ORIGINAL ARTICLE

Head geometric morphometrics of two Chagas disease vectors from Venezuela

Ana María Oropeza[1], Carlos Arturo Perri Fernández[1], Jonathan Liria[2,3], Ana Soto-Vivas[4,5] *

[1]*Escuela de Bioanalisis, Facultad de Ciencias de la Salud, Universidad de Carabobo, Venezuela*
[2]*Universidad Regional Amazónica IKIAM, km7 vía Muyuna, Ecuador*
[3]*Centro de Estudios en Zoología Aplicada. Facultad Experimental de Ciencias y Tecnología, Universidad de Carabobo, Venezuela*
[4]*Centro de Estudios de Enfermedades Endémicas y Salud Ambiental. Instituto de Alto Estudios "Dr. Arnoldo Gabladon", Venezuela*
[5]*Carrera de Cs. Biológicas y Ambientales, Facultad de Ciencias Biológicas, Universidad Central del Ecuador, Ecuador*
Correspondence author, E-mail: aysoto@uce.edu.ec

Abstract   Triatominae species are considered the main vectors of Chagas disease or American Trypanosomiasis. In Venezuela, the principal vectors are *Rhodnius prolixus* (Stål, 1959) and *Triatoma maculata* (Erichson, 1848), which are belonged to the tribe Rhodniini and Triatomini, respectively. The head conformation and size development of these species can reflect ontogenetic changes which contribute with the vectors biology studies, as well to support of instars determination. The goal of the paper is to the application of geometric morphometric techniques for describing head conformation and size of instars of these species. We photographed 140 heads in *R. prolixus*: First instar (I: 16), second instar (II: 17), third instar (III: 18), fourth instar (IV: 21), fifth instar (V: 21), adult female (F: 26) and adult males (M: 21); in *T. maculata* heads of 136 specimens were photographed, I: 20, II: 17, III: 26, IV: 15, V: 19, F: 20 and M: 19. Landmark coordinate (x, y) configurations were registered and aligned by Generalized Procrustes Analysis. Covariance Analyses were implemented with proportions of re-classified groups and MANOVA. Statistical analyses of variance found not significant differences in head isometric size (Kruskal–Wallis) among IV and V instars in both species. The *a posteriori* re-classification was almost perfect in *R. prolixus* (82%) and *T. maculata* (86%); the main head differences occurs in antenniferous tubercles, postocular and preocular. Our study using quantitative tools for describing the shape differences contributes to explain the morphology variability and development of Chagas disease vectors.

Key words   Instars, Rhodniini, Triatomini, conformation, centroid size.

1   Introduction

Chagas disease or American Trypanosomiasis is a complex anthropozoonosis, caused by *Trypanosoma cruzi* (Chagas, 1909). Reduviidae, specifically Triatominae species are its main vectors. These insects are characterized by their hematophagic diet (in both nymphs and adults). The subfamily comprises six tribes and 138 species (Galvão *et al.*, 2003), and all the species are potentially available to transmit *T. cruzi*. These species are restricted to sylvatic habitats associated with small mammals and birds; however, others are adapted to domestic habitats, feeding on domestic animals and humans. In Venezuela, the principal vector species are included in two tribes: Rhodniini with *Rhodnius prolixus* (Stål, 1959), and Triatomini with *Triatoma maculata* (Erichson, 1848). *Rhodnius prolixus* is responsible for maintenance of domestic transmission, and the enzootic cycle between arboreal mammals and humans; and *Triatoma maculata* is considered

peridomestic and a secondary vector because it is close to rural houses found predominantly in chicken coops and animal pens (Pífano, 1969). The head conformation and size development of these species can reflect ontogenetic changes which contribute with the vectors biology studies, as well to support of instars determination. The traditional morphometrics and geometric morphometrics are important tools in Triatominae studies: population differentiation (Dujardin *et al.*, 1997, 1998; Feliciangeli *et al.*, 2007; Soto-Vivas *et al.*, 2007), sexual dimorphism (Jaramillo *et al.*, 2002), for inferring phylogenetic hypothesis (Soto-Vivas *et al.*, 2011), and recently in ontogenetic studies (Goncalves *et al.*, 2016). Due to this, we proposed the application of geometric morphometric techniques for describing the variation of conformation and size between instars in two Chagas disease vector species in Venezuela.

2   Material and methods

2.1   Specimens source

Rhodnius prolixus specimens were collected in a peridomiciliary area surrounded by *Acrocomia aculeta* (Jacq.) or "Corozo" palms, in Santa Ana (9.3065°S, 64.6629°W), Santa Ana municipality, Anzoátegui State, Venezuela (01.IV.2009); and *Triatoma maculata* specimens were collected in the domiciliary and peridomiciliary habits from rural houses in Tucupido (9.2727°S, 65.7699°W), José Félix Ribas municipality, Guárico State, Venezuela (02.IV.2009). All specimens were transported to the laboratory and sorted by instars following immature descriptions of Lent and Wygodzinsky (1979). Then, the insects were maintained to 27±1°C, HR 70±10%, photoperiod 12:12, and feed every eight days following protocols of Gomez-Nuñez and Fernández (1963) and Soto-Vivas and Molina de Fernández (2001).

2.2   Head dissection and mounting

The cephalic tagma was dissected using minutien pins. Small heads (I and II instars) were mounted on slide using euparal media; the remaining instars (III to adults) were mounted on card fixed on a pin. We photographed 140 heads in *R. prolixus:* first instar (I): 16, second instar (II): 17, third instar (III): 18, fourth instar (IV): 21, fifth instar (V): 21, adult female (F): 26 and adult males (M): 21; 136 specimens were photographed for *T. maculata*: I: 20, II: 17, III: 26, IV: 15, V: 19, F: 20 and M: 19.

2.3   Geometric morphometrics

Eight anatomical landmarks (LM1-LM8) (Figs 1A–B) were selected and digitized for each images, all following Bookstein (1991) type I and II criteria: (1) interception between the anteclypeus and postclypeus (right side), (2) external region of antenniferous tubercles (right side), (3) preocular (right side), (4) postocular (right side), (5) postocular (left side), (6) preocular (left side), (7) external region of the antenniferous tubercles (left side), and (8) interception between the anteclypeus and postclypeus (left side). From 276 matrix configurations we performed the Generalized Procrustes Analysis using the CoordGen program (Sheets, 2011a) for Procrustes superimposition and then was extracted a matrix variable conformation (Partial warps = Pw) and centroid size (CS). The Pw matrix was used for a Canonical Variates Analysis (CVA) and Multivariate ANOVA (MANOVA) with CVAGen (Sheets, 2011b) to determine whether pre-defined groups (instars by species) can be statistically distinguished based on multivariate data. Finally, we analyzed the CS differences by means of a non-parametric ANOVA with Kruskal-Wallis test (*P* 0.05), using Bonferroni correction, with PAST statistical program (Hammer & Harper, 2001).

3   Results

3.1   Centroid size

The Figure 2A shows the *Rhodnius prolixus* head CS between the five instars; the specimens grow gradually from the first instar to adults. The I instar specimens were the smallest 1.23 mm (1.19–1.29), followed by II instar 1.27 mm (1.25–1.30), III instar 2.30 mm (2.17–2.43), IV 3.12 mm (2.86–3.33), V instar 3.14 mm (2.88–3.36), male adult 3.56 mm (3.39–3.78) and female adult 3.67 mm (3.47–3.84). We found significant differences (Kruskal-Wallis: χ^2 129.8 p < 0.001) between all instars, with the exception of IV and V. The Figure 2B shows *Triatoma maculata* head CS between instars; the specimens growth gradually from the first instar to adults. The I instar specimens were the smallest 0.92 mm (0.88–0.96), followed by II 1.22 mm (1.18–1.28), III 2.23 mm (2.07–2.39), IV 2.90 mm (2.75–3.01), V 2.93 mm (2.81–3.10), female

adult 3.09 mm (2.83–3.23) and male adult 2.92 mm (2.77–3.09). We found significant differences (Kruskal-Wallis: χ^2 118 p < 0.001) between the instars, with an exception of IV, V and adults.
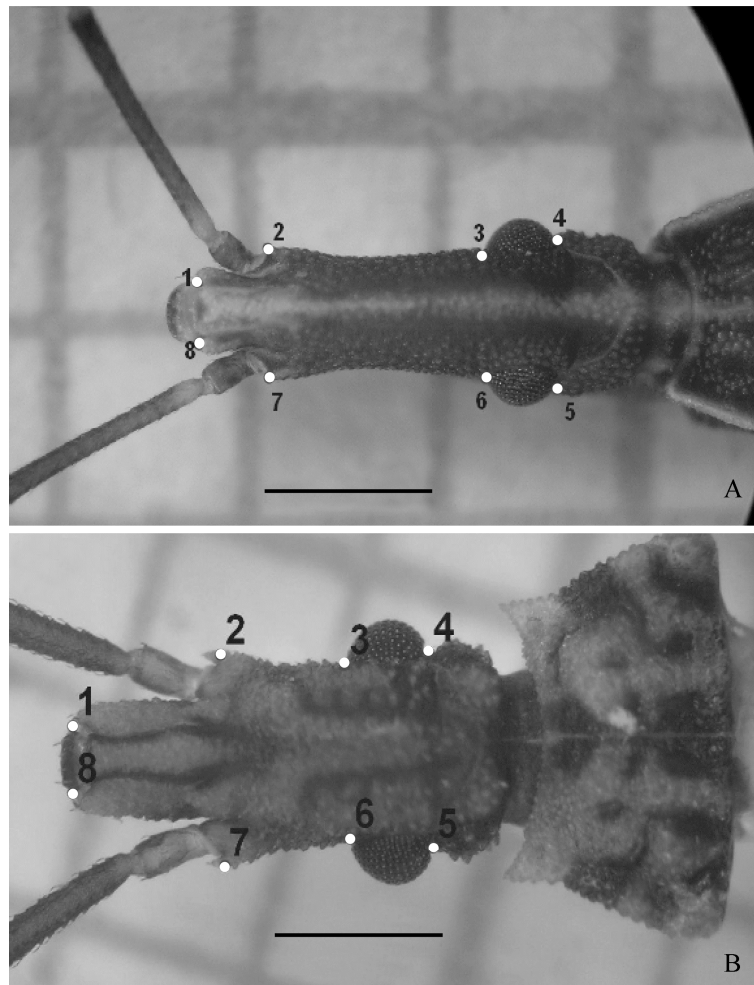

Figure 1. Landmarks head selection. A. *Rhodnius prolixus*. B. *Triatoma maculata*. Scale bar = 1 mm.

3.2   Conformation

We present the CVA statistics and assignation test results, based on *a priori* group definitions from instars morphological identification, and *a posteriori* assignment based on Mahalanobis distances between each specimen and the instar mean. In *R. prolixus*, Axis 1 Λ = 0.0001, χ^2 = 1144.32, df 72, p < 0.0001 and Axis 2 Λ = 0.0168, χ^2 = 529.11, df 55, p < 0.0001; the specimens were 82% correctly classified: I instar (100%), II (88.24%), III (88.89%), IV (61.90%), V (80.95%), female adult (76.92%) and male adult (76.19%). In *T. maculata*, Axis 1 Λ = 0.0003, χ^2 = 1002.99, df 72, p < 0.0001 and Axis 2 Λ = 0.0319, χ^2 = 432.37, df 55, p < 0.0001; the specimens were 86% correctly classified: I instar (100%), II (100%), III (84.62%), IV (66.67%), V (78.95%), female adult (85.00%) and male adult (84.21%). The thin-plate spline deformation grid shows the differentiation between instars and species: In *R. prolixus* (Figs 3A–C), the conformation differences between adult and V nymphs (Fig. 3A) correspond to LM3 and LM6 diagonal displacements to the postocular region, and LM4 and LM5 displace to the postocular region, which corresponds to an interocular region compression; finally, LM2 and LM7 separate and displaced to the anterior region. In I instar and adults (Fig. 3B), LM3 and LM6 displaced to the postocular region, LM4 and LM5 displaced to the preocular region, and LM2 and LM7 moved corresponding to a preocular region elongation. Finally, the difference between II and III nymphs (Fig. 3C) correspond to LM1 and LM8 displacement, causing clypeus compression, and the displacement of antenniferous tubercles (LM2 and LM7). In *T. maculata* (Figs 4A–C), the conformation differences between adult and V nymphs (Fig. 4A) correspond to LM3 and LM6 diagonal displacements to the postocular region, LM4 and LM5 displace to the postocular region, which corresponds to an interocular region compression; finally, LM2 and LM7 separate and displaced to the anterior region. In I

instar and adults (Fig. 4B), LM3 and LM6 displaced to the postocular region, LM4 and LM5 displaced to the preocular region, and LM2 and LM7 moved corresponding to a preocular region elongation. Finally, the difference between II and III nymphs (Fig. 3C) correspond to LM2 and LM7 displacement, causing an anteocular elongation, and LM3 and LM6 diagonal displacement to the postocular region.
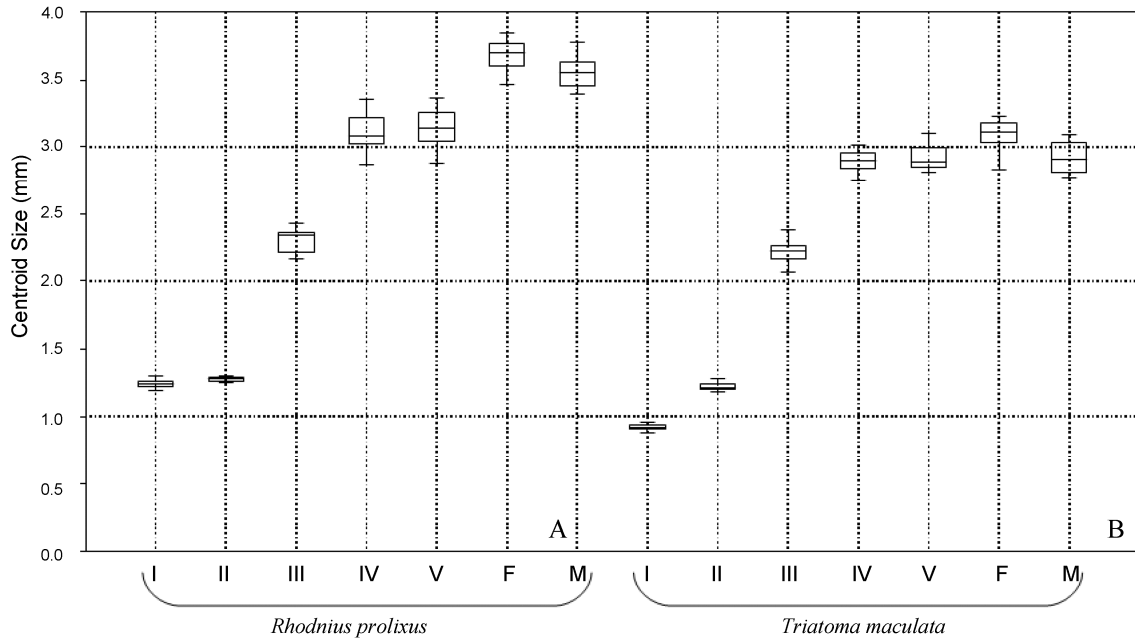

Figure 2. Box-plot head centroid size. A. *Rhodnius prolixus* instars. B. *Triatoma maculata* instars. Abbreviation: I—First instar; II—Second instar; III—Third instar; IV—Fourth instar; V—Fifth instar; F—Adult female; M—Adult male.

4   Discussion

The geometric morphometrics is proven to be a useful tool for describing ontogenetic changes in Hemiptera, Coreidae (Rodrigues, 2005). In Triatominae, Galvão *et al.* (2005) describing eggs and nymphs of *Linshcosteus karupus* (Galvão, Patterson, Rocha & Jurberg, 2002), and Rocha *et al.* (2005) studied instars differences in *Belminus herreri* (Lent & Wygodzinsky, 1979); both investigations concluded the importance of morphometric tools that quantified anatomical changes. More recently, Raigorodschi *et al.* (2011) determined ontogenetic differences in *Triatoma costalimai* Verano & Galvâo, 1959, considering that main change occurs between I and II instars, and III and IV; head elongation, increasing the distance between anteclypeus and antenniferous tubercles, head width decreasing, and eye size increasing. Later, Goncalves *et al.* (2016) described conformation and size differences in *Psammolestes arthuri* (Pinto, 1926), reporting a progressive growth from I to V instar, with head size reduction in the adults. These authors concluded that sized head reduction in adults could be attributed to landmarks selection and insect development. Our study showed a typical hemimetabolous insect development, with a gradual growth from I instar to the adults. Also, we found significant differences between head size in males and females in both species. Several studies in Triatominae confirm the sexual dimorphism, the females are larger than males (Jaramillo *et. al.*, 2002; Jaramillo & Caro-Riaño, 2005; Soto-Vivas *et al.*, 2007; Aldana *et al.*, 2011). Dujardin *et al.* (1999) comparing *Rhodnius robustus* Larrousse, 1927 specimens from sylvatic and domestic habits and suggested that population density affected the sexual dimorphism in the habitat transition. At high population density, which occurs in domestic or laboratory populations, each individual would get less blood because of competition and would then be smaller, especially among the females with greater food requirements. These authors concluded that due to higher survivorship in domestic or laboratory colonies, smaller individuals would survive and the average size decrease, especially in females.

Recently, Nattero *et al.* (2013) used head geometric morphometrics for exploring the phenotypic plasticity in peridomiciliary *Triatoma infestans* (Klug, 1834), feed on two food sources (pigeon and guinea pig) from Argentina. They reported significant differences between adults and food sources. In particular, adult specimens feed on guinea pig showed

to be larger than those feed on pigeon; and in both food sources, females were larger than males. However, in Triatominae, the sexual dimorphism is evident in sylvatic populations, and because this, the results exposed by these authors are difficult to attribute to the food source. Several studies reveal, that are necessary various generations for dimorphism lost (Dujardin *et al.*, 1999; Jaramillo *et al.*, 2002). Also, the little variation among head size instars (I to V), contrast with our results and any bug growth: in Triatominae, as typical hemimetabolous insects, each size increment is the product of following existing size (Goncalves *et al.*, 2016). Finally, our study using quantitative tools for describing the shape differences contributes to explain the morphology variability in Chagas disease vectors.
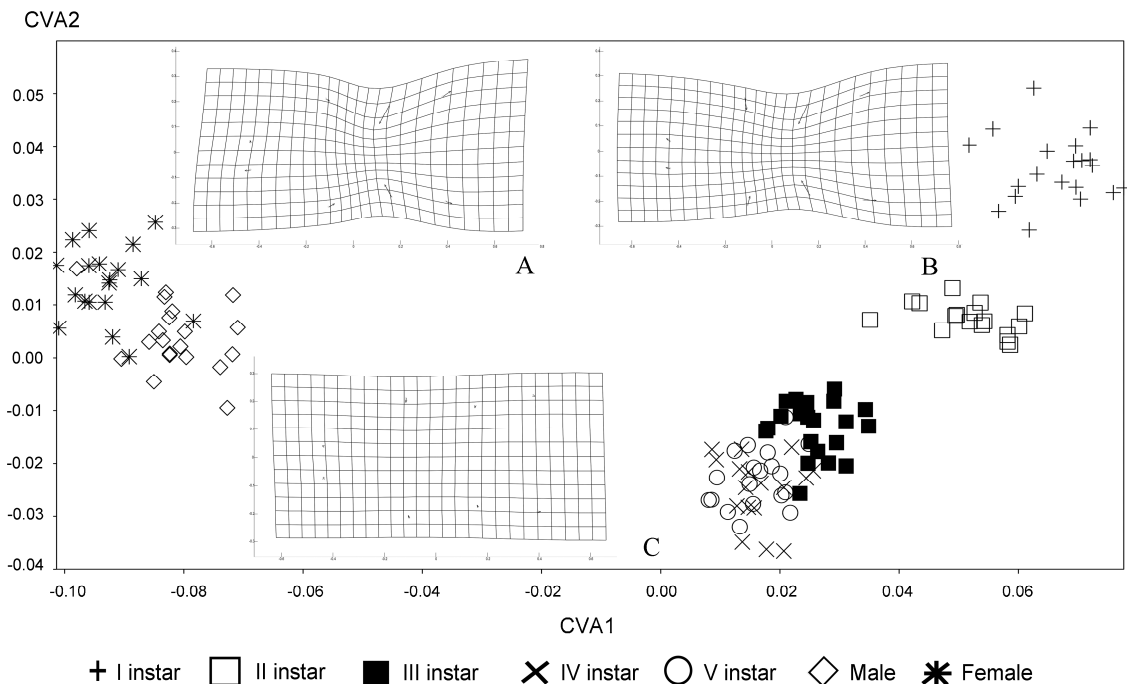

Figure 3. Canonical Variates Analysis head conformation diagram for 140 *Rhodnius prolixus* specimens and thin-plate deformation grids. A. V instar–Adults. B. I instar–Adults. C. II instar–III instar.
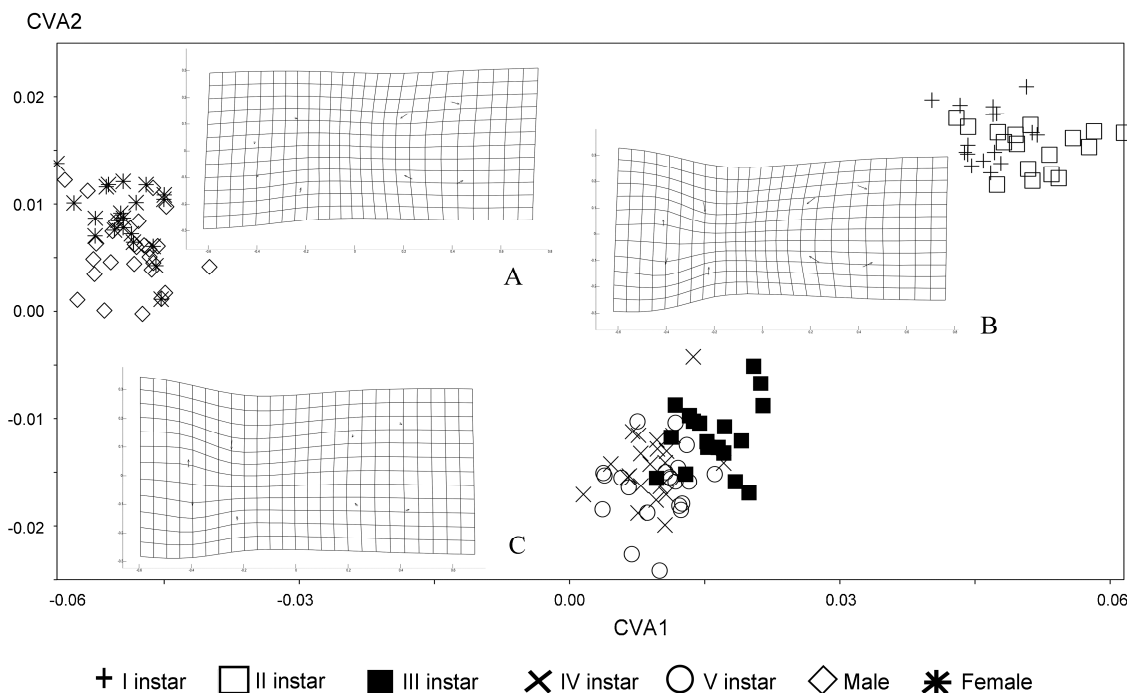

Figure 4. Canonical Variates Analysis head conformation diagram for 136 *Triatoma maculata* specimens and thin-plate deformation grids. A. V instar–Adults. B. I instar–Adults. C. II instar–III instar.

References

Aldana, E., Heredia-Coronado, E., Avendaño-Rangel, F., Lizano, E., Concepción, J., Bonfante-Cabarcas, R., Rodríguez-Bonfante, C., Pulido, M.M. 2011. Análisis morfométrico de *Panstrongylus geniculatus* de Caracas, Venezuela. *Biomedica*, 31: 108–117.

Bookstein, F.L. 1991. *Morphometric tools for landmark data: Geometry and Biology*. Cambridge, EEUU. 435pp.

Dujardin, J.P., Bermúdez, H., Casini, C., Schofield, C.J., Tibayrenc, M. 1997. Metric differences between sylvatic and domestic *Triatoma infestans* (Hemiptera: Reduviidae) in Bolivia. *Journal of Medical Entomology*, 34: 544–551.

Dujardin, J. P., Forgues, G., Torrez, M., Martinez, E., Cordoba, C., Gianella, A. 1998. Morphometrics of domestic *Panstrongylus rufotuberculatus* in Bolivia. *Annals of Tropical Medicine and Parasitology*, 92: 219–228.

Dujardin, J. P., Steindel, M., Chavez, T., Machane, M., Schofield, C.J. 1999. Changes in the sexual dimorphism of Triatominae in the transition from natural to artificial habitats. *Memórias do Instituto Oswaldo Cruz*, 94: 565–569.

Feliciangeli, M.D., Sanchez-Martin, M., Marrero, R., Davies, C., Dujardin, J.P. 2007. Morphometric evidence for a possible role of *Rhodnius prolixus* from palm trees in house reinfestation in the State of Barinas (Venezuela). *Acta Tropica*, 101: 169–177.

Galvão, C., Carvallo, R., Da Silva, D., Jurberg, J. 2003. A Checklist of the current valid species of subfamily Triatominae Jeannel 1919 (Hemiptera, Reduviidae) and their geographical distribution, with nomenclatural and taxonomic notes. *Zootaxa*, 202: 1–36.

Galvão, C., Mcallon, M., Rocha, D., Schaefer, C., Paterson, J., Jurberg, J. 2005. Description of Eggs and Nymphs of *Linshcosteus karupus* (Hemiptera: Reduviidae: Triatominae). *Annals of the Entomological Society of America*, 98: 861–872.

Goncalves, L. Liria, J., Soto-Vivas, A. 2016. Ontogenetic morphometrics in *Psammolestes arthuri* (Pinto, 1926) (Reduviidae, Triatominae) from Venezuela. *Journal of Entomology and Zoology Studies*, 4: 369–373.

Gomez-Nuñez, J.C., Fernandez, J.M. 1963. La colonia de *Rhodnius prolixus* en el Instituto Venezolano de Investigaciones Cientificas. *Boletin de la Dirección de Malariología y Saneamiento Ambiental*, 3: 132–137.

Hammer, Ø., Harper, D.A.T. 2011. PAST: Palaeontological Statistics, Version 2.10. Available from http://folk.uio.no/ohammer/past. (accessed 19 Oct. 2015).

Jaramillo, N., Caro-Riaño, H. 2005. Diferencias morfométricas entre Rhodnius prolixus Stål, 1859 y R. pallescens Barber, 1932. *In*: Guhl, F. (ed.). *Primer Taller Internacional sobre Control de la Enfermedad de Chagas. SSA-ES, Tripanosomiasis Update*. CDIA, Uniandes, Bogotá. pp. 69–79. Available from http://cdiaec.uniandes.edu.co/(accessed 20 May. 2016).

Jaramillo. N., Castillo, D., Wolf, M. 2002. Geometric morphometric differences between *Panstrongylus geniculatus* from field and laboratory. *Memórias do Instituto Oswaldo Cruz*, 97: 667–73.

Lent, H., Wygodzinsky, P. 1979. Revision of the Triatominae (Hemiptera, Reduviidae), and their significance as vectors of Chagas' disease. *Bulletin of the American Museum of Natural History*, 63: 408.

Nattero, J., Malerba, R., Rodríguez, C. S., Crocco, L. 2013. Phenotypic plasticity in response to food source in *Triatoma infestans* (Klug, 1834) (Hemiptera, Reduviidae: Triatominae). *Infection, Genetics and Evolution*, 19: 38–44.

Pífano, F. 1969. *Algunos Aspectos en la Ecología y Epidemiología de las Enfermedades Endémicas con Focos Naturales en el Area Tropical, Especialmente en Venezuela*. Archivos del Ministerio de Sanidad y Asistencia Social, Caracas. 297pp.

Raigorodschi, R., Rocha, D., Jurberg, J., Galvão, C. 2011. Description and ontogenic morphometric of eggs and instars of *Triatoma costalimai* Verano and Galvão, 1959 (Hemiptera, Reduviidae, Triatominae). *Zootaxa*, 3062: 13–24.

Rodrigues, D., Sanfelice, D., Monteiro, L., Moreira, G. 2005. Ontogenetic trajectories and hind tibia geometric morphometrics of *Holymenia clavigera* (Herbast) and *Anisoscelis foliacea marginella* (Dallas) (Hemiptera: Coreidae). *Neotropical Entomology*, 34: 769–776.

Rocha, D., Patterson, J., Sandoval, C., Jurberg, J., Angulo, V., Esteban, L. Galvão, C. 2005. Description and ontogenetic morphometrics of nymphs of *Belminus herreri* Lent and Wygodzinsky (Hemiptera: Reduviidae, Triatominae). *Neotropical Entomology*, 34: 491–497.

Sheets, H.D. 2011a. CoordGen7, Coordinate Generation program for calculating shape coordinates. Available from http://www3.canisius. edu/~sheets/imp7.htm (accessed 28 May 2016).

Sheets, H.D. 2011b. CVAGen7, Canonical Variates Analysis program for the analysis of shape, based on partial warp scores. Available from http://www3.canisius.edu/~sheets/imp7.htm (accessed 28 May 2016).

Soto-Vivas, A., Molina de Fernández, D. 2001. Toxicidad de cinco insecticidas en una cepa de laboratorio de *Rhodnius prolixus* Stål, 1859 (Hemiptera: Reduviidae) de Venezuela. *Entomotropica*, 16: 187–190.

Soto-Vivas, A., Rodríguez, C., Bonfante-Cabaraca, R., Aldana, E. 2007. Morfometría geométrica de *Triatoma maculata* (Erichson, 1848) de ambientes doméstico y peridoméstico, estado Lara, Venezuela. *Boletin de Malariología y Salud Ambiental*, 47: 232–235.

Soto-Vivas, A., Liria, J., de Luna, E. 2011. Morfometría geométrica y filogenia en Rhodniini (Hemiptera, Reduviidae) de Venezuela. *Acta Zoológica Mexicana*, 27: 87–102.