Sexual, ontogenetic, and geographic variation of the Neotropical freshwater ostracod *Cytheridella ilosvayi*

Claudia Wrozyna[1,2*] , Juliane Meyer[1], Martin Gross[3], Maria Ines F. Ramos[4] and Werner E. Piller[1]

Abstract

Background: The Neotropics are considered to represent one of the most biologically diverse areas on Earth. Nonetheless, many species are assumed to have widespread distributions and occur in the entire Neotropical range. However, many freshwater invertebrates such as ostracods challenge this contradiction since they live in discrete habitats and possess no active dispersal abilities. The freshwater ostracod *Cytheridella* is a prime example for this paradigm. From three extant species, only one is described to occur ubiquitously within the Neotropics. Examination of morphological variability is the prerequisite for identification of environmentally induced variations, estimation of inter- and intraspecific variability, and, ultimately, the distinction of species. This study focuses on the quantitative investigations of the appendages of *Cytheridella* from several living populations sampled in Florida, Mexico, Colombia, and Brazil.

Results: The morphological traits including podomere ratios and limb-limb ratios, showed that the largest variability occurs at the antennae, and proved a geographical structure. Soft parts reflect the morphological divergence of regional *Cytheridella* priorly demonstrated by valves shape variability.
Additionally, allometric coefficients, limb dimensions and body proportions revealed sexual and female biased size dimorphism. Large variability ranges of A-1 juveniles with reproductive anlagen could be the result of temporary deformations due to imminent moulting.

Conclusion: The geographical structure in the morphological variability allows the conjecture how widespread (freshwater invertebrate) species in the Neotropics may have arisen. Passive dispersal via e.g., birds can constitute the maximum ranges of species. If a population has adapted to local ecological conditions and may have occupied all available niches it may impede colonisation through (occasionally) other species. Relatively recent speciation(s) could explain why morphological divergence is not recognizable in qualitative investigations.

Keywords: Neotropics, Ostracoda, Widespread species, Morphological variability

Background

Tropical areas, and the Neotropics in particular, are considered to represent the most diverse areas on Earth [1–5]. The known historic climatic oscillations and geological events [5–11] have likely configured the ranges of most Neotropical species. This contradicts the assumption that many species have a widespread distribution,

e.g., [5, 12–14]. Especially, freshwater invertebrates such as ostracods live in discrete habitat patches and depend on passive dispersal. Although overland dispersal is presumed to be frequent and widespread in most freshwater taxa, is stated that generalizations are not valid and accurate ecology assessments require specific information for each taxon, and the temporal and spatial scales relevant to the process of interest [15].

The common Neotropical freshwater ostracod *Cytheridella* comprises the three extant species *C. ilosvayi*, *C. argentinensis*, and *C. boldii* of which the latter two species are only described from their type localities in Venezuela (*C. boldii*) and Argentina (*C. argentinensis*)

* Correspondence: claudia.wrozyna@uni-leipzig.de
[1]University of Graz, Institute of Earth Sciences, NAWI Graz Geocenter, Heinrichstraße 26, 8010 Graz, Graz, Austria
[2]University of Leipzig, Institute for Geophysics and Geology, Talstraße 35, 04109 Leipzig, Germany
Full list of author information is available at the end of the article

[16, 17]. Based on traditional morphological investigations is *C. ilosvayi* assumed as ubiquitous and reported from the entire Neotropical range [5].

Within the recent decade, morphometric methods were disclosed as effective tools for the identification of species and to investigate intra- and interspecific morphological variability either in case where traditional taxonomic approaches provide insufficient resolution, or being complementary to genetic methods [18, 19]. Understanding the morphological variability of an organism is crucial for taxonomic decisions, which are in either case the base for any (paleo-)ecological, phylogenetic and biostratigraphic consideration. The increasing number of cryptic species suggests that the resolution of traditional morphological techniques may be insufficient for taxonomical research. Resulting from this it is assumed that morphological stasis represents an evolutionary constant and cryptic metazoan diversity does predictably affect estimates of earth's animal diversity [20]. Exhaustive morphological investigations using, e.g., multivariate statistical approaches, indicated that some assumed cryptic species indeed revealed considerable differences [21].

With this study, we want to examine the soft part morphology of *Cytheridella* quantitatively as extension to comprehensive morphometric investigations of their hard parts [22–24]. This contributes to the understanding of the links between soft and hard part morphology, a strongly underrepresented integrated approach in ostracodology.

Since ostracod valves are easily and abundantly preserved in various sediments they became popular model organisms for palaeoenvironmental and –climatological studies [25]. The investigation of ostracod hard parts has therefore a long tradition and has increased with the rise of morphometric techniques, e.g., [22, 26–28]. The most striking result of the investigation of the valves of *C. ilosvayi* was the disclosure of a geographic pattern provided by shape variations. The rough regional pattern displayed an apportionment into Florida, South America and Mexico with a conspicuous divergence of the Mexican populations [23, 24]. The appendages provide plenty of morphological information but they are predominantly used for classification of Recent ostracods (e.g., 29). There are only few approaches that use dimensions of e.g., limbs or podomeres to characterize the soft part variability. Our study includes analyses of the variability of the appendages in terms of podomere and limb/limb ratios of the antennas and the first two thoracopods, and investigates if they reflect the geographical pattern as revealed by the valve shapes. Studies on allometry and body size dimensions (e.g., sexual size dimorphism) can unveil evolutionary patterns [29] but are rare for crustaceans and almost missing for ostracods. Therefore, we included analyses of regionally allometric coefficients and sexual size dimorphism in order to analyse similarities and differences within and

between the regions in which *Cytheridella* occur and discuss possible linkages to speciation and/or environmental influences.

Results

The data for this study were obtained from 102 specimens and extended to 134 specimens by data from a previous study from Northern Brazil [30]. The data set comprises 60 females (48 this study), 36 males [26], 31 A-1 juveniles [23], 6 A-2 juveniles [4], and 1 A-3 juvenile. Detailed information about the specimen number of each region, sex, and instar are summarised in the supporting material (Additional file 1: Table S1). Our analyses are based on ratios of podomere and setae lengths of the antennas A1 and A2, and first two thoracopods T1, and T2 of *Cytheridella*. These ratios indicate first the limb on which the measurements were obtained, followed by the considered podomere. For instance, A1EIV/EIII refers to the ratio between the third and the second podomere on the first antenna. Limb-limb ratios, such as e.g., A1/A2, are composed of the sum of podomere lengths of one limb in relation to the length of another limb. For details on the methodological approach see section material and methods and [30].
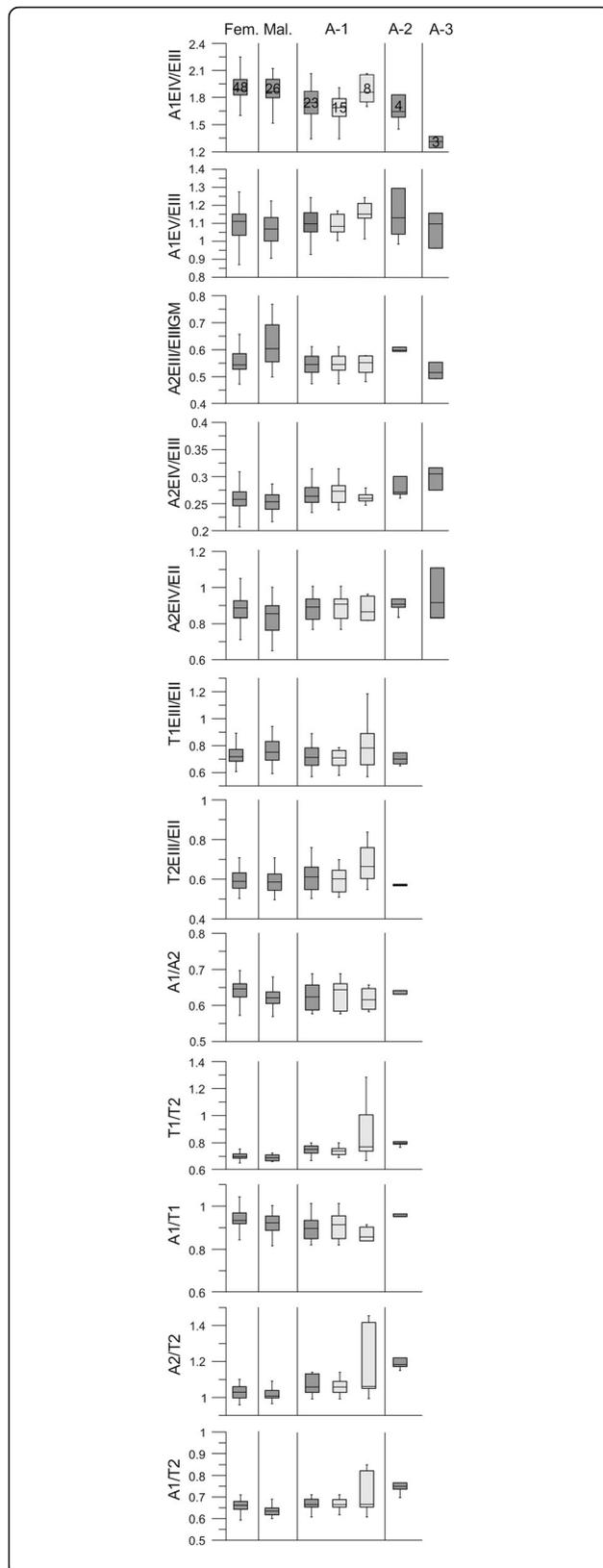
Qualitative characteristics and quantitative variability of appendages

The limb and limb-limb ratios reveal a characteristic variability pattern between geographically defined groups, which has been already observed by [30]. Most of the limb ratios shows relatively small variation ranges observable in adults and all instars, e.g., A2EIV/EIII, A1/A2 (Fig. 1). Largest variability occurs at the ratios A1EIV/EIII, T1EIII/EII and A2 /T2, respectively. The majority of limb-limb ratios reveal low variance.

All 48 females were investigated for the number of eggs and larvae carried in their brood pouches. Eggs were found in 39 females of which 17 carried also larvae. The number of eggs was very variable and ranged from 1 to 30 eggs (Additional file 1: Table S1). Populations with highest egg numbers (28 and 30, respectively) are from Colombia and Southern Brazil. Maximum egg numbers of Mexican and Floridian females are 14 and 17, respectively. Some females yielded no eggs although their reproductive organs were fully developed. In addition, some females, which displayed dense cover by microbes exposed no or very few eggs. The number of larvae is also relatively variable ranging from 0 up to 7 larvae. The ratio between number of eggs and larvae in females ranges from 0.4 to 9 (Additional file 1: Table S1).

The masticatory processes showed no variability in terms of numbers of teeth and setose teeth.

One male from Loxahatchee River (FL LX 4 14), Florida, and two females from Lagoa Itapeva (BR ITA 4 15) exhibited malformed appendages. The male specimen
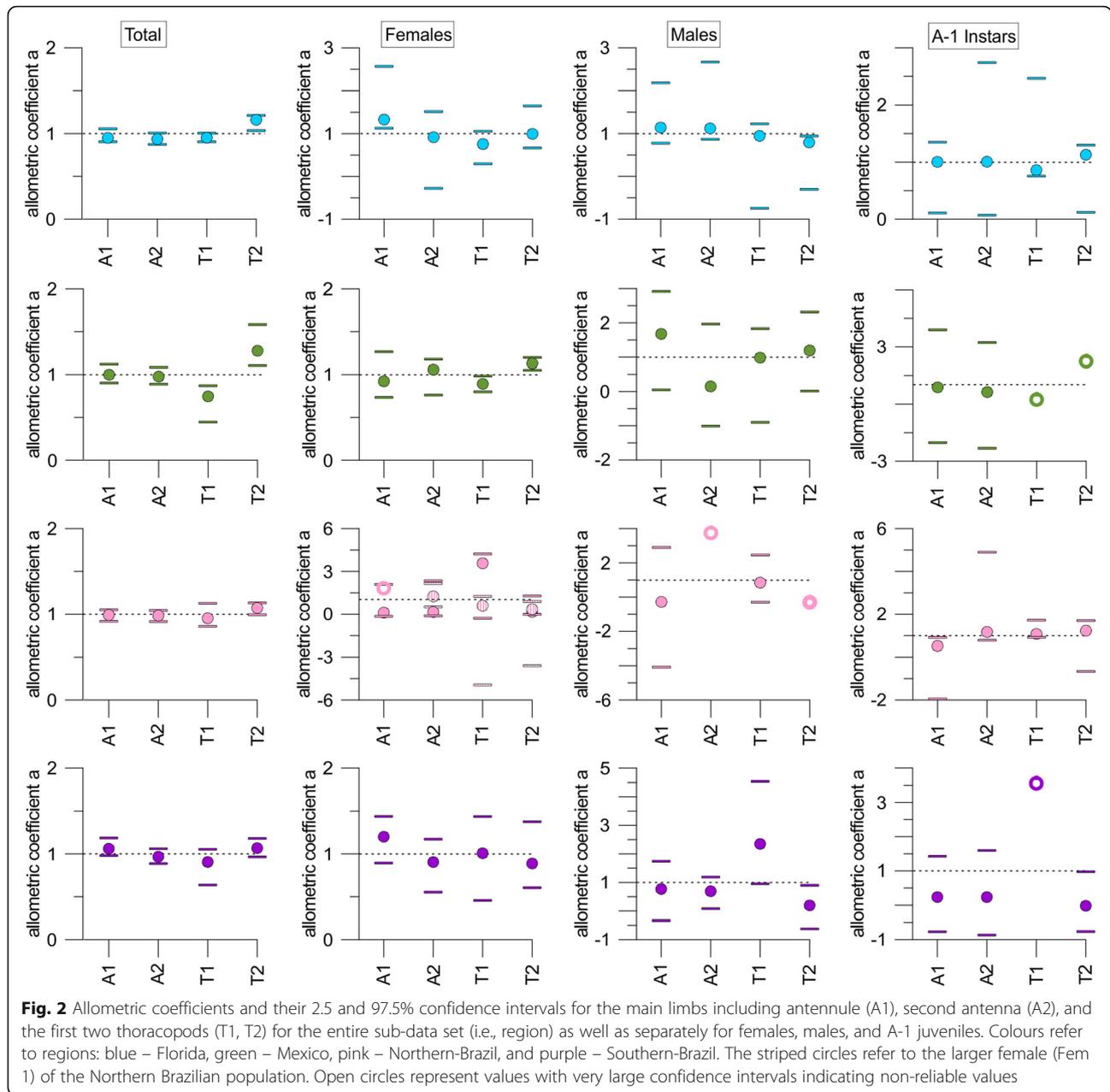
Wrozyna *et al. BMC Zoology*   (2019) 4:4

Page 3 of 19

possessed one antennule with a malformed EIV. One female from Brazil displayed malformations on the second antenna where the last podomere EIV was missing and the claws deformed. The other female revealed an insufficient separation of the podomeres at the first thoracopod.

Allometric patterns

Some ratios expose increasing or decreasing values from juveniles to adults. The most conspicuous trend is seen in A1EIV/EIII. Other ratios such as A2EIV/EIII, T1/T2, A1/T1, and A2/T2 reveal less pronounced trends. The ratios A1/T2, and to a lesser degree A2/T2, are relatively similar for adults and A-1 whereas A-2 juveniles display much higher ratios. The differentiation of A-1 juveniles into specimens with and without proto-reproductive organs uncovers a significantly higher variability of ratios related to thoracopods (T1EIII/EII, T2EIII/EII) and contributing to large variations of inter-appendages ratios (e.g., T1/T2, A2/T2) in specimens with reproductive anlagen (Fig. 2).

Analyses of multivariate allometry revealed that for all regional data sets (total, females, males, A-1) the variation accounted for by PC1 was > 87%. The antennas (A1, A2) display for the total data set values of the allometric coefficients a around 1 with low deviations between the regions. The first thoracopod (T1) is characterized by values of a < 1 with lowest values most distinctly displayed by Mexicans and S-Brazilians. The second thoracopod (T2) reveals for all regions allometric coefficients of a > 1. The coefficients of females are relatively similar for the different regions except of Northern Brazil which is characterised by co-occurrence of two females primarily separated by size [30] (Fig. 2). Both N-Brazilian females define the maximum (positive and negative, respectively) variation ranges and possess for A1, A2 and T1 opposite values. The smaller females (fem 2) exhibit the most positive values (a = 3.6) for T1, while females of the other regions show lower values. Male coefficients show some differences to females and between regions. The coefficients of A1 indicate positive allometry shown by Floridian and Mexican males contrary to Northern and Southern Brazilian males which exhibit negative allometry. Floridian males display for all limbs, values around 1. Northern Brazil displays very high coefficients (e.g., a = 3.7 for A2) which represent,

Fig. 2 Allometric coefficients and their 2.5 and 97.5% confidence intervals for the main limbs including antennule (A1), second antenna (A2), and the first two thoracopods (T1, T2) for the entire sub-data set (i.e., region) as well as separately for females, males, and A-1 juveniles. Colours refer to regions: blue – Florida, green – Mexico, pink – Northern-Brazil, and purple – Southern-Brazil. The striped circles refer to the larger female (Fem 1) of the Northern Brazilian population. Open circles represent values with very large confidence intervals indicating non-reliable values

except for T1, the maximum values in the diagram. Southern Brazil is characterized by intermediate values and rather resembling the Floridian coefficient with exception of T1 where it displays a > 2 while the others display values around 1. Mexican males show opposite values to the Brazilian specimens (except for T1) but with smaller excursion.

A-1 juveniles show another pattern. Southern Brazil shows the maximum values with an exceptionally high coefficient for T1 (a = 3.5). Juveniles from Florida and Northern Brazil display similar values around 1. Mexican A-1 display intermediate values with a ≤ 1 for A1, A2, T1 and a = 2.2 for T2.
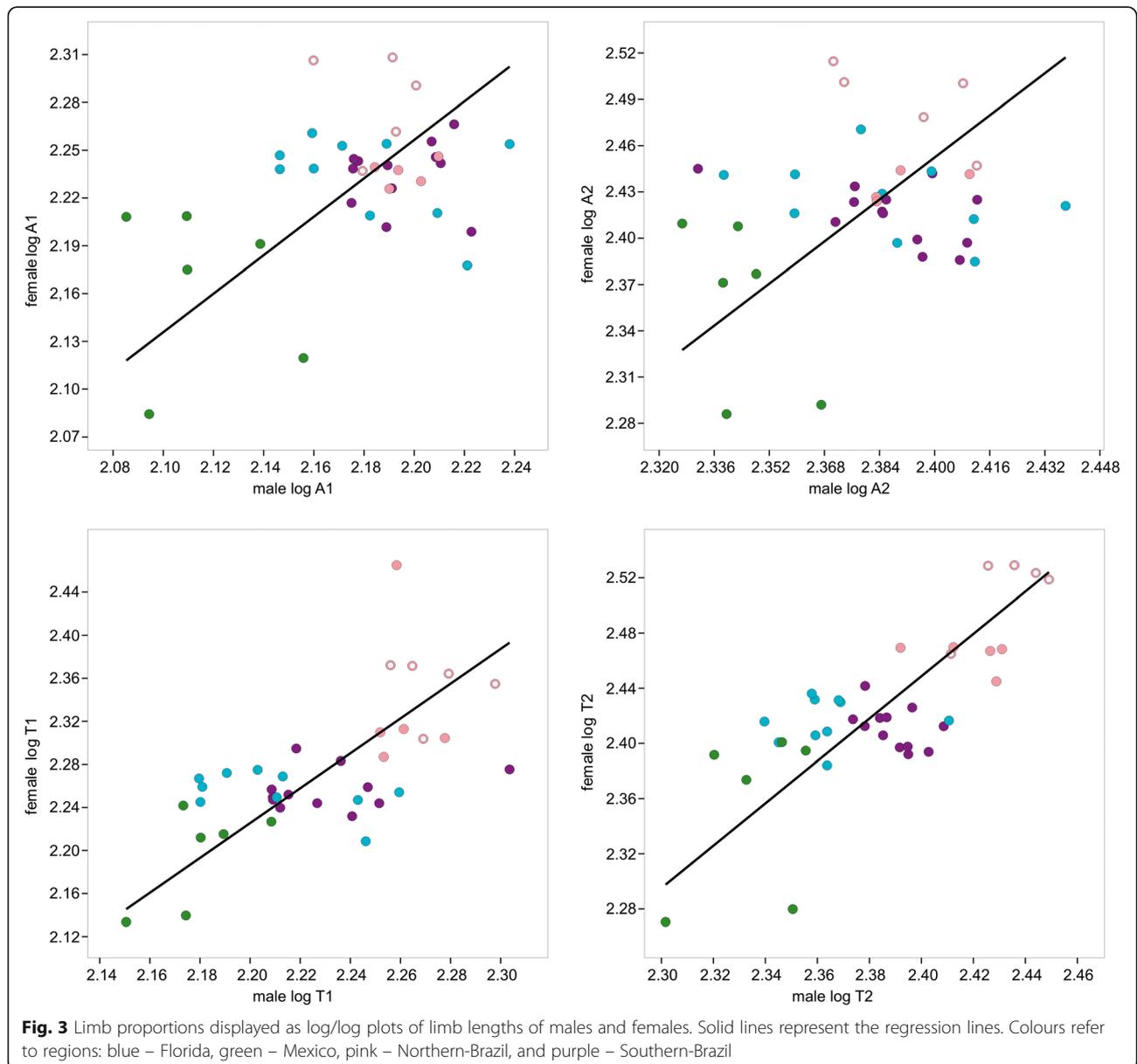
Sexual (size) dimorphism

The Hoteling's t^2 test revealed that ratios between females and males are significantly different (t^2: 63.357, F = 3.328, $p < 0.001$). This can be also seen by a virtual inspection of the boxplots of all ratios Exceptions representing more similar ratios for both sexes are T2EIII/EII, A2EIII/EIIIGM, T1/T2, T1EIII/EII, and A1/A2 (Fig. 1). The largest deviation between females and males occurs at A2EIV/EII. Other ratios show lower differences but display not a simple pattern in which, e.g., males exhibit generally lower or higher values. For instance, males display lower A1EV/EIII values than females but higher values for A2EIII/EIIIGM (Fig. 1).

Eight of 23 A-1 specimens exhibited anlagen of reproductive organs (7 proto-females, 1 proto-male). These anlagen occur predominantly in Mexican populations and in two specimens from Florida and Brazil.

All slopes of male-female Reduced Major axis regression analyses of all limb traits have values ß ≥1 (Fig. 3). Table 1 summarizes the results of the regression analyses for all specimens and for the population means. The female and male thoracopods (T1, T2) are significantly correlated ($r^2 \geq 0.65$, $p = 0.0001$) while correlations for A1 are lower ($r^2 = 0.4$, $p = 0.008$). No correlation was found for A2 ($r^2 = 0.22$, $p = 0.202$).

The log/log-plots of population means of female and male lengths for the antennae and legs (Fig. 4) show also that all traits have regression slopes of ß > 1. The smaller females (fem 2) show for the antennae very similar SSDs to Southern Brazil and the population from the Loxahatchee River (FL LX 4) contrary to the larger females (fem 1) which deviates strongly from the other females and regions. The other Floridian population (FL PR 1) shows largest antennae compared to females. Both Mexican populations exhibit the smallest limb lengths. Thoracopods lengths are more evenly distributed than antennae with the largest lengths shown in Northern Brazil, and the smallest presented by the Mexican populations. Accordingly, Floridian and

Fig. 3 Limb proportions displayed as log/log plots of limb lengths of males and females. Solid lines represent the regression lines. Colours refer to regions: blue – Florida, green – Mexico, pink – Northern-Brazil, and purple – Southern-Brazil

Wrozyna *et al. BMC Zoology* (2019) 4:4

Page 6 of 19

Table 1 Results of Reduced Major Axis (RMA) regressions of log (male size) on log (female size) for raw data (all females vs. all males) and population means

| Raw data Females vs Males					
Limb	Slope β	Error	95% Interval	r^2	p
A1	1.207	0.182	[0,.7474; 1.714]	0.430	0.0081
A2	1.704	0.277	[0,.9983; 5.105]	0.216	0.2021
T1	1.619	0.206	[1.068; 2.105]	0.646	0.0001
T2	1.535	0.165	[1.189; 1.931]	0.764	0.0001
Population Means					
Limb	Slope β	Error	95% Interval	r^2	p
A1	1.084	0.202	[0.5776; 4,.801]	0.792	0.0031
A2	1.683	0.473	[0.5055; 6.663]	0.526	0.0418
T1	1.391	0.224	[0.6445; 1.946]	0.847	0.0013
T2	1.336	0.233	[0.3382; 1.799]	0.816	0.0021

Southern Brazilian populations display intermediate values (Fig. 4).

Mean SDI (Sexual Dimorphism Index) values as, quantitative measure for sexual dimorphism,are all positive ranging from 0.005 (A2, T1) to 0.047 (A1). Large females of Northern Brazil occupy maximum values accompanied by a strong deviation from the other regions (Fig. 5). The other regions show similar values and no pattern.

Geographical variability

Most ratios show distinct ranges for the different regions (Brazil, Colombia, Mexico, and Florida) (Figs. 6 and 7). The general pattern is similar for females, males, and A-1 juveniles. There are, however, specific variability patterns for each ratio, region, the developmental stage and sex, respectively. For instance, Colombian females display the highest A1EIV/EIII and A2EIV/EII ratios but do not show similar strong deviations from the other females in e.g., A2EIII/EIIIGM. Mexican females and males exhibit an example for differences between sexes at A1EIV/EIII. The females show higher ratios than males. A contrasting relationship is displayed by the A1/T2 ratio of Floridian specimens with distinctly higher ratios revealed by the males. Differences between adults and juveniles are represented by, e.g., higher T1/T2 ratios of Mexican A-1 compared to the adults.

There is a rough pattern dividing *Cytheridella* into North-Central American and South American morphotypes. Colombian females reveal an intermediate position exhibiting partial coincidences with Florida and Mexico (e.g., A1EIV/EIII, T1EIII/EII, A1/T1) and in some cases higher accordance with Northern and Southern Brazil (e.g., T2EIII/EII). The Colombian females show large variation ranges in T1EIII/EII. Northern Brazil provides, however, strongest deviations from the other regions displayed by both females, males and A-1
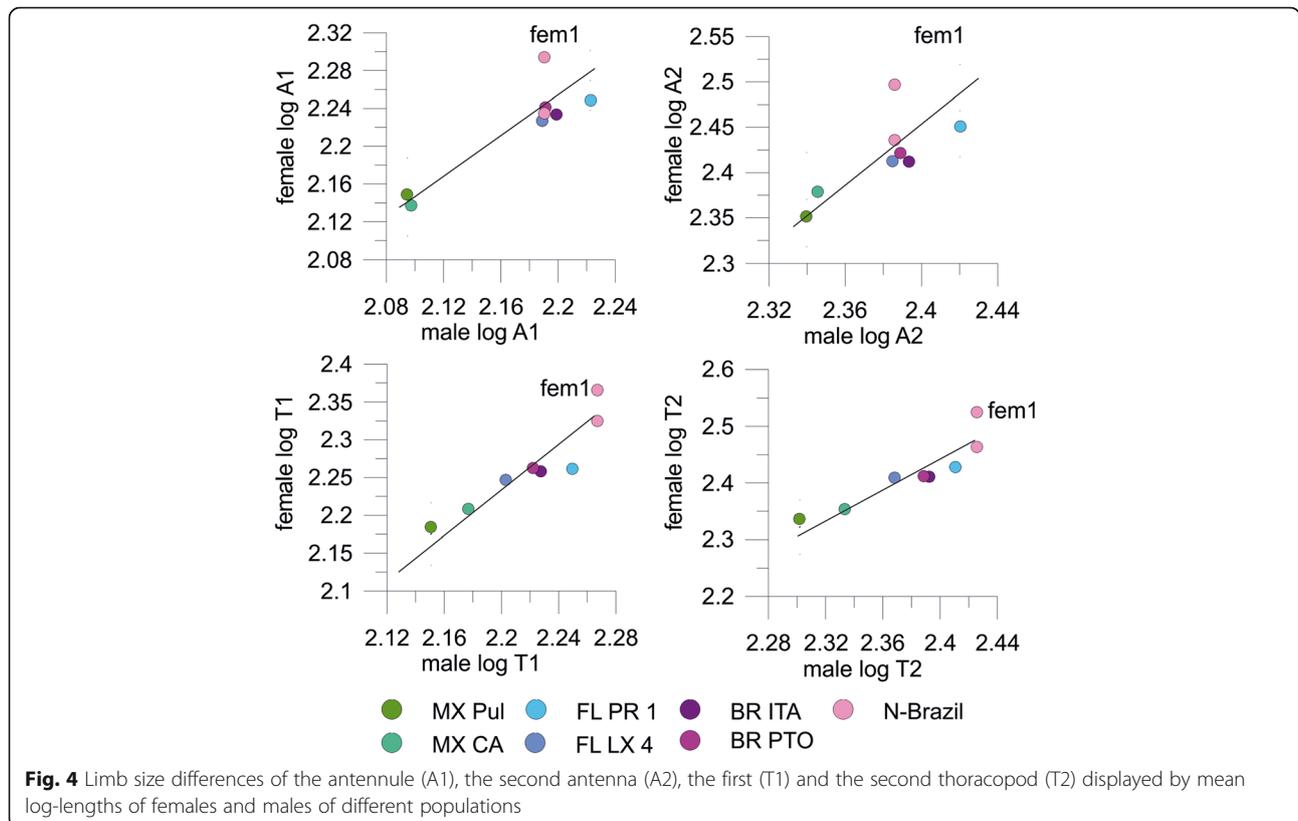
Fig. 4 Limb size differences of the antennule (A1), the second antenna (A2), the first (T1) and the second thoracopod (T2) displayed by mean log-lengths of females and males of different populations
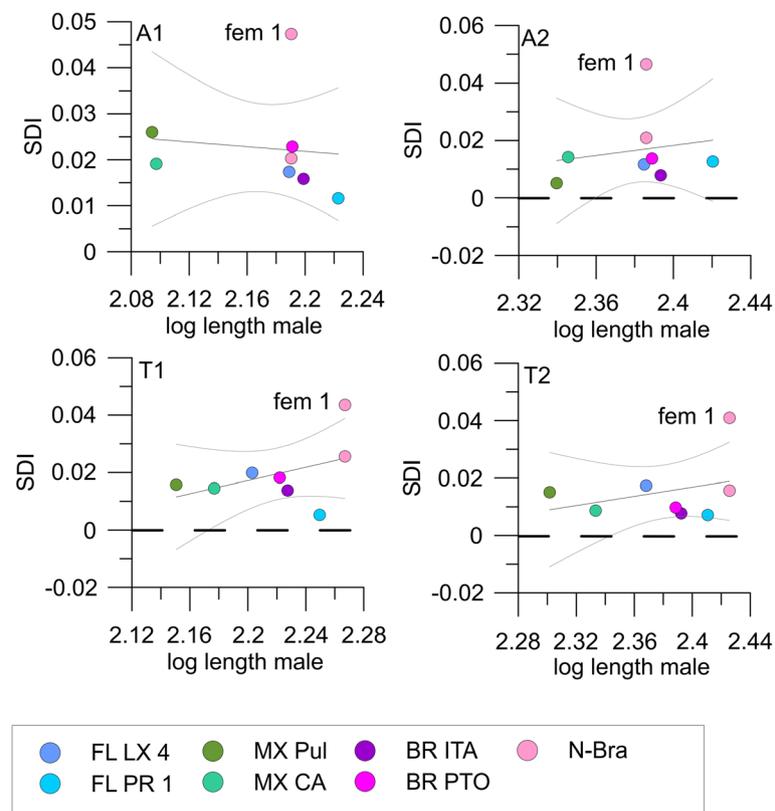
Fig. 5 Sexual dimorphism index (SDI) values versus log-lengths of males for the antennule (A1), the second antenna (A2), the first (T1) and the second thoracopod (T2) of different populations. Isometric growth is indicated by the dashed line

(e.g., A2EIV/EII, A1/T1) (Fig. 7). There is also no clear pattern shown by the two female types of northern Brazil revealing higher similarity of one of them with the other females. The Mexican populations deviate also from Floridian populations especially in limb-limb ratios (A1/T1, A1/T2, and A1/A2) and in A2EIV/EII. Mexican specimens exhibit distinctly shorter antennules and longer T2EIII podomeres. Juveniles generally reflect the (regional) pattern and values of the adults. One exception to that represents the T1/T2 ratio. While the values for adult females and males are very similar for the regions, the juveniles exhibit generally higher values with highest values displayed by the Mexicans.
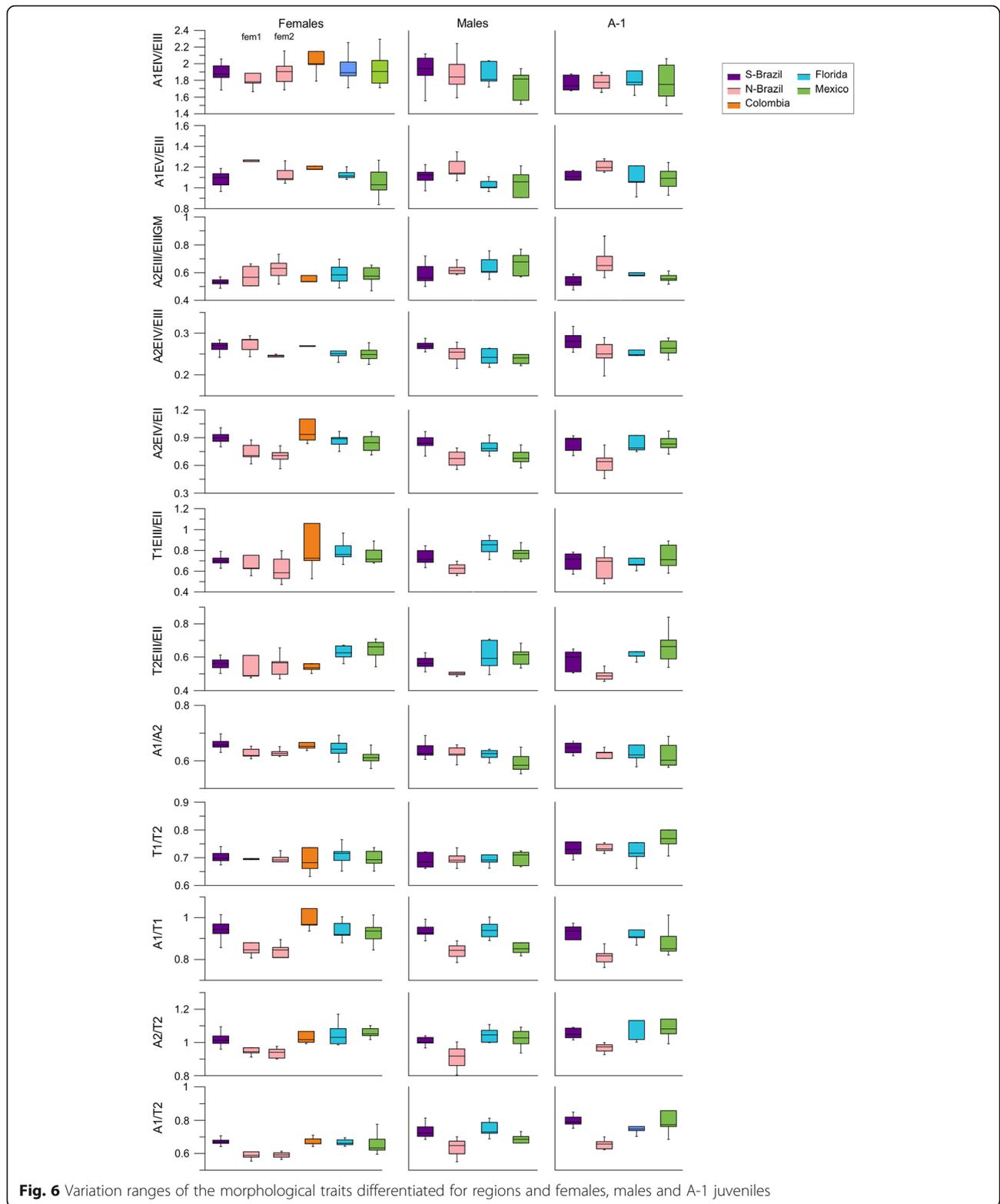
Principal component analyses of soft parts ratios

The PCA enables to investigate the variability pattern of the total data set (all specimens), and separately for adults (females, males) and juveniles (A-1). The results of the analyses are summarized in Table 2. In all analyses the first three components explain > 71% of the total variability (total dataset: 71%, females: 80.1%, males: 82.3%, and A-1: 78,3%). Largest coefficients on PC1 are provided by the ratio of the antennule (A1EIV/EIII) for the analyses of the total data set, females and males. The

highest loading on PC1 of the juveniles is the limb-limb ratio A2/T2. Highest coefficients on PC2 are A2EIV/EII (total, females, and males) and T1EIII/EII (total and males). A1EIV/EIII provides the highest coefficient on PC2 for the juveniles. The third component is more or less associated with the same ratios: A2EIV/EII (total, males) and T1EIII/EII (total, females, males, A-1).
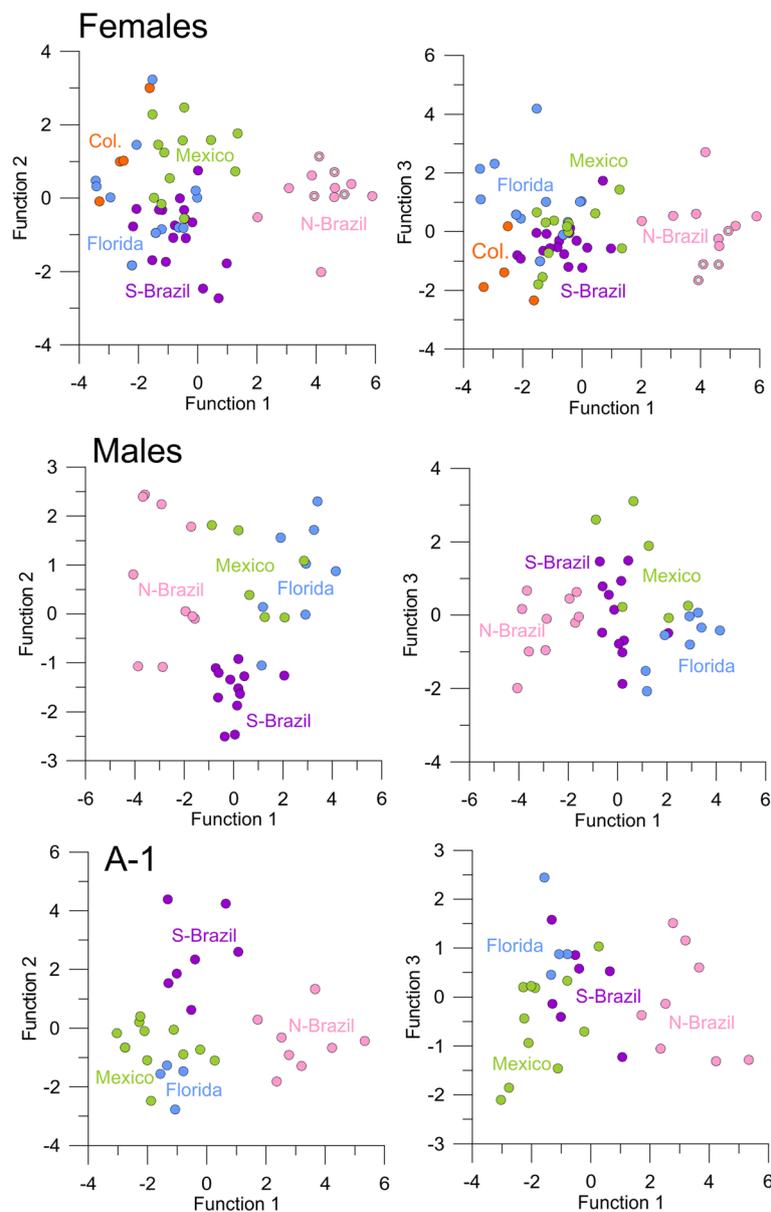
Canonical variates analyses of soft part ratios

The CVA which was used to investigate the significance of the geographical pattern, achieved partial delimitations of regional morphotypes. The overall MANOVA statistics confirmed that some group means are significantly different (females: Wilk's lambda test: Λ_{Wilks} = 0.057, F = 6.555, $p < 0.001$, Pillais trace test: Λ_{Pillai} = 1.683, F = 4.632, $p < 0.001$; males: Wilk's lambda test: Λ_{Wilks} = 0.0532, F = 5.33, p < 0.001, Pillais trace test: Λ_{Pillai} = 1.716, F = 4.512, p < 0.001; A-1: Wilk's lambda test: Λ_{Wilks} = 0.0392, F = 4.77, p < 0.001, Pillais trace test: Λ_{Pillai} = 1.779, F = 3.825, p < 0.001). Accordingly, the majority of females is significantly separated from each other. This is most distinct for Southern Brazil which reveals significant (p < 0.001) different group means compared to the other regions. Colombia cannot be separated from Southern Brazil, Florida, and

Fig. 6 Variation ranges of the morphological traits differentiated for regions and females, males and A-1 juveniles

Mexico. Due to low specimen number of Florida, some comparisons of the group means could not be achieved. Group means of Floridian and Mexican females are not separated ($p = 0.91$). This pattern is illustrated in the scatter plot (Fig. 7). The most conspicuous differentiation is displayed by Northern Brazilian specimens from the other

Fig. 7 Canonical variates analyses (CVA) of soft part ratios of *Cytheridella* for females, males, and juveniles (A-1). Groups were defined according to regions. The open circles represent the larger females (fem1) of the Northern Brazilian population [31]

regions associated with most positive scores on Function 1 (p < 0.001). The other regions are assembled at more negative values between 2 and – 4 on Function 1. Although there is some overlap, Function 2 discriminates Southern Brazil from Colombian, Mexican, and Floridian females. No conspicuous differentiation of the regions is indicated at Function 3. Males and A-1 instars reflect the differentiation of the regions as shown by the females. Both analyses confirm a high conformity between Florida and Mexico (each with *p* = 1) which corresponds to a strong overlap in the scatter plots (Fig. 7). The significant separation of Northern Brazil from the other regions is displayed by

most negative (positive) scores on Function 1 for males (A-1 juveniles). Less significant separations between Southern Brazil and the other regions (*p*-values > 0.001) are displayed by less deviating scores on the functions.

Discussion
Intraspecific variation of appendages
Quantitative analyses of the soft parts of *Cytheridella* were done according to the approach by [30] focusing on limb traits represented by podomere proportions and limb lengths. The most varying ratios are related to the first and second antenna (A1EIV/EIII, A1EV/EIII, A2EIII/EIIIGM,

Table 2 Results of the Principal component analysis (PCA)

		PC1	PC2	PC3
Total	A1EIV/EIII	0.8853	0.2104	0.08226
	A1EV/EIII	0.4008	−0.1478	−0.174
	A2EIII/EIIIGM	0.0433	−0.3212	0.4299
	A2EIV/EIII	−0.007781	0.03588	−0.0647
	A2EIV/EII	−0.06342	0.5725	−0.464
	T1EIII/EII	0.0075	0.449	0.619
	T2EIII/EII	−0.07469	0.2568	0.2991
	A1/A2	0.01959	0.038	−0.1567
	T1/T2	−0.108	−0.0151	0.07886
	A1/T1	0.00221	0.3177	−0.1209
	A2/T2	−0.1588	0.2891	0.2202
	A1/T2	−0.0814	0.2188	−0.02705
Eigenvalue		0.0323686	0.0219078	0.0138004
% variance		33.759	22.849	14.393
Females	A1EIV/EIII	0.8659	−0.1277	0.01202
	A1EV/EIII	0.4086	−0.273	−0.2309
	A2EIII/EIIIGM	−0.03511	−0.4115	0.3737
	A2EIV/EIII	0.00995	0.06442	−0.05384
	A2EIV/EII	0.1998	0.6912	−0.2112
	T1EIII/EII	0.1601	0.2225	0.7789
	T2EIII/EII	−0.02112	0.02975	0.2519
	A1/A2	−0.01829	0.1216	−0.1242
	T1/T2	−0.0933	−0.08056	−0.07786
	A1/T1	0.08119	0.3601	0.1004
	A2/T2	0.01223	0.1245	0.2398
	A1/T2	−0.01064	0.2028	0.01834
Eigenvalue		0.0317675	0.0216854	0.00994066
% variance		40.137	27.398	12.56
Males	A1EIV/EIII	0.8394	0.1203	0.3032
	A1sEV/EIII	0.4492	−0.1676	0.009439
	A2EIII/EIIIGM	−0.1491	−0.3769	0.4256
	A2EIV/EIII	0.02381	0.02137	−0.05588
	A2EIV/EII	0.07824	0.5562	−0.5003
	T1EIII/EII	−0.1292	0.4741	0.5425
	T2EIII/EII	−0.07431	0.2339	0.291
	A1/A2	0.0935	0.04018	−0.1306
	T1/T2	−0.0273	−0.03406	0.0264
	A1/T1	−0.002958	0.3399	0.02481
	A2/T2	−0.1797	0.255	0.2734
	A1/T2	−0.02478	0.2066	0.03717
Eigenvalue		0.0384331	0.0183375	0.0130082
% variance		45.342	21.634	15.347
A-1	A1EIV/EIII	0.249	0.728	0.1598
	A1EV/EIII	−0.04079	0.3499	−0.1392

Table 2 Results of the Principal component analysis (PCA) (Continued)

		PC1	PC2	PC3
	A2EIII/EIIIGM	−0.167	0.2975	−0.08096
	A2EIV/EIII	0.02485	−0.04553	−0.01597
	A2EIV/EII	0.2517	−0.4608	0.1339
	T1EIII/EII	0.3331	0.1223	0.6975
	T2EIII/EII	0.422	−0.08638	0.1268
	A1/A2	−0.04893	−0.008404	0.03144
	T1/T2	0.4345	0.04905	−0.5011
	A1/T1	−0.06218	−0.1277	0.3512
	A2/T2	0.537	−0.04488	−0.211
	A1/T2	0.2686	−0.03747	−0.0883
Eigenvalue		0.0387642	0.026016	0.0232999

A2EIV/EIII, respectively) and to the first thoracopod (T1EIII/EII). Except for the influence of the thoracopod this pattern was similarly observed by [30] which analysed the variability within a population from Northern Brazil. Base for this study was a dataset including adults and juveniles revealing that antenna related ratios were most important for describing the observed variability. Here we found these ratios important for the whole data set as well as separately for females, males, and to a minor degree also for juveniles. The variability of the antennas (and of the thoracopod) occurs, thus, independently of scale (i.e., during ontogeny, within and among populations and regions).

The antennule fulfils both sensory and locomotory functions while the second antenna is the main locomotory limb for walking, climbing, digging or swimming [31]. In cytheroids the first thoracopod represents a walking limb [31]. The high variability of limbs with functions for locomotion and sensory abilities could be related to adaptations to the (micro) habitat or more precisely the substrate type and/or the macrophyte cover. If this is the case one could assume a similar high variability of the walking legs. Accordingly, the ratio of the second thoracopod should also contribute to the observed variability. The ratio T2EIII/EII evinces, however, no significant high coefficients on one component of the performed PCA analyses (Table 1). This could indicate that variability of the antennae has a different source than other limbs during morphogenesis. It is postulated that different variabilities of setae could be related to different ontogenetic developments [32]. If this would also apply for limbs or podomeres one could expect a similar variability pattern by other cytheroidean species. This is, however, not the case.

The irregular pattern that implies a higher variability of some limbs is also apparent in juveniles, and indicates therefore a stronger genetic fixation, and might be caused by differing functional and/or adaptive features.

For instance, antennae are assumed to fulfil additional functions for the reproductive behaviour [33] (for additional discussion see the following chapter). Other comparable studies found highest variabilities by claws and setae [32, 34]. Specimens reared in laboratory cultures revealed a strong correlation with salinity and temperature [32]. The limb traits included for the analyses of the soft part variability for this study were chosen according to normal distribution of the ratios [30]. Accordingly, all setae related ratios were excluded except the ratio relating to the claw GM of A2. This could be simply due to inaccurate measurements since especially setae are relatively difficult to evenly arrange on the slide. Contrary, one could hypothesize that claws and setae are generally more sensitive to environmental changes than, e.g., podomeres.

The few available studies use different morphological characters, e.g., different podomeres and number of podomeres in total, what hampers an assessment of coinciding variability patterns among species or the degree of sensitivity to external factors of claws/setae vs. podomeres.

The wide geographic range and different habitat types of the studied populations probably covers different food sources and compositions which might lead to adaptations in the mandibles, e.g., [35]. The low variability of the masticatory processes, however, points to very low environmental influence.

Malformations in ostracods are poorly studied. It is known that many environmental pollutants have toxic effects and can affect normal limb regeneration and moulting in crustaceans [36]. Pollution of the habitat can have physiological, developmental, and behavioural effects on crustaceans depending on the pollutant such as metals, organic compounds, nutrients, or hypoxia [37]. In order to identify the causes for the observed malformations repeated sampling in combination with extended hydrochemical analyses is required.

Concluding, although relatively disregarded in ostracodology, quantitative examinations of appendages have the potential to shed light on ostracod species biology in terms of morphogenetic differences of limbs and adaptive responses to environmental changes. Expanding the approach to other genera and families will help to identify the position of the variability pattern within the systematic framework and evaluate its taxonomic information.

Sexual (size) dimorphism

Sexual dimorphism in ostracods is variegated and can affect sensory features, copulatory behaviour, and female brooding [38]. Our data show that sexual dimorphism in *Cytheridella* is also expressed in limb dimensions and can be proven in all examined soft part ratios. The largest differences occur at the second antenna shown by higher A2EIII/EIIIGM ratios of males compared to females. Differing values and variability ranges of allometric coefficients indicate an additional discrimination between the sexes (Fig. 2). Limb dimensions considered by log-transformed lengths of males against females reveal sexual size dimorphism (Figs. 3, 4). The limb lengths are characterized by a relatively high variation in both sexes (Fig. 3) resulting in lower correlations compared to analyses of mean limb lengths of selected populations (Fig. 4).

Patterns of interspecific variation in body size are expected to reflect patterns of adaptive divergence [39]. Differences in body size is also one of the main characters distinguishing females and males. Sexual size dimorphism varies greatly in direction and degree, both among and within clades [40, 41]. Studies on sexual size dimorphism (SSD) are predominantly related to total body dimensions (e.g., total length) or parameters directly linked to it (e.g., body mass). For ostracods, this is hardly to apply for soft parts that are almost completely encompassed by their calcitic valves. Therefore, we considered dimensions of the body in terms of length of the main limbs (antennae, walking legs).

The most common approach for the determination of SSD is documented by regression slopes deviating from 1 (Figs. 3, 4) of log-transformed lengths of female vs. male appendages. The slopes of all limbs exhibit $\beta > 1$ indicating a stronger variability by males [39] and decreasing SSD with body size [42]. Allometric coefficients as well as SSDs differ between the geographical regions (Figs. 2, 3) which could indicate different strategies for growth [43] due to different selection preferences and/or environmental differences. [42] interprets different SSDs among populations by genetic divergence in overall body size.

The SDI is another approach to quantify SSD and has the advantage to provide symmetrical results around zero regardless of which sex is the larger [44]. Per convention are positive SDI values related to species with larger females [45] and proves that for all observed limbs and regions. Although there are slight differences between the regions, *Cytheridella* displays females-biased sexual dimorphism in all populations. The larger female (fem 1) of the Northern Brazilian population represents an outlier to the 95% confidence intervals indicating that female size relative to that of males is even greater. The SSDs of the smaller females (fem 2) are within the confidence intervals and display similar values to Florida and Southern Brazil. The observed greater variance of male allometric coefficients (Fig. 2) (and generally larger females) corresponds to what has been formulated as Rensch's Rule (RR) [39, 46]. This rule relates sexual dimorphism to body size and predicts that sexual size dimorphism will be positively correlated with mean body size in taxa in which males are the larger sex and

negatively correlated with mean body size in taxa in which females are the larger sex [47].

Explanations for Rensch's Rule comprise the hypothesis that in species exhibiting female-biased dimorphism females compete with each other [41]. Natural selection through ecological processes may also determine dimorphism as, for example, resource poor habitats may favour smaller males [48]. Plenty of studies deal with SSD in different organisms and cover different taxonomic levels. Crustacean studies are, however, rare. Across diverse copepod clades, isometry is found to be almost universal providing little support for Rensch's Rule [49]. An extensive study of a range of insect orders revealed that RR consistently applies to one order and a suborder, but not to any other insect group. The authors conclude that the mechanisms causing the pattern are unevenly distributed among taxa [40]. Evidences based on avian data sets were found, that Rensch's Rule is driven by a correlated evolutionary change in females to directional selection on males. The degree of differential size selection operating on the sexes, mediated primarily through intrasexual competition for mating opportunities, are considered to best explain variances in size allometry [50]. Another meta-study dealing with > 150 insect species suggests that environmental conditions may strongly affect the degree, though not the direction of SSD within species [48]. [48] propose also a strong environmental influence especially at the intraspecific level, which could explain differences between the regions.

The mating process of ostracods starts with the mate recognition phase, which is in presumably the majority of non-marine ostracods initiated by the male touching the female attempting to stimulate her for accepting him for copulation [33]. Morphological and behavioural signalling is used for this. The combination of morphological characters (valves, limbs) and behaviour of the male (position of the valves, touching the female parts by antennae, etc.) are assumed to be key criteria for females to determine whether the potential mate is conspecific (recognition) and whether or not this mate is preferable (selection) within the variation of the species [33]. If this process takes also place in *Cytheridella* this would indicate that female selection is the major cause for sexual (size) dimorphism. The involvement of the antennae in the mating process could also explain why antennae dimensions provide the highest variability of all soft parts and on all scales – from the population to regional scale.

Deduced from laboratory cultures [39] found that traits that are most closely associated with reproductive fitness show lowest allometric slopes. It is suggested that these traits are "adaptively canalized" [39]. Commonly, fecundity models predict that larger females are able to produce more or larger offspring and directly increases their reproductive success [51]. Accepting this, the higher mean egg number of the South American females could be evidence for this. However, too few specimens preclude reliable evaluation.

Juvenile morphological variability: relationships to the moult cycle?

There are two main findings of the analyses of the juvenile variability: some ratios display ontogenetic trends, and A-1 juveniles possessing anlagen of reproductive organs exhibit the highest variability ranges (Fig. 1). The presence of anlagen of reproductive organs in juveniles is documented for several ostracod species and instars. Many podocopid species develop sexual organs through ontogeny of the last instar, in A-1 [52, 53]. The specimens of this study showed that not all A-1 specimens of a population displayed reproductive anlagen (Additional file 1: Table S1). This implicates that the reproductive organs are not developed in the preceding instar A-2. Therefore, formation of the reproductive organs must take place in A-1, and their presence points to an advanced stage of ontogeny or moult cycle compared to A-1 specimens without these characteristics. Attention of ontogenetic changes of ostracods comprised the general body plan (i.e., successive addition of limbs or setae) e.g., [52, 54] but no information is available on the exact timing and development of copulatory organs. Moreover, there is no information about the connection to the moult cycle. Moulting in crustaceans is a complex process including hormonal as well as external (e.g., temperature, food) controls [55] and each moult is composed of different stages including preecdysis, ecdysis, postecdysis, and intermolting stages [56]. Already in 1950 it was stated that nearly nothing is known about the biology of the moulting process [57], and since then not much progress has been achieved. Following studies predominantly dealt with aspects of calcification of hard parts [58, 59]. It is known, that each moulting is quickly followed by a renewal of the tissues, before new covering of chitin is secreted. In the juvenile instars new appendages and organs are added, and the structure of previous appendages is drastically changed [31].

The determination of the moult stage was applied to many commercially used crustaceans (e.g., lobsters, shrimps) using different criteria such as degree of setae development or epidermal retraction [60, 61]. Comparable information are not available for ostracod moult cycles. In a previous study a `swelling´ of the ostracods prior and during ecdysis is reported [60]. Although this is a very imprecise note about moulting, it could be a hint that moulting causes also (temporarily) distortion or at least modifications on podomere proportions, which might explain the high variability of soft part

ratios (Fig. 1) of specimens with copulatory anlagen. It could be therefore possible that specimens with copulatory anlagen have completed the A-1 intermoult period or started the preecdysis stage, whereas the individuals without anlagen are in the intermoult stage.

The life history of many species, at least in shallow surface waters, depends on climatic conditions and is therefore found to vary slightly inter-annually and between geographic areas [31].

Based on coincidences of stable isotopes signatures between a theoretical calcite and ostracod carbonate [62] found that calcification of Floridian *Cytheridella* is seasonally restricted and takes place in spring (April) before the onset of the rainy season which lasts from May to October. Therefore, *Cytheridella* is assumed to display a seasonal life cycle, which could be related to hydrological conditions. Considering the study areas, covering almost the north-south extension of the Neotropics, a variety of climate regimes and hydrological gradients occur [63, 64]. Therefore, *Cytheridella* might have deviating life history patterns in different regions.

Sampling in Mexico was carried out within a couple of days after sampling in Florida (Table 3). Thus, the studied populations could have been caught at the same point in their life histories. The majority of specimens with reproductive anlagen occurred, however, in Mexico. Regionally, specific life (and according moult) cycles could explain that.

On the other hand, Mexican localities deviate from the other study areas by distinctly higher salinities and hydrochemical compositions [22]. From other crustaceans such as *Artemia* it is known that external factors (e.g., temperature) can affect the duration of pre-reproductive periods [65]. Thus, ecological stress may trigger earlier sexual maturity in Mexican habitats.

Combined investigations of life history and moult cycles would enable to characterize population specific age structures and potential external controls. The understanding of moult cycles, in particular the duration of each moult phase, can provide important information relevant to any application of ostracod species as (palaeo-)environmental proxy. Thus, it could be possible to designate the timing (generation(s) per year) and

Table 3 Overview about the sample material and information about localities including names, access dates, coordinates, and a short habitat description, modified from [23]. Copyright (2018) by the Society of Freshwater Science. Reprinted with permission

Sample	Locality	Date	Country	N (S)	E (W)	Habitat
BB 0109	Barro Branco	27.09.2009	Brazil	06°50′18.3″	69°45′37.0″	root zone in an abandoned channel
LG 01 09	Lago Comprido	27.09.2009	Brazil	06°43′52.7″	69°44′33.9″	littoral with abundant leaf litter
BR-CU-1-15	Custódia Lagoon	04.09.2015	Brazil	30°02′15.2″	050°10′20.2″	lagoon
BR-MN-3-15	Rio de Relógio	04.09.2015	Brazil	30°04′10.3″	050°12′20.8″	inflow to Manoel Nunes Lagoon
BR-PL-1-15	Passos da Lagoa	03.09.2015	Brazil	29°51′16.1″	050°06′57.9″	littoral of a lake, dense macrophytes
BR-ITA-4-15	Itapeva Lagoon	06.09.2015	Brazil	29°22′32.6″	049°47′39.2″	lagoon, next to inflow
BR-EM-3-15	Emboaba Lagoon	03.09.2015	Brazil	29°57′52.8″	050°13′27.4″	lagoon with temporary conection to the ocean
BR-PTO-4-15	Laguna Passos de Torres	07.09.2015	Brazil	29°18′39.6″	049°42′32.8″	lagoon, floating plants
CO-ET-1a-15	Estero Texas	04.02.2015	Colombia	04°24′31.2″	71°58′44.5″	Phytal sample (Eichhornia)
CO-ET-1b-15	Estero Texas	04.02.2015	Colombia	04°24′31.2″	71°58′44.5″	spillway channel, permanent flooded
FL-PG-3-13	Shell Creek, Peace River	28.11.2013	Florida, US	26°58′26.99″	81°53′21.8″	littoral of an artifical slack water, with dense terrestrial macrophytes
FL-LX-4-15	Loxahatchee River	31.07.2014	Florida, US	26°56′46.91″	80°10′15.42″	root zone littoral of a river
FL-CAL-14-3	Caloosahatchee River	06.08.2014	Florida, US	26°50′22.4″	81°04′51.8″	artifical littoral with large stones and sand lenses
FL-CAL-14-4	Caloosahatchee River	06.08.2014	Florida, US	26°50′09.8″	81°05′14.4″	littoral, parts cased
FL-PR-15a/b-14	Shell Creek, Peace River	08.08.2014	Florida, US	26°58′26.99″	81°53′21.81″	littoral of an artifical slack water, with dense terrestrial macrophytes
FL-EG-3a/b-14	Everglades	02.08.2014	Florida, US	25°26′2.0″	80°45′12.3″	marsh
MX-CA-1-14	Cenote Azúl	12.08.2014	Mexico	18°48′43.3″	90°38′48.1″	hardground littoral of a cenote
MX-SiNo-1a-14	Siiji No-Ha Cenote	11.08.2014	Mexico	19°28′33.5″	88°03′15.6″	littoral, cenote
MX-CG-1-14	Cenote Galeana	11.08.2014	Mexico	19°27′45.6″	88°1′46.3″	littoral, cenote
MX-BC-1a-14	Laguna Bacalar	11.08.2014	Mexico	18°39′5.8″	88°24′33.07″	littoral of a lake, slight artificially altered
MX-Pul-1-14	Punta Laguna	13.08.2014	Mexico	20°38′49.4″	87°38′04.1″	littoral, karstified blocks proximate to thick layer of leaf litter and reed belt
MX-LG-1-14	Lake "Las Garantias"	13.08.2014	Mexico	18°27′11.7″	89°00′42.0″	steep littoral, hard ground
MX-Sil-1-14	Lake Silvituc	12.08.2014	Mexico	18°38′29.8″	90°16′28.1″	littoral of a lake

duration of ontogenetic development (i.e., intermoult period) allowing to characterize the time when the ostracod formed its calcitic valve.

Geographical variability of appendages

Many ostracod groups are regarded as species-poor with cosmopolitan distribution and little geographic structure [66]. Quantitative studies on intra- and interspecific variability of ostracod soft parts are rare. But it is generally accepted that soft parts are more conservative to environmental influences than hard parts [67]. Despite some overlap, allowed variability patterns of limb traits the discrimination of populations of a groundwater ostracod from different localities in western and south-eastern Europe [34]. As possible causes, the authors consider this variability patterns as micro-evolutionary changes and, besides founder and repeated colonization effects, organismically cued selectively neutral changes. However, juveniles were not incorporated into their analyses.

The presence of a geographical pattern revealed also by juvenile *Cytheridella* may point to a longer persistence of this pattern since it affects the whole morphology and is integrated into the ontogenetic development, too. Allometric coefficients and SSD values show also a certain geographical pattern. This pattern corresponds to that expressed by shape variability of the valves. In-depth analyses of valve shape variability of *Cytheridella* [23, 24] revealed that variability patterns exhibit a geographic structure and valve shape variation is correlated with environmental conditions (temperature, precipitation, hydrochemistry).

Fossil records of *Cytheridella* cover more or less the maximum geographical ranges of extant species and encompass more or less the Neotropical realm. It is hypothesized that passive dispersal via water birds has caused the wide distribution within the Neotropics. Founder events may have developed new habitat adaptions accompanied by successive morphological separation from other populations. Consistent with the monopolisation hypothesis all niches are occupied by the established species, although occasionally colonization events can occur [68]. Accordingly, the morphological (and genetic?) differentiation is higher within populations that are more distant since the interconnection possibility between water bodies is less probable than for closer populations. This could be a possible mechanism explaining this pattern of species with an assumed wide geographic range. As stated above, valves are assumed to be more sensitive to environmental influences than soft parts. The presence of a geographical structure in the variability of appendages therefore robustly reflects a differentiation of regional *Cytheridella* morphotypes.

The long-range dispersal as well as the differentiation have probably happened on longer (geological) time scales. Based on genetic divergence rates it is estimated

that two *Physocypria* species which can be morphologically distinguished by pigmentation and size may evolved within a lake between one and two million years ago [65].

The next necessary step to learn more about tempo and mode of speciation of *Cytheridella* in the Neotropics is the incorporation of fossil records from different (palaeo) habitats and geographical areas.

Hard and soft part variability of *Cytheridella*

This study shows that soft parts mirror the pattern of hard parts although there are some deviations regarding morphological similarities between particular regions especially Northern Brazil and Mexico. Valve analyses reveal a distinct separation of (some) Mexican populations from the other regions such as Florida. Contrarily, based on soft part traits the most distinct separation shows the Northern Brazilian population. Although the Mexican specimens display a relatively wide variability range (Figs. 6, 7) no distinct separation into two groups appears, as observed by valve shapes (Wrozyna et al., unpublished data). The differences between valve and soft parts derived patterns may result from a higher sensitivity of the valves to environmental influences [68, 69].

Actually, some other ostracod groups show the pattern as observed in *Cytheridella* with a high variability or disparity of valves in shape and/or ornamentation and slightly fewer variable limbs [32, 34]. Regional morphotypes of *Cytheridella* show variability by the same characters (e.g., A1, A2, T3) but are significantly discriminated indicating a certain degree of separation. It has to be tested by molecular analyses if and how much they are also genetically separated. The example of *Limnocythere inopinata* [32] shows, however, that the relationship between morphological variability, genotype, and environment is far from being straightforward. In this example, three clones from freshwater and saline field populations were cultured in the laboratory under various temperature and salinity conditions. Morphological traits including valve shape and size, and soft parts (limb setae) showed that some characters such as limb setae are surprisingly strongly affected by environmental conditions while other characters such as noding, which is traditionally considered ecophenotypic, seem to be predominantly influenced by the genotype. Although there were certain morphologies linked to specific ecologies, there were also morphologies occurring in opposite ecologies. Therefore, the authors concluded that all clones should be included in one (semi-)continuous morphological cluster representing one species. Contrary, [34] defined a new species based on quantitative differences of the valve shape and hypothesize the presence of more new species within the studied lineage and concluded the probability of the existence of several

geographically differentiated species rather than just only one widely dispersed species.

Here we show that *Cytheridella* can be discriminated on quantitative morphological characters into regional morphotypes implying a certain degree of genetic separation. Traditionally should species be defined by gaps within the morphological variation of a trait within a polymorphic lineage [70].

The large females of Northern Brazil provide the largest gap from the other morphotypes best displayed by the CVA (Fig. 7). The remaining smaller morphotypes represent also distinct clusters with different degrees of differentiation. Within this, Northern Brazil is significantly differentiated implying a rather isolated (endemic?) species. The largest overlap in the clusters, pointing to a relatively close relationship, is presented by Florida and Mexico. Surprisingly, Southern Brazil (and Colombia) show relatively small gaps to Florida/Mexico, which could point to much more recent divergence than, e.g., Northern Brazil.

If accepting the morphotypes as species this would provide evidence that *Cytheridella ilosvayi* is not as widespread as assumed in the Neotropics and there are more extant species as currently described.

Resulting from this, one could hypothesize that: (1) Carapaces can reliably reflect (beside possible environmental controls) genetic divergence and combined analyses with soft parts help to test the stability of the pattern. (2) The strength of the variability and affected limb characters might be the result of "degree of species differentiation" (although modulated environmentally). (3) Conclusions about the morphological variability strongly depend on the considered scale(s) and should ideally cover investigations within and among, as well as close and distant populations.

Due to low specimens' number of the single populations preventing reliable statistical analyses we summarized populations into regional groups. These groups could be composed of more morphologically discernible groups covering smaller geographical scales or even single water bodies.

Evidence has accumulated that many putatively widespread species in the Neotropics could in fact be complexes of cryptic taxa e.g., [13, 71]. This study shows that quantitative morphological analyses are powerful enough to reveal subtle differences in proportion or dimension of appendages (and podomeres) that are invisible by traditional (qualitative) observation. Investigations of relationships and systematics of (cryptic) species complexes require therefore morphometric approaches that provide sufficient resolution for identifying species.

Conclusions

Quantitative investigations of appendages (proportions, dimensions) represent an effective approach to obtain plenty of information about, e.g., ontogenetic development, sexual dimorphism, intra- and/or interspecific morphological divergence of an ostracod.

Largest variability of the appendages of *Cytheridella* adults and juveniles is shown by the antennae (A1, A2) and to a minor degree by the first thoracopod (T1). It has to be tested if this variability pattern is species or genus specific, respectively, or represents an additionally characteristic on a higher taxonomic level.

Size differences, soft part ratios, allometric coefficients, and body proportions display sexual dimorphism of *Cytheridella*. Limb dimensions and limb-limb proportions reflect female biased size dimorphism, which could indicate that female selection is the major cause for sexual size dimorphism. Accordingly, the large variability of antennae could be explained by the involvement into the mating process. Further, sex specific SSDs indicate that sexual selection affects limb traits.

Some soft part ratios display ontogenetic trends that allow to comprehend morphological modifications during larval development. Juveniles with reproductive anlagen exhibited very large variability ranges, which could be related to imminent moulting. *Cytheridella* could possess regionally varying life (and moulting) cycles indicated by the finding that specimens with reproductive anlagen were predominantly present in Mexican populations.

The variability of the appendages reflects a morphological divergence of regional morphotypes complimentary to the valve shape variability.

The geographical structure in the morphological variability allows the conjecture how widespread (freshwater invertebrate) species in the Neotropics may have arisen. Passive dispersal via e.g., birds can constitute the maximum ranges of a particular species. If a population has adapted to local ecological conditions and may have occupied all available niches it may impede colonisation through other species. Here, we found evidence for at least regional constraints in the distribution of the species but higher resolution sampling may reveal even smaller distributional ranges. Much higher number of species probably might indicate that *Cytheridella ilosvayi* represents a species complex. Relatively recent speciation(s) could explain why morphological divergence is not recognizable in qualitative investigations. This study highlights the benefits of the utilization of quantitative morphometric approaches for the investigation of widespread species and (cryptic) species complexes complementary to traditional qualitative considerations.

Methods
Soft part preparation
This study provides quantitative morphological investigations of the appendages of *Cytheridella*.

Sampling of *Cytheridella* populations were carried out in Florida, Mexico, Colombia, and Southern Brazil (Fig. 8). The analysed ostracod specimens were sampled in several environments on public land during 2013 to 2015 covering the geographical range of Neotropical *Cytheridella*. Details of the sample locations are summarized in Table Error! Reference source not found.. Data from Northern Brazilian specimens are derived from [30]. No *Cytheridella* species is listed in the IUCN Red List of Threatened Species (http://www.iucnredlist.org/) [72]. No permits were required for the described study, which complied with all relevant regulations.

Soft parts were detached from both valves using entomological needles, dissected, and mounted with Hydro-Matrix® (Micro-Tech-Lab, Graz, Austria) onto cover slides. Light photographs for analysis of soft parts were taken using a Zeiss Axioplan 2 and a digital camera Leica DFC 320. S1 Table gives an overview about the total specimen number and number from the different localities.

Additionally, the data set of a previous study [30] from a northern Brazilian population was included in this study since the most striking finding was the discovery of two female morphotypes, primarily separated by size, and one male. All investigated specimens were found to correspond to the species description by [16].

Quantification of morphological characteristics was performed according to [30] using ratios of podomere lengths and setae of the first and second antenna (A1, A2, respectively), and the first and second thoracopods (T1 and T2, respectively). In total, 16 characters were measured as linear distances (lengths). Limb lengths are compiled from the sum of the podomere lengths of the limb. The measurements were converted into 12 ratios that were selected in the previous study [30] based on normal distribution, homogeneity of variance and significance of differences of variable means to provide the reproducibility of the data.

The number of eggs and larvae in female specimens were also counted. For the investigation of the variability of the mandibles the masticatory process was considered in terms of shape and number of teeth. The presence of anlagen for reproductive organs in juvenile specimens were noted. SEM photographs of valves were taken in order to classify the juveniles to their developmental stage according to their valve lengths.

Statistical analyses

A PCA was performed in order to investigate the variances of the variables. Analyses were performed for the total dataset as well as separately for females, males, and juveniles (A-1) based on 12 soft part ratios according to [30].

Hotelling's T^2 test was used in order to test for significant differences of appendage lengths between sexes using data from all localities pooled together and population means.
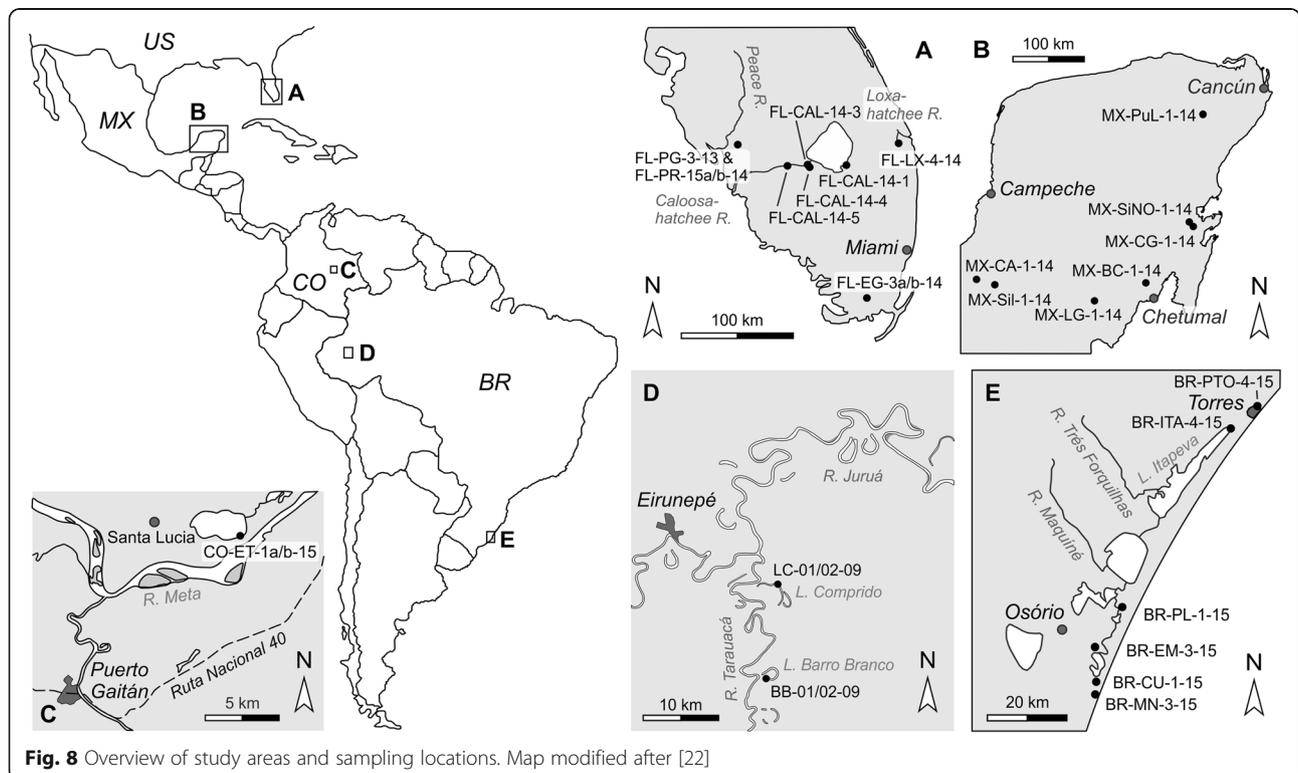
Fig. 8 Overview of study areas and sampling locations. Map modified after [22]

In order to assess whether populations differ in appendage dimensions we applied MANOVA) and CVA. Definitions of groups were based on geographic entities according to regions since the specimen numbers were too unevenly distributed which precluded comparisons of populations. All analyses were conducted on log-transformed ratios and performed separately for females, males and A-1. *P*-values of pairwise comparisons in MANOVA were Bonferroni-corrected.

Allometric analyses were performed on log-transformed lengths of the limbs (antennae A1 and A2, and thoracopods T1 and T2). Allometric coefficients were computed for each variable and region for all specimens and separately for females, males, and juveniles (A-1) based on a PCA in PAST version 2.11The first principal component (PC 1) was regarded as size axis when the variation accounted for by PC 1 was large (≥80%). Allometric coefficients are estimated by dividing the PC 1 loading for that variable by the mean loading over all variables [73].

Log-transformed limb lengths served as base to estimate sexual size dimorphism (SSD) in a log/log-plot from the slope of the regression line (ß) (compare [39, 74]. The slopes were estimated using RMA in PAST version 2.11.

This was done for all females and males as well as for means of two populations with high specimen number (≥ 50 specimens) of each region. The more accurate SDI was calculated according to [44] with SDI = (mean size of females/mean size males)-1.

Additional file

Additional file 1: Table S1. Overview of soft part ratios of *Cytheridella* with additional information about number of eggs and larvae found in female brood pouches and juveniles with anlagen of reproductive organs. Asterisks refer to specimens obtained from [31]. (XLSX 70 kb)

Abbreviations
A1: First antenna; A1EV-EIII: Fifth to third podomere of the first antenna; A2: Second antenna; A2EIIIGM: Apical claw of the second antenna; A2EIV-EII: Fourth to second podomere of A2; CVA: Canonical Variates Analysis; MANOVA: Multivariate Analysis of Variance; PCA: Principal Component Analysis; RMA: Reduced major axis regression; SDI: Sexual Dimorphism Index; SSD: Sexual Size Dimorphism; T1: First thoracopod; T1EIII-EII: Third to second podomere on T1; T2: Second thoracopod; T2EIII-EII: Third to second podomere on T2 (according to 29,54)

Availability of data and materials
Data of soft part ratios and limb lengths are provided in the supplementary information file.

Authors' contributions
CW, JM, and MG collected the data, JM, MIFR and MG discussed and commented on the manuscript. CW and WEP were major contributors in writing the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Publisher's Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details
[1]University of Graz, Institute of Earth Sciences, NAWI Graz Geocenter, Heinrichstraße 26, 8010 Graz, Graz, Austria. [2]University of Leipzig, Institute for Geophysics and Geology, Talstraße 35, 04109 Leipzig, Germany. [3]Universalmuseum Joanneum, Department for Geology & Palaeontology, Weinzöttlstrasse 16, 8045 Graz, Graz, Austria. [4]Museu Paraense Emilio Goeldi, Coordenacao de Ciencias da Terra e Ecologia, Avenida Perimetral, s/n Terra Firme, Belem-PA 66077-830, Brazil.

References
1. Carrillo JD, Forasiepi A, Jaramillo C, Sanchez-Villagra MR. Neotropical mammal diversity and the great American biotic interchange: spatial and temporal variation in South America's fossil record. Front Genet. 2014;5:451.
2. Basset Y, Cizek L, Cuenoud P, Didham RK, Guilhaumon F, Missa O, et al. Arthropod diversity in a tropical forest. Science. 2012;338(6113):1481–4.
3. Jaramillo C, Rueda MJ, Mora G. Cenozoic plant diversity in the Neotropics. Science. 2006;311:1893–6.
4. Toussaint A, Charpin N, Brosse S, Villeger S. Global functional diversity of freshwater fish is concentrated in the Neotropics while functional vulnerability is widespread. Sci Rep. 2016;6:22125.
5. Peréz L, Lorenschat J, Bugja R, Brenner M, Scharf B, Schwalb A. Distribution, diversity and ecology of modern freshwater ostracods (Crustacea), and hydrochemical characteristics of Lago Petén Itzá, Guatemala. J Limnol. 2010; 69(1):146–59.
6. O'Dea A, Lessios HA, Coates AG, Eytan RI, Restrepo-Moreno SA, Cione AL, et al. Formation of the isthmus of Panama. Sci Adv. 2016;2(8):e1600883.
7. Escobar J, Hodell DA, Brenner M, Curtis JH, Gilli A, Mueller AD, et al. A ∼43-ka record of paleoenvironmental change in the central American lowlands inferred from stable isotopes of lacustrine ostracods. Quat Sci Rev. 2012;37: 92–104.
8. Hoorn C, Wesselingh FP, ter Steege H, Bermudez MA, Mora A, Sevink J, et al. Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. Science. 2010;330(6006):927–31.
9. Hodell DA, Anselmetti FS, Ariztegui D, Brenner M, Curtis JH, Gilli A, et al. An 85-ka record of climate change in lowland Central America. Quat Sci Rev. 2008;27(11–12):1152–65.
10. Assine ML, Soares PC. Quaternary of the Pantanal, west-Central Brazil. Quat Int. 2004;114(1):23–34.
11. Colinvaux PA, de OPE. Amazon plant diversity and climate through the Cenozoic. Palaeogeogr Palaeoclim Palaeoecol. 2001;166:51–63.
12. Pérez L, Frenzel P, Brenner M, Escobar J, Hoelzmann P, Scharf B, et al. Late Quaternary (24–10 ka BP) environmental history of the Neotropical lowlands inferred from ostracodes in sediments of Lago Petén Itzá, Guatemala. J Paleolimnol. 2011;46(1):59–74.
13. Fouquet A, Gilles A, Vences M, Marty C, Blanc M, Gemmell NJ. Underestimation of species richness in neotropical frogs revealed by mtDNA analyses. PLoS One. 2007;2(10):e1109.
14. Bergmann PJ, Russell AP. Systematics and biogeography of the widespread Neotropical gekkonid genus *Thecadactylus* (Squamata), with the description of a new cryptic species. Zoolo J Linnean Soc. 2007;149(3):339–70.

15. Bohonak AJ, Jenkins DG. Ecological and evolutionary significance of dispersal by freshwater invertebrates. Ecol Lett. 2003;6(8):783–96.

16. Colin JP, Danielopol DL. Sur La Morphologie, La Systématique, La Biogéographie Et L'Évolution Des Ostracodes Timiriaseviinae (Limnocytheridae). Paleobiol Continentale 1980; 11(1):1–51.

17. Purper I. Cytheridella boldii Purper, sp. nov. (Ostracoda) from Venezuela and a revision of the genus Cytheridella Daday, 1905. An Acad Bras Ciênc. 1974; 46:635–62.

18. Baylac M, Villemant C, Simbolotti G. Combining geometric morphometrics with pattern recognition for the investigation of species complexes. Biol J Linnean Soc. 2003;80(1):89–98.

19. Edwards DL, Knowles LL. Species detection and individual assignment in species delimitation: can integrative data increase efficacy? Proc Biol Sci. 2014;281(1777):20132765.

20. Pfenninger M, Schwenk K. Cryptic animal species are homogeneously distributed among taxa and biogeographical regions. BMC Evol Biol. 2007;7: 121.

21. Lajus D, Sukhikh N, Alekseev V. Cryptic or pseudocryptic: can morphological methods inform copepod taxonomy? An analysis of publications and a case study of the Eurytemora affinis species complex. Ecol Evol. 2015;5(12):2374–85.

22. Wrozyna C, Neubauer TA, Meyer J, Ramos MIF, Piller WE. Significance of climate and hydrochemistry on shape variation – a case study on Neotropical cytheroidean Ostracoda. Biogeosciences. 2018;15(17):5489–502.

23. Wrozyna C, Meyer J, Gross M, Ramos MIF, Piller WE. Definition of regional ostracod (Cytheridella) morphotypes by use of landmark-based morphometrics. Freshwater Sci. 2018;37(3):573–92.

24. Wrozyna C, Neubauer TA, Meyer J, Piller WE. Shape variation in Neotropical Cytheridella (Ostracoda) using Semilandmarks-based geometric Morphometrics: a methodological approach and possible biogeographical implications. PLoS One. 2016;11(12):e0168438.

25. Horne DJ. Microfossils-Ostracoda. In: Selley RC, Cocks LRM, Plimer IR, editors. Encyclopedia of geology. Amsterdam, Boston, Heidelberg: Elsevier Academic Press; 2005. p. 453–463.

26. van der Meeren T, Verschuren D, Ito E, Martens K. Morphometric techniques allow environmental reconstructions from low-diversity continental ostracode assemblages. J Paleolimnol. 2010;44(4):903–11.

27. Baltanas A, Brauneis W, Danielopol DL, Linhart J. Morphometric methods for Apllied Ostracodology: tools for outline analysis of nonmarine Ostracodes. In: Park LE, Smith AJ, editors. Bridging the gap: trends in the Ostracode biological and geological sciences: Palaeontological society papers 9. New Haven: Paleontological Society; 2003. p. 101–18.

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

29. Okie JG, Boyer AG, Brown JH, Costa DP, Ernest SKM, Evans AR, et al. Effects of allometry, productivity and lifestyle on rates and limits of body size evolution. Proc Biol Sci. 2013;280(1764):20131007.

30. Wrozyna C, Piller WE, Gross M. Morphotypes of Cytheridella ilosvayi (Ostracoda) detected by soft and hard part analyses. Crustaceana. 2014; 87(8–9):1043–71.

31. Meisch C. Crustacea, Ostracoda. Heidelberg: Spektrum Akademischer Verlag; 2000.

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

33. Horne DJ, Danielopol DL, Martens K. Reproductive behaviour. In: Martens K, editor. Sex and parthenogenesis: evolutionary ecology of reproductive modes in non-marine ostracods. Leiden: Backhuys Publishers; 1998. p. 157–95.

34. Iepure S, Namiotko T, Danielopol DL. Morphological diversity and microevolutionary aspects of the lineage Cryptocandona vavrai Kaufmann, 1900 (Ostracoda, Candoninae). Ann Limnol-Int J Lim. 2008;44(2):151–66.

35. Sainte-Marie B. Morphological adaptations for carrion feeding in four species of littoral or circalittoral lysianassid amphipods. Can J Zool. 1984; 62(9):1668–74.

36. Weis JS, Cristini A, Rao KR. Effects of pollutants on molting and regeneration in Crustacea. Amer Zool. 1992;32(3).

37. Weis JS. Physiological, developmental and behavioral effects of marine pollution. Dordrecht, s.l. Netherlands: Springer; 2014.

38. Cohen AC, Morin JG. Patterns of reproduction in Ostracodes: a review. J Crustacean Biol. 1990;10(2):184–211.

39. Fairbairn DJ. Allometry for sexual size dimorphism: pattern and process in the coevolution of body size in males and females. Annu Rev Ecol Syst. 1997;28(1):659–87.

40. Blanckenhorn WU, Meier R, Teder T. Rensch's rule in insects: patterns among and within species. In: Fairbairn DJ, Blanckenhorn WU, Szekely T, editors. Sex, size and gender evolutionary studies of sexual size dimorphism. Oxford: Oxford University Press; 2007. p. 60–70.

41. Szekely T, Freckleton RP, Reynolds JD. Sexual selection explains Rensch's rule of size dimorphism in shorebirds. Proc Natl Acad Sci U S A. 2004;101(33): 12224–7.

42. Fairbairn DJ. Allometry for sexual size dimorphism: testing two hypotheses for Rensch's rule in the water strider Aquarius remigis. Am Nat. 2005;166:69–84.

43. Hirose GL, Fransozo V, Tropea C, López-Greco LS, Negreiros-Fransozo ML. Comparison of body size, relative growth and size at onset sexual maturity of Uca uruguayensis (Crustacea: Decapoda: Ocypodidae) from different latitudes in the South-Western Atlantic. J Mar Biol Ass. 2013;93(03):781–8.

44. Lovich JE, Gibbons JW. A review of techniques for quantifying sexual size dimorphism. Growth Dev Aging. 1992;56:269–81.

45. Fairbairn DJ, Blanckenhorn WU. Szekely T, editors. Sex, size and gender evolutionary studies of sexual size dimorphism. Oxford: Oxford University Press; 2007.

46. Rensch B. Die Abhängigkeit der relativen Sexualdifferenz von der Körpergrösse. Bonn Zool Beitr. 1950;1:58–69.

47. Abouheif E, Fairbairn DJ. A comparative analysis of Allometry for sexual size dimorphism: assessing Rensch's rule. Am Nat. 1997;149(3):540–62.

48. Teder T, Tammaru T. Sexual size dimorphism within species increases with body size in insects. Oikos. 2005;108(2):321–34.

49. Hirst AG, Kiørboe T. Macroevolutionary patterns of sexual size dimorphism in copepods. Proc Biol Sci. 2014;281(1791):20140739.

50. Dale J, Dunn PO, Figuerola J, Lislevand T, Szekely T, Whittingham LA. Sexual selection explains Rensch's rule of allometry for sexual size dimorphism. Proc Biol Sci. 2007;274(1628):2971–9.

51. Fairbairn DJ. Introduction: the enigma of sexual size dimorphism. In: Fairbairn DJ, Blanckenhorn WU, Szekely T, editors. Sex, size and gender evolutionary studies of sexual size dimorphism. Oxford: Oxford University Press; 2007. p. 1–10.

52. Smith RJ, Kamiya T. The ontogeny of Loxoconcha japonica Ishizaki, 1968 (Cytheroidea, Ostracoda, Crustacea). Hydrobiologia. 2003;490:31–52.

53. Smith RJ, Martens K. The ontogeny of the cyprid osttacod Eucypris virens (Jurine, 1820) (Crustacea, Ostracoda). Hydrobiologia. 2000;419:31–63.

54. Karanovic I. Recent freshwater ostracods of the world: Crustacea, Ostracoda, Podocopida. Berlin. New York: Springer; 2012.

55. Hartnoll RG. Growth in Crustacea - twenty years on. Hydrobiologia. 2001; 449:111–22.

56. Skinner DM. Interacting factors in the control of the crustacean molt cycle. Am Zool. 1985;25(1):275–84.

57. Kesling RV. The morphology of ostracod molt stages. Ill Biol Monogr. 1951; 21(1–3):1–364.

58. Yamada S, Keyser D. Calcification of the marginal infold in podocopid ostracods. Hydrobiologia. 2010;638(1):213–22.

59. Turpen JB, Angell RW. Aspects of molting and calcification in the ostracod Heterocypris. Biol Bull. 1971;140(2):331–8.

60. de Oliveira C, Renato J, Zhao B, Malecha S, Ako H, Yang J. Morphological and biochemical changes in the muscle of the marine shrimp Litopenaeus vannamei during the molt cycle. Aquaculture. 2006;261(2):688–94.

61. Isaacs G, Cockcroft AC, Gibbons MJ, Villiers de CJ. Determination of moult stage in the south African west coast rock lobster Jasus lalandii (h. Milne Edwards) (Crustacea: Decapoda). South Afr J M Sci. 2000;22(1):177–83.

62. Meyer J, Wrozyna C, Leis A, Piller WE. Modeling calcification periods of Cytheridella ilosvayi from Florida based on isotopic signatures and hydrological data. Biogeosciences. 2017;14(21):4927–47.

63. Bush MB, Metcalfe SE. Latin American and the Caribbean. In: Metcalfe SE, Nash DJ, editors. Quaternary Environmental Change in the Tropics. Chichester, UK: John Wiley & Sons, Ltd; 2012.

64. Punyasena SW, Eshel G, McElwain JC. The influence of climate on the spatial patterning of Neotropical plant families. J Biogeogr. 2007;35(1):117–30.

65. Karanovic I. Barcoding of ancient lake ostracods (crustacea) reveals cryptic speciation with extremely low distances. PLoS One. 2015;10(3):e0121133.

66. Taylor DJ, Finston T, Hebert PDN. Biogeography of a widespread freshwater crustacean: Pseudocongruence and cryptic endemism in the north American Daphnia laevis complex. Evolution. 1998;52(6):1648–70.

67. Park LE, Ricketts RD. Evolutionary history of the Ostracoda and the origin of nonmarine faunas. In: Park LE, Smith AJ, editors. Bridging the gap: trends in the Ostracode biological and geological sciences: Palaeontological society papers 9; 2003. p. 11–36.

68. de ML, Gómez A, Okamura B, Schwenk K. The monopolization hypothesis and the dispersal–gene flow paradox in aquatic organisms. Acta Oecol. 2002;23(3):121–35.

69. Park LE, Martens K, Cohen AS. Phylogenetic relationships of *Gomphocythere* (Ostracoda) in Lake Tanganyika, east Afria. J Crust Biol. 2002;22(1):15–27.

70. Balakrishnan R. Species concepts, species boundaries and species identification: a view from the tropics. Syst Biol. 2005;54(4):689–93.

71. Camargo A, de SRO, Heyer WR. Phylogenetic analyses of mtDNA sequences reveal three cryptic lineages in the widespread neotropical frog *Leptodactylus fuscus* (Schneider, 1799) (Anura, Leptodactylidae). Biol J Linnean Soc. 2006;87(2):325–41.

72. IUCN. IUCN Red list of threatened species; 2017. Available from: URL: www. iucnredlist.org.

73. Hammer Ø, Harper DAT, Hammer Ø, Harper DAT. Palentological data analysis. Malden, MA: Blackwell Publ; 2006.

74. Fairbairn DJ, Preziosi RF. Sexual selection and the evolution of Allometry for sexual size dimorphism in the water strider, *Aquarius remigis*. Am Nat. 1994; 144(1):101–18.