DOI: https://doi.org/10.1016/j.actatropica.2022.106383

Acta Tropica 229 (2022) 106383

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

New in morphometry: Geometric morphometry of the external female genitalia of Triatominae (Hemiptera: Reduviidae)

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ARTICLE INFO

Keywords:
Morphometry
Geometric morphometric
Phenotype
Operational homology

ABSTRACT

The study of geometric morphometry has an impact on Triatominae studies. Currently, several taxonomic and systematic studies use this approach. The Triatominae subfamily comprises three fossil species and 154 extant species potentially capable of transmitting *Trypanosoma cruzi*, the causative agent of Chagas disease. This study aims to evaluate the external female genitalia of adult triatomines using multivariate geometric morphometric approaches, not only for validation but also for systematic inferences. Specimens belonging to the genera *Panstrongylus, Psammolestes, Rhodnius,* and *Triatoma* were evaluated, in addition to two species previously included in *Triatoma: T. longipennis* and *T. phyllosoma*. The results show that the external female genitalia have operational morphology and allow characterization of the species and the genera of the Triatominae. In addition, the multivariate technique enabled delimitation of the phylogenetic relationships of the subfamily, presenting results consistent with systematic studies. It can be concluded that the external female genitalia evaluated by geometric morphometry is a useful character for the taxonomy and systematics of Triatominae.

1. Introduction

Triatominae Jeannel, 1919 is a diverse group of blood-sucking insects of vertebrates, including man (Lent and Wygodzinsky, 1979) that comprises three fossil species and 154 extant species (Zhao et al., 2021; Dale et al., 2021; Paiva et al., 2022). Some are proven or potential vectors of *Trypanosoma cruzi* (Chagas, 1909), the causative agent of Chagas disease. Currently, 18 genera are recognized, of which three are relevant to public health: *Panstrongylus* Berg, 1879, *Rhodnius* Stål, 1859, and *Triatoma* Laporte, 1832.

Published as an extensive review, the systematization of triatomines was supported by morphological characters (Lent and Wygodzinky, 1979). Phylogenetic relationships in the subfamily are not fully resolved although evidence points to Reduviidae predators as ancestors and to the monophyletic origin of the subfamily, there is no consensus (Justi et al., 2014, 2016; Zhang et al., 2016; Kieran et al., 2021). Morphological, behavioral, and sympatry similarities are common in the subfamily and these biological characteristics, in addition to making phylogenetic reconstruction difficult, also make specific characterization arduous (Galvão et al., 2021).

In addition to the phylogenetic difficulties, correct identification of triatomines is critical and essential for the control and surveillance of entomology. This requires accessible and easy-to-apply identification methods such as morphometric methods (Dujardin, 2008). Morphometry is frequently explored in studies with Triatominae, being applied through the classical form also called linear morphometry (Patterson et al., 2001; Obara et al., 2007; Rivas et al., 2014; Oliveira et al., 2015; Belintani et al., 2021a), as well as through multivariate strategies such as geometric morphometry (Patterson et al., 2001; Oliveira et al., 2017; Belintani et al., 2020; 2021b; Kamimura et al., 2020; Zhao et al., 2021). Geometric morphometry is used to assess the variables of shape and size of the hemelytra (Gurgel-Gonçalves et al., 2011; Mendonça et al., 2016; Belintani et al., 2020; 2021a; Kamimura et al., 2020), heads (Oliveira et al., 2017; Falcone et al., 2020; Zhao et al., 2021), eggs (Santillán--Guayasamín et al., 2017), ontogenetic studies (Raigorodschi et al., 2005; Rocha et al., 2005, 2020), ecological and fitness-related studies (Nattero et al., 2013; Gutiérrez-Cabrera et al., 2021).

In the last century, few studies have evaluated female genitalia (Lent, 1948; Abalos and Wygodzinsky 1951; Sherlock and Serafim, 1967; Lent and Jurberg, 1969). However, the taxonomic validity of the characters that compose the external female genitalia of the Triatominae, first evidenced by Rosa et al. (2010) were confirmed by

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https://doi.org/10.1016/j.actatropica.2022.106383
Received 29 November 2021; Received in revised form 16 February 2022; Accepted 18 February 2022

several other authors (Rosa et al., 2014; Rivas et al., 2017; Oliveira et al., 2020; Teves et al., 2020; Belintani et al., 2021a). As these authors explored the morphological characters of the external female genitalia, the current study tested whether these characters have taxonomic validity through geometric morphometry.

Approaches based on morphometric assessments are useful and widely used in vector studies (Dujardin, 2008). Morphometric geometry, when compared to other methods, is useful for this purpose, as it does not require highly technological devices or specialized techniques (Monteiro and Reis, 1999). For Triatominae, morphometric study of the hemelytron and head has significantly contributed to taxonomic evaluations (Gurgel-Gonçalves et al., 2011; Mendonça et al., 2016; Oliveira et al., al.,2017; Belintani et al., 2020). Thus, this geometric morphometric study of the female external genitalia is a new methodology that may provide valuable contributions to the specific characterization, as well as the phylogenetic analysis of Triatominae.

2. Methods

2.1. Specimens and identification

The specimens used in this work were obtained from the Triatominae Insectarium of São Paulo State University “Julio de Mesquita Filho”, School of Pharmaceutical Sciences, Araraquara, São Paulo, Brazil (https://www2.fcfar.unesp.br/#!/triatominae). Specimens were identified from descriptions and identification keys (Lent and Wygodzinsky, 1979). In this study *Psammolestes tertius* Lent and Jurberg, 1965*, Panstrongylus lignarius* Walker, 1873, *Panstrongylus megistus (Burmeister, 1835), Rhodnius prolixus Stål, 1859, Rhodnius neglectus* Lent, 1954, *Triatoma infestans (Klug, 1834), Triatoma longipennis* Usinger, 1939*, Triatoma phyllosoma* (Stål, 1872), and *Triatoma. vitticeps* (Stål, 1859) were evaluated.

2.2. Geometric morphometry of the external female genitalia

To evaluate the potential of the method and the systematic signal of the structure, all statistical analyses were applied independently between species and genera of the Triatominae.

2.3. Obtaining images and landmarks

Geometric morphometry (GM) was used to assess variations in the shape and size of external female genitalia (EFG). In total, 15 adult specimens were selected from each species analyzed. External female genitalia images were acquired using a stereoscopic microscope Leica DMC 2905 coupled to a Leica M205AC digital camera with Leica Application Suite (LAS) software. The images followed the same configurations, aiming to standardize the capture.

The anatomical landmarks were selected through careful evaluation of the operational homology of the structure (Monteiro and Reis, 1999). Thus, we selected nine reference landmarks of type 1 (supplementary material). The landmarks were collected and processed with the TPS package utilities (Rohlf, 2006): TPSdig 2.3.2 and TPSutil 1.81. The reference landmarks of all populations were collected 3 times, seeking to minimize collector effects (Monteiro and Reis, 1999). The collected data generate a text file (TPS extension) containing the raw coordinates, and this file was used in downstream evaluations. Raw coordinates are used for generalized Procrustes analysis (GPA) in MorphoJ (Klingenberg, 2011). GPA is a statistical analysis method that is performed to delete all information related to size, position, and orientation (Rohlf, 1998). Subsequently, the generated matrix is projected onto a Euclidean space to generate a set of scores: partial warps (Bookstein, 1991). An average setting, known as a "consensus" is calculated and allows the average variation between the raw data to be determined (Bookstein, 1991). All additional statistics were performed using Procrustes residues to analyze differences in size and shape.

2.4. Analysis of size and shape variables

A factorial map was generated using MorphoJ (Klingenberg, 2011) by the general patterns of morphological variation in the multidimensional data obtained with principal component analysis (PCA). PCA allows exploration of variations in the shape (Monteiro and Reis, 1999). The PCA was analyzed through the covariance matrix of the Procrustes coordinates obtained after the GPA of the original reference coordinates. Procrustes ANOVA (Klingenberg and Leamy, 2001) was performed to assess shape variations using MorphoJ (Klingenberg, 2011), and is used to infer differences between groups as in Belintani et al. (2020).

To determine the size variables, we used the isometric estimator defined as centroid size (CS) (Monteiro and Reis, 1999). CS is derived from raw coordinate data (Bookstein, 1991) generated in MorphoJ (Klingenberg, 2011). Furthermore, the Procrustes Distances were obtained through the relation with the significant distribution of Goodall’s F-test (Goodall, 1991) being obtained by a permutation test. The relationship between CS and discrimination of shapes between groups (allometry) was estimated through a multivariate regression between the coordinates of Procrustes (dependent variables) and CS (independent variable). Mahalanobis distances between pairs of species were calculated for shape measurements and their significance was assessed using a non-parametric test based on permutations (bootstrap, 10.000 replicates) using MorphoJ (Klingenberg, 2011). In addition, using Mahalanobis distance data, neighboring trees (NJ) were retrieved using PAST v.3.25 (Hammer et al., 2001).

2.5. Morphological variation among groups

To determine the relationships between species and genera, canonical variate analysis (CVA) was performed using MorphoJ (Klingenberg, 2011). Canonical variate analysis (CVA) is a method used to find the shape features that best distinguish among multiple groups of specimens. Group membership is assumed to be known a priori (Klingenberg, 2011). Multivariate statistics were performed using Procrustes coordinates (Klingenberg and Monteiro, 2005). The CVA was performed associated with a resampling method (10.000 replicates) to build regions of confidence in relation to the mean centroid sizes of the species. A factorial map of the first two canonical factors was generate using MorphoJ (Klingenberg, 2011) to illustrate the main results for species and genera.

In addition, we used discriminant function analysis (DFA) to examine the separation between two groups of problems, which are known a priori. An analysis is like a CVA; however, the test is performed between two groups.

2.6. Mapping onto phylogeny

To map the shapes in a phylogenetic tree we used the MorphoJ resource (Klingenberg, 2011). MorphoJ uses squared-change parsimony (Maddison, 1991) for mapping data onto phylogenies. As a result, the locations of internal nodes of the phylogeny can be reconstructed. This information can be used to interpret the diversification of related species in direct relation to their phylogenetic history. MorphoJ provides various plots for visualizing reconstructed ancestral shapes, reconstructed evolutionary trajectories through shape space, and the trees themselves.

To assess the effects of phylogenetic uncertainty on the results of the evaluation of phylogenetic signals in morphological data, we used the “collect statistics on tree set” tool from MorphoJ. Phylogenies can be estimated with greater or lesser degrees of uncertainty. One method of quantification and evaluation is to use a few different trees with an expression of uncertainty about the tree as described by Klingenberg and Gidaszewski (2010).

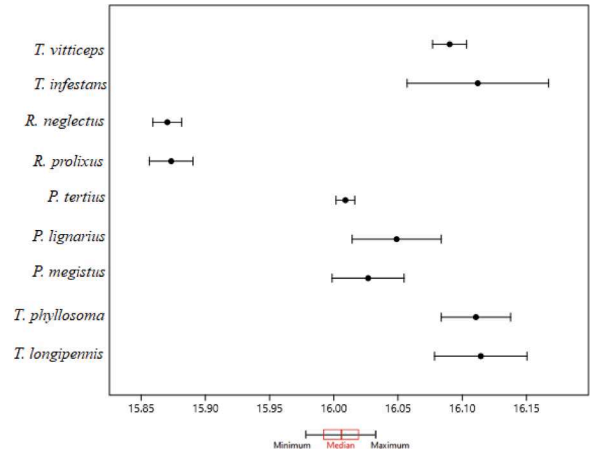

Fig. 1. Variation in CS of external female genitalia among species (in pixel). Box plot: mean and standard deviation, 95% interval.

3. Results

135 external female genitalia of nine species of Triatominae were digitized and the anatomical coordinates collected. Of these, approximately 10 samples were considered as "outliers" and excluded from the analysis. The outliers are observations that, due to operational factors, show great variance from the others in the series, which is why they are excluded, as they can compromise the results. To validate and demonstrate that the external female external genitalia of triatomines is a useful character for geometric morphometric analysis, we evaluated several species and genera of the Triatominae. We tried to sample two species for each genus, with *Psammolestes* showing only one representative. *Triatoma longipennis* and *T. phyllosoma* were transferred to *Triatoma* [the validity of *Meccus* genus has been discussed (Martínez-Ibarra et al., 2011; Cesaretto et al., 2021)] and for this reason we chose to insert the specimens to corroborate the descriptions.

3.1. Analysis of size and shape variables between species

The CS means obtained from the GPA show the size variables between species. The size variation is illustrated with quantiles (Fig. 1). The highest means recorded are *T. infestans, T. longipennis, T phyllosoma*, and *T. vitticeps* all belonging to *Triatoma*. The smallest means were retrieved for *Rhodnius neglectus* and *R. prolixus* followed by *P. tertius. P.s lignarius* and *P. megistus* maintained approximate means. All species had approximate means allowing for group species/genus by the mean values of CS. The size ratio was *T. infestans* > *T. longipennis* > *T. phyllosoma* > *T. vitticeps* > *P. lignarius* > *Pa. megistus* > *P. tertius* > *R. prolixus* > *R. neglectus.* The CS ANOVA between populations showed differences in the size of the EFG. Values showed that size means have significant differences $F_{(8113)} = 478.15$ $p < 0.001$. The CS size shows a morphometric relationship between the analyzed species, as the phylogenetically related species maintained approximate values (Fig. 1).

The Mahalanobis distance values retrieved from the pairwise comparison showed size differences between the EFGs ($p < 0.001$, Table. 1). *Rhodnius neglectus* and *R. prolixus* had the highest values, that is, the greatest power of discrimination in pairwise comparisons. In addition, we also used the Procrustes distance to describe the differences between the reference point configurations. All values were significant ($p < 0.001$, Table 1).

To illustrate the differences, we used the Mahalanobis distance

Table 1

Mahalanobis *distances* and Procrustes distances (values in bold) between pairs of species were performed for shape variables of female external genitalia (10.000 rounds of permutation). The estimate between groups was significant for all species ($p < 0.001$).

Species	1	2	3	4	5	6	7	8	9
1. P. lignarius	0.00	0.1929	0.2040	0.4200	0.3630	0.1837	0.1306	0.0850	0.1254
2. P. megistus	4.1946	0.00	0.2968	0.5788	0.5213	0.2621	0.1705	0.1472	0.2813
3. P. tertius	8.1197	12.3472	0.00	0.3257	0.2664	0.0815	0.1922	0.1702	0.2813
4. R. neglectus	23.2782	26.0509	11.521	0.00	0.0749	0.2664	0.4296	0.4465	0.1067
5. R. prolixus	20.4673	23.1352	8.5633	4.8191	0.00	0.2082	0.3744	0.3846	0.272
6. T. infestans	6.6728	5.7946	10.9816	22.9359	19.6973	0.00	0.2399	0.2271	0.1210
7. T. longipennis	8.1498	13.5188	6.1191	18.8547	16.0765	11.2683	0.00	0.0953	0.1715
8. T. phyllosoma	10.1747	10.0912	14.394	21.0735	18.4746	5.6695	13.0169	0.00	10.3168
9. T. vitticeps	9.7448	11.9775	10.5909	18.6712	15.0424	9.6975	5.046	10.3168	0.00

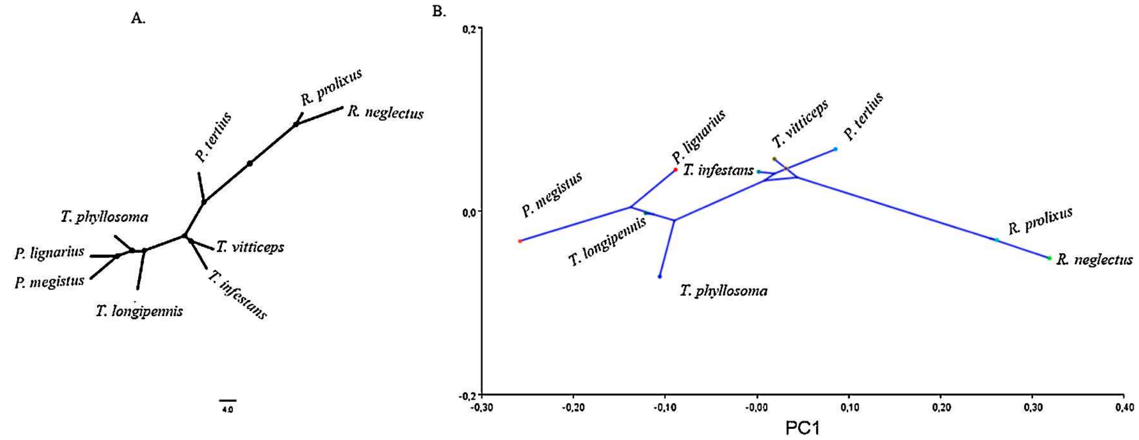

Fig. 2. A. Neighbor-joining trees derived from Mahalanobis distances of shape measurements for the external female genitalia in populations of nine species of the Triatominae. B. Mapped phylogeny retrieved by the main components (PC1 and PC2) and by the NJ tree. The Tree maps shape variables through a phylogenetic tree of the 9 species of the Triatominae.

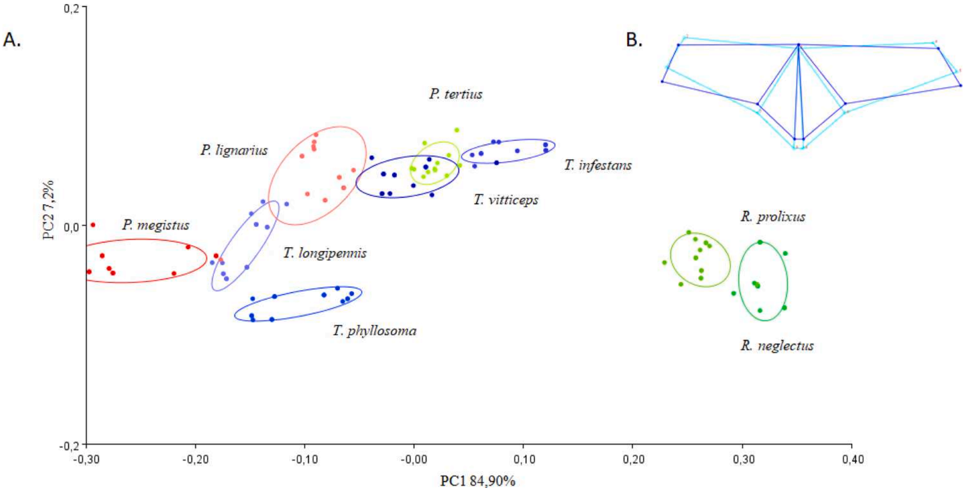

Fig. 3. A. Scatter plot of the two main components of the shape variation scores of the external female genitalia of the 9 species studied. The percentage contribution of each component to the total variation of the shape is shown in the axes (PC1 84.90% and PC2 7.2%). B. Visualizations of shape changes with wireframes.

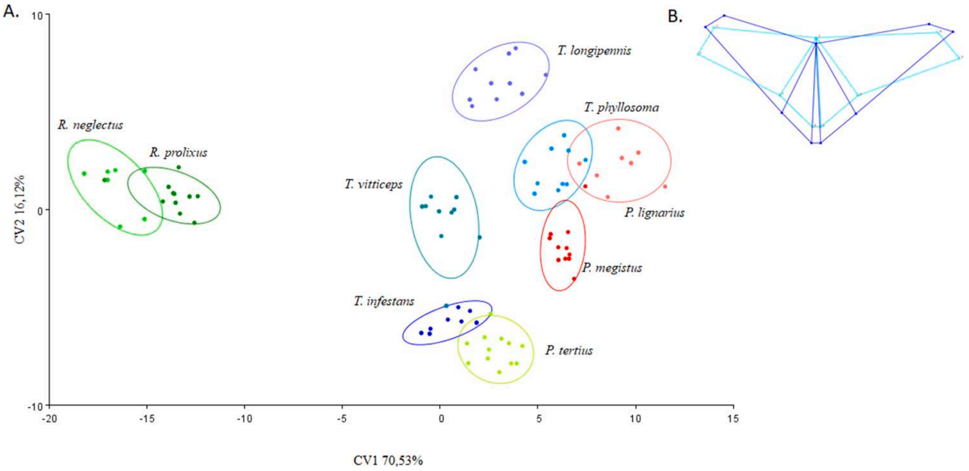

Fig. 4. A. Projection of the nine species studied in the space of canonical axes 1 and 2 (CVA1 and CVA2) resulting from the canonical analysis carried out from the coordinates of Procrustes. The ellipses represent the projection of each species in this study. B. Visualizations of shape changes with wireframes.

values to reconstruct an NJ phylogenetic tree (Fig. 2A). The NJ recovered well-defined clades consistent with the available phylogenies for Triatominae. In addition, we mapped the shapes using a phylogenetic tree (Fig. 2B). The mapped phylogeny retrieves a topology coherent with the NJ tree and with other phylogenies of Triatominae. The statistical results of the set of trees showed that applying a bootstrap of 250 generations gave a positive sign ($p < 0.001$), that is, the data mapped and presented graphically are coherent with the phenotypic evaluations of each species.

The main components (PCA1 and PCA2) show the variability of shape between groups. PCA1 explains 84.90% of the differences, while PCA2 explains only 7.2%, respectively (Fig. 3). The main components together explain 100% of the differences. The Procrustes ANOVA obtained significant values ($F_{(112.1582)} = 153.24\ p < 0.001$, supplementary material) revealing that the shape of the EFG is a determining factor to differentiate the groups.

The projection of the nine species onto the space defined by canonical axes 1 (CVA1) and 2 (CVA2) provides a description of the differences between the groups specified in the multivariate dataset. The CVA provides variables that explain 100% of the discrimination between groups. The first two variables explain 70.53% and 16.12%, respectively (Fig. 4). The projection of the ellipses in the space of the canonical axis (CVA1 and CVA2) shows the organizational profile of the groups, thus

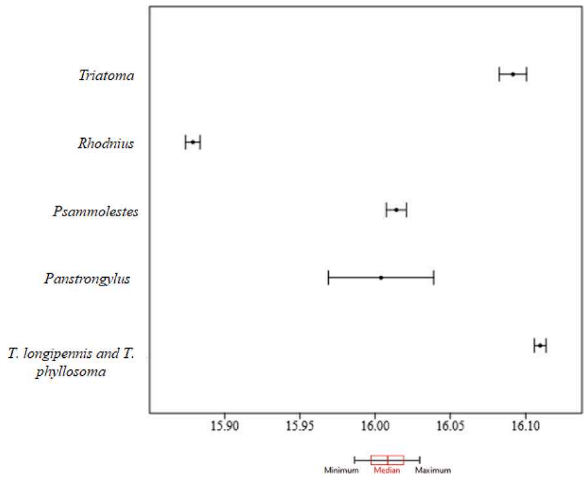

Fig. 5. Variation in CS of external female genitalia among genera (in pixel). Box plot: mean and standard deviation, 95% interval.

Table 2
Mahalanobis distances and Procrustes distances (bold values) between pairs of genera were performed for shape variables of female external genitalia (10.000 permutation rounds). The estimate among groups was significant for all species ($p < 0.0001$).

Genera	1	2	3	4	5
1. Panstrongylus	0.00	0.2268	0.4499	0.2269	0.0736
2. Psammolestes	6.6988	0.00	0.2601	0.0625	0.2002
3. Rhodnius	12.2832	4.1243	0.00	0.2531	0.4028
4. Triatoma	4.5651	9.2362	8.4665	0.00	0.1955
5. T. longipennis/ T. phyllosoma	4.095	7.5178	11.502	6.3224	0.00

allowing group populations with similar values, that is, related species can be clearly visualized (Fig. 4).

3.2. Analysis of size and shape variables between genera

The CS means obtained from the GPA shows the size variables between the *Panstrongylus, Psammolestes, Rhodnius*, and *Triatoma*. In these results, we present the species *T. longipennis* and *T. phyllosoma* separated from *Triatoma*, so that we can clearly assess the relationships of these triatomines with the others evaluated. The size variation is illustrated with quantiles (Fig. 5). The highest means are *T. longipennis/ T. phylossoma* and *Triatoma* species, followed by the species of *Panstrongylus, Psammolestes*, and *Rhodnius*. The size relationship: *T. longipennis/ T. phyllosoma* > *Triatoma* > *Panstrongylus* > *Psammolestes* > *Rhodnius*. The

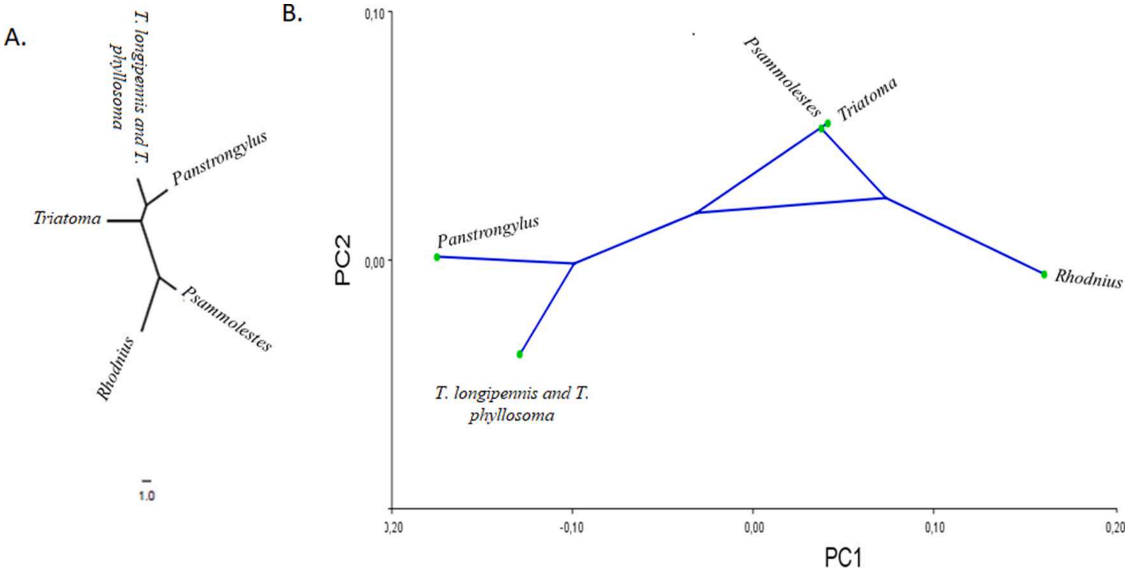

Fig. 6. A. Neighbor-joining trees derived from Mahalanobis distances of shape measurements for the external female genitalia in genera of the five genera of the Triatominae. B. Mapped phylogeny retrieved by the main components (PC1 and PC2) and by the NJ tree. The Tree maps shape variables through a phylogenetic tree of the five genera of the Triatominae.

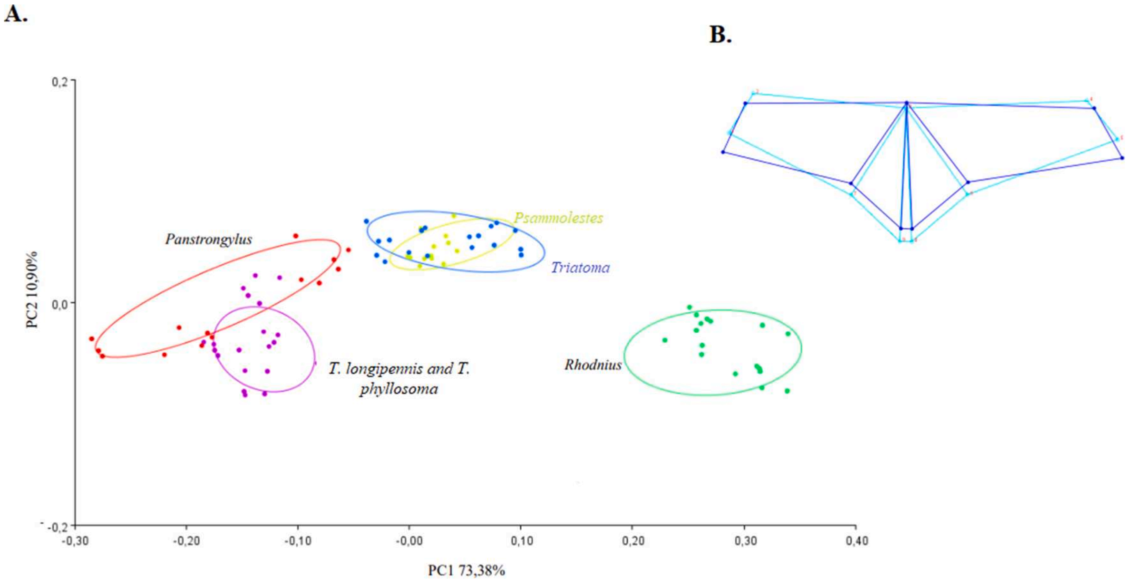

Fig. 7. Scatter plot of the two main components of the shape variation scores of the external female genitalia of the five genera of the Triatominae. The percentage contribution of each component to the total variation of the shape is shown in the axes (PC1 73.38% and PC2 10.90%). B. Visualizations of shape changes with wireframes.

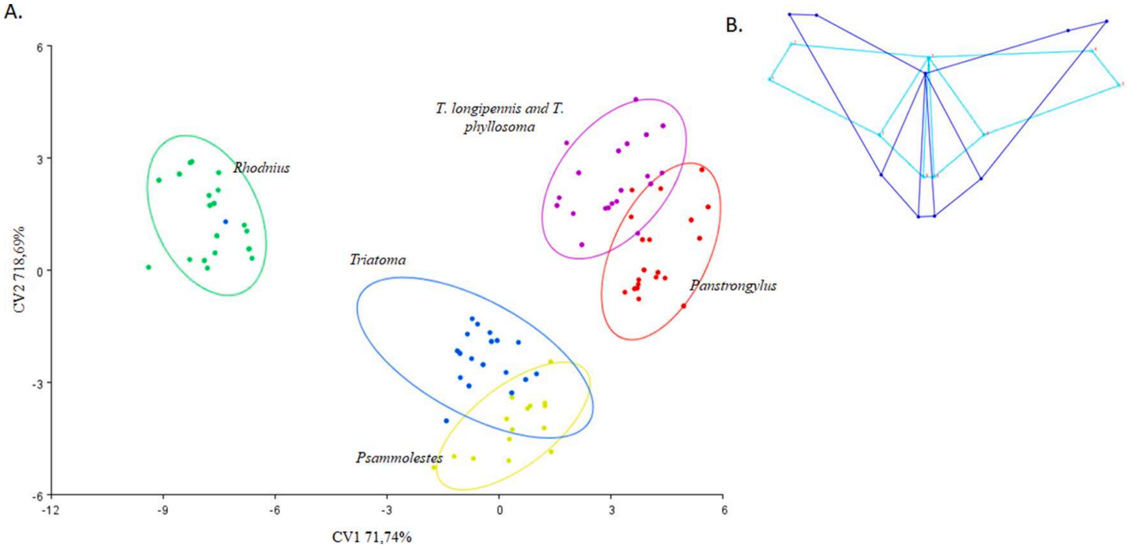

Fig. 8. Projection of the nine species studied in the space of canonical axes 1 and 2 (CVA1 and CVA2) resulting from the canonical analysis carried out from the coordinates of Procrustes. The ellipses represent the projection of each genus in this study. B. Visualizations of shape changes with wireframes.

CS ANOVA between populations shows differences in the size of the EFG. The values show that the means have significant differences ($F_{(4125)} = 278.56$, $p < 0.001$).

The values retrieved from the Mahalanobis distances show size differences between the EFGs ($p < 0.001$, Table. 2). *Rhodnius* obtained high values compared to the other genera. Respectively all genera were well-differentiated by values. We also used another estimator to assess the variations: the Procrustes distance allowed us to describe the differences between the configurations of the reference points. All values were significant ($p < 0.001$, Table. 2). However, they are not consensual with the other results, as they do not offer a clear correlation with the Triatominae systematics. The NJ tree retrieves well-defined clades, which are consistent with the available phylogenies for Triatominae. (Fig. 6A). Likewise, we map the shapes through a phylogenetic tree, with the values of the principal components (PCA1 and PCA2) and the coordinates of the NJ tree retrieved by the Mahalanobis distances (Fig. 6B). The mapped phylogeny retrieves a topology coherent with the NJ tree recovered in this study and with the phylogeny of Justi et al. (2014). We evaluated the phylogenetic signal, and the results show that applying a bootstrap of 250 generations gave a positive signal ($p < 0.001$), values consistent with the phenotypic differences of each genus.

The main components (PCA1 and PCA2) show the variability of shape between groups. The first main component, responsible for most of the differentiation between genera, alone explains 73.38% of the differences, while PCA2 explains only 10.90%, together explaining 100% (Fig. 7). The Procrustes ANOVA shows significant values ($F_{(56.1750)} = 100.78$, $p < 0.001$, supplementary material) revealing that the shape is crucial to differentiate the EFG. The projection of the genera in the space defined by canonical axes 1 (CVA1) and 2 (CVA2) provides a description of the differences between the groups specified in the multivariate dataset. Both explain 100% of the variations between the a priori groups. The first variable explains 70.53% and the second is responsible for 16.12%, respectively. The projection of the ellipses clearly shows the groups (Fig. 8). Genera separation was also evaluated using the DFA. The DFA performed pair by pair clearly separates all species when confronted (DFA_results in material supplementary). The DFA results corroborate the groups recovered from the stroke and are consistent with the other analyses in this study.

4. Discussion

The results show that the external female genitalia are a useful resource to discriminate species through geometric morphometric (GM). The analyses between species allowed a clear description of the shape variables, in addition to allowing specific delimitation of all the populations studied. Furthermore, the size variables were statistically significant in all analyses and, when used to reconstruct a phylogeny, they were informative, that is, they allowed us to assess the degree of phylogenetic relatedness between the studied species.

Although the morphological characters of the female genitalia of triatomines are useful for taxonomy (Rosa et al., 2010, 2014), they have been explored in few studies (Lent, 1948; Abalos and Wygodzinsky 1951; Sherlock and Serafim, 1967; Lent and Jurberg, 1969). This is not only the case for Triatominae, but also for Insecta Linnaeus, 1758, female genitalia have been underused in studies with geometric morphometry, with very few published works (Polihronakis, 2006; Noboa et al., 2017; Khan et al., 2020). In an unprecedented way, our results show that these characters are also useful for morphometric studies and corroborate recent morphological descriptions (Rosa et al., 2014; Oliveira et al., 2020; Teves et al., 2020; Belintani et al., 2021). The framework also proved to be useful for application in systematic studies.

PCA is widely used for exploratory analyses of shape (Rohlf et al., 1993). The technique commonly applied in geometric morphometry allows the use of multivariate data to estimate macro-evolutionary events (Valente et al., 2001; Adams et al., 2011; Anderson et al., 2007). The results showed that the shape variables of the EFG are decisive for the differentiation between the taxa. The principal components (PCA) explain 100% of the shape variables across species. The distribution of ellipses on the Cartesian axis of PCA1 and PCA2 provides valuable information that contributes to the phylogenies retrieved in this study by NJ. Furthermore, our trees are consistent with molecular phylogenies (Lyman et al., 1999; Dujardin et al., 1999; Monteiro et al., 2000, 2002; Hypša et al., 2002; Justi et al., 2014).

In addition to recovering the topology of phylogenetic trees, quadratic change parsimony (Maddison 1991) was used to map the phylogenetic signal of the morphometric analysis. The results confirm that EFGs from triatomines are useful to delimit systematic relationships. The data generated and analyzed from the EFG allowed us to describe the ancestry relationships. Therefore, in addition to validating the character for taxonomic studies based on morphometric approaches, the method can also corroborate discussions on the systematics of triatomines, and, thus, we suggest that the method be applied to other species.

The phylogenetic relationship between *Psammolestes* and *Rhodnius* is often discussed (Monteiro et al., 2002; Weirauch and Munro, 2009; Kieran et al., 2021). The data presented here show the proximity of *Psammolestes* to the two studied *Rhodnius* species. The morphology of *Psammolestes* is unique among other Triatominae genera. However, phylogenetic studies are inconclusive, and the validity of the genus is disputed. Likewise, the validity of the genus *Meccus* [in this study we accept the synonymy with *Triatoma*] engenders discussions. Reproductive compatibility studies show the possibility of gene flow between these genera (Martínez-Ibarra et al., 2011; Cesaretto et al., 2021). The results corroborate the synonymy, as it clearly shows the proximity of *T. longipennis* and *T. phyllosoma*, to *Triatoma*.

The phylogenetic relationships of the Triatomini tribe are controversial and available estimates reveal polytomies and unnatural groups (Justi et al., 2014). Some species of *Triatoma* and *Panstrongylus* possess a paraphyletic ancestral history (Hypša et al., 2002; Hwang and Weirauch, 2012; Justi et al., 2014). A canonical variable analysis (CVA) is a widely used method in morphometric studies to analyze group structure in multivariate data. It is equivalent to a one-way multivariate analysis of variance and is often called canonical discriminant analysis (Monteiro and Reis, 1999). In this study, the analysis of canonical variables was applied to assess the correlation between the data from the observations and thus trace those that are statistically similar. We show that despite the inconclusive systematic relationships, the method applied in this study allows for a clear separation of all genera, even the paraphyletic ones.

This is the first study using external female genitalia for taxonomic identification through geometric morphometry. The method was effective to discriminate and delimit Triatominae species, moreover, all results are consistent with the morphology. The use of size as a diagnostic character is effective for differentiation, as well as the shape profile. The results demonstrate the enormous potential of geometric morphometric analysis as a taxonomic tool for triatomines using external female genitalia. Given the epidemiological importance of the group and the robustness of the results, the validation of the technique and its application in studies with other species and genera of Triatominae are suggested.

Funding

TB, VFP and JAR: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001, CAPES Organization (Ministry of Education, Brazilian Government, Brazil), process number 23038.005285/2011-12. J.A.R Conselho Nacional de Desenvolvimento Científico e Tecnológico CNPq, PQ-2, process 307 398/2018-8. Financial support had neither role in study design, collection, analysis, interpretation, and manuscript writing.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Acknowledgments

The authors are grateful to the Universidade Estadual Paulista (Unesp), Faculdade de Ciências Farmacêuticas and to its collaborators who help in the maintenance of the Triatominae insectarium especially the Tacachi Hatanaka. The authors appreciate the researchers and students of the Parasitology Laboratory at the same institution. We thank the members of the Biology Institute of the Universidade Estadual de Campinas (Unicamp). We appreciate Professor Dr. Mauro Toledo Marelli and Dr. Walter Ceretti of the Faculty of Public Health, University of São Paulo, Laboratory of Entomology in Public Health, Department of Epidemiology, São Paulo, Brazil.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.actatropica.2022.106383.

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