Evolution of sexual size dimorphism and its relationship with sex ratio in carabid beetles of Genus *Ceroglossus* Solier

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Abstract Although the degree of mate competition, given extreme differences in sex ratio, explains much of the pattern of male-biased size dimorphism among diverse taxa, it fails for some species which have potential for intense male competition for mates and yet exhibit little or no sexual size dimorphism (SSD). This fact suggests that species with low SSD should express the effect of evolutionary pressure in non-obvious geometrical shape promoted by sex ratio in an evolutionary time scale. To evaluate this hypothesis we used phylogenetic comparative method in a Bayesian framework to investigate the evolution of SSD and the role of sex ratio at inter-specific level in the species of *Ceroglossus* (Coleoptera: Carabidae). In our results the proportion farthest from 1:1 is associated with more disparate body shape, even though the entire group has minimum variation in sex ratio, which is an intrinsic life history character of this group considering its phylogenetic conservatism or phylogenetic signal. We suggest that the sex ratio has determined the dimorphism degree during evolution of this group, since both traits have increased or decreased together during the species divergence (i.e. positive phylogenetic correlation: $r^2 \approx 0.85$). We suggest that morphological studies of SSD will benefit from using comparative method with Bayesian approaches to assess the effect of phylogenetic history and its uncertainty. Finally, this will be allow to researchers to quantify the uncertainty of specific evolutionary hypotheses accounting for observed sexual dimorphism patterns [Current Zoology 59 (6): 769–777, 2013].

Keywords Macroevolution, Geometric Morphometrics, Bayesian approach, Sexual Dimorphism, Sex Ratio, *Ceroglossus*

Theory predicts that microevolutionary processes, such as sexual selection, acting on males by different reproductive strategies of sexes during their lifetime, lead to more intense intrasexual competition for access to mates (Andersson, 1994). This will favour exaggeration of the targeted male traits, promoting the evolution of increased male size relative to females, as well as more complex structures, if individuals with such traits gain an advantage over competitors and achieve higher mating and reproductive success (Moczek and Emlen, 2000; Simmons and Emlen, 2008).

The direction and degree of sexual differences in body size and shape vary greatly among taxa (Andersson, 1994). This phenomenon has launched a large number of studies devoted to explain evolutionary mechanisms underlying in among-species patterns of sexual size dimorphism (SSD) and sexual shape dimorphism (SShD) (e.g. Hurlburt, 1987; Fairbairn, 1997; Monnet and Cherry, 2002; Gidaszewski et al., 2009). The evidence sustains that sexual fecundity, sexual selection and sex ratio have been shown to drive the evolution of SSD in numerous comparative studies (Cox et al., 2003; Lindenfors et al., 2007; Szekely et al., 2007; Cox and Calsbeck, 2009), and given that, the intensity of sexual selection by male-male competition is correlated with population sex ratio (Emlen and Oring, 1977; Crespi, 1989). Male-biased sex ratios may provide additional evidence for sexual selection as the cause of dimorphism (Kumano et al., 2010; Wong-Muñoz et al., 2011). Although the degree of mate competition, given extreme differences in sex ratio, explains much of the pattern of male-biased size dimorphism among diverse taxa, it fails for some species which have potential for intense male competition for mates and yet exhibit little
or no size dimorphism of the sexes (e.g. muriqur monkeys, Strier, 1990; lemurs, Kappeler, 1991; equids, Linklater, 2000; hyraxes, Koren et al., 2006; beetles, Benitez et al., 2010b). Species with low dimorphic variation can be especially revealing to the researchers because they generally imply morphological specializations for alternative behavioral or ecological situations. Thus for an optimal detection, it is recommended to use more complex measurement techniques which include, for example, the geometrical shape (Gidaszewski et al., 2009; Benítez et al., 2011b). Actually, empirical studies of shape in carabid beetles of the species Ceroglossus chilensis have demonstrated that morphological sexual dimorphism is much reduced, hence the similarity of males and females is directly associated with the sex ratio of this species (Benitez et al., 2010b; Benitez et al. unpublished data). In terms of geometric morphometrics these little differences are visible in two body regions: 1- the abdomen of females, whose variation has been reported to have an adaptive value due to the presence of eggs; and 2- changes in the pronotum of the thorax in males, which has been attributed to intrasexual competition in this species as a microevolutionary process (Benitez et al., 2010a,b; 2011b).

However, considering that differences in dimorphism and sex ratio are evident among the species of this genus (Jiroux, 2006), we suggest that historic macroevolutionary processes occurring during diversification in this genus have played a substantial role to generate variability in the sexual characteristics observed (Jaffrézic and Rataj, 2006).

The evolution of sexual dimorphism has been extensively studied, but most studies have dealt with dimorphism of size at intra and inter-specific levels (Fairbairn et al., 2007; Corl et al., 2010; Stillwell and Davidowitz, 2010). Here we address this issue using a phylogenetic comparative method, investigating the evolution of SSD by centroid size and the role of sex ratio in body shape at inter-specific level using species of Ceroglossus genus as study model. The aim of this study was to assess the effect of macroevolutionary processes on inter-specific variability in dimorphism and sex ratio in this group, considering the general hypothesis: Inter-specific sexual differentiation patterns based on shape are promoted by sex ratio of insects ruled by the evolutionary phylogenetic heritage, and therefore the Ceroglossus species sharing a most recent common ancestor will tend to be more similar in its phenotypic expression than those species having greater divergence (Harvey and Pagel, 1991; Martins and Hansen, 1996; 1997).

1 Materials and Methods

1.1 Samples and shape analysis

We installed 12 barber traps, separated by approximately 5 m, for 3 days and 3 nights (Benitez et al., 2011) and the samples were collected for each species at the different seasons; spring-summer and winter. We collected more than 1000 individuals of the 8 species currently described, from which we used a random selection of 100 individuals approximately per species, as far as possible given the availability of samples: Ceroglossus buqueti (53 females; 47 males), C. chilensis (63 females; 37 males), C. guerini (56 females; 44 males), C. ochsenii (56 females; 47 males), C. magellanicus (49 females; 51 males), C. suturalis (56 females; 44 males), C. darwinii (18 females; 22 males), C. speciosus (2 females; 2 males).

The sex of individuals was determined by observation of the carenae of the antennae present in the 5th antennal segment (Benitez et al., 2010a, b) using an optical microscope. Based on this information we determined the sex ratio between female and male (i.e. female over males) (Table 1).

The geometric analysis considered exclusively varia-

<table>
<thead>
<tr>
<th>Specie</th>
<th>Male</th>
<th>Female</th>
<th>n</th>
<th>Sex Ratio</th>
<th>Centroid size F*</th>
<th>Centroid size M**</th>
<th>dif CS F-M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceroglossus buqueti</td>
<td>47</td>
<td>53</td>
<td>100</td>
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<td>944.4181064</td>
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<td>66.43277624</td>
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<td>56</td>
<td>100</td>
<td>0.273</td>
<td>1179.364186</td>
<td>1142.759059</td>
<td>36.60512694</td>
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<tr>
<td>Ceroglossus ochsenii</td>
<td>47</td>
<td>56</td>
<td>103</td>
<td>0.191</td>
<td>1063.871009</td>
<td>1049.717203</td>
<td>14.15380591</td>
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<tr>
<td>Ceroglossus magellanicus</td>
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<td>49</td>
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<td>1058.024992</td>
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<td>58.49720218</td>
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<tr>
<td>Ceroglossus darwinii</td>
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<td>945.5537985</td>
<td>907.7180855</td>
<td>37.93571307</td>
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<tr>
<td>Ceroglossus speciosus</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>0</td>
<td>838.6989833</td>
<td>960.333728</td>
<td>-121.6347447</td>
</tr>
</tbody>
</table>

*F: Female, **M: Male
tion in shape, and it was performed using a photograph in ventral view of males and females with an Olympus X-715 digital camera; using the methodology of Alibert et al., 2001, we digitized 22 landmarks (LMs, anatomical homologous points) on every picture, by TpsDig 2.10 (Rohlf, 2008) (Fig. 1). All analyses were then run using MorphoJ software version 1.05a (Klingenberg, 2011).

Once the Cartesian x-y coordinates were obtained for all landmarks, the shape information was extracted with a full Procrustes fit (GPA, Rohlf and Slice, 1990; Dry-}

den and Mardia, 1998), taking into account the object symmetry of the structure. Procrustes superimposition is a procedure that removes the information of size, position and orientation to standardize each specimen according to centroid size. Because of the symmetry of the structure, reflection is removed by including the original and mirror images of all configurations in the analysis and simultaneously superimposing all of them (Klingenberg et al., 2002). To examine the differences in shape among species and the amount of symmetric variation and asymmetry we used Procrustes ANOVA to assess studies on object symmetry (Klingenberg and McIntyre, 1998; Klingenberg et al., 2002; Klingenberg and Monteiro, 2005).

Finally, to determine the SSD of the *Ceroglossus* species, we use the average of centroid size between females and males. Centroid size corresponds to a geometrical measure of the size of the measured object, defined as the square root of the sum of the squared distance between each landmark and the centre of gravity of the measured object (Zelditch et al., 2012).

**1.2 Molecular Phylogeny by BMCMC**

We used DNA aligned sequence data of the NADH deshigrogenas gene (ND5) of 9 species (i.e. 6 ingroup and 3 outgroup) to reconstruct phylogenetic relationships of *Ceroglossus* genus (Table 2). The alignment was performed using SEQUENCHER v5.0 (Gene Codes Corporation) and visually. Since the *Ceroglossus* genus shows a high diversification, the selected molecular marker could be saturated and provide spurious phylogeny. Therefore, we evaluated whether the sequences were saturated and hence if they were useful for the phylogenetic analysis, using Xia’s test (Xia et al., 2003) implemented in DAMBE v5.1.5 (Xia and Xie, 2001). This is an entropy-based index that estimates a substitution saturation index (Iss) and compares it to a critical substitution saturation index (Iss.c) via a ran-
domination process with 95% confidence intervals (Xia and Xie, 2001; Xia and Lemey, 2009).

The molecular marker used in this study comes from different species that have diversified rapidly, so their patterns and rates of nucleotide substitution are potentially different. For these reasons, we applied a general likelihood-based mixture model (hereafter MM) of gene-sequence evolution as described by Pagel and Meade, 2004a, 2005a. This model, based on the general time-reversible (GTR) model (see Rodriguez et al., 1990), accommodates cases in which different sites in the alignment evolved in qualitatively distinct ways, but does not require prior knowledge of these patterns or partitioning of the data. The MM model, implemented in a BMCMC framework, was used to estimate the probability a posteriori of the phylogenetic trees to include this information in the comparative method analyses used in this study. The Reversible-Jump Markov Chain Monte Carlo (hereinafter RJMCMC) procedure (Pagel and Meade, 2008; Pagel and Meade, 2006) was used with the purpose of integrating results of all the patterns, producing an MM that summarizes the sequence evolution, using BayesPhylogenies 1.1 software (http://www.evolution.rdg.ac.uk/BayesPhy.html). This approach enables researchers to explore the variety of possible models and parameters, converging towards the model that best fits the data in the posterior tree sample (Pagel and Meade, 2008). Three independent BMCMC analyses were run using 78,350,000 generations of phylogenetic trees, sampling every 10,000th tree to assure that successive samples were independent. We used the chain with the highest marginal likelihoods. From this sample of trees the first 100 trees of the sample were removed to avoid including trees sampled before the convergence of the Markov Chain, and we re-sampled every 15 trees to obtain a final sample of 522 trees, with no significant autocorrelation in the ln-likelihood of the sample. These independent sample of trees were used for the Bayesian phylogenetic comparative analyses.

1.3 Evolution of SSD and sex ratio

In this study we evaluate the role of historical processes in originating the actual variability of sexual variables (i.e. SSD and sex ratio) in Ceroglossus genus using the phylogenetic generalized least squares model (PGLS) (Martins and Hansen, 1997; Pagel, 1997, 1999a, b, 2002) implemented in a Maximum likelihood framework. We first evaluated the tempo and mode of sexual dimorphisms and sex ratio using the three phylogenetic scaling parameters defined by Pagel (1999a, b; 2002) (lambda $\lambda$ and kappa $\kappa$; estimated from species data and the BMCMC sample of phylogenetic trees), to determine three aspects of trait evolution: (1) We evaluated whether a random-walk (Model A) or directional change model (Model B) was the most appropriate model to explain the evolution of sex variables. Model A corresponds to the standard constant-variance ($\sigma^2$) random-walk model. In this model the $\sigma^2$ parameter of evolution is determined by choosing a value of $\alpha$ from the random-walk model, where $\alpha$ is the trait value assigned to the root of the tree based on the tip data (Pagel, 2002). Model B is a directional random-walk model, which has two parameters: the $\sigma^2$ parameter as in Model A, plus the directional change parameter $\beta$. This latter parameter effectively measures the regression of trait values across species against the total path length (from the root of the tree to the tips), which is interpreted as the direction and magnitude of change in a character per unit of divergence (Pagel, 2002); (2) We evaluated the influence of phylogeny or phylogenetic signal using the phylogeny scaling parameter $\lambda$. In this approach, $\lambda$ detects whether the shared evolutionary histories as specified by the phylogeny produce the patterns of similarity observed in the data. If a trait is not evolving according to phylogenetic relationships, this parameter will take value 0, values $0 < \lambda < 1$ correspond to traits being less similar among species than expected from their phylogenetic relationships, and $\lambda > 1$ suggests the reverse. If traits are evolving as expected, given the tree topology and branch lengths, $\lambda$ takes the value of 1; (3) Next, we contrasted punctuational versus gradual trait evolution using the branch-length scaling parameter $\kappa$. In this test $\kappa$ scales the relationship between individual branch lengths and trait evolution (Pagel, 1994, 2002). If $\kappa$ is 1, trait evolution is directly proportional to branch length and, hence, the gradual mode of trait evolution is better supported. Values of $\kappa$ greater than 1 indicate proportionally more evolution in longer branches. Values of $\kappa$ less than 1 indicate proportionally more evolution in shorter branches. In the extreme case of $\kappa = 0$, trait evolution is independent of branch length, which is consistent with a punctuational mode of evolution.

To evaluate the evolution of sex variables we estimated the phylogenetic scaling parameters (i.e. $\lambda$, and $\kappa$) using a maximum likelihood framework, estimating the parameter values from each tree of the Bayesian sample. We used the distribution of parameter values over the sample of trees to evaluate the deviation of the estimated parameters from the null model of pure Brownian motion (i.e. constant-variance model; with $\lambda$ and $\kappa$ equal
to 1), and a non-historical model (i.e. \( \lambda \) equal to 0). These analyses were conducted using the Continuous module implemented in BayesTrait 1.0 software (Pagel and Meade, 2007). We used the Bayes factor (Gelman et al., 1995) to compare the observed model (i.e. constant-variance model; with \( \lambda \) and \( \kappa \) calculated in a Bayesian framework) with the pure Brownian motion and non-historical models.

Finally, we evaluated the correlated evolution between sexual dimorphism and sex ratio. To do this, we evaluated the significance of the relationships between the pair of characters using a measure of correlated evolution in an ML framework implemented in BayesTrait 1.0 software (Pagel and Meade, 2007). As the null hypothesis we used a model in which the covariance between characters was set to zero (i.e. complete character independence), and the alternative hypothesis was, then, the observed covariance between characters (Pagel, 1999a, b). We used the Bayes factor (Gelman et al., 1995) to compare these hypotheses. We summarized the parameters of all selected models using the mean and 95% highest posterior density interval (HPD).

2 Results

2.1 Morphometric analyses

The morphological variation among beetles determined by Procrustes ANOVA indicates that variation in size and shape and the symmetric pattern of shape (fluctuating asymmetry and directional asymmetry) among species and sexes are highly significant (Table 3). Because of the lack of specimens for Ceroglossus speciosus we run the analysis only for the other seven species.

The PCA for the symmetric component (individual variation) and asymmetric component shows sexual differences among the seven species analyzed. The first three PCs account for 53.48 % (PC1 + PC2 + PC3 = 27.678 % + 15.56 % + 10.25 %) of the total shape variation and provide a reasonable approximation of the total amount of variation, with the other PC components, accounting each for no more than 8% of the variation. In the ventral view, the landmarks with greatest contribution were numbers 4, 5 and 7 (0.14681, 0.5269 and 0.10013, respectively), indicating that males have a less robust abdomen than females; this is related to a wider proepisternum in males and wider abdominal sternites in females.

2.2 Phylogenetic analyses

The Xia test (Xia et al., 2003; Xia and Lemey, 2009) shows that the substitution saturation index (Iss = 0.112) is significantly lower than the substitution saturation index calculated (ISS.c = 0.749) (Iss << Iss.c; \( p = 0.00001 \)). This result shows that the data set has low substitution saturation and is useful for phylogenetic analyses. The analysis conducted under BMCMC approach indicates that the Ceroglossus genus is monophyletic, considering the high posterior probability values (hereinafter pp) (pp \( \approx 1.0 \), see Fig. 2). This phylogenetic reconstruction showed high pp values in the nodes defining the six species of Ceroglossus genus included in this study (Fig. 2).

2.3 Comparative method analyses

The comparative method results showed that the best predictor of evolution of sex variables (i.e. SSD and sex ratio) in the Ceroglossus genus was a random-walk change model (Bayes Factor = 0.73 (Sexual dimorphism) and 0.74 (Sex ratio). Both characters were significantly determined by phylogenetic relationships (\( \lambda = 1.01 \) and \( \lambda = 1.02 \), respectively; Table 4, Fig. 3). The evolution of sexual dimorphisms and sex ratio was not consistent with the gradual or punctuational model of evolution (\( \kappa = 2.9 \); Table 4, Fig. 3), since the observed \( \kappa \) is greater than 1, proportionally more evolution occurred in larger branches of the trees (Table 4; Fig. 3).

Table 3  Procrustes ANOVA for both centroid size (CS) and shape (SH) of Ceroglossus, characterized by object symmetry

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>MS</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS</td>
<td>4631129.030831</td>
<td>771854.838472</td>
<td>6</td>
<td>361.33</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SD</td>
<td>170457.984124</td>
<td>170457.984124</td>
<td>1</td>
<td>79.80</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FA</td>
<td>1303061.924972</td>
<td>2136.167090</td>
<td>610</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SH</td>
<td>0.09525422</td>
<td>0.0007937852</td>
<td>120</td>
<td>43.59</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SD</td>
<td>0.04446880</td>
<td>0.0022234399</td>
<td>20</td>
<td>122.11</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FA</td>
<td>0.22214164</td>
<td>0.0000182083</td>
<td>1220</td>
<td>1.21</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>DA</td>
<td>0.02228653</td>
<td>0.001143265</td>
<td>20</td>
<td>73.82</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Sums of squares (SS) and mean squares (MS) are in units of Procrustes distances (dimensionless). SV = Species variation; FA = fluctuating asymmetry; DA = directional asymmetry.
Fig. 2  The consensus tree of the 522 phylogenetic trees obtained by means of the Bayesian approach
Values above nodes correspond to the posterior probability of the nodes with its corresponding grid of morphological consensus for SSD in the 6 species of *Ceroglossus* according to the respective order of precedence in the tree (The species *C. ochsenii* and *C. guerinii* were not included in the analysis because there were no sequences available).

Table 4  Bayes factors used to test the observed versus expected values of phylogenetic scaling parameters for different models of trait evolution

<table>
<thead>
<tr>
<th>Observed values of phylogenetic scaling parameters</th>
<th>Sexual Dimorphism</th>
<th>Bayes Factor</th>
<th>Sex Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>λ</strong> Mean (95% HPD)</td>
<td>1.01 (1–1.04)</td>
<td>1.3E+22</td>
<td>6.8</td>
</tr>
<tr>
<td><strong>κ</strong> Mean (95% HPD)</td>
<td>2.9 (2.11–3)</td>
<td>5.0E+6</td>
<td>5.2</td>
</tr>
</tbody>
</table>

The observed λ were contrasted with values expected under the hypotheses of no phylogenetic signal (λ = 0) and the pure Random Walk model (λ = 1). The observed κ were contrasted with expected values for punctuated evolution (κ = 0), and the pure Random-Walk model (κ = 1).

The correlated evolution analysis showed that both traits evolve positively correlated along the phylogeny (mean $r^2 = 0.85$, 95% HPD = 0.79 – 0.90, BF = 4.8E+22), indicating the existence of a significant historical relationship between the characters.

3 Discussion

The analysis of evolution in variation of sex variables in *Ceroglossus*, revealed a striking congruence between SSD and sex ratio. In our results, the proportion farthest from 1:1 was associated with more disparate body shape, even though the entire group had minimum variation in sex ratio, which is an intrinsic life history character of this group considering its phylogenetic conservatism or phylogenetic signal (i.e. λ). This result reinforces the idea that the commonly observed sexual selection as a microevolutionary process at intra-specific level (e.g. Cox and Calsbeeck, 2009; Blackburn et al., 2010; House et al., 2011) is also very important at inter-specific level as a macroevolutionary process. We propose that the intensity of sexual selection by male-male competition is a species-specific process that depends on sex ratio.
having a high phylogenetic signal (Fig. 3), then the male-biased sex ratio may provide evidence for sexual selection as the cause of dimorphism at macroevolutionary framework. This means that sex ratio is an indirect measure of sexual selection at both micro and macroevolutionary levels. Actually, this could explain why the shape changes involved in SShD consist of the same features that are widely observed in variation within or between populations of *Ceroglossus* (Benitez et al., 2010a, b; 2011b), and also between species. As observed in the ventral view, males tend to have a less robust abdomen than females; this is related to a wider proepisternum in males and wider abdominal sternites in females. The SShD in different *Ceroglossus* species, compared using PCA, suggested that variation is distributed over many dimensions rather than being concentrated primarily in one or few dimensions. Moreover, the scatter of SShD in shape space is irregular with no dominant patterns or strongly preferred directions. This suggests that evolutionary divergence may combine a set of body shape features to make up the SShD for each species as shown in Fig. 2.

In this context, results from the phylogenetic comparative method support the hypothesis sustaining that the phylogenetic divergence degree has largely determined the SSD and sex ratio, and it’s correlation between species in the *Ceroglossus* genus. This is based on the fact that divergence patterns in this genus are related to the inter-specific differentiation patterns regarding sexual variables. Those species sharing a closest common ancestor tend to resemble more at the sexual dimorphism and sex ratio levels than those species with greater divergence.

Considering the taxonomic level and time scale that determine the sexual variable evolution mode (Fig. 3), we suggest that the sex ratio has determined the dimorphism degree in this group, since both traits have increased or decreased together during the species divergence (i.e. positive correlation; \( r^2 \approx 0.85 \)). In fact, divergence patterns in this group affected evolution of both variables (\( \lambda_{\text{mean}} = 1.01 \) and 1.02 for SSD and sex ratio, respectively), and the major evolutionary change has occurred in those species having larger genetic divergence (i.e. longest tree branches; \( \kappa_{\text{mean}} = 2.9 \)).
Finally, we suggest that morphological studies of SSD will benefit from using the comparative method with Bayesian approaches to assess the effect of phylogenetic history and its uncertainty. Moreover, these approaches provide an explicit way to evaluate the predictions of sexual selection in a macroevolutionary scale using sex ratio and Bayesian comparative method, which will allow researchers to quantify the uncertainty of specific evolutionary hypotheses accounting for observed sexual dimorphism patterns. For example, the idea that large males and pronounced forms often have advantages in male-male competition and female choice (Thornhill and Alcock, 1983), or on the other hand, the idea of natural selection acting on female fecundity and being stronger than the action of sexual selection on males, hypothesis that is necessary to be evaluate in future works.

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