SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

Ontogenetic Trajectories and Hind Tibia Geometric Morphometrics of Holymenia clavigera (Herbst) and Anisoscelis foliacea marginella (Dallas) (Hemiptera: Coreidae)

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Trajetórias Ontogenéticas e Morfometria Geométrica das Tibias de Holymenia clavigera (Herbst) e Anisoscelis foliacea marginella (Dallas) (Hemiptera: Coreidae)

RESUMO - Holymenia clavigera (Herbst) e Anisoscelis foliacea marginella (Dallas) (Hemiptera: Coreidae: Anisoscelini) são hemípteros pouco estudados e que ocorrem no sul do Brasil. Observações preliminares indicam alta coexistência no uso de suas plantas hospedeiras (passifloráceas). Adicionalmente, há uma acentuada semelhança morfológica dos ovos e ninhas. Objetiva-se caracterizar e comparar as suas trajetórias ontogenéticas através de curvas de crescimento e da alometria ontogenética multivariada, uma vez que a única diferença aparente entre as espécies é uma dilatação da tíbia no terceiro par de pernas de A. foliacea marginella. Por não apresentarem as formas das tíbias distintas visualmente nos primeiro e segundo instares, as diferenças entre as espécies foram quantificadas pela função spline de placas finas, com o relaxamento do algoritmo para pontos em contorno. Em todos os instares, H. clavigera foi significativamente maior que A. foliacea marginella. As trajetórias ontogenéticas dos coreídeos foram estatisticamente diferentes, e as estruturas mensuradas apresentaram coeficientes alométricos significativamente distintos. A forma das tíbias de H. clavigera e A. foliacea marginella não foi estatisticamente diferente no primeiro, mas sim no segundo instar. No terceiro instar, a forma da tíbia desses coreídeos torna-se distinta a olho nu. Ou seja, embora sejam espécies com imaturas quase idênticas morfológicamente, seus padrões de crescimento e tamanho dife rem de forma significativa. Assim, o tamanho apresenta-se como parâmetro útil para a diferenciação desses estágios.

PALAVRAS-CHAVE: Alometria, Anisoscelini, crescimento, estágio imaturo, análise da forma

ABSTRACT - Holymenia clavigera (Herbst) and Anisoscelis foliacea marginella (Dallas) (Hemiptera: Coreidae) are distributed in southern Brazil and belong to the poorly studied tribe Anisoscelini. Preliminary observations indicate an ample coexistence of these species in terms of host-plant use. There is also a strong similarity regarding egg and nymph morphology. We characterized and compared their ontogenetic trajectories by means of growth curves and multivariate ontogenetic allometry, since the only apparent difference between these species is a remarkable growth in the hind tibia of A. foliacea marginella. Because their hind tibia is similar in the early instars, the shape variation of this structure was quantified by the thin plate splines function with the relaxed algorithm for semilandmarks. H. clavigera was significantly larger than A. foliacea marginella in all stages. Their ontogenetic trajectories were significantly different, and the allometric vectors did not present a significant correlation. Hind tibia shape was not statistically different in the first instar, but was different in the second instar. In the third instar, this difference became visually apparent. Thus, although these bugs are almost morphologically identical in the immature stages, their growth patterns and general size are different. We found size to be a useful parameter for species differentiation at these stages.

KEY WORDS: Allometry, Anisoscelini, growth, immature stage, shape analysis
Insects have been historically recognized as an important group for the study of size variation among structures, individuals and species (see Huxley 1932). Insect size and growth has also been studied through the Brooks-Dyar rule, which assumes that the head will grow constantly and exponentially during successive molts (Dyar 1890; for a revision, see Daly 1985). Morphometrics constitutes a relevant tool for characterizing insect phylogenetic relationships (Pretorius & Scholtz 2001), intra-specific morphological variation (Querino et al. 2002), sibling species and sexual dimorphism (e.g., Adams & Funk 1997), morphological adaptations (e.g., Moran 1986, Medeiros & Moreira 2002), and instar identification (Daly 1985). Within Insecta, previous studies of hemipterans have investigated static allometry in the hind tibia of Acanthocephala decilvis guatemalana Distant, and their role in sexual selection (Eberhard 1998, also see Mitchell 1980), as well as studies concerning immature multivariate growth patterns (e.g. Klingenberg & Zimmermann 1992a, b), and size and shape dimorphism in gerrids (Fairbain 1992).

The anisoscelins Holymenia clavigera Herbst and Anisoscelis foliacea marginella Dallas are distributed in southern Brazil and present a pronounced coexistence in abiotic conditions (14h light: 10h darkness; 25 ± 2ºC). These structures seem to have major importance when considering hemipteran body design (see examples in Rodrigues & Moreira 2005). Excepting the fifth instar, nymphs are very similar. H. clavigera adult stage probably is either an ichneumonid batesian mimic or belongs to a related mimetic ring (Lima 1940, Rodrigues 2003), whereas A. foliacea marginella bears morphological features typical of the Anisoscelini tribe, including a remarkable expansion in the hind tibia (see Osuna 1984, Schuh & Slater 1995, Mitchell 2000).

This study aims, from a comparative perspective, to characterize and discriminate nymphs of H. clavigera and A. foliacea marginella in relation to their growth patterns of size and shape. First, we compared growth patterns of body structures of H. clavigera and A. foliacea marginella. Second, we calculated and compared the allometric coefficients and the ontogenetic trajectories of both species. Finally, we used geometric morphometrics to examine the instar at which hind tibia shape of A. foliacea marginella becomes significantly different from H. clavigera.

**Material and Methods**

**Insects.** H. clavigera and A. foliacea marginella adults were collected from the vicinity of the Departamento de Zoologia of Universidade Federal do Rio Grande do Sul (UFRGS), as well as from a yard located in an urban area of Porto Alegre Municipality (30º05’S 51º10’W), Rio Grande do Sul State (RS). Adults were kept separately per species in cages (20 x 20 x 93 cm; maximum 12 adult/cage) under controlled abiotic conditions (14h light: 10h darkness; 25 ± 2°C). Coreids were fed ad libitum with Passiflora suberosa (Passifloraceae) shoots, bearing both vegetative and reproductive structures. Tap water placed on petri dishes with wet cotton was also offered to the adults. Shoots came from a cultivation existing at the Departamento de Zoologia of UFRGS, and from plants collected from Fundação Zoobotânica, Porto Alegre Municipality. Shoots were placed in plastic bottles provided with 50-cm high wooden frame support (maximum three shoots per bottle), and replaced when wilted. Cages were checked daily, and eggs were collected and placed on petri dishes, lined with moist filter paper.

To obtain specimens with known ages, newly hatched nymphs were reared in transparent plastic pots (8.5 x 8.5 x 8.5 cm) covered with a mesh cloth. Plant structures were inserted into cylindrical pots with water (3 x 5 cm), and were placed inside the pots to feed the nymphs. Nymphs were fixed with Dietrich’s fluid (n = 20 / instar / species). Since nymphs came from eggs that were obtained from several adults according to the method above mentioned, they did not necessarily share the same parents.

**Growth Trajectories Comparison.** Growth curves describe the growth of a given structure in relation to age. On the other hand, multivariate allometric analysis compares the size variation of a given structure relative to a general size factor, which is a linear combination of measurements from all body parts. Consequently, both methods are useful and appropriate approaches to compare growth patterns of H. clavigera and A. foliacea marginella.

H. clavigera and A. foliacea marginella nymphs were fixed and later measured through a stereomicroscope equipped with a micrometric scale in relation to antennal, rostrum and body length, as well as head and hind tibia width. These structures seem to have major importance when considering hemipteran body design (see examples in Klingenberg 1996).

For both species, the linear regressions were tested through Fisher’s F test (α = 0.05). To compare the growth trajectories of H. clavigera and A. foliacea marginella structures, we performed a one-way ANCOVA, which tests the parallelism of slopes Y on X of the linear regressions, and the homogeneity of the Y-intercepts (P < 0.05) (Sokal & Rohlf 1995). To perform the ANCOVA, data were log-transformed. For the purpose of regression, age (instars 1 to 5) was considered the independent variable. The tests mentioned above were performed using Statistica software (STATSOFT 1997).

The growth rate of head width of H. clavigera and A. foliacea marginella was assessed by dividing postmoult size / premoult size, which provides the Brooks-Dyar constancy rate. Additionally, we determined whether the best-fit model for head width growth pattern was linear or exponential for both species (α = 0.05). We used the Graph Pad Prism software (Motulsy 1999) to determine the corresponding head growth patterns.

**Multivariate Ontogenetic Allometry.** Because of the difficulty imposed by the inaccuracy of measurements associated with articulation of body parts in insects, geometric morphometrics were not used for the study of allometric patterns of body shape. The variables used for this analysis were traditional linear measurements, which are
not dependent on the articulation of body parts.

To calculate the allometric coefficients of *H. clavigera* and *A. foliacea marginella* nymphs, we analysed the principal components. Thirteen body part measurements were taken: body length (from tylus to last abdominal segment), head length, rostrum length, the lengths of the four antennomeres, femur length, tibia length and pronotum length, as well as head width, hind tibia width, and pronotum width. These measurements were log-transformed, and the multivariate allometric coefficients were calculated by dividing each coefficient from the first principal component by the isometry estimate – the mean of all coefficients (Cochard 1985, Monteiro 1997). If the observed allometric coefficient for a given variable was larger or smaller than 1, the corresponding allometry was considered positive or negative, respectively, in relation to general size (in this case, the linear combination of all variables represented by the first principal component). On the other hand, if such a coefficient was not significantly different from 1, the structure was considered isometric in relation to general size (see Gould 1966, Reis et al. 1988). To calculate this significance, the standard error of each allometric coefficient was multiplied by 1.96 and then summed to (allometric coefficients smaller than one) or subtracted from (allometric coefficients larger than one) the corresponding coefficient. The structure is significantly allometric if the resulting result was smaller or larger than one ($\alpha = 0.05$). The specimens used and the method for obtaining size measurements were the same as those described in the previous section. The multivariate allometric coefficients and their standard errors were calculated by a jackknife procedure (Sokal & Rohlf 1995) using the JACKIE2 Program (freely available from M.J. Cavalcanti, http://life.bio.sunysb.edu/morph).

In order to detect if the allometric coefficients from different species are significantly correlated, we calculated a correlation coefficient between allometric vectors and the corresponding confidence interval (95%). To estimate such a confidence interval, we used the bootstrap method (1000 resamplings). The allometric vectors were considered correlated (i.e., vector correlation significantly different from zero) if the estimated confidence interval did not include 0. Both the correlation coefficient and the 95% confidence interval were calculated by the R-System (freely available from http://www.r-project.org).

**Hind Tibia Geometric Morphometrics.** Hind tibiae from first and second instars ($n = 20$ / instar / species), which were not apparently different, were cut off from specimens fixed with Dietrich's fluid, and then individually photographed by a digital camera connected to a stereomicroscope. Right hind tibiae were photographed in posterior view, and digitalized through tpsdig program (freely available from F.J. Rohlf, http://life.bio.sunysb.edu/morph). For detecting hind tibia shape differences between species, we used the thin plate splines relaxed algorithm for semilandmarks (points along a contour) (see Bookstein 1997). This method allows for some of the configuration points (semilandmarks) to slide along tangent vectors relative to the contour so as to minimize the shape differences between the target and reference configuration. Four landmarks and twenty semilandmarks were used, at the hind tibia contour in the posterior view. The four landmarks chosen were located on the proximal and distal tibial corners, at the articulation with femur and tarsus. Semilandmarks were placed equidistantly around all hind tibia outline on the photographic plane. To our knowledge, this is one of the first studies that use this morphometric method in an evolutionary context (to examine some of these studies, see for example Monteiro et al. 2004).

We used TpsRelw program to calculate the partial warps ($\alpha = 0$, uniform components included) and the corresponding tibia consensus configuration for each instar (freely available from F.J. Rohlf, http://life.bio.sunysb.edu/morph). In order to reduce the dimensionality of the data set, the space spanned by the partial warps was summarized by principal component vectors (relative warps), and the first three relative warps were used as shape variables in the posterior statistical tests. To evaluate hind tibia shape differences between species in a given instar, the shape variables (relative warps) were tested through a randomization version of Hotteling's $T^2$ test by LINDAW Program (freely available from M.J. Cavalcanti, http://life.bio.sunysb.edu/morph), comparing the observed results with 1000 random permutations of the original data.

**Results**

**Growth Trajectories Comparison.** For both coreids, head width was better adjusted to an exponential growth rate ($R^2 = 0.9809$ and 0.9718 to exponential growth, and 0.9639 and 0.9585 to linear growth in *H. clavigera* and *A. foliacea marginella*, respectively). In general, postmoult / premoult rates were relatively constant, being $1.23, 1.34, 1.27$, and $1.26$ for *H. clavigera* and $1.26, 1.32, 1.29$ and $1.24$ for *A. foliacea marginella*, respectively. Although *H. clavigera* and *A. foliacea marginella* structures did not differ significantly in the test of parallelism, all of them presented growth trajectories significantly different between species (homogeneity of the $Y$-intercepts test, one-way ANCOVA) (Fig. 1; Table 1). The hind tibia of *A. foliacea marginella* had a more pronounced growth rate when compared to all body parts, including hind tibia of *H. clavigera* (particularly after the third instar). For all instars, antenna, body and rostrum length, and head width of *H. clavigera* were significantly larger than *A. foliacea marginella* (one-way ANCOVA) (Table 1).

**Multivariate Ontogenetic Allometry.** Allometric coefficients of the size measurements of *H. clavigera* and *A. foliacea marginella* and the corresponding standard errors are shown in Table 2. All structures were significantly allometric in relation to general size (data not shown). Despite the allometry detected here, body, rostrum length, and antennomeres in general presented allometric coefficients very close to 1 for both species, indicating nearly isometric growth of these structures. In contrast, head measurements were strongly negatively allometric in relation to general size. For *H. clavigera*, tibia width and pronotum length showed a negative allometric growth, whereas the hind tibia of *A. foliacea
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**Figure 1.** Growth of *H. clavigera* (---) and *A. foliacea marginella* (**••**) in the nymph stage (mean ± standard error).

*marginella* showed the more pronounced positive allometric growth when compared to all body structures of both coreids (Table 2). Standard errors calculated for each allometric coefficient by a jackknife procedure were small (Table 2).

The correlation coefficient calculated for *H. clavigera* and *A. foliacea marginella* allometric vectors was 0.242, and the corresponding confidence 95% interval was -0.318 : 0.947. This means that this vectorial correlation is not different from zero; thus, the multivariate allometric coefficients are not correlated, and are considered significantly different.

**Hind Tibia Geometric Morphometrics.** Fig. 2 shows the consensus configuration of *H. clavigera* and *A. foliacea marginella* hind tibia at first and second instars, with the corresponding landmarks and semilandmarks. In *A. foliacea marginella* second instar consensus configuration, the region where the expansion is originated seems different when compared to all other configurations. Only the first three relative warps (= principal components) were used for statistics, since they represent almost all hind tibia shape variation. At first instar, the first three relative warps accounted for 36.80, 21.23 and 10.56% of the total shape variation, respectively. At second instar, such values were 42.67, 18.20, and 7.67%, respectively. Hind tibia shape of *H. clavigera* and *A. foliacea marginella* did not differ significantly in the first, but in the second instar (Hotteling’s $T^2$ test) (Table 3).

**Discussion**

As already reported for several insects, head width of *H. clavigera* and *A. foliacea marginella* grow in an exponential and constant rate, although less pronounced than in other groups such as lepidopterans. Consequently, these coreids grow according to the predictions summarized in the Brooks-Dyar rule. However, some works report the lack of applicability of this rule for some insects, including hemipterans (Daly 1985), and related studies for this order remain scarce (e.g., Matsuda 1961). Alternative multivariate analyses of this rule were performed on some aquatic Heteroptera (e.g. Klingenberg & Zimmermann 1992a).

Due to the larger size of *H. clavigera* for several parameters such as egg volume, egg height (Rodrigues & Moreira 2005), rostrum length and body length (this study) when compared to *A. foliacea marginella*, size probably is under stronger selection than general morphology (i.e., appearance and coloration; see Rodrigues & Moreira 2005). In turn, morphological similarity may be an important factor that maintains the coexistence and the common use of host plants and tissues. At least four hypotheses may explain the strong morphological similarity between *H. clavigera* and *A. foliacea marginella* immatures: absence of selection in the immature stage (parsimony if they are sibling species), evolutionary convergence, Müllerian mimicry or genetic drift. Further studies would be necessary to clarify this issue, since shape differences between these species were still not investigated.

*H. clavigera* and *A. foliacea marginella* have distinct ontogenetic trajectories; i.e. they grow differently. Their allometric coefficients are not significantly correlated, which...
means that the trajectories have less in common than could be expected by chance. However, most structures present very similar allometric coefficients between species. As expected, body length shows a virtually isometric growth in relation to general size for both species. Other structures such as the rostrum and some antennomeres present the same pattern. On the other hand, the head grows negatively in relation to general size. Such a result reflects the pattern known for coreids in general, where medium-to-large size adults have relatively small heads (see Schuh and Slater 1995). The most conspicuous difference between species was the growth of tibia width, whose allometry is strongly positive for A. foliacea marginella, and slightly negative for H. clavigera. In fact, tibia is the structure that allows species differentiation at the immature stage in the field. Besides tibia width, pronotum length also grows differently between species, and presents a similar pattern to the one described for the tibia width. In conclusion, tibia width and pronotum length are responsible for the absence of correlation between the allometric vectors, being the structures that generate the divergence in the ontogenetic trajectories of H. clavigera and A. foliacea marginella.

Two factors may be important to determine differences regarding growth trajectories of H. clavigera and A. foliacea marginella nymphs. First, this difference may occur due to different growth rates of H. clavigera and A. foliacea marginella. Second, this difference may occur due to different growth rates of H. clavigera and A. foliacea marginella.

| Table 1. Regression equations of growth trajectories of H. clavigera and A. foliacea marginella. |
|---|---|---|---|---|---|
| Structure | Species | Equation | R² | F(1) | F(2) |
| Antenna | H. clavigera | y = 1.461x + 2.204 | 0.9665 | 2823.4 | |
| | A. foliacea | y = 0.861x + 2.410 | 0.9648 | 2572.3 | 25.03 |
| Head | H. clavigera | y = 0.335x + 0.297 | 0.9639 | 2617.2 | |
| | A. foliacea | y = 0.307x + 0.274 | 0.9585 | 2260.7 | 29.14 |
| Rostrum | H. clavigera | y = 0.609x + 1.665 | 0.9632 | 2567.7 | |
| | A. foliacea | y = 0.488x + 1.353 | 0.9773 | 4218.2 | 11.14 |
| Body | H. clavigera | y = 0.653x + 2.730 | 0.9673 | 2898.4 | |
| | A. foliacea | y = 0.660x + 2.453 | 0.9396 | 1545.4 | 24.47 |
| Tibia | H. clavigera | y = 0.159x + 0.113 | 0.8690 | 649.83 | |
| | A. foliacea | y = -0.381x + 0.391 | 0.8756 | 690.02 | 121.71 |

¹Linear regression; significant for all regressions; P < 0.0001
²One-way ANCOVA; significant difference between species; P < 0.01

| Table 2. Allometric coefficients (AC) and standard errors (SE) for body structures of H. clavigera and A. foliacea marginella nymphs. |
|---|---|---|---|---|---|
| Structure (measurement) | H. clavigera | A. foliacea |
| | AC | SE | AC | SE |
| Body (length) | 1.1314 | 0.0010 | 0.9206 | 0.0012 |
| Head (length) | 0.7686 | 0.0062 | 0.5869 | 0.0021 |
| Head (width) | 0.8046 | 0.0062 | 0.6766 | 0.0022 |
| rostrum (length) | 1.1309 | 0.0070 | 0.9295 | 0.0026 |
| Antennomer 1 (length) | 0.9666 | 0.0071 | 0.9174 | 0.0030 |
| Antennomer 2 (length) | 0.9718 | 0.0071 | 0.9351 | 0.0042 |
| Antennomer 3 (length) | 0.8725 | 0.0071 | 0.8471 | 0.0042 |
| Antennomer 4 (length) | 1.0412 | 0.0079 | 0.8752 | 0.0050 |
| Femur (length) | 1.2109 | 0.0079 | 1.1988 | 0.0050 |
| Tibia (length) | 1.1981 | 0.0079 | 1.1035 | 0.0051 |
| Tibia (width) | 0.8761 | 0.0086 | 1.4869 | 0.0051 |
| Pronotum (length) | 0.6267 | 0.0094 | 1.1198 | 0.0053 |
| Pronotum (width) | 1.1932 | 0.0105 | 1.0803 | 0.0079 |
nymphs, even if they have the same direction. Second, organisms may present different trajectories, but their growth rates are the same. These factors are not mutually exclusive (Zelditch et al. 2003). For a real understanding of allometric growth in shape disparity between *H. clavigera* and *A. foliacea marginella* (either tibial or general shape), other factors besides allometric growth must be taken into account, as for example initial shape and the corresponding growth rates (Zelditch et al. 2003, 2004). Thus, studies concerning shape modelling of *H. clavigera* and *A. foliacea marginella* are necessary, where the three variables above mentioned can be either controlled or fixed separately.

We did not find any study that used similar method to detect ontogenetic allometry in insects. For Hemiptera, current works have used multivariate methods as principal components analysis to evaluate shape and size polymorphisms (Fairbain 1992), as well as to compare the three kinds of allometry (static, ontogenetic and evolutionary) in some Gerridae (Klingenberg & Zimmermann 1992b). Thus, it is difficult to discuss our findings in conjunction with other allometric studies. However, in this study bivariate and multivariate analysis provide complementary and at some point similar results, corroborating related works (e.g. Shea 1985).

Hind tibial shapes of *H. clavigera* and *A. foliacea marginella* are not statistically different in the first instar, indicating that these coreids are morphologically identical when they hatch. In contrast, hind tibial shape becomes different at the second instar. As pointed above, hind tibia presents the most conspicuous ontogenetic change when comparing both species, and can be used for visual species differentiation from the third instar on. However, this study did not find any adequate criteria for species differentiation in the early instars. As further research, characterization of ontogenetic and evolutionary allometries in the hind tibia of other anisoscelins should be considered, since it may contribute to understand their taxonomy and evolutionary radiation.

The developmental basis for allometry in insects points that the final size of any structure depends on the organism’s general magnitude. It is controlled by a hormonal system that leads to changes at both cellular and higher levels (Stern & Emlen 1999). When considering *A. foliacea marginella* and other coreids that show hind tibia expansion, allometry itself can explain how structures develop during the ontogeny and how different they are spatially concerning a given developmental stage. In addition, shape disparity during ontogeny is a potential factor that increases morphological and species diversity. Some of the structures here considered (e.g. tibia, rostrum) may be considered important traits (sensus Bernays 1991) due to their ecological functions (e.g., feeding, camouflage, speciation). Finally, the factors that may explain selection and evolutionary success in coreids remain poorly investigated, especially sexual selection (Aldrich et al. 1982, Eberhard 1998) and camouflage (see Schuh & Slater 1995).

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Table 3. Hotteling’s $T^2$ test for comparing hind tibia shape of *H. clavigera* and *A. foliacea marginella*. Asterisk indicates statistical significance (1000 randomizations). DF, degrees of freedom for rows and lines of the corresponding matrix, respectively (n = 20 / instar / species).

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**Literature Cited**


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