerates a large range of air temperatures (summer from 6 to 26 °C and winter from -15 to 12°C, Horne and Mezquita 2008). It has been found in Europe, Turkey, Middle East and North America (Meisch 2000), in the Neotropics (Coviaga et al., 2018) and as invasive species also in Western Australia (Koenders et al. 2012, 2016). Female valve length varies from 1.1 to 1.5 mm. The collected adult specimens precipitated their shells between December and the beginning of January under temperatures of 4°C (± 2°C).

Herpetocypris brevicaudata (Kaufmann, 1900) specimens were collected in a small and very shallow river (less then 20 cm deep), with continuous running water, that is fed by groundwater in the Padul lake area (Granada-Spain). This species was found in Europe and North Africa and prefers springs, slowly flowing streams, and the littoral zone of lakes. It tolerates a large range of air temperatures (summer from 16 to 33 °C (July) and winter from -1 to 16°C (January) (Horne and Mezquita 2008)). Female valve length varies from 1.3 to 2.2 mm. Little is known about the life cycle of H. *brevicaudata* but it is supposedly similar to that of its closest congener, *H. reptans* (Baird, 1835), which produce two generations per year (April/August and November/overwinter). The collected adults precipitated their shells between November and the beginning of February, which is a period during which the water temperature is constant at 12°C (± 1°C).

All the collected ostracods were euthanized and preserved in 70% ethanol.

REFERENCES

Coviaga, C., Cusminsky, G., and Pérez, P., 2018, Ecology of freshwater ostracods from Northern Patagonia and their potential application in paleo-environmental reconstructions: Hydrobiologia, v. 816, p. 3–20, doi:10.1007/s10750-017-3127-1.

Horne, D.J., and Mezquita, F., 2008, Palaeoclimatic applications of large databases: developing and testing methods of palaeotemperature reconstruction using nonmarine ostracods: Senckenbergiana lethaea, v. 88, p. 93–112, doi:10.1007/BF03043981.

Koenders, A., Schön, I., Halse S., and Martens, K., 2016, Valve shape is not linked to genetic species in the *Eucypris virens* (Ostracoda, Crustacea) species complex. Zoological Journal of the Linnean Society 80 (1), 36-46 (doi: 10.1111/zoj.12488)

Koenders, A., Martens, K., Halse, S., and Schön, I., 2012, Cryptic species of the *Eucypris virens* species complex (Ostracoda, Crustacea) from Europe have invaded Western Australia. Biological Invasions 14(10): 2187-2201. (DOI 10.1007/s10530-012-0224-y)

Meisch, C. (Ed.), 2000, Ostracoda: Heidelberg Berlin, Spektrum Akad. Verl, Süßwasserfauna von Mitteleuropa / begr. von A. Brauer. Hrsg. von J. Schwoerbel Bd. 8/3, 522 p.

The raw data are available upon request and will be deposited after publication in EarthChem.

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