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Article

Morphometric Analysis of a Trapdoor Spider (Araneae, Idiopidae) across Different Brazilian Biomes Reveals the Geographic Variation of Spiders from the Caatinga Biome

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Abstract: Widespread species are exposed to different environmental drivers and can consequently present variations in body shape and/or size. Trapdoor spiders of the genus *Idiops* are generally associated with a sedentary lifestyle and limited dispersion. However, the species *Idiops pirassununguensis* has a wide distribution, occurring in a diverse range of distinct environmental conditions, with their presence recorded in the Amazon, Caatinga, and Cerrado Brazilian biomes. We investigated how their morphological variation is structured regarding the biomes in which they occur through a morphometric analysis of the linear measurements and morphogeometric shapes of 64 specimens. Combining different methods proved to be a valuable approach to understanding how the spider's morphology varies in different environments. The results were congruent and complementary, indicating intraspecific geographic variation, with the Caatinga specimens being distinct from their biome conspecifics. In Caatinga, a biome with periods of severe drought and warm climate, *I. pirassununguensis* specimens were found to be smaller, in addition to having narrower and elongated sternums and shorter legs. The morphological structuring herein is consistent with the results found comparing animals from Caatinga and other Brazilian biomes. Despite differences in their non-sexual structures, the specimens share a set of diagnostic sexual characteristics for the species, allowing all individuals to be classified as belonging to the same species.

Keywords: morphometry; *Idiops*; Mygalomorphae; idiopinae; landmarks; Fourier analysis; discriminant analysis

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1. Introduction

The existence of morphological, ecological, and/or behavioral differences between populations distributed throughout a species' natural range is known as geographic variation [1–3]. Therefore, individuals of the same species that are exposed to different environmental drivers may present variations in body shape and/or size, which are often a reflection of these organisms adapting to different environments [2,4]. Due to its large dimensions, Brazil encompasses a wide range of environmental conditions, from grasslands (e.g., Pampa) and savannas (i.e., Cerrado), to dry (i.e., Caatinga) and humid (Amazon and Atlantic) forests. These biomes are shaped by both abiotic (e.g., temperature, precipitation, aridity, and relief and soil characteristics) and biotic (e.g., vegetation cover and the availability of organic resources) characteristics [5,6]. These heterogeneous environments might influence geographic variation both at an intraspecific level, especially in the case of widely distributed species, and at an interspecific level [1].

When assessing the geographic variation within a species, the most common tools require measurements of body sizes and shapes, the core of morphometric analysis [7]. These analyses are generally performed using two main types of analytical methods, which are directly related to the nature of the analyzed structures. The first is traditional or linear morphometry, based on the analysis of linear measurements, such as the length, width, and height of the body, and other morphological traits [8]. The second, more recent, is geometric morphometry, which aims to analyze and quantify the differences between complex morphological shapes, through multivariate analysis and the visualization of variation in a morphospace [8,9]. Based on these morphometric tools, it is possible to explore several biological factors, ranging from the recognition and delimitation of possible cryptic or morphologically related species [10,11] to the exploration of patterns of ecogeographic variation [12–14].

Based on a combined perspective, which involves measurements of both linear and geometric structures, few studies have investigated the possible patterns of geographic variation between different environments [15–17]. These tools have been used to assess several Brazilian taxa, with conflicting results. A study carried out with bee populations throughout the country did not find evidence of strong morphological variation between the analyzed populations [18]. However, similar studies with grasshoppers [19], beetles [20], and bats [21] found geographically structured populations.

In spiders, few studies have explored morphometric variation throughout the range of a species [22–28]. The majority of the existing morphometric studies are focused on species delimitation and on the investigation of sexual dimorphism [22–24,28–34]. There are also some studies that focus on habitat preference and foraging ecology [35,36], and others that investigate the morphological variation of specific structures in a phylogenetic context [36–39]. Despite the diversity of approaches, morphometric analyses based on multivariate and morphogeometric data have only been applied to Mygalomorph spiders one a few occasions [28,29,38–40].

Mygalomorph trapdoor spiders generally show marked morphological homogeneity (i.e., a reduced number of relevant taxonomic characteristics) and are typically associated with a sedentary lifestyle, limited dispersal capabilities, narrow geographic distribution, and high levels of environmental specificity [30,41–45]. These characteristics suggest that trapdoor spiders are excellent models for morphometric studies [30,39–41]. Here, through a broad analysis based on linear and geometric morphometric methods, we investigated the patterns of geographic variation of the trapdoor spiders of the species *Idiops pirassununguensis* Fukami and Lucas, 2008 (Idiopidae). The sampled populations are from different biomes in Brazil, including a tropical rainforest (Amazon), a savanna (Cerrado), and a seasonally dry tropical forest (Caatinga) [46].

Considering the species' wide distribution, which is unusual among trapdoor spiders, the presence of records of the target species in significantly different biomes creates an uncertain expectation regarding its interpopulation differences in body size. The size of adult spiders might be smaller in warmer biomes (i.e., Caatinga), following the temperature–size rule [47–49] or the starvation resistance hypothesis [50]. On the other hand, the water conservation hypothesis suggests that higher desiccation rates might be observed in smaller individuals [51,52], resulting in the expectation of larger spiders in warmer biomes. Therefore, we tested these hypotheses by morphometrically comparing the differences among the specimens of *I. pirassununguensis* from the Amazon, Caatinga, and Cerrado biomes, a wet–dry cline.

2. Materials and Methods

For the morphometric analysis, we selected 64 specimens of *I. pirassununguensis*, previously identified during the review of neotropical species of the genus *Idiops* [46]. Only males were used in the analysis, as they represent more than 90% of the material available for examination. As such, the number of available females would not be sufficient for the statistical analysis. Additionally, some specimens were damaged owing to the burning of the Butantan Institute's arachnid collection (see Kumar, 2010 [53]) and were thus excluded from the present study. Specimens from 24 of the 34 localities with records for the species from Brazil were included (Table S1), covering its entire distribution range: the Amazon (*n*: 20), Caatinga (*n*: 18) and Cerrado (*n*: 26) biomes (Figure 1). The definition of the biomes of the localities with records for the species was based on the official Brazilian biomes' limits, provided by the Instituto Brasileiro de Geografia e Estatística [54]. To perform the morphometric analysis, the specimens were photographed and measured using a Leica M205C stereomicroscope equipped with a Leica DFC295 digital camera.

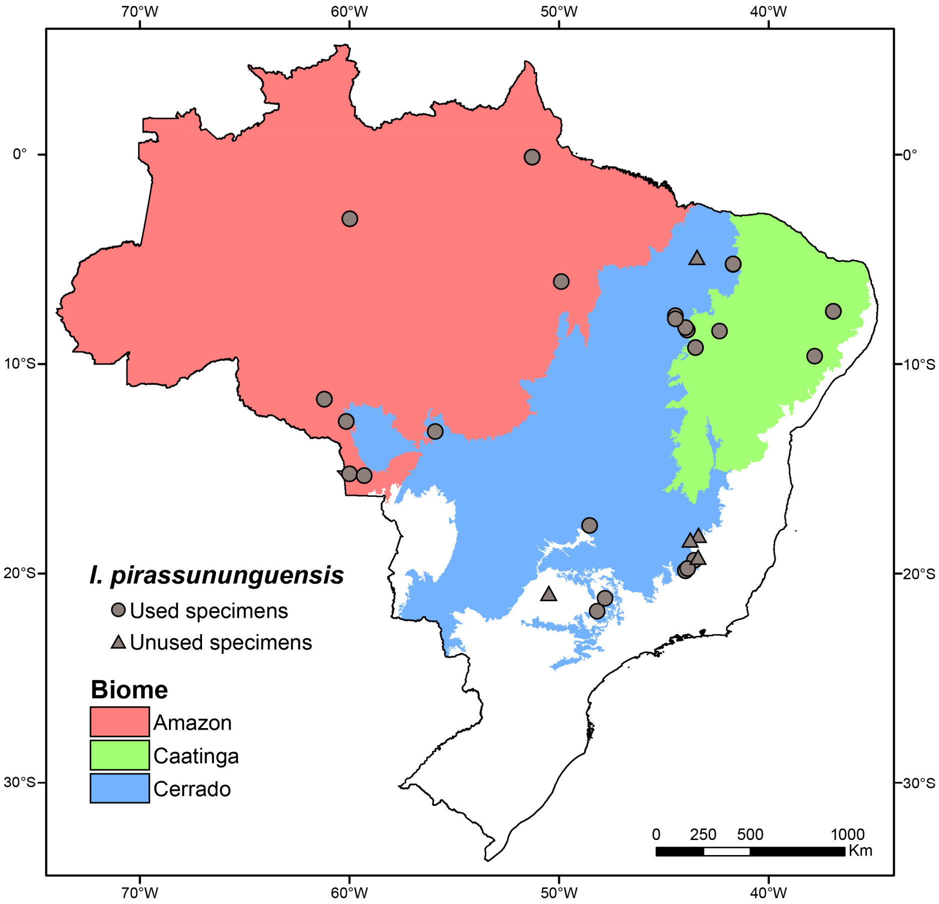

Figure 1. Known geographic distribution of *I. pirassununguensis*, highlighted by Brazilian biomes, categorized by specimens used (circles) and not used (triangles) in the statistical analyses.

The material examined is deposited in the following Brazilian zoological collections: Coleção Aracnológica Diamantina, Rio Claro, São Paulo (CAD; curator J. P. L. Guadanucci); Coleção de História Natural, Universidade Federal do Piauí, Floriano (CHNUFPI; curator J.F. Vilela); Instituto Butantan, São Paulo (IBSP; curator A.D. Brescovit); Instituto Nacional de Pesquisas da Amazônia, Manaus (INPA; curator M. L. Oliveira); Museu de Ciências e Tecnologia, Pontifícia Universidade Católica, Porto Alegre (MCTP; curator R. Teixeira); Museu Paraense Emilio Goeldi, Belém (MPEG; curator A.B. Bonaldo); Museu de Zoologia, Universidade de São Paulo, São Paulo (MZSP; curator R. Pinto-da-Rocha); Centro de Coleções Taxonômicas, Universidade Federal de Minas Gerais, Belo Horizonte (UFMG; curator A.J. Santos); and Coleção de Arachnida, Universidade Federal do Mato Grosso, Cuiabá (UFMT; A. Chagas Junior).

2.1. Definition and Measurement of Morphometric Variables

To explore the variation in the linear structures in relation to each biome, nine measurements of different body parts were taken: the carapace length (CL), carapace width (CW), sternum length (SL), sternum width (SW), leg 1 length (LL1), leg 2 length (LL2), leg 3 length (LL3), leg 4 length (LL4), and the pedipalp length (PpL) (Figure 2A–C). The definition of the linear measurements used was based on the structures that showed morphological variation among different groups of spiders in previous morphometric studies [28,35,36].

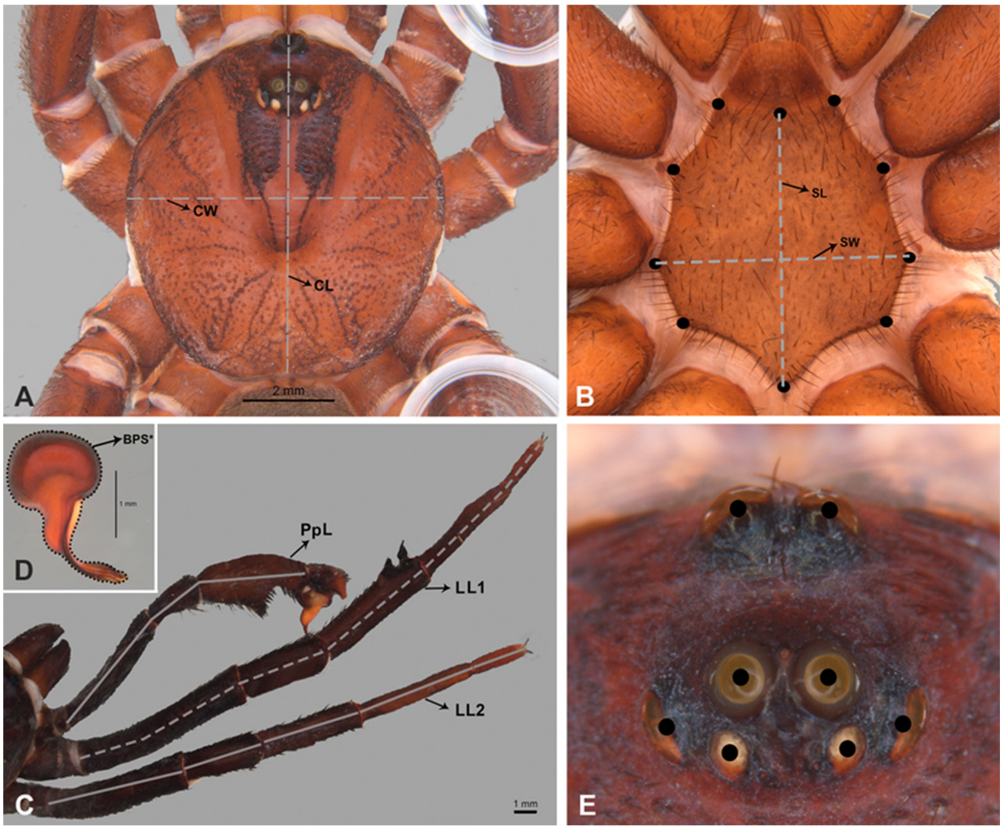

Figure 2. Morphometric variables of *I. pirassununguensis*. (**A**) Carapace length and width. (**B**) Length and width of the sternum; sternum landmarks. (**C**) Length of legs and pedipalp (legs 3 and 4 not visible in the image). (**D**) Shape and representation of the contour of the copulatory bulb. (**E**) Eye landmarks.

In addition to linear measurements, to detect geometrically significant variations of specimens in relation to biomes, geometric morphometric analysis was performed based on images of the dorsal copulatory bulb (Figure 2D), the sternum (Figure 2B), and the eye arrangement (Figure 2E). These structures were analyzed according to their biological characteristics: contours for the copulatory bulb and landmarks for the sternum and eye arrangement.

2.2. Linear Morphometric Analysis

First, the mean, the standard deviation, and the minimum and maximum values were generated for each linear measurement obtained. To perform the morphometric analysis of the linear measurements, seven relative measurements were generated from the ratio between the length and width of the carapace and sternum and between the length of each of the legs and the pedipalp by the width of the carapace. These ratios were used because they better characterize the proportional morphological variation among different groups, effectively demonstrating the adaptation of the specimens to different environments [35].

Then, the relative variables were subjected to a series of multivariate analyses. To explore and visualize possible patterns of similarity among the specimens, the relative variables were subjected to a principal coordinate analysis (PCoA) calculated with the Gower coefficient of similarity. Finally, to investigate whether there is structuring of the specimens in relation to the biomes, two complementary analyses were carried out. First, a canonical variate analysis (CVA) was performed to visualize the structuring patterns of the specimens among the biomes (i.e., Amazon, Caatinga and Cerrado). This analysis maximizes the variation among the previously defined groups by comparing the samples classified a priori and calculating the probability of individuals belonging to a different group [55]. Then, a permutational multivariate analysis of variance (PERMANOVA) was used to test whether there are significant differences among the analyzed groups [56]. The PERMANOVA was based on the Gower similarity index, with 10,000 permutations. p-values were corrected for pairwise comparisons using Bonferroni's correction; p-values < 0.01 were considered statistically significant. All multivariate analyses of linear measurements were conducted using PAST (Paleontological Statistics) version 4.03 [57].

2.3. Geometric Morphometric Analysis

2.3.1. Geometric Morphometric Analysis Based on Outlines

To investigate the variation in the shape of the copulatory bulb (Figure 2D), which has a curvilinear shape that is difficult to replicate using landmarks, Fourier elliptic functions were used. These analyses describe and characterize the outlines of the analyzed structures in a quantifiable way [58]. To perform the analysis, initially, the images of the copulatory bulbs were converted into silhouettes, using Photoshop CC 2018 software. The following analyses were performed on the RStudio platform (R software v. 4.2).

First, using the R package Momocs v. 1.3.0, a series of procedures necessary to perform the elliptic Fourier analysis was performed [59]. For this, the contours of the copulatory bulb images were extracted and smoothed. Then, from the function 'calibrate_harmonicpower_efourier', a series of harmonics were generated; these are functions resulting from the accumulation of sine and cosine functions, detecting an increasing form of outline details. These harmonics predict the number of contours needed to achieve 95% to 99% of the shape variation, which is called Fourier power. With the number of harmonics defined, the Fourier elliptics were analyzed using the 'eFourier' function, which normalizes all images with respect to rotation, size, and orientation [59].

The generated coefficients were submitted to principal component analysis (PCA), to visualize the distribution of the copulatory bulb outlines in morphospace (the R base 'PCA' function) and characterize their variation, based on the values of the first two principal components (R base 'PCcontrib' function). Finally, as a way of visualizing the variation in the shape of the copulatory bulb in relation to the biome and calculating the hit rate in relation to the groups defined a priori, a CVA was performed using the R package MASS ('Ida' function).

2.3.2. Geometric Morphometric Analysis Based in Landmarks

To investigate the variation in the shape of the sternum (Figure 2B) and eye arrangements (Figure 2E), which present well-defined geometric structures and allow for the insertion of replicable reference points, morphometric analyses based on landmarks were performed.

First, image libraries were built using the TPSUtil software. Then, the landmarks were inserted, with the help of the TPSDig2 v. 1.31 [60]. Ten landmarks were defined for the sternum (Figure 2B) and eight landmarks for the ocular arrangement, referring to the central position of each eye (Figure 2E).

The following morphometric and statistical analysis were performed using MorphoJ v. 1.06 [61]. First, information regarding shape was extracted through generalized procrustes analysis (GPA). This analysis removes information related to size, orientation, and position, keeping only information regarding the shape (i.e., the Procrustes coordinates); it standardizes each specimen to a single unit centroid size [8,59]. Then, for each structure, a PCA was performed, based on the covariance matrix of the Procrustes forms, followed by a CVA. The significance of the CVA was evaluated through the values of the Procrustes and Mahalanobis distances, as well as the respective *p*-values, with 10,000 permutation replicates; *p* values < 0.01 were considered statistically significant. Finally, to investigate the influence of size on shape (the allometric effect) and the size variation, a multivariate regression was performed between the centroid size (the independent variable) and the Procrustes coordinates (the dependent variable), followed by a permutation test of 10.000 interactions.

3. Results

3.1. Linear Morphometry

The total variation among the specimens for each linear morphometric variable and the variation among biomes are presented in Table 1. In relation to the multivariate analysis of traditional relative measures, the PCoA resulted in seven axes, of which the first two described 59.8% of the variation (Axis 1: 50.1%, Axis 2: 9.7%; Figure S1, Table S2A) Regarding the CVA, the scatter plot (Figure 3A) also shows the differentiation of the Caatinga specimens, which are separated from the others and distributed along the left portion of the graph, while the Amazon and Cerrado specimens are superimposed in the right portion of the graph. The differentiation among the groups was confirmed by the general PERMANOVA (F = 11.55, $p < 0.01$). The separation of the Caatinga specimens in relation to specimens from other biomes was evidenced by a pairwise PERMANOVA, both between the Caatinga specimens and the Amazon specimens (F = 14.74, $p < 0.01$) and between the Caatinga specimens and the Cerrado specimens (F = 14.6, $p < 0.01$). There was no significant difference between specimens from the Amazon and those from Cerrado (Table 2a).

Table 1. Mean, standard deviation, and minimum and maximum values for each linear measurements of *I. pirassununguensis*, according to the biome and the total variation.

Measurements	Amazon (*n*: 20)	Caatinga (*n*: 18)	Cerrado (*n*: 26)	Total (*n*: 64)
Carapace length	6.96 ± 0.14 (5.53–8.04)	7 ± 0.11 (5.94–8.12)	7.08 ± 0.12 (5.64–8.2)	7 ± 0.07 (5.53–8.2)
Carapace width	6.51 ± 0.16 (4.97–7.56)	6.5 ± 0.11 (5.39–7.31)	6.65 ± 0.16 (5.07–8)	6.56 ± 0.08 (4.97–8)
Sternum length	4 ± 0.09 (3.10–4.60)	4.09 ± 0.07 (3.35–4.73)	4.02 ± 0.07 (3.45–4.8)	4.07 ± 0.04 (3.10–4.8)
Sternum width	3.68 ± 0.09 (2.66–4.34)	3.53 ± 0.07 (2.82–4.09)	3.62 ± 0.07 (2.94–4.4)	3.61 ± 0.05 (2.66–4.4)
Leg 1 length	23.4 ± 0.58 (18.36–27.1)	23 ± 0.27 (21.17–25.63)	23.52 ± 0.5 (17.89–27.08)	23.34 ± 0.28 (17.89–27.08)
Leg 2 length	20 ± 0.5 (15.51–23.13)	17.5 ± 0.6 (13.92–21.54)	20 ± 0.43 (14.48–23.1)	19.31 ± 0.3 (13.92–23.13)
Leg 3 length	17.6 ± 0.46 (12.68–20.6)	15.37 ± 0.58 (12.23–20.46)	17.47 ± 0.36 (13.32–20.45)	16.93 ± 0.28 (12.23–20.6)
Leg 4 length	23.95 ± 0.62 (18.26–27.96)	20.56 ± 0.72 (16.62–26.84)	23.67 ± 0.5 (18.15–28.15)	22.88 ± 0.38 (16.62–28.15)
Pedipalp length	12.96 ± 0.27 (10.25–14.9)	11.08 ± 0.42 (9.01–14.85)	12.7 ± 0.25 (10.48–15.16)	12.33 ± 0.2 (9.01–15.16)

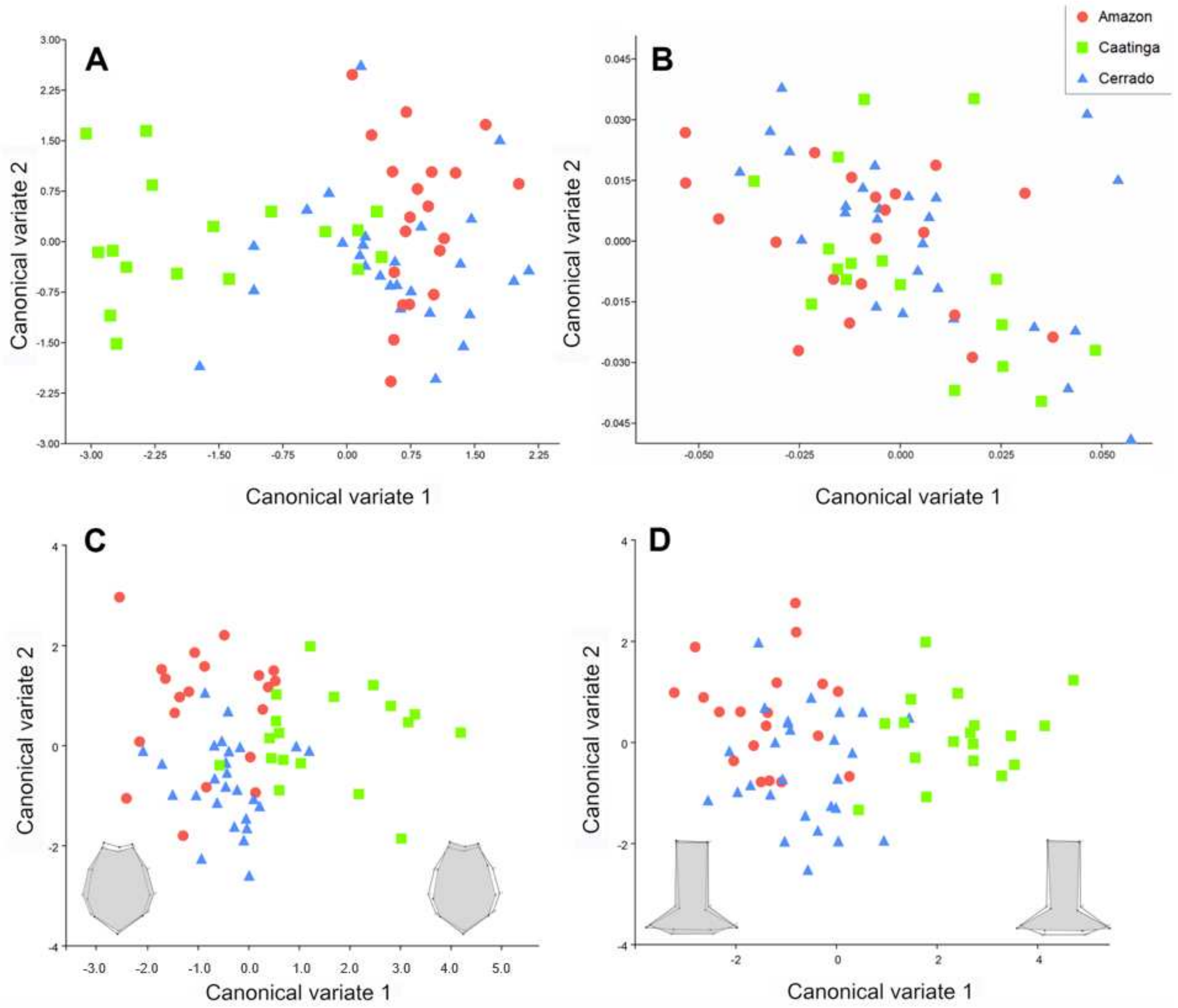

Figure 3. Canonical variate analysis (CVA) scatterplots of different morphometric variables of *I. pirassununguensis* in relation to the biome of occurrence. (**A**) Linear measurements. (**B**) Bulb copulatory. (**C**) Sternum shape (transformation grids at the bottom of the scatterplot). (**D**) Eye arrangement (transformation grids at the bottom of the scatterplot). The different biomes are represented by the following symbols and colors: Amazon (red dots), Caatinga (green squares), Cerrado (blue triangles).

3.2. Geometric Morphometry

3.2.1. Copulatory Bulb

The Fourier elliptic analysis required seven harmonics, which explained 99% of the variation in the dorsal shape of the copulatory bulb. The PCA resulted in 28 PC axes, of which the first 5 contributed 90.6% of the variation (Table S2B). The first two PCs contributed 75.8% of the total shape variation (PC1: 52.6%, PC2: 23.2%; Figure S2), with the variation in the first principal component (PC1) mainly related to the tegulum width and the variation in the second principal component (PC2) mainly related to the angulation of the embolus.

In relation to the CVA (Figure 3B), the scatter plot does not indicate a clear separation of specimens related to biome, as the plots belonging to the three biomes are mostly overlapping. The average hit rate per biome was high (>70%), and only the Cerrado values showed relevant percentages of specimens being incorrectly hit (22% for Caatinga; 25% for the Amazon; see Table 2b).

Table 2. Statistical results of the discriminant analysis in relation to the biomes of occurrence of *I. pirassununguensis*. (**a**) Linear measurements (pairwise PERMANOVA). (**b**) Bulb copulatory (contingency table). (**c**) Sternum (Mahalanobis distance). (**d**) Sternum (Procrustes distance). (**e**) Eye arrangement (Mahalanobis distance). (**f**) Eye arrangement (Procrustes distance). For A, the values on the bottom of the diagonal represent F values while those on the upper represent p values. For C and E, the Mahalanobis distance values are on the bottom of the diagonal and their respective p-values are on the upper part. The same goes for D and F in relation to the values of the Procrustes distances. Significant values (p-value < 0.01) were highlighted with an asterisk.

a. Linear measurements (PERMANOVA)				**b. Bulb copulatory** (contingency table)			
Biome	Amazon	Caatinga	Cerrado	Biome	Amazon	Caatinga	Cerrado
Amazon	-	<0.01	0.914	Amazon	65%	10%	25%
Caatinga	14.74 *	-	<0.01	Caatinga	0%	78%	22%
Cerrado	1.16	14.16 *	-	Cerrado	11.50%	11.50%	77%
c. Sternum (Mahalanobis distance)				**d. Sternum** (Procrustes distance)			
Biome	Amazon	Caatinga	Cerrado	Biome	Amazon	Caatinga	Cerrado
Amazon	-	<0.01	0.0135	Amazon	-	<0.01	0.583
Caatinga	2.49 *	-	<0.01	Caatinga	0.03 *	-	<0.01
Cerrado	1.56	2.2 *	-	Cerrado	0	0.03 *	-
e. Eye arrangement (Mahalanobis distance)				**f. Eye arrangement** (Procrustes distance)			
Biome	Amazon	Caatinga	Cerrado	Biome	Amazon	Caatinga	Cerrado
Amazon	-	<0.01	0.0912	Amazon	-	<0.01	0.324
Caatinga	3.86 *	-	<0.01	Caatinga	0.03 *	-	<0.01
Cerrado	1.3	3.17 *	-	Cerrado	0.02	0.03 *	-

3.2.2. Sternum

The PCA resulted in 16 PC axes, of which the first 5 contributed 76.8% of the sternum shape variation (Table S2C). The first two PCs contributed 54.5% of the total shape variation (PC1: 44.4%, PC2: 10.1%; Figure S3), with the variation in the first principal component (PC1) mainly related to sternum width and length and the variation in the second principal component (PC2) related to the shape of the anterior portion of the sternum.

According to the CVA scatter plot (Figure 3C), specimens from the Caatinga group differ from those from the Amazon and Cerrado, which are more similar to each other. CV1 explained 70.9% of the variation, which was related to a greater narrowing and elongation of the sternum in individuals from the Caatinga group compared to the others. When compared with the Amazon and Cerrado groups, the Caatinga specimens showed significantly higher values of Procrustes distances and Mahalanobis distances (Table 2c,d), which evidence the distinction between these groups.

The multiple regression showed that, despite the allometry effect having a significant permutation value ($p < 0.01$), size contributed only 8.8% of the shape variation. Specimens from the Caatinga area tend to be smaller than their conspecifics from the other analyzed biomes (Figure 4).

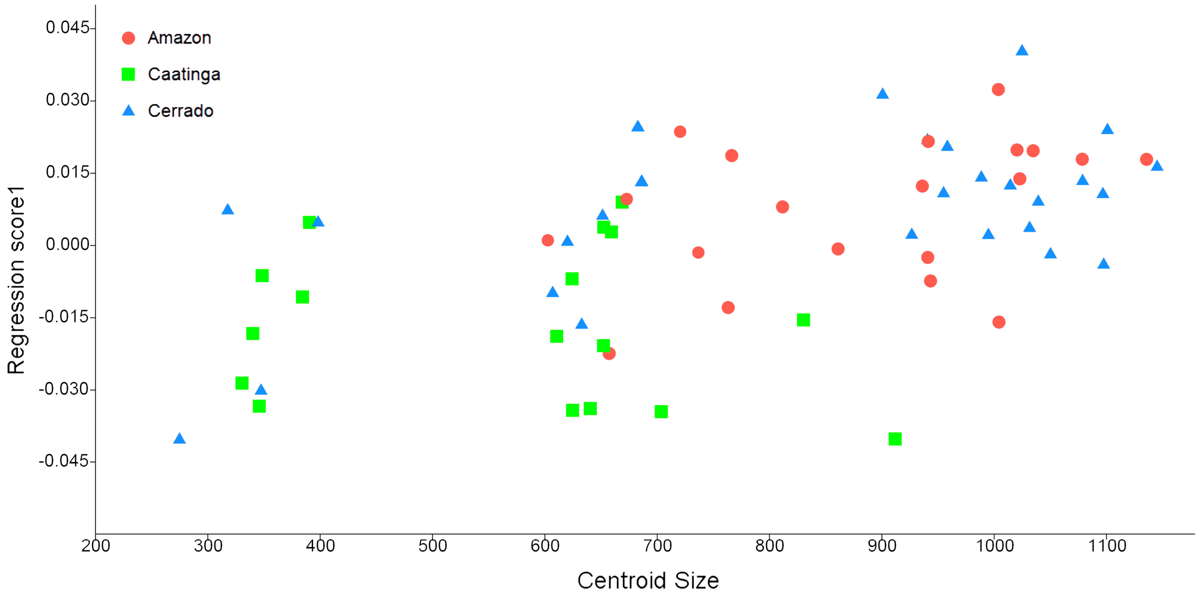

Figure 4. Multivariate regression of sternum shape on the sternum centroid size of *I. pirassununguensis*. The different biomes are represented by the following symbols and colors: Amazon (red dots), Caatinga (green squares), Cerrado (blue triangles).

3.2.3. Eye Arrangement

Regarding the shape of the eye arrangement, PCA resulted in 12 PC axes, of which the first 5 contributed to 80.3% of the shape variation (Table S2D). The first two PCs contributed 51.6% of the total shape variation (PC1: 28.7%, PC2: 22.9%; Figure S4). The first principal component (PC1) is mainly related to the length of the eye tubercle and the distance of the eye tubercle in relation to the anterior lateral eyes. The second principal component (PC2) explains the part of the variation related to the arrangement of the anterior median eyes and the posterior lateral eyes.

According to the CVA results (Figure 3D), the Caatinga specimens are separated from those from the Amazon and the Cerrado, as observed for the sternum shape (Figure 3C). These differences are statistically significant ($p < 0.01$) for the Mahalanobis and Procrustes distances (Table 2d,e). CV1 explained 92.1% of the variation, which was related to a greater narrowing of the eye tubercle and the distance between the anterior lateral eyes of Caatinga individuals when compared to specimens from the other biomes.

Although the multiple regression analysis indicates that 3% of the size variation is related to the shape, this analysis did not provide significant support in the permutation test (p-value = 0.06).

4. Discussion

This study constitutes the first morphometric investigation of an Idiopidae trapdoor spider species. The results of the analysis, which was based on the traditional morphometry of linear measurements and the geometric morphometry of the sternum and the eye arrangement, were congruent and complementary. The specimens from the Caatinga are morphologically distinct from their conspecifics from the Amazon and the Cerrado, which showed no significant variation from each other. Regarding the results of the morphogeometric analysis of the copulatory bulb, there was no separation of the specimens in relation to the biomes.

Individuals from the Caatinga tend to be smaller when compared to specimens from the Amazon and Cerrado. Those specimens shared a narrower and more elongated sternum, shorter legs and pedipalps, a narrower eye tubercle, and anterior lateral eyes that were furthest from the eye tubercle. This differentiation of the Caatinga specimens supports the temperature–size rule [47–49] or the starvation resistance hypothesis [50]. First, the temperature–size rule postulates that warmer environments harbor smaller species and/or specimens [47–49]. As such, the observed differences may be a byproduct of morphological and physiological adaptations related to the thermoregulation and water balance of spiders due to the restrictions imposed by the arid and semi-arid environment [62,63]. This would be congruent with the Caatinga climatic conditions. This biome is a nucleus of the South American seasonally dry tropical forests (SDTF) [64], being characterized by low levels of annual precipitation, high seasonality (precipitation concentrated in a few consecutive months), high average annual temperatures, low annual thermal amplitude, and low relative humidity [65,66]. The second hypothesis (i.e., starvation resistance) relates to ecological limitations, which are associated with the lower availability of food resources, water resources, and shelter, common in arid and open environments affected by drought; such circumstances are typical of the Caatinga [65,67]. In any case, it is not possible to make a distinction between these two hypotheses with the currently available data.

Our results corroborate those of a recent study of Brazilian scorpions from a temperature cline zone (i.e., Caatinga and Atlantic Forest sites), in which high temperatures showed a significantly negative correlation with body size [49]. In an intraspecific context, Lira et al. [48] suggested that the reduction in the size of the specimens from Caatinga may be related to the molting process and the physiological restrictions imposed by it. Similarly, specimens of the trunk-dwelling lizard *Gonatodes humeralis* (Guichenot, 1855) from the Caatinga exhibited smaller body sizes when compared to Amazonian populations [68]. This variation was interpreted as a result of both abiotic (e.g., temperature and humidity; the so-called temperature–size rule), and biotic (e.g., the availability of food resources; the so-called starvation hypothesis) factors, and their interactions [68]. Finally, laboratory-raised *Acheta domesticus* (Linnaeus, 1758) crickets that were subjected lower hydration conditions were significantly smaller than those submitted to high hydration conditions [69].

However, the smaller body size in arid conditions is not a rule of thumb. For example, gymnophthalmid lizards that inhabit more arid regions are generally more elongated [70]. This result was generally thought to be a byproduct of the association between the habitat structure, lizard behavior, environmental conditions, and lower resource availability [70]. Although the target species of our study is a trapdoor spider, which does not build orb webs, body elongation may be related to heat transfer, since males are wanderers and spend part of their lives outside of burrows looking for females, occasionally being exposed to the sun. Because the environments of the Caatinga biome are characterized by open, seasonally dry, and deciduous tropical phytophysiognomies, this might have resulted in specimens with more elongated bodies, as observed in the sternums of specimens from the Caatinga, possibly reducing desiccation.

Some of these morphological variations, which are mainly associated with arid and semi-arid environments, may also be related to the evolutionary and biogeographic history of *I. pirassununguensis* [45]. Phylogeographic studies of different taxa from the Caatinga showed the genetic structuring associated with the Caatinga, the Cerrado, and the Chaco [5,71], biomes that are biogeographically and climatically related. Studies of spiders [72], lizards [73,74], amphibians [75], and birds [76,77] found similar patterns of diversification and structuring, mainly related to the Pliocene and Pleistocene periods, showing genetic lineages with occurrence restricted to the Caatinga.

The current distribution of *I. pirassununguensis* over a large geographic area shows an unusual pattern that is distinct from that observed for other trapdoor spider species, which, in general, have a restricted range and strong geographic and genetic structuring, as observed, for example, in spiders from the families Euctenizidae [30,41,78], Antrodiaetidae [42,79,80], Migidae [43,81,82], Halonoproctidae [83], and even Idiopidae [84]. It is remarkable that, even though *I. pirassununguensis* has a wide distribution across different biomes and presents significant variation in relation to the size and shape of non-sexual structures, the specimens share a series of diagnostic sexual characteristics for the species, related to the copulatory bulb, pedipalp, and tibial apophysis [46]. This finding supports the hypothesis that, based on various species of insects and spiders [85], male genitalia show less variation than other body parts, indicating a case of intraspecific variation.

The use of different morphometric methods herein demonstrates the importance of combined analysis in quantifying variation and identifying morphologically distinct groups. Our results indicate distinctions in the size and shape of the Caatinga specimens when compared to their conspecifics from the Amazon and Cerrado. These results provide support for future investigations involving an integrative taxonomy using time-calibrated molecular data and species distribution modeling. This approach can lead to a deeper understanding of the diversification patterns of *I. pirassununguensis* across Brazilian biomes and yield insights into possible adaptive processes associated with the evolution of these spiders in semi-arid environments.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15070861/s1, Figure S1: Linear measurements PCoA; Figure S2: Bulb copulatory PCA; Figure S3: Sternum PCA; Figure S4: Eye arrangement PCA; Table S1: Sample information; Table S2: Table S2: Variance percentage PCoA-PCA.

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