

RESEARCH ARTICLE

# Morphometric Wing Characters as a Tool for Mosquito Identification

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## Abstract

Mosquitoes are responsible for the transmission of important infectious diseases, causing millions of deaths every year and endangering approximately 3 billion people around the world. As such, precise identification of mosquito species is crucial for an understanding of epidemiological patterns of disease transmission. Currently, the most common method of mosquito identification relies on morphological taxonomic keys, which do not always distinguish cryptic species. However, wing geometric morphometrics is a promising tool for the identification of vector mosquitoes, sibling and cryptic species included. This study therefore sought to accurately identify mosquito species from the three most epidemiologically important mosquito genera using wing morphometrics. Twelve mosquito species from three epidemiologically important genera (*Aedes*, *Anopheles* and *Culex*) were collected and identified by taxonomic keys. Next, the right wing of each adult female mosquito was removed and photographed, and the coordinates of eighteen digitized landmarks at the intersections of wing veins were collected. The allometric influence was assessed, and canonical variate analysis and thin-plate splines were used for species identification. Cross-validated reclassification tests were performed for each individual, and a Neighbor Joining tree was constructed to illustrate species segregation patterns. The analyses were carried out and the graphs plotted with TpsUtil 1.29, TpsRelw 1.39, MorphoJ 1.02 and Past 2.17c. Canonical variate analysis for *Aedes*, *Anopheles* and *Culex* genera showed three clear clusters in morphospace, correctly distinguishing the three mosquito genera, and pairwise cross-validated reclassification resulted in at least 99% accuracy; subgenera were also identified correctly with a mean accuracy of 96%, and in 88 of the 132 possible comparisons, species were identified with 100% accuracy after the data was subjected to reclassification. Our results showed that *Aedes*, *Culex* and *Anopheles* were correctly distinguished by wing shape. For the lower hierarchical levels (subgenera and species), wing geometric morphometrics was also efficient, resulting in high reclassification scores.

## Introduction

### Mosquito importance

Insects that are obligate blood feeders, such as the Culicidae family, can have a major impact on human and animal health, primarily because they transmit infectious agents, and their bites constitute a significant inconvenience. The more urbanized an area is, the lower the mosquito richness and the higher the abundance of a few, epidemiologically important mosquito species that are well adapted to urban environments [1–3]. Mosquitoes of the *Aedes*, *Culex* and *Anopheles* genera are responsible for the transmission of highly important infectious diseases such as dengue, filariasis, West Nile virus disease and malaria. Together, these diseases cause millions of deaths every year and endanger approximately 3 billion people around the world living in endemic areas. Expansion of the geographic distribution of mosquitoes is followed by the (re-) emergence of diseases, making it vital to identify epidemiologically important mosquito species correctly [4–18].

Precise species identification of mosquitoes is crucial for an understanding of epidemiological patterns of disease transmission, which are associated with the vector mosquito's abundance, infectivity and vector capacity and competence. In addition, morphologically indistinguishable species complexes such as the *An. gambiae* and *Cx. pipiens* complexes may have distinct epidemiological roles, making correct identification of the species in these complexes crucial to establish an effective vector mosquito control initiative [19–23].

The most common method of mosquito identification relies on the use of morphological taxonomic keys [14,24–26], a laborious process that requires intensive training and, most importantly, that the mosquito be undamaged. If for any reason the mosquito specimen of interest is damaged, morphological identification may not be possible. Moreover, some species can only be identified by quantitative differences in the male genitalia, making it impossible to identify adult females, or may even be morphologically indistinguishable (e.g. *An. gambiae* and *Cx. pipiens* complex) [19,27]. The vector species *An. cruzii*, *An. homunculus* and *An. bellator*, considered difficult to identify using only taxonomic keys, have been successfully identified based only on adult female wing morphometry [28]. Jaramillo-O et al. [29] were able to distinguish eleven *Anopheles* mosquito species from the *Nyssorhynchus* subgenus using wing geometric morphometrics.

Mosquito wing geometric morphometrics is an established mosquito identification technique and is inexpensive and reliable [30–33]. It can be used to identify vector mosquitoes of epidemiological importance, sibling species, cryptic species and females in some species whose identification using other techniques has proved problematic [20,23,28]. The objective of this study was to accurately identify mosquito species from the three main epidemiologically important mosquito genera using wing morphometrics.

## Material and Methods

### Mosquito sampling and identification

Twelve species of mosquitoes from the three most epidemiologically important genera (*Ae. aegypti*, *Ae. albopictus*, *Ae. fluviatilis*, *Ae. scapularis*, *An. cruzii*, *An. darlingi*, *An. strodei*, *Cx. chidesteri*, *Cx. dolosus*, *Cx. eduardoi*, *Cx. nigripalpus* and *Cx. quinquefasciatus*) were collected from different sampling locations (Table 1) as previously described by Medeiros-Sousa et al. [2]. Adults were collected using CO<sub>2</sub>-baited CDC light traps, and immature forms were collected in natural and artificial breeding sites with larval dippers or suction tubes. The mosquitoes were identified with the aid of taxonomic keys [24] and stored by species in 1.5 mL Eppendorf tubes with silica gel at room temperature until the wings were removed. The study

**Table 1. Mosquito species, collection sites and geographic coordinates.**

Taxon	N*	Collection site	Coordinate	Collection year
<i>Aedes (Stegomyia) aegypti</i> (Linnaeus, 1762)	28	São Paulo	S-23°24'54", W-46°47'6"	2013
<i>Aedes (Stegomyia) albopictus</i> (Skuse, 1895)	23	São Paulo	S-23°31'40" W-46°34'14"	2013
<i>Aedes (Ochlerotatus) fluviatilis</i> (Lutz, 1904)	30	São Paulo	S-23°34'49" W-46°43'33"	2013
<i>Aedes (Ochlerotatus) scapularis</i> (Rondani, 1848)	23	São Paulo	S-23°31'40" W-46°34'14"	2013
<i>Anopheles (Kerteszia) cruzii</i>	22	São Paulo	S-23°82'69" W-46°72'70"	2015
<i>Anopheles (Nyssorhynchus) darlingi</i>	30	Manaus	S-3°11'89" W-60°02'15"	2015
<i>Anopheles (Nyssorhynchus) strodei</i> Root, 1926	28	São Paulo	S-23°24'54", W-46°47'6"	2013
<i>Culex (Culex) chidesteri</i> Dyar, 1921	28	São Paulo	S-23°31'40" W-46°34'14"	2012
<i>Culex (Culex) dolosus</i> (Lynch Arribáizaga, 1891)	29	São Paulo	S-23°34'40" W-46°43'37"	2012
<i>Culex (Culex) eduardoi</i> Casal & García, 1968	14	São Paulo	S-23°45'29" W-46°46'23"	2013
<i>Culex (Culex) nigripalpus</i> Theobald, 1901	29	São Paulo	S-23°49'25" W-46°76'17"	2013
<i>Culex (Culex) quinquefasciatus</i> Say, 1823	28	São Paulo	S- 23°37'55" W-46°43'17"	2013

\*Number of specimens used

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was approved by the Ethical Committee of the University of São Paulo (FSP/USP—Project 000304), and collection permits were provided by the Department of the Environment and Green Areas (Permit 345/2010).

### Material preparation and data acquisition

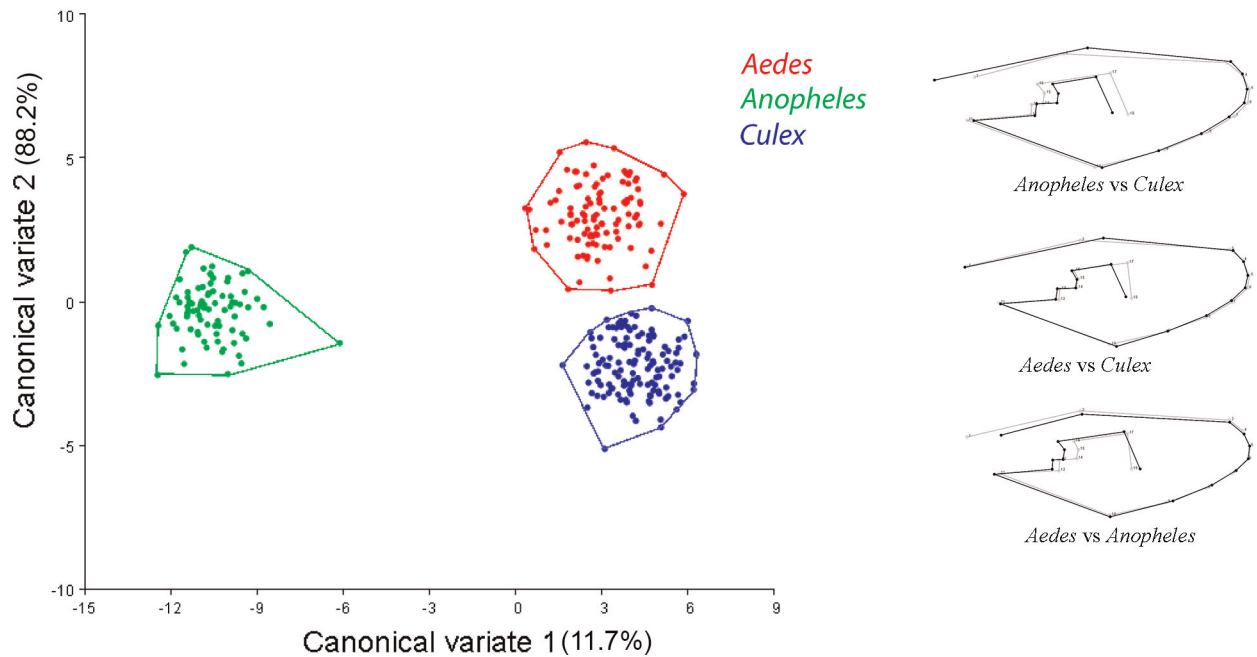
The right wing of each adult female mosquito was removed and mounted on a microscope slide with a cover slip. The wings were then photographed under 40x magnification with a Leica DFC320 digital camera coupled to a Leica S6 microscope. On each wing image, 18 landmarks were digitized by one of the authors (ROC) using TpsDig V1.40 software [S1 Fig] [28,30,34,35].

### Geometric morphometric analysis

The allometric influence of wing size on wing shape was assessed by multivariate regression of the Procrustes coordinates against centroid size using a permutation test with 10000 randomizations. Discriminant analysis was performed to explore the degree of wing shape dissimilarity among species in a morphospace produced by canonical variate analysis (CVA) and to calculate the Mahalanobis distance. Thin-plate splines were obtained by regression analysis of CVA scores against wing shape variation to visualize the shape disparity among the species compared. Each individual was then reclassified using the cross-validated reclassification test based on the Mahalanobis distance [28,36,37]. A Neighbor Joining tree was constructed with 1000 bootstrap replicates based on the Mahalanobis distance to illustrate species segregation patterns (20 specimens of *Wyeomyia oblita*, were used as an outgroup). The analyses were carried out and graphs plotted with TpsUtil 1.29 [38], TpsRelw 1.39 [38], MorphoJ 1.02 [39] and Past 2.17c [40].

### Results

Although small, the allometry effect was significant (5.91%,  $p < 0.0001$ ). However, it was not removed from the analysis because we consider allometric size variation part of the species identification process [37]. CVA of wing shape in *Aedes*, *Anopheles* and *Culex* genera showed three distinct clusters in morphospace and correctly distinguished between the three mosquito



**Fig 1. Morphological space produced by CVA of the three mosquito genera.** Shape variations in a wireframe graph are shown to the right of the morphological space.

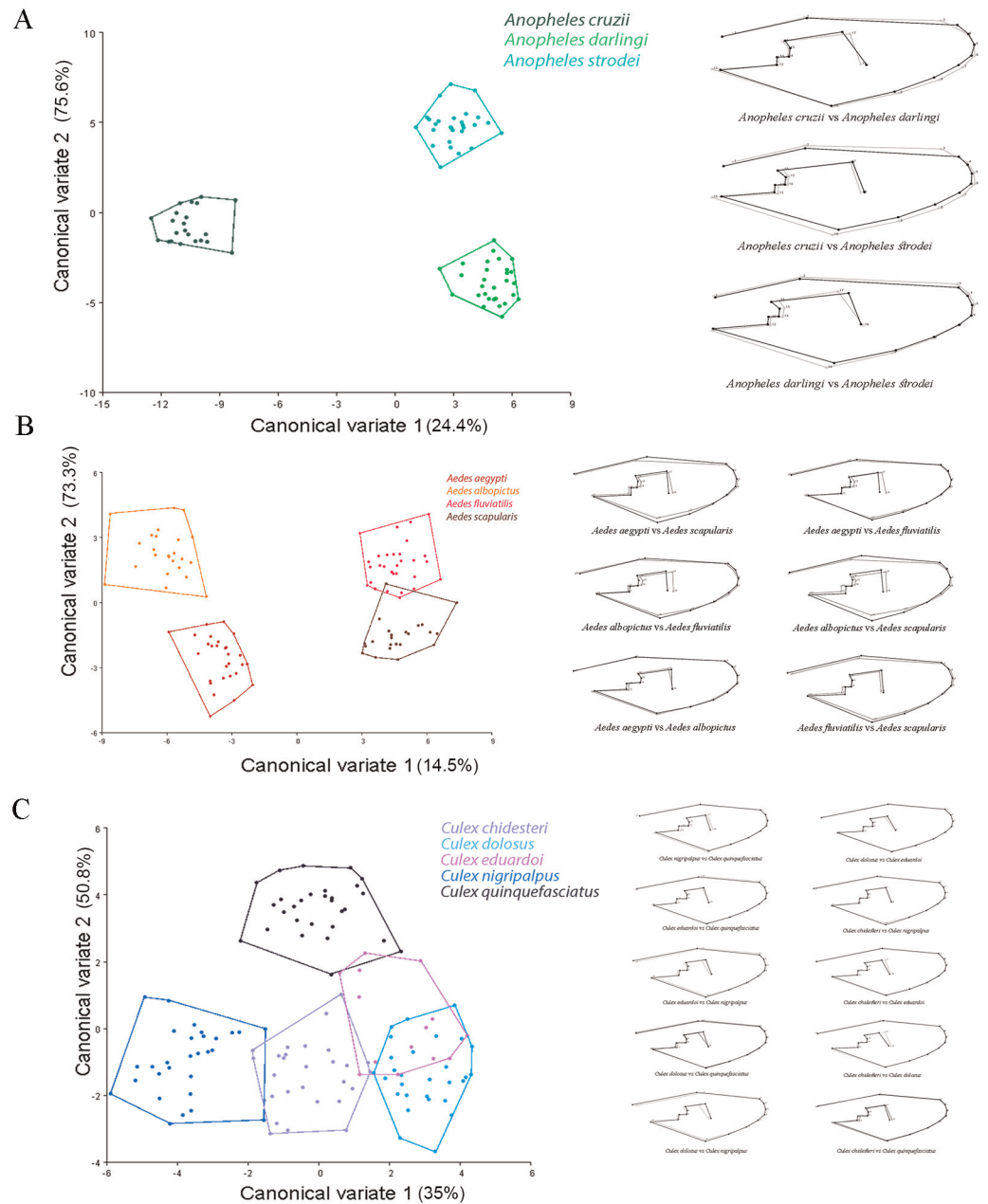
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genera. Subsequent pairwise comparison of thin-plate splines between genera showed that quantitative landmarks are associated with differentiation among mosquitoes from each genus (Fig 1, S2 Fig). A pairwise cross-validated reclassification test comparing the three genera (*Aedes*, *Anopheles* and *Culex*) was also performed and resulted in scores with an accuracy of at least 99%.

The results obtained within each genus show that subgenera were identified when present. The *Anopheles* genus was correctly segregated into two main clusters, the *Kerteszia* and *Nyssorhynchus* subgenera (Fig 2A), and the *Aedes* genus was also divided into two clusters, the *Stegomyia* and *Ochlerotatus* subgenera (Fig 2B).

CVA was carried out among the species of each genus followed by a thin-plate spline pairwise comparison and yielded the following results: in the genus *Anopheles*, wing shape CVA successfully separated the species (*An. cruzii*, *An. darlingi* and *An. strodei*), and the subgenera *Kerteszia* and *Nyssorhynchus* segregated perfectly along the canonical variate 1 (CV1) axis (Fig 2A). In the genus *Aedes*, *Ae. aegypti*, *Ae. albopictus*, *Ae. fluviatilis* and *Ae. scapularis* were successfully separated, and the subgenera *Stegomyia* and *Ochlerotatus* (Fig 2B) segregated with a small degree of overlapping along the CV1 axis. In the genus *Culex*, wing-shape CVA revealed structuring within the genus; *Cx. nigripalpus* was the most segregated species, and *Cx. dolosus* and *Cx. eduardoi* overlapped (Fig 2C). The Neighbor Joining tree shows the segregation of the three mosquito genera, subgenera and respective species with high bootstrap values in most branches (Fig 3).

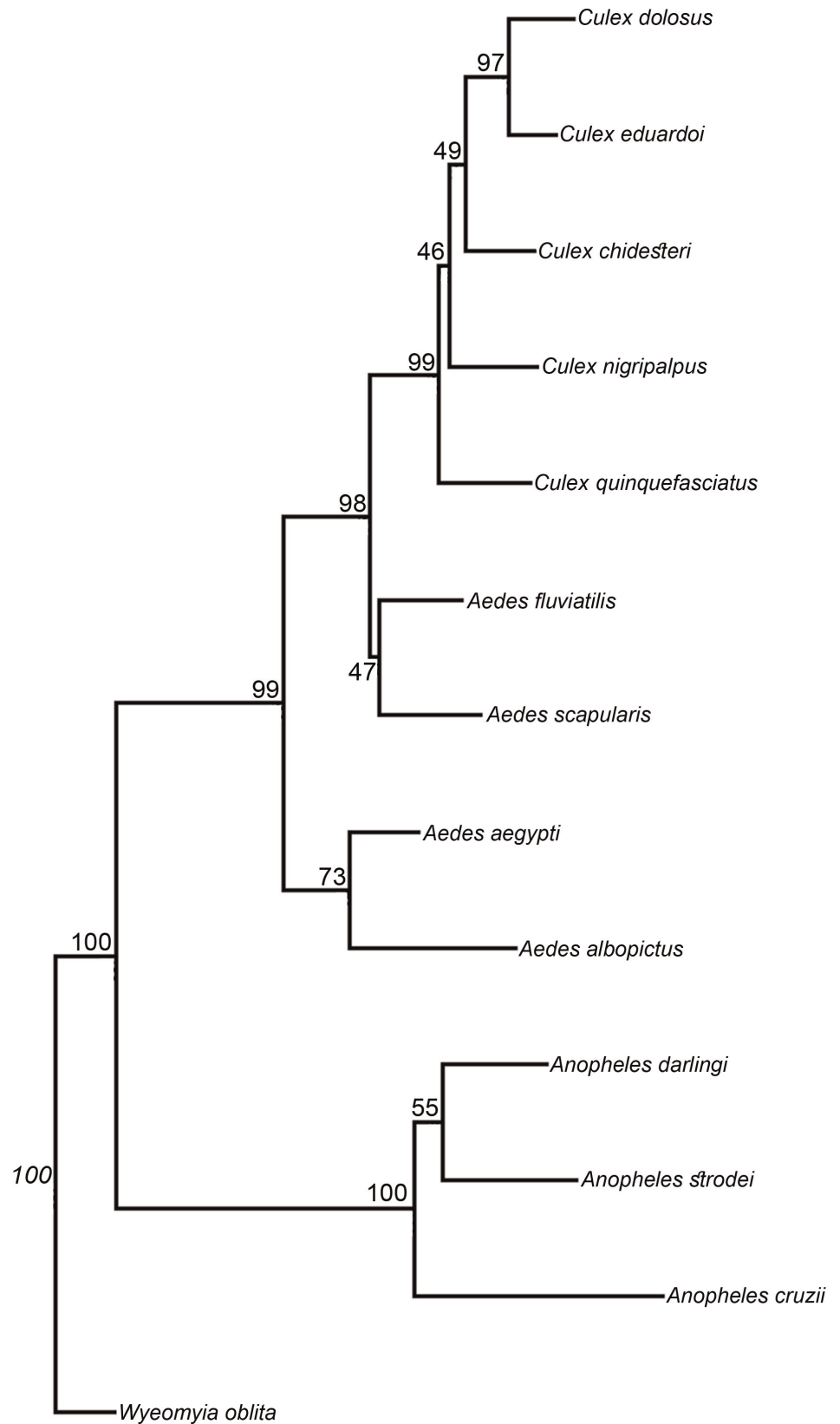
The genera were correctly identified by morphometric analysis with at least 99% reliability after a reclassification test, indicating a high reliability rate. The reclassification test also had a high identification power when each genera was analyzed separately and was able to distinguish subgenera correctly when these were present.



**Fig 2. Morphological space produced by CVA of species and shape variations in a wireframe graph. A. *Anopheles* genus. B. *Aedes* genus. C. *Culex* genus.** Shape variations in a wireframe graph are shown to the right of each morphological space.

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Mean species-identification reliability after the data were subjected to a reclassification test was 96%, and in 88 out of 132 comparisons accuracy was 100%. Comparisons of the results of pairwise cross-validated species reclassification tests between species (*Ae. aegypti*, *Ae. albopictus*, *Ae. fluviatilis*, *Ae. scapularis*, *An. cruzii*, *An. darlingi*, *An. strodei*, *Cx. chidesteri*, *Cx. dolosus*, *Cx. eduardoi*, *Cx. nigripalpus* and *Cx. quinquefasciatus*) yielded high identification rates in the majority of the comparisons. *Ae. aegypti*, *An. darlingi*, *An. strodei* and *Cx. quinquefasciatus* had a reclassification score of 100% when they were compared with mosquito species from different



**Fig 3. Neighbor-Joining tree based on Mahalanobis distance with 1000 bootstrap replicates.**

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**Table 2. Results of pairwise cross-validated species reclassification tests.**

		Group 2											
		<i>Ae. aegypti</i>	<i>Ae. albopictus</i>	<i>Ae. fluviatilis</i>	<i>Ae. scapularis</i>	<i>An. cruzii</i>	<i>An. darlingi</i>	<i>An. strodei</i>	<i>Cx. chidesteri</i>	<i>Cx. dolosus</i>	<i>Cx. eduardoi</i>	<i>Cx. nigripalpus</i>	<i>Cx. quinquefasciatus</i>
Group 1	<i>Ae. aegypti</i>	x	85%	100%	96%	100%	100%	100%	100%	100%	96%	100%	100%
	<i>Ae. albopictus</i>	83%	x	100%	95%	100%	100%	100%	100%	100%	95%	100%	100%
	<i>Ae. fluviatilis</i>	100%	100%	x	86%	100%	100%	100%	93%	96%	93%	93%	100%
	<i>Ae. scapularis</i>	95%	100%	91%	x	100%	100%	100%	95%	100%	82%	95%	100%
	<i>An. cruzii</i>	100%	100%	100%	100%	x	100%	100%	100%	100%	100%	100%	100%
	<i>An. darlingi</i>	100%	100%	100%	100%	100%	x	100%	100%	100%	96%	100%	100%
	<i>An. strodei</i>	100%	100%	100%	100%	100%	100%	x	100%	100%	96%	100%	100%
	<i>Cx. chidesteri</i>	100%	100%	96%	100%	100%	100%	100%	x	92%	85%	85%	96%
	<i>Cx. dolosus</i>	100%	100%	100%	100%	100%	100%	100%	96%	x	68%	100%	96%
	<i>Cx. eduardoi</i>	100%	85%	78%	64%	78%	100%	100%	85%	57%	x	85%	71%
	<i>Cx. nigripalpus</i>	100%	100%	96%	96%	100%	100%	100%	82%	93%	82%	x	100%
<i>Cx. quinquefasciatus</i>	100%	100%	96%	93%	100%	100%	100%	89%	93%	82%	100%	x	

Values below the diagonal correspond to mosquitoes from group 1 compared with group 2 and correctly identified; values above the diagonal correspond to mosquitoes from group 2 compared with group 1 and correctly identified. P-value (parametric): <0.0001.

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genera. When *Cx. eduardoi* was compared with *Cx. dolosus*, its reclassification score was only 57%, the lowest score in all the comparisons of species carried out, although its mean reclassification score was 80%. The overall mean reclassification score for both groups 1 and 2 in the pairwise cross-validated reclassification tests was 96%, indicating a high species-identification reliability (Table 2).

The minimum number of landmarks required to distinguish genera with more than 90% accuracy in the cross-validated reclassification test was five (1, 2, 14, 15, 16). Using seven landmarks (1, 2, 14, 15, 16, 17, 18), 96% accuracy was achieved, and with nine (1, 2, 12, 13, 14, 15, 16, 17, 18) the lowest score was 97% (S3 Fig, S1 Table).

## Discussion

Although morphological analysis of characters is the gold standard for mosquito identification, geometric morphometrics based on quantitative analysis of mosquito wing venation characters has proved to be a reliable tool in mosquito identification as well, showing a high identification power when distinguishing morphologically similar species [28,41].

Our results indicate that the three most epidemiologically important mosquito genera, *Aedes*, *Culex* and *Anopheles*, were correctly distinguished by wing shape. When lower hierarchical levels (subgenera and species) were analyzed, wing geometric morphometrics was also efficient, yielding high reclassification scores for most of the mosquito species analyzed. In light of this, the technique can be considered a valid alternative for identification of the twelve mosquito species studied here when identification by taxonomic methods is not possible.

The morphospace pattern formed by the four *Aedes* species allows identification of the subgenus *Stegomyia* and the species within this subgenus in this study, i.e., *Ae. albopictus* and *Ae. aegypti*, the two most important vectors of dengue globally, although in Latin America the role of *Ae. albopictus* in dengue transmission is still unknown. Subgenus *Ochlerotatus*, represented by two species native to Brazil, was also successfully identified. Hence, identification of species of secondary epidemiological importance, about which less information is available, is also possible with the geometric morphometrics used here [42].

The *Anopheles* species used in this study are epidemiologically very important because they are responsible for malaria transmission in Latin America. *An. darlingi* is the primary vector of this disease in the Amazon region, and *An. cruzii* and *An. strodei* are responsible for malaria transmission in the Atlantic Forest region, accounting for hundreds of thousands of malaria cases every year [15,17].

Our results showed that wing geometric morphometrics is a reliable technique for identification of the *Anopheles* species used in this study at the genus, subgenus and species level. The three species tested formed a clear pattern in morphospace and exhibited significant morphometric singularities. Wing morphometrics was previously used by Lorenz et al. [28], who were able to identify the species *An. cruzii*, *An. homunculus* and *An. bellator* using wing morphometry. These species can usually only be identified by examination of male genitalia, making identification of females problematic.

Species from the *Culex* genus may be undergoing microevolution and speciation [43], leading to species complexes and even hybrid mosquitoes as a result of the mating of sibling species [41,44]. The species complex formed by *Cx. quinquefasciatus* and *Cx. pipiens*, which are adapted to tropical and temperate climates, respectively, is found in an overlapping tropical/temperate climate zone in Brazil, where the two species can mate, producing hybrid mosquitoes.

This phenomenon has been observed using two different approaches: wing morphometrics and microsatellite markers [41,44]. A similar finding was reported for *Cx. pipiens* and *Cx. torrentium*, which are only distinguishable by the characteristics of their male genitalia; although not even molecular tools such as COI barcodes are sufficiently sensitive to identify specific *Culex* species, wing morphometrics has been successfully used to identify species in this genus [20,45].

In the present study, we were able to correctly identify epidemiologically important *Culex* species, including *Cx. quinquefasciatus*, one of the main vectors of West Nile virus in the Americas [46], *Cx. nigripalpus*, a neglected mosquito native to South America that is very abundant in the urban environment [43,47], and *Cx. chidesteri*, which is also native to South America but less adapted to urban environments. As expected, *Cx. dolosus* and *Cx. eduardoi* overlapped in the CVA analysis and had the lowest reclassification scores. However, these species can only be identified by morphological differences in larval stages, and adults are indistinguishable.

The Neighbor Joining tree produced here corroborated the results of the wing shape CVA and reclassification test, showing that wing morphometrics clearly identified genus, subgenus and species and yielded results comparable to those obtained with classic morphological analysis [43,48–50].

The use of wing morphometrics for mosquito identification has a number of advantages over classical species identification based on morphological traits and genetic characterization. One such advantage is that wing characters respond rapidly to microevolutionary events. Thus, small but significant variations in sibling species or even hybrid mosquitoes can be identified, allowing them to be correctly identified [20,21,32,41,51,52].

Furthermore, variation in wing shape among species has proven to be a reliable marker that is consistent regardless of the geographic origin of the mosquitoes and does not produce significant interspecific overlapping in morphospace [32].

The use of wing morphometric characters can be an important tool in the identification of mosquito species that are not easily identified by morphological characters or specimens that are damaged during collection, making morphological identification unfeasible. There are freely accessible databases, such as the CLIC morphometrics database ([mpl.ird.fr/morphometrics/clic/index.html](http://mpl.ird.fr/morphometrics/clic/index.html)) and WINGBANK ([winngbank.com.br](http://winngbank.com.br)), that researchers can use to



obtain photos of wings of different mosquito species for use as reference and for further analysis.

As long as one wing is preserved, specimens can be identified by morphometric techniques. These techniques are fast and relatively low-cost, especially compared with genetic characterization, and can be performed in the field without advanced equipment.

## Conclusion

We were able to successfully identify all the species analyzed in this study, including even cryptic species that cannot be identified by morphological characters or species whose males can be identified by their genitalia but whose females are indistinguishable. These findings suggest that wing geometric morphometrics is a reliable tool for mosquito identification.

## Supporting Information

**S1 Fig. Wireframe showing the 18 landmarks on wings of female *Aedes*, *Anopheles* and *Culex* mosquitoes selected for geometric morphometrics analysis.**

(TIF)

**S2 Fig. Morphological space produced by CVA of *Ae. aegypti*, *Ae. albopictus*, *Ae. fluviatilis*, *Ae. scapularis*, *An. cruzii*, *An. darlingi*, *An. strodei*, *Cx. chidesterei*, *Cx. dolosus*, *Cx. eduardoi*, *Cx. nigripalpus* and *Cx. quinquefasciatus*.**

(TIF)

**S3 Fig. Morphological space produced by CVA of the three mosquito genera using A. Five landmarks (1, 2, 14, 15, 16); B. Seven landmarks (1, 2, 14, 15, 16, 17, 18); and C. Nine landmarks (1, 2, 12, 13, 14, 15, 16, 17, 18).**

(TIF)

**S1 Table. Comparisons of the three genera (*Aedes*, *Anopheles* and *Culex*) using the pairwise cross-validated reclassification test and five, seven and nine landmarks.**

(DOCX)

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## Author Contributions

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**Methodology:** ABBW ROC LCM POV.

**Project administration:** ABBW MTM.

**Resources:** MTM.

**Supervision:** ABBW MTM.

**Validation:** ABBW POV.

**Writing – original draft:** ABBW LCM POV.

**Writing – review & editing:** ABBW LCM POV MTM.

## References

1. Ceretti-Júnior W, Medeiros-Sousa AR, Wilke ABB, Strobel RC, Dias Orico L, Souza Teixeira R, et al. Mosquito Faunal Survey in a Central Park of the City of São Paulo, Brazil. *J Am Mosq Control Assoc.* 2015; 31: 172–176. doi: [10.2987/14-6457R](https://doi.org/10.2987/14-6457R) PMID: [26181694](https://pubmed.ncbi.nlm.nih.gov/26181694/)
2. Medeiros-Sousa AR, Ceretti W, Urbinatti PR, de Carvalho GC, de Paula MB, Fernandes A, et al. Mosquito fauna in municipal parks of São Paulo City, Brazil: a preliminary survey. *J Am Mosq Control Assoc.* 2013; 29: 275–9. doi: [10.2987/12-6304R.1](https://doi.org/10.2987/12-6304R.1) PMID: [24199502](https://pubmed.ncbi.nlm.nih.gov/24199502/)
3. Chaves LF, Koenraadt CJM. Climate change and highland malaria: fresh air for a hot debate. *Q Rev Biol.* 2010; 85: 27–55. doi: [10.1086/650284](https://doi.org/10.1086/650284) PMID: [20337259](https://pubmed.ncbi.nlm.nih.gov/20337259/)
4. Harbach RE. Mosquito Taxonomic Inventory. 2013. Available: <http://mosquito-taxonomic-inventory.info>
5. Cartaxo MFS, Ayres CFJ, Weetman D. Loss of genetic diversity in *Culex quinquefasciatus* targeted by a lymphatic filariasis vector control program in Recife, Brazil. *Trans R Soc Trop Med Hyg.* 2011; 105: 491–499. doi: [10.1016/j.trstmh.2011.05.004](https://doi.org/10.1016/j.trstmh.2011.05.004) PMID: [21737112](https://pubmed.ncbi.nlm.nih.gov/21737112/)
6. Dibo MR, Menezes RMT de, Ghirardelli CP, Mendonça AL, Chiaravalloti Neto F. The presence of Culicidae species in medium-sized cities in the State of São Paulo, Brazil and the risk of West Nile fever and other arbovirus infection. *Rev Soc Bras Med Trop.* 2011; 44: 496–503. doi: [10.1590/S0037-86822011000400019](https://doi.org/10.1590/S0037-86822011000400019) PMID: [21860898](https://pubmed.ncbi.nlm.nih.gov/21860898/)
7. Honório NA, Nogueira RMR, Codeço CT, Carvalho MS, Cruz OG, de Avelar Figueiredo Mafrá Magalhães M, et al. Spatial Evaluation and Modeling of Dengue Seroprevalence and Vector Density in Rio de Janeiro, Brazil. *PLoS Negl Trop Dis.* 2009; 3: e545. doi: [10.1371/journal.pntd.0000545](https://doi.org/10.1371/journal.pntd.0000545) PMID: [19901983](https://pubmed.ncbi.nlm.nih.gov/19901983/)
8. Restrepo AC, Baker P, Clements ACA. National spatial and temporal patterns of notified dengue cases, Colombia 2007–2010. *Trop Med Int Health.* 2014; 19: 863–871. doi: [10.1111/tmi.12325](https://doi.org/10.1111/tmi.12325) PMID: [24862214](https://pubmed.ncbi.nlm.nih.gov/24862214/)
9. Tipayamongkholgul M, Fang CT, Klinchan S, Liu CM, King CC. Effects of the El Niño-southern oscillation on dengue epidemics in Thailand, 1996–2005. *BMC Public Health.* 2009; 9: 422. doi: [10.1186/1471-2458-9-422](https://doi.org/10.1186/1471-2458-9-422) PMID: [19930557](https://pubmed.ncbi.nlm.nih.gov/19930557/)
10. Chaves LF, Morrison AC, Kitron UD, Scott TW. Nonlinear impacts of climatic variability on the density-dependent regulation of an insect vector of disease. *Glob Chang Biol.* 2012; 18: 457–468. doi: [10.1111/j.1365-2486.2011.02522.x](https://doi.org/10.1111/j.1365-2486.2011.02522.x)
11. Sang S, Gu S, Bi P, Yang W, Yang Z, Xu L, et al. Predicting unprecedented dengue outbreak using imported cases and climatic factors in Guangzhou, 2014. *PLoS Negl Trop Dis.* 2015; 9: e0003808. doi: [10.1371/journal.pntd.0003808](https://doi.org/10.1371/journal.pntd.0003808) PMID: [26020627](https://pubmed.ncbi.nlm.nih.gov/26020627/)
12. Descloux E, Mangeas M, Menkes CE, Lengaigne M, Leroy A, Tehei T, et al. Climate-based models for understanding and forecasting dengue epidemics. *PLoS Negl Trop Dis.* 2012; 6: e1470. doi: [10.1371/journal.pntd.0001470](https://doi.org/10.1371/journal.pntd.0001470) PMID: [22348154](https://pubmed.ncbi.nlm.nih.gov/22348154/)
13. Do TT, Martens P, Luu N, Wright P, Choisy M. Climatic-driven seasonality of emerging dengue fever in Hanoi, Vietnam. *BMC Public Health.* 2014; 14: 1078. doi: [10.1186/1471-2458-14-1078](https://doi.org/10.1186/1471-2458-14-1078) PMID: [25323458](https://pubmed.ncbi.nlm.nih.gov/25323458/)
14. Consoli RAGB, Lourenço-de-Oliveira R. Principais mosquitos de importância sanitária no Brasil. *Cadernos de Saúde Pública.* 1st ed. Rio de Janeiro: FIOCRUZ; 1994.
15. World Health Organization. World Malaria Report. 2014. Available: <http://www.who.int/mediacentre/factsheets/fs094/en/>
16. World Health Organization. Dengue and severe dengue. WHO Fact Sheet. 2012. Available: [www.who.int/mediacentre/factsheets/fs117/en/index.html](http://www.who.int/mediacentre/factsheets/fs117/en/index.html)
17. World Health Organization. World Malaria Report 2015. *Nature.* 2015. Available: [www.who.int](http://www.who.int)
18. World Health Organization. Global programme to eliminate lymphatic filariasis (GPELF): Progress Report 2000–2009 and Strategic Plan 2010–2020; 2010

19. Farajollahi A, Fonseca DM, Kramer LD, Marm Kilpatrick A. "Bird biting" mosquitoes and human disease: A review of the role of *Culex pipiens* complex mosquitoes in epidemiology. *Infect Genet Evol.* 2011; 11: 1577–1585. doi: [10.1016/j.meegid.2011.08.013](https://doi.org/10.1016/j.meegid.2011.08.013) PMID: [21875691](https://pubmed.ncbi.nlm.nih.gov/21875691/)
20. Börstler J, Lühken R, Rudolf M, Steinke S, Melaun C, Becker S, et al. The use of morphometric wing characters to discriminate female *Culex pipiens* and *Culex torrentium*. *J Vector Ecol.* 2014; 39: 204–212. doi: [10.1111/j.1948-7134.2014.12088.x](https://doi.org/10.1111/j.1948-7134.2014.12088.x) PMID: [24820574](https://pubmed.ncbi.nlm.nih.gov/24820574/)
21. Calle L DA, Quiñones ML, Erazo HF, Jaramillo O N. Morphometric discrimination of females of five species of *Anopheles* of the subgenus *Nyssorhynchus* from Southern and Northwest Colombia. *Mem Inst Oswaldo Cruz.* 2002; 97: 1191–1195. doi: [10.1590/S0074-02762002000800021](https://doi.org/10.1590/S0074-02762002000800021) PMID: [12563488](https://pubmed.ncbi.nlm.nih.gov/12563488/)
22. Laurito M, Almirón WR, Ludueña-Almeida FF. Discrimination of four *Culex* (*Culex*) species from the Neotropics based on geometric morphometrics. *Zoomorphology.* Springer Berlin Heidelberg; 2015; 1611: 447–455. doi: [10.1007/s00435-015-0271-x](https://doi.org/10.1007/s00435-015-0271-x)
23. Vidal PO, Peruzin MC, Suesdek L. Wing diagnostic characters for *Culex quinquefasciatus* and *Culex nigripalpus* (Diptera, Culicidae). *Rev Bras Entomol.* 2011; 55: 134–137. doi: [10.1590/S0085-56262011000100022](https://doi.org/10.1590/S0085-56262011000100022)
24. Forattini OP. *Culicidologia Médica: Identificação, Biologia e Epidemiologia.* 2nd ed. São Paulo: EdUSP; 2002.
25. Harbach RE, Kitching IJ. Phylogeny and classification of the Culicidae (Diptera). *Syst Entomol.* 1998; 23: 327–370. doi: [10.1046/j.1365-3113.1998.00072.x](https://doi.org/10.1046/j.1365-3113.1998.00072.x)
26. Reinert JF. List of abbreviations for currently valid generic-level taxa in family Culicidae (Diptera). *Eur Mosq Bull.* 2009; 27: 68–76.
27. Mayagaya V, Ntamatungiro A, Moore S, Wirtz R, Dowell F, Maia M. Evaluating preservation methods for identifying *Anopheles gambiae* s.s. and *Anopheles arabiensis* complex mosquitoes species using near infra-red spectroscopy. *Parasit Vectors.* 2015; 8: 60. doi: [10.1186/s13071-015-0661-4](https://doi.org/10.1186/s13071-015-0661-4) PMID: [25623484](https://pubmed.ncbi.nlm.nih.gov/25623484/)
28. Lorenz C, Marques T, Sallum MA, Suesdek L. Morphometrical diagnosis of the malaria vectors *Anopheles cruzii*, *An. homunculus* and *An. bellator*. *Parasit Vectors. Parasites & Vectors;* 2012; 5: 257. doi: [10.1186/1756-3305-5-257](https://doi.org/10.1186/1756-3305-5-257) PMID: [23148743](https://pubmed.ncbi.nlm.nih.gov/23148743/)
29. Jaramillo-O N, Dujardin JP, Calle-Londoño D, Fonseca-González I. Geometric morphometrics for the taxonomy of 11 species of *Anopheles* (*Nyssorhynchus*) mosquitoes. *Med Vet Entomol.* 2015; 29: 26–36. doi: [10.1111/mve.12091](https://doi.org/10.1111/mve.12091) PMID: [25393150](https://pubmed.ncbi.nlm.nih.gov/25393150/)
30. Louise C, Vidal PO, Suesdek L. Microevolution of *Aedes aegypti*. *PLoS One.* 2015; 10: e0137851. doi: [10.1371/journal.pone.0137851](https://doi.org/10.1371/journal.pone.0137851) PMID: [26360876](https://pubmed.ncbi.nlm.nih.gov/26360876/)
31. Virginio F, Oliveira Vidal P, Suesdek L. Wing sexual dimorphism of pathogen-vector culicids. *Parasit Vectors.* 2015; 8: 1–9.
32. Henry A, Thongsripong P, Fonseca-Gonzalez I, Jaramillo-Ocampo N, Dujardin JP. Wing shape of dengue vectors from around the world. *Infect Genet Evol.* 2010; 10: 207–214. doi: [10.1016/j.meegid.2009.12.001](https://doi.org/10.1016/j.meegid.2009.12.001) PMID: [20026429](https://pubmed.ncbi.nlm.nih.gov/20026429/)
33. Dujardin J, Slice DE. Contributions of morphometrics to medical entomology. *Encycl Infect Dis.* 2006; 433–446.
34. Dujardin JP, Kaba D, Henry AB. The exchangeability of shape. *BMC Res Notes.* 2010; 3: 266. doi: [10.1186/1756-0500-3-266](https://doi.org/10.1186/1756-0500-3-266) PMID: [20964872](https://pubmed.ncbi.nlm.nih.gov/20964872/)
35. Fruciano C. Measurement error in geometric morphometrics. *Dev Genes Evol.* 2016; 139–158. doi: [10.1007/s00427-016-0537-4](https://doi.org/10.1007/s00427-016-0537-4) PMID: [27038025](https://pubmed.ncbi.nlm.nih.gov/27038025/)
36. Vidal PO, Suesdek L. Comparison of wing geometry data and genetic data for assessing the population structure of *Aedes aegypti*. *Infect Genet Evol.* 2012; 12: 591–596. doi: [10.1016/j.meegid.2011.11.013](https://doi.org/10.1016/j.meegid.2011.11.013) PMID: [22178147](https://pubmed.ncbi.nlm.nih.gov/22178147/)
37. Dujardin JP. Morphometrics applied to medical entomology. *Infect Genet Evol.* 2008; 8: 875–890. doi: [10.1016/j.meegid.2008.07.011](https://doi.org/10.1016/j.meegid.2008.07.011) PMID: [18832048](https://pubmed.ncbi.nlm.nih.gov/18832048/)
38. Rohlf FJ. tpsDig, digitize landmarks and outlines, version 2.05. Dep Ecol Evol State Univ New York Stony Brook. 2008; (c) 2005 by F. James Rohlf.
39. Klingenberg CP. MorphoJ: an integrated software package for geometric morphometrics. *Mol Ecol Resour.* 2011; 11: 353–357. doi: [10.1111/j.1755-0998.2010.02924.x](https://doi.org/10.1111/j.1755-0998.2010.02924.x) PMID: [21429143](https://pubmed.ncbi.nlm.nih.gov/21429143/)
40. Hammer Ø, Harper DATT, Ryan PD. PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontol Electron.* 2001; 4: 9.
41. Morais SA de, Moratore C, Suesdek L, Marrelli MT. Genetic-morphometric variation in *Culex quinquefasciatus* from Brazil and La Plata, Argentina. *Mem Inst Oswaldo Cruz.* 2010; 105: 672–676. doi: [10.1590/S0074-02762010000500012](https://doi.org/10.1590/S0074-02762010000500012) PMID: [20835615](https://pubmed.ncbi.nlm.nih.gov/20835615/)

42. Multini LC, Marrelli MT, Wilke ABB. Microsatellite loci cross-species transferability in *Aedes fluviatilis* (Diptera: Culicidae): a cost-effective approach for population genetics studies. *Parasit Vectors*. 2015; 8: 635. doi: [10.1186/s13071-015-1256-9](https://doi.org/10.1186/s13071-015-1256-9) PMID: [26667177](https://pubmed.ncbi.nlm.nih.gov/26667177/)
43. Harbach RE, Kitching IJ, Culverwell CL, Dubois J, Linton YM. Phylogeny of mosquitoes of tribe culicini (Diptera: Culicidae) based on morphological diversity. *Zool Scr*. 2012; 41: 499–514. doi: [10.1111/j.1463-6409.2012.00546.x](https://doi.org/10.1111/j.1463-6409.2012.00546.x)
44. Wilke A, Vidal P, Suesdek L, Marrelli M. Population genetics of neotropical *Culex quinquefasciatus* (Diptera: Culicidae). *Parasit Vectors*. 2014; 7: 468. doi: [10.1186/s13071-014-0468-8](https://doi.org/10.1186/s13071-014-0468-8) PMID: [25280576](https://pubmed.ncbi.nlm.nih.gov/25280576/)
45. Laurito M, Oliveira TM de, Almiron WR, Sallum MAM. COI barcode versus morphological identification of *Culex (Culex)* (Diptera: Culicidae) species: a case study using samples from Argentina and Brazil. *Mem Inst Oswaldo Cruz*. 2013; 108: 110–122. doi: [10.1590/0074-0276130457](https://doi.org/10.1590/0074-0276130457)
46. Eastwood G, Kramer LD, Goodman SJ, Cunningham A. West Nile Virus Vector Competency of *Culex quinquefasciatus* Mosquitoes in the Galapagos Islands. *Am J Trop Med Hyg*. 2011; 85: 426–433. doi: [10.4269/ajtmh.2011.10-0739](https://doi.org/10.4269/ajtmh.2011.10-0739) PMID: [21896799](https://pubmed.ncbi.nlm.nih.gov/21896799/)
47. Medeiros-Sousa AR, Ceretti-Junior W, de Carvalho GC, Nardi MS, Araujo AB, Vendrami DP, et al. Diversity and abundance of mosquitoes (Diptera: Culicidae) in an urban park: Larval habitats and temporal variation. *Acta Trop*. 2015; 150: 200–209. doi: [10.1016/j.actatropica.2015.08.002](https://doi.org/10.1016/j.actatropica.2015.08.002) PMID: [26259817](https://pubmed.ncbi.nlm.nih.gov/26259817/)
48. Wilkerson RC, Linton Y-M, Fonseca DM, Schultz TR, Price DC, Strickman DA. Making mosquito taxonomy useful: A stable classification of tribe Aedini that balances utility with current knowledge of evolutionary relationships. *PLoS One*. 2015; 10: e0133602. doi: [10.1371/journal.pone.0133602](https://doi.org/10.1371/journal.pone.0133602) PMID: [26226613](https://pubmed.ncbi.nlm.nih.gov/26226613/)
49. Harbach RE, Kitching IJ. Reconsideration of anopheline mosquito phylogeny (Diptera: Culicidae: Anophelinae) based on morphological data. *Syst Biodivers*. 2005; 3: 345–374. doi: [10.1017/S147720000500174X](https://doi.org/10.1017/S147720000500174X)
50. Harbach RE. Classification within the cosmopolitan genus *Culex* (Diptera: Culicidae): The foundation for molecular systematics and phylogenetic research. *Acta Trop*. 2011; 120: 1–14. doi: [10.1016/j.actatropica.2011.06.005](https://doi.org/10.1016/j.actatropica.2011.06.005) PMID: [21741350](https://pubmed.ncbi.nlm.nih.gov/21741350/)
51. Motoki MT, Suesdek L, Bergo ES, Sallum MAM. Wing geometry of *Anopheles darlingi* Root (Diptera: Culicidae) in five major Brazilian ecoregions. *Infect Genet Evol*. Elsevier B.V.; 2012; 12: 1246–1252. doi: [10.1016/j.meegid.2012.04.002](https://doi.org/10.1016/j.meegid.2012.04.002) PMID: [22522004](https://pubmed.ncbi.nlm.nih.gov/22522004/)
52. Cornel A, Lee Y, Fryxell RT, Siefert S, Nieman C, Lanzaro G. *Culex pipiens* Ssensu Lato in California: A complex within a complex? *J Am Mosq Control Assoc*. 2012; 28: 113–121. doi: [10.2987/8756-971X-28.4s.113](https://doi.org/10.2987/8756-971X-28.4s.113) PMID: [23401951](https://pubmed.ncbi.nlm.nih.gov/23401951/)