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Influence of seasonality on wing morphological variability in populations of *Mansonia amazonensis* (Theobald) (Diptera: Culicidae)

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Abstract

Mansonia amazonensis (Theobald, 1901) is one of 15 species of the subgenus *Mansonia* Blanchard, 1901. It is essentially a Neotropical species, recorded in Bolivia, Peru and Brazil. In the highly seasonal Amazon region, *Mansonia* species find ideal environmental conditions for reproduction, development and establishment. Considering that climate significantly influences the size and behavior of mosquitoes, and insects in general, we analyzed the influence of seasonality on wing morphological variability in populations of *Ma. amazonensis*. Captures were carried out near the banks of the Madeira River in Rondônia State, Brazil during the dry, rainy and transition periods between seasons during 2018 and 2019. Morphometric characters of 181 wings were analyzed using morphological methods. The results show that wing size of *Ma. amazonensis* increases following a relatively gradual trend, from smaller wings during the dry period to larger ones in the rainy season. This study provides the first evidence, detected using geometric morphometry, of seasonally associated phenotypic variability in the wing conformation of *Ma. amazonensis*.

Key words: Amazon, dry season, rainy season, Mansoniini, mosquito, taxonomy

Introduction

Mansonia amazonensis (Theobald, 1901) is among 15 species of the subgenus *Mansonia* Blanchard, 1901 (Harbach 2022). It is essentially a Neotropical species, recorded in Bolivia, Peru and Brazil (Lane 1953; Klein *et al.* 1992; Pecor *et al*. 2000; Assumpção 2009). Females of these mosquitoes deposit their eggs in an aggregate on the leaves of aquatic plants. When the eggs hatch, the larvae cross the water column and settle on the roots of the plants (Ferreira & Nunes de Mello 1999; da Silva Ferreira *et al*. 2020). The immature stages have the spiracular apparatus adapted to perforate the aerenchyma of certain aquatic macrophytes, allowing gas exchange without shifting to the surface of the water (Ferreira *et al*. 2003; Service 2008). Adult females feed on a wide range of blood sources, feeding even on reptiles (Viana *et al*. 2010).

Mansonia species are voracious during blood feeding, capable of causing medical and social problems when in high densities (Rehena & Matdoan 2020; Pedro *et al*. 2021). These species have nocturnal habits but can be found abundantly during the day, biting at any time in forests near their larval habitats (Lima 1929; Consoli & Oliveira 1994). They are exophilic and can be found in altered environments, expanding their area of occurrence to the vicinity of urban environments (Lima 1929; Navarro-Silva *et al.* 2004). In the Amazon, *Mansonia* species find ideal larval habitats for reproduction, development and establishment, becoming the more frequent genus of hematophagous mosquitoes in floodplains (Ferreira 1999). *Mansonia amazonensis* is not considered a vector of etiological agents.

However, viruses and helminths have been detected in many other *Mansonia* species, suggesting their participation in sylvatic transmission cycles (Burton 1967; Turell *et al*. 2000; Lutomiah *et al*. 2011; Beranek *et al.* 2018).

Seasonal variation contributes to dynamic and variable ecosystems, and the Amazon region is highly seasonal, with two well-defined seasons: the Amazon winter, a regional name for the rainy season despite it being summer at this time of the year in most of the Southern Hemisphere, and the Amazon summer, a popular name used by locals to identify the dry season, a period of warmer weather (Brando *et al*. 2010; Costa *et al.* 2013; Caldas 2016; Marengo *et al*. 2016; Rente Neto & Furtado 2015; Coutinho *et al.* 2018). Climatic factors can explain variation in the wing size of several mosquito species (Da Silva *et al*. 2020; Mathania *et al*. 2020). Considering the lack of biological and ecological studies on *Ma. amazonensis*, and the epidemiological importance of the taxonomic group to which it belongs, we aimed to analyze the influence of seasonality on the variation of wing morphology in populations of this species.

Material and methods

Females of *Ma. amazonensis* were captured near the banks of the Madeira River, located in Rondônia State, Brazil: 9° 13′ 53.7″ S; 64° 28′ 20.6″ W (Fig. 1). The captures were carried out during three periods: July 2018 and 2019 (dry season), October and December 2018 and April and November 2019 (rainy season), and September 2018 and 2019 (intermediate transition period) (Fisch *et al*. 1998). The captures were conducted between 18:00 and 20:00 on three alternate days using Shannon light traps. Mosquitoes were also captured with a manual suction tube when they were occasionally attracted to team members, landed on the trap or on the surrounding vegetation. According to Koeppen (1948), the region's climate can be classified as type Aw, tropical with dry winter. In the last 10 years, the average annual temperature in Rondônia State was around 25ºC, with the coldest monthly average less than 16ºC and the warmest monthly average above 34ºC. Precipitation ranged between 1,300 and 2,600 mm/year for the same 10-year period (INMET 2021).

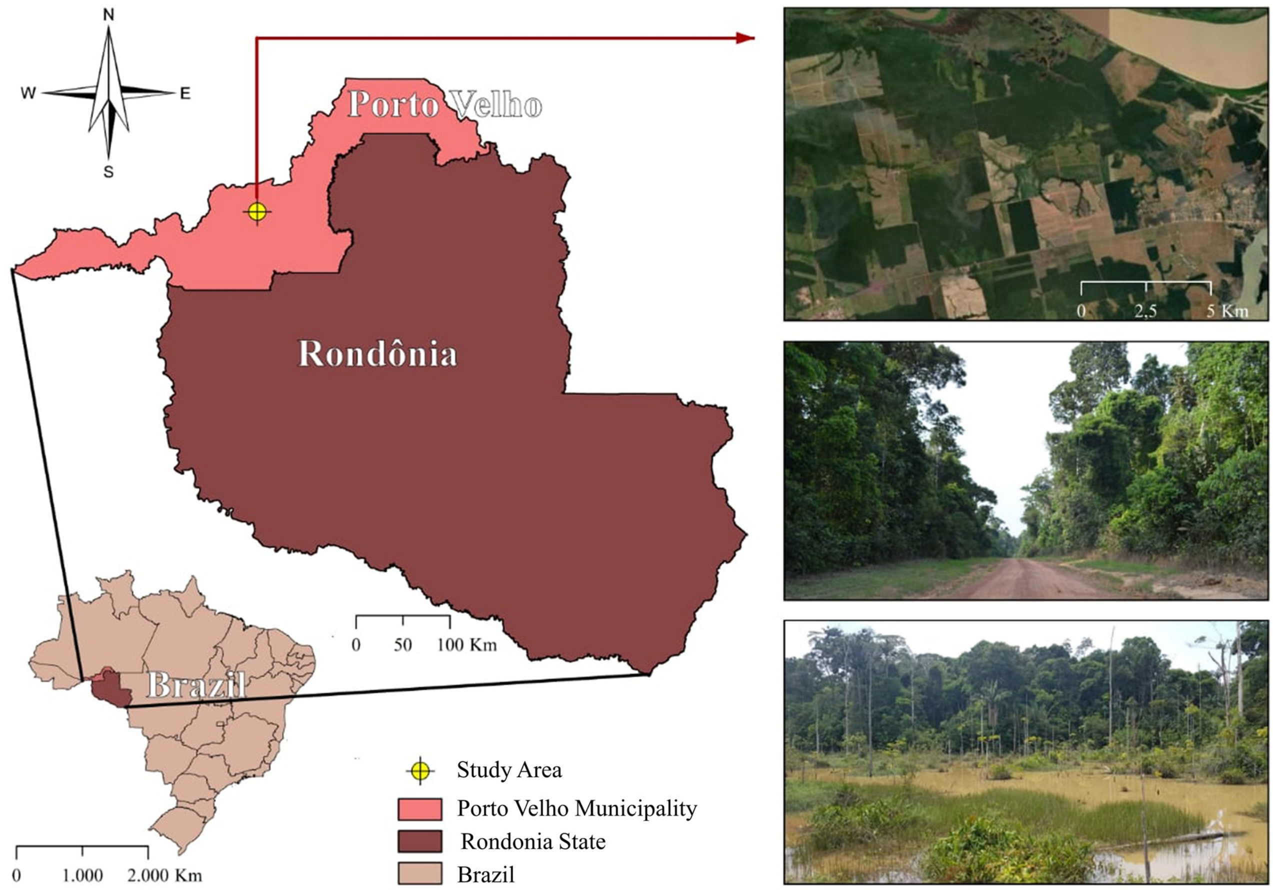

FIGURE 1. Sampling point for populations of *Mansonia amazonensis* in the area of the Amazon Hydroelectric Development Unit of Jirau (UHE-Jirau), Rondônia State, Brazil, during the dry, rainy and transition periods of 2018 and 2019.

Geometric morphometric analyses were carried out on 181 specimens (Table 1). We analyzed right wings of females that were carefully removed from the thorax using entomological forceps. The wings were mounted between a slide and a four-corner adhesive coverslip. A piece of paper marked in millimeters (26 x 76 mm) added below the wings served as a scale for photographic records. The wings were photographed using a Leica EZ4 stereo microscope with 35x magnification and an HD 3 MP camera attached. The images were digitalized using Leica LAS EZ 1.6v 2013 software. Thirteen anatomical type I landmarks were chosen (Bookstein 1997), characterized by vein connections and intersections (Fig. 2, Table 2). These landmarks were digitized using the free program tpsDig32 version 2.31 (http://www.sbmorphometrics.org/soft-dataacq. html).

TABLE 1. Number of right wings from females of *Mansonia amazonensis* captured in the area of the Amazon Hydroelectric Development Unit of Jirau (UHE-Jirau), Rondônia State, Brazil, during the dry, rainy and transition periods of 2018 and 2019.

Collection year	Month	Season	N
2018	July	Dry	25
	October	Rainy	24
	December	Rainy	6
2019	April	Rainy	25
	July	Dry	55
	September	Transition	6
	November	Rainy	40
Total	7	3	181

TABLE 2. Description of the 13 anatomical landmarks of the wings of *Mansonia amazonensis* captured in the area of the Amazon Hydroelectric Development Unit of Jirau (UHE-Jirau), Rondônia State, Brazil, during the dry, rainy and transition periods of 2018 and 2019.

Anatomical landmark	Abbreviation	Location of anatomical landmarks
1	1A	axillary incision
2	CuA	cubitus anterior
3	M_{3+4}	media-three-plus-four
4	M_2	media-two and cell
5	M_1	media-one and cell
6	R_{4+5}	radius-four-plus-five
7	R_3	radius-three
8	R_2	radius-two
9	R_1	radius-one
10	Sc	subcostal
11	-	Intersection between veins R_2 and R_3
12	-	Intersection between M_2 and M_1 veins
13	-	Intersection between CuA and M_{3+4} veins

Size variation estimation. Isometric estimator centroid size (CS), derived from Cartesian coordinate data, was used to compare the size of the wings. Centroid size is defined by the square root of the sum of the squared distances between the center of the waypoint configuration and each landmark (Bookstein 1997).

Conformation variation estimation. Conformation variables define the positional changes in each landmark regarding consensus conformation. These variables were obtained using the Generalized Procrustes Analysis (GPA) superposition algorithm (Rohlf 1998, 1999). The method estimates the least-squares criterion on superimpositions of a group of individual samples, eliminating the effects of scale, orientation and position of objects. We assessed the between groups significant differences and reclassified individuals using discriminant functions analysis and Procrustes coordinates (Rohlf 1999).

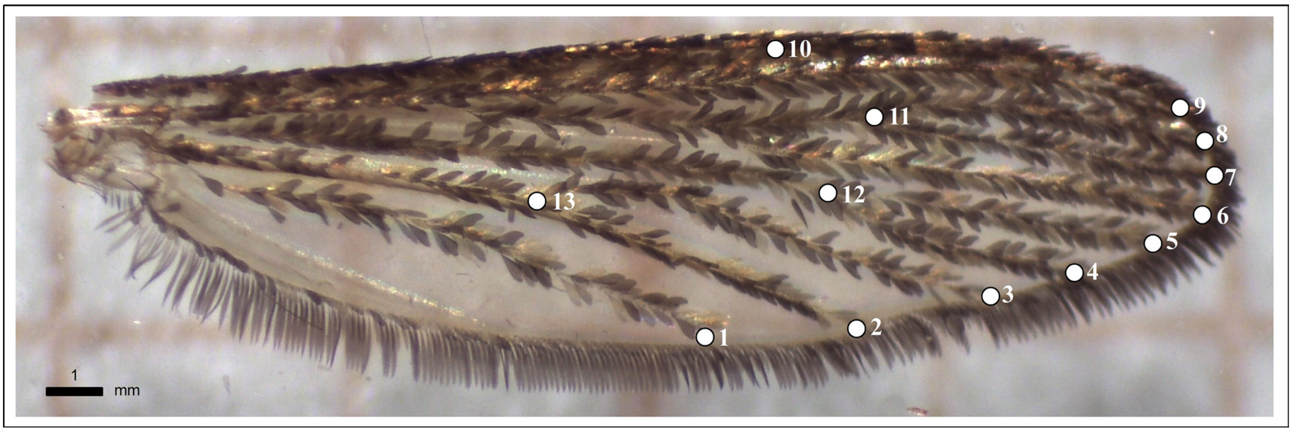

FIGURE 2. Dorsal view of the right wing of a female of *Mansonia amazonensis* captured in the area of the Amazon Hydroelectric Development Unit of Jirau (UHE-Jirau), Rondônia State, Brazil. The numbers represent the position of the landmarks. Scale = 1 mm.

Allometry estimation. Allometry is responsible for changes in conformation resulting from size variation (Shingleton *et al*. 2007). We assessed allometry by estimating the relationship between CS and conformation discrimination between groups using multivariate regression analysis between the Procrustes coordinates (dependent variables) and the CS (independent variable) (Debat *et al.* 2003).

Statistical analysis. The Kruskal-Wallis test was used to test the statistical difference between *Ma. amazonensis* populations. A significance level of 5% was adopted for rejection of the null hypothesis ($p < 0.05$). The isometric size between the groups was compared using the Kruskal-Wallis test (Kruskal & Wallis 1952). The conformation variables were compared using multivariate analysis following the methodology proposed by Dujardin (2008). We also characterized the conformation-related variation by applying principal component analysis (PCA) on the wing-shape covariance matrix. A canonical variable analysis (CVA) was performed to analyze the conformational variation and detect differences in wing shape between seasons. Mahalanobis distances with their respective statistical significance values (p) were calculated after a permutation test (10,000 runs). The relationship between CS and shape discrimination between populations (allometry) was estimated using multivariate regression between conformation variables (dependent variables) and CS (independent variable).

Centroid size, conformation variables and allometric analyzes were conducted using the free MorphoJ program (Klingenberg 2011). The Kruskal-Wallis non-parametric test and cross-reclassification analyzes were performed using the free Infostat program (Di Rienzo 2020).

Results

The results show a relatively gradual trend of increasing wing size in individuals of *Ma. amazonensis* collected during the dry, transition and rainy seasons of 2018 and 2019. This trend is from smaller wing sizes during the dry period to larger sizes in the rainy season (Table 3). Despite the increased wing size detected in the rainy season of both collection years, these sizes were significantly larger in individuals collected in 2018 than in individuals collected in 2019 (Kruskal-Wallis test, gl = 6, $p < 0.001$) (Table 3).

Principal component analysis (PCA) on the covariance matrix of conformation variables reveal variabilities of 43.69% for the first component (CP1), 18.19% for the second (CP2) and 9.67% for the third (CP3). Thus, approximately 72% of the accumulated variation was concentrated in these first three components. To locate the magnitude of the change in the conformation, we extracted the consensus conformation for the different groups collected in different periods. By this means, and based on the dorsal view of the wings, the diagram with the trend of wing-shape variation of populations of *Ma. amazonensis* for Major Component 1 differs from the consensus mainly in the location of landmarks 1 (axillary incision), 10 (subcostal vein) and 13 (intersection between veins CuA and M_{3+4}) (Fig. 3, Table 2). The magnitude of the consensus conformation changes for Major Component 2 for the groups collected during different periods show that the diagram with the trend of populations of *Ma. amazonensis* differs from the consensus mainly in the location of landmarks 1 (anal vein), 11 (intersection between veins R_2 and R_3) and 13 (intersection between veins CuA and M_{3+4}) (Fig, 3).

TABLE 3. Comparisons of the median centroid size of wings of females of *Mansonia amazonensis* captured in the area of the Amazon Hydroelectric Development Unit of Jirau (UHE-Jirau), Rondônia State, Brazil, during the dry, rainy and transition periods of 2018 and 2019.

Period	Jul/18 (D)$^+$	Oct/18 (R)$^+$	Dec/18 (R)$^+$	Apr/19 (R)$^+$	Jul/19 (R)$^+$	Sep/19 (T)$^+$	Dec/19 (R)$^+$	Median
July/18 (D)	-	0.18	0.05	0.0001*	0.0900	0.200	0.480	2.94
October/18 (R)	-	-	0.28	0.0001*	0.0001*	0.030*	0.420	3.07
December/18 (R)	-	-	-	0.0001*	0.0001*	0.010*	0.110	3.1
April/19 (R)	-	-	-	-	0.0001*	0.060	0.001*	2.66
July/19 (R)	-	-	-	-	-	0.670	0.001*	2.87
September/19 (T)	-	-	-	-	-	-	0.080	2.83
December/19 (R)	-	-	-	-	-	-	-	2.99

D = dry, R = rainy, T = transition.

$^+$P values. *Statistically significant median differences between periods, $p \leq 0.05$.

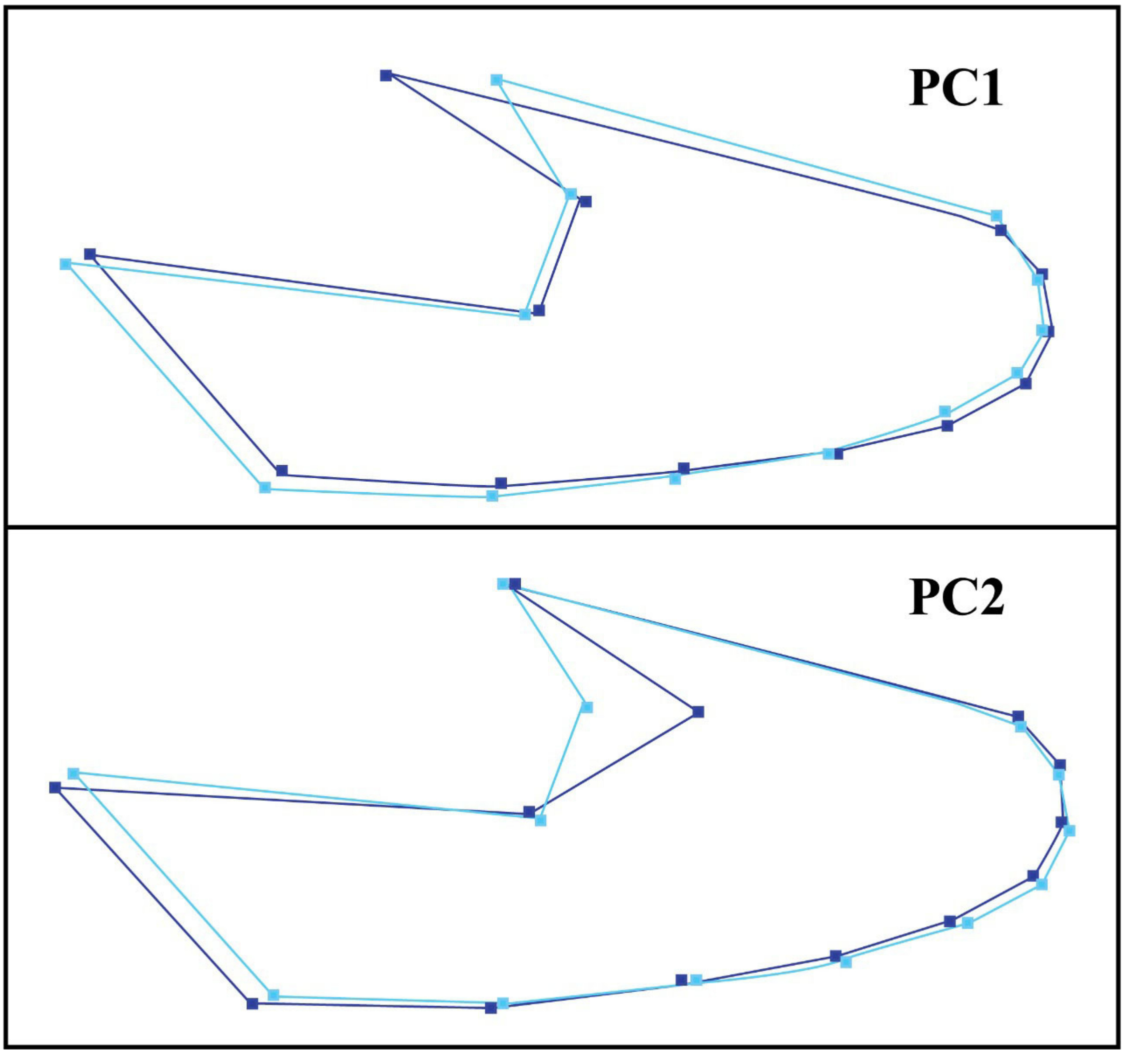

FIGURE 3. Diagrams of conformation variation between landmarks for Principal Component 1 (PC1) and 2 (PC2). Light blue lines represent consensus conformations and dark blue lines correspond to the conformation trends.

Canonical variable analysis (CVA) showed a slight separation between the groups corresponding to the collection periods, mainly for specimens collected during the two periods of the rainy season of 2018 (Fig. 4). The results obtained for the Mahalanobis distances indicate that there are significant differences between the groups (permutation test, $p < 0.01$) (Table 4). Based on the Mahalanobis distances, the biggest difference occurred between the populations sampled during the rainy seasons of 2018 (4) and 2019 (8). In contrast, the biggest morphological proximity occurred between populations from the 2019 rainy and dry seasons (5 and 6). The percentages of correctly assigned individuals were reasonably good for all groups, with an average of 58.6%. The populations of the periods July/18 (Dry), October/18 (Rainy), September/19 (Transition) and December/19 (Rainy) produced the best scores of correctly assigned individuals, reaching 64%, 79%, 66% and 70%, respectively. The scores were lower for populations sampled in December/18 (Rainy), April/19 (Rainy) and July/19 (Dry), for which the correct reclassifications were 50%, 44% and 35%, respectively.

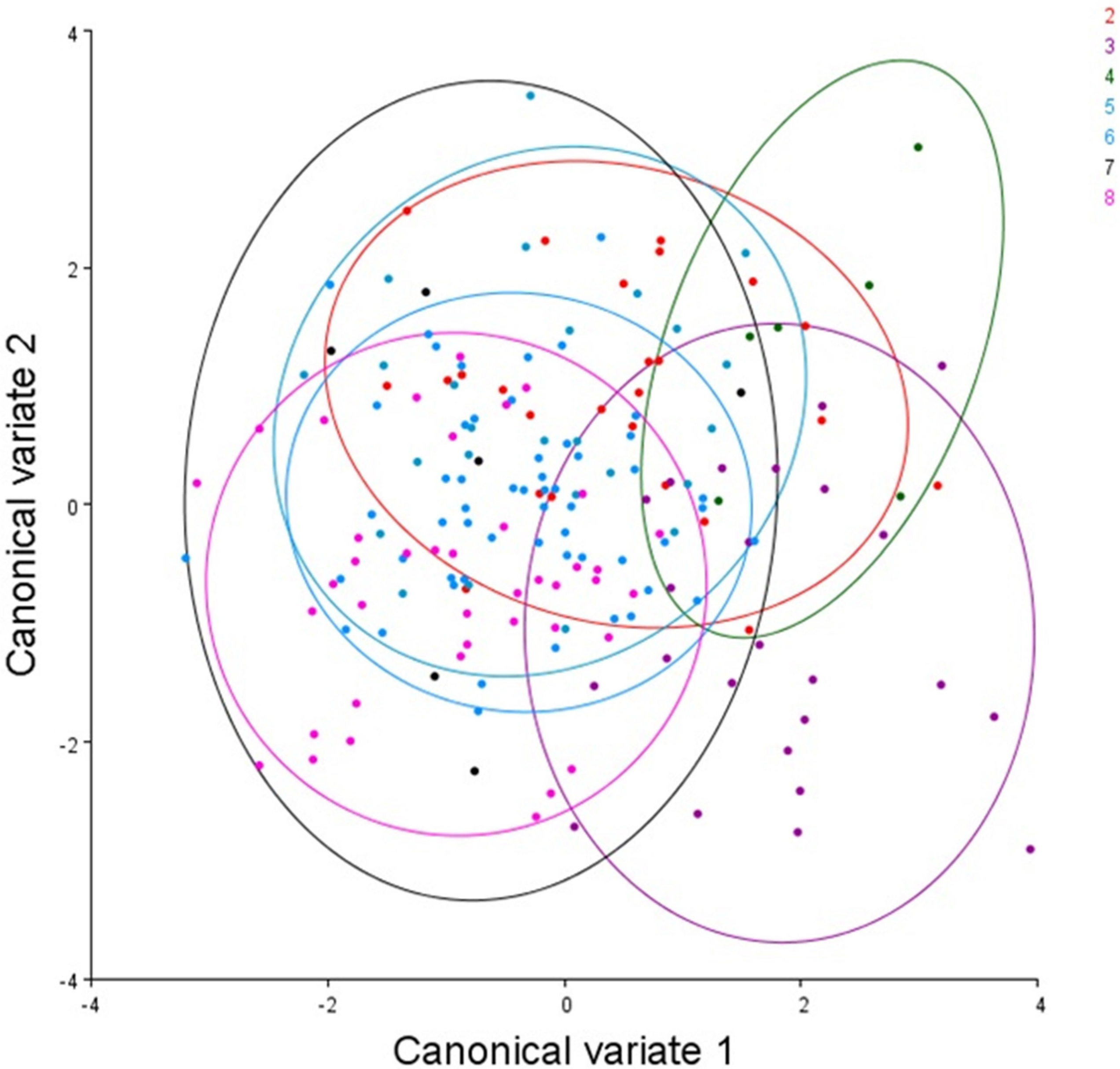

FIGURE 4. Canonical analysis of shape variables of 181 specimens of *Mansonia amazonensis* according to dry, rainy and transition periods of 2018 and 2019 in the area of the Amazon Hydroelectric Development Unit of Jirau (UHE-Jirau), Rondônia State, Brazil. Each point indicates an individual, and the ellipses indicate the confidence interval (90%). Numbers identify groups of specimens collected during different periods: (2) July/18, dry season; (3) October/18, rainy season; (4) December/18, rainy season; (5) April/19, rainy season; (6) July/19, dry season; (7) September/19, transition period; (8) December/19, rainy season.

TABLE 4. Mahalanobis distances between groups of *Mansonia amazonensis* captured in the area of the Amazon Hydroelectric Development Unit of Jirau (UHE-Jirau), Rondônia State, Brazil, during the dry, rainy and transition periods of 2018 and 2019. The p values were obtained by permutation test (10,000 runs). Significant values are indicated by an asterisk (*).

Period/Year	Dry/18	Rainy/18	Rainy/18	Rainy/19	Dry/19	Transition/19
Rainy/18	2.6197 (< 0.001*)					
Rainy/18	3.8244 (0005)	3.7091 (0.0046)				
Rainy/19	1.6633 (0.0350)	2.8342 (< 0.0001*)	3.579 (0.0003*)			
Dry/19	1.8117 (< 0.0001*)	2.5661 (< 0.0001*)	3.9182 (< 0.0001*)	1.4245 (0168)		
Transition/19	2.6446 (0.0948)	3.2383 (0.132)	4.2842 (0.029)	1.8731 (0.7269)	1.8915 (0.5079)	
Rainy/19	2.4321 (< 0.0001*)	2.8844 (< 0.0001*)	4.3018 (< 0.0001*)	1.8581 (<0.0001*)	1.4605 (< 0.0001*)	2.0733 (0.2312)

Regression analysis between conformation variables and CS for each group, based on the permutation test (10,000 iteration runs), was not statistically significant ($p \geq 0.3814$). The value of the influence of allometry was relatively low (0.4%).

Discussion

This study shows the influence of seasonality on the size and conformation of the wings of females of *Ma. amazonensis* captured in the Amazon Hydroelectric Development area of Rondônia State of Brazil. The results show that the wing size of individuals captured during the dry, transition and rainy seasons of 2018 and 2019 increases gradually from smaller during the dry period to larger in the rainy season.

Wing size can be considerably influenced by environmental factors, such as temperature, relative humidity, habitat, diet and larval density, although some researchers argue that it is a potentially heritable trait (Dujardin *et al*. 1997, 1999; Scott *et al*. 2000; Tsuda & Takagi 2001; Smith & Mullens 2003; Jirakanjanakit *et al.* 2007; Morales-Vargas *et al*. 2010; Gurgel-Gonçalves *et al*. 2011). In other species, such as *Aedes aegypti* (Linnaeus, 1762), wing size variation between populations is associated with different vector capacities (Jirakanjanakit *et al.* 2007). Geometric morphometry allows the use of a robust wing size estimator to describe the effects of environmental factors. A recent study on *Ae. aegypti* adults showed that wing size grows as larval density increases (Clements 1963; Silva *et al*. 2020). Clements (1963) reported that temperature influences the body size of adult mosquitoes raised from larvae bred under ideal environmental and feeding conditions.

The wing shape of the specimens of *Ma. amazonensis* analyzed in this study indicates a significant variation, with considerable heterogeneity in the populations studied, mainly during the rainy season of 2018. Similar results of intra-population variability were obtained on the diversity of wing conformation of *Culex coronator* Dyar & Knab, 1906 among seven populations of southern and southeastern Brazil (Demari-Silva *et al.* 2014). Likewise, populations of *Aedes scapularis* (Rondani, 1848) captured in urban park areas of São Paulo showed high levels of variation in wing shape (Demari-Silva *et al*. 2014). The results of allometric analysis show a non-significant influence of size on wing conformation, with a relatively low value of allometry indicating that size would not influence the conformation.

The populations of *Ma. amazonensis* analyzed came from areas with extensive forest cover under evident decline, as easily visualized in satellite images (Prates & Bacha 2011). Hence, it is possible to hypothesize that new geographic barriers produced by the absence of cover of vegetation may prevent migration through flight, directly influencing gene flow and causing genotypic instability, increasingly accelerating the differences and reducing the similarities between the populations of *Ma. amazonensis* from the study area (Slatkin 1987). Multini *et al*. (2019) pointed to urbanization as a determining factor in the variation of wing conformation of *Anopheles cruzii* Dyar &

Knab, 1908. Similarly, morphometric analyzes of *Anopheles darlingi* Root, 1926 suggested that variation of wing conformation could be related to the different selective pressures present in the Cerrado and the inland and coastal regions of the Atlantic Forest.

In summary, we conducted the first geometric morphometric study of *Ma. amazonensis* that shows phenotypic variability associated with seasonality for the conformation and size of the wings. We hypothesize that variability could be related to the strong selective pressures occurring in the Amazon region. Although it was not possible from the present study to infer an association between detected wing polymorphisms and selective pressures resulting from the devastation of environment, further research on populations of *Ma. amazonensis* in the native forest on the left bank of the Madeira River in Rondônia State is being conducted to further explore this hypothesis.

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