Impact of climate and hydrochemistry on shape variation – a case study on Neotropical cytheroidean Ostracoda

Claudia Wrozyna[1], Thomas A. Neubauer[2,3], Juliane Meyer[1], Maria Ines F. Ramos[4,] Werner E. Piller[1]

[1] Institute of Earth Sciences, NAWI Graz Geocenter, University of Graz, Graz, 8010, Austria

5 [2] Department of Animal Ecology & Systematics, Justus Liebig University, Giessen, 35392, Germany

[3] Naturalis Biodiversity Center, Leiden, 2300 RA, The Netherlands

[4] Coordenação de Ciências da Terra e Ecologia, Museu Paraense Emílio Goeldi, 66077-830, Brazil

Correspondence to: Claudia Wrozyna (claudia.wrozyna@uni-graz.at)

10

Abstract. How environmental change affects a species' phenotype is crucial not only for taxonomy and biodiversity assessments but also for its application as (paleo-)ecological indicator. Previous investigations addressing the impact of climate and hydrochemical regime on ostracod valve morphology have yielded contrasting results. Frequently identified ecological factors influencing carapace shape are salinity, cation and sulphate concentrations and alkalinity. Here, we present a thorough

15 approach integrating data from carapace outline and surface details of the ubiquitous Neotropical cytheroidean ostracod species *Cytheridella ilosvayi*, as well as several climatic and hydrochemical variables, in order to investigate a potential link between morphology and environmental conditions. A previous study lately demonstrated considerable biogeographical variation in valve morphology among Floridian, Mexican and Brazilian populations of this species. We hypothesize that the climatic differences between the regions it inhabits and associated differences in hydrochemical regimes have influenced valve

20 morphology and eventually led to biogeographically distinctive groups. Generalized least-squares Procrustes Analyses based on outline and structural features were applied to left and right valves of adult females and males. The analyses identified relative carapace length and shape symmetry as most important morphological characteristics representing shape differences across all datasets. Two-block partial least-squares analyses and multiple regressions indicate strong relationships between morphological and environmental variables, specifically with temperature seasonality, annual precipitation and chloride and

25 sulphate concentrations. We hypothesize that increased temperature seasonality slowed down growth rates during colder months, potentially triggering the development of shortened valves with well-developed brood pouches. Differences in chloride and sulphate concentrations, related to fluctuations in precipitation, are considered to affect valve development via controlling osmoregulation and carapace calcification. These factors represent hitherto unknown drivers for ostracod ecophenotypy and emphasize that environmental predictors for morphological variability are not consistent across non-marine

30 ostracods.

1 Introduction

Understanding how species respond to environmental change is crucial for their application as proxies for past climate fluctuations as well as forecasting future dynamics and distribution of species. Morphological diversity represents a key character for the interpretation of faunal changes (Wagner and Erwin, 2006) and ecological shifts (Mahler et al., 2010) and urges discussions about speciation and extinction processes through time (e.g., Ciampaglio, 2004). Differences in shape and size among species have been shown to relate with changes of environmental parameter, in particular, differences in temperature across various clades (e.g., Loehr et al., 2010; Maan and Seehausen, 2011; Danner and Greenberg, 2015). Within freshwater invertebrates, ecophenotypic response has been documented for a variety of species, both recent and fossil (e.g., Hellberg et al., 2001; Zieritz and Aldridge, 2009; Inoue et al., 2013; Neubauer et al., 2013; Clewing et al., 2015).

Ostracods represent a popular proxy group for climate and ecosystem changes due to their occurrence in various habitats, ranging from most inland waters to marine and interstitial and even (semi-)terrestrial environments (e.g., Horne, 2004). Their distribution is controlled by ecological factors such as salinity, temperature, and ion composition of the ambient water (e.g., Ruiz et al., 2013). The study of ecophenotypical variation in response to environmental change (Anadón et al., 2002; Frenzel et al., 2012; Fürstenberg et al., 2015; van der Meeren et al., 2010) demonstrates another approach using them for palaeoenvironmental studies. Due to their calcitic valves, they have an excellent fossil record and are utilized as palaeoenvironmental and biostratigraphic indicators (Anadón et al., 2002). A number of studies has shown that ornamentation, noding, sieve pore shape, and carapace size are linked to environmental factors, e.g., salinity, temperature, water depth and nutrient availability (van Harten, 1975; Yin et al., 1999; Majoran et al., 2000; van Harten, 2000; Anadón et al., 2002; Frenzel and Boomer, 2005; Medley et al., 2007; Marco-Barba et al., 2013; Meyer et al., 2016; Boomer et al., 2017). Especially with the rise of morphometric techniques, investigations also dealing with carapace shape variation in relation to environmental variables have increased (Yin et al., 1999; Baltanas et al., 2002; Baltanas et al., 2003; van der Meeren et al., 2010; Ramos et al., 2017; Grossi et al., 2017). Yet, the use of morphological data, even those based on morphometric analyses (Baltanas et al., 2002; Baltanas et al., 2003; van der Meeren et al., 2010; Grossi et al., 2017), has been restricted to either landmark-based or outline-based studies but have rarely used a combination of both (e.g., Ramos et al., 2017). Few studies integrate geographic gradients into their statistical analyses and corresponding climate variables or a reduced number of predictor variables. Moreover, shape-environment relationships are commonly identified based on simple linear regressions or qualitative observations on multivariate ordination methods.

Here, we apply a thorough approach integrating data from carapace outline and surface details, as well as several climatic and hydrochemical variables, in order to investigate a potential link between morphology and environmental conditions. Subject of study are valves of the Neotropical cytheroidean ostracod species *Cytheridella ilosvayi* Daday, 1905. Wrozyna et al. (2016)

and Wrozyna et al. (under review) lately demonstrated considerable biogeographical variation in valve morphology among Floridian, Mexican and Brazilian populations of that species. Morphological differences in populations of *C. ilosvayi* are discernible for both valves and appendages, for adult and juvenile (A-1 to A-3) stages and across sexes, suggesting that morphological divergence is a result of long-term biogeographic isolation (Wrozyna et al., submitted). While the morphological aspects of the biogeographic variability in *C. ilosvayi* are well understood, the causes for the regional differences have not been investigated. We hypothesize that the climatic differences between the regions inhabited by *Cytheridella ilosvayi* and associated differences in hydrochemical regimes have influenced valve morphology and finally led to biogeographically distinctive groups. We apply two-block partial least squares analyses and multiple regression analyses in order to test for covariation between the two sets of parameters (morphology, environment) and to identify the morphological characteristics and environmental variables that contribute most to the relationship.

2 Material and Methods

2.1 Material

Specimens of *C. ilosvayi* derive from several sampling campaigns in Florida, Mexico and Brazil during 2009–2015 (Fig. 1). A detailed list of the sampled localities is available in Supplementary Table 1. Only adult valves were utilized in this study, providing a sufficient number of left and right valves for both sexes. Right and left valves were investigated separately due to dimorphism in size and shape (Wrozyna et al., 2014). Beyond that, females and males were analyzed separately because a large part of within-valve variation has been shown to depend on sexual differences (such as the presence of brood pouches in females; Wrozyna et al., 2016).

2.2 Predictor variables

Altogether, 15 variables were included in the analyses. Simultaneously to water sampling, field variables (electrical conductivity, water temperature and pH) were measured *in situ* at all sample sites using a WTW multi-sensor probe (Multi 3420 Set C). Water samples were taken with plastic bottles and promptly filtrated using a syringe filter with a filter pore size of 0.45 µm and stored in a freezer until analysis. Major ions were measured at the laboratory center of Joanneum Research in Graz by ion chromatography (Dionex ICS-3000). As the variables measured per sampling station only provide a snapshot of the local ecological conditions, the set of variables was supplemented with bioclimatic data from the WorldCLIM database (WorldClim, 2017), providing data on monthly to yearly scales. From the many variables available we included annual mean temperature [°C] (BIO1), mean diurnal range [°C] (BIO2; mean of monthly maximum-minimum temperature), temperature seasonality [°C] (BIO4; standard deviation *100), annual precipitation [mm] (BIO12) and precipitation seasonality (BIO15; coefficient of variation), each with a spatial resolution of 30″. We chose not to include all bioclimatic variables because many

3

of them are highly correlated, causing issues for the regressions. Bioclimatic variables and occurrence data were linked in ESRI ArcGIS v. 10.4 with the tool "Extract Multi Values to Points". Environmental variables are provided in Appendix S2.

2.3 Methods

2.3.1 Generalized Procrustes Analysis

Valve morphology was captured using a combination of landmarks and semilandmarks. Eight landmarks (LM) were chosen to characterize anterior pore tubuli (LM 1–5, type-I) and the dorsal dip point of the posterior curvature (LM 6, type-II), as well as to delimitate maximum anterior and posterior curvatures (LM 7–8, type-III). Carapace outline was defined by two curves between LM 7 and 8, each comprising 30 equidistantly spaced semilandmarks (see also Wrozyna et al., 2016). All points were set on digitized SEM images using the program TpsDig v. 2.17 (Rohlf, 2013). The sliders file determining sliding direction of the semilandmarks during the Procrustes alignment was created in TpsUtil v. 1.58 (Rohlf, 2015). A Generalized least-squares Procrustes Analysis, computing consensus configuration, partial warps and relative warps (RW), was performed in TpsRelw v. 1.65 (Rohlf, 2016). Thin-plate spline deformation grids were used to visualize deviations of selected configurations from the mean and to identify morphological characteristics that account for differences among geographic regions. For details on the method see Rohlf and Slice (1990) and Bookstein (1996).

We ran preliminary analyses for each dataset to identify major outliers that may bias the morphometric analyses by overemphasizing particular directions in the morphospace (and associated morphological characteristics). Such distortion may severely impede sound interpretation of follow-up statistical analyses.

2.3.2 Statistics

In order to study the covariance between shape variation and environmental variables, two-block partial least-squares analyses (PLS) were performed using software PAST 3.18 (Hammer et al., 2001). As a great advantage over other ordination methods such as principal components analysis, this method disregards within-block variation that may mask between-block covariance (Mitteroecker and Bookstein, 2008, 2011). Using all RWs in the PLS might severely bias the pattern because – contrary to their descending significance in terms of explaining shape variation – they would be treated equally by the analysis. Therefore, we restricted the morphological block to RW 1–20, which account for at least 98.6% of the total shape variation in all four datasets. The environmental variables were log10-transformed to constrain the orders of magnitude involved. PLS analyses were computed based on correlation matrices.

The PLS analysis provides an idea of the overall strength of the relationships with between shape and environment. To identify the parameter(s) that affect specific morphological traits or combination of traits, multiple regression analyses were conducted on selected RWs in the statistical environment R v. 3.3.2 (R Core Team, 2016). Only warps 1) along which biogeographic differentiation was observed, 2) with an amount of shape variation higher than 10% of the total variation, and 3) with PLS

loading values higher than the mean loading value (based on absolute values) were considered. These selection criteria were chosen in order to prevent from misinterpreting seemingly strong relationships between shape and environmental variables. Since the environmental parameters are likely to be highly correlated, eventual regression models including all variables might be strongly skewed and susceptible to misinterpretation. Therefore, we employed a stepwise selection of variables based on the variance inflation factor (VIF), which is an estimator of multicollinearity among variables (Quinn and Keough, 2002). As a rule of thumb, VIF values greater than ten indicate the presence of multicollinearity (Quinn and Keough, 2002); some authors even consider values above five evidences of collinearity (Heiberger and Holland, 2004). The applied function iteratively removes collinear variables by calculating the VIF of variables against each other (for the script, see Ijaz, 2013); R package 'fmsb' v. 0.5.2 (Nakazawa, 2015) is required for this procedure. VIF values were calculated with package 'HH' v. 3.1-32 (Heiberger, 2016). To enhance the models further, multiple regressions using backward stepwise selection by evaluation of the Akaike Information Criterion (AIC) were performed with the remaining set of factors. Normality of model residuals was tested with Shapiro-Wilk tests. In case normality was not achieved, residual distributions were assessed qualitatively using Q-Q-plots; only if the majority of cases match the expected distribution, a model was considered significant. Finally, we used the R package 'hier.part' v. 1.0-4 (Walsh and Mac Nally, 2013) to evaluate the independent contribution of each predictor to the (reduced) models.

3 Results

The relative warps analysis (RWA) yielded different results for males and females, while patterns were largely consistent within sexes (Fig. 2, 3). Along the first three relative warps, Mexican females have little overlap with Brazilian/Floridian ones. Only some of the specimens from Punta Laguna in northern Yucatan seem to be morphologically closer to the Floridian group and cluster apart in the analyses of both valves. Brazilian and Floridian individuals have a distinctly higher overlap and differentiate only little along RW 2. A clear differentiation within both clusters, like in the Mexican group, is lacking. Group differentiation in male valves is quite contrary: Floridian specimens have little overlap with Brazilian ones in both valves along RW 1, while Mexican specimens are hardly separable from either group along any of the first 3 RWs. However, the differentiation between some Punta Laguna valves and remaining Mexican carapaces along RW 1 is comparable to the patterns observed for females. Mexican and Brazilian males show slight biogeographic differentiation along RW 2 (left valves) and RW 3 (right valves), respectively. No clustering is observed for higher warps in either sex or valve.

Similar to the patterns posed by the scatter plots, the thin-plate splines indicate that shape variation along RWs is largely consistent within valves but differs slightly between sexes. (Here we discuss only axes along which biogeographic discrimination is observed. See Wrozyna et al., 2016 for within-group variation.) The most important morphological characteristic representing shape differences along RW 1 in both females and males and right and left valves, is relative carapace length (Fig. 2, 3). However, the exact expression differs between sexes: valve outline in males varies between elongate-elliptical and short asymmetrical with slightly inflated anterior part, and between elongate-elliptical and short

5

asymmetrical with distinctly inflated posterior region (i.e., brood pouch) in females. In addition to outline differences, the position of the anteriormost pore conulus (LM 2) shifts in dorso-ventral direction consistently in both valves and sexes. In females, also the position of the dorsal dip point of the posterior curvature (LM 6) varies in dorso-ventral direction. Shape variation along RW 2 is in females similar as for RW 1 but with a different combination of traits: negative scores correspond
5 to elongate valves with inflated posterior and slightly shifted LM 2 and LM 6 in dorso-ventral direction. In male *Cytheridella*, only left valves show weak biogeographic differentiation along RW 2, representing shape differences from elongate-elliptical to slightly asymmetrical with higher dorsal margin, and the dorsal dip point of the posterior curvature (LM 6) shifts towards posterior. The only differentiation along RW 3 is for male right valves, corresponding mostly to shell elongation and a little to the relative positions of pore conuli. The relative warp scores of all datasets are provided in Supplementary Table 2.

10 The PLS analyses indicate relationships between morphological and environmental variables, yet with different results for males and females. The first PLS axis explains between 68.7% and 77.9% of the total variation, whereas values are consistently higher for females (LV: 77.5%; RV: 77.9%) than for males (LV: 68.7%; RV: 71.5%). In all four analyses, Brazilian specimens are widely separated from Floridian/Mexican ones along PLS axis 1, corresponding to a clear differentiation along both environmental and morphological scores. Left valves of females and left and right valves of males of Brazilian specimens
15 exhibit negative scores on both PLS axes corresponding to shape and environmental variables. Females display inverse distributions for Brazilian and Mexican specimens. Floridian and Mexican groups overlap little but consistently in all analyses, while the specimens of Florida tend to have smaller variation ranges than Mexican groups (Fig. 4). Permutation tests indicate however that PLS analyses are hardly significant for male valves (LV: P = 0.126, RV: P = 0.135). Yet, the low significance levels do not necessarily imply lacking relationships between shape and environment, they rather witness the difficulties in
20 finding clear relationships in multifactorial analyses. In fact, the overall picture provided by the PLS might mask individual relationships between selected shape traits and environmental parameters, which is why a closer look is required by using multiple regressions. Nonetheless, the PLS analysis are useful to examine the overall strength of the relationships, which seem to be stronger and clearer in females than in males.

For PLS axis 2, low relationships between shape and environment are yielded for all four datasets, and none of them are
25 significant (see Supplementary Table 3).

The loadings for morphological variables in the PLS analyses yield constantly high values for RW 1; RW 2 shows loading values higher than the mean (based on absolute values) in all analyses except male right valves; RW 3, in turn, contributes above average to variation in all cases but females right valves. Other warps were not considered because of their minor influence on shape variation (low loading values) or the lack of biogeographic separation. See Table 1 for a summary of the
30 results.

Following warps fulfil the selection criteria defined in the Methods section for consideration in the multiple regressions: RW 1 for all four datasets; RW 2 for female right and left valves; RW 3 for female left valves and male right and left valves. Hence, nine regression analyses were carried out. Shapiro-Wilks tests of model residuals indicate normality for four of the nine analyses (Table 1). Inspection of Q-Q-plots yielded, however, that in all models the majority of cases match the expected

6

distributions, which is why the remaining models are still considered significant (see Supplementary Figure 1). Eight out of nine models are significant ($P < 0.05$); the model for RW 3 for male left valves is not ($P = 0.074$).

Only a limited set of predictor variables is retained out of the originally 15 variables in each model after elimination of collinear parameters and backward stepwise selection (see Supplementary Table 4). Seven parameters do not contribute to any models:

5   Na^+, Ca^{2+}, Mg^{2+}, HCO_3^-, conductivity, mean annual temperature, and precipitation seasonality. Of the remaining factors, temperature seasonality is one of the most important predictors in almost all models, accounting for at least 28.7% in all models with RW 1. Temperature seasonality is highest in Florida, closely followed by Brazil, and considerably less in Mexico, reflecting the distinction between Mexican and Floridian/Brazilian populations along RW 1. Similarly, annual precipitation and the anions Cl^- and SO_4^{2-} contribute significantly to many models, corresponding to differences in the hydrological regimes.

10   Less explanation power is provided by pH, K^+, water temperature, and mean diurnal temperature range. It is noteworthy that anions, represented by Cl^- and SO_4^{2-}, are obviously much more important than cations.

4 Discussion

Variation in temperature seasonality, annual precipitation and anions (Cl^-, SO_4^{2-}) explain a large portion of shape variation in *Cytheridella*, which is mostly related to relative carapace length and outline shape. Narrow elongate shapes, such as those

15   occurring in Mexico, correspond to relatively low seasonality and precipitation but high anion concentrations. Opposite conditions seem to favor the formation of short, asymmetrical valves typical for specimens from Florida and Brazil. Secondary shape variations differentiating between elongated valves with slightly wider posterior and short, symmetrical valves (i.e., RW2) are attributed to higher and lower annual precipitation, respectively.

The link between shape variation and environmental conditions is a well-studied branch of ostracodology, but studies have

20   yielded quite contrasting results. Frequently identified ecological factors are salinity (Yin et al., 1999; Yin et al., 2001; Grossi et al., 2017) and hydrochemical regime, mirrored by Mg^{2+}, Ca^{2+} and K^+ contents (Ramos et al., 2017) or alkalinity and sulphate, respectively (van der Meeren et al., 2010). Morphological response to the same environmental factor may even differ between environments (e.g., Yin et al., 1999), complicating straightforward explanation models.

4.1 Potential environmental drivers of valve shape variation

25   The mechanisms that control the relationship between valve shape and environmental gradients in *Cytheridella* are not understood at present. Like many non-marine ostracods, *Cytheridella* is characterized by a benthic life style. Growth and proliferation of an individual and a population, respectively, might benefit from changes in carapace shape with respect to different habitats. For instance, a more elongated shape could be advantageous in more densely vegetated environments because of increased motility. For the present study, we sampled various habitats within each region, differing in vegetation

30   cover and composition (Supplementary Table 1). If shape differences were indeed functional adaptations to varying habitat conditions, we would expect much higher morphological variability within each region and smaller differences between

specimens from similar habitat types than shown by the analyses. We rather suppose that shape difference in *Cytheridella ilosvayi* has a physiological origin that mirrors the varying environmental conditions.

The geographical range of *Cytheridella* coincides with the Neotropical region, which spans a wide latitudinal range from ~30°N to ~30°S. This range involves a latitudinal decline in mean annual temperature, which mainly corresponds to differences in annual minimum temperature (Lewis, 1996). Florida and southern Brazil are characterized by higher annual temperature gradients compared to Mexico. Annual minimum and maximum temperatures range between 16°C and 30°C in Florida and 10°C and 30°C in southern Brazil, respectively. Minimum and maximum temperatures vary between 19°C and 33°C (Climate-Data.org, 2017).

Temperature has a direct effect on other environmental parameters such as salinity and oxygenation of the water. Water temperature is one of the most important variables affecting metabolism, oxygen consumption, growth, molting and survival of crustaceans (Le Moullac and Haffner, 2000 and references therein). Increases in temperature can result in significantly shortened intermolt periods, higher molting rates (Roca and Wansard, 1997; Mezquita et al., 1999; Brylawski and Miller, 2006), increased growth increments (Martens, 1985; Iguchi and Ikeda, 2004) and reduction in maturation time (Pöckl, 1992). We expect that higher temperature seasonality induced prolonged molt cycles in populations of *C. ilosvayi* by extending intermolt periods during colder seasons. We hypothesize that the changed molt cycles affected calcification patterns and led to the observed differences in shape. Since the physiological processes involved in the secretion of ostracod valves are poorly known (and not at all for *Cytheridella*), this hypothesis cannot be tested at present.

Precipitation causes declines in nutrients and promotes physical disturbance of the water column (Figueredo and Giani, 2009). Moreover, changes in precipitation directly influence hydrochemical composition, input of sediments, organic components and contaminants and lake level (Mortsch and Quinn, 1996; Whitehead et al., 2009). Indirect influence poses, e.g., the control on aquatic plants, which represent important (micro)habitats and/or food sources (Lacoul and Freedman, 2006). The annual cycle of precipitation over most of South America is monsoon-like with great contrasts between winter and summer (Grimm et al., 2007). The peak rainy season in the Brazilian sample region is the austral winter. The rainfall is caused by frontal penetration associated with migratory extratropical cyclones (Grimm et al., 1998). The amount of rainfall in Yucatan is associated with seasonal migration of the Intertropical Convergence Zone and less by spatially oriented tropical convective activity (e.g., Hodell et al., 2008). Florida, in particular Southern Florida, where most of our samples derive from, receives maximum precipitation during northern hemisphere summer from convectional and tropical storms (Schmidt et al., 2001). The annual precipitation amounts for the sampled areas are with 1396–1492 mm per year in Brazil higher on average than in Florida and Yucatan, with 1185–1430 mm and 1125–1359 mm, respectively. Since the annual amounts of the regions are very similar it might be more plausible that precipitation seasonality has an influence on carapace shape of *Cytheridella* through seasonally restricted nutrient inputs or changes of the hydrochemistry. Annual precipitation should be therefore considered with caution since it is difficult to deduce a causal relationship with carapace shape.

Ionic composition of the host water is vital for calcification and growth rates of ostracods (Mezquita et al., 1999). The relationship between hydrochemistry and phenotypic variability is poorly understood, however. A study of Kim et al. (2015)

shows that increased levels of pH account for decreased carapace growth rates, i.e., prolonged intermolt periods, and smaller carapaces. Carapace shape differences have been moreover associated with changes in Ca^{2+}, Mg^{2+} and pH (Ramos et al., 2017). Our analyses, however, revealed correlations neither with ions related to formation of carbonate, such as HCO_3^-, Ca^{2+}, and Mg^{2+}, nor with pH. Only chloride and sulfate contents significantly correlate with carapace shape variation.

5   Natural sources of Cl^- in freshwater derive from marine sprays transferring NaCl into the atmosphere and are either transported as aerosol by wind or are washed out by precipitation and the weathering of rocks. Additionally, large amounts of chloride are derived anthropogenically from farming and waste water production (Müller and Gächter, 2012). Sulfate can derive through runoff from mining and agricultural areas, mobilization from pyrite deposits by oxygen intrusion during desiccation and weathering of rocks containing sulphur (Holmer and Storkholm, 2001; Lamers et al., 2002). Sulfate contents in groundwaters

10   and surface waters result from dissolution of gypsum and anhydrite occurrences (Perry et al., 2002) and from mixing with seawater (Sacks et al., 1995). In Yucatan, a gypsum-rich stratigraphic unit occurs providing a solution-enhanced subsurface drainage pathway for a broad region extending along the eastern coast and from east to west in the southern part (Perry et al., 2009). The chloride content of groundwater is the result of mixing with seawater (Mondal et al., 2010). Additionally, a Cl^- gradient extends from southeast to northwest providing generally higher chloride contents (Perry et al., 2009). Concentration

15   gradients of SO_4^{2-} and Cl^- in Florida occur from inland to coastal areas as well as with depth (Sacks et al., 1995), explaining the relatively higher amount of chloride and sulfate. The comparably low values for south Brazilian sampling locations is not surprising given that such coastal water bodies are often fed by groundwater (Santos et al., 2008) that is dominated by bicarbonate waters and low chloride and sulfate contents (Gianesella-Galvão and Arcifa, 1988; Viero et al., 2009). The detected relationship between morphotypes and chloride and sulfate contents, respectively, could thus mirror the hydrochemical

20   compositions resulting from different hydrogeological conditions of the regions.

    Van der Meeren et al. (2010) found ostracod valve shape variability to be significantly correlated with the ratio between alkalinity and sulfate. As the ratio was inversely related to solute concentration, the authors hypothesized that carapace shape may be linked to changes in the lake water balance or relative climatic moisture, or changes in the sources of solutes delivered to the environment. Varying anionic composition has also been considered to affect osmoregulation and calcification (Mezquita

25   et al., 1999). As hyperosmotic organisms, freshwater ostracods are obliged to pump ions inwards (mainly Na^+ and Cl^-) and water outwards to maintain a stable internal ionic concentration higher than that of the ambient water (Weihrauch et al., 2004). Chloride is obtained from the environment through a HCO_3^-/Cl^- antiport pump. The organism needs to precipitate calcite but also pump HCO_3^- outwards to maintain the internal Cl^- concentration (Mezquita et al., 1999). These authors assumed that even small genetic differences affect varied ecophysiological responses to temperature and water chemistry, which may be a key

30   factor for the explanation of different biogeographical patterns of non-marine ostracods. Especially the trade-off between ionic regulation and calcification is considered to play a key role in ostracod speciation (Mezquita et al., 1999).

    One of the best-studied phenomenon in ostracods is variable noding (hollow outward flexions on the lateral surfaces on the valves) in *Cyprideis* (e.g., Vesper, 1975; van Harten, 2000). A connection between node formation and salinity was noted early, but the reported salinity limits are partly contradictory (Keyser and Aladin, 2004). Frenzel et al. (2012) deduced from a

combination of mesocosm cultures and field studies that noding in *Cyprideis torosa* valves is pathologically and caused by osmotic problems under lower salinities and lacking Ca^{2+} during molting. From the same species it is known that increasing salinity corresponds with decreasing proportion of rounded sieve pores of the valves (Frenzel et al., 2017). Considering that the variability of discrete valve traits such as noding or sieve pore shape is related to complex physiological processes, we

5   hypothesize that the relationship between carapace shape and ionic composition detected by our analyses could be a result of complex interplay of different physiological processes affecting valve calcification. Understanding the physiological processes involved requires more detailed studies.

4.2 Genetic diversity or ecophenotypic plasticity?

10   Phenotypic variation in ostracods is considered to reflect either genotypic or ecophenotypic variability or a combination of both (Martens et al., 1998; Yin et al., 1999; Anadón et al., 2002; Frenzel and Boomer, 2005; Boomer et al., 2017; Grossi et al., 2017). A recent study on valve outline variability of a non-marine ostracod demonstrated that differences in carapace shape do not correspond to genetic clades (Koenders et al., 2016). However, caution is advised when comparing patterns among species, since different species react differently and have different potentials for ecophenotypic variation (Anadón et al., 2002;

15   Frenzel and Boomer, 2005). The relationship between genotype and environment might differ among species, geographical regions and through time (see, e.g., Sanchez-Gonzalez et al., 2004; Koenders et al., 2016). Our results clearly imply that morphological disparity in *Cytheridella* is controlled by environmental factors. However, the distribution and the variation range of regional clusters reveal some opposing implications. For instance, a part of the Mexican populations comprises specimens with similarly shortened valves as are found in the Floridian group. Both shortened and elongated morphotypes co-

20   occur in one lake (i.e., Punta Laguna) (Wrozyna et al., under review). On the one hand, this co-occurrence could suggest the presence of microhabitats with specific environmental conditions, posing differential impact on valve calcification in the very same ecosystem. On the other hand, this discrepancy might be considered evidence for genetic differentiation. An integrated study combining genetic and morphometric data is required to further explore this case.

5 Conclusion

25   The comparison of our results and a large number of previous studies witnesses the difficile nature of ecophenotypic response to varying climatic and ecological conditions in freshwater ostracods. Shape variation in *Cytheridella*, mostly related to relative carapace length and outline shape, is mainly explained by temperature seasonality, annual precipitation and chloride and sulfate compositions. Increased temperature seasonality, characteristic for Florida and south Brazil, are considered to account for slower growth rates during colder months and may have triggered the development of shortened valves with well-developed

30   brood pouches. We propose that differences in chloride and sulfate concentrations, which are related to fluctuations in precipitation, might have affected valve development via controlling osmoregulation and carapace calcification. These

10

explanation models are, however, tentative as physiological studies on the influence of changing ecological conditions in non-marine ostracods are still scanty. A more detailed picture will require mesocosm experiments and field observations.

Temperature *per se*, salinity (expressed as electrical conductivity) and pH have surprisingly little or no effect on shape variation in *C. ilosvayi*, although these factors have been discussed as important drivers of ostracod ecophenotypy, variably affecting size, ornamentation and shape. The discrepancies in explanation models suggest that environmental predictors for valve shape are not consistent across non-marine ostracods. The nature of the phenotype–environment relationship likely depends on the choice of the model taxon and ecosystem. On a larger scale, this lack of a general pattern complicates reconstruction of paleoenvironments based on ecophenotypic variation.

Data availability

All relevant data are presented within the manuscript or in supplementary material.

Supplement link

Author contribution

C. Wrozyna, J. Meyer and W. E. Piller carried out sampling of *Cytheridella* populations. C. Wrozyna and J. Meyer prepared ostracod material for morphometric analyses. T. A. Neubauer performed statistical analyses. The manuscript was written by C. Wrozyna and T. A. Neubauer. M. I. F. Ramos contributed to the discussion of the results and provided taxonomic advice.

Competing interests

The authors declare that they have no conflict of interest.

References

Anadón, P., Gliozzi, E., and Mazzini, I.: Palaeoenvironmental Reconstruction of Marginal Marine Environments From Combined Palaeoecological and Geochemical Analyses on Ostracods, in: The Ostracoda: Applications in Quaternary Research, Holmes, J. A. and Chivas, A. R. (Eds.), Geophys. Monogr. Ser., 22–247, doi:10.1029/131GM12, 2002.

5 Baltanas, A., Alcorlo, P., and Danielopol, D. L.: Morphological disparity in populations with and without sexual reproduction: a case study in *Eucypris virens* (Crustacea: Ostracoda), Biol. J. Linn. Soc., 75, 9–19, 2002.

Baltanas, A., Brauneis, W., Danielopol, D. L., and Linhart, J.: Morphometric Methods For Applied Ostracodology: Tools for Outline Analysis Of Nonmarine Ostracodes, in: Bridging the Gap: Trends in the Ostracode Biological and Geological Sciences, Park, L. E., and Smith, A. J. (Eds.), Palaeont. Soc. Papers, 9, 101–118, 2003.

10 Bookstein, F. L.: Biometrics, biomathematics and the morphometric synthesis, Bull. Math. Biol., 313–365, 1996.

Boomer, I., Frenzel, P., and Feike, M.: Salinity-driven size variability in *Cyprideis torosa* (Ostracoda, Crustacea), J. Micropalaeontol., 36, 63–69, doi:10.1144/jmpaleo2015-043, 2017.

Brylawski, B. J. and Miller, T. J.: Temperature-dependent growth of the blue crab (*Callinectes sapidus*): A molt process approach, Can. J. Fish. Aquat. Sci., 63, 1298–1308, doi:10.1139/f06-011, 2006.

15 Ciampaglio, C. N.: Measuring changes in articulate brachiopod morphology before and after the Permian mass extinction event: do developmental constraints limit morphological innovation?, Evol. Dev., 6, 260–274, doi:10.1111/j.1525-142X.2004.04031.x, 2004.

Clewing, C., Riedel, F., Wilke, T., and Albrecht, C.: Ecophenotypic plasticity leads to extraordinary gastropod shells found on the „Roof of the World", Ecol. Evol., 5, 2966–2979, doi:10.1002/ece3.1586, 2015.

20 Climate-Data.org: Climate data for cities worldwide - Climate-Data.org: https://en.climate-data.org/, last access: 13 June 2017.

Danner, R. M. and Greenberg, R.: A critical season approach to Allen's rule: Bill size declines with winter temperature in a cold temperate environment, J. Biogeogr., 42, 114–120, doi:10.1111/jbi.12389, 2015.

Figueredo, C. C. and Giani, A.: Phytoplankton community in the tropical lake of Lagoa Santa (Brazil): Conditions favoring a
25 persistent bloom of *Cylindrospermopsis raciborskii*, Limnologica, 39, 264–272, doi:10.1016/j.limno.2009.06.009, 2009.

Frenzel, P. and Boomer, I.: The use of ostracods from marginal marine, brackish waters as bioindicators of modern and Quaternary environmental change, Palaeogeogr. Palaeocl., 225, 68–92, 2005.

Frenzel, P., Schulze, I., and Pint, A.: Noding of *Cyprideis torosa* valves (Ostracoda) – a proxy for salinity? New data from field observations and a long-term microcosm experiment, Int. Rev. Hydrobiol., 97, 314–329,
30 doi:10.1002/iroh.201211494, 2012.

Frenzel, P., Ewald, J., and Pint, A.: Salinity-dependent sieve pore variability in *Cyprideis torosa*: an experiment, J. Micropalaeontol., 36, 57-62, doi:10.1144/jmpaleo2016-009, 2017.

Fürstenberg, S., Frenzel, P., Peng, P., Henkel, K., and Wrozyna, C.: Phenotypical variation in *Leucocytherella sinensis* Huang, 1982 (Ostracoda): A new proxy for palaeosalinity in Tibetan lakes, Hydrobiologia, 751, 55–72,
35 doi:10.1007/s10750-014-2171-3, 2015.

Gianesella-Galvão, S. M. F. and Arcifa, M. S.: The ionic composition of ten reservoirs in southern Brazil, Rev. Hydrobiol. Trop., 21, 93–99, 1988.

Grimm, A. M., Ferraz, S. E. T., and Gomes, J.: Precipitation anomalies in Southern Brazil associated with El Niño and La Niña Events, J. Clim., 11, 2963-2880, 1998.

40 Grimm, A. M., Pal, J. S., and Giorgi, F.: Connection between Spring Conditions and Peak Summer Monsoon Rainfall in South America: Role of Soil Moisture, Surface Temperature, and Topography in Eastern Brazil, J. Clim., 20, 5929–5945, doi:10.1175/2007JCLI1684.1, 2007.

12

Grossi, F., Prato, S. D., and Gliozzi, E.: Is the occurrence of a sigmoidal ventral border in *Cyprideis torosa* (Jones) valves linked to salinity?: A morphometrical analysis approach, J. Micropalaeontol., 36, 70–79, doi:10.1144/jmpaleo2016-018, 2017.

Hammer, Ø., Harper, D. A. T., and Ryan, P. D.: PAST: Palaeontological statistics software package for education and data analysis, Palaeontol. Electron., 4, 9, 2001.

Heiberger, R. M.: HH: Statistical Analysis and Data Display: Heiberger and Holland: R package version 3.1-32, http://CRAN.R-project.org/package=HH, 2016.

Heiberger, R. M. and Holland, B.: Statistical analysis and data display: An intermediate course with examples in S-plus, R, and SAS, Springer, New York, 2004.

Hellberg, M. E., Balch, D. P., and Roy, K.: Climate-driven range expansion and morphological evolution in a marine gastropod, Science, 292, 1707–1710, doi:10.1126/science.1060102, 2001.

Hodell, D. A., Anselmetti, F. S., Ariztegui, D., Brenner, M., Curtis, J. H., Gilli, A., Grzesik, D. A., Guilderson, T. J., Müller, A. D., and Bush, M. B.: An 85-ka record of climate change in lowland Central America, Quat. Sci. Rev., 27, 1152–1165, doi:10.1016/j.quascirev.2008.02.008, 2008.

Holmer, M. and Storkholm, P.: Sulphate reduction and sulphur cycling in lake sediments: a review, Freshwater Biol., 46, 431–451, 2001.

Horne, D. J., Ostracoda, *in*: Microfossils, Selley, R.C., Cocks, M. and Plimer I. R. (eds): Encyclopedia of Geology, Amsterdeam, Boston, Heidelberg, Elsevier Academic Press, 453-463, 2005.

Iguchi, N. and Ikeda, T.: Effects of temperature on metabolism, growth and growth efficiency of *Thysanoessa longipes* (Crustacea: Euphausiacea) in the Japan Sea, J. Plankton Res., 27, 1–10, doi:10.1093/plankt/fbh146, 2004.

Ijaz, U. Z.: Collinearity and stepwise VIF selection: R script vs. 0.1, http://www.r-bloggers.com/collinearity-and-stepwise-vif-selection, 2013.

Inoue, K., Hayes, D. M., Harris, J. L., and Christian, A. D.: Phylogenetic and morphometric analyses reveal ecophenotypic plasticity in freshwater mussels *Obovaria jacksoniana* and *Villosa arkansasensis* (Bivalvia: Unionidae), Ecol. Evol., 3, 2670–2683, doi:10.1002/ece3.649, 2013.

Kim, Y., Mo, H.-h., Son, J., Lee, Y.-S., Lee, S.-E., and Cho, K.: Interactive effects of water pH and hardness levels on the growth and reproduction of *Heterocypris incongruens* (Crustacea: Ostracoda), Hydrobiologia, 753, 97–109, doi:10.1007/s10750-015-2199-z, 2015.

Koenders, A., Schön, I., Halse, S., and Martens, K.: Valve shape is not linked to genetic species in the *Eucypris virens* (Ostracoda, Crustacea) species complex, Zool. J. Linn. Soc., doi:10.1111/zoj.12488, 2016.

Lacoul, P. and Freedman, B.: Environmental influences on aquatic plants in freshwater ecosystems, Environ. Rev., 14, 89–136, doi:10.1139/A06-001, 2006.

Lamers, L. P. M., Falla, S.-J., Samborska, E. M., Dulken, Ivo A. R. van, van Hengstum, G., and Roelofs, J. G. M.: Factors controlling the extent of eutrophication and toxicity in sulfate-polluted freshwater wetlands, Limnol. Oceanogr., 47, 585–593, doi:10.4319/lo.2002.47.2.0585, 2002.

Le Moullac, G. and Haffner, P.: Environmental factors affecting immune responses in Crustacea, Aquaculture, 191, 121–131, 2000.

Lewis, M. L., jr: Tropical lakes: how latitude makes a difference, in: Perspectives in Tropical Limnology, Schiemer, F., and Boland, K. T. (Eds.), SPB Academic Publishing, Amsterdam, 43–64, 1996.

Loehr, J., Carey, J., O'Hara, R. B., and Hik, D. S.: The role of phenotypic plasticity in responses of hunted thinhorn sheep ram horn growth to changing climate conditions, J. Evol. Biol., 23, 783–790, doi:10.1111/j.1420-9101.2010.01948.x, 2010.

Maan, M. E. and Seehausen, O.: Ecology, sexual selection and speciation, Ecol. Lett., 14, 591–602, doi:10.1111/j.1461-0248.2011.01606.x, 2011.

13

Mahler, D. L., Revell, L. J., Glor, R. E., and Losos, J. B.: Ecological opportunity and the rate of morphological evolution in the diversification of Greater Antillean anoles, Evolution, 64, 2731–2745, doi:10.1111/j.1558-5646.2010.01026.x, 2010.

Majoran, S., Agrenius, S., and Kucera, M.: The effect of temperature on shell size and growth rate in *Krithe praetexta praetexta* (Sars), Hydrobiologia, 419, 141–148, doi:10.1023/A:1003943617431, 2000.

5   Marco-Barba, J., Mesquita-Joanes, F., and Miracle, M. R.: Ostracod palaeolimnological analysis reveals drastic historical changes in salinity, eutrophication and biodiversity loss in a coastal Mediterranean lake, Holocene, 23, 556–567, doi:10.1177/0959683612466752, 2013.

Martens, K.: Effects of Temperature and Salinity on Postembryonic Growth in *Mytilocypris henricae* (Chapman) (Crustacea, Ostracoda), J. Crustacean Biol., 5, 258–272, 1985.

10   Martens, K., Rossetti, G., and Geiger, W.: Intraspecific morphological variability of limbs, in: Sex and Parthenogenesis: Evolutionary Ecology of Reproductive Modes in Non-Marine Ostracods, Martens, K. (Ed.), Backhuys Publishers, Leiden, 143–155, 1998.

Medley, P., Tibert, N., Patterson, W., Curran, H., Greer, L., and Colin, J.-P.: Paleosalinity history of middle Holocene lagoonal and lacustrine deposits in the Enriquillo Valley, Dominican Republic based on pore morphometrics and isotope
15   geochemistry of ostracoda, Micropaleontology, 53, 409–419, doi:10.12691/aees-414, 2007.

Meyer, J., Wrozyna, C., Gross, M., Leis, A., and Piller, W. E.: Morphological and geochemical variations of *Cyprideis* (Ostracoda) from modern waters of the northern Neotropics, Limnology, doi:10.1007/s10201-016-0504-9, 2016.

Mezquita, F., Roca, J. R., and Wansard, G.: Moulting, survival and calcification: the effects of temperature and water chemistry on an ostracod crustacean (*Herpetocypris intermedia*) under experimental conditions, Arch. Hydrobiol., 146,
20   219–238, 1999.

Mitteroecker, P. and Bookstein, F.: The evolutionary role of modularity and integration in the hominoid cranium, Evolution, 62, 943–958, doi:10.1111/j.1558-5646.2008.00321.x, 2008.

Mitteroecker, P. and Bookstein, F.: Linear discrimination, ordination, and the visualization of selection gradients in modern morphometrics, J. Evol. Biol., 38, 100–114, 2011.

25   Mondal, N. C., Singh, V. P., Singh, V. S., and Saxena, V. K.: Determining the interaction between groundwater and saline water through groundwater major ions chemistry, J. Hydrol., 388, 100–111, doi:10.1016/j.jhydrol.2010.04.032, 2010.

Mortsch, L. D. and Quinn, F. H.: Climate change scenarios for Great Lakes Basin ecosystem studies, Limnol. Oceanogr., 41, 903–911, 1996.

Müller, B. and Gächter, R.: Increasing chloride concentrations in Lake Constance: Characterization of sources and
30   estimation of loads, Aquat. Sci., 74, 101–112, doi:10.1007/s00027-011-0200-0, 2012.

Nakazawa, M.: fmsb: Functions for Medical Statistics Book with some Demographic Data, R package version 0.5.2. http://CRAN.R-project.org/package=fmsb, 2015.

Neubauer, T. A., Harzhauser, M., and Mandic, O.: Phenotypic evolution in a venerid bivalve species lineage from the late Middle Miocene Central Paratethys Sea: A multi-approach morphometric analysis, Biol. J. Linn. Soc., 110, 320–334,
35   doi:10.1111/bij.12120, 2013.

Perry, E., Paytan, A., Pedersen, B., and Velazquez-Oliman, G.: Groundwater geochemistry of the Yucatan Peninsula, Mexico: Constraints on stratigraphy and hydrogeology, J. Hydrol., 367, 27–40, doi:10.1016/j.jhydrol.2008.12.026, 2009.

Perry, E., Velazquez-Oliman, G., and Marin, L.: The Hydrogeochemistry of the Karst Aquifer System of the Northern Yucatan Peninsula, Mexico, Int. Geol. Rev., 44, 191–221, doi:10.2747/0020-6814.44.3.191, 2002.

40   Pöckl, M.: Effects of temperature, age and body size on moulting and growth in the freshwater amphipods *Gammarus fossarum* and *G. roeseli*, Freshwater Biol., 27, 211–225, doi:10.1111/j.1365-2427.1992.tb00534.x, 1992.

Quinn, G. P. and Keough, M. J.: Experimental Design and Data Analysis for Biologists, Cambridge University Press, Cambridge, 537 pp., 2002.

R Core Team: R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, http://www.R-project.org, 2016.

Ramos, L., Cusminsky, G., Schwalb, A., and Alperin, M.: Morphotypes of the lacustrine ostracod *Limnocythere rionegroensis* Cusminsky & Whatley from Patagonia, Argentina, shaped by aquatic environments, Hydrobiologia, 786, 137–148, doi:10.1007/s10750-016-2870-z, 2017.

Roca, J.R., and Wansard, G.: Temperature influence on development and calcification of *Herpetocypris brevicaudata* Kaufmann, 1900 (Crustacea: Ostracoda) under experimental conditions, Hydrobiologia, 347, 91–95, 1997.

Rohlf, F. J.: tpsDig: digitize landmarks and outlines, Department of Ecology and Evolution, State University of New York at Stony Brook, http://life.bio.sunysb.edu/morph, 2013.

Rohlf, F. J.: tpsRelw: relative warps analysis, Department of Ecology and Evolution, State University of New York at Stony Brook, http://life.bio.sunysb.edu/morph, 2015.

Rohlf, F. J.: tpsRelw: relative waprs analysis, Department of Ecology and Evolution, State University of New York at Stony Brook, http://life.bio.sunysb.edu/morph, 2016.

Rohlf, F. J. and Slice, D.: Extensions of the Procrustes Method for the Optimal Superimposition of Landmarks, Syst. Zool., 39, 40, doi:10.2307/2992207, 1990.

Sacks, L. A., Herman, J. S., and Kauffman, S. J.: Controls on High Sulfate Concentrations in the Upper Floridan Aquifer in Southwest Florida, Water Resour. Res., 31, 2541–2551, doi:10.1029/95WR02105, 1995.

Sanchez-Gonzalez, J. R., Baltanas, A., and Danielopol, D. L.: Patterns of morphospace occupation in recent Cypridoidea (Crustacea, Ostracoda), Rev. Esp. Micropaleontol., 36, 13–27, 2004.

Santos, I. R., Niencheski, F., Burnett, W., Peterson, R., Chanton, J., Andrade, C. F., Milani, I. B., Schmidt, A., and Knoeller, K.: Tracing anthropogenically driven groundwater discharge into a coastal lagoon from southern Brazil, J. Hydrol., 353, 275–293, doi:10.1016/j.jhydrol.2008.02.010, 2008.

Schmidt, N., Lipp, E. K., Rose, J. B., and Luther, M. E.: ENSO Influences on Seasonal Rainfall and River Discharge in Florida, J. Clim., 14, 615–628, 2001.

van der Meeren, T., Verschuren, D., Ito, E., and Martens, K.: Morphometric techniques allow environmental reconstructions from low-diversity continental ostracode assemblages, J. Paleolimnol., 44, 903–911, doi:10.1007/s10933-010-9463-z, 2010.

van Harten, D.: Size and environmental salinity in the modern euryhaline ostracod *Cyprideis torosa* (Jones, 1850), a biometrical study, Palaeogeogr. Palaeocl., 17, 35–48, 1975.

van Harten, D.: Variable noding in *Cyprideis torosa* (Ostracoda, Crustacea): an overview, experimental results and a model from Catastrophe Theory, Hydrobiologia, 419, 131–139, 2000.

Vesper, B.: Ein Beitrag zur Ostracodenfauna Schleswig-Holsteins, Mitt. Hamb. Zool. Mus. Inst., 72, 97–108, 1975.

Viero, A. P., Roisenberg, C., Roisenberg, A., and Vigo, A.: The origin of fluoride in the granitic aquifer of Porto Alegre, Southern Brazil, Environ. Geol, 56, 439, doi:10.1007/s00254-008-1273-5, 2009.

Wagner, P. J. and Erwin, D. H.: Patterns of convergence in general shell form among Paleozoic gastropods, Paleobiology, 32, 316–337, doi:10.1666/04092.1, 2006.

Walsh, C. and Mac Nally, R.: hier.part: Hierarchical Partitioning: R package version 1.0-4, http://CRAN.R-project.org/package=hier.part, 2013.

Weihrauch, D., McNamara, J. C., Towle, D. W., and Onken, H.: Ion-motive ATPases and active, transbranchial NaCl uptake in the red freshwater crab, *Dilocarcinus pagei* (Decapoda, Trichodactylidae), J. Exp. Biol., 207, 4623–4631, doi:10.1242/jeb.01333, 2004.

Whitehead, P. G., Wilby, R. L., Battarbee, R. W., Kernan, M., and Wade, A. J.: A review of the potential impacts of climate change on surface water quality, Hydrolog. Sci. J., 54, 101–123, doi:10.1623/hysj.54.1.101, 2009.

WorldClim: WorldClim-Global Climate Data: http://worldclim.org/version2, last access: 25 March 2017.

15

Wrozyna, C., Meyer, J., Gross, M., Ramos, M. I. F., and Piller, W. E.: Cryptic morphospecies of the *C. ilosvayi* group in the Neotropics, PlosONE, under review.

Wrozyna, C., Meyer, J., Gross, M., Ramos, M. I. F., and Piller, W. E.: Definition of regional ostracod (*Cytheridella*) morphotypes by use of landmark-based morphometrics, Freshwater Sci., under review.

5   Wrozyna, C., Neubauer, T. A., Meyer, J., and Piller, W. E.: Shape Variation in Neotropical *Cytheridella* (Ostracoda) Using Semilandmarks-Based Geometric Morphometrics: A Methodological Approach and Possible Biogeographical Implications, PlosONE, 11, e0168438, doi:10.1371/journal.pone.0168438, 2016.

Wrozyna, C., Piller, W. E., and Gross, M.: Morphotypes of *Cytheridella ilosvayi* (Ostracoda) detected by soft and hard part analyses, Crustaceana, 87, 1043–1071, doi:10.1163/15685403-00003342, 2014.

10   Yin, Y., Geiger, W., and Martens, K.: Effects of genotype and environment on phenotypic variability in *Limnocythere inopinata* (Crustacea: Ostracoda), Hydrobiologia, 400, 85–114, 1999.

Yin, Y., Li, W., Yang, X., Wang, S., Li, S., and Xia, W.: Morphological responses of *Limnocythere inopinata* (Ostracoda) to hydrochemical environment factors, Sci. China Ser. D, 44, 316–323, 2001.

Zieritz, A. and Aldridge, D. C.: Identification of ecophenotypic trends within three European freshwater mussel species

15   (Bivalvia: Unionoida) using traditional and modern morphometric techniques, Biol.J. Linn. Soc., 98, 814–825, doi:10.1111/j.1095-8312.2009.01329.x, 2009.
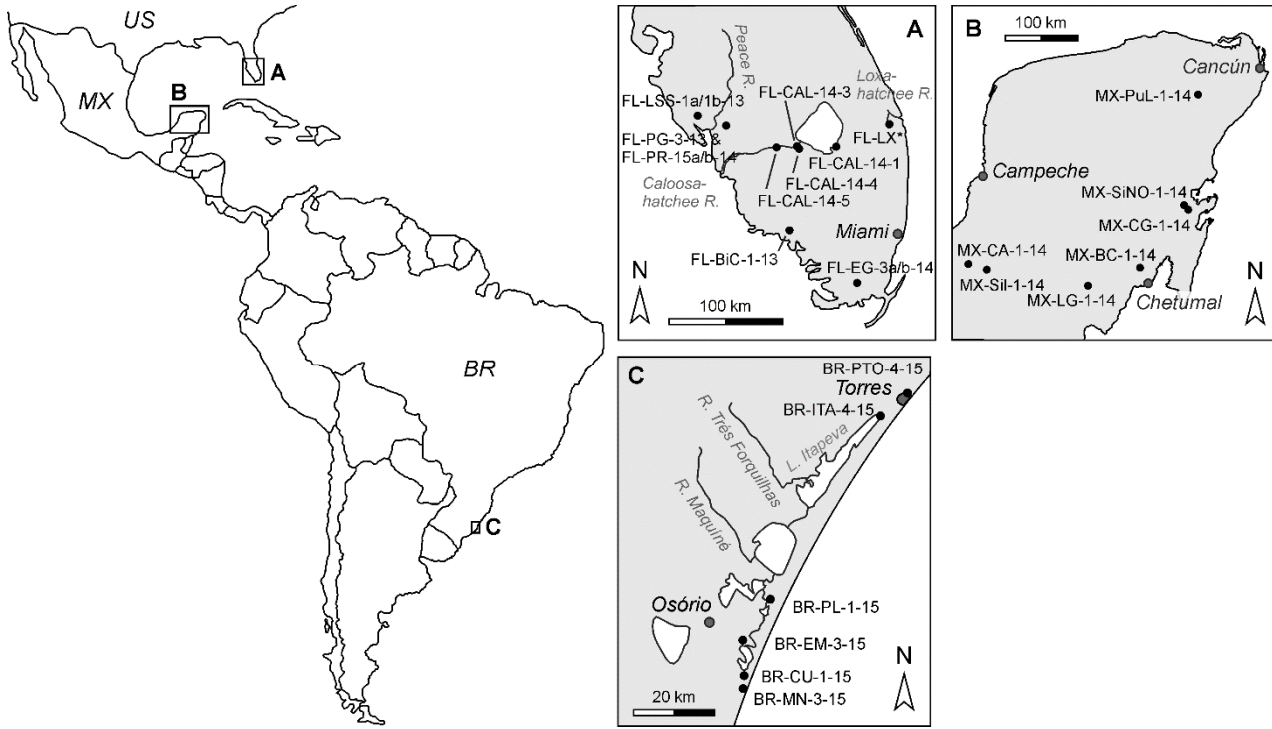
Figure 1: Geographic overview of the sampled populations (modified from Wrozyna et al., 2016). The label FL-LX* in map A comprises samples FL-LX-1-14 to FL-LX-6-14. For details, see Table S1.

Figure 2: Relative Warps Analyses of left and right valves of females of the first three warps and the associated thin-plate splines at minimum and maximum scores. Colors refer to the different regions: blue – Florida, green – Mexico, pink – Brazil.

5

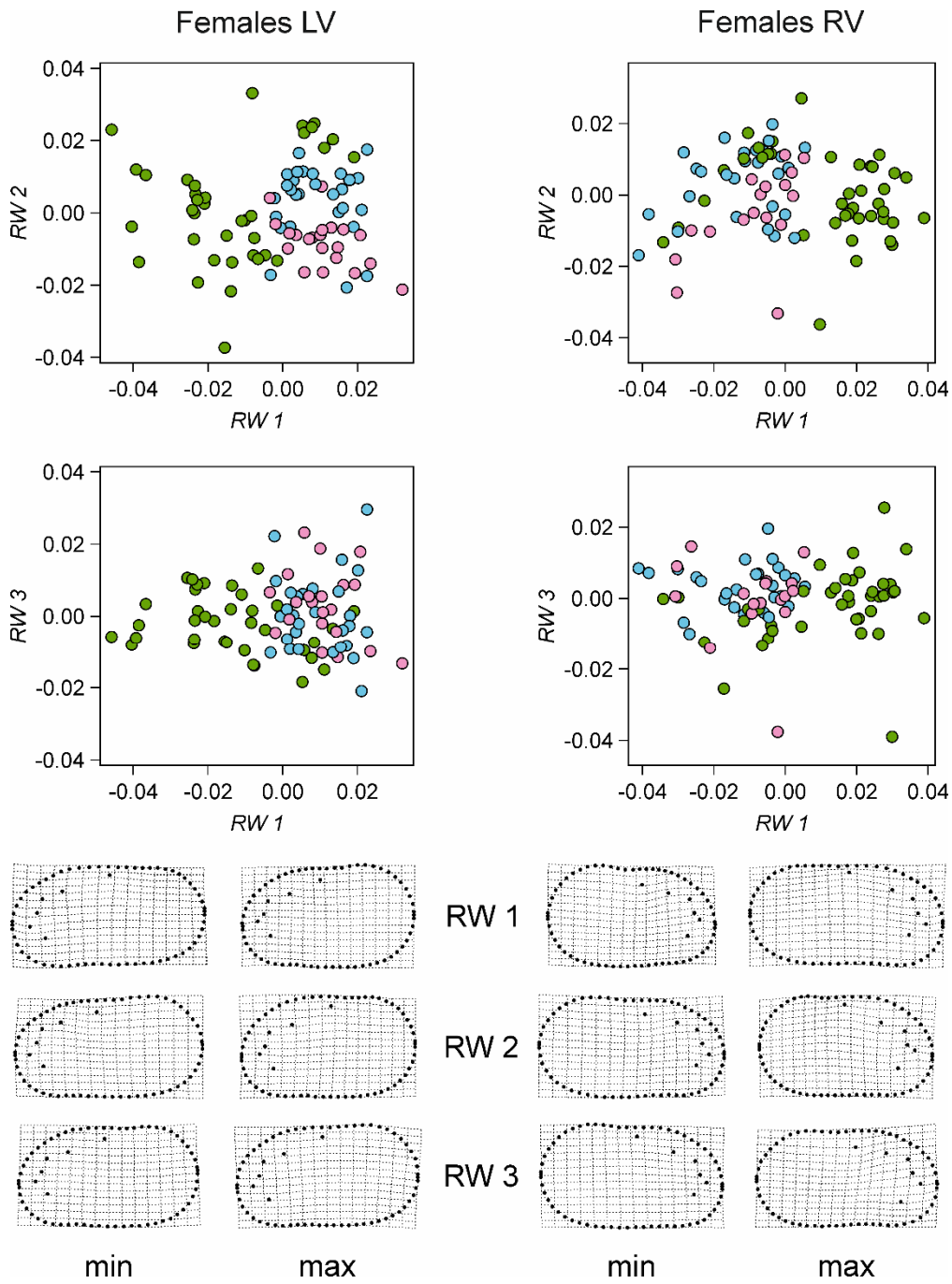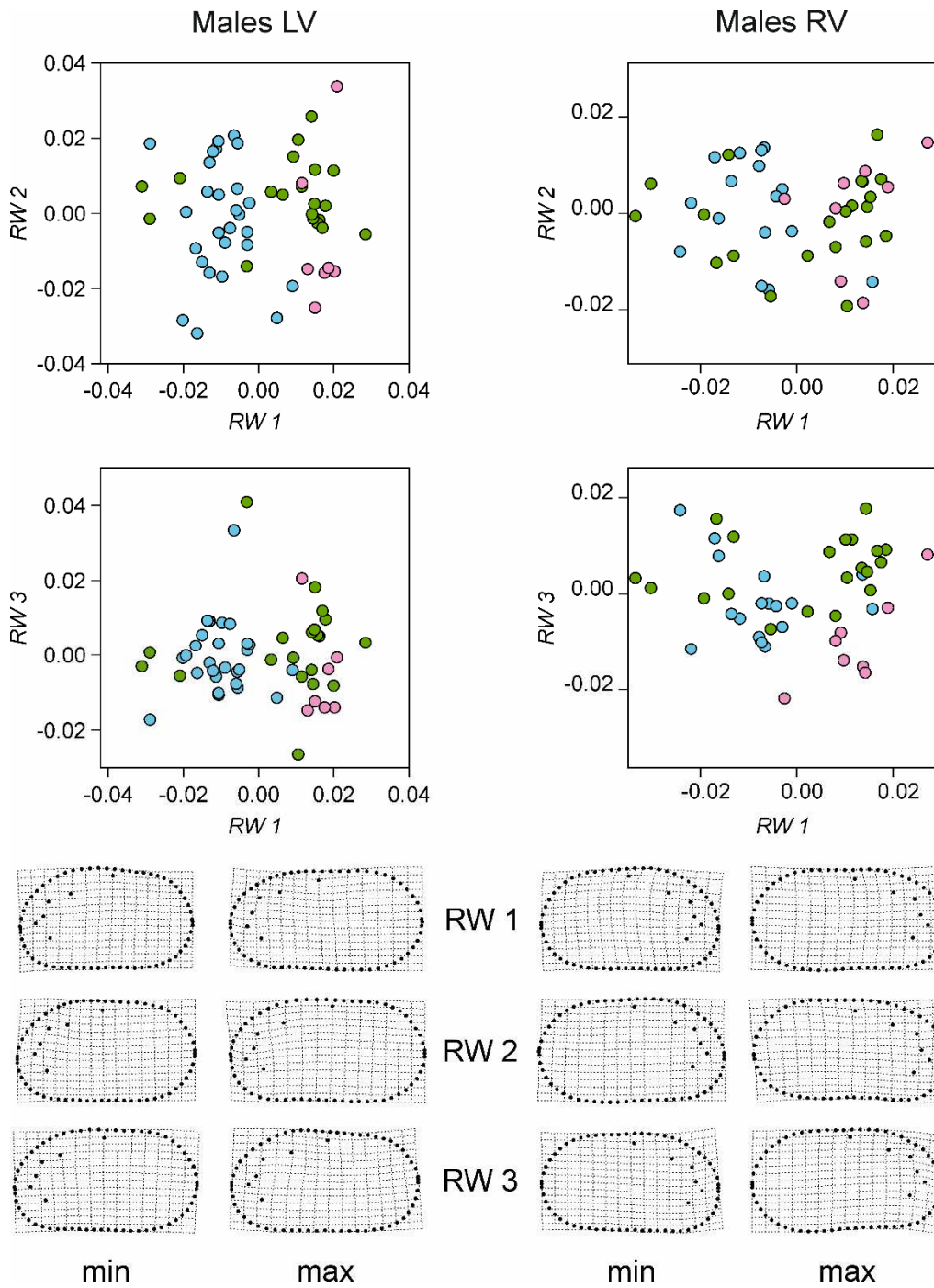**Figure 3: Relative Warps Analyses of left and right valves of males of the first three warps and the associated thin-plate splines at minimum and maximum scores. Colors refer to the different regions: blue – Florida, green – Mexico, pink – Brazil.**
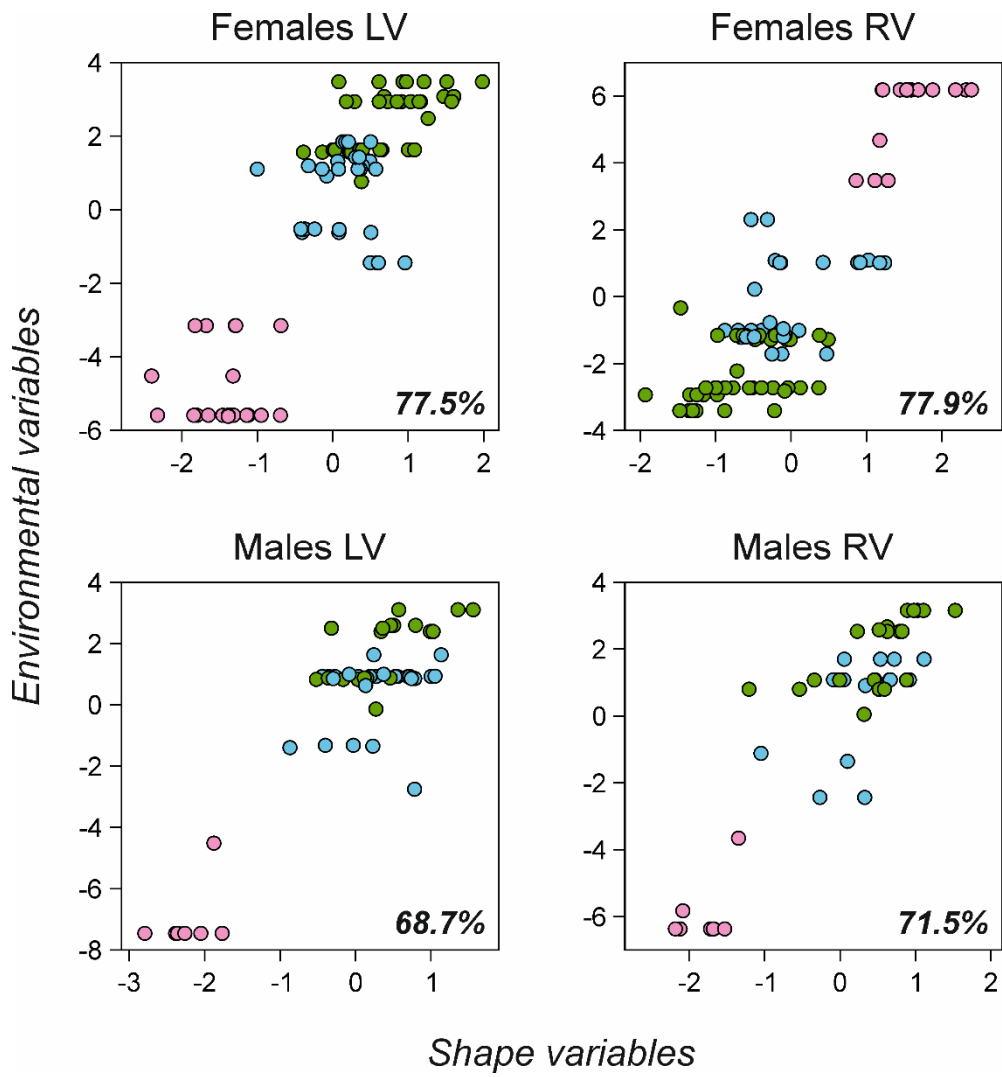
Figure 4: First axis of the PLS analysis of carapace shape and environmental variables. Colors refer to the different regions: blue – Florida, green – Mexico, pink – Brazil.

Table 1. Summary statistics of the RWA, PLS analyses and multiple regression analyses for RWs 1–3 for the four datasets. The reification column summarizes shape variation along the respective warp. For the multiple regressions, percentage values are indicated for predictor variables with relative contributions over 10%. BIO2 – Mean diurnal temperature range, BIO4 – Temperature seasonality, BIO12 – Annual precipitation. See Methods chapter for details.

Females LV	*RWA*		*PLS*	*Multiple Regressions*			
	% var. explained	**Reification**	**Loadings axis 1 (77.55%)**	**R^2_{adj}**	**P**	**Shapiro-Wilks test (P)**	**Predictor variables**
RW 1	33.22	elongate-elliptical vs. short asymmetrical with distinctly inflated posterior region; position of pore conuli	-0.5637	0.632	<0.001	0.0200	BIO4 (46.2%), SO_4 (19.3%), pH (13.0%), Cl^-, Temp., BIO12
RW 2	19.00	elongate-elliptical with slightly inflated posterior region vs. short; position of pore conuli	0.4798	0.510	<0.001	0.0063	BIO12 (43.9%), Cl^- (32.9%), K (11.7%), SO_4, BIO4
RW 3	10.72	minor deviations of outline; small differences in position of pore conuli	-0.1850	0.142	0.002	0.3338	SO_4 (33.3%), Temp. (27.5%), Cl (24.6%), BIO4 (14.6%)
Females RV	*RWA*		*PLS*	*Multiple Regressions*			
	% var. explained	**Reification**	**Loadings axis 1 (77.95%)**	**R^2_{adj}**	**P**	**Shapiro-Wilks test (P)**	**Predictor variables**
RW 1	40.39	elongate-elliptical vs. short asymmetrical with distinctly inflated posterior region; position of dorsal dip point of the posterior curvature; position of pore conuli	-0.3892	0.593	<0.001	0.0079	BIO4 (44.5%), Cl^- (20.4%), SO_4 (18.1%), Temp., K, BIO12
RW 2	14.89	elongate with slightly inflated posterior region vs. short; position of dorsal dip point of the posterior curvature; position of pore conuli	-0.2373	0.257	<0.001	0.0040	BIO12 (48.4%), SO_4 (23.5%), Cl^- (20.1%), BIO4
RW 3	10.50	elongate-elliptical vs. short asymmetrical with inflated posterior region; position of dorsal dip point of	0.1003	*n/a*			

		the posterior curvature; position of pore conuli					
Males LV	*RWA*		*PLS*	*Multiple Regressions*			
	% var. explained	**Reification**	**Loadings axis 1 (67.74%)**	**R^2_{adj}**	**P**	**Shapiro-Wilks test (P)**	**Predictor variables**
RW 1	24.14	elongate-elliptical vs. short asymmetrical with slightly inflated anterior part; position of pore conuli	-0.4063	0.638	<0.001	0.3948	BIO4 (37.3%), Cl⁻ (34.5%), BIO12 (15.0%), SO₄ (10.8%), pH
RW 2	22.25	elongate vs. short with higher dorsal margin; position of pore conuli	0.2359	*n/a*			
RW 3	13.92	minor deviations of outline; position of pore conuli	0.2792	0.042	0.074	0.0017	(not significant)
Males RV	*RWA*		*PLS*	*Multiple Regressions*			
	% var. explained	**Reification**	**Loadings axis 1 (71.55%)**	**R^2_{adj}**	**P**	**Shapiro-Wilks test (P)**	**Predictor variables**
RW 1	29.88	elongate-elliptical vs. short-asymmetrical with slightly inflated anterior part; position of pore conuli	-0.3798	0.318	0.001	0.2221	BIO12 (31.8%), BIO4 (28.7%), Cl⁻ (15.8%), pH (12.5%), K (11.2%)
RW 2	12.81	elongate vs. short with higher dorsal margin; position of dorsal dip point of the posterior curvature; position of pore conuli	0.0157	*n/a*			
RW 3	11.98	shape of anterior part; position of pore conuli	0.5502	0.286	<0.001	0.2978	BIO4 (57.2%), BIO2 (42.8%)