Wing diagnostic characters for *Culex quinquefasciatus* and *Culex nigripalpus* (Diptera, Culicidae)

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ABSTRACT. Wing diagnostic characters for *Culex quinquefasciatus* and *Culex nigripalpus* (Diptera, Culicidae). *Culex quinquefasciatus* and *Culex nigripalpus* are mosquitoes of public health interest, which can occur sympatrically in urban and semi-urban localities. Morphological identification of these species may be difficult when specimens are not perfectly preserved. In order to suggest an alternative taxonomical diagnosis, wings of these species were comparatively characterized using geometric morphometrics. Both species could be distinguished by wing shape with accuracy rates ranging from 85–100%. Present results indicate that one can identify these species relying only on wing characters when traditional taxonomical characters are not visible.

KEYWORDS. Culicid; geometry; species diagnosis; vector.

RESUMO. Caracteres alares diagnósticos de *Culex quinquefasciatu*s and *Culex nigripalpus* (Diptera, Culicidae). *Culex quinquefasciatus* e *Culex nigripalpus* são mosquitos de interesse à saúde pública e que podem ocorrer em simpatria em ambientes urbanos e semi-urbanos. A identificação morfológica dessas espécies pode ser difícil nos casos em que os espécimes não estão bem preservados. Com o intuito de sugerir um método diagnóstico taxonômico, asas de indivíduos dessas espécies foram caracterizadas comparativamente utilizando-se morfometria geométrica. Ambas as espécies puderam ser distinguidas pela forma alar com índices de confiabilidade entre 85–100%. Os resultados presentes indicam que é possível identificar essas espécies baseando-se apenas no formato alar, nos casos em que os caracteres taxonômicos tradicionais não são visíveis.

PALAVRAS-CHAVE. Culicídeos; geometria; diagnose de espécie; vetor.

C*ulex quinquefasciatus* Say, 1823 is the main vector of bancroftian filariasis in Brazil and because it can transmit arboviruses between animals and human beings. Its geographical distribution is pantropical, and in the Americas it occurs from the central and southern regions of the U.S.A. to Argentina (Forattini 2002). Adults and larvae have synanthropic habits and can develop in urban environments where populations often show tolerance to pollutants and insecticides (Natal & Ueno 2004). This species is adapted to urban areas and benefits from the processes of industrialization and urbanization.

Another mosquito species found in urban environments in the Americas is *Culex nigripalpus* Theobald, 1901. It is a species of public health interest and occurs from the Tropic of Cancer, North America, to the Tropic of Capricorn, South America. It is able to reproduce in artificial breeding containers rich in organic matter, such as polluted streams connected to sewers (Forattini *et al.* 1998; Forattini 2002). This species is a vector of human encephalitis arboviruses in tropical region (Mitchell *et al.* 1979; Forattini *et al.* 1995; Day & Curtis 1999).

These two species are mostly found in sympatry and in many cases the larvae live syntopically in the same breeding site, occupying similar ecological niches (Forattini 2002). Although these species are easily distinguished when in good condition, the concurrence of these requires caution in taxonomic identification, especially when the specimens are damaged or preserved in ethanol.

This paper is intended to define wing characters that will allow workers to distinguish between *Cx. quinquefasciatus* and *Cx. nigripalpus*, which could expedite identification of poorly-preserved culicids. To that purpose, wings were described by geometric morphometrics tools.

MATERIAL AND METHODS

Specimen collection. Samples were collected in the urban area of São Paulo city (State of São Paulo, Brazil). *Cx. nigripalpus* specimens were collected from the Parque Ecológico do Tietê (April/2007) and *Cx. quinquefasciatus* from the banks of the Pinheiros River (May/2007). Specimens were collected via entomological aspirators, taxonomically identified to species and stored in 70% ethanol.

Wings preparation. Wing of *Cx. nigripalpus* (males n = 56, females n = 31) and *Cx. quinquefasciatus* (males n = 55, females n = 32) were mounted on glass microscope slides with Entellan medium (Merck, NJ-USA). Images of wings were captured by a Leica DFC320 digital camera coupled to a Leica S6 stereoscope equipped with plain lenses which avoid image distortion (Fig. 1A). For each wing, coordinates of 18 evolutionary informative landmarks (firstly described by Jirakanjanakit 2007) were digitized (Fig. 1B) and assembled into matrices.

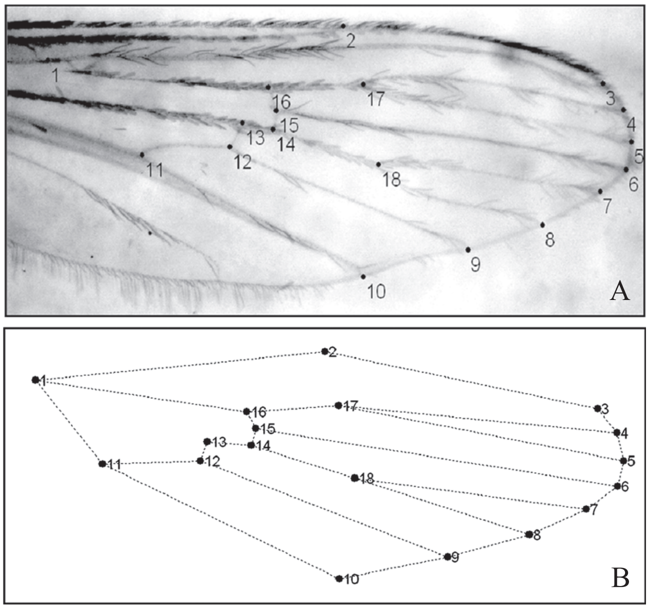


Fig. 1. (A) Wing of *Cx. quinquefasciatus* showing the landmarks. (B) Graphical representation of the consensus formed by 18 landmarks. Points are linked by lines to facilitate viewing of wing coverage.

Morphometrics. Morphometrical analyzes were done in general accordance to Rohlf (1990, 1996, 1999) and are summarized as follows. Within each sex, two parameters were compared between the two species: overall wing size and wing shape. For wing sizes we used the isometric estimator "centroid size", defined by the square root of the sum of the squared distances between the center of the configuration of landmarks and each separate landmark (Bookstein 1991). For wing shape, relative warps were computed and their principal components were plotted in graphs to describe the morphological space for each sample comparison. Besides that, Procrustes superimposition of shape data was applied to compare wings of both species in deformation grids.

Reclassification. To test the accuracy of present morphometric classification, each individual was reclassified according to its wing similarity to the average shape of each species. Mahalanobis distances were used to estimate metric distance. To perform a validating procedure, distances were computed on discriminant axes estimated without the specimen to be classified. In this "validated classification", each wing to be classified was then introduced as supplementary data. Landmark digitizing, data analyzes and graphs were done using softwares BAC and PAD (http://www.mpl.ird.fr/morphometrics) and TPS software pack (http://life.bio.sunysb.edu/morph/).

RESULTS

Size: Wing centroid sizes (Fig. 2) of *Cx. quinquefasciatus* (males) ranged from 2.7 mm to 2.92 mm (mean = 2.81 mm) and for females the range was 3.43–3.64 mm (mean = 3.54 mm). Centroid sizes of *Cx. nigripalpus* ranges were 2.54–2.79 mm (mean = 2.66 mm) in males and 2.78–3.24 mm (mean = 3.01 mm) in females. Parametric comparisons revealed statistically significant interspecific distinction within each sex (T-test, two-tailed, $P < 0.0001$).

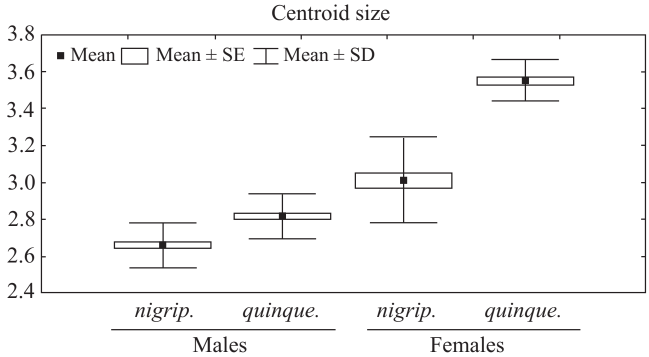


Fig. 2. Boxplot graph illustrating the mean of centroid size of wings of females and males of *Cx. quinquefasciatus* and *Cx. nigripalpus* and their standard errors and deviations. Values in the Y axis are in millimeters.

Shape: Shape analyzes performed separately for each sex showed the individuals arranged in distinct groups in the morphospace defined by principal components 1 and 2 (females Fig. 3; males Fig. 4). Each group corresponded to one species, with a slight intersection between them.

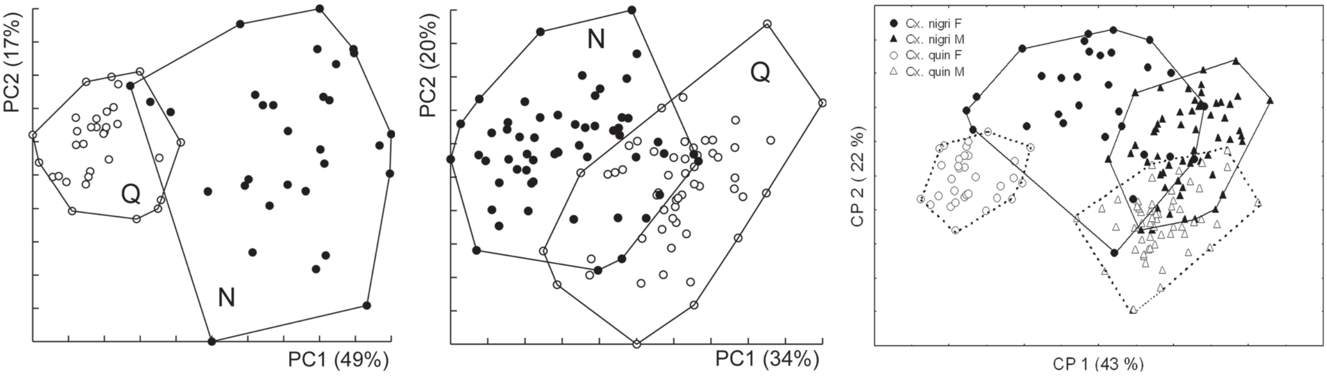
Classification of females based on the Mahalanobis distances was 100% accurate for both species before validation. After validation, 100% of the *Cx. quinquefasciatus* and 90% of the *Cx. nigripalpus* were correctly reclassified. Among males, for *Cx. quinquefasciatus* and *Cx. nigripalpus* respectively, accuracy rates were 98% and 94% before validation, and 90% and 85% after validation.

When both sexes were analyzed simultaneously in a morphospace (Fig. 5), species overlapping was minimal (females) or about 40% (males). Consistently, Mahalanobis distance between species was higher in females than in males (respectively 4.08 and 3.29). Reclassification accuracy scores after validation were: *Cx. nigripalpus* females = 67%; males = 80%, *Cx. quinquefasciatus* females = 96%; males = 87%.

Mean wing configurations plotted in deformation grids after Procrustes superimposition showed that in females (Fig. 6), distances between landmarks 18–7 and between 17–5 were proportionally lower in *Cx. nigripalpus* than in *Cx. quinquefasciatus*, whereas distances between landmarks 2–17 were higher in *Cx. nigripalpus*. Species also differed regarding the ratio obtained by dividing the length of segment 13–14 by the length of segment 12–13 (Figs. 6, 7). Mean ratio values were 1.40 and 1.67 for *Cx. nigripalpus* and 2.15 and 2.48 for *Cx. quinquefasciatus* (males and females, respectively).

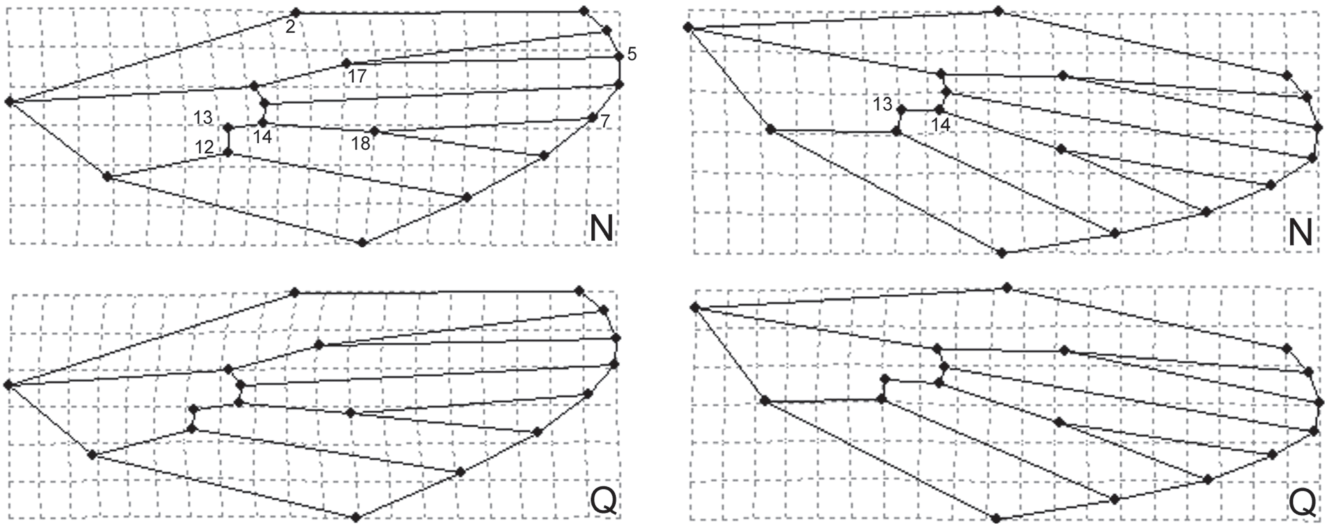
DISCUSSION

Present results indicate that wings are distinct in *Cx. quinquefasciatus* and *Cx. nigripalpus* what lead us to believe



3 4 5

Figs. 3–5. Graphical presentation of morphological space of *Cx. nigripalpus* and *Cx. quinquefasciatus*: 3, females; 4, males; 5, males and females. Contribution of each principal component (PC1 and PC2) to the variation is indicated between brackets.



6 7

Figs. 6–7. Mean configuration of (6) female and (7) male wings of *Cx. nigripalpus* (N) and *Cx. quinquefasciatus* (Q) after Procrustes superimposition. Grid deformations (dotted lines) describe deviation of each landmark from an intermediate configuration between species. Landmarks are numbered as in Fig. 1 (only some numbers shown).

that wing characters may be of some help in diagnosing these species. Besides being often sympatric, *Cx. quinquefasciatus* and *Cx. nigripalpus* are morphologically quite similar and are mainly recognized by coloration of some head scales, presence or absence of thoracic and abdominal scales, characters which are easily lost or damaged.

Regarding the wing shape, relative warps revealed a natural arrangement of the two species into two groups, which was more conspicuous in females. The relative position of landmarks 2, 5, 7, 13, 12, 14, 17, 18 were more informative. Reclassification analyzes before and after validation showed that our diagnostic method reached high levels of accuracy (generally over 90%), mainly when applied to females. When both sexes were analyzed together reclassification accuracy was lower, but even though, recognition rates of *Cx. quinquefasciatus* were 87% or higher. Our interpretation indicates that from this point on a researcher who is not familiarized with morphometrics could distinguish accurately these two species upon observing, say, medial-cubital and M_1+M_2 wing veins, where the taxonomically informative landmarks 12–14 are placed.

Overall wing size, which was higher in *Cx. quinquefasciatus*, may also contribute to diagnose the two species, mainly if combined with shape analysis. However, size may suffer plasticity (Dujardin 2008) and should be used with caution.

Nonetheless, it is necessary to highlight that up to the moment, the suitability of this diagnosis is limited to samples containing only these two species. Moreover, this diagnosis is intended to be a complimentary tool and does not substitute traditional identification methods when specimens are perfectly preserved.

Species of *Culex* are usually identified using traditional taxonomical techniques based primarily on the use of morphological dichotomous keys, a procedure that requires specialized skill and a well-preserved set of specimens. For these reasons, alternative paths to identification of *Culex* species are frequently desired and addressed. Sanogo *et al.* (2007) performed identification of *Cx. nigripalpus* and *Cx. quinquefasciatus* (among other culicids) using Real Time PCR. These two species were also characterized using isoenzyme analyses by Knight & Nayar (2004). Moreover, successful attempts to diagnose *Cx. nigripalpus* and *Cx. salinarius* larvae have also been undertaken (Darsie *et al.* 2006).

The use of geometric morphometrics to diagnose species in medical entomology has been strongly encouraged (Dujardin *et al.* 2003). Wing shape is recognizably useful to such purpose, since they are determined by quantitative heritage (Bitner-Mathé & Klaczko 1999; Jirakanjanakit *et al.* 2007, 2008; Dujardin 2008).

Arguably, our work represents a helpful step toward development of a cheap and rapid diagnosis of species, which is viable even when only wings are available. In such context, another paper on identification of *Culex* species using geometric morphometrics by our group was recently published (Morais *et al.* 2010). Possibly the application of geometric morphometrics for taxonomic purposes can be extended to other species of Culicidae, as it has been true for other insects (Dujardin 2008; Marsteller *et al.* 2009).

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