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Ontogenetic morphometrics in *Psammolestes arthuri* (Pinto 1926) (Reduviidae, Triatominae) from Venezuela

Lisseth Goncalves, Jonathan Liria, Ana Soto-Vivas

Abstract
Psammolestes arthuri is a secondary Chagas disease vector associated with bird nests in the peridomicile. We studied the head architecture to describe the size changes and conformation variation in the *P. arthuri* instars. Were collected and reared 256 specimens associated with *Campylohynochus nucalys* nests in Guarico state, Venezuela. We photographed and digitized ten landmarks coordinate (x, y) on the dorsal head surface; then the configurations were aligned by Generalized Procrustes Analysis. Canonical Variates Analysis (CVA) was implemented with proportions of re-classified groups (=instars) and MANOVA. Statistical analysis of variance found significant differences in centroid size (Kruskal-Wallis). We found gradual differences between the 1st instar to 5th and a size reduction in the adults; the CVA showed significant separation, and *a posteriori* re-classification was 50-78% correctly assigned. The main differences could be associated with two factors: one related to the sampling protocol, and another to the insect morphology and development.

Keywords: Instars, conformation, Rhodniini, centroid size, Venezuela

1. Introduction
The Chagas disease or American Tripanosomiases is mainly distributed between the 42º and 46º of North latitude and South, respectively [1]. The vectors of this disease are bugs included in Triatominae subfamily and comprises six tribes. In particular, Rhodniini with the genera *Rhodnius* (16 spp.) and *Psammolestes* with three species: *P. coreodes* Bergroth, 1911, *P. arthuri* Pinto, 1926 and *P. tertius* Lent & Jurberg, 1965 [2]. Some authors suggested that these species are specialized to bird nests microhabitats in open forests [3, 4]. *Psammolestes arthuri* is widely distributed in Venezuela and Colombia [5-7]; mainly associate to the peridomicile [8] in the Venezuelan Llanos and Venezuelan Coast Biogeographic Province [9]. In recent years, was reported the presence of sylvatic Triatominae in rural houses, possibly attracted by light houses or by food source; between them: *Triatoma maculata*, *Panstrongylus geniculatus, Eratyrus mucronatus* Stal 1859, *R. robustus* Larrouse 1927, *T. nigromaculata* Stal 1872 and *Psammolestes arthuri* Pinto 1926 [3, 5, 10, 11].
The geometric morphometrics [12-15] are widely used in Triatominae: Studies of sylvatic and domestic differentiation [16-19], variation in field and laboratory populations [20], or between geographic regions [21, 22], ontogeny [23], as taxonomic tool [24] or in a phylogenetic approach [25]. In *Psammolestes* the studies were restricted to traditional morphometrics: eggs and nymphs descriptions in *P. salazari* (=*P. arthuri*) [26], in *P. tertius* bionomics [27], and finally in combination with molecular tools in *P. tertius* [28]. However, the geometric morphometrics was not evaluated in *P. arthuri*, because that we propose to study the ontogenetic differences, in order to contribute to future research in taxonomy and population variability.

2. Materials and methods
2.1 Biological material and data acquisition: The *P. arthuri* was obtained in nest of *Campylohynochus nucalys* (Stripe-backed Wren) in the locality Bersuga, Guarico state, at 383 masl, 9º46'2.1'' N and 67º37'40.8'' W, in March 2008. This locality is included in the physiogeographic category of Sabanas Piemontanas Arbustivas according to [29, 30]. Then, the specimens were transported to the insectary and posteriorly sorted into the five instars (I-V) using the Lent and Wygodzinsky [3] criteria. Finally, all the specimens (by instars and sex) were reared in the insectary [31, 32] until complete the necessary individuals for the morphometric analysis. Then, were photographed, selected and digitized, ten anatomical

landmarks (LM1-LM10), all according Bookstein [12] type I and II criteria (Figure 1): 1) Interception between the anteclipeous and postclipeous, 2) internal region of antennal tubercle, 3) external region of antennal tubercle, 4) preocular, 5) postocular, 6) postocular, 7) preocular, 8) external region of the antennal tubercle (left side), 9) internal region of antennal tubercle (left side) and 10) interception between the anteclipeous and postclipeous (left side).

Fig 1: Head of *Psammolestes arthuri* showing the landmarks (1-10) disposition. The polygon enclosed by the points conform the configurations analyzed.

2.2. Geometric morphometrics: From 256 matrix configurations (first instar; I: 24, second; II: 40, third III: 24, fourth; IV: 44, fifth V:79, adult; A:45), we perform the Generalized Procrustes Analysis, with the Coord Gen program [33] for Procrustes superimposition and then was extracted a matrix variable conformation (Partial warps = Pw) and centroid size (CS). The Pw matrix was used for an Canonical Variates Analysis (CVA) and Multivariate ANOVA (MANOVA) with CVAGen [34] to determine whether pre-defined groups (instars) can be statistically distinguished based on multivariate data. Finally, we analyzed the CS differences by means of a non-parametric ANOVA with Kruskall-Wallis test (*P* 0.05), using Bonferroni correction, with PAST statistical program [35].

3. Results

The Figure 2 shows the box plot for CS head between instars; the *P. arthuri* head size, gradually increased from the first instar to the fifth, and subsequently reduced in the adults. These results were significantly (Kruskal-Wallis: $\chi 2 = 244.2$, $p<0.001$), the first instar specimens were small 0.85 mm (0.76-0.96), followed by the second instar 1.01 mm (0.90-1.10), third instar 1.40 mm (1.08-1.60), fourth 1.80 mm (1.46-2.08), and fifth 2.31 mm (1.68-2.54). However, no statistically significant difference was found between the adult 1.85 mm (1.61-2.04) and fourth instar nymph. The first CVA axes explained the 94% of variance (CV1= 85%, CV2= 9%) with lambda Wilks 0.012; all the axes showed significative discrimination (Table 1). The Table 2 shows the assignment *a posteriori* re-classification; the instars were poorly classificated (50-78%), the best assignment was in adults (males: 78% and females: 72%). The two axes diagram form CVA for *P. arthuri* instars showed (Figure 2) the separation into two mainly groups: one comprises the adults (males nd females) and another with the remaining instars (I-V). The last group showed a slight separation between the V instar nymphs (males and females) from the remaining instars (I-IV). The thin-plate spline deformation grid show the differentiation between instars: In V nymphs and adults (Figure 2A) the differences correspond with the diagonal displacement of LM4 and LM7 to the clipeal area, displacement of LM2 and LM9 to the anteocular region, and finally the separation between LM3 and LM8; in I and II nymphs and V nymphs (Figure 2B) the mainly deformations occur in LM4-LM7 associated with eye displacements, corresponding to preocular and preocular areas.

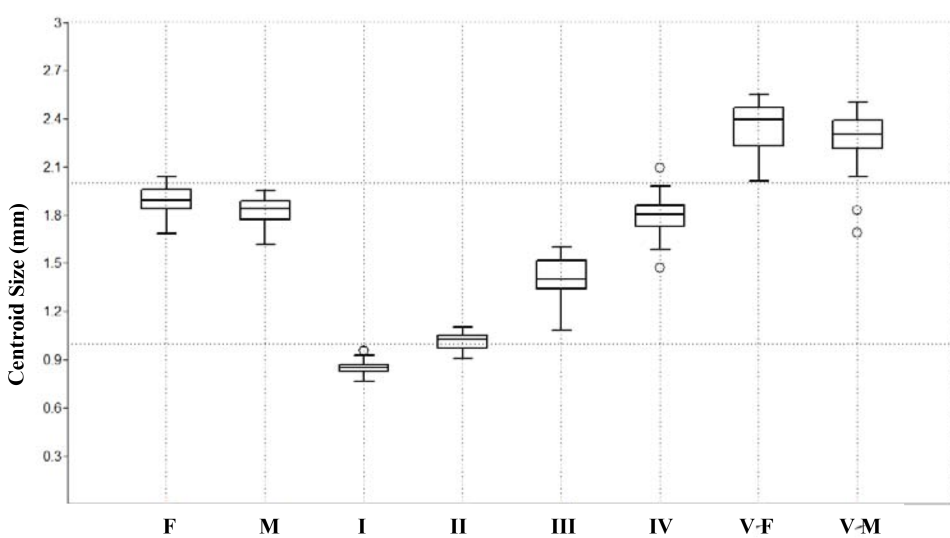

Fig 2: Box plot for the *Psammolestes arthuri* head centroid size and instars: **F.** Adult females; **M.** Adult males; **I.** 1st instar nymph; **II.** 2nd instar nymph; **III.** 3rd instar nymph; **IV.** 4th instar nymph; **V F.** 5th instar female nymph; **V M.** 5th instar male nymph.

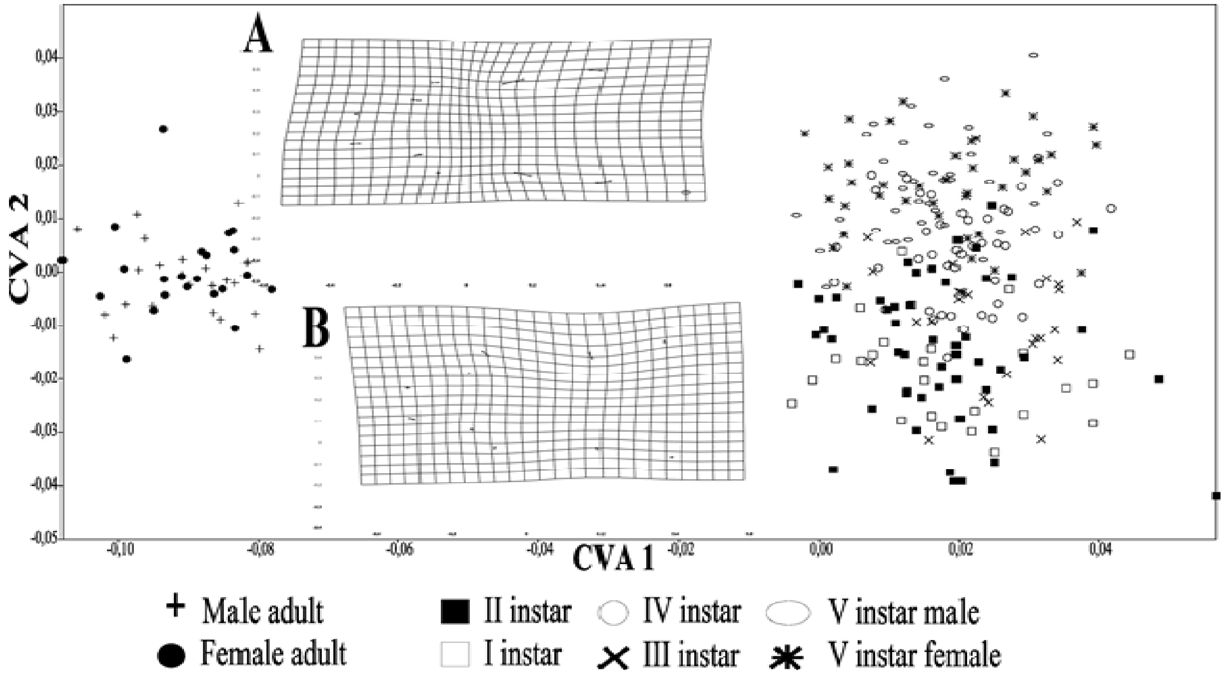

Fig 3: Canonical Variates Analysis diagram of two canonical axes from 256 *Psammolestes arthuri* specimens, and thin-plate spline grid deformations between: A) V nymphs and adults (♀,♂) and B) I and II nymphs and V nymphs (♀,♂).

Table 1: Lambda (Λ) values for Wilk's test for each CVA axis.

Axis	Lambda	χ^2	g.l	*P*
1	0.0011	1138.12	112	< 0.0001
2	0.1947	413.75	90	< 0.0001
3	0.5020	174.35	70	< 0.0001
4	0.6726	98.10	52	< 0.0001
5	0.7948	58.11	36	< 0.0001

Table 2: Results of statistical testing of group assignments for total sample of 256 *Psammolestes arthuri* specimens, based on the *a posteriori* assignments using the CV axes and Mahalanobis distances between each specimen and the instars mean.

***a priori* assignment**	***a posteriori* assignments**								
Instars	**♀ adult**	**♂ adult**	**I**	**II**	**III**	**IV**	**♀ V**	**♂ V**	**%**
♀ adult	**16**	6							72.0
♂ adult	5	**18**							78.0
I			**16**	8					66.0
II				**27**	3	8	1	1	67.5
III			2	2	**16**	1	1	2	66.6
IV				2	9	**22**	6	5	50.0
♀ V			2	1	0	2	**25**	11	60.9
♂ V						6	10	**22**	60.1

4. Discussion

The geometric morphometric tools have been recently used in ontogenetic studies for the instar description in *Holymenia clavigera* (Herbast) and *Anisoscelis foliacea marginella* (Dallas) (Hemiptera: Coreidae) [36]. These authors suggested that quantitative studies among nymphal instar could be a useful tool for the character's determination during the species development. In Triatominae we only found two studies: the eggs and nymph descriptions in 61 specimens of *Linshcosteus karupus* Galvão, Patterson, Rocha & Jurberg, 2002 [37] and the immature descriptions of 17 specimens *Belminus herreri* Lent & Wygodzinsky, 1979 [23]. Both studies concluded that the geometric morphometrics is an informative tool for detection of anatomical variation. However, these investigations used a low specimens representation for the statistical analysis of conformation differences (e.g. CVA or Discriminant Analysis), and according to Strauss [38] and Webster and Sheets [39] the total sample size must be larger than the variables analyzed (number of landmarks * dimensions: 2D or 3D) in order to obtain a reliable estimate of the variance-covariance structure in the data. In our analysis, the differences found in the head size among *P. arthuri* instars could be associated with two factors: In the first case, the sampling protocol was transversal, the insects were collected in different bird nests and possibly the specimens come from different cohorts. Klingenberg and Zimmermann [40] reports the inconvenience about these sampling protocols, and concluded that the growth rate could be affected to the transversal sampling. On the other hand, Jaramillo *et al.* [20] showed that are necessary at least five generations in *Panstrongylus geniculatus*, for quantify the size variations among instars. In the second case, in hemimetabolous insects the growth is a gradual process and occurs in several nymphal stages; each size increment is product of following existing size [40]. The size increment occurs because the old cuticle are replaced by means of the ecdysis process and is regulated by hormonal mechanisms [40, 41]. In our study the head size reduction in adults could by explain in terms of the landmark selection, in particular those anatomical points in the intersection between the anteclipeal and the postclipeal area; in the fifth instar occurs an increment in the head size due to the ocellus rise, and then in the next instar (adults) the centroid size decreased because the landmarks displace toward the anterior part of the head.

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