

# Rapid differentiation and asynchronous coevolution of male and female genitalia in stink bugs

B. C. GENEVCIOUS\*†, D. S. CAETANO‡ & C. F. SCHWERTNER\*

\*Department of Ecology and Evolutionary Biology, Graduate Program in Ecology and Evolution, Federal University of São Paulo, Diadema, SP, Brazil

†Museum of Zoology (MZUSP), University of São Paulo, São Paulo, SP, Brazil

‡Department of Biological Sciences, University of Idaho, Moscow, ID, USA

## Keywords:

*Chinavia*;  
intersexual conflict;  
morphometrics;  
Pentatomidae;  
sexual selection;  
sperm competition.

## Abstract

Despite claims that genitalia are among the fastest evolving phenotypes, few studies have tested this trend in a quantitative and phylogenetic framework. In systems where male and female genitalia coevolve, there is a growing effort to explore qualitative patterns of evolution and their underlying mechanisms, but the temporal aspect remains overlooked. An intriguing question is how fast male and female genitalia may change in a coevolutionary scenario. Here, we apply a series of comparative phylogenetic analyses to reveal a scenario of correlated evolution and to investigate how fast male and female external, nonhomologous and functionally integrated genitalia change in a group of stink bugs. We report three findings: the female gonocoxite 8 and the male pygophore showed a clear pattern of correlated evolution, both genitalia were estimated to evolve much faster than non-genital traits, and rates of evolution of the male genitalia were twice as fast as the female genitalia. Our results corroborate the widely held view that male genitalia evolve fast and add to the scarce evidence for rapidly evolving female genitalia. Different rates of evolution exhibited by males and females suggest either distinct forms or strengths of selection, despite their tight functional integration and coevolution. The morphological characteristics of this coevolutionary trend are more consistent with a cooperative adjustment of the genitalia, suggesting a scenario of female choice, morphological accommodation, lock-and-key or some combination of the three.

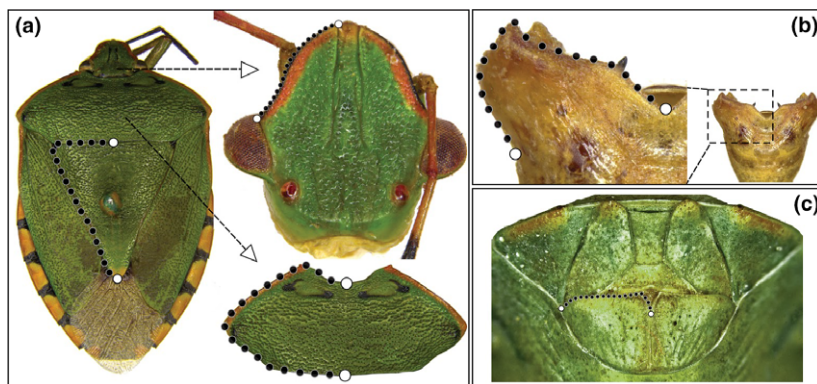
## Introduction

Extreme among-species divergence of genitalia is one of the most prevalent and noticeable trends of morphological evolution (Eberhard, 1985; Arnqvist, 1998). Despite increasing efforts to understand how selection drives this trend, studies have been disproportionately focused on male genitalia alone (Méndez & Córdoba-Aguilar, 2004; Puniamoorthy *et al.*, 2010). This male bias historically characterizes the research field and has resulted in much less reports with detailed investigations of the female genitalia. Two frequent, but questionable, presumptions supporting the lesser value of the female

genitalia to comparative studies are the dominant role played by males in the copulatory process and the inherent lack of morphological variation of the female genitalia (Ah-King *et al.*, 2014). Overall, few model groups such as *Drosophila* flies (e.g. Fritz & Turner, 2002; Jagadeeshan & Singh, 2006) and seed beetles (e.g. Polihronakis, 2006; Rönn *et al.*, 2011; Hotzy *et al.*, 2012) are exceptions to this bias, and most studies using these models illustrate the crucial role of female genitalia in the selective pressures driving the evolution of genitalia as a whole.

Despite the frequently neglected role of female sexual traits in evolution, evidence for male–female genital coevolution is strong for a variety of taxa (Brennan & Prum, 2015). Similar patterns of male–female genital covariation can result from both sexual and natural selection. For instance, natural selection through lock-and-key may boost fast codifferentiation of male and

Correspondence: Bruno C. Genevcius, Av. Nazaré, 481, Ipiranga, São Paulo (SP), Brazil. zip code: 04263-000.  
Tel.: +55(11) 2065-8100; fax: +55(11) 2065-8100;  
e-mail: bgenevcius@gmail.com



**Fig. 1** Morphological traits used in the geometric morphometric analyses.

Somatic traits (a): left half of head (top right), scutellum (left) and pronotum (bottom right). Male genitalia (b): left posterolateral angle of the genital capsule. Female genitalia (c): posterior margin of left gonocoxite 8. White dots are fixed landmarks, and black dots are sliding semilandmarks.

female genitalia as means of species reinforcement (Kameda *et al.*, 2009). Likewise, coevolution should be expected as an inherent consequence of cycles of intersexual conflict over mating frequencies (Bergsten & Miller, 2007). Under cryptic female choice, male and female genitalia may adapt to each other in a cooperative fashion to guarantee a proper fit of the genitalia (Huber, 2005; Eberhard & Huber, 2010). Regardless of the mechanism involved, genital coevolution is apparently more widespread than previously thought (Yassin, 2016) and can be expected particularly when the genitalia show direct and tight interaction during copula (Simmons, 2014; Brennan & Prum, 2015). In quantitative terms, male and female genitalia have been shown to covary with respect to size (e.g. Brennan *et al.*, 2007), complexity (e.g. Kuntner *et al.*, 2009) and shape (e.g. Evans *et al.*, 2011). However, we are unaware of studies that have estimated and compared the degree of morphological change exhibited by primary genital components of male and female in coevolutionary systems.

Rates of differentiation of primary genitalia have only been assessed separately for the sexes (e.g. Takami & Sota, 2007; Puniamoorthy *et al.*, 2010; Rowe & Arnqvist, 2012). McPeck *et al.* (2009) compared evolutionary rates of male primary genitalia and a female secondary sexual trait in damselflies, revealing a scenario of coevolution and similar modes and rates of differentiation. However, one cannot always expect similar rates of evolution in coevolutionary systems because two traits mechanically engaged in copula may experience distinct forms or strengths of selection (Rowe & Arnqvist, 2012). Furthermore, different mechanisms of both pre- and post-copulatory selection may yield indistinguishable patterns of rates of evolution, thus making rates of change hard to predict even when the mechanism is known *a priori*. Nonetheless, estimates of evolutionary rates of two coevolving genitalia may provide novel and valuable information about the temporal aspect of genital coevolution, a topic that has been broadly overlooked. By comparing rates of evolution, it is possible to investigate whether two sets of

traits may respond similarly to a selective pressure in terms of amount of phenotypic change. Here, we address this subject using a clade of Neotropical stink bugs as model.

The stink bugs (Hemiptera, Pentatomidae) comprise a diverse family of true bugs with polygamous mating system (McLain, 1992; Wang & Millar, 1997). Their external male genitalia are complex and formed by a series of folds and projections that comprise the genital capsule (pygophore). The pygophore is characterized by striking among-species diversity and very low within-species variation (Rolston, 1974; Greve *et al.*, 2013; Genevicius & Schwertner, 2014). In contrast, the external genitalia of females are relatively simple (Scudder, 1959; McDonald, 1966), comprising five plates whose variation is mainly bidimensional (Fig. 1). Among-species variation in female plates has been overlooked in pentatomids (Garbelotto *et al.*, 2013), possibly due to the absence of remarkable structures such as folds, projections and hooks, commonly found on male genitalia (Sharp, 1890). The copulatory mechanism of the external parts of the genitalia is far from completely understood in Heteroptera as a whole (Forero & Weirauch, 2012). The only study describing the external genital coupling in the Pentatomidae is very simplistic and overlooked or superficially explored numerous structures (Leston, 1955). However, at least one pair of female plates, the gonocoxites 8, have been suggested to interact with the pygophore in the genital coupling during mating, what has been also observed in other related families (e.g. Bonhag & Wick, 1953). The gonocoxites 8 are firmly pressed against the folds of the pygophore acting as a bridge, allowing the male phallus to penetrate the female tract where sperm transfer takes place (Leston, 1955).

Since male pygophores are highly species specific, most likely the fastest evolving phenotypes in Pentatomidae, and operate exclusively on the external attachment with the female gonocoxites 8, we hypothesize these two traits should show some degree of evolutionary covariation. If both genitalia diversify under a common selective pressure or in response to evolutionary

changes experienced by the other sex, we predict that the male pygophore and the female gonocoxites 8 should evolve faster than nonsexually selected traits. Herein we test whether male and female external genitalia of stink bugs coevolve. Further, we estimate the rates of evolution of male and female genitalia to test whether these traits change at distinct rates despite their coevolution and functional integration. Finally, we assessed how fast the genitalia change by comparing their rates with those of three nonsexually selected traits.

## Material and methods

### Study system and species sampling

Pentatomids are good systems for studies on sexual selection and evolution of genitalia for numerous reasons. Generations are relatively short, copula and oviposition occur at high frequencies, and they have a wide variety of sexual behaviours (Wang & Millar, 1997; Zahn *et al.*, 2008; Silva & Panizzi, 2009; Fortes & Consoli, 2011). Most importantly, males and females are promiscuous and both have internal and external genitalia composed by various components that may foster interesting ecological and evolutionary questions. Male and female external genitalia are well developed, rigid and sclerotized, facilitating their handling, preservation and measurements (Singh-Pruthi, 1925; Scudder, 1959). Nonetheless, the majority of studies with these insects are focused on agronomic aspects and traditional taxonomy; thus, little is known about their evolution.

Recently, Genevicius & Schwertner (2014) published a phylogenetic hypothesis based on morphological data with 28 species of the genus *Chinavia* (Insecta: Hemiptera: Pentatomidae) from the Afrotropical, Nearctic and Neotropical regions (TreBASE access: <http://purl.org/phylo/treebase/phyloids/study/TB2:S20305>). We selected the Neotropical clade with strongest phylogenetic support plus three outgroups to represent well the morphological diversity spread across the phylogeny. We did not use all species because either males or females are completely unknown for some of these species. Some species are also known from two or three individuals only, to which we did not have access. Our sampling criterion resulted in 13 species, and at least three specimens of each species were analysed. We believe three individuals of each species are fairly reasonable for the following reasons. Characters of external genitalia are the most putative and reliable to distinguish between species in Pentatomidae. Virtually, all taxonomic studies in the family reveal considerable and remarkable differences even between sister species (e.g. Genevicius *et al.*, 2012; Greve *et al.*, 2013; Fernández-Aldea *et al.*, 2014), implying that within-species variation may not be quite significant. Studies with species

groups and complexes in the family give support to this assertion (Rolston, 1974; Memon *et al.*, 2006). There is also evidence of low or null populational variation exhibited by pygophores and female plates (Cruz *et al.*, 2011; Peredo, 2013; Coudron *et al.*, 2015).

### Morphology and morphometric data

To test for coevolution and to evaluate how fast male and female genitalia evolve, we estimated and compared the patterns of evolution of three nongenital (somatic) and two genitalic traits. Somatic structures were the expanded dorsal region of the head (i.e. juga), scutellum and pronotum (Fig. 1a). We used three criteria to choose these somatic traits. First, we selected structures whose variations were comprehensively bidimensional, therefore better represented as 2D shape variables (i.e. length-based traits such as legs and antennae were not considered). Second, we chose structures most likely unrelated to any form of sexual selection to avoid possible evolutionary codependence among somatic and genitalia characters. Behaviour studies do not report any role of those traits in courtship or mating behaviour in the Pentatomidae (Gamboa & Alcock, 1973; Kon *et al.*, 1988; Krupke *et al.*, 2008; Zahn *et al.*, 2008). Third, we sought for a set of structures that might comprise the fastest evolving structures among somatic traits, thereby offering the most conservative reference to test whether the genital traits evolve relatively fast. Although there is no direct evidence for rapidly evolving structures in the Pentatomidae, these three somatic structures are consistently the most variable and informative in taxonomic and phylogenetic studies at the species level with respect to shape (e.g. Bernardes *et al.*, 2009). Studies with species complexes in the family have shown the potential of such characters to differentiate even among species with probable recent divergence (Genevicius *et al.*, 2012; Fernández-Aldea *et al.*, 2014). In particular, the shape of head is remarkably divergent in phytophagous hemipterans as a result of shifts in feeding habits and habitat (Torres *et al.*, 2010; Zahniser & Dietrich, 2015).

The genital traits include the left posterolateral angle of the male pygophore (Fig. 1b) and the posterior margin of the female left gonocoxite 8 (Fig. 1c). Although the exact interaction between the pygophore and the gonocoxites in copula has not been described in detail by Leston (1955), the posterolateral angle and the posterior margin are basically the two parts of the external genitalia that has been shown to vary in males and females, respectively (Grazia *et al.*, 2006; Genevicius *et al.*, 2012; Genevicius & Schwertner, 2014).

Landmark-based geometric morphometrics was used to capture phenotypic variation of three nongenital and two genital traits. To capture shape variation of the studied traits, we used 20 landmarks for each structure. The first and the last landmarks were fixed in each

structure and all the remaining were sliding semilandmarks (Fig. 1). We obtained all landmarks from photographs taken in dorsal view using a stereomicroscope Leica MZ135 and the software tpsDig2 (Rohlf, 2006). We performed a generalized Procrustes analysis using the R package 'geomorph' (Adams & Otárola-Castillo, 2013) to remove the effects of position, scale and rotation, also allowing the semilandmarks to slide along their tangent directions based on minimizing bending energy. Mean coordinates of each species were used for further analyses (see Fig. S1 for shape means, standard errors and detailed samples sizes).

### Evolutionary allometry

Evolutionary allometry in the context of shape analysis is the evolutionary change in shape that is associated with evolutionary change in size (see Klingenberg & Marugán-Lobón, 2013 for a short review). Body size can often have a significant and nonlinear effect in traits, including shape, and especially in the presence of sexual selection (Eberhard, 2009). Thus, we performed phylogenetic regression of the shape data in function of the log-transformed centroid size using phylogenetic generalized least squares (PGLS) (Adams, 2014) to test whether the evolutionary change in shape is associated with change in size. We repeated all subsequent analyses with the residuals from the phylogenetic multivariate regression of shape on size for all traits that showed significant effect of evolutionary allometry.

### Tests of correlated evolution

To test whether the shape of the male and female genitalia is evolutionarily correlated, we employed phylogenetic two-block partial least-squares (PLS) analyses of the shape coordinates (Adams & Felice, 2014). Since this analysis is based on PGLS rather than phylogenetic independent contrasts (PIC) (Rohlf & Corti, 2000; Klingenberg & Marugán-Lobón, 2013), we were able to investigate the patterns of evolutionary integration using the species data, which facilitates biologically relevant interpretations. Because our phylogenetic tree does not show branch lengths proportional to time, we tested for correlation between male and female genitalia using both a phylogeny with all branch lengths equal to 1 and an ultrametric tree with branch lengths calculated under Grafen's (1989) method (for similar approaches see Midford *et al.*, 2008; Kuntner *et al.*, 2009; Lichter-Marck *et al.*, 2015; Sheehy *et al.*, 2016). Following, we used the covariance ratio coefficient (CR) (Adams, 2016) estimated based on the phylogenetic contrast of the shape data (Klingenberg & Marugán-Lobón, 2013) to test whether the genitalia of males and females are evolutionarily correlated with the somatic traits. This analysis can help discard the possibility that an eventual

morphological correlation between male and female genitalia would be a result of strong correlation among all measured structures due to other factors that are not directly relevant to our study. The CR coefficient is a measure of modularity and represents the ratio of the covariance between the groups relative to the covariance within the groups (Adams, 2016). If the shape of male and female genitalia shows correlated evolution independent of the somatic traits, this analysis would yield support to two modules: one formed by the genitalia and the other by the somatic traits. We used the recently described CR coefficient (Adams, 2016) instead of the frequently applied RV coefficient (e.g. Klingenberg & Marugán-Lobón, 2013) because the RV coefficient can be biased by the imbalance in the number of variables, whereas the CR coefficient is robust to this effect. We repeated the CR coefficient test using the phylogeny with both configurations of branch lengths.

### Estimates of evolutionary rates

We estimated the evolutionary rates of the traits using the Brownian motion rate parameter for multidimensional data ( $\sigma_{\text{mult}}^2$ ; Adams, 2014). This approach is more suitable for geometric morphometric data than other methods that rely on the estimation of the evolutionary covariance matrix  $R$  (Revell & Harmon, 2008; Adams, 2014). To test whether the genitalia shape evolved faster than somatic traits, we independently estimated  $\sigma_{\text{mult}}^2$  for the male and female genitalia and for the male and female scutellum, pronotum and juga. Then, we used the ratio between the rates of evolution of the genitalia (for males and females separately) and each of the three somatic traits ( $\sigma_{\text{mult.G}}^2/\sigma_{\text{mult.S}}^2$ ). Finally, we used the ratio between the rate of evolution of the male and female genitalia ( $\sigma_{\text{mult.M}}^2/\sigma_{\text{mult.F}}^2$ ) to test whether male genitalia evolve faster than the female genitalia. For each test, we performed Monte Carlo simulations by shuffling shape coordinates between the two groups 1000 times (genitalia and somatic traits or male and female genitalia) and computing the  $P$ -value as the quantile of the observed ratio in function of the resulting null distribution (Adams, 2014; Denton & Adams, 2015).

Since our estimates of  $\sigma_{\text{mult}}^2$  are based on a tree with arbitrary branch lengths, we performed simulations to test whether the lack of branch lengths proportional to time could hinder our ability to estimate and compare rates of trait evolution. For this, we simulated birth-death trees constrained to match the number of species included in our data (13 spp.). We set the root state as the mean shape across all species for the male and female genitalia and scutellum. We used a homogeneous Brownian motion model to generate data under the simulated trees following the same approach as Adams (2014). We simulated two sets of multivariate



traits with the same rate ( $R_1$  and  $R_2$  = diagonal rate matrices of 0.5) and different rates ( $R_1$  = diag 0.5 and  $R_2$  = diag 1.0). Finally, we arbitrarily transformed the branch lengths of the simulated birth–death trees using two configurations: all branches equal to 1 and branch lengths computed using Grafen’s (1989) method. We used the simulated data and trees to estimate the ratio of rates ( $\sigma_{\text{mult.1}}^2/\sigma_{\text{mult.2}}^2$ ) and check whether results vary in function of different sets of branch lengths. If arbitrary branch lengths do not bias our results, we would expect no difference across treatments. We performed all comparative analyses and simulations in the statistical environment R (R Development Core, 2016) using custom code and the R package ‘geomorph’ (Adams & Otárola-Castillo, 2013). Data and scripts to reproduce all analyses, simulations and graphs are available at [https://github.com/Caetanods/Geniculata\\_rates](https://github.com/Caetanods/Geniculata_rates).

## Results

### Genitalia show no effects of evolutionary allometry

The PGLS regression of male and female genitalia shape on log-transformed centroid size resulted in no correlation independent of the choice of branch lengths (Table 1). Among the somatic traits, only pronotum and scutellum of females showed an effect of evolutionary allometry (Table 1). All subsequent analyses including any of these somatic traits were repeated with the residuals of the phylogenetic correlation of shape on log-transformed size.

### Male and female genitalia show correlated evolution

The shape of male and female genitalia showed significant correlated evolution according to the phylogenetic two-block partial least-squares (PLS) analyses using both the ultrametric tree ( $P = 0.018$ ; Fig. 2) and the phylogeny with branch lengths equal to 1 (Fig. S2).

**Table 1** Results from evolutionary allometry tests. Table shows significance levels for phylogenetic regression of shape on log-transformed centroid size. Unit tree has all branch lengths equal to 1 and Grafen tree has branch lengths computed with Grafen’s (1989) method. The ‘\*’ marks each significant result at the 5% threshold level.

Shape	Unit tree	Grafen tree
Male genitalia	0.286	0.477
Female genitalia	0.224	0.081
Male scutellum	0.062	0.127
Female scutellum	0.034*	0.09
Male pronotum	0.182	0.47
Female pronotum	0.009*	0.03*
Male juga	0.536	0.782
Female juga	0.509	0.529

Partial least-squares analysis reduces the dimensionality of the data by generating singular axes that are oriented towards the major axes of covariation among the traits in the data. The first PLS axis corresponds to 75% of the covariation and the second axis to 15%, and combined they explain 95% of the covariation in the data (Fig. 2). Both axes show strong correlation (PLS1  $r = 0.85$  and PLS2  $r = 0.84$ ). The analysis of modularity gives further support to the conclusion that male and female genitalia are evolutionarily correlated. The estimated CR coefficient between the phylogenetic contrast of shape coordinates of the genitalia and somatic traits is smaller than expected by chance independent of the set of branch lengths (both  $P < 0.001$ ; Fig. 3). Since some of the somatic traits showed a significant effect of evolutionary allometry, we repeated the analyses of modularity controlling for size, but results are quantitatively similar.

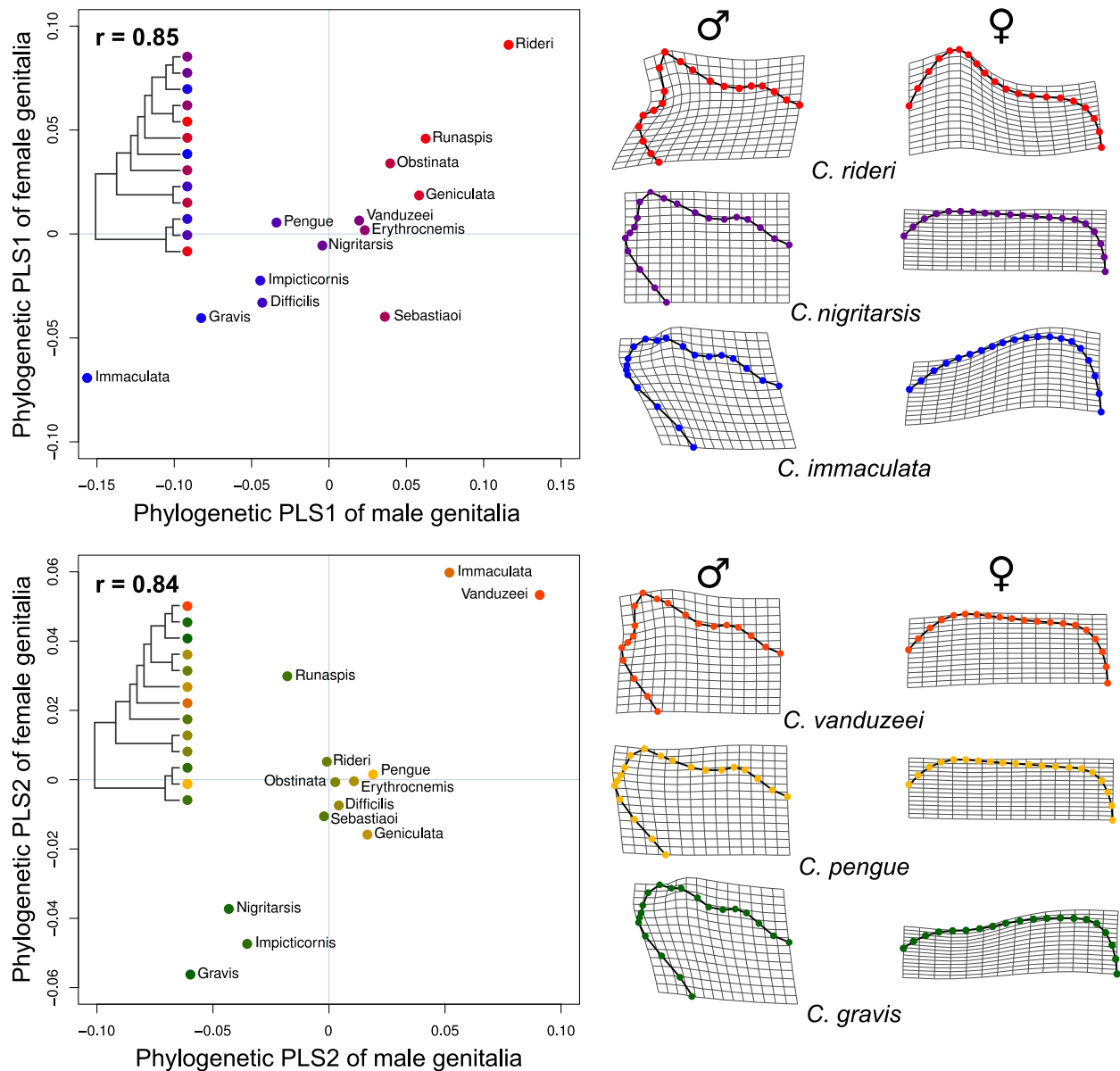
### Both genitalia evolve fast, but male genitalia evolve faster

Male and female genitalia showed faster rates of evolution when compared with somatic traits independently of the choice of branch lengths (in all cases  $P < 0.01$ , Table 2). This means that, regardless of the degree of phylogenetic relatedness, both genitalia are much more variable among species than any of the somatic traits (Fig. 4). These results were also unaffected by evolutionary allometry. Simulations showed no bias in the estimates of the ratio of  $\sigma_{\text{mult}}^2$  produced by the choice of arbitrary sets of branch lengths. We were able to recover accurate estimates of the ratios under all sets of simulated branch lengths (Fig. 5).

The shape of male genitalia showed the fastest evolutionary rates, approximately twice as fast as the female genitalia, and much faster than the somatic traits (Table 2). When setting all branch lengths equal to 1, male genitalia were 2.36 times as fast as the female genitalia using the raw data and 2.22 times faster using data corrected for evolutionary allometry. With tips lined up in time, male genitalia were 1.74 times faster using raw data, whereas this difference rises to 1.89 using the data corrected for the effects of evolutionary allometry.

## Discussion

Our comparative analyses of male and female genitalia of stink bugs revealed three interesting results. First, male and female external genitalia showed a clear pattern of correlated evolution. Second, both genitalia were estimated to evolve much faster than nongenital traits. Third, rates of evolution of the male genitalia were approximately twice as fast as the female genitalia. Below we discuss the evolutionary trends of the genitalia in the group, the observed differences in

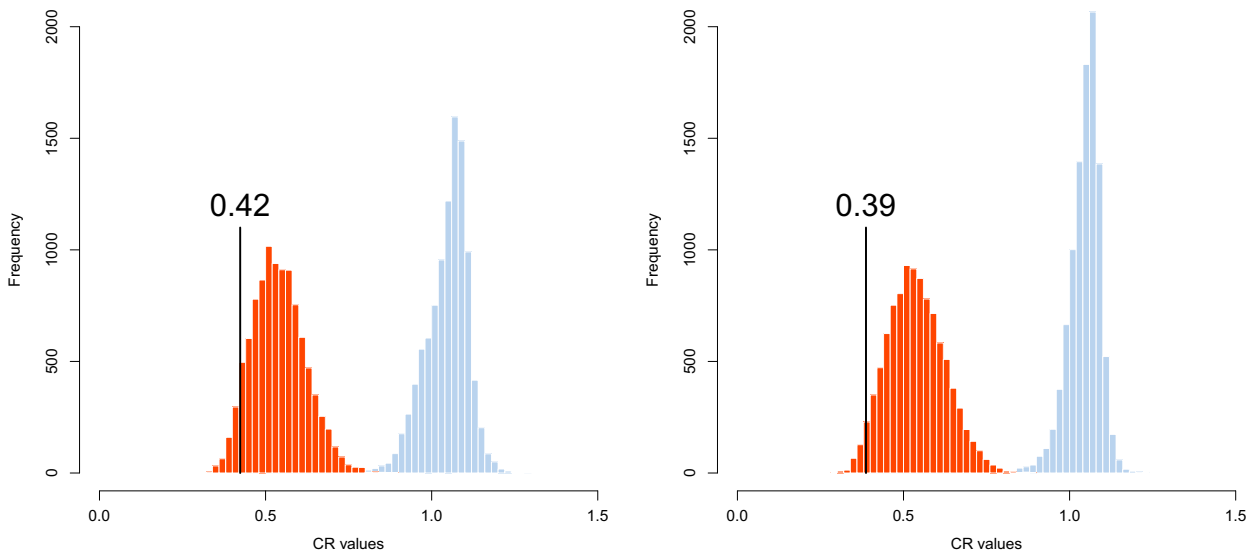


**Fig. 2** Correlated evolutionary changes of male and female genitalia. Left figures show the results of phylogenetic two-block partial least-squares (PLS) analysis between the male and female genitalia shape ( $P = 0.018$ ) using branch lengths computed with Grafen's method. First and second PLS axes explain, respectively, 75% and 15% of the evolutionary covariance in shape. Figures on the right show landmark configurations of species mean shape of male and female genitalia for some selected species spread along the PLS axes (see Fig. S3 for configurations of all species). Left column of landmarks is the left posterolateral angle of the genital capsule of males and right column is the posterior margin of left gonocoxite 8 of females (see Figure 1).

evolutionary rates and the probable mechanisms driving the evolution of these traits.

As predicted, we revealed a scenario of intersexual genital covariation. This pattern is supported by two independent analyses, the phylogenetic PLS and the modularity test. Although we view these results as robust evidence of coevolution, one could argue that this same scenario could also be the outcome of an

ontogenetic dependence among the traits. This may be true for homologous traits that show strong cross-sex genetic correlation (Lande, 1980; Poissant *et al.*, 2010). However, although the genetic basis of these two traits has not been studied in the group, their developmental architecture is presumably different since the pygophore is derived from the ninth abdominal segment and the gonocoxites 8 are derived from the eighth



**Fig. 3** Analysis of evolutionary modularity. CR coefficient test between the phylogenetic independent contrasts of genitalia and somatic traits. Left plot shows results based on phylogenetic tree with all branch lengths equal to 1 and right plot shows results from ultrametric tree (both  $P = 0.0001$ ). Null distributions were generated by calculating CR values from 1000 permutations of landmarks between the genitalia and somatic groups and are represented in blue. Red distributions show results from bootstrap simulations and the vertical lines mark the estimate of the CR coefficient.

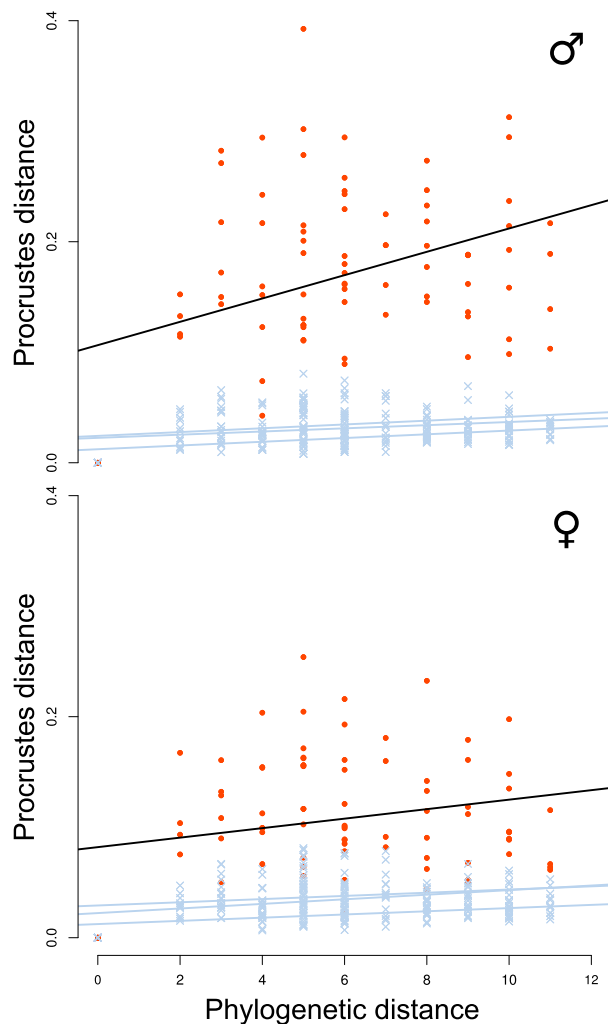
**Table 2** Estimates of evolutionary rates between genitalia and somatic traits. Columns show results of  $\sigma^2_{\text{mult}}$  ratio tests and  $P$  values with raw shape data and size corrected data. Size correction was performed by using the residuals of a phylogenetic regression of shape on log-transformed centroid size. Unit tree has all branch lengths equal to 1 and Grafen tree has branch lengths computed with Grafen's (1989) method. Values are the estimated ratio between  $\sigma^2_{\text{mult}}$  rates, followed by the respective significance level computed using Monte Carlo simulations.

Rate ratio	Raw data		Size corrected data	
	Unit tree	Grafen tree	Unit tree	Grafen tree
$\sigma/\varnothing$ genitalia	2.36 (0.001)	1.74 (0.003)	2.22 (0.001)	1.89 (0.003)
$\sigma$ genitalia/ $\sigma$ scutellum	34.66 (0.001)	25.547 (0.001)	57.152 (0.001)	42.27 (0.001)
$\sigma$ genitalia/ $\sigma$ pronotum	18.54 (0.001)	12.194 (0.001)	21.192 (0.001)	14.224 (0.001)
$\sigma$ genitalia/ $\sigma$ juga	17.097 (0.001)	11.13 (0.001)	20.446 (0.001)	17.8 (0.001)
$\varnothing$ genitalia/ $\varnothing$ scutellum	19.232 (0.001)	19.421 (0.001)	25.754 (0.001)	22.39 (0.001)
$\varnothing$ genitalia/ $\varnothing$ pronotum	8.412 (0.001)	7.9 (0.001)	9.55 (0.001)	7.533 (0.001)
$\varnothing$ genitalia/ $\varnothing$ juga	6.382 (0.001)	7.42 (0.001)	9.213 (0.001)	9.43 (0.001)

segment (Grimaldi & Engel, 2005; Tsai *et al.*, 2011). Results from the modularity tests also show that the genitalia and the somatic traits constitute two structured evolutionary groups that are relatively independent of each other (i.e. evolutionary modules), suggesting a lack of evolutionary covariation between genitalia and somatic traits. This indicates that male–female genitalia coevolution cannot be simply explained by overall morphological integration due to allometry, pleiotropy, ontogenetic constraints or other processes unrelated to the morphological integration of genitalia in copula (Klingenberg, 2008; Voje *et al.*, 2014; Macagno & Moczek, 2015). For these reasons, we

believe the pattern we found is better explained by an evolutionary influence of one genitalia on the evolution of the other, directly or indirectly, as result of the functional integration between the traits.

Diversification in male and female genitalia has been quantified only in separate studies using distinct systems. For instance, male genitals in ground beetles and *Anolis* lizards have been estimated to evolve faster than nongenital traits (Takami & Sota, 2007; Klaczko *et al.*, 2015). In sepsid flies, there is evidence that female genitalia may evolve rapidly when contrasted with behavioural features (Puniamoorthy *et al.*, 2010). What is common to such studies is that the genital traits in



**Fig. 4** Pairwise Procrustes distances between the shape of male and female genitalia and somatic characters in function of the phylogenetic distance. Red circles and black lines represent pairwise distances between genitalia shapes, whereas crosses and lines in blue show pairwise distances between somatic shapes. Phylogenetic distance is the sum of branch lengths between species and was computed with branch lengths all equal to 1.

question are exclusively internal and intromittent. Because internal genitalia are directly associated with sperm transfer, reception and storage, it is intuitive that post-mating sexual selection should be responsible for fast divergence in such cases (Polak & Rashed, 2010), whereas nonintromittent genitalia would be subject to other selective mechanisms (Lupše *et al.*, 2016). External and nonintromittent sexual characters have been previously suggested to be more evolutionarily constrained when they act to maintain a tight fit in copula (Eberhard, 2006). Although this prediction has not been explicitly tested, Rowe & Arnqvist (2012) showed that nonintromittent genitalia are slightly more

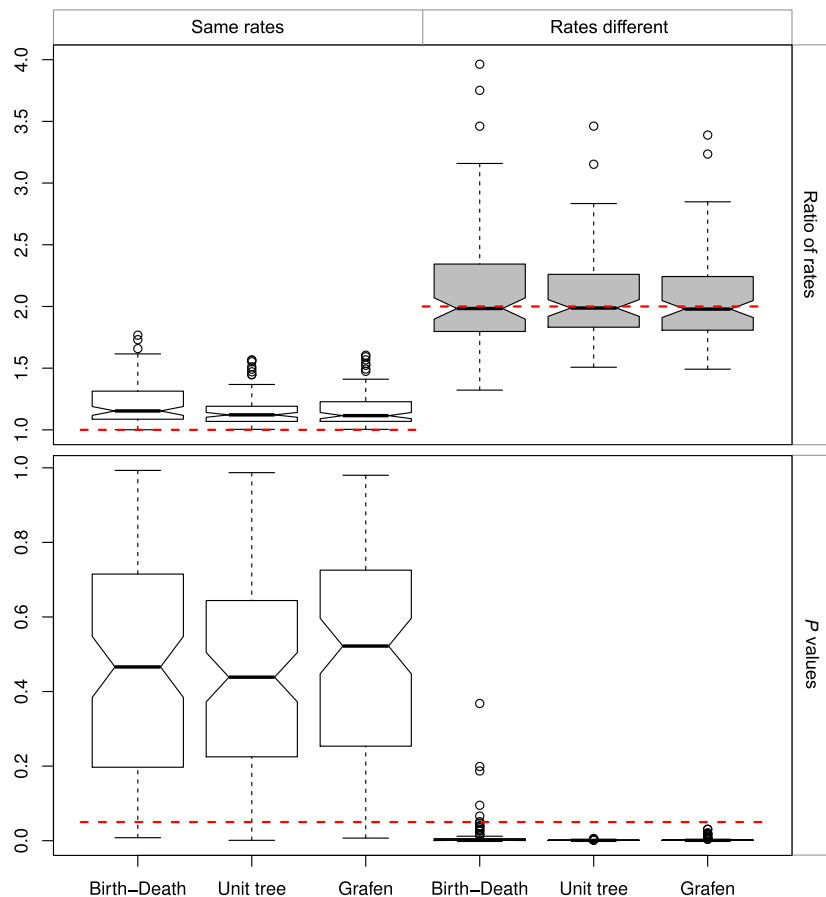
divergent and complex than somatic structures, whereas intromittent genitalia are strongly more divergent and complex than both. Contrary to this prediction, we show that two exclusively external and nonintromittent genital structures evolve strikingly fast. We are unaware of other somatic structures in pentatomids that could potentially show more among-species variation than the observed for juga, pronotum and scutellum. These three structures, together with some colouration aspects, correspond to the majority of non-genital morphological information used to define and recognize species in *Chinavia* and Pentatomidae as a whole (e.g. Schwertner & Grazia, 2007; Fernández-Aldea *et al.*, 2014; Genevcious & Schwertner, 2014), thus being most likely among the fastest evolving somatic structures across the family.

We show that the pygophore evolved approximately twice as fast as the gonocoxite 8, despite their evolutionary and mechanical integration, and that both genital traits show higher rates of evolution in comparison with the somatic traits. Although branch lengths proportional to time could have strengthen our results by allowing more detailed interpretations of the absolute value of parameter estimates, our simulations provide evidence that the lack of informative branch lengths does not implicate in misleading estimates of relative rates of evolution. This happens because the effect of the arbitrary change in branch lengths is shared by all structures when rates are estimated in a given tree, and is counterbalanced when the ratio of rates is calculated. We caution that further studies are necessary to understand whether such disparate rates in coevolutionary systems can also be observed at broader scales, especially considering that the phylogeny used here is morphology-based and reasonably small. We strongly encourage similar investigations using larger groups and other models with robust phylogenies estimated from molecular data and calibrated with molecular clocks and fossils.

The observed differences in rates of evolution are possible in three scenarios: differing strengths of selection in each genitalia, distinct balances of selection and constraint or completely distinct evolutionary mechanisms (Rowe & Arnqvist, 2012). It is difficult to address with our data which sex triggers the observed evolutionary responses on the other because we do not know which of the traits is primarily subjected to some form of selection. In systems where female traits are known to evolve in response to males, there is evidence at inter- and intrapopulation scales that sexually selected male traits start to diverge first and accumulate changes in shorter intervals when compared to female traits (Wigby & Chapman, 2004; Cayetano *et al.*, 2011). This mechanism of delayed coevolution could help explain why females often show relatively less amount of change in the macroevolutionary scale such as in our study. A plausible reason for such asynchronous



**Fig. 5** Results of simulations showing that estimates of ratio between rates under Brownian motion are not biased by the lack of informative branch lengths. We simulated data using 100 single rate birth–death trees and estimated the rates ratio under different configurations of branch lengths while maintaining the topology constant. Upper plots show pooled estimates of the ratio between rates and red horizontal lines mark the true value of the simulations. The minimum ratio is 1 because the method by Adams (2014) calculates only the ratio between the larger and smaller rates. Bottom plot shows the distribution of *P* values for simulations when the rates are equal (left) or different (right) and the red line marks the threshold value of 0.05. Birth–death are the set of ‘true’ trees with branch lengths simulated under a constant rate birth–death model; unit tree are trees with all branch lengths equal to 1; Grafen are trees with branch lengths computed with Grafen’s (1989) method.



coevolution is that female genitalia could be evolutionarily constrained due to some other mechanical function in addition to the attachment during copula. Several heteropteran lineages possess well-developed tube-like ovipositors (e.g. Davis, 1955; Esaki & Miyamoto, 1959) that participate in both genital coupling and oviposition, potentially representing targets of alternative selection processes. Although this is not the case of the Pentatomidae, which have simple external genitalia with reduced ovipositors (Schuh & Slater, 1995), female genital plates may be used to evaluate the oviposition substrate prior to oviposition (Panizzi *et al.*, 2004) and to hold eggs in position after oviposition (Owusu-Manu, 1980). Such potential secondary functions in oviposition may represent a source of stabilizing selection which may constrain female plates to particular regions of the morphospace.

Published illustrations of Pentatomidae genitalia are simplistic and detailed reports about how genitalia interact during copula are scarce. Even though the gonocoxite 8 and the pygophore have been suggested to interact in copula (Leston, 1955), the specific parts of these two structures that touches one another were unknown. For this reason, we predicted that the parts that should coevolve were those that show the higher

levels of variation across species. Indeed, the posterior margin of the gonocoxite 8 and the posterolateral angle of the pygophore comprise virtually all morphological variation exhibited by external genitalia in the genus (Frey-da-Silva & Grazia, 2001; Grazia *et al.*, 2006; Genevicius *et al.*, 2012). As predicted, these two traits showed correlated evolution in the group. Two species that clearly illustrate this trend are *C. rideri* and *C. immaculata*, which exhibit the most pronounced disparities in both traits (Fig. 2). The left gonocoxite 8 of *C. rideri* is strongly projected in its left portion, followed by the pygophore with the most invaginated lateral margin among all species. At the other extreme, the same portion of the pygophore in *C. immaculata* is curved towards the outside, whereas the left portion of the posterior margin of the gonocoxite is strongly retracted. In summary, we show that an exaggeration of one trait towards the outside is correlated with a retraction of the other to the inside, suggesting these two structures are under selection to adjust to each other while in copula. We view this scenario as more consistent with a cooperative fit of the genitalia rather than with a defensive-aggressive pattern generally observed in systems experiencing sexually antagonistic coevolution (Huber, 1999).

Apart from the seemingly cooperative mode of interaction between the genitalia, we believe sexually antagonistic coevolution (SAC) is unlikely for other two reasons. First, there is no evidence of defensive-like structures or damaged genitalia on females of hundreds of specimens studied by the first author or reported in the taxonomic literature. Such signals have been recognized as signatures of SAC in other groups (Kuntner *et al.*, 2009; Macagno *et al.*, 2011). Second, antagonistic behaviours such as forced copulation, resistance, harassment and physical struggles, which are common in systems under SAC (Alexander *et al.*, 1997), are absent in the Pentatomidae (e.g. Harris & Todd, 1980; Owusu-Manu, 1980; Kawada & Kitamura, 1983; Kon *et al.*, 1988; Silva *et al.*, 2012). In fact, females seem to be fairly passive and receptive to several sexual attempts from the same male (Zahn *et al.*, 2008). We encourage, however, further studies to conduct experimental measurements of mating costs in the family to investigate potential intersexual conflicts even in the absence of antagonist behaviours.

Nevertheless, sexual conflict could also be thought here in terms of avoidance of sperm competition. Males of pentatomoids are usually capable of holding females for several hours during mating in order to avoid male–male sperm competition (McLain, 1985; Carroll, 1991; Hosokawa & Suzuki, 2001). The mechanism through which males hold females is unclear, but secondary sexual traits with this function, such as the forelegs of male gerrids (Arnqvist & Rowe, 1995), have never been observed in Pentatomidae. Although both internal and external genitalia are candidates to this function, we have observed in individuals during copula that the internal connection (i.e. the inflated phallus and the spermathecae; Konstantinov & Gapon, 2005) is much tighter and more robust than the external connection (B.C. Genevcious & C.F. Schwertner, unpublished data). In the seed bug *Nysius huttoni*, whose general aspect of the external genitalia are reasonably similar to those of pentatomids, duration of copula in situations of male-biased sex ratio has been attributed to the internal parts (Wang *et al.*, 2008). In this light, we hypothesize that any antagonistic processes, if existent in the Pentatomidae, are more likely to be acting over internal genital structures.

Females of pentatomids exercise mate choice, and there is some evidence that males may be rejected after an initial contact of the genitalia (Wang & Millar, 1997; Zahn *et al.*, 2008). Moreover, both male and female external genitalia are covered with sensory setae (see illustrations in Thomas & Yonke, 1990; Schwertner & Grazia, 2012; Greve *et al.*, 2013). These aspects together with the passive precopulatory behaviour of females are in line with predictions from models of ‘nonantagonistic selection’, such as morphological accommodation, lock-and-key, cryptic female choice or some combination of the three (Eberhard, 2010; Brennan & Prum,

2015). We caution that distinguishing putatively among these mechanisms is only possible with more detailed studies on functional morphology and experimental approaches.

## Conclusions

Much progress has been achieved recently with respect to which mechanisms of sexual selection drive genitalia diversification, but most of the evidence comes from a few arthropod model systems. We provide novel evidence that female genitalia evolve fast, and male and female genitalia evolve in different rates despite their mechanical entanglement and coevolution. The patterns shown here are fundamental results concerning the tempo of evolution of genitalia, which will guide further investigations and most likely establish a new model system for the study of genital evolution under morphological and functional integration among insects. The pentatomids are unique in having both male and female external genitalia well developed, rigid and composed by multiple parts, which make them ideal for objective morphometrical analyses and useful to investigate a series of broad questions about the evolution of genital traits. Pentatomids comprise an understudied group of insects in terms of evolution, but we aim to establish this group as a new model system for studies of genitalia. At the macroevolutionary level, future research should aim to explore multiple parts of a single genitalia to test hypotheses of evolutionary integration and modularity and to investigate the prevalence of the pattern that we observed here across other lineages of animals. Untangling lock-and-key, female choice and even intersexual conflict will be feasible with population-level approaches and experimental studies. Specifically to Pentatomidae, crucial next steps include understanding the functional morphology of the internal and external parts of the genitalia in more detail as well as the patterns of diversification of other genital components at broader levels.

## Acknowledgments

We wish to thank the curators of all museums for the access to the specimens; M.N Rossi for providing access to the stereomicroscope in which all photographs were taken; and J.M.B. Alexandrino, F.R. Amaral, G. Machado, M. Maldonado-Coelho and C.J.E. Lamas for constructive criticisms throughout the development of the study. Special thanks to G. Zhang and P.E.C. Peixoto for valuable comments on the manuscript. BCG was supported with fellowships by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) procs. n. 2012/24620-5 and 2014/21104-1). DSC was supported with a fellowship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES: 1093/12-6). This project was partially supported by the

Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil (CNPq, Edital Universal proc. n. 478056/2010-9).

## References

- Adams, D.C. 2014. Quantifying and comparing phylogenetic evolutionary rates for shape and other high-dimensional phenotypic data. *Syst. Biol.* **63**: 166–177.
- Adams, D.C. 2016. Evaluating modularity in morphometric data: challenges with the RV coefficient and a new test measure. *Methods Ecol. Evol.* **7**: 565–572.
- Adams, D.C. & Felice, R.N. 2014. Assessing trait covariation and morphological integration on phylogenies using evolutionary covariance matrices. *PLoS ONE* **9**: 1–8.
- Adams, D.C. & Otárola-Castillo, E. 2013. geomorph: an R package for the collection and analysis of geometric morphometric shape data. *Method. Ecol. Evol.* **4**: 393–399.
- Ah-King, M., Barron, A.B. & Herberstein, M.E. 2014. Genital evolution: why are females still understudied? *PLoS Biol.* **12**: 1–7.
- Alexander, R., Marshall, D. & Cooley, J. 1997. Evolutionary perspectives on insect mating. In: *The Evolution of Mating Systems in Insects and Arachnids* (J. Choe & B. Crespi, eds), pp. 4–31. Cambridge Univ. Press, Cambridge.
- Arnqvist, G. 1998. Comparative evidence for the evolution of genitalia by sexual selection. *Nature* **393**: 784–786.
- Arnqvist, G. & Rowe, L. 1995. Sexual conflict and arms races between the sexes: a morphological adaptation for control of mating in a female insect. *Proc. R. Soc. Lond. B Biol. Sci.* **261**: 123–127.
- Bergsten, J. & Miller, K.B. 2007. Phylogeny of diving beetles reveals a coevolutionary arms race between the sexes. *PLoS ONE* **2**: 1–6.
- Bernardes, J.L., Schwertner, C.F. & Grazia, J. 2009. Cladistic analysis of *Thoreyella* and related genera (Hemiptera: Pentatomidae: Pentatominae: Proclitini). *Zootaxa* **2310**: 1–23.
- Bonhag, P.F. & Wick, J.R. 1953. The functional anatomy of the male and female reproductive systems of the milkweed bug, *Oncoleptus fasciatus* (Dallas) (Heteroptera: Lygaeidae). *J. Morphol.* **93**: 177–283.
- Brennan, P.L. & Prum, R.O. 2015. Mechanisms and evidence of genital coevolution: the roles of natural selection, mate choice, and sexual conflict. *Cold Spring Harb. Perspect. Biol.* **7**: 1–21.
- Brennan, P.L., Prum, R.O., McCracken, K.G., Sorenson, M.D., Wilson, R.E. & Birkhead, T.R. 2007. Coevolution of male and female genital morphology in waterfowl. *PLoS ONE* **2**: 1–6.
- Carroll, S.P. 1991. The adaptive significance of mate guarding in the soapberry bug, *Jadera haematoloma* (Hemiptera: Rhopalidae). *J. Insect Behav.* **4**: 509–530.
- Cayetano, L., Maklakov, A.A., Brooks, R.C. & Bonduriansky, R. 2011. Evolution of male and female genitalia following release from sexual selection. *Evolution* **65**: 2171–2183.
- Coudron, T.A., Meeds, A., Bailey, C. & Meihls, L.N. 2015. Viable progeny from crosses between geographically isolated populations of podisus (say) 1 indicate a single species. *Southwest. Entomol.* **40**: 677–689.
- Cruz, L.M.L.D., Torres, M.A.J., Barrion, A.T., Joshi, R.C., Sebastian, L.S. & Demayo, C.G. 2011. Geometric morphometric analysis of the head, pronotum and genitalia of the rice black bug associated with selected rice types. *Egypt. Acad. J. Biolog. Sci. A. Entomol.* **4**: 21–31.
- Davis, N.T. 1955. Morphology of the female organs of reproduction in the Miridae (Hemiptera). *Ann. Entomol. Soc. Am.* **48**: 132–150.
- Denton, J.S.S. & Adams, D.C. 2015. A new phylogenetic test for comparing multiple high-dimensional evolutionary rates suggests interplay of evolutionary rates and modularity in lanternfishes (Myctophiformes; Myctophidae). *Evolution* **69**: 2425–2440.
- Eberhard, W.G. 1985. *Sexual Selection and Animal Genitalia*. Harvard Univ. Press, Cambridge.
- Eberhard, W.G. 2006. Sexually antagonistic coevolution in insects is associated with only limited morphological diversity. *J. Evol. Biol.* **19**: 657–681.
- Eberhard, W.G. 2009. Static allometry and animal genitalia. *Evolution* **63**: 48–66.
- Eberhard, W.G. 2010. Rapid divergent evolution of genitalia. In: *The Evolution of Primary Sexual Characters in Animals* (J.L. Leonard & A. Córdoba-Aguilar, eds), pp. 40–78. Oxford Univ. Press, Oxford.
- Eberhard, W.G. & Huber, B.A. 2010. Spider genitalia. In: *The Evolution of Primary Sexual Characters in Animals* (J. Leonard & A. Córdoba-Aguilar, eds), pp. 249–284. Oxford University Press, New York, NY.
- Esaki, T. & Miyamoto, S. 1959. A new or little known *Hypselosoma* from Amami-Oshima and Japan, with the proposal of a new tribe for the genus (Hemiptera). *Sieboldia* **2**: 109–120.
- Evans, J.P., Gasparini, C., Holwell, G.I., Ramnarine, I.W., Pitcher, T.E. & Pilastro, A. 2011. Intraspecific evidence from guppies for correlated patterns of male and female genital trait diversification. *Proc. R. Soc. Lond. B Biol. Sci.* **278**: 2611–2620.
- Fernández-Aldea, A.F., Barão, K.R., Grazia, J. & Ferrari, A. 2014. An integrative approach to the taxonomy of *Oenopiella* Bergroth (Hemiptera: Heteroptera: Pentatomidae: Pentatominae: Carpocorini) with the description of two new species from Argentina and Southern Brazil. *Ann. Entomol. Soc. Am.* **107**: 364–381.
- Forero, D. & Weirauch, C. 2012. Comparative genitalic morphology in the New World resin bugs Apiomerini (Hemiptera, Heteroptera, Reduviidae, Harpactorinae). *Dtsch. Entomol. Z.* **59**: 5–41.
- Fortes, P. & Cônsoli, F.L. 2011. Are there costs in the repeated mating activities of female Southern stink bugs *Nezara viridula*? *Physiol. Entomol.* **36**: 215–219.
- Frey-da-Silva, A. & Grazia, J. 2001. New species of *Acrosternum* subgenus *Chinavia* (Heteroptera, Pentatomidae, Pentatomini). *Iheringia. Ser. Zool.* **90**: 107–126.
- Fritz, A.H. & Turner, F.R. 2002. A light and electron microscopical study of the spermathecae and ventral receptacle of *Anastrepha suspensa* (Diptera: Tephritidae) and implications in female influence of sperm storage. *Arthropod Struct. Dev.* **30**: 293–313.
- Gamboa, G. & Alcock, J. 1973. The mating behavior of *Brochymena quadrapustulata* (Fabricius). *Psyche* **80**: 225–270.
- Garbelotto, T.D.A., Campos, L.A. & Grazia, J. 2013. Cladistics and revision of *Alitocoris* with considerations on the phylogeny of the Herrichella clade (Hemiptera, Pentatomidae, Discocephalinae, Ochlerini). *Zool. J. Linn. Soc.* **168**: 452–472.

- Genevcious, B.C. & Schwertner, C.F. 2014. Review and phylogeny of the *geniculata* group, genus *Chinavia* (Heteroptera: Pentatomidae), with notes on biogeography and morphological evolution. *Zootaxa* **3847**: 33–56.
- Genevcious, B.C., Grazia, J. & Schwertner, C.F. 2012. Cladistic analysis and revision of the *obstinata* group, genus *Chinavia* Orian (Hemiptera: Pentatomidae). *Zootaxa* **3434**: 1–30.
- Grafen, A. 1989. The phylogenetic regression. *Philos. Trans. R. Soc. Lond. B.* **326**: 119–157.
- Grazia, J., Schwertner, C.F. & Ferrari, A. 2006. Description of five new species of *Chinavia* Orian (Hemiptera, Pentatomidae, Pentatominae) from western and northwestern South America. *Denisia* **19**: 423–434.
- Greve, C., Schwertner, C.F. & Grazia, J. 2013. Cladistic analysis and synopsis of *Chloropepla* Stål (Hemiptera: Heteroptera: Pentatomidae) with the description of three new species. *Insect Syst. Evol.* **44**: 1–43.
- Grimaldi, D. & Engel, M.S. 2005. *Evolution of the Insects*. Cambridge Univ. Press, Cambridge.
- Harris, V.E. & Todd, J.W. 1980. Temporal and numerical patterns of reproductive behavior in the southern green stink bug, *Nezara viridula* (Hemiptera: Pentatomidae). *Entomol. Exp. App.* **27**: 105–116.
- Hosokawa, T. & Suzuki, N. 2001. Significance of prolonged copulation under the restriction of daily reproductive time in the stink bug *Megacopta punctatissima* (Heteroptera: Plataspididae). *Ann. Entomol. Soc. Am.* **94**: 750–754.
- Hotzy, C., Polak, M., Rönn, J.L. & Arnqvist, G. 2012. Phenotypic engineering unveils the function of genital morphology. *Curr. Biol.* **22**: 2258–2261.
- Huber, B.A. 1999. Sexual selection in pholcid spiders (Araneae, Pholcidae): artful chelicerae and forceful genitalia. *J. Arachnol.* **27**: 135–141.
- Huber, B.A. 2005. High species diversity, male-female coevolution, and metaphyly in Southeast Asian pholcid spiders: the case of *Belisana* Thorell 1898 (Araneae, Pholcidae). *Zoologica* **155**: 1–126.
- Jagadeeshan, S. & Singh, R.S. 2006. A time-sequence functional analysis of mating behaviour and genital coupling in *Drosophila*: role of cryptic female choice and male sex-drive in the evolution of male genitalia. *J. Evol. Biol.* **19**: 1058–1070.
- Kameda, Y., Kawakita, A. & Kato, M. 2009. Reproductive character displacement in genital morphology in Satsuma land snails. *Am. Nat.* **173**: 689–697.
- Kawada, H. & Kitamura, C. 1983. The reproductive behavior of the brown marmorated stink bug, *Halyomorpha mista* Uhler (Heteroptera: Pentatomidae) I. Observation of mating behavior and multiple copulation. *App. Entomol. Zool.* **18**: 234–242.
- Klaczko, J., Ingram, T. & Losos, J. 2015. Genitals evolve faster than other traits in *Anolis* lizards. *J. Zool.* **295**: 44–48.
- Klingenberg, C.P. 2008. Morphological integration and developmental modularity. *Ann. Rev. Ecol. Evol. Syst.* **39**: 115–132.
- Klingenberg, C.P. & Marugán-Lobón, J. 2013. Evolutionary covariation in geometric morphometric data: analyzing integration, modularity, and allometry in a phylogenetic context. *Syst. Biol.* **62**: 591–610.
- Kon, M., Oe, A., Numata, H. & Hidaka, T. 1988. Comparison of the mating behaviour between two sympatric species, *Nezara antennata* and *N. viridula* (Heteroptera: Pentatomidae), with special reference to sound emission. *J. Ethol.* **6**: 91–98.
- Konstantinov, F.V. & Gapon, D.A. 2005. On the structure of the aedeagus in shield bugs (Heteroptera, Pentatomidae): 1. Subfamilies Discocephalinae and Phyllocephalinae. *Entomol. Rev.* **85**: 221–235.
- Krupke, C.H., Brunner, J.F. & Jones, V.P. 2008. Factors influencing mate choice in *Euschistus conspersus* Uhler (Heteroptera: Pentatomidae). *Environ. Entomol.* **37**: 192–197.
- Kuntner, M., Coddington, J.A. & Schneider, J.M. 2009. Intersexual arms race? Genital coevolution in nephilid spiders (Araneae, Nephilidae). *Evolution* **63**: 1451–1463.
- Lande, R. 1980. Sexual dimorphism, sexual selection and adaptation in polygenic characters. *Evolution* **34**: 292–305.
- Leston, D. 1955. The function of the conjunctiva in copulation of a shieldbug, *Piezodorus lituratus* (Fabricius) (Hemiptera, Pentatomidae). *J. Soc. Brit. Entomol.* **5**: 101–105.
- Lichter-Marck, I.H., Wylde, M., Aaron, E., Oliver, J.C. & Singer, M.S. 2015. The struggle for safety: effectiveness of caterpillar defenses against bird predation. *Oikos* **124**: 525–533.
- Lupše, N., Cheng, R.C. & Kuntner, M. 2016. Coevolution of female and male genital components to avoid genital size mismatches in sexually dimorphic spiders. *BMC Evol. Biol.* **16**: 1–9.
- Macagno, A.L. & Moczek, A.P. 2015. Appendage-patterning genes regulate male and female copulatory structures in horned beetles. *Evol. Dev.* **17**: 248–253.
- Macagno, A.L., Pizzo, A., Parzer, H.F., Palestini, C., Rolando, A. & Moczek, A.P. 2011. Shape - but not size - codivergence between male and female copulatory structures in *Onthophagus* beetles. *PLoS ONE* **6**: 1–10.
- McDonald, F.J.D. 1966. The genitalia of north american Pentatomidae (Hemiptera: Heteroptera). *Quaest. Entomol.* **2**: 7–150.
- McLain, D.K. 1985. Male size, sperm competition, and the intensity of sexual selection in the southern green stink bug, *Nezara viridula* (Hemiptera: Pentatomidae). *Ann. Entomol. Soc. Am.* **78**: 86–89.
- McLain, D.K. 1992. Preference for polyandry in female stink bugs, *Nezara viridula* (Hemiptera: Pentatomidae). *J. Insect Behav.* **5**: 403–410.
- McPeck, M.A., Shen, L. & Farid, H. 2009. The correlated evolution of three-dimensional reproductive structures between male and female damselflies. *Evolution* **63**: 73–83.
- Memon, N., Meier, R., Manan, A. & Su, K.F.Y. 2006. On the use of DNA sequences for determining the species limits of a polymorphic new species in the stink bug genus *Halys* (Heteroptera: Pentatomidae) from Pakistan. *Syst. Entomol.* **31**: 703–710.
- Méndez, V. & Córdoba-Aguilar, A. 2004. Sexual selection and animal genitalia. *Trends Ecol. Evol.* **19**: 224–225.
- Midford, P.E., Garland, T. & Maddison, W. 2008. PDAP: PDTree package for Mesquite, version 1.13. [http://mesquiteproject.org/pdap\\_mesquite/](http://mesquiteproject.org/pdap_mesquite/)
- Owusu-Manu, E. 1980. Observations on mating and egg-laying behaviour of *Bathycorbia thalassina* (Herrich-Schaeffer) (Hemiptera: Pentatomidae). *J. Nat. Hist.* **14**: 463–467.
- Panizzi, A.R., Berhow, M. & Bartelt, R.J. 2004. Artificial substrate bioassay for testing oviposition of southern green stink bug conditioned by soybean plant chemical extracts. *Environ. Entomol.* **33**: 1217–1222.
- Peredo, L.C. 2013. Life cycle and geographic variation of *Pharypia nitidiventris* (Stål) (Hemiptera: Heteroptera: Pentatomidae: Pentatomini). *Dtsch. Entomol. Z.* **60**: 25–32.
- Poissant, J., Wilson, A.J. & Coltman, D.W. 2010. Sex-specific genetic variance and the evolution of sexual dimorphism: a



- systematic review of cross-sex genetic correlations. *Evolution* **64**: 97–107.
- Polak, M. & Rashed, A. 2010. Microscale laser surgery reveals adaptive function of male intromittent genitalia. *Proc. R. Soc. Lond. B Biol. Sci.* **277**: 1–6.
- Polihronakis, M. 2006. Morphometric analysis of intraspecific shape variation in male and female genitalia of *Phyllophaga hirticula* (Coleoptera: Scarabaeidae: Melolonthinae). *Ann. Entomol. Soc. Am.* **99**: 144–150.
- Puniamoorthy, N., Kotrba, M. & Meier, R. 2010. Unlocking the “Black box”: internal female genitalia in Sepsidae (Diptera) evolve fast and are species-specific. *BMC Evol. Biol.* **10**: 1–21.
- R Development Core Team 2016. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna.
- Revell, L.J. & Harmon, L.J. 2008. Testing quantitative genetic hypotheses about the evolutionary rate matrix for continuous characters. *Evol. Ecol. Res.* **10**: 311–331.
- Rohlf, F.J. 2006. *tpsDig, version 2.10*. Department of Ecology and Evolution, State University of New York, Stony Brook.
- Rohlf, F.J. & Corti, M. 2000. The use of two-block partial least squares to study covariation in shape. *Syst. Biol.* **49**: 740–753.
- Rolston, L.H. 1974. Revision of the genus *Euschistus* in Middle America (Hemiptera, Pentatomidae, Pentatomini). *Entomol. Am.* **48**: 1–102.
- Rönn, J.L., Katvala, M. & Arnqvist, G. 2011. Correlated evolution between male and female primary reproductive characters in seed beetles. *Funct. Ecol.* **25**: 634–640.
- Rowe, L. & Arnqvist, G. 2012. Sexual selection and the evolution of genital shape and complexity in water striders. *Evolution* **66**: 40–54.
- Schuh, R.T. & Slater, J.A. 1995. *True Bugs of the World (Hemiptera: Heteroptera): Classification and Natural History*. Cornell Univ. Press, Ithaca, NY.
- Schwertner, C.F. & Grazia, J. 2007. O gênero *Chinavia* Orian (Hemiptera, Pentatomidae, Pentatominae) no Brasil, com chave pictórica para os adultos. *Rev. Bras. Entomol.* **51**: 416–435.
- Schwertner, C.F. & Grazia, J. 2012. Review of the neotropical genus *Aleixus* McDonald (Hemiptera: Heteroptera: Pentatomidae: Procteticini), with description of a new species and cladistic analysis of the tribe Procteticini. *Entomol. Am.* **118**: 252–262.
- Scudder, G.G.E. 1959. The female genitalia of the heteroptera: morphology and bearing on classification. *Trans. R. Entomol. Soc. Lond.* **111**: 405–467.
- Sharp, D. 1890. On the structure of the terminal segment in some male Hemiptera. *Trans. R. Entomol. Soc. Lond.* **3**: 399–427.
- Sheehy, C.M., Albert, J.S. & Lillywhite, H.B. 2016. The evolution of tail length in snakes associated with different gravitational environments. *Funct. Ecol.* **30**: 244–254.
- Silva, F.A. & Panizzi, A.R. 2009. Oviposition of the neotropical brown stink bug *Euschistus heros* (Heteroptera: Pentatomidae) on artificial and on natural substrates. *Fla Entomol.* **92**: 513–515.
- Silva, C.C.A., Laumann, R.A., Ferreira, J.B.C., Moraes, M.C.B., Borges, M. & Čokl, A. 2012. Reproductive biology, mating behavior, and vibratory communication of the brown-winged stink bug, *Edessa meditabunda* (Fabr.) (Heteroptera: Pentatomidae). *Psyche* **2012**: 1–9.
- Simmons, L.W. 2014. Sexual selection and genital evolution. *Austral Entomol.* **53**: 1–17.
- Singh-Pruthi, C.A. 1925. The morphology of the male genitalia in Rhynchota. *Trans. R. Entomol. Soc. Lond.* **1–2**: 127–267.
- Takami, Y. & Sota, T. 2007. Rapid diversification of male genitalia and mating strategies in *Ohomopterus* ground beetles. *J. Evol. Biol.* **20**: 1385–1395.
- Thomas, D.B. & Yonke, T.R. 1990. Review of the genus *Banasa* (Hemiptera: Pentatomidae) in South America. *Anna. Entomol. Soc. Am.* **83**: 657–688.
- Torres, M.A.J., Lumansoc, J. & Demayo, C.G. 2010. Variability in head shapes in three populations of the Rice Bug *Leptocoris oratorius* (Fabricius) (Hemiptera: Alydidae). *Egypt. Acad. J. Biol. Sci.* **3**: 173–184.
- Tsai, J.F., Yang, M.M., Rédei, D. & Yeh, G.F. 2011. *Jewel Bugs of Taiwan (Heteroptera: Scutelleridae)*. Chung Hsing Univ., Taichung.
- Voje, K.L., Hansen, T.F., Egset, C.K., Bolstad, G.H. & Pélabon, C. 2014. Allometric constraints and the evolution of allometry. *Evolution* **68**: 866–885.
- Wang, Q. & Millar, J.G. 1997. Reproductive behavior of *Thyanta pallidovirens* (Heteroptera: Pentatomidae). *Ann. Entomol. Soc. Am.* **90**: 380–388.
- Wang, Q., Yang, L. & Hedderley, D. 2008. Function of prolonged copulation in *Nysius huttoni* White (Heteroptera: Lygaeidae) under male-biased sex ratio and high population density. *J. Insect Behav.* **21**: 89–99.
- Wigby, S. & Chapman, T. 2004. Female resistance to male harm evolves in response to manipulation of sexual conflict. *Evolution* **58**: 1028–1037.
- Yassin, A. 2016. Unresolved questions in genitalia coevolution: bridging taxonomy, speciation, and developmental genetics. *Org. Div. Evol.* **16**: 681–688.
- Zahn, D.K., Girling, R.D., McElfresh, J.S., Cardé, R.T. & Millar, J.G. 2008. Biology and reproductive behavior of *Murgantia histrionica* (Heteroptera: Pentatomidae). *Ann. Entomol. Soc. Am.* **101**: 215–228.
- Zahniser, J.N. & Dietrich, C.H. 2015. Phylogeny, evolution, and historical biogeography of the grassland leafhopper tribe Chiasmini (Hemiptera: Cicadellidae: Deltocephalinae). *Zool. J. Linn. Soc.* **175**: 473–495.

## Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article: **Figure S1** Intra and interspecific variation of female (left) and male (right) genitalia represented by the first two principal components, showing means and standard errors of each species (sample sizes in parentheses).

**Figure S2** Correlated evolutionary changes of male and female genitalia.

**Figure S3** landmark configurations of species mean shape of male and female genitalia for all species in the phylogeny.

Received 14 July 2016; revised 27 October 2016; accepted 7 December 2016