

Host effect on morphology of the fruit fly *Anastrepha zenildae* (Diptera: Tephritidae) from the Semi-Arid region of Rio Grande do Norte

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Abstract. *Anastrepha zenildae* Zucchi is an important pest in the Semi-Arid region of Rio Grande do Norte and other states in Brazil, with Guava being its main host. Environment induced morphological changes in adult fruit flies can lead to mating incompatibilities among populations within a species. Furthermore, sexual isolation between these populations can be one of the first steps in the speciation process, as described to other tephritid fly species. Here, we compared several body measurements through analysis of variance and geometric morphometrics to assess significant morphological differences between sympatric flies from different hosts: Guava and Jua. We found significant differences in body size for both sexes of flies from the different fruit hosts, with flies from Guava being the larger. Different degrees of sexual size dimorphism of flies from each fruit has also been detected. Flies from different fruits also exhibited different wing shapes, even though the pattern of sexual shape dimorphism remained the same for both fruits. These results show the influence of the host fruits on adult fruit fly morphology, a phenomenon that must be considered when implementing pest control programs such as the Sterile Insect Technique (SIT). Further studies are needed to explore mating preference and genetic structure between these populations.

Keywords. Fruit flies; Guava; Jua; Semi-Arid; Geometric Morphometry.

INTRODUCTION

Tephritidae is one of the largest families of the Diptera order, with nearly 5,000 species (Norrbom *et al.*, 2012). Insects belonging to this family are considered to be important agricultural pests, comprising 38% of the species that feed on fruits during the larval stage, especially the genera *Anastrepha* Schiner, 1868, *Bactrocera* Macquart, 1835, *Ceratitis* Macleay, 1829 *Dacus* Fabricius, 1805, and *Rhagoletis* Loew, 1862 (White & Elson-Harris, 1992). *Anastrepha*, well distributed throughout the neotropic with over 250 species described, is the largest fruit fly genus in the Americas where several species are noteworthy pests, like the South American fruit fly *Anastrepha fraterculus* Wiedemann, 1830, since many of its hosts are shipped to many countries around the world. (Norrbom *et al.*, 2015).

The species *Anastrepha zenildae* Zucchi, 1971, like *A. fraterculus*, *A. obliqua* (Macquart, 1835) and *A. sororcula* Zucchi, 1979, has great economic importance in several states in Brazil, with Guava (*Psidium guajava*) often being their main host (Araujo *et al.*, 2005, Querino *et al.*, 2014). A few studies have been done in the state of Rio Grande do Norte, mainly with Guava in the Semi-Arid region, and it's been consistently shown that *A. zenildae* is the main parasite of this fruit in the genus *Anastrepha* (Araujo & Zucchi, 2003; Araujo *et al.*, 2013). Others fruit hosts of this species in the Semi-Arid region of the Rio Grande do Norte state are the Cajarana (*Spondias* sp.), Seriguela (*Spondias purpurea*), Castanhola (*Terminalia catappa*), Acerola (*Malpighia emarginata*), Kumquat (*Citrus japonica*) and Jua (*Ziziphus joazeiro*), with the latter only having *A. zenildae* as parasite in the genus (Araujo *et al.*, 1996; Araujo *et al.*, 2005).

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Both traditional and, more frequently, geometric morphometry have often been used as a tool for identifying closely related insect species and also species complexes. In Diptera, wings are one of the most frequently used body parts that are used for comparison among different species, since they show a lot of plasticity and have very clear landmark structures (Macedo, 2017; Perre *et al.*, 2014; Soto *et al.*, 2010; Souza *et al.*, 2020). These methods can also be used effectively to analyze how different environmental conditions can affect morphology within a species, as discussed below.

Hernández-Ortiz *et al.* (2015), using linear and geometric morphometrics to analyze 40 different populations of the *Anastrepha fraterculus* complex in several countries (Mexico, Guatemala, Panama, Venezuela, Colombia, Ecuador and Peru), found that the flies differed in wing, ovipositor and thorax shape. Similarly, Bomfim *et al.* (2011), also using linear and geometric morphometrics, comparing *Anastrepha pickeli* Lima, 1934 flies from Paraguay, Bolivia and four different states in Brazil (Bahia, Espírito Santo, Manaus and Rio Grande do Norte), showed that they differed in wing shape, wing size and ovipositor shape, suggesting that *A. pickeli* could also be part of a cryptic species complex, whose species were later described (Canal *et al.*, 2013).

Some studies using the same methods, showed that adult flies also exhibit a certain phenotypic plasticity, manifesting morphometric differences induced by the larval diet (Gomez-Cendra *et al.*, 2016; Navarro-Campos *et al.*, 2011; Perre, 2016; Pereira-Rego *et al.*, 2011; Pieterse *et al.*, 2017). While others authors showed that, on average, both larger and more symmetrical flies were most successful in mating (Almeida *et al.*, 2013; Burk & Webb, 1983; Sicuriano *et al.*, 2007), which demonstrates that host induced differences can affect aspects of the life history of these insects.

In this study, we utilized several body measurements as well as geometric morphometry of the wings in order to verify if adult flies of *Anastrepha zenildae* infesting Guava and Jua fruits, in a sympatric condition in the Semi-Arid region of Rio Grande do Norte, present morphological differences.

MATERIAL AND METHODS

Study area and data collection

All fruits were collected in April of 2019, in the municipalities of Ipanguaçu (05°32'56.7"S, 36°52'50.4"W), Florânia (06°05'38.6"S, 36°48'52.0"W), Santana do Matos (06°03'37.8"S, 36°36'01.1"W) and Lagoa Nova (06°06'10.6"S, 36°26'35.4"W) (Fig. 1). Both Jua and Guava fruits were collected directly from the trees, as well as on the ground beneath them, placed in plastic trays containing a layer of vermiculite and then covered in voile until transported to the laboratory.

Once in the lab, the fruits were weighted and stored again in labeled trays layered with vermiculite, where they remained at 30°C and 70% humidity for three

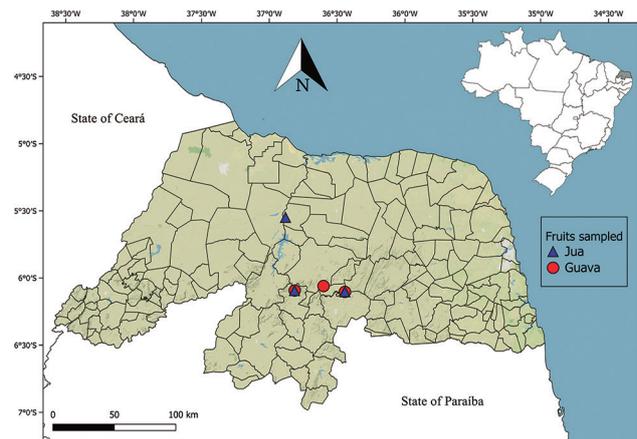


Figure 1. Map of the Guava and Jua sample sites in the state of Rio Grande do Norte.

weeks. The vermiculite was sieved periodically and the recovered puparium placed in small plastic containers containing vermiculite until the adults' emergence. After species identification, flies were stored in 2 ml Eppendorf labeled tubes containing 70% alcohol. Subsequent analyses consisted on 100 fly individuals from each host (50 males and 50 females) in order to assess size and shape variations between hosts and sexes. All material is deposited at the Fruit Fly Laboratory and Adalberto A. Varela-Freire Entomological Collection (CEAAVF) at Universidade Federal do Rio Grande do Norte, Natal, Brazil.

Body measurements

The *A. zenildae* flies were dissected in a petri dish under a stereomicroscope (Motic SMZ-168). The forewings (and ovipositors in the females) were sectioned and placed between slides and cover slides. The heads were severed from the thorax, and both were placed horizontally on the petri dish. Images were captured by a single researcher (Canejo, R.P.R.) using a Motic camera (MotiCam 5MP) attached to the microscope, with standardized position and distance from the object. Optical and digital zoom were not used. All measurements were performed through the Motic Images Plus 2.0 program.

The chosen measurements for the size comparison between flies (Fig. 2) were right wing length (RWL), left wing length (LWL), right wing width (RWW), left wing width (LWW), thorax length (TH), head width (HW) and face width (FW). For female flies, the ovipositor length (OVP) was also measured. Wing length was determined as the distance between the apex of the basal medial cell (bm) and the intersection of the R4+5 vein and the wing margin. Wing width was determined as the distance between the intersection of the costal (C) and subcostal (Sc) veins and the distal end of vein A1+Cu2.

To analyze the size difference between flies, each body measurement was compared through a One-Way ANOVA in the R© Software (R Core Team, 2021). First, the flies were compared between sexes within each fruit, to see if any existing size differences between males and females are consistent between fruits. Then, flies of the

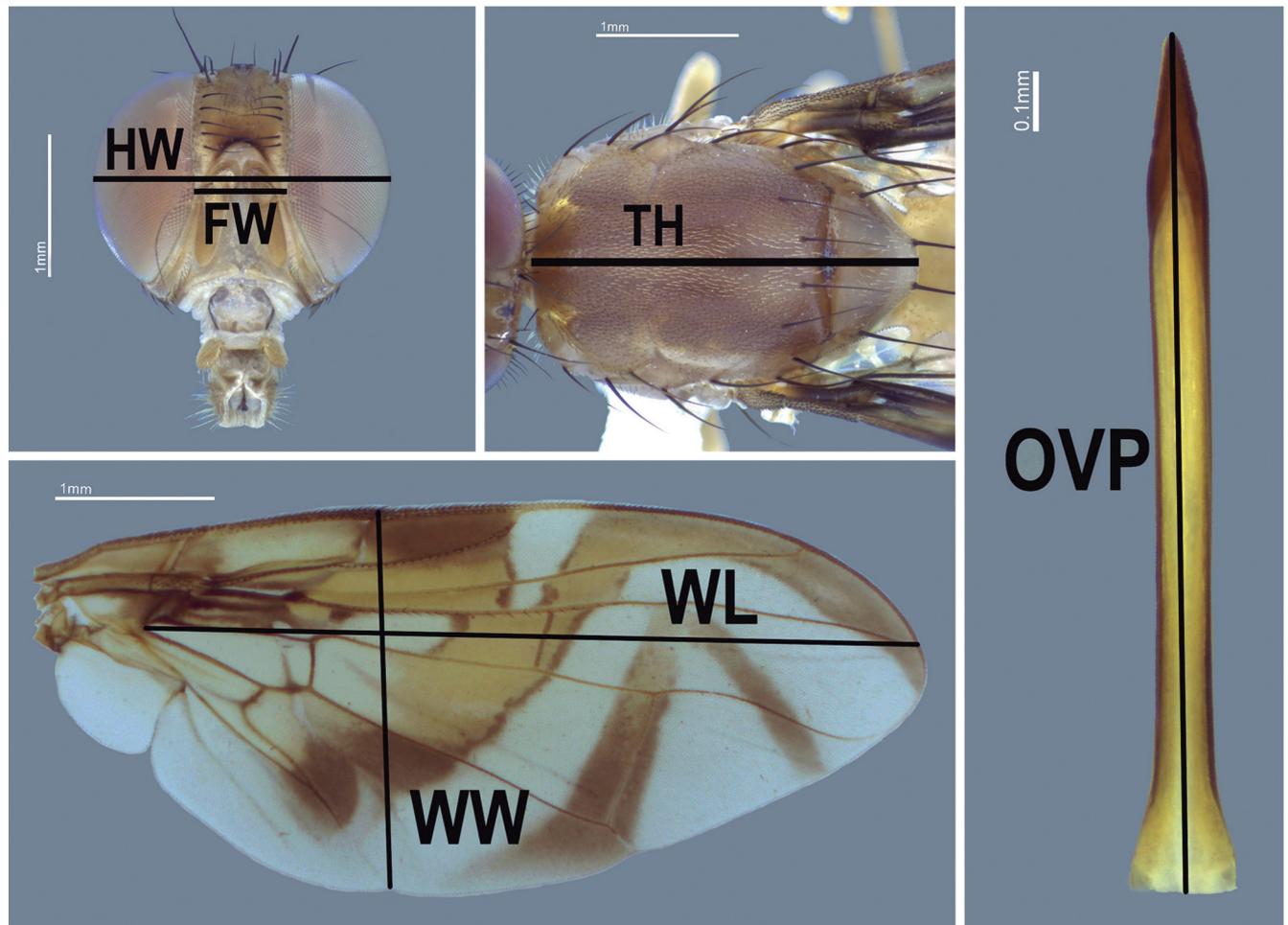


Figure 2. Female of *Anastrepha zenildae* showing body measurements used to compare flies between hosts and sexes. HW = Head width; FW = Face width; TH = Thorax length; WL = Wing length; WW = Wing width; OVP = Ovipositor length.

same sex were compared between each fruit, in order to know the host fruit effect on the size.

Geometric Morphometric

The right wings of the *A. zenildae* flies were used as a biological structure for shape abstraction. Wing images, captured by a single researcher using a Motic camera attached to the microscope (see previous topic), were ordered in TPS format by tpsUtil software (Rohlf, 2008). Seventeen landmarks (Fig. 3) were applied in the vein intersections using the software tpsDig2.31 (Rohlf, 2010).

Generalized Procrustes Analysis (Rohlf & Slice, 1990) was performed to remove aspects of position, scale, and rotation (Dryden & Mardia, 1998). A Multivariate Regression (pooled across hosts and sex) of Procrustes coordinates (shape) on centroid size (size) was performed to correct for the allometric effect (Drake & Klingenberg, 2008). The residuals from this regression were used in subsequent analyses to demonstrate only the variation in shape. Principal Component Analysis (PCA) (Jolliffe, 2002) was used to observe the variation around the wing shape of adult *A. zenildae* flies (males and females) from Guava and Jua. We then performed Canonical Variable Analysis (CVA) (Albrecht, 1980) to identify statistical dif-

ferences between groups (hosts vs. sex) and inspection of shape variation among the canonical variables. Finally, Discriminant Function Analysis (DFA) (Timm, 2002) was performed to test the shape abstraction between sex pairs (males and females) in each host (Guava and Jua) and we estimated the correct percentages of the cross-validation matrix to assess the possible correct identification between flies.

Generalized Procrustes analysis, multivariate regression analysis, PCA, CVA and DFA, and wireframe plots were performed in MorphoJ 2.06 d^o software (Klingenberg, 2011).

RESULTS

Size

The analysis (one-way ANOVA) comparing the body measurements between the sexes within each fruit, showed that flies from Guava differed significantly in every body trait but one, which was Face Width (FW). Meanwhile, flies from Jua did not differ significantly in most traits, except for Right Wing Length (RWL) and Head Width (HW), which were still only marginally significant, with p values being 0.03 and 0.04 respectively (Table 1).

These results showed that, overall, flies emerging from Guava had more accentuated sexual dimorphism when compared to flies that emerged from Jua, with females being the larger sex.

The comparison of measurements of flies belonging to the same sex but from different fruit hosts showed the existence of significant differences ($p < 0.001$), in both males and females (Table 2). In general terms, flies from Guava fruits had higher mean values than flies from Jua,

showing that the overall size of the flies differs between fruits.

Shape

The PCA morphospace had 19.63% representation in its first component (PC1) and 13.19% in its second component (PC2), demonstrating the overall variation in the

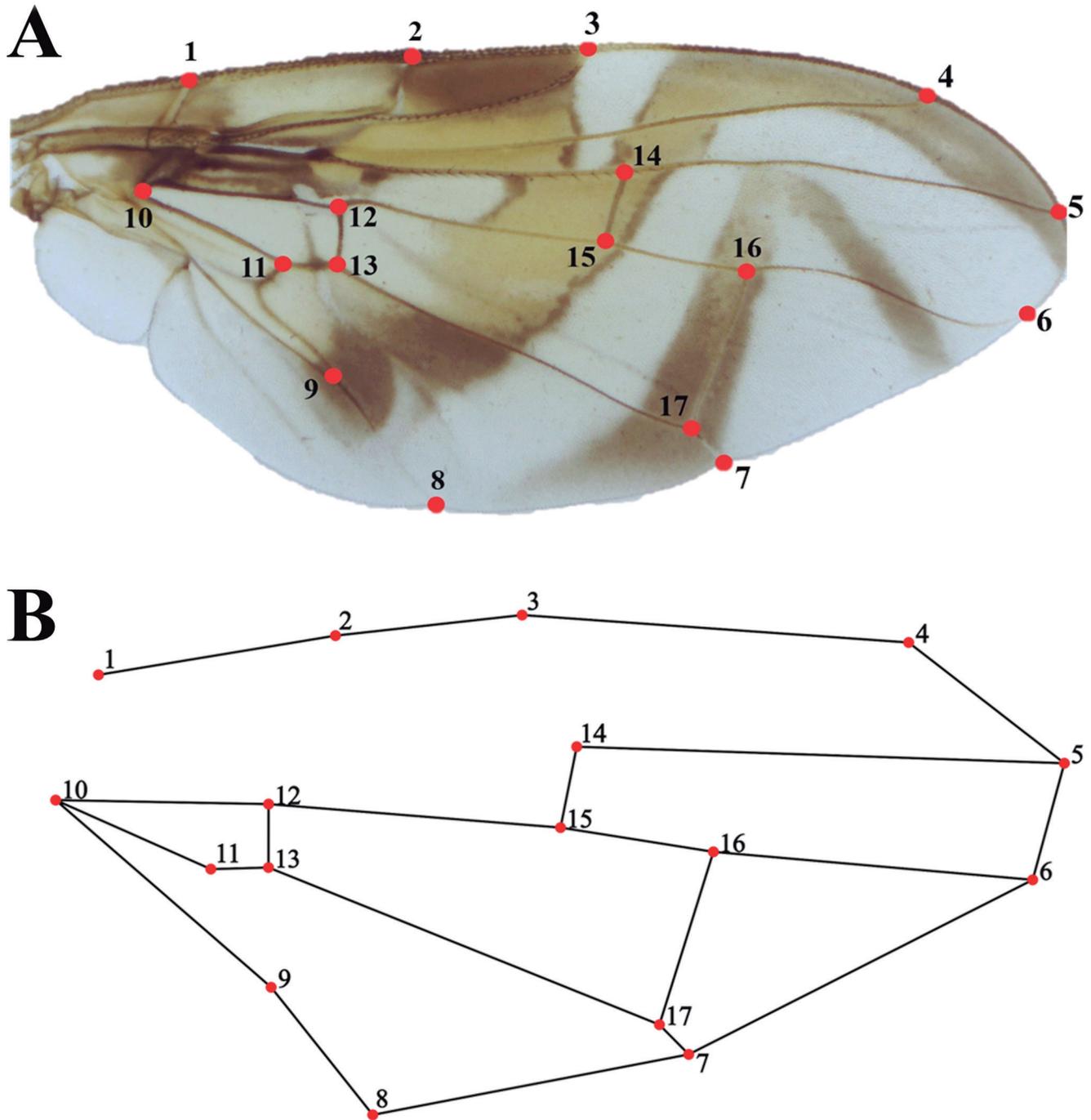


Figure 3. Representation of landmarks in wing structure and consensus shape applied to *Anastrepha zenilidae* with landmarks. 1 = intersection of humeral and costal veins; 2 = intersection of subcostal and costal veins; 3 = intersection of R1 and costal veins; 4 = intersection of R2+3 and costal veins; 5 = intersection of R4+5 and costal veins; 6 = intersection of M vein with wing margin; 7 = intersection of vein Cu1 with wing margin; 8 = intersection of vein A1+Cu2 and wing margin; 9 = intersection of A1 and Cu2 veins; 10 = intersection of M vein and base of bm cell; 11 = intersection of Cu1 and Cu2 veins; 12 = intersection of M and bm-cu veins; 13 = intersection of Cu1 and bm-cu veins; 14 = intersection of r-m and R4+5 veins; 15 = intersection of r-m and M veins; 16 = intersection of M and dm-cu veins; 17 = intersection of Cu1 and dm-cu veins.

Table 1. One-way ANOVA demonstrating size variation between female and male *Anastrepha zenilldae* from the same host, in the semi-arid region of Rio Grande do Norte. JUAF = Females from Jua; JUAM = Males from Jua; GUAF = Females from Guava; GUAM = Males from Guava; SD = Standard deviation; RWL = Right wing length; LWL = Left wing length; RWW = Right wing width; LWW = Left wing width; TH = Thorax length; HW = Head width; FW = Face width. Values in bold indicate statistical significance ($p < 0.05$).

	JUAF	JUAM	F	p		GUAF	GUAM	F	p
	Mean \pm SD	Mean \pm SD				Mean \pm SD	Mean \pm SD		
RWL	5.12 \pm 0.27	5.00 \pm 0.27	5.07	< 0.05	RWL	5.51 \pm 0.17	5.30 \pm 0.24	24.39	< 0.001
LWL	5.11 \pm 0.27	5.00 \pm 0.34	3.30	0.07	LWL	5.51 \pm 0.18	5.31 \pm 0.23	23.34	< 0.001
RWW	2.42 \pm 0.15	2.41 \pm 0.14	0.22	0.63	RWW	2.63 \pm 0.09	2.58 \pm 0.15	4.74	< 0.05
LWW	2.43 \pm 0.15	2.41 \pm 0.14	0.70	0.40	LWW	2.64 \pm 0.09	2.59 \pm 0.15	4.74	< 0.05
TH	2.82 \pm 0.16	2.76 \pm 0.18	3.68	0.05	TH	3.08 \pm 0.10	2.94 \pm 0.16	23.46	< 0.001
HW	1.98 \pm 0.10	1.94 \pm 0.10	4.12	< 0.05	HW	2.12 \pm 0.07	2.06 \pm 0.10	8.97	< 0.01
FW	0.58 \pm 0.04	0.56 \pm 0.04	3.73	0.05	FW	0.62 \pm 0.03	0.61 \pm 0.04	1.33	0.25

Table 2. One-way ANOVA demonstrating the size variation between male and female *Anastrepha zenilldae* from different hosts, in the semi-arid region of Rio Grande do Norte. JUAF = Females from Jua; JUAM = Males from Jua; GUAF = Females from Guava; GUAM = Males from Guava; SD = Standard deviation; RWL = Right wing length; LWL = Left wing length; RWW = Right wing width; LWW = Left wing width; TH = Thorax length; HW = Head width; FW = Face width. OVP = Ovipositor length. Values in bold indicate statistical significance ($p < 0.05$).

	GUAM	JUAM	F	p		GUAF	JUAF	F	p
	Mean \pm SD	Mean \pm SD				Mean \pm SD	Mean \pm SD		
RWL	5.31 \pm 0.24	5.00 \pm 0.27	35.60	< 0.001	RWL	5.52 \pm 0.18	5.12 \pm 0.27	74.09	< 0.001
LWL	5.31 \pm 0.23	5.00 \pm 0.34	33.07	< 0.001	LWL	5.52 \pm 0.18	5.11 \pm 0.27	60.09	< 0.001
RWW	2.58 \pm 0.15	2.41 \pm 0.15	27.49	< 0.001	RWW	2.64 \pm 0.10	2.42 \pm 0.15	76.06	< 0.001
LWW	2.59 \pm 0.15	2.41 \pm 0.15	34.75	< 0.001	LWW	2.65 \pm 0.10	2.44 \pm 0.15	62.32	< 0.001
TH	2.95 \pm 0.17	2.76 \pm 1.18	27.91	< 0.001	TH	3.08 \pm 0.11	2.83 \pm 0.17	80.61	< 0.001
HW	2.07 \pm 0.11	1.94 \pm 0.10	35.28	< 0.001	HW	2.12 \pm 0.08	1.98 \pm 0.11	58.82	< 0.001
FW	0.61 \pm 0.04	0.57 \pm 0.04	28.31	< 0.001	FW	0.62 \pm 0.04	0.58 \pm 0.05	18.68	< 0.001
—	—	—	—	—	OVP	1.89 \pm 0.05	1.79 \pm 0.07	68.47	< 0.001

shape abstraction of the flies (males and females) from Jua and Guava fruits (Fig. 4). CVA magnified the effects indicated by PCA and had 53.30% representation in the first component and 34.60% in the second component (Fig. 5).

The main variation in the data (CV1) demonstrated the strong presence of sexual dimorphism in the *A. zenil-*

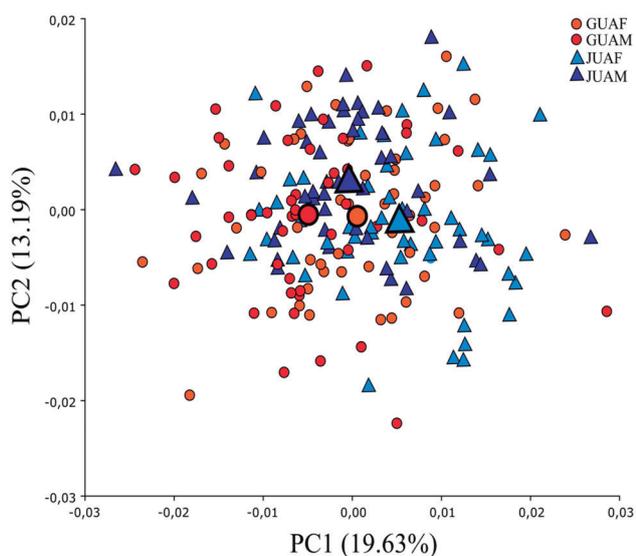


Figure 4. Principal component analysis (PCA) of the adult flies of *Anastrepha zenilldae* (Males and females) from Guava and Jua fruits. GUAF = Females from Guava; GUAM = Males from Guava; JUAF = Females from Jua; JUAM = Males from Jua. Larger symbols: Centroids of each dataset.

dae species, as the shape of the females of Jua and Guava were more similar to each other, just as the males of Jua and Guava were more similar to each other (Fig. 5). In CV1, the landmarks responsible for the variation in shape abstraction between the sexes were 1 (intersection of humeral and costal veins), 2 (intersection of subcostal and costal veins), 5 (intersection of R4+5 and costal veins) e 6 (intersection of M vein with wing margin). The morpho space along the CV component showed two clusters: the first represented by male Jua and Guava flies (CV1+) and the second represented by female Jua and Guava flies (CV1-). Therefore, males had the anterior wing margin more expanded, represented by the intersection of humeral and costal veins and the intersection of subcostal and costal veins. In addition, they also obtained the shape of the narrowest apex, represented by the intersection of the R4+5 and costal veins and the intersection of the M vein with wing margin (CV1+). In contrast, females showed the opposite vector displacement to that previously reported (CV1-).

CV2 showed that, in addition to the sexual dimorphism shown in CV1, individuals also exhibit shape distinctions with respect to host type (Fig. 5). The landmarks responsible for the variation in fly wing shape abstraction between hosts were the 8 (intersection of vein A1+Cu2 and wing margin), 10 (intersection of M vein and base of bm cell), 16 (intersection of M and dm-cu veins) and 17 (intersection of Cu1 and dm-cu veins). The morpho space along this CV component showed two other clusters: the

first represented by flies from the Jua host (CV2+) and the second by flies from the Guava host (CV2-). Therefore, Jua flies had the posterior wing margin more reduced, represented by the intersection of veins A1+Cu2. It was

also shown that the intersections between the M vein and the base of the bm cell, the M and dm-cu veins and Cu1 and dm-cu veins were all distally displaced (CV2+). Flies from the Guava host, on the other hand, showed

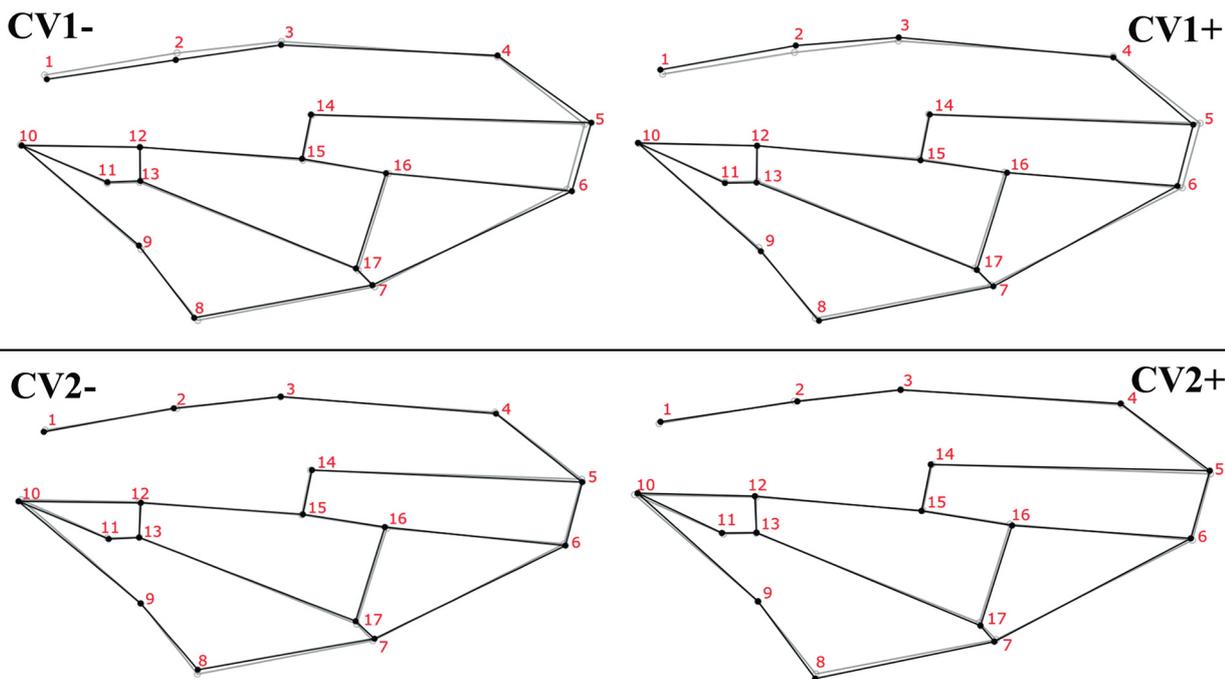
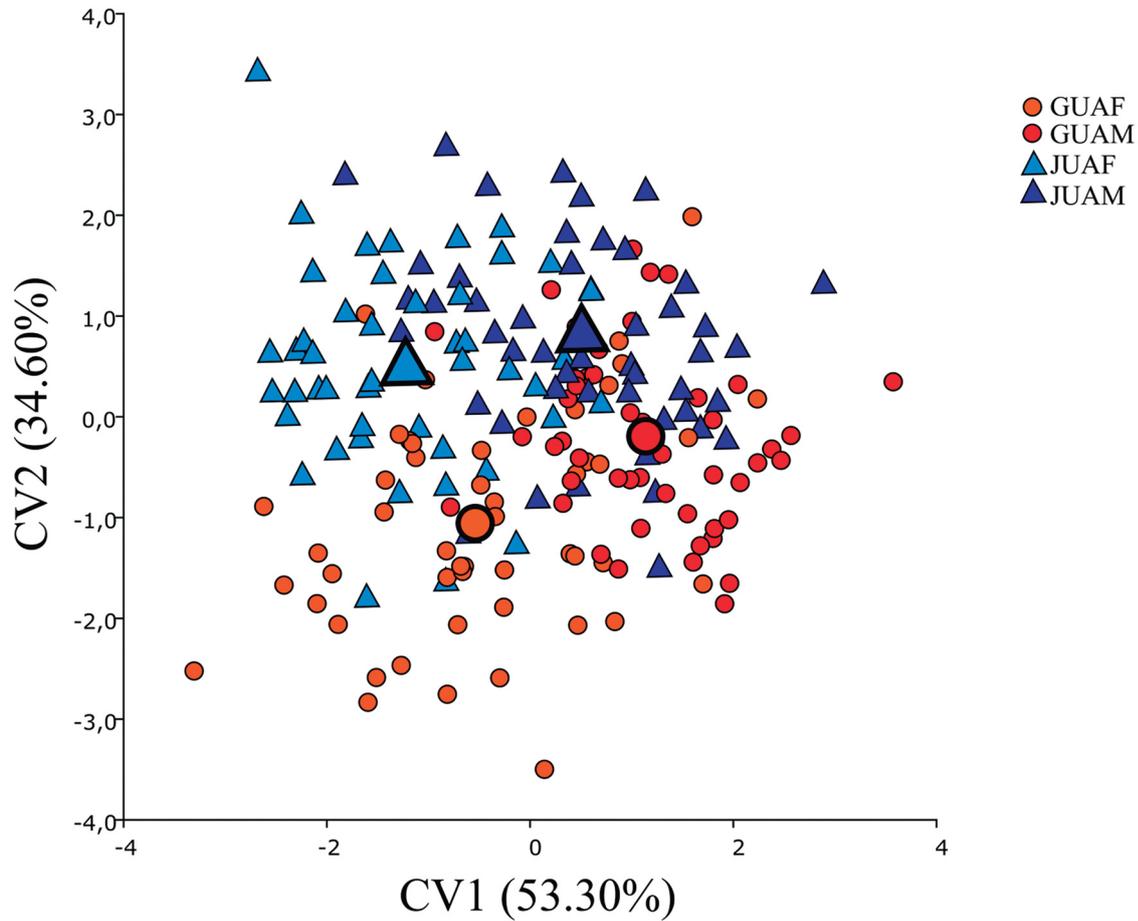


Figure 5. Canonical Variable Analysis (CVA) of the wings of adult *Anastrepha zenilldae* (Males and females) from Guava and Jua fruits (above), and the wireframes of wing shape for each investigated component (below). GUAF = Females from Guava; GUAM = Males from Guava; JUAF = Females from Jua; JUAM = Males from Jua. Wireframes in black represent displacement for each CV.

Table 3. Statistical results of Canonical Variable Analysis for adult flies of *Anastrepha zenildae* (Males and females) from Guava and Jua fruits, in the semi-arid region of Rio Grande do Norte. GUAF = Female host flies of Guava; GUAM = Male host flies of Guava; JUAF = Female host flies of Jua; JUAM = Male host flies of Jua; CV's = Components; Eig = Eigenvalues; %Var = CVA variation percentage; %Cum = cumulative percentage variation; Pcr = Procrustes Distance; p-value = values in bold indicate statistical significance ($p < 0.05$).

CV's	Eig	%Var	%Cum	Pcr/p-value	GUAF	GUAM	JUAF	JUAM
					GUAF	<.0001	0.0491	<.0001
1	0.82799	53.30	53.30		GUAM	0.0093	<.0001	0.0021
2	0.53745	34.60	87.89		JUAF	0.0056	0.0115	<.0001
3	0.18806	12.11	100.00		JUAM	0.0086	0.0066	0.0097

Table 4. Statistical results of Discriminant Function Analysis and cross-validation matrix for adult flies of *Anastrepha zenildae* (Males and females) from Guava and Jua fruits, in the semi-arid region of Rio Grande do Norte. GUAF = Female host flies of Guava; GUAM = Male host flies of Guava; JUAF = Female host flies of Jua; JUAM = Male host flies of Jua; Pcr = Procrustes distance; %AC = Percentual assimilation correct of cross-validation matrix. Values in bold indicate statistical significance ($p < 0.05$).

Discriminant Function Analysis	Cross-validation matrix					
Pcr	0.0093		GUAF	GUAM	Total	%AC
Pcr (p-value)	<.0001	GUAF	31	19	50	0.62
T-square	104.53	GUAM	14	36	50	0.72
T-square (p-value)	0.002					
Pcr	0.0056		GUAF	JUAF	Total	%AC
Pcr (p-value)	0.046	GUAF	32	18	50	0.64
T-square	108.91	JUAF	18	32	50	0.64
T-square (p-value)	<.0001					
Pcr	0.0086		GUAF	JUAM	Total	%AC
Pcr (p-value)	<.0001	GUAF	33	17	50	0.66
T-square	104.35	JUAM	19	31	50	0.62
T-square (p-value)	0.002					
Pcr	0.0114		GUAM	JUAF	Total	%AC
Pcr (p-value)	<.0001	GUAM	43	7	50	0.86
T-square	240.76	JUAF	10	40	50	0.80
T-square (p-value)	0.002					
Pcr	0.0066		GUAM	JUAM	Total	%AC
Pcr (p-value)	<.0001	GUAM	34	16	50	0.68
T-square	75.375	JUAM	21	29	50	0.58
T-square (p-value)	0.029					
Pcr	0.0097		JUAF	JUAM	Total	%AC
Pcr (p-value)	<.0001	JUAF	36	14	50	0.72
T-square	128.81	JUAM	12	38	50	0.76
T-square (p-value)	0.002					

the opposite vectorial displacement to that previously reported (CV2-).

Procrustes distance confirmed that the abstraction of wing shape between sexes is more similar than between hosts (Table 3). The distance between hosts of the species (Table 4, Fig. 6A) were: Jua (Pcr: 0.0097, $p < 0.05$), Guava (Pcr: 0.0093, $p < 0.05$). However, when comparing the sexes pairwise between the hosts (Table 4, Fig. 6B), we find that there is greater similarity of wing shape between females of Jua and Guava (Pcr: 0.0056, $p < 0.05$, cross validation matrix: 64%) and between males of Jua and Guava (Pcr: 0.0066, $p < 0.05$, cross validation matrix: 58% to 68%).

DISCUSSION

The analyses showed that *A. zenildae* flies from different fruit hosts had not only their overall individual size and wing shape affected, but also showed different levels of sexual dimorphism.

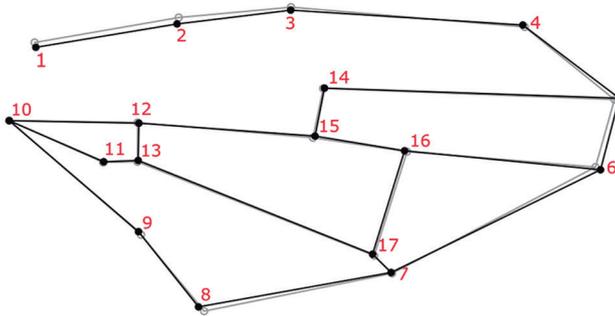
Anastrepha zenildae flies from both sexes that emerged from Guava were larger in all measured traits when compared to flies that emerged from Jua (Table 2). These host induced differences in size are consistent with other tephritid flies studies. Studying the effects of the host fruit on *Ceratitis capitata* (Wiedemann, 1824), Krainacker et al. (1987) demonstrated that the host fruit affected pupal diameter, with flies emerging from Tomatoes having the smallest pupae, and the ones emerging from Lychee having the largest. Navarro-Campos et al. (2011), also studying the effects of host fruit on *C. capitata* under laboratory and field conditions, found that in the lab, flies emerging from Apricot and Peach had a larger wing area than ones emerging from Plum and Oranges. Meanwhile, in fruits collected in the field, an opposite trend was found, with flies that emerged from Oranges having larger wings than the ones that emerged from Peach and Plum. This difference was attributed to the seasonal change during the periods in which the fruits matured, since the same study found that the temperature during larval development affects the adult size on both sexes, with larger flies being found at lower temperatures.

The *A. zenildae* flies also exhibited wing shape differences among the fruit hosts (Figs. 5-6). This effect has also been observed by some degree in other fruit fly species. Another study using *C. capitata* and *Bactrocera dorsalis* (Hendel, 1912) found that the former differed in wing shape when reared in Apples, Nectarines, Plums and Pears, and the latter differed when reared in Apples, Oranges, Nectarines, Plums and Pears (Pieterse et al., 2017). As for the genus *Anastrepha*, Oroño et al. (2013) and Gomez-Cendra et al. (2016), using *Anastrepha* sp.1 *affinis fraterculus* in Argentina, showed that individuals reared on Peach were different in six morphometric traits from the ones reared on Guava and Walnut, and flies from all three fruits were genetically different from each other.

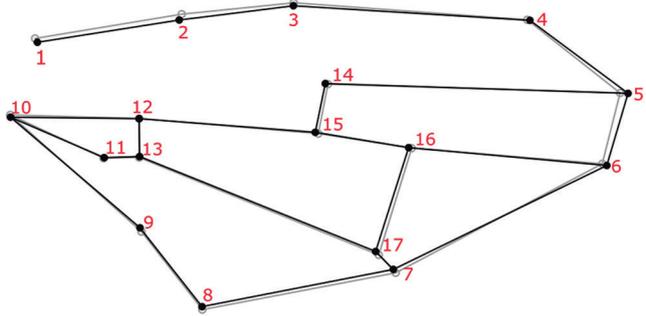
Perre (2016), studying the same species, found that flies reared on several fruit hosts differed in wing shape, size and also symmetry on frontal and post-orbital setae. It is clear that for these phytophagous flies, the larval diet greatly affects adult morphology. Exploring alternate hosts can lead to the appearance of host races, which are in some way morphologically and/or genetically different between populations (Dres & Mallet, 2002). Although these host races are usually just a case of polymorphism within the same species induced by the larval diet on different hosts, this size difference between individuals from different populations can have consequences when mating occurs across them, as it has been demonstrated that larger males have higher copulatory success in *C. capitata* (Anjos-Duarte et al., 2011; Churchill-Stanland et al., 1986; Neto et al., 2012; Orozco & Lopez, 1993; Ro-

(A)

GUAF — GUAM

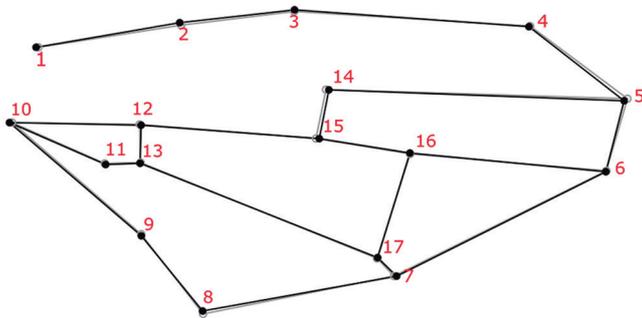


JUAF — JUAM

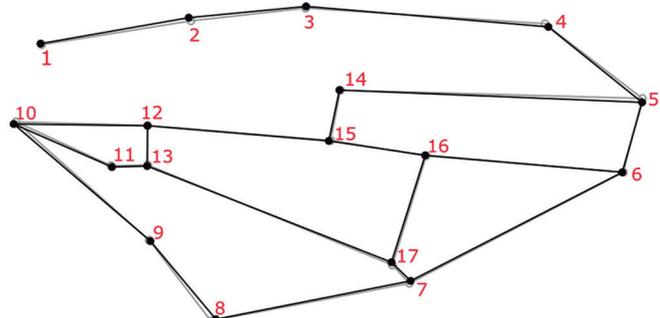


(B)

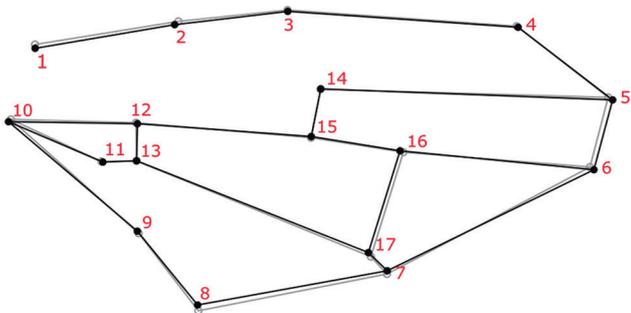
GUAF — JUAF



GUAM — JUAM



GUAF — JUAM



GUAM — JUAF

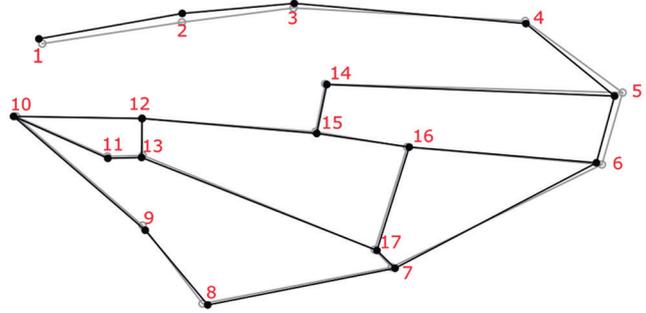


Figure 6. Wireframe Comparison of Discriminant Function Analysis of the wings of *Anastrepha zenilidae* (Males and females) from Guava and Jua fruits. (A) Wing shape variation between female and male flies of the same host, in a semi-arid region. (B) between female and male flies from different hosts. GUAF = Females from Guava; GUAM = Males from Guava; JUAF = Females from Jua; JUAM = Males from Jua. Larger symbols: Centroids of each dataset.

driguero et al., 2002; Taylor & Yuval, 1999) and several *Anastrepha* species (Almeida et al., 2013; Burk & Webb, 1983; Sicuriano et al., 2007).

The *A. zenilidae* flies from our study also showed wing shape sexual dimorphism. Males had shorter and broader wings and the females had longer and narrower ones. However, host fruit had no influence on that aspect, as flies from both hosts exhibited the same differences between sexes. These shape patterns for males and females have also been observed to some extent in *C. capitata* (Churchill-Stanland et al., 1986; Lemic et al., 2020; Reis et al., 2021) and in other generalist *Anastrepha* species (Chang, 2020; Sivinski & Dodson, 1992).

Those authors attribute these differences to the fact that longer and narrower wings on females are advantageous for long distance flights in search for fruit hosts, and shorter and broader wings in males would be more effective during courtship in 'Lek' presenting species, as they are better at displacing more air and produce a louder sound.

The flies from our work also showed sexual dimorphism in size for Guava, but not for Jua, with females being significantly larger than males in the former host, but not in the latter. Sexual dimorphism in body size has already been reported for several tephritid fly species, with female flies being larger than males (Dodson, 1985;

Lemic et al., 2020; Shelly, 2018; Zhou et al., 2020). This size difference between sexes can be attributed to several factors. One of them is the possible faster growth rate in females, whose adults tend to emerge in less time than males as was shown in *Anastrepha suspensa* (Loew, 1862) (Sivinski & Calkins, 1990). A tendency towards larger females can also be explained by increased offspring size (Rollinson & Rowe, 2015) and increased fecundity, as was demonstrated that larger *A. suspensa* females laid more eggs than smaller ones (Sivinski & Dodson, 1992). These parameters however, have not been studied in *A. zenildae*, and still need verification. As to the difference in the degree of sexual dimorphism between fruits, it could be a case of an inversion of Rensch's Rule, which dictates that in closely related taxa, sexual size dimorphism increases with body size in taxa where males are larger, and decreases with size in taxa where females are larger (Abouheif & Fairbairn, 1997; Rensch, 1950). As this study demonstrated, *A. zenildae* exhibit female biased sexual dimorphism. Therefore, by an inversion of the rule, the flies with smaller body size that emerged from Jua, show decreased sexual size dimorphism, and, the larger flies that emerged from Guava, have it more accentuated.

Overall, our study with *A. zenildae* reinforces the evidence that, for fruit flies, the exploration of different hosts by sympatric populations can lead to morphological changes induced by the larval diet. As was shown in several other tephritid fly species, these morphological differences can cause reproductive isolation through assortative mating (Dres & Mallet, 2002) and host specific mating (Feder & Forbes, 2010), which is the case for *A. zenildae* (Almeida et al., 2013). Mating compatibility among populations is also an important factor to be studied when integrating pest management programs such as the Sterile Insect Technique (SIT) (Cladera et al., 2014). In addition, different fruit compounds can cause changes in pheromone composition and emission (Bachmann et al., 2015; Kumaran et al., 2014; Robledo & Azuffi, 2012) and differences in genetic structure (Mopper, 1996) that over time may lead to speciation, which is what is suggested to have happened to the *A. fraterculus* species complex (Cáceres et al., 2009; Selivon et al., 2004, Vera et al., 2006). Further studies are needed to verify if the aforementioned phenomena apply to *A. zenildae*.

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