WING SHAPE VARIATION IN THE TAXONOMIC RECOGNITION OF SPECIES OF *Diachlorus* OSTEN-SACKEN (DIPTERA: TABANIDAE) FROM COLOMBIA

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Trabajo de Grado para optar al título de
Biólogo

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To my family by its support, especially to my mother, Martha T. Galvis; and to my daughter, Salomé Torres P.

ACKNOWLEDGMENTS

We thank Dr. Augusto Loureiro Henriques and Dr. Tiago Kutter Krolow for helping with specimens identification; to Dr. Carlos Eduardo Sarmiento Monroy (ICN-UNAL) and "Instituto de Investigación de Recursos Biológicos Alexander von Humboldt (IavH)" for kindly providing the specimens used in this study. We thank Dr. Santiago Catalano and two anonymus reviewers for their helpful comments on the manuscript.

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AUTOR: AMBROSIO TORRES GALVIS**
PALABRAS CLAVE: *Diachlorus*, TABANIDAE, TAXONOMÍA, MORFOMETRÍA GEOMÉTRICA, COLOMBIA.

Utilizamos una configuración de 15 landmarks, sobre el ala derecha de 601 individuos, para evaluar que tanto la variación intra- e interespecífica de la forma alar discriminaba taxonómicamente seis especies de *Diachlorus* Osten-Sacken, distribuidas en seis áreas protegidas de Colombia. Realizamos análisis de forma geométrica, integrando métodos de Procrustes, componentes principales, análisis de agrupamiento, funciones discriminantes lineal y cuadrática, y evaluación de cambios en la forma. En *Diachlorus*, las alas derecha e izquierda no presentaron asimetría direccional. Es posible determinar la especie y en algunos casos el origen de los individuos usando la forma del ala; la especie mejor asignada fue *Diachlorus leticia* Wilkerson & Fairchild, mientras la peor fue *Diachlorus jobbinsi* Fairchild, que también tuvo la mayor cantidad de variación intraespecífica, *Diachlorus fuscistigma* Lutz presentó la menor variación intraespecífica. El par más similar de especies entre sí, fue *D. fuscistigma* y *Diachlorus leucotibialis* Wilkerson & Fairchild, mientras el más diferente fue *D. leucotibialis* y *Diachlorus nuneztovari* Fairchild & Ortiz. Los individuos con la forma del ala más diferente con respecto a los demás, pertenecieron a Chocó, (especialmente los de *D. jobbinsi*) el área geográfica más alejada del resto de áreas en este estudio; sin embargo, no se evidenció correlación entre las distancias geográficas y geométricas. La función discriminante lineal fue mejor que la no-lineal (cuadrática) para asignar los individuos a la categoría (especie) correcta, mientras que para asignar los individuos a el área que provenian, la función cuadrática fue mejor. Dados los resultados de esta investigación, nosotros recomendamos utilizar la forma del ala como herramienta para discrimar taxonómicamente las demás especies del género *Diachlorus*, no utilizadas en este estudio.

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*Trabajo de Grado.

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ABSTRACT

TITLE: WING SHAPE VARIATION IN THE TAXONOMIC RECOGNITION OF SPECIES OF *Diachlorus* OSTEN-SACKEN (DIPTERA: TABANIDAE) FROM COLOMBIA*

AUTHOR: AMBROSIO TORRES GALVIS**

KEYWORDS: *Diachlorus*, TABANIDAE, TAXONOMY, GEOMETRIC MORPHOMETRY, COLOMBIA.

We used a configuration of 15 landmarks from the right wings of 601 specimens to evaluate what extent the intra- and interspecific variation of the wing shape discriminated taxonomically six species of *Diachlorus* Osten-Sacken distributed in six protected areas of Colombia. We performed geometric analyses, integrating Procrustes methods, principal component analyses, cluster analyses, linear- and quadratic discriminant analyses, and evaluation of shape changes. In *Diachlorus*, left and right wings did not present directional asymmetry. And it is possible to determine the species and in some cases the origin of the specimens using the variation of wing shape; the best assigned species was *Diachlorus leticia* Wilkerson & Fairchild, while the worst was *Diachlorus jobbinsi* Fairchild, that also had the highest intraspecific variation, while *Diachlorus fuscistigma* Lutz had the lowest. The most similar pair of species were *D. fuscistigma* and *Diachlorus leucotibialis* Wilkerson & Fairchild, while the most different were *D. leucotibialis* and *Diachlorus nuneztovari* Fairchild & Ortiz. The specimens with the most different wing shape belonged to Chocó, (especially those of *D. jobbinsi*) the geographically farthest area from the others in study; however, there is not a correlation between geometric and geographical distances. Linear discriminant was better than non-linear (quadratic) discriminant analysis in predicting species membership, while for predicting area membership was the opposite. Given the results of this research, we hypothesize that other species of *Diachlorus* could also be taxonomically discriminated using wing shape.

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*Bachelor Thesis.

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1. INTRODUCTION

Tabanidae (Diptera: Brachycera) is a monophyletic family of nearly 4400 described species (Wiegmann *et al* 2000, Morita 2008, Roskov *et al* 2014), with 27% (1205 species) distributed in the Neotropical region (Coscarón & Papavero 2009a, Henriques *et al* 2012). At the present day, there are 234 species of Tabanidae registered for Colombia (Coscarón & Papavero 2009a, Henriques *et al* 2012). The taxonomic classification scheme used in Tabanidae includes four subfamilies (Chrysopsinae, Pangoniinae, Scepsidinae, and Tabaninae), with further division into tribes (Mackerras 1954, Mackerras 1955, Coscarón & Philip 1979). This classification and the principal taxonomical keys are based on external and genital morphological information of adults, and external morphology of known inmature stages (Coscarón & Papavero 2009b, 2014). Despite the increasing of the number of studies performed during the last years in Brazil (Henriques 2006, Krolow & Henriques 2009, Limeira-de-Oliveira *et al* 2009, Krolow & Henriques 2010, Krolow *et al* 2010, Turcatel *et al* 2010, Krolow *et al* 2012, Rafael *et al* 2012, Henriques & Krolow 2013, Gorayeb *et al* 2013, Gorayeb 2014, Krolow *et al* 2015), Chile (González 2004, González 2006, González 2009, González 2014) and Ecuador (Buestán *et al* 2007, Cárdenas *et al* 2009), taxonomic researches on Neotropical Tabanidae still are few.

The genus *Diachlorus* Osten-Sacken (Tabaninae: Diachlorini) is a group of small flies with yellow and black coloration; its pleura have a patch of pearly pollinosity; the wings present dark patterns of spots, mainly at the apex; and the eyes have characteristic patterns of lines very similar to those presented in the genus *Chrysops* Meigen (Fairchild 1969). Fairchild (1972) and Wilkerson & Fairchild (1982) presented the taxonomical keys used at the present day. Henriques & Rafael (1999) described two species, reaching to 29 the number of recognized species (Coscarón & Papavero 2009a). The *Diachlorus* species range from southeastern United States and the Bahamas to Argentina, but they are absent from Chile and the Caribbees (Fairchild 1969, Coscarón & Papavero 2009a). The species *Diachlorus anduzei* Stone, *Diachlorus curvipes* (Fabricius), *Diachlorus fuscistigma* Lutz, *Diachlorus jobbinsi* Fairchild, *Diachlorus leticia* Wilker-

son & Fairchild, *Diachlorus pechumani* Fairchild and *Diachlorus xynus* Fairchild, are distributed in Colombia, mainly in the amazonic region (Wilkerson & Fairchild 1982, Coscarón & Papavero 2009a).

Morphological interspecific variation has not been quantified in Tabanidae, Although Cárdenas *et al* (2013), using classical morphometry, measured the body size, thorax volume, wing area and wing loading in specimens of *Stypommisa* sp. (Diachlorini), along an altitudinal gradient to evaluate the correlation of those variables with flying activity. The quantification of symmetrical differences (e.g. directional asymmetry, biased departures from perfect symmetry across individuals) is important given that right and left sides can tell different histories about morphological variation, adaptation and development of traits, and it can define if it is necessary the diferential use of structures on the two body sides in morphological studies (Swaddle 1997, Tomkins & Kotiaho 2001, Adams *et al* 2013). Also, right and left sides can play different roles in taxonomic, evolutionary, and ecological understanding (Klingenberg *et al* 1998, Runyon & Hurley 2004, Savriama & Klingenberg 2011).

Regarding morphological variation, *Diachlorus pechumani* Fairchild, presents intraspecific variation that generates some taxonomic recognition problems, and it has been divided in two species (Wilkerson & Fairchild 1982). Also, some species of the genus (e.g. *Diachlorus fuscistigma* Lutz, *Diachlorus jobbinsi* Fairchild) present intraspecific variation in their external morphology, that in some cases makes the taxonomic identification complicated (Wilkerson & Fairchild 1982). In Diachlorini, there are other genera that present intraspecific variation, for example the species of the genus *Chlorotabanus* Lutz, show high regional variation (Krolow & Henriques 2010). The genus *Dichelacera* Macquart, also present high morphological variation, and some of their species are very similar to each other (e.g. *Dichelacera costaricana* (Fairchild) and *Dichelacera apicalis* (Fairchild)) (Fairchild & Philip 1960, Wilkerson 1981, Henriques & Rafael 1993).

With the advances in computation and image handling in the last years, the use of geometric morphometrics has been increasing, and today is considered an efficient tool to register shape changes that cannot be easily detected with the naked eye, being useful to conduct taxonomical, ecological and phylogenetic studies (Rohlf 1990, Rohlf & Marcus 1993, Weaver *et al* 2014). Insect wings are almost transparent and flat, and have many comparable regions. These features avoid the subjectivity on geometric data capture in two dimensions (Lorenz & Suesdek 2013).

In the present study we evaluated the directional asymmetry between right and left wings, and quantified the intraspecific and interspecific variation of the wing shape, for

determining at what extent the geometrical variation discriminates the species of the genus *Diachlorus* from Colombia.

2. MATERIALS AND METHODS

2.1. SPECIES

The biological material examined were females of four previously registered species for Colombia (*Diachlorus curvipes* Fabricius, *Diachlorus fuscistigma* Lutz, *Diachlorus jobbinsi* Fairchild and *Diachlorus leticia* Wilkerson & Fairchild), and two previously not registered species, *Diachlorus leucotibialis* Wilkerson & Fairchild, from Amazonas, Caquetá, Putumayo and Vaupés; and *Diachlorus nuneztovari* Fairchild & Ortiz, from Amazonas and Putumayo. Althought there are other *Diachlorus* species in Colombia, we did not include them because of the number of available specimens (lower than 10 specimens by park for *Diachlorus pechumani* Fairchild and *Diachlorus xynus* Fairchild, and none for *Diachlorus anduzei* Stone).
Females (most of them blood-feeding) of Tabanidae are easier to collect using Malaise traps and are more abundant than males (nectar-feeding), due the sexual dimorphism in feeding habits (Coscarón & Papavero 2009b, Lessard *et al* 2013). Given the above, we only used females in this research as our main source of specimens was Malaise trapping.

2.2. LOCALITIES

The specimens included in this study belong to the collections of Tabanidae of the "Instituto de Investigación de Recursos Biológicos Alexander von Humboldt (IavH)", collected in 23 protected areas of Colombia during the project "Diversidad de Insectos en Colombia" (Campos & Fernández 2002); and of the "Instituto de Ciencias Naturales (ICN-UNAL-Bogotá)", collected in two protected areas of the south of Colombia. A total of six protected areas had the minimum number ($n > 29$) of specimens defined

(see "Material and Methods: Data" section) (Table 1).
The "Parque Nacional Natural (PNN) Amacayacu" (AMA), located in the department of Amazonas (3°17'28"S 70°9'21"W), is a tropical rainforest ecosystem with flooded forests and an average temperature of 27°C; the "PNN Serranía de Chiribiquete" (CAQ), between the departments of Caquetá and Guaviare (0°43'0"N 72°43'59"W), is composed by large rock formations surrounded by dense rainforests with an average temperature of 24°C; the "PNN Utría" (CHO) is in Chocó (6°1'14"N 77°14'2"W), and is a cove in the Pacific ocean, covered by tropical rainforests with 27°C of average temperature; the "PNN Sierra de la Macarena" (MET) is located in the department of Meta (2°36'32"N 73°33'44"W), and is composed of rainforests and Amazon savanna with 27°C of average temperature; the "PNN La Paya" (PUT), in Putumayo (0°13'8"N 75°14'46"W), corresponds principally to tropical rainforest with an average temperature of 26°C; finally, the "Estación Biológica (EB) Mosiro Itajura-Caparú" (VAU), in the department of Vaupés (1°04'0"S 69°30'34"W), is in the border with Brazil, and its ecosystem is Amazon jungle and 27°C average. "Sistema de Parques Nacionales Naturales de Colombia" and "Sistema Nacional de Áreas protegidas de Colombia (SINAP)". http://www.parquesnacionales.gov.co/ accessed 27 Jan 2015.

2.3. DATA

The specimens were previously determined using the taxonomic keys by Fairchild (1972), Wilkerson (1979), Wilkerson & Fairchild (1982) and Coscarón & Papavero (2009b). In order to gather geometric shape data, we randomly took a subsample (30 specimens by each species) from 601 total specimens (Table 1), taking photographs of left and right wings in dorsal view. We also took photographs only of the right wing in dorsal view for each one of the 601 specimens (Table 1). For the photographs, we placed the wings over microscope slides without dissecting of wings from the body. After that, we took the photographs using a stereoscope attached to a camera of 10.1 Megapixels.
We chose a minimal size of specimens in a sample ($n > 29$) (Table 1), taking into account the within-group variation relative to differences between groups (data not shown), the question and the taxonomic rank we addressed, and previous studies which suggest

some ways to evaluate the sample size (Polly 2005, Cardini & Elton 2007). Over each photograph we digitalized a configuration of 15 type I landmarks (Bookstein 1991) (Fig 1). We chose this configuration avoiding type II and III landmarks and representing the greatest surface of the wing, with the maximum variation, using fewer number of landmarks.

2.4. MORPHOMETRIC ANALYSES

First, we performed a Generalized Procrustes Analysis (GPA) for each species, using the least squares criterion (Gower 1975, Rohlf & Slice 1990) over the subsamples of the left and right wings to obtain shape variables and consensus shapes. To quantify the asymmetric shape differences we used the Riemmanian distance (Kendall 1984, Savriama & Klingenberg 2006). We tested for statistical directional asymmetry (for each specimen) through the F test of Goodall (Goodall 1991, Dryden & Mardia 1998, Savriama & Klingenberg 2006). We use geometric morphometrics to register shape changes between left and right wings, but not to evaluate the differential presence/absence of some structures or body parts.

Subsequently, we performed a GPA (Gower 1975, Rohlf & Slice 1990) for the entire set of right wings. With the aligned configurations, we performed a Principal Component Analysis (PCA) to reduce the dimensionality of the data and to observe the tendency of the interspecific variation. From the set of shape principal components (PC) (80% of cumulative explained variance) we determined the relevant number of clusters in the entire data set using the Bayesian information criterion (BIC) for model-based clustering (McLachlan & Peel 2000, Fraley & Raftery 2002, Fraley & Raftery 2007), the "gap" statistic (Tibshirani *et al* 2001) and the Calinski and Harabasz (CH) index (Calinski & Harabasz 1974). We conducted an UPGMA hierarchical cluster analysis (Sokal & Sneath 1963, McQuitty 1966, Sneath & Sokal 1973) using Mahalanobis distances computed from the set of principal components.

To quantify the geometric shape differences among the species we used the Riemmanian distance (Kendall 1984), and we tested the statistical significance of the geometric distances among species, using F test of Goodall (Goodall 1991, Dryden & Mardia 1998). We visualized the shape changes among the species through relative warps: thin-

plate splines transformation grids and displacement vectors (Bookstein 1989, Bookstein 1991, Rohlf & Bookstein 2003). We followed the same protocol described above for interspecific variation, for the measurement of intraspecific variation, but we only used the species that were present in more than one area (*D. curvipes*, *D. fuscistigma*, *D. jobbinsi*, *D. leucotibialis* and *D. nuneztovari*), to compare for each species the wing geometrical configurations of specimens from different areas. We used the Mantel test (Mantel 1967) to evaluate the correlation between the matrix of Riemmanian distances and the matrix of geographical distances. We performed the analyses separately for each species and the unities of differentiation were the areas where the species are distributed.

Finally, with the calculated Mahalanobis distances from the shape PCA we carried out the discriminant analysis (Fisher 1936, Slice 2007) (linear and quadratic) to predict both, species membership and area (locality) membership, we used cross-validation to obtain the posterior classification probabilities in which each individual is allocated to its closest group without being used to determine the centroid of the group (Venables & Ripley 2002, Tuffery 2011).

2.5. SOFTWARE

We used, for the gathering of landmark data, the R software environment for statistical computing and graphics (R Development Core Team 2014), with the R package "geomorph" (Adams & Otarola-Castillo 2013). We used for the described statistical analysis and for creating the graphics, the R software (R Development Core Team 2014), with the packages "colorspace" (Ihaka *et al* 2013), "cluster" (Maechler *et al* 2014), "DiscriMiner" (Sanchez 2013), "ellipse" (Murdoch & Chow 2013), "geomorph" (Adams & Otarola-Castillo 2013), "mclust" (Fraley & Raftery 2002), "NbClust" (Charrad *et al* 2014), "shapes" (Dryden 2013) and "vegan" (Oksanen *et al* 2013). For graphics handling, we used the "ggplot2" package (Wickham 2009). The morphometric and statistical analyses that we present in this study can be reproduced using the code and data available in the repository of github (https://github.com/atorresgalvis/Diachlorus_Colombia-Wing_shape).

3. RESULTS

3.1. WINGS ASYMMETRY

The asymmetry between left and right wings of the species analized was non-significant. The mean Riemmanian distances between left and right wings ranged from 0.003413 (*Diachlorus leucotibialis* Wilkerson & Fairchild, G = 0.297, p = 0.99) to 0.005972 (*Diachlorus curvipes* Fabricius, G = 0.785, p = 0.63).

3.2. INTERSPECIFIC WING VARIATION

The ordination of the specimens in the morphospace defined by the principal component 1 (PC1) and the principal component 2 (PC2) (cummulative variance = 56%) could efficiently help to differentiate at least four of the six species of *Diachlorus* in the study (Fig 2). However, it was necessary to use the first seven principal components of the PCA of the genus *Diachlorus* to retain 80% of the cumulative explained variance (Table 2). The optimal number of clusters (K) for the wing geometry data, based on the BIC, the "gap" statistic and the CH index, was five, with an ellipsoidal, equal volume, shape and orientation ('EEE') Gaussian mixture model (see Electronic Supplementary Material). These five K-groups represented in an efficient way the species *Diachlorus curvipes* Fabricius, *Diachlorus fuscistigma* Lutz, *Diachlorus jobbinsi* Fairchild, *Diachlorus leucotibialis* Wilkerson & Fairchild and *Diachlorus nuneztovari* Fairchild & Ortiz, in the Hierarchical Cluster Analysis (Fig 3). Specimens of *Diachlorus leticia* Wilkerson & Fairchild, were clustered together, though nested within *D. jobbinsi* (Fig 3); meaning that the cluster of *D. jobbinsi* is in fact *D. jobbinsi* + *D. leticia*.

The differences among all species were statistically significant (P > 0.05 for all species). *D. nuneztovari* was the species geometrically most distant to the other species, while

D. curvipes was the least distant from the others (Table 3). The most similar pair of species were *D. fuscistigma* and *D. leucotibialis*, while the most different were *D. leucotibialis* and *D. nuneztovari* (Table 3).

3.3. INTRASPECIFIC WING VARIATION

The Goodall's F test showed significant shape differences ($P < 0.05$) among groups of specimens from different areas in all species, except between Amazonas and Putumayo for the species *D. curvipes* and *D. fuscistigma*, and between Caquetá and Vaupés for *D. fuscistigma* (Table 4). Riemmanian distances (Table 4) showed that for *D. curvipes*, the specimens from Chocó were the most different in relation with the specimens from the other areas, while specimens from Putumayo were the least different; for *D. fuscistigma*, Meta and Vaupés were the most and least different, respectively; in *D. jobbinsi*, Chocó was the most different and Caquetá the least different; and for *D. leucotibialis*, Caquetá was the most different area and Putumayo the least different. The Mantel test denoted that the Riemmanian distances were not significantly correlated to geographical distances between the areas, *D. jobbinsi* had the highest r2 (0.3460, $p = 0.217$) for correlation of matrices, while *D. leucotibialis* had the lowest r2 (0.2331, $p = 0.667$) (See Supplementary Material to see te complete values of the Mantel test).
The cumulative explained variance of the first two components (PC) for all species was low (38% in average), such that, for example in *D. curvipes*, it is necessary to use the first ten components to retain 80% of explained variance (Table 2). In *Diachlorus*, it is not possible to determine the geographic origin of the specimens, based on the morphospace defined by the PC1 and PC2 of wing shape (only one K-group was determined by BIC, the "gap" statistic and the CH index with a diagonal multivariate normal 'XXI' model, for each species, see Electronic Supplementary Material). The only exception is *D. jobbinsi*, where the specimens from Chocó are well separated from the other parks (Fig 4); the optimal number of clusters (K), was three, with a diagonal, equal volume and shape ('EEI') model. The hierarchical clustering of *D. jobbinsi* showed that more than a half of the specimens from Chocó were grouped together into one K-group, but the specimens from the other parks were mixed into the other two groups (Fig 5).

3.4. SHAPE VARIATION AND TAXONOMIC DISCRIMINATION

D. leticia was the best assigned species, reaching 100% of accuracy; while *D. jobbinsi* (89.6%) had the lowest accuracy; the percentages of accuracy were not always higher using quadratic discriminant functions. In fact, the accuracy was worse with quadratic discriminant in *D. nuneztovari* (Table 5). The specimens of *D. jobbinsi* and *D. leucotibialis* were the best assigned to the areas from where they come; while *D. fuscistigma* presented the worst assignment (Table 6).

In summary, linear discriminant analysis was better than quadratic in predicting species membership, while quadratic discriminant analysis overcame linear in predicting area membership.

3.5. VISUALIZATION OF SHAPE CHANGES

Diagrams of interspecific variation (Fig 6a, 6b) showed large displacements upward and backward on the apex of the wing (intersections of the veins Radials and Costal; apical and costal wing border), downward and forward on posterior/anal wing section (ends of veins M3, M4 and CuA; intersection of veins CuA and A1), and backward on the intersection of veins R4 and R5. In intraspecific deformations (Fig 6c, 6d), as expected, the diagram of minimum variation showed almost no changes, while the diagram of maximum variation showed displacements forward on the apex of the wing, and changes on the separation distance between the intersection of veins CuA and A1, and the posterior wing border. In summary, the intraspecific and interspecific changes were not the same along the wing, these differences regarding to variation were represented not only in amount of change, but in morphological localization on the wing.

4. DISCUSSION AND CONCLUSIONS

The present research indicated that, as there is no significant symmetrical variation in left/right wings, any of them could be used in geometric morphometrics studies. We demostrated that geometric morphometrics is a powerful complement to external morphology in the taxonomy of *Diachlorus*. *Diachlorus nuneztovari* Fairchild & Ortiz, was similar to *Diachlorus jobbinsi* Fairchild (Fig 2, Table 3). This result agrees with the analysis of external morphology that put these species very close, mainly in terms of coloration of thorax and legs, making harder their separation in some occasions using external morphology (Fairchild 1972, Wilkerson & Fairchild 1982); *Diachlorus fuscistigma* Lutz and *Diachlorus leucotibialis* Wilkerson & Fairchild, were quite similar to each other, contrasting with the analysis of external morphology that allows to clearly differentiate these species based on coloration characters of thorax and abdomen (Wilkerson & Fairchild 1982). Concerning *Diachlorus curvipes* Fabricius and *Diachlorus leticia* Wilkerson & Fairchild, these species can be distinguished very well from the rest of the species under study (Fig 3).

Chocó, the geographically farthest area from the others in this study, presents also the specimens that are the most different in wing shape. This can be seen in the two species presented in Chocó, *D. jobbinsi* and *D. curvipes* (Table 4), in which the specimens from Chocó have the most different wing geometry among the areas where these species are present. Furthermore, this could suggest that the wing shape is more similar in geographically closer areas; however, this is not evidenced by Mantel test that showed non-significant correlation between Riemmanian distances and geographical distances. This finding disagreed with those obtained in other groups of Diptera for many morphological structures (Dujardin *et al* 1999, Franco *et al* 2008) and it could indicate that morphological intraspecific variation, wing shape of *Diachlorus* in this case, could be related to other variables (not taken into account in this study), as environmental and ecological conditions , genetic variability, adaptative variables, etc., and not only to the geographic proximity (Lane & Marshall 1981, Soto *et al* 2007, Franco *et al* 2008). Besides, the groups formed in cluster analysis for *D. jobbinsi*, did not always corre-

spond to predefined groups of specimens from each area (Fig 4, Fig 5). This means that intraspecific variation is due not only to geographic distribution, but to different variables which can be genetic or ecological (Lane & Marshall 1981, Bolnick *et al* 2011). Regarding the distribution for each species, the smaller-ranged species have low intraspecific morphological variation given that these tend to occupy smaller niches (Oney *et al* 2013). This pattern is not supported in the present study where the widest-ranged species, *D. curvipes* and *D. fuscistigma* have the lowest intraspecific variation, while smaller-ranged species, *D. jobbinsi*, *D. leucotibialis* and *D. nuneztovari* have the highest intraspecific variation (Fig 4, Table 4). Further studies with more species and localities are needed to evaluated and test in a better way the mechanisms of morphological intraspecific variation.

The interspecific variation in *Diachlorus* requires fewer principal components and variables to describe its structure and it fits in a finest way to a linear discriminant function; while intraspecific variation requires to add more principal components and it fits better to a quadratic discriminant function (Table 5, Table 6). Linear discriminant analysis is conceptually simple and has been used broadly (Dujardin *et al* 1999, De la Riva *et al* 2001, Sheets *et al* 2006, Jaramillo *et al* 2014). However, it has some limitations: it requires at least one of the scatter matrices to be nonsingular, it can not easily capture a nonlinearly clustered structure and it allows low dimensional representations of the data (Roth & Steinhage 1999).

Interspecific variation can be interpreted as cumulative intraspecific changes (Solé *et al* 1999, Jablonski 2000, Simons 2002). It means that mechanisms of intraspecific variation and interspecific variation are the same. Then, the only difference between intra- and interspecific variation would be the amount of change that would tend to be higher in interspecific variation than intraspecific variation. We did not observe this pattern in *Diachlorus*; its intraspecific and interspecific mechanisms of wing variation are different in direction, amount and place of the changes in the wing (Fig 6).

The effectiveness of determining the species and the area of origin in the genus *Diachlorus* can be viewed as an inverse relationship of between the amount of intraspecific variation and amount of interspecific variation, because in those species which is more accurate predicting species membership, predicting area membership is less accurate (*D. curvipes*, *D. fuscistigma*); but in species in which is more accurate predicting area membership, is less accurate predicting species membership (*D. jobbinsi*, *D. leucotibialis*) (Table 5, Table 6).

Given that we showed in this research that geometric morphometrics techniques are

efficient in the taxonomic understanding of *Diachlorus*, we hypothesize that the rest of the species of *Diachlorus*, that are not in Colombia can also be taxonomically discriminated using geometric morphometrics of the wings. The expansion of the range of data collection could define more accurately the dynamics of regional variation. For these reasons, further morphometric studies of populations (not only of *Diachlorus*, but other genera of Tabanidae) along the entire distribution range would help to evaluate in a better way morphological variation in Tabanidae.

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FIGURES

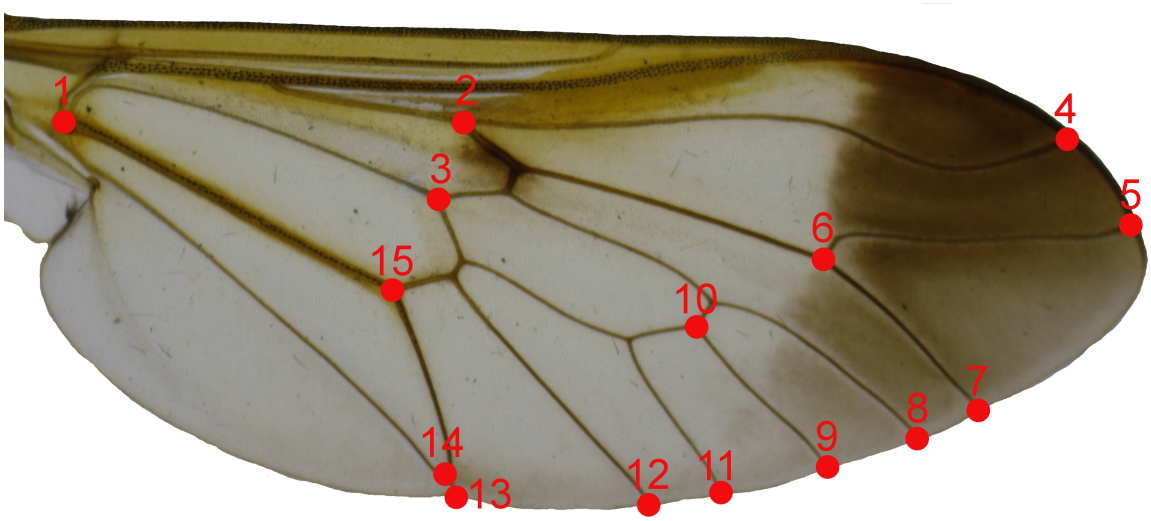

Figure 1. Wing of *Diachlorus jobbinsi* from Caquetá, "PNN Serranía del Chiribiquete" (CAQ), with the configuration of landmarks. 1 = start of second basal cell; 2 = start of vein R 2+3 ; 3 = start of discal cell; 4 = intersection of the veins R 2+3 and costal; 5 = intersection of veins R 4 and costal; 6 = intersection of veins R 4 y R 5 ; 7 = end of vein R 5 ; 8 = end of vein M 1 ; 9 = end of vein M 2 ; 10 = end of discal cell; 11 = end of vein M 3 ; 12 = end of vein M 4 ; 13 = end of vein CuA; 14 = intersection of veins CuA and A 1 ; 15 = start of vein CuA.

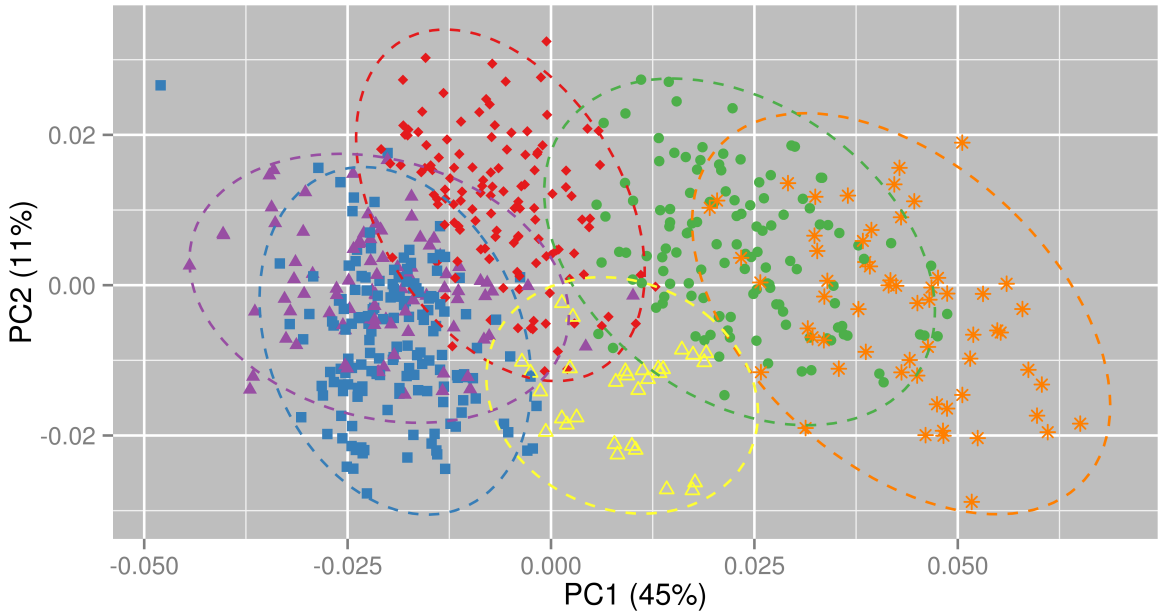

Figure 2. Dispersion graph of genus *Diachlorus* from Colombia in the bidimensional space of principal components PC1 and PC2. The covariance error ellipses (95% of confidence) are drawn. Red diamonds = *D. curvipes*; blue squares = *D. fuscistigma*; green points = *D. jobbinsi*; yellow empty triangles = *D. leticia*; violet solid triangles = *D. leucotibialis*; orange asteriks = *D. nuneztovari*.

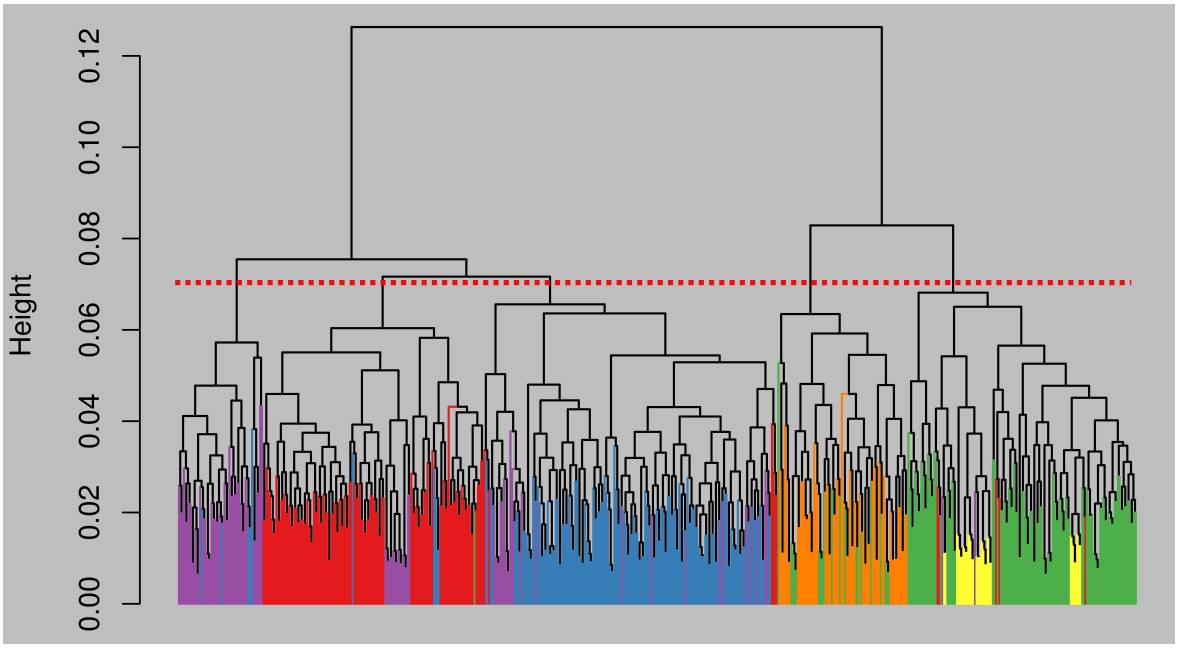

Figure 3. Dendrogram of genus *Diachlorus* (K = 5) from Colombia by Hierarchical Cluster Analysis (UPGMA). The red dashed line cut the dendrogram into the K-groups defined according to the Bayesian Information Criterion, the "gap" statistic and Calinski and Harabasz (CH) index. Red = *D. curvipes*; blue = *D. fuscistigma*; green = *D. jobbinsi*; yellow = *D. leticia*; violet = *D. leucotibialis*; orange = *D. nuneztovari*.

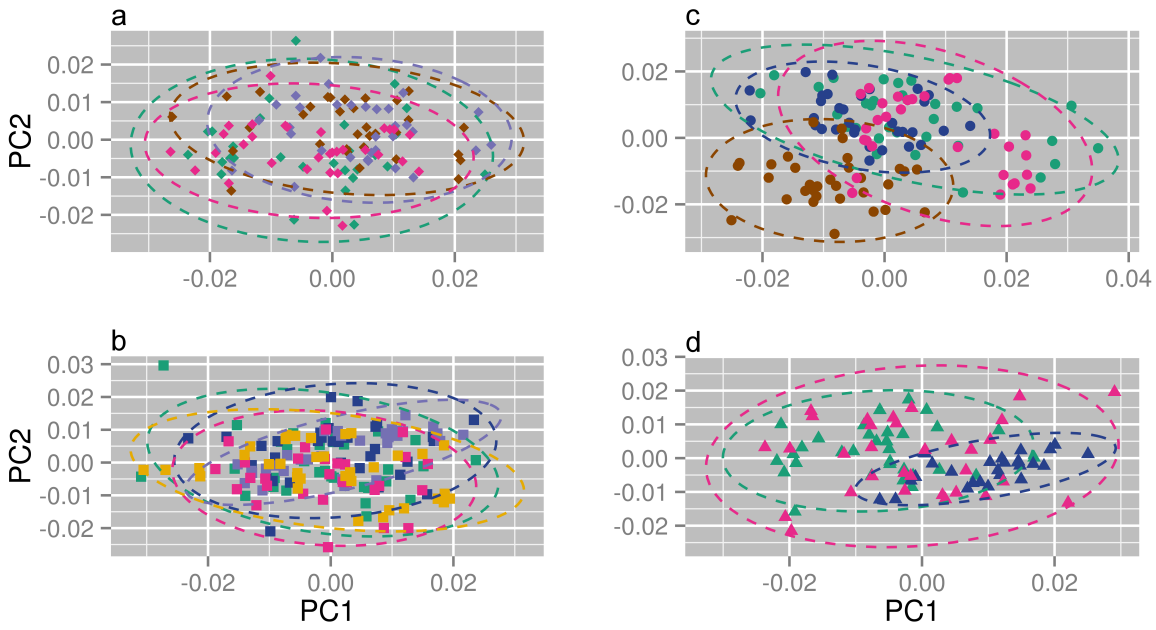

Figure 4. Dispersion graphs of four *Diachlorus* species from different areas of Colombia in the bidimensional space of principal components PC1 and PC2. The covariance error ellipses (95% of confidence) are drawn. a (diamonds) = *D. curvipes* (PC1: 23%, PC2: 11.7%); b (squares) = *D. fuscistigma* (PC1: 23%, PC2: 13%); c (points) = *D. jobbinsi* (PC1: 20%, PC2: 19%); d (triangles) = *D. leucotibialis* (PC1: 24%, PC2: 11.4%). Aquamarine green = Amazonas; dark blue = Caquetá; brown = Chocó; ligth purple = Meta; pink = Putumayo; dark yellow = Vaupés (see Supplementary Material to look over the PCA of *D. nuneztovari*).

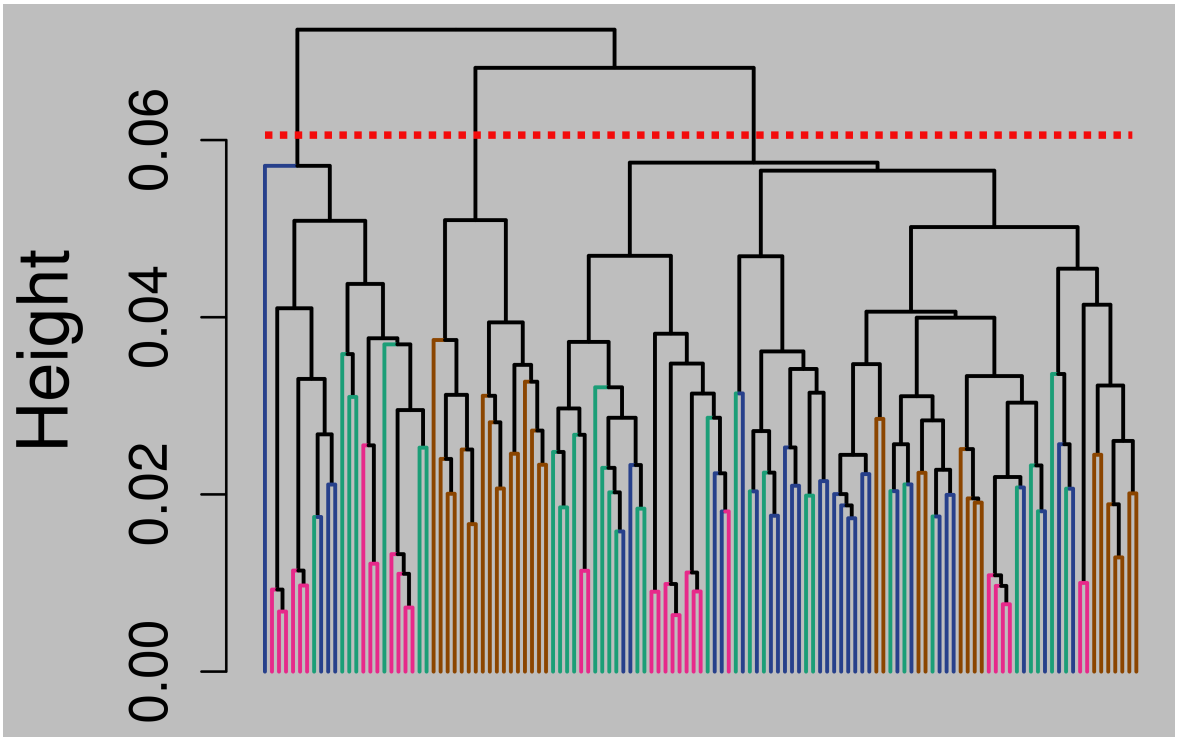

Figure 5. Dendrogram of *D. jobbinsi* (K = 3) from different areas of Colombia by Hierarchical Cluster Analysis (UPGMA). The red dashed line cut the dendrogram into the K-groups defined according to the Bayesian Information Criterion, the "gap" statistic and Calinski and Harabasz (CH) index. Aquamarine green = Amazonas; dark blue = Caquetá; brown = Chocó; pink = Putumayo.

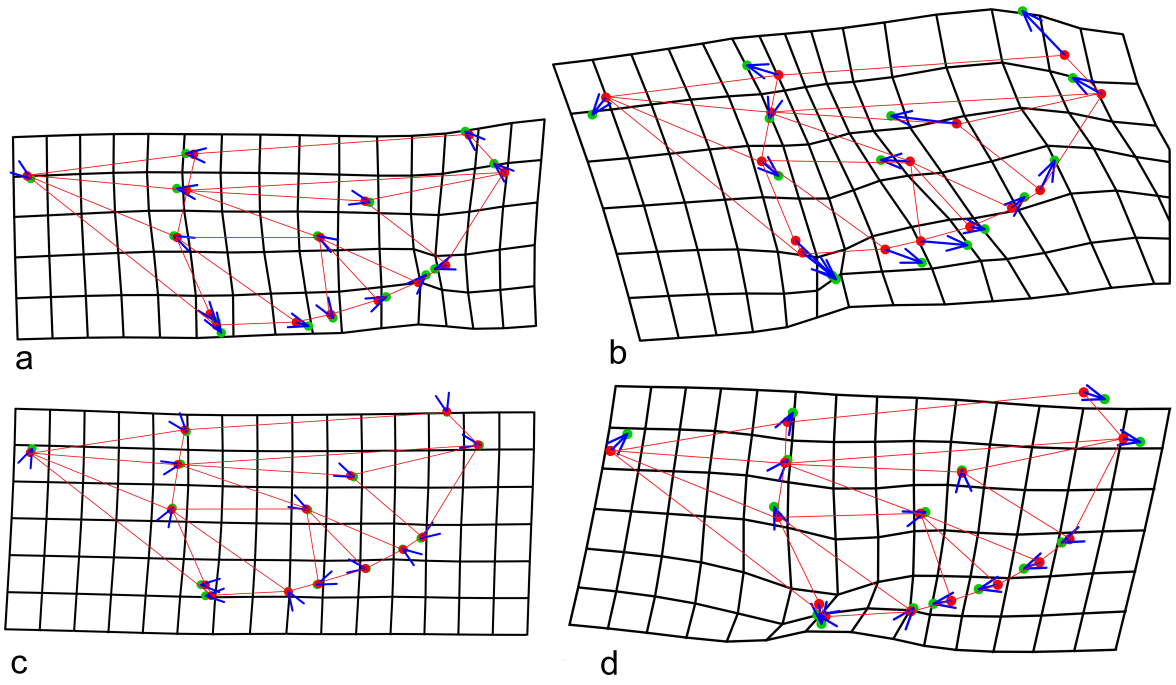

Figure 6. Deformation diagrams, using thin plate spline grids over consensus shapes. a = from *D. leucotibialis* to *D. fuscistigma* (minimum interspecific variation); b = from *D. leucotibialis* to *D. nuneztovari* (maximum interspecific variation); c = *D. fuscistigma*, from Vaupés to Putumayo (minimum intraspecific variation) and d = *D. jobbinsi* from Amazonas to Chocó (maximum intraspecific variation). Red points are landmark configuration of source consensus shape, while green points are landmark configuration of target consensus shape; blue arrows (or blue arrowheads) indicate directions and ammount of shape changes from source to target consensus shapes.

TABLES

Table 1. Species and number of specimens by area (locality) used in this study. Amazonas, "PNN Amacayacu" (AMA); Caquetá, "PNN Serranía de Chiribiquete" (CAQ); Chocó, "PNN Utría" (CHO); Meta, "PNN Sierra de la Macarena" (MET); Putumayo, "PNN La Paya" (PUT); Vaupés, "EB Mosiro Itájura Caparú" (VAU).

	AMA	CAQ	CHO	MET	PUT	VAU	Total
Diachlorus curvipes	30		32	32	32		126
Diachlorus fuscistigma	32	32		32	32	35	163
Diachlorus jobbinsi	32	32	32		30		126
Diachlorus leucotibialis[a]	32	30			32		94
Diachlorus leticia						30	30
Diachlorus nuneztovari[a]	30				32		62
Total	186	94	64	64	190	65	601

[a] Species not previously registered in Colombia

Table 2. Cumulative percentages (%) of variance explained of the first ten components in PCAs by species and genus.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
Genus *Diachlorus*	45.0	56.0	63.9	70.0	75.5	79.7	83.0	85.7	87.7	89.4
Diachlorus curvipes	23.0	34.7	46.0	55.0	61.0	66.3	71.5	75.4	78.7	82.0
Diachlorus fuscistigma	23.0	36.0	46.3	55.5	62.8	68.5	73.0	76.4	79.7	82.6
Diachlorus jobbinsi	20.0	39.0	52.0	61.7	67.3	72.7	76.4	80.0	83.0	85.7
Diachlorus leucotibialis	24.0	35.4	46.2	55.0	62.3	68.3	73.0	76.8	80.0	83.0
Diachlorus nuneztovari	30.0	44.0	54.0	62.0	69.0	73.8	78.0	81.0	84.0	86.7

Table 3. Riemmanian distances among *Diachlorus* species from Colombia, based on wing shape.

	Diachlorus curvipes	*Diachlorus fuscistigma*	*Diachlorus jobbinsi*	*Diachlorus leucotibialis*	*Diachlorus leticia*
Diachlorus fuscistigma	0.02587*				
Diachlorus jobbinsi	0.03292*	0.04486*			
Diachlorus leucotibialis	0.02566*	**0.01807*[a]**	0.04619*		
Diachlorus leticia	0.03685*	0.03366*	0.02783*	0.03925*	
Diachlorus nuneztovari	0.05359*	0.06362*	0.02695*	**0.06568*[b]**	0.04453*

* ($p < 0.05$) in the F test of Goodall

Bold values are the minimum ([a]) and maximum ([b]) Riemmanian distances

Table 4. Riemmanian distances among groups of specimens from different areas. a = *Diachlorus curvipes*; b = *D. fuscistigma*; c = *D. jobbinsi*; d = *D. leucotibialis*. Amazonas (AMA); Caquetá (CAQ); Chocó (CHO); Meta (MET); Putumayo (PUT); Vaupés (VAU). For *D. nuneztovari*, not present in the table, the Riemmanian distance between AMA and PUT is 0.01596*.

a

	AMA	CHO	MET
CHO	**0.01239*[b]**		
MET	0.01200*	0.00872*	
PUT	**0.00594[a]**	0.01128*	0.01159*

c

	AMA	CAQ	CHO
CAQ	0.01189*		
CHO	**0.02497*[b]**	0.02138*	
PUT	**0.01135*[a]**	0.01660*	0.02426*

b

	AMA	CAQ	MET	PUT
CAQ	0.00894*			
MET	**0.01321*[b]**	0.01235*		
PUT	**0.00816[a]**	0.01095*	0.01276*	
VAU	0.00894*	0.00820	0.01235*	0.01095*

d

	AMA	CAQ
CAQ	**0.01977*[b]**	
PUT	**0.01077*[a]**	0.01587*

* ($p < 0.05$) in F test of Goodall

Bold values are the minimum ([a]) and maximum ([b]) Riemmanian distances

Table 5. Confusion matrix of Cross-validated classification of the *Diachlorus* species. The percentages of assignments to the species are presented. Bold values are the correct assignments. Misclassification global error rate for linear function is 0.101; and for quadratic function is 0.406.

	F^u	*Diachlorus curvipes*	*Diachlorus fuscistigma*	*Diachlorus jobbinsi*	*Diachlorus leucotibialis*	*Diachlorus leticia*	*Diachlorus nuneztovari*
Diachlorus curvipes	Lin	**97.6**		1.60	0.80		
	Qua	**98.4**		1.60			
Diachlorus fuscistigma	Lin	0.70	**96.8**		2.50		
	Qua		**96.8**		3.10		
Diachlorus jobbinsi	Lin			**85.6**			14.4
	Qua			**89.6**			10.4
Diachlorus leucotibialis	Lin	1.10	7.70		**90.1**	1.10	
	Qua	1.10	7.70		**90.1**	1.10	
Diachlorus leticia	Lin					**100**	
	Qua					**100**	
Diachlorus nuneztovari	Lin			3.30			**96.7**
	Qua			5.00			**95.0**

F^u - Discriminant functions: Linear (Lin) and quadratic (Qua)

Table 6. Cross-validated classification of the areas where four *Diachlorus* species are distributed: Amazonas (AMA); Caquetá (CAQ); Chocó (CHO); Meta (MET); Putumayo (PUT); Vaupés (VAU). The percentages of assignments to areas are presented. Bold values are the correct assignments. Misclassification global error rate for discriminant functions are: a = *Diachlorus curvipes* (Lin = 0.648; Qua = 0.472); b = *D. fuscistigma* (Lin = 0.660; Qua = 0.553); c = *D. jobbinsi* (Lin = 0.288; Qua = 0.224); d = *D. leucotibialis* (Lin = 0.439; Qua = 0.230). (*D. nuneztovari* confusion matrix is in Electronic Supplementary Material).

a

	F^u	AMA	CHO	MET	PUT
AMA	Lin	**51.8**	6.80	10.3	31.1
	Qua	**75.6**		10.3	13.8
CHO	Lin	3.10	**62.6**	21.8	12.5
	Qua	3.10	**84.5**	9.30	3.10
MET	Lin	9.30	12.5	**71.9**	6.30
	Qua	3.10	9.30	**84.4**	3.20
PUT	Lin	18.7	12.5	15.6	**53.2**
	Qua	18.7	12.5		**68.8**

c

	F^u	AMA	CAQ	CHO	PUT
AMA	Lin	**64.5**	19.3		16.2
	Qua	**83.9**	9.70		6.40
CAQ	Lin	6.30	**84.4**		9.30
	Qua	18.8	**81.2**		
CHO	Lin		3.20	**96.8**	
	Qua			**100**	
PUT	Lin	16.7			**83.3**
	Qua				**100**

b

	F^u	AMA	CAQ	MET	PUT	VAU
AMA	Lin	**50.0**	9.30	3.10	12.6	25.0
	Qua	**65.7**	9.30		6.20	18.8
CAQ	Lin	15.6	**53.2**	9.30	6.30	15.6
	Qua	6.20	**78.1**	3.10	6.30	6.30
MET	Lin			**90.6**		9.40
	Qua			**100**		
PUT	Lin	21.4	21.4	10.8	**25.0**	21.4
	Qua	14.3	14.3	3.50	**53.6**	14.3
VAU	Lin	28.6	22.8	2.80	14.2	**31.6**
	Qua	11.4	11.4		8.60	**68.6**

d

	F^u	AMA	CAQ	PUT
AMA	Lin	**78.2**		21.8
	Qua	**100**		
CAQ	Lin		**96.3**	3.70
	Qua		**100**	
PUT	Lin	6.30	12.5	**81.2**
	Qua	3.10		**96.9**

F^u - Discriminant functions: Linear (Lin) and quadratic (Qua)