Definition of regional ostracod (*Cytheridella*) morphotypes by use of landmark-based morphometrics

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Abstract: Geometric morphometric analyses were performed on the Neotropical ostracod *Cytheridella* including recent populations from Florida, Yucatán, Colombia, and Brazil. Generalized least-squares Procrustes analyses were applied to left and right valves of adult females and males and to A-1 to A-3 juveniles. The analyses show that the prevailing shape variability is in the outline of the valves. Further characters defining the variability range are the anterior pore conuli 2 and 4 (LM 2, LM 4) and the connection point between transversal sulcus and the extension of the posterior lobe (LM 6). Relative Warps Analysis enabled delineation of the whole-shape disparity and revealed a geographical pattern in the morphological variability that is apparent in adults and juveniles. Females show greatest morphological disparity, whereas juveniles are more variable on the population scale. Differences in hard- and soft-part morphology identify the specimens from Yucatán as new a morphospecies. Different hydrological or hydrochemical conditions may have led to the evolution of the new species. *Cytheridella* has occurred in the Neotropics since the Oligocene with a similar distribution to that in the Recent. Avian dispersal may repeatedly reintroduce *C. ilosvayi* to Yucatánian populations possibly explaining the sympatric occurrence of 2 *Cytheridella* morphospecies. Our findings promote the benefit of investigating morphological variability quantitatively to detect regional morphotypes and ultimately to contribute to evaluations of biodiversity.

Key words: Neotropics, geometric morphometrics, ostracods, morphological variability

The Neotropics are considered to be the most diverse tropical area on Earth (Jaramillo et al. 2006, Pérez et al. 2010, Basset et al. 2012, Carrillo et al. 2014, Toussaint et al. 2016). The ranges of most Neotropical species probably arose from historic climatic oscillations and geological events (Colinvaux and Oliveira 2001, Assine and Soares 2004, Hodell et al. 2008, Hoorn et al. 2010, Escobar et al. 2012, O’Dea et al. 2016), in contrast to the assumption that many species occur ubiquitously (e.g., Pérez et al. 2010, 2011, Bergmann and Russell 2007, Fouquet et al. 2007). Freshwater invertebrates, such as ostracods, live in discrete habitat patches and depend on passive dispersal. Overland dispersal is presumed to be common in most freshwater taxa, but generalizations are not accurate and proper ecological assessments require specific information for each taxon (Bohonak and Jenkins 2003).

The common Neotropical freshwater ostracod *Cytheridella* comprises 3 extant species: *C. ilosvayi*, *C. argentinensis*, and *C. boldii*. The latter 2 species are described only from their type localities in Venezuela (*C. boldii*) and Argentina (*C. argentinensis*) (Purper 1974, Colin and Danielopol 1980). *Cytheridella ilosvayi* is assumed to ubiquitous and has been reported from the entire Neotropical range (Pérez et al. 2010). Knowledge of morphological variability within and between populations of ostracods is still very vague. Investigations of morphological variation on a (large) continuous geographical and ecological range are the prerequisite for identification of environmentally induced vari-

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Morphological variability is most obvious at the antennule A1. The penultimate segment is substantially shorter for males and females from Mexico than from Florida.
and Brazil (Fig. 3A–F). In addition, the hemipenes are very variable with differences in the shape of the proximal lobe. The shape is flatter for Mexican than for Floridian or Brazilian specimens, which have cuspidal shapes (Fig. 4A–F).

**RWA of carapaces**

**Carapace shape variability of females** The major shape variation at RW1 differentiated between slender elongated outlines (positive scores) and shortened outlines with prominent rounded posterior lobes (negative scores) (Fig. 5A, B, Tables S1, S2). Shape variation also affected the position of the connecting point between the extension of the posterior lobe and the transversal sulcus (LM 6), respectively (Fig. 5 thin plate splines). In the elongated forms, this point was close to the dorsal margin and slightly shifted posteriorly. The shortened females exhibited this point closer to the center of the valve and closer to the anterior pore conuli. At RW2, shape variation also differed between elongated outline shapes with well-defined posterior lobes (positive scores) and
| Sample identifier | Locality | Date          | Country   | N (S) | E (W) | Habitat                                                                 |
|-------------------|----------|---------------|-----------|-------|-------|-------------------------------------------------------------------------|
| BB 01 09          | Barro Branco | 27.09.2009   | Brazil    | 06'50' 18.31' | 69'45' 37.03' | Root zone in an abandoned channel                                      |
| LG 01 09          | Lago Comprido | 27.09.2009   | Brazil    | 06'43' 52.77' | 69'44' 33.99' | Littoral, abundant leaf litter, abandoned channel                     |
| MX Sil 1 14       | Lake Silvius | 12.08.2014   | Mexico    | 18'38' 29.89' | 90'16' 28.11' | Littoral of a lake                                                      |
| BR CU 1 15        | Costaódia Lagoon | 04.09.2015  | Brazil    | 30'02' 15.22' | 050'10' 20.22' | Lagoon                                                                |
| BR MN 3 15        | Rio do Relógio | 04.09.2015   | Brazil    | 30'04' 10.37' | 050'12' 20.87' | Inflow to lake Mañuel Nuñez Lagoon                                  |
| BR PL 1 15        | Passos da Lagoo | 03.09.2015   | Brazil    | 29'51' 16.11' | 050'06' 57.99' | Littoral of a lake, dense macrophytes                                 |
| BR ITA 4 15       | Lagoa Itapeva  | 06.09.2015   | Brazil    | 29'22' 32.67' | 049'47' 39.22' | Lagoon, next to inflow                                               |
| BR EM 3 15        | Embobua Lagoon | 03.09.2015   | Brazil    | 29'57' 52.87' | 050'13' 27.44' | Lagoon, temporary connection to the ocean                             |
| BR PTO 4 15       | L. Passos de Torres | 07.09.2015 | Brazil    | 29'18' 39.67' | 049'42' 32.87' | Lagoon, floating plants                                              |
| CO ET 1a 15       | Estero Texas  | 04.02.2015   | Colombia  | 04'24' 31.27' | 71'58' 44.54' | Phytal of 1b                                                          |
| CO ET 1b 15       | Estero Texas  | 04.02.2015   | Colombia  | 04'24' 31.27' | 71'58' 44.54' | Spillway channel, permanent flooded                                    |
| FL PG 3 13        | Shell Creek, Peace R. | 28.11.2013  | Florida, USA | 26'58' 26.99' | 81'53' 21.89' | Littoral of an artificial slack water, dense terrestrial macrophytes |
| FL LS 1a/1b 13    | Little Salt Spring | 26.11.2013  | Florida, USA | 27'4' 29.37' | 82'14' 53.77' | Spring with outflow                                                    |
| FL Bc 1 13        | Big Cyprus National Reserve | 29.11.2013 | Florida, USA | 25'53' 29.53' | 81'16' 14.52' | Swamp                                                                  |
| FL LX 1 14        | Locohatchee R. | 31.07.2014   | Florida, USA | 26' 56' 03.07' | 80'10' 36.47' | Root zone littoral of a river                                         |
| FL LX 2 14        | Locohatchee R. | 31.07.2014   | Florida, USA | 26' 56' 32.57' | 80'10' 19.27' | Littoral of a river                                                   |
| FL LX 3 14        | Locohatchee R. | 31.07.2014   | Florida, USA | 26' 56' 40.28' | 80'10' 15.94' | Littoral of a river                                                   |
| FL LX-4 15        | Locohatchee R. | 31.07.2014   | Florida, USA | 26' 56' 46.91' | 80'10' 15.42' | Root zone littoral of a river                                         |
| FL LX 5 15        | Locohatchee R. | 31.07.2014   | Florida, USA | 26' 56' 49.87' | 80'10' 12.47' | Littoral of a river                                                   |
| FL LX 6 15        | Locohatchee R. | 31.07.2014   | Florida, USA | 26' 56' 52.57' | 80'10' 11.14' | Root zone littoral of a river                                         |
| FL CAL 4 3        | Caloosahatchee R. | 06.08.2014  | Florida, USA | 26'50' 22.41' | 81'04' 51.18' | Artificial littoral, large stones and sand lenses                     |
| FL CAL 4 4        | Caloosahatchee R. | 06.08.2014  | Florida, USA | 26'50' 09.98' | 81'05' 14.44' | Littoral, parts cased                                                  |
| FL CAL 4 5        | Caloosahatchee R. | 06.08.2014  | Florida, USA | 26'47' 21.77' | 81'18' 33.64' | Stillwater area, floating macrophytes                                 |
| FL PR 15a/b 14    | Shell Creek, Peace R. | 08.08.2014  | Florida, USA | 26'58' 26.99' | 81'53' 21.81' | Littoral of an artificial slack water, dense terrestrial macrophytes |
| FL EG 3a/b 14     | Everglades   | 02.08.2014   | Florida, USA | 25'26' 2.07'  | 80'45' 12.33' | Marsh                                                                  |
| MX CA 1 14        | Cenote Azul  | 12.08.2014   | Mexico    | 18'48' 43.33' | 90'38' 48.14' | Hardground littoral of a cenote                                       |
| MX SNo 1a 14      | Siüi No Há Cenote | 11.08.2014   | Mexico    | 19'28' 33.55' | 88'03' 15.65' | Littoral, cenote                                                      |
| MX CG 1 14        | Cenote Galáena | 11.08.2014   | Mexico    | 19'27' 45.67' | 88'19' 46.37' | Littoral, cenote                                                      |
| MX BC 1 14        | Laguna Bacalar | 11.08.2014   | Mexico    | 18'39' 5.87'  | 88'24' 33.07' | Littoral of a lake                                                    |
| MX P Zhang 14     | Punta Laguna | 13.08.2014   | Mexico    | 20'38' 49.47' | 87'38' 04.13' | Littoral, karstified blocks near thick layer of leaf litter and reed belt |
| MX LG 114         | Lake Las Garantias | 13.08.2014  | Mexico    | 18'27' 11.77' | 89'00' 42.00' | Steep littoral, hard ground                                          |

Table 1. Details on sampling localities with sample identifier, name of the locality, sampling date, country, coordinates, a short habitat description, and physicochemical field data. EC = electrical conductivity, Temp = temperature, L. = Laguna, R. = river, nd = no data.
rather ovate compressed outlines and an upward shifted pore conulus (LM 1) (Fig. 5A, B and thin plate splines). Positive scores at RW3 were related to elongated outline shapes with less developed posterior lobes and subrectangular outlines with a slight depression mid-dorsally (LV) and a relatively well-defined posterior lobe (RV) (negative scores) (Fig. 5C, D).

The Mexican populations, characterized by positive scores, were clearly separated from the Floridian and South American populations along RW1 and plotted at higher scores. One Mexican population from Punta Laguna (MX Pul114) was divided. Some females plotted adjacent to the Floridian/Brazilian cluster and the others in the Mexican cluster. Brazilian populations were differentiated from other South American and Floridian populations along RW2, but the difference was better expressed at RV (Fig. 5B).

**Carapace shape variability of males** The major shape variation at RW1 was strongly related to the position of LM 6 (Fig. 6 thin plate splines). At LV it was shifted slightly ventrally at negative scores (6A, C, Tables S3, S4). At positive scores, it was shifted dorsal-posteriorly and almost reached the outline (Fig. 6B, D). The most positive scores at RV described elongated outlines with a rounded posterior, which is wider than the anterior (positive scores). The differences at RW2 were related to outline shapes and pore conuli, which led to elongated outlines (positive scores at LV, negative scores at RV) and more compressed outlines (negative scores at LV, positive scores at RV). At LV, pore conuli 2 and 4 changed between narrower (positive scores) and more distant positions (negative scores) whereas at RV, only pore conulus 2 displayed a slight shift up-down (positive scores) (negative scores). Shape variation at RW3 was related predominantly to the anterior lobe and discriminated between steeper (positive scores) and flatter slopes (negative scores) (Fig. 6B, D). Aside from a distinct cluster of Floridian males with positive scores at RW1 of LV and a weaker separation of the Mexican populations at RV at RW2, the distribution of the populations did not show a clear pattern (Fig. 6A–D).

**Carapace shape variability of A-1 instars** Shape variability of A-1 instars was primarily related to the position of the dip point of the posterior lobe, which differed between a more centrodorsal position (negative scores) and a shift posterodorso (positive scores) (Fig. 7A–D, Tables S5, S6). Variations along RW2 affected the outline and separated elongated shapes (positive scores, LV; negative scores, RV) and more rounded outlines with a slightly broader anterior (negative scores, LV; positive scores, RV) (Fig. 7 thin plate splines). The position of the pore conuli 2 and 4 switched between more distal (negative scores, LV; positive scores, RV) and more central positions (opposite to before mentioned scores). RW3 described changes between the position of the points of maximum curvature at the anterior and posterior ends. A shift downward was related to elongated and slightly ventrally curved outlines (negative scores, LV; positive scores, RV) and a shift upward was connected to more shortened and anteriorly and posteriorly well-rounded outlines (positive scores, LV; negative scores, RV) (Fig. 7C, D).

Northern Brazilian populations (LC 1 09/BB 01 09) had their own cluster at higher positive scores at RV, but no other separations occurred among populations at RW1 (Fig. 7A, B). Mexican populations were relatively separated at positive scores at RW2 (LV) and RW3 (RV) (Fig. 7A, D). The Everglades (Florida) population was relatively close at negative scores of RW1 (RV), but the other Floridian populations did not show a clear pattern in their distribution and were widely scattered on RW1 and RW2 (Fig. 7A, B).

**Carapace shape variability of A-2 instars** Maximal shape variations at RW1 of A-2 instars were primarily related to LM 6 (Fig. 8A–D, Tables S7, S8). LM 2 and 4 displayed relatively large variation and shifted between inward (nega-
Table 2. Overview of valve material analyzed from the different sampling localities. RV = right valve, LV = left valve, A-1, A-2, A-3 = juvenile instars, Σ = total individuals sampled.

| Sample   | Females | Males | A-1 | A-2 | A-3 | Σ  |
|----------|---------|-------|-----|-----|-----|----|
|          | LV      | RV    | LV  | RV  | LV  | RV | LV  | RV  | LV  | RV  | LV  | RV  | LV  | RV  | LV  | RV  | LV  | RV  | LV  | RV  | Σ    |
| LC 1 09  | 10      | 9     | 2   | 2   | 5   | 5  | 1   | 1   | 35  |     |     |     |     |     |     |     |     |     |     |     |     |
| BB 01 09 | 7       | 6     | 1   | 0   | 0   | 1  | 1   |     | 16  |     |     |     |     |     |     |     |     |     |     |     |
| FL BiC 1 13 | 0     | 1     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| FL PG 3 13 | 2     | 3     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| FL LSS 1 13 | 0     | 1     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| FLPR 15a/b 14 | 1       | 3     | 1   | 1   | 1   | 1  |     |     | 11  |     |     |     |     |     |     |     |     |     |     |     |
| FL LX 6 14 | 0       | 1     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| FL LX 5 14 | 1       | 1     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| FL LX 4 14 | 3       | 1     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| FL LX 3 14 | 2       | 3     | 2   | 1   | 15  | 14 | 15  | 17  | 6   | 2   | 77  |     |     |     |     |     |     |     |     |
| FL LX 2 14 | 1       | 10    | 13  | 11  | 13  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| FL LX 1 14 | 3       | 4     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| FL EG 3a/b 14 | 4       | 2     | 1   | 2   | 6   | 5  | 1   | 1   | 22  |     |     |     |     |     |     |     |     |     |     |
| FL CAL 1 14 | 2       | 1     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| FL CAL 4 14 | 4       | 3     | 2   | 4   | 2   | 1  |     |     | 16  |     |     |     |     |     |     |     |     |     |     |
| FL CAL 1 14 | 6       | 6     | 13  | 9   | 18  | 13 | 10  | 9   | 84  |     |     |     |     |     |     |     |     |     |
| FL CAL 1 14 | 2       | 2     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| MX BC 1 14 | 8       | 6     | 3   | 5   | 5   | 2  | 1   |     | 30  |     |     |     |     |     |     |     |     |     |
| MX CA 1 14 | 9       | 8     | 5   | 4   | 13  | 9  | 2   | 8   | 7   | 7   | 72  |     |     |     |     |     |     |
| MX CG 1 14 | 0       | 1     | 2   | 1   | 1   | 1  |     |     | 6   |     |     |     |     |     |     |     |     |
| MX CT 1 14 | 1       | 1     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| MX LG 1 14 | 4       | 5     | 3   | 4   | 2   |     |     |     | 18  |     |     |     |     |     |     |     |
| MX Pul 1 14 | 10      | 13    | 3   | 4   | 6   | 3  | 2   | 1   | 42  |     |     |     |     |     |     |     |
| MX SiNo 1 14 | 3       | 5     | 3   | 3   | 5   | 2  | 1   |     | 22  |     |     |     |     |     |     |     |
| MX Sil 1 14 | 1       | 1     | 1   | 1   |     |     |     |     | 4   |     |     |     |     |     |     |     |
| BR CU 1 15 | 2       | 0     |     |     |     |     |     |     | 2   |     |     |     |     |     |     |
| BR EM 3 15 | 1       | 1     |     |     |     |     |     |     | 2   |     |     |     |     |     |     |
| BR ITA 4 15 | 14      | 13    | 6   | 7   |     |     |     |     | 40  |     |     |     |     |     |     |
| BR MN 3 15 | 1       |     |     |     |     |     |     |     | 1   |     |     |     |     |     |     |
| BR PL 1 15 | 1       |     |     |     |     |     |     |     | 1   |     |     |     |     |     |
| BR PTO 4 15 | 5       | 2     | 1   | 1   | 2   | 2  |     |     | 13  |     |     |     |     |     |     |
| CO ET 1b 15 | 3       | 2     |     |     |     |     |     |     | 7   |     |     |     |     |     |     |
| Σ         | 107     | 104   | 60  | 52  | 94  | 77 | 41  | 54  | 14  | 11  | 614 |     |     |     |     |     |     |

tive scores) and outward (positive scores) directed positions (Fig. 8 thin plate splines). Shape variations at RW2 described a change from a more pointed anterior part and a ventrally shifted LM 2 (negative scores, LV) to a more pointed posterior part with a dorsally shifted LM 2 (positive scores) (Fig. 8A, B). At RV, the variation was primarily related to the outline and distinguished between more elongated with ventrally shifted LM 2 (positive scores) and more shortened with dorsally shifted LM 2 (negative scores) outlines (Fig. 8B). RW3 also affected the outline, which differed between those with elongated straight outlines (negative scores) and outlines with a smaller posterior becoming wider to the anterior (positive scores) (Fig. 8C, D). The distribution of the populations showed no specific pattern. Some populations (e.g., FL-LX-3-14) were widely scattered on each warp, and others (e.g., FL-CAL-14-3) were more concentrated (Fig. 8A–D).

**Carapace shape variability of A-3 instars** Negative scores at RW1 were associated with a kidney-like outline with a rounded dorsal part (Fig. 9A–D, Tables S9, S10). The maximum posterior was slightly shifted downward, and the ventral outline was slightly concave (Fig. 9 thin plate splines).
LM 6 was situated near the mid-length of the valve. At positive scores, the outline was more oval with a straight ventral outline and a less rounded dorsal outline. LM 6 was behind the mid-length of the valve. RW2 predominantly differentiated between more a compressed triangular outline with a smaller posterior (positive scores) and more elongated and more ovate outlines (negative scores) (Fig. 9A, B). RW3 was connected to shape variations similar to those at RW2 but also involved the position of the 2nd pore tubercle (LM 2) (Fig. 9C, D). The triangular forms (positive scores, LV; negative scores, RV) showed an LM 2 shifted ventrally and vice versa in the more elongated forms. Mexican and Floridian populations tended to be separated at RW1, but the number of specimens was low (Fig. 9A–D).

DFA of females

Discrimination of the relative warps of the females showed significant separation of the specimens belonging to the different populations (Wilk’s Lambda = 0.0 [LV, RV], $\chi^2 = 778.580$ [LV], $636.931$ [RV], $p = 0.00$ [LV, RV]). Classification results showed that 94.1% (LV) and 92.5% (RV), respectively, of the specimens were correctly classified. The first 3 canonical functions accounted for 46.9, 14.7, and 12.1% of the explained variation in LV and 54.1, 14.4, and 7.3% of the explained variability in RV (Tables S11, S12). The most obvious separation was between the Mexican and the other populations along function 1 (Fig. 10A–D). For LV, 2 conspicuous clusters of northern Brazil (negative scores) and southern Brazil (positive scores) populations occurred along function 2 (Fig. 10A). The Floridian populations overlapped with the 2 Brazilian clusters. Populations from Caloosahatchee River and, to some extent, from the Everglades plotted adjacent to the southern Brazilian cluster, and the population from Loxahatchee was situated near the northern Brazilian cluster. This pattern was not conspicuous for RV (Fig. 10C).

Function 3 separated the populations into large regions: South America and Florida (Fig. 10B, D). This pattern was distinct only for LV (Fig. 10B). A pattern also could be seen in the Mexican cluster. Populations from Laguna Bacalar (MX BC 1 14) and Sijill No Há (MX SiNo 1 14) had the most positive scores on function 1 (LV) followed by Cenote Azul (MX CA 1 14) with scores between 2 and 4. The pop-
ulation from Punta Laguna was divided with some specimens coinciding with the southern Brazilian cluster.

DISCUSSION

Shape variation of *Cytheridella*

Some ostracod species show minimal morphological variability even at large geographic ranges (Rossetti and Martens 1996), whereas other species display high intraspecific variability, (e.g., Reyment 1993, Yin et al. 1999, do Carmo et al. 1999, Minati et al. 2008, Frenzel et al. 2012, Wrozyna et al. 2014). Studies of morphological variability are generally rare and follow different approaches. Investigators deal either with soft parts (e.g., Maddocks 2000) or qualitative (Martens et al. 1998) or quantitative characters (Aiello et al. 2007, Hunt 2007), and they usually include adults only (Iepure et al. 2007).

Our results show that adult *Cytheridella* show a greater morphological disparity than juveniles as reflected by the splines of maximum scores of the RWs. Juveniles are relatively variable on the population scale. Primary shape variation is related to outline, as has been observed in several other species (Baltanás and Geiger 1998, Tanaka 2009).

Females display the most striking variability in (outline) shape, which ranges from elongated narrow forms in Mexico to shortened forms with well-developed brood pouches (posterior lobes) in Florida. Subordinate shape changes differentiate between shorter and elongated outlines both with curved posterior lobes and separate northern and southern Brazilian populations (Fig. 5A–D). Large overlapping areas at RW2 and RW3 are apparent in populations outside Mexico. This result indicates that the shapes of the populations are relatively variable and the similarity between populations is higher. The shape disparity (or morphological diversity) of males is not as high as in females and is primarily related to the position of the transversal sulcus (LM 6) (Fig. 6A–D). They reflect, however, the same pattern of regional and local shape variation as females.
Figure 5. Relative Warps Analysis (RWA) of female left (LVs) (A, C) and right valves (RVs) (B, D) with RWs 1 and 2 and 1 and 3 and the associated thin plate splines at maximum and minimum scores.
The primary shape variation in A-1 is similar to the variation shown by the males and is related to the position of LM 6 resulting in anteriorly or posteriorly shifted transversal sulci. In A-1, the differing elongated and shortened outlines are connected to RW2 and are of secondary importance. The high variability of Floridian A-1 also is represented in A-2. The question is whether this is an expression of genotypic features or is under environmental control. The fact that the variability occurs within populations points to a relatively high genotypic variability. Most

Figure 6. Relative Warps Analysis (RWA) of male left (LVs) (A, C) and right valves (RVs) (B, D) with RWs 1 and 2 and 1 and 3 and the associated thin plate splines at maximum and minimum scores.
of the Mexican and Brazilian populations were sampled from lakes or ponds, whereas the Floridian populations frequently were sampled in rivers (Table 1) where the probability of genetic exchange might be higher. Ecophenotypic variability (Wrozyna et al. 2017, in review) might be masked to some extent by the high within-population variability. To test this possibility, further studies including environmental data are necessary. The variability also might be explained to some extent by slightly different morphospaces occupied by immature females and males. Many ostracod species develop sexual organs throughout ontogeny from the last instar A-1 to adults. Therefore, juvenile appendages must be investigated to check if they exhibit differences in carapace shape variability that might be related to phase of the molt cycle.

Morphological variability also is reflected by the position of pore conuli, especially LM 2 and, less frequently,
LM 4. The pore setae extruding from the conuli are thought to possess a sensory function (Karanovic 2012). An adaptation to different environmental conditions (e.g., change in vegetation cover) could result in decrease or increase in number of sensory setae. Thus, the small shifts of the position of the pore conuli might not influence the sensory ability. Since the small observed variations occur within populations and no pattern is recognizable, these variations may not be ecologically induced but rather the expression of genetic variability.

Species differentiation

The Mexican populations form a clearly separated cluster represented in both sexes and all instars down to A-3 (Figs 5A–D to 9A–D). This clear differentiation points to separation on the species level. Hints for a new morpho-
species also are provided by soft parts: different proportions of the 4th podomere at A1 and the difference in the hemipenis morphology (Figs 3A–F, 4A–F). Differences in the hemipenes might indicate reproductive isolation (Martens 2003). The bipartite clusters of the population from Punta Laguna, Mexico (Figs 3A–F, 4A–F) indicate the existence of 2 discernible morphotypes in 1 sample. One morphotype is more similar to that from Florida and Brazil and is assigned to C. ilosvayi; the other one represents a new (hitherto undescribed) species.

Ostracods are known for a high degree of intralacustrine speciation (Martens 1994). For example, Gomphocythere, a member of the subfamily Timiriaseviinae to which Cythereidella belongs, is known for high speciation rates in Afri-

Figure 9. Relative Warps Analysis (RWA) of juvenile A-3 left (LVs) (A, C) and right valves (RVs) (B, D) with RWs 1 and 2 and 1 and 3 and the associated thin plate splines at maximum and minimum scores.
can lakes Tanganyika (Park and Martens 2001) and Malawi (Martens 2003) and shows a complex distributional pattern. Martens (2003) argued that, at least for the Malawian Gomphocythere species, evolution and speciation are controlled by both environmental and sexual selection. Sexual selection is indicated by significant differences of the copulatory complex of the hemipenes and parapatric speciation by the exclusive occurrence of species at specific water depths. In the case of Cytheridella, environmental conditions (e.g., conductivity or hydrological conditions) differ between Florida and Yucatán and may have driven speciation ecologically. However, sympatric speciation is also known for ostracods and may explain the co-occurrence of 2 Cytheridella morphospecies in Punta Laguna, which may occupy different microhabitats.

**Biogeography**

Shape is the result of a complex interaction of genotype and environment (Baltanás and Geiger 1998), but the application of shape analysis enabled us to identify strikingly evident biogeographical patterns on various scales for adult and juvenile Cytheridella. Our data show a rough regional differentiation of morphotypes between Mexico, Florida, and South America along RW1 and RW2, respectively (Fig. 5A, B). The DFA revealed separation patterns on regional and populational scales (Fig. 10A–D). A unique feature of the Timiriaseviinae is that they possess brood care, which is discussed in terms of dispersal advantage (Horne et al. 1998). Therefore, the presence of a geographical pattern in the morphological variability of Cytheridella might be the result of complex interactions of dispersal, reproductive strategies, and environmental conditions.

The first doubtful records of the genus Cytheridella are reported from the Upper Jurassic of North America (Swain 1999). Well-documented Cytheridella occurred during the Oligocene at the northern and southern margins of the present geographical range (Fig. 1). North and South America were not connected by a land bridge before 2.8 Ma (O’Dea et al. 2016), so the Oligocene distribution supports the hypothesis of avian dispersal of Cytheridella as already discussed for extant Cytheridella by Wrozyna et al. (2016). Neogene records are restricted to the Miocene of northern Brazil (Gross et al. 2013), late Miocene/Pliocene of Argentina (Marengo 2015), and the Pliocene of the Dominican Republic and Venezuela (van den Bold 1975, 1986). Pleistocene records cover more or less the distributional range of recent Cytheridella (Fig. 1). All recent and Pleistocene Cytheridella occurrences relate to C. ilosvayi except those from Venezuela, which belongs to C. boldii and one locality in Argentina, which refers to C. argentinensis (Fig. 1, Table 3).

According to our morphological data, the Yucatán specimens do not belong to C. ilosvayi (except for Punta Laguna) but represent a new undescribed species. Wrozyna et al. (2016) discussed whether the distinct morphological separation of the Yucatánian Cytheridella might be the result of longer isolation, but the high similarity of the 2 Cytheridella species may point to a relatively recent separation. The presence of 2 morphotypes, of which one is considered to belong to C. ilosvayi, contradicts continuous isolation. In our data set, comprising 9 localities in Yucatán, Lake Punta Laguna is the only locality that yielded both species. Different ecological conditions (i.e., higher salinities or hydrological composition) may have led to the evolution of a new Cytheridella species in Yucatán. Dispersal by birds may occasionally introduce C. ilosvayi into the same habitats, but ecological barriers could prevent C. ilosvayi from establishing where the new species lives. This scenario is what de Meester et al. (2002) described in their ‘monopolization hypothesis.’ Rapid population growth and local adaptation upon colonization of a new habitat result in the effective monopolization of resources, thereby yielding a strong priority effect. Once a population is locally adapted, the presence of a large resting propagule bank provides a powerful buffer against newly invading genotypes and enhances priority effects.
| Number | Locality                  | Country | Stratigraphic age | Species          | Reference                          |
|--------|---------------------------|---------|-------------------|------------------|------------------------------------|
| 1      | Pruett Formation, Texas   | USA     | Oligocene         | C. alpinensis    | Swain 1999                         |
| 2      | Tampa Bay                 | USA     | Pleistocene       | C. ilosvayi      | Willard et al. 2007                |
| 3      | Little Salt Spring        | USA     | Holocene          | C. ilosvayi      | Alvarez-Zarikian et al. 2005       |
| 4      | MX Pul 1 14               | Mexico  | Recent            | C. sp. nov/      |                                    |
|        |                            |         |                   | C. ilosvayi      |                                    |
| 5      | Lake Punta Laguna         | Mexico  | Holocene          | C. ilosvayi      | Curtis et al. 1996                 |
| 6      | Cenote Aktun Ha           | Mexico  | Holocene          | C. ilosvayi      | Gabriel et al. 2009                |
| 7      | MX-SiNo 1 14              | Mexico  | Recent            | C. sp. nov       |                                    |
| 8      | MX CG 1 14                | Mexico  | Recent            | C. sp. nov       |                                    |
| 9      | MX BC 1 14                | Mexico  | Recent            | C. sp. nov       |                                    |
| 10     | MX LG 1 14                | Mexico  | Recent            | C. sp. nov       |                                    |
| 11     | MX Sil 1 14               | Mexico  | Recent            | C. sp. nov       |                                    |
| 12     | MX CA 1 14                | Mexico  | Recent            | C. sp. nov       |                                    |
| 13     | Cobweb Swamp              | Belize  | Holocene          | C. alosa*        | Alcala-Herrera et al. 1994         |
| 14     | Laguna Tuspán             | Guatemala| Holocene         | C. ilosvayi      | Fleury et al. 2014                 |
| 15     | Lake Petén Itza           | Guatemala| Recent          | C. ilosvayi      | Pérez et al. 2010                  |
| 16     | Lake Petén Itza           | Guatemala| Pleistocene    | C. ilosvayi      | Escobar et al. 2012, Pérez et al. 2013 |
| 17     | Lake Güija                | Guatemala/El Salvador| Recent | C. ilosvayi      | Pérez 2010                         |
| 18     | Lake Amatitlán            | Guatemala| Recent          | C. ilosvayi      | Pérez et al. 2010                  |
| 19     | Lake Nicaragua            | Nicaragua| Recent         | Metacypris       | Swain 1976                         |
|        |                            |         |                  | onomatopensis*   |                                    |
| 20     | Wallywash Pond            | Jamaica | Pleistocene       | C. ilosvayi      | Holmes 1998                        |
| 21     | Wallywash Pond            | Jamaica | Recent           | C. ilosvayi      | Holmes 1997                        |
| 22     | Jimani Formation          | Dominican Republic| Pliocene | C. ilosvayi?    | van den Bold 1975                 |
| 23     | Lago Enriquillo           | Dominican Republic| Holocene      | C. ilosvayi      | Medley et al. 2007                 |
| 24     | Lajas Valley              | Puerto Rico| Pleistocene   | C. ilosvayi?    | cited by van den Bold 1975         |
| 25     | Lake Valencia             | Venezuela| Recent         | C. boldii        | Purper 1974                        |
| 26     | Lake Valencia             | Venezuela| Pleistocene     | C. boldii        | Binford 1982                      |
| 27     | Siqueire Formation        | Venezuela| Pliocene       | C. boldii        | van den Bold 1986                  |
| 28     | Talparo Formation         | Trinidad| Pliocene        | C. ilosvayi?     | cited in van den Bold 1975         |
| 29     | Pebas Formation           | Peru    | Miocene          | C. danielopoli   | Muñoz-Torres et al. 2006           |
| 30     | Solimões Formation        | Brazil  | Miocene          | C. danielopoli   | Gross et al. 2013                  |
| 31     | Paraná River Floodplain   | Brazil  | Recent           | C. ilosvayi      | Higuti et al. 2007                 |
| 32     | Paraná River Floodplain   | Brazil  | Recent           | C. ilosvayi      | Mormul et al. 2010                 |
| 33     | Tremembé Formation        | Brazil  | Oligocene        | C. sp.           | Berge et al. 2015                  |
| 34     | Rio Paraguay              | Argentina| Recent        | C. ilosvayi      | Poi de Neiff 2003                  |
| 35     | Villa Escolar             | Argentina| Pleistocene    | C. ilosvayi      | Zamudio 2013                       |
| 36     | Rio Negro                 | Argentina| Recent        | C. ilosvayi      | Pieri Damborsky et al. 2012        |
| 37     | Florianópolis Island      | Brazil  | Recent           | C. ilosvayi      | Lisboa et al. 2011                 |
| 38     | Porto Alegre              | Brazil  | Recent           | C. ilosvayi      | Purper 1974                        |
| 39     | Rio Grande do Sul         | Brazil  | Recent           | C. ilosvayi      | Stener et al. 2012                 |
| 40     | Santa Fe                  | Argentina| Recent        | C. argentinensis**| Ferguson 1967                     |
| 41     | Pinamar                   | Argentina| Recent        | C. ilosvayi      | Laprida 2006                       |
| 42     | Pozo del Tigre Member,    | Argentina| Late Miocene to Pliocene | C. ilosvayi | Marengo 2015                     |
|        | Chaco Formation           |         |                  |                  |                                    |
In general, hydrochemical conditions (i.e., salinity) control ostracod species distribution (Curry 1999). The (calculated) optimum conductivity of Cytheridella in Yucatán is ~700 μS/cm (Pérez 2010) but it has been reported at conductivities up to 5960 μS/cm (~3.7 psu) (Pérez et al. 2013), which is a rather large range. Most species prefer smaller conductivities (~<200 μS/cm) of the South American sampling localities might favor the occurrence of C. ilosvayi. Lack of ecological barriers and persistent interconnection through, e.g., avian dispersal may explain the great morphological resemblance between Floridian and South American Cytheridella.

Relevance for freshwater biodiversity and ecology assessments

Virtually all empirical ecological studies require species identification during data collection (Taberlet et al. 2012). In the recent decades, genetic analysis has become popular and has undergone impressive improvements (Taberlet et al. 2012). Some authors consider DNA-based tools as surrogate for traditional morphological species identification (e.g., Smith et al. 2005, Pfrender et al. 2010). Future projections of diversity change undoubtedly require consideration of current diversity patterns and temporal change (Bunnell and Huggard 1999, Willis and Whittaker 2002). Fossil records provide an historical perspective to the present and can contribute to key questions in conservation and management, such as habitat naturalness, biological invasions, disturbance regimes, natural variability, and ecosystem health (Willis and Birks 2006). However, molecular data cannot be obtained from fossil material because preservation of soft-part material is rare. Our geometric morphometric approach is not restricted to modern material because it is applicable to living and fossil ostracod valves.

Our study highlights the relevance of quantitative morphological investigations of ostracods for characterization of morphological differences for the discrimination of species or morphotypes, which probably would be overlooked in traditional morphological investigations. Our findings show that quantitative data of morphological variability reveal ontogenetically fixed differentiations of a widespread Neotropical taxon into several species or morphotypes with regional or local distributions. If this higher taxonomic resolution applies for other ostracod species, it implies higher rates of freshwater ostracod species diversity in the Neotropics than estimated thus far (see e.g., Martens and Behen 1994, Pérez-Gelabert 2008). The Neotropics are known for great biodiversity in aquatic and terrestrial ecosystems, but neither data that quantify this diversity exactly nor a sound understanding of species ecology and distribution exist (Hoorn et al. 2010). Ecological models and reconstructions of climatic and environmental history of the Neotropics are based predominantly on botanical indicators (e.g., van der Hammen and Hooghiemstra 2000, Baker and Fritz 2015). Ostracods offer the possibility to achieve insights into recent Neotropical freshwater diversity and ecology and to integrate fossil and modern data sets, thereby enabling investigation of diversity changes and patterns on multiple temporal scales. This capability would enable assessment of the influences of long-term climatic fluctuations or geological events (see e.g., Hubert and Renno 2006).

The higher taxonomic resolution of Cytheridella also has relevance for ecological assessments. Widespread species like C. ilosvayi typically are characterized by wide ecological ranges (see discussion above). Changing the taxonomic scale of a biotic-assemblage data set influences our ability to detect ecological patterns (Jones 2008). False or imprecise deductions of ecological conditions may result from the integration of several species with potentially different or narrower ecological preferences. The observed biogeographical patterns in the morphology of Cytheridella might provide a base on which spatially scaled revision of ecological preferences can be prepared if detailed morphological and ecological investigations are coupled with, e.g., genetic analyses critical for a final documentation of species richness (Hajibabaei et al. 2006).

Conclusion

Our study yielded insight on the variability of hard parts of adults and instars of Cytheridella on different geographical scales based on geometric morphometrics. The dominant feature that is subject to the shape variability is the outline of the valves. Other characters describing the variability range are the anterior pore conuli 2 and 4 (LM 2, LM 4) and the connection point between the transversal sulcus and extension of the posterior lobe (LM 6). RWA enabled us to identify the overall shape disparity and revealed a regional pattern of the morphological variability (Florida, Yucatán, and South America). DFA identified subregional (e.g., northern and southern Brazil) and local (populations) patterns.

The Mexican specimens are identified as a new species based on distinct differences in hard (outline shape) and soft parts (short penultimate segment of the antennula, different distal and proximal lobes at the hemipenis). The evolution of a new species in Yucatán could have been caused by the specific hydrochemical conditions (i.e., higher salinity). The occurrence of 2 species in Lake Punta Laguna indicates that C. ilosvayi might be introduced occasionally but may not be able to establish there.

Our study highlights the advance of geometric morphometrics for the differentiation of species or morphotypes,
which is the prerequisite for estimations of freshwater biodiversity. It refutes the assumption of the ubiquitous distribution of *Cytheridella* species and indicates a much higher, yet not detected, diversity of freshwater ostracods in the Neotropics.

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Author contributions: CW and WEP conceived the study. MG and MIFR provided data collection and analysis guidance. CW, WEP, and JM collected and analyzed the data and prepared the manuscript.

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