MORPHOLOGY, HISTOLOGY, AND FINE STRUCTURE

Cryptic Species of the *Anastrepha fraterculus* Complex (Diptera: Tephritidae): A Multivariate Approach for the Recognition of South American Morphotypes

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ABSTRACT Although a large amount of data have been published in past years on the taxonomic status of the *Anastrepha fraterculus* (Wiedemann) species complex, there is still a need to know how many species this complex comprises, the distribution of each one, and their distinguishing features. In this study, we assessed the morphometric variability of 32 populations from the *A. fraterculus* complex, located in major biogeographical areas from the Neotropics. Multivariate techniques for analysis were applied to the measurements of 21 variables referring to the mesonotum, aculeus, and wing. For the first time, our results identified the presence of seven distinct morphotypes within this species complex. According to the biogeographical areas, populations occurring in the Mesoamerican dominion (Mexico, Guatemala, and Panama) were clustered within a single natural entity labeled as the ‘Mexican’ morphotype; whereas in the northwestern South American dominion, samples fell into three distinct groups: the ‘Venezuelan’ morphotype with a single population from the Caribbean lowlands of Venezuela, the ‘Andean’ morphotype from the highlands of Venezuela and Colombia, and the third group or ‘Peruvian’ morphotype comprised the samples from the Pacific coastal lowlands of Ecuador and Peru. Three additional groups were identified from the Chacoan and Paranaense sub-regions: the morphotype “Brazilian-I” was recognized as including the Argentinean samples with most pertaining to Brazil, and widely distributed in these biogeographical areas; the morphotype “Brazilian-2” was recognized as including two samples from the state of Sao Paulo (Ilha-Bela and São Sebastião); whereas the morphotype “Brazilian-3” included a single population from Botucatu (state of Sao Paulo). Based on data published by previous authors showing genetic and karyotypic differentiation, as well as reproductive isolation, we have concluded that such morphotypes indeed represent natural groups and distinct taxonomic entities.

RESUMEN A pesar de la gran cantidad de información publicada en los últimos años sobre el estatus taxonómico de la especie nominal *Anastrepha fraterculus* (Wiedemann), aun persiste la necesidad de conocer cuantas especies conforman este complejo2, cual es su distribución o cómo podemos distinguirlos?. En este estudio se evaluó la variabilidad morfométrica de 32 poblaciones del complejo *A. fraterculus*, procedentes de las principales zonas biogeográficas del Neotrópico. Mediante el uso de técnicas multivariadas, se evaluaron las mediciones de 21 variables del mesonotum, aculeus y el ala. Nuestros resultados identificaron por primera vez la presencia de siete morfotipos distintos dentro de este complejo de especies. Las poblaciones provenientes del dominio Mesoamericano (México, Guatemala y Panamá) fueron agrupadas en una sola entidad natural denominada morfotipo “Mexicano”; en cambio, las muestras localizadas en el dominio Noroeste Sudamericano fueron segregadas en tres grupos distintos: el morfotipo “Venezolano”, representado por una sola población de las tierras bajas del Caribe en Venezuela; el morfotipo “Andino” que agrupó todas las poblaciones de las tierras altas de Venezuela y Colombia; mientras que un tercer grupo denominado morfotipo “Peruano” fue caracterizado con las muestras procedentes de las tierras bajas costeras del Pacífico en Ecuador y Perú. En las sub-regiones Chaqueña y Paranaense se observaron tres grupos adicionales: el morfotipo

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The genus Anastrepha Schiner comprises >230 valid species and thus represents the most widely diversified group of all Neotropical fruit flies, including some economically important species that reproduce in cultivated fruits (Norrbom et al. 1999, Norrbom 2004, Hernández-Ortiz 2007, Norrbom and Korytkowski 2011). One of these species is Anastrepha fraterculus (Wiedemann), commonly known as the “South American fruit fly” recorded from southern Texas throughout eastern Mexico, Central America, and South America (Stone 1942, Hernández-Ortiz 1992, Hernández-Ortiz and Ahuja 1993, Foote et al. 1993). For the last 70 yr, sufficient information has been documented to suggest that the nominal species A. fraterculus in fact represents a cryptic species complex (AF complex). The first documented evidence appeared in the most comprehensive taxonomic revision of the genus Anastrepha by Stone (1942), who described extensive morphological variation (especially in wing pattern) among specimens from Mexico through to South America, and considered these samples to constitute geographical races. Shortly thereafter, Mexican populations were referred to as the “Mexican form” because of differences in host plant preferences that contrasted with other populations occurring in South America (Baker et al. 1944, Baker 1945).

Since then, a large number of articles have addressed this issue. For example, Mendes (1958) and Bush (1962) described karyotypic differences between Mexican and Brazilian populations, likewise there are descriptions of three different karyotypes within the AF complex, referring to the Brazilian populations from the states of Sao Paulo and Bahia (Solferrini and Morgante 1987). Isozyme analyses performed for samples from several Brazilian regions suggested the presence of four distinct groups (Morgante et al. 1980). Steck (1991) reported sufficient genetic differentiation among several populations of A. fraterculus to distinguish two groups; one including samples from northeastern Brazil (Bahia), the Venezuelan lowlands, Costa Rica and Mexico, and a second group was recognized comprising samples from southern Brazil, the Venezuelan highlands, and Peru.

Studies based on DNA analysis, using restriction fragment-length polymorphism techniques made by Steck and Sheppard (1993), reported high levels of polymorphism in two Venezuelan populations (lowlands of Caracas versus highlands of Mérida), and also when compared two others from Brazil (Bahia in the north versus Sao Paulo in the south). Results by Smith-Caldas et al. (2001) using DNA sequences of the COI gene for 16 samples (mostly from South America), provided additional evidence of multiple gene pools within the nominal A. fraterculus, suggesting the presence of a new species at high elevations in the Andes. Additional morphometric evidence showed that Mexican populations of A. fraterculus could be distinguished from others in Colombia, northern Argentina, and southern Brazil. In fact, samples from Brazil and Argentina were different from Colombian specimens, possibly indicating the existence of two other species (Hernández-Ortiz et al. 2004).

The problem of the AF complex in Brazil is of particular interest, because previous studies have suggested the existence of three species within the complex. These entities were characterized by a combined analysis of isozymes, karyotypes, morphometry, and egg morphology, and were referred to as Anastrepha sp.1 aff. fraterculus, Anastrepha sp.2 aff. fraterculus, and Anastrepha sp.3 aff. fraterculus (Selivon and Perondini 1998, 2007; Selivon et al. 2004, 2005). Likewise, the crossing of Anastrepha sp.1 aff. fraterculus with Anastrepha sp.2 aff. fraterculus resulted in decreased egg hatching and a sex-ratio deviation, in accordance with Haldane’s rule, indicating a degree of reproductive isolation between them (Selivon et al. 1999, 2005). Selivon et al. (2004) also recognized the Anastrepha sp.4 aff. fraterculus in specimens from Guayaquil (Ecuador) on the basis of egg-shell morphology and chromosomes.

Experiments testing mating compatibility among different South American populations have indicated that some of these are not mutually compatible and thus they are reproductively isolated (Vera et al. 2006); paired combinations between the Peruvian sample (La Molina) with populations from Brazil (Piracicaba), Colombia (Ibagué), and Argentina (Concordia and Tucuman) showed a high degree of isolation. According to sexual behavior these populations could be classified into three groups: early morning maters (samples from Concordia, Tucumán, and Piracicaba); noon or midday maters (La Molina and Piura); and late afternoon maters (Ibagué). Recently, Cáceres et al. (2009) assessed mating compatibility, sex pheromones, hybridization, and cytology of two laboratory strains from Argentina and Peru, concluding that these two populations constitute different biological entities.

Clear understanding of taxonomy and subsequent recognition of cryptic species within this complex would have practical implications, because colonies of this pest must be derived from the target population.
if they are to be of use in any project involving the sterile insect technique (Vera et al. 2006). In spite of the current knowledge of the AF complex, including morphology, genetics, and mating incompatibility, important questions still need to be addressed: 1) how many species are represented within this complex?, 2) what taxonomic methods and diagnostic characteristics should be used to identify the cryptic species?, and 3) what are the current distributions of the individual species?. The goal of our study was to assess the morphometric variability of this species complex, by analyzing natural populations distributed from Mexico to Argentina. We also explore the use of multivariate techniques to be incorporated in further diagnostic protocols, for identifying taxonomic entities within the complex and mapping their current distributions within the Neotropics.

Materials and Methods

Sources of Material. We examined 419 specimens in total from 32 populations distributed throughout nine countries within tropical America (Fig. 1). It is well known that morphological variability within the AF complex appears with different ecological conditions and the contrasting host preferences of samples found...
throughout the area. Therefore, the samples compared in this study were selected by location during a single collection event, to ensure the common origin of the specimens as follows:

Argentina: 1) [ARG-Conc] (n = 15): Entre Ríos, Concordia (31°23′13″ S, 58°01′12″ W), ex: Psidium guajava. 2) [ARG-HMoL] (n = 15): Tucumán, Horco Molle (26°46′37″ S, 63°19′49″ W), ex: Psidium guajava. 3) [ARG-Mis] (n = 15): Misiones, Posadas (27°23′59″ S, 55°56′01″ W), ex: Psidium guajava. 4) [ARG-Tucu] (n = 10): Tucumán (27°02′18″ S, 65°19′13″ W), ex: Psidium guajava. Brazil: 5) [BRA-BoTu] (n = 10): Sao Paulo, Botucatu (22°56′18″ S, 45°18′25″ W), ex: Psidium guajava. 6) [BRA-FlIh] (n = 15): Sao Paulo, Ilhabela (23°45′22″ S, 45°21′54″ W), ex: Psidium guajava. 7) [BRA-Pirac] (n = 15): Sao Paulo, Piracicaba 520m (22°43′35″ S, 47°38′48″ W), ex: Psidium guajava. 8) [BRA-SCat] (n = 10): Santa Catarina, Caçador, 986 m (26°46′52″ S, 51°00′52″ W), McPhail trap. 9) [BRA-S pau] (n = 10): Sao Paulo 790 m (23°23′52″ S, 46°37′51″ W), Lab. Colony. 10) [BRA-SSeb] (n = 15): Sao Paulo, Sao Sebastiao 55 m (23°45′42″ S, 45°24′11″ W), ex: Terminalia catappa. 11) [BRA-Ubat] (n = 12): Sao Paulo, Ubatuba (22°46′24″ S, 45°41′52″ W), ex: Terminalia catappa. 12) [BRA-Uber] (n = 15): Minas Gerais, Uberlândia (18°56′56″ S, 48°13′55″ W), ex: Psidium guajava. Colombia: 13) [COL-Cund] (n = 10): Cundinamarca, La Mesa (4°38′50″ N, 74°27′21″ W), McPhail trap. 14) [COL-To] (n = 15): Ibagué, Tolima, Vereda Gamboa 1,600 m (4°26′11″ N, 75°11′29″ W), Lab. Colony originated from Coffea arabica. Ecuador: 15) [ECU-Gua] (n = 15): Guayaquil (2°12′13″ S, 79°53′50″ W), ex: Psidium guajava. Guatemala: 16) [GUA-City] (n = 15): Guatemala City? (14°36′51″ N, 90°32′22″ W), ex: Psidium guajava. Mexico: 17) [MEX-Apaz] (n = 10): Veracruz, Apazapan, Apazapan, 250 m (19°17′00″ N, 96°39′23″ W), McPhail trap. 18) [MEX-Chis] (n = 10): Chiapas, San Vicente 1,400 m (16°11′50″ N, 92°02′57″ W), ex: Psidium guajava. 19) [MEX-Coat] (n = 10): Veracruz, Coatpecate 1,200 m (19°27′25″ N, 96°37′29″ W), ex: Syzygium jambos. 20) [MEX-Jica] (n = 10): Veracruz. Emiliano Zapata, “La Jucayana” 400 m (19°21′44″ N, 96°39′23″ W), McPhail trap. 21) [MEX-QB] (n = 10): Quintana Roo, Chunuhubb, 30 m (19°37′39″ N, 85°35′56″ W), McPhail trap. 22) [MEX-Teoc] (n = 10): Veracruz, Teocelo, Tejería, 980 m (19°23′14″ N, 96°36′59″ W), ex: Psidium guineense. 23) [MEX-Tuxt] (n = 10): Veracruz, Est. Biol. Los Tuxtlas, 160 m (15°35′06″ N, 95°04′12″ W) ex: Psidium guajava. Panama: 24) [PAN-BCol] (n = 17): Barro Colorado Is. 125 m (9°09′05″ N, 79°50′47″ W), ex: Eugenia uniflora. 25) [PAN-LCam] (n = 15): La Campana 61 m (8°44′16″ N, 79°51′20″ W), ex: Psidium guajava. Peru: 26) [PER-LmO] (n = 15): La Molina (Coord. No data). Lab. colony originated from Annona cherimola. 27) [PER-Piru] (n = 15): Piura (7°40′23″ S, 79°12′40″ W). Lab. colony originated from Mangifera indica, Psidium guajava, or both. Venezuela: 28) [VEN-Corr] (n = 15): Zulia, Corrales, 40 m (10°44′35″ N, 71°21′10″ W), McPhail trap. 29) [VEN-DDiaz] (n = 15): Trujillo, Sector Diego Diaz, 1,640 m (Coord. No data), ex: Eriobotrya japonica. 30) [VEN-LMt] (n = 15): Trujillo, Loma Mitimbis, 1,570 m (9°16′57″ N, 70°14′59″ W), ex: Rubus glaucus. 31) [VEN-SDom] (n = 15): Mérida, Santo Domingo, 2,500 m (8°57′37″ N, 71°02′54″ W), ex: Coffea arabica. 32) [VEN-Tig] (n = 15): Trujillo, Tiguanín, 1,900 m (Coord. No data) ex: Psidium candelatum.

Morphometry. Structures such as the mesonotum, aculeus and wing, comprising 21 morphometric traits in total, were assessed for each specimen, using methods described by Hernández-Ortiz et al. (2004). Measurements were expressed as linear distances between two points, also expressed as ratios between two or more variables and qualitative features in wing pattern also were considered. Mesonotal variables were measured directly using a Zeiss stereo microscope and ocular micrometer, whereas the aculeus and wing structures were mounted on permanent slides, before observations. Female terminalia were cleaned in a boiling solution, consisting of 10% sodium hydroxide. Measurements were obtained with a digital camera adapted to the microscope, and the resulting digitized images then were analyzed using Scion Image software (Scion Corporation 2000).

Mesonotum Variables. M1) mesonotal length; M2) mesonotal width at level of poststural supra-alar seta; M3) length from the apex of scutellum to left poststural supra-alar seta (Fig. 2).

Aculceus Variables. A1) total aculeus length; A2) basal width of the aculeus tip: A3) width at beginning of serrated section; A4) basal tip length of nonserrated section; A5) apical tip length of serrated section; A6) length from basal left side to aculeus apex; A7) mean of lateral teeth by side; A8) aculeus tip length (=A4+A5); A9) ratio of the length of nonserrated section versus length of serrated section (=A4/A5); A10) ratio of aculeus tip length versus aculeus length (=A8/A1); A11) ratio of nonserrated section versus aculeus tip length (=A4/A8) (Fig. 3).

Wing Variables. W1) wing length; W2) wing width at R1 apex; W3) width of apical section of S-band (from juncture of S-band and vein R4 + 5 perpendicular to Costal vein); W4) distance from proximal end of proximal arm of V-band on posterior wing margin to apex of vein Cu5; W5) S- and V-band connection between R2 + 3 and R4 + 5 (1 = present; 2 = absent); W6) V-band anterior connection of proximal and distal arms between R1 + 5 and M (1 = present; 2 = absent); W7) ratio of wing width versus wing length (W2/W1) (Fig. 4).

Data Analysis. Discriminant function analyses (DFA) were applied to the entire data set, grouping specimens by their locality of origin in all instances, but samples were analyzed in three separate sets according to their biogeographical location within the major areas of the Neotropical Region (sensu Morrone 2006). Measurements of mean distances between samples were derived from pairwise comparisons of group centroids expressed as Squared Mahalanobis Distances (MD). Average and standard deviations for all variables were calculated for each morphotype found, and functions were tested.
by applying canonical correlation analysis, to assess the significance of the discriminatory power of the model, as well as the specific variables responsible for the segregation of groups. All statistical analyses were carried out using the Statistica software program (StatSoft 2006). For reference purposes, specimens and mounting slides of studied samples were deposited in the Entomological Collections of the Instituto de Ecología AC, Xalapa, Mexico (IEXA), except for the samples from Panama, which were loaned by the United States National Museum, Washington, DC (USNM).

Results

Populations were classified according to their geographical distribution defined by major biogeographical areas in the Neotropical region, resulting in three groups: the Mesoamerican dominion represented by samples from Mexico (7), Guatemala (1), and Panama (2); the Northwestern South American dominion that included samples from Venezuela (5), Colombia (2), Ecuador (1), and Peru (2); and the Chacoan and Paranaense sub-regions that were represented by samples from Argentina (4) and Brazil (8).

Mesoamerican Dominion. Discriminant function analysis was applied to ten samples from Mexico, Guatemala, and Panama, indicating significant differences between samples (Wilks’ Lambda: 0.01383, approx. F(90,695) = 5.9267 P < 0.0000). The discriminant function only included 11 out of the 21 variables considered, whereas pairwise comparisons of morphological similarity among Mexican populations indicated that group centroid distances were very close (MD = 2.5–12.8), except in the case of the MEX-Tuxt sample that showed greater distances with respect to others (MD = 17.4–22.9). Distances between other paired comparisons were close, for instance populations from Mexico versus Guatemala (MD = 8.3–20.5), Mexico versus Panama (MD = 12.3–32.3), and Panama versus Guatemala (MD = 10.9–16.9). The scatterplot contrasting the first two canonical roots for all Mesoamerican samples showed some inter-population variability, but this was not so significantly important that it indicated the existence of more than one morphotype in this biogeographical area (Fig. 5).

Northwestern South American Dominion. A second DFA of samples from the NW-South American dominion comprised ten populations located in Venezuela, Colombia, Ecuador and Peru. Our results yielded a significant level of differentiation for three well-defined groups (Wilks’ Lambda: 0.00011, approx. F(171,974) = 11.698, P < 0.0000). This model included 19 of the 21 variables considered (excluding W2 and W5). A scatterplot of factor scores indicated three separate groups: the first one included all Venezuelan (VEN-LMit, VEN-Tig, VEN-SDom, and VEN-DDiaz)
and Colombian (COL-Cund, COL-Tol) highland populations; a second group comprised a single lowland Venezuelan sample (VEN-Corr); while a third group comprised lowland samples from Ecuador (ECU-Guay) and Peru (PER-Piura, PER-LMol) (Fig. 6). The squared Mahalanobis distances showed high ranges of differentiation among group centroids. For instance, the lowland Venezuelan population (VEN-Corr) appeared well separated from all highland Venezuelan populations (MD = 119.1–129.9), but also from those of Colombia, Ecuador and Peru (MD = 105.5–147.7); similarly, samples from Peru and Ecuador were grouped together, but differentiated from the highland populations of Colombia (MD = 44.7–79.9) and Venezuela (MD = 62.8–114.7).

Chacoan and Paranaense Sub-regions. The third DFA was carried out by comparing 12 populations from Argentina and Brazil, all of them pertaining to the biogeographical sub-regions of Chacoan and Paranaense. The results also evidenced significant differences among samples (Wilks’ Lambda: 0.00113, approx. F(187,1222) = 7.0370, P < 0.0000). Here, the model was produced using 17 out of the 21 morphometric variables (excluding A5, A8, A10, and M3). In this case three discrete groups were recognized; the first one comprising all Argentinian populations (ARG-Tucu,
ARG-HMolle, ARG-Conc, and ARG-Mis) plus most of those from Brazil (BRA-Pir, BRA-SPau, BRA-SCat, BRA-Uber, BRA-Botu). All of them manifested a high degree of morphological similarity, reflected in the Squared Mahalanobis distances that were closer among group centroids (MD = 7.4–26.1). However, other Brazilian populations could be sufficiently differentiated to form two other groups; one comprising the samples Ilha-Bela and Sao Sebastiao (BRA-Ilha and BRA-SSeb), whereas the last one was represented by a single population from Ubatuba (BRA-Ubat) (Fig. 7).

Morphotypes. To demonstrate the accuracy of the natural groups encountered, further analyses of morphological similarities were implemented for all 32 populations. For this we performed a cluster analysis using the Squared Mahalanobis distance matrix, which resulted in the assembly of seven clusters or morphotypes defined as follows: 1) “Mexican” comprising samples from Mexico, Guatemala and Venezuela; 2) “Venezuelan” represented by a single Venezuelan lowland population (VEN-Corr); 3) “Andean” including samples belonging to the highland Andean mountains located in both Colombia and Venezuela; 4) “Peruvian” grouped lowland samples from Peru (PER-LMol, PER-Piura) plus a single one from Ecuador (ECU-Guay); 5) “Brazilian-1” comprising all Argentinian samples (except ARG-Tucu) and most of those from Brazil (BRA-Botu, BRA-SCat, BRA-SPau, BRA-Pira, BRA-Uber); 6) “Brazilian-2” included the samples BRA-Ilha and BRA-SSeb; and 7) “Brazilian-3” represented by a single population from Ubatuba (BRA-Ubat). The average and standard deviations were calculated for the 21 morphometric variables analyzed for all groups (Fig. 8; Table 1).

Further DFA applied to regrouped samples according to morphotypes indicating highly significant differences (Wilks’ Lambda: 0.00120, approx. F(126,2280) = 39.656, P < 0.0000). The segregation among settled groups exposed by previous analyses was sustained, and it was emphasized in the results that the model was produced using all of the 21 variables, 20 of which contributed significantly to the definition of morphotypes. The 3D scatterplot based on the first three canonical variables, showed the average distances for the centroid of each morphotype as well as their relative proximity (Fig. 9; Table 2). The Squared Mahalanobis distances revealed that the Mexican morphotype was closer to the Brazilian-1, Brazilian-2, and Brazilian-3 (MD = 37.03, 34.5, and 31.4 respectively); whereas greater distances were observed in paired comparisons of the Andean morphotype with the Mexican, Brazilian-3, and Venezuelan (MD = 75.3, 75.7, and 80.9, respectively) (Table 3).

Finally, we explored the relationship between expected and observed individual classifications within morphotypes, where our results exhibited a very high percentage of correct classifications according to the seven groups. The classification matrix showed that only a few individuals (3.6%) were classified incorrectly in a group other than that expected. The Mexican morphotype correctly grouped 92.3% of the specimens, whereas the Venezuelan morphotype properly assigned 100%; similarly, most specimens from the Andean and Peruvian morphotypes were accurately classified with 98.8% and 97.8% of cases respectively, whereas the classification of individuals from Argentina and Brazil also produced high accuracy levels within respective morphotypes: Brazilian-1 (98.3%), Brazilian-2 (96.7%) and the Brazilian-3 (91.7%) (Table 4).
Fig. 8. Cluster analysis produced with the Squared Mahalanobis distances matrix of 32 populations of the *A. fraterculus* complex. Clustering method unweighted pair group average.

Key to the Recognition of the *A. fraterculus* Complex Morphotypes (Based on Variables used in the Discriminant Analyses).

1. S- and V-bands connected along vein R4 + 5 (W5), or at least evidence of slight connection; and apical section of S-band wide (W3 = 0.437–0.440 mm) (Figs. 17 and 23) .................................. 2

2. S- and V-bands always separated along vein R4 + 5 (W5); and apical section of S-band narrow (W3 = 0.300–0.380 mm) (Figs. 18–22), if wider, the aculeus tip with ~14 marginal teeth per side .................................................. 3

Table 1. Mean and standard deviations for 21 variables assessed in morphotypes of the *Anastepha fraterculus* complex on the basis of 32 populations from the American tropics

| Variables | Mexican | Andean | Venezuelan | Peruvian | Brazilian-1 | Brazilian-2 | Brazilian-3 |
|-----------|---------|--------|------------|----------|-------------|-------------|-------------|
| A1        | 1.769 ± 0.10 | 1.542 ± 0.10 | 1.945 ± 0.05 | 1.660 ± 0.07 | 1.645 ± 0.07 | 1.795 ± 0.12 | 1.603 ± 0.09 |
| A2        | 0.123 ± 0.01 | 0.125 ± 0.01 | 0.131 ± 0.01 | 0.115 ± 0.01 | 0.116 ± 0.01 | 0.125 ± 0.01 | 0.122 ± 0.01 |
| A3        | 0.087 ± 0.01 | 0.080 ± 0.01 | 0.093 ± 0.01 | 0.076 ± 0.01 | 0.075 ± 0.01 | 0.077 ± 0.01 | 0.081 ± 0.01 |
| A4        | 0.117 ± 0.01 | 0.125 ± 0.01 | 0.142 ± 0.01 | 0.111 ± 0.01 | 0.112 ± 0.01 | 0.122 ± 0.02 | 0.102 ± 0.00 |
| A5        | 0.161 ± 0.02 | 0.126 ± 0.01 | 0.178 ± 0.01 | 0.129 ± 0.01 | 0.141 ± 0.01 | 0.143 ± 0.01 | 0.129 ± 0.01 |
| A6        | 0.285 ± 0.02 | 0.261 ± 0.02 | 0.328 ± 0.02 | 0.246 ± 0.02 | 0.259 ± 0.02 | 0.273 ± 0.03 | 0.237 ± 0.01 |
| A7        | 11.79 ± 1.52 | 10.30 ± 1.10 | 14.13 ± 0.77 | 12.66 ± 0.96 | 9.59 ± 1.00 | 10.19 ± 1.18 | 9.33 ± 0.69 |
| A8        | 0.278 ± 0.02 | 0.254 ± 0.01 | 0.320 ± 0.01 | 0.240 ± 0.01 | 0.252 ± 0.02 | 0.266 ± 0.03 | 0.230 ± 0.01 |
| A9        | 0.734 ± 0.09 | 1.023 ± 0.11 | 0.903 ± 0.06 | 0.864 ± 0.09 | 0.797 ± 0.08 | 0.861 ± 0.11 | 0.793 ± 0.06 |
| A10       | 0.157 ± 0.01 | 0.135 ± 0.01 | 0.165 ± 0.01 | 0.144 ± 0.01 | 0.153 ± 0.01 | 0.148 ± 0.01 | 0.144 ± 0.01 |
| A11       | 0.422 ± 0.03 | 0.504 ± 0.03 | 0.445 ± 0.02 | 0.462 ± 0.02 | 0.442 ± 0.03 | 0.461 ± 0.03 | 0.442 ± 0.02 |
| W1        | 6.265 ± 0.51 | 6.817 ± 0.45 | 7.103 ± 0.26 | 6.367 ± 0.37 | 6.540 ± 0.41 | 6.165 ± 0.55 | 6.274 ± 0.62 |
| W2        | 2.669 ± 0.24 | 2.815 ± 0.21 | 2.903 ± 0.12 | 2.758 ± 0.22 | 2.758 ± 0.19 | 2.737 ± 0.25 | 2.633 ± 0.29 |
| W3        | 0.440 ± 0.04 | 0.300 ± 0.04 | 0.411 ± 0.03 | 0.358 ± 0.03 | 0.374 ± 0.04 | 0.358 ± 0.03 | 0.437 ± 0.05 |
| W4        | 1.404 ± 0.13 | 1.212 ± 0.17 | 1.459 ± 0.10 | 1.291 ± 0.17 | 1.475 ± 0.15 | 1.305 ± 0.10 | 1.397 ± 0.14 |
| W5        | 1.145 ± 0.35 | 1.500 ± 0.00 | 1.933 ± 0.26 | 2.000 ± 0.00 | 1.983 ± 0.13 | 2.000 ± 0.00 | 1.417 ± 0.51 |
| W6        | 1.000 ± 0.00 | 1.906 ± 0.29 | 1.000 ± 0.00 | 2.000 ± 0.00 | 1.861 ± 0.50 | 1.000 ± 0.00 | 1.000 ± 0.00 |
| W7        | 0.426 ± 0.01 | 0.413 ± 0.01 | 0.413 ± 0.01 | 0.433 ± 0.02 | 0.422 ± 0.01 | 0.442 ± 0.01 | 0.416 ± 0.01 |
| M1        | 2.572 ± 0.24 | 2.917 ± 0.23 | 3.159 ± 0.12 | 2.962 ± 0.16 | 2.729 ± 0.18 | 2.915 ± 0.32 | 2.648 ± 0.30 |
| M2        | 1.888 ± 0.15 | 1.893 ± 0.16 | 2.103 ± 0.09 | 1.947 ± 0.12 | 1.841 ± 0.12 | 1.857 ± 0.19 | 1.753 ± 0.21 |
| M3        | 1.906 ± 0.15 | 1.824 ± 0.16 | 2.007 ± 0.08 | 1.635 ± 0.12 | 1.753 ± 0.11 | 1.825 ± 0.20 | 1.748 ± 0.29 |

Valid N 117 55 15 45 115 30 12

Measurements for all variables are expressed in millimeters, except the mean for teeth (A7); ratios between two variables (A9, A10, A11, W7); and qualitative characters (W5, W6).
of serrated section; marginal teeth big and strong, mean number of 9.33 ± 0.7 (A7) (Fig. 16). Specimens from Sao Paulo, Brazil (in part) .......................... Brazilian-3

3. Arms of V-band slender and separated along cell r₄₊₅ (W6) (Figs. 19–20), if diffusely connected, the ratio of A₄/A₅ is near 1.023 ± 0.11 (A9) .......................... 4

Arms of V-band usually broad and always connected along cell r₄₊₅ (W6) (Figs. 18, 21 and 22), if diffusely separated (e.g., some specimens from Argentina), the ratio of A₄/A₅ always <0.800 (A9) .......................... 4

4. Nonserrated section almost as long as the serrated section (A₉ = 1.023 ± 0.11); ratio between nonserrated section and the aculeus tip length of 0.504 ± 0.03 (A11) (Fig. 12). Specimens from Andean highlands of Venezuela and Colombia .......................... Andean

Nonserrated section always <0.9 times as long as the serrated section (A₉ = 0.864 ± 0.09); ratio between nonserrated section and the aculeus tip length of 0.462 ± 0.02 (A11) (Fig. 13); arms of V-band completely separated along cell r₄₊₅ for a distinctive hyaline area (Fig. 20). Specimens from Peru and Ecuador .......................... Peruvian

5. Aculeus tip provided with numerous teeth (A₇ = 14.13 ± 0.77 serrations), without an obvious constriction just before the beginning of serrations; A₃ = 0.093 ± 0.01 mm width; aculeus tip length (A₈) of 0.320 ± 0.01 mm (Fig. 11). Specimens from Caribbean lowlands of Venezuela .......................... Venezuelan

Aculeus tip provided with fewer teeth (A₇ = 9.59–10.19 ± 1.18 serrations), and with an evident constriction just before the beginning of serrations; A₃ = 0.075–0.077 ± 0.01 mm width; aculeus tip length (A₈) < 0.290 mm (Figs. 14–15) .......................... 5

6. Proximal and distal arms of V-band slender (especially the external one), and usually a weak and sometimes diffuse connection on cell r₄₊₅ (e.g., some specimens from Argentina) (Fig. 21). Ratio between length of the nonserrated section and the toothed section of 0.861 ± 0.11 (A9) (Fig. 14). Specimens from Argentina and most of Brazil .......................... Brazilian-1

Table 2. Means of canonical variables resulting from the discriminant function analysis for seven morphotypes of the *Anastrepha fraterculus* complex

| Morphotype    | Root 1 | Root 2 | Root 3 | Root 4 | Root 5 | Root 6 |
|---------------|--------|--------|--------|--------|--------|--------|
| Mexican       | 4.090  | 1.216  | -0.621 | -0.306 | 0.122  | -0.199 |
| Andean        | -4.528 | 1.484  | -1.559 | -0.121 | -0.072 | 0.033  |
| Venezuelan    | 0.763  | 2.586  | 1.647  | 4.451  | 1.280  | 1.037  |
| Peruvian      | -1.968 | 1.089  | 3.432  | -1.237 | 0.762  | -0.000 |
| Brazilian-1   | -0.395 | -2.504 | -0.088 | 0.330  | 0.350  | -0.254 |
| Brazilian-2   | 0.054  | -0.074 | 1.558  | 0.592  | -3.191 | -0.108 |
| Brazilian-3   | 2.266  | -2.625 | -0.869 | -1.726 | -0.425 | 3.114  |
Proximal and distal arms of V-band broad and widely connected on cell r4.5 (Fig. 22). Ratio between length of the non serrated section and the toothed section of 0.797 ± 0.08 (A9) (Fig. 15). Specimens from Sao Paulo, Brazil (in part) ....................... Brazilian-2

Discussion

The Mexican morphotype previously characterized by Hernández-Ortiz et al. (2004) was confirmed in this research, observing populations from Mexico together with others from Guatemala and Panama. Morphologic similarities among Mesoamerican samples seem to be correlated with genetic similarities resulting from the ITS-1 DNA sequences for three of these (MEX-Teoc, MEX-Apaz, GUA-City) when compared with South American samples (Prezotto et al., unpublished data). Thus, the Mexican morphotype is currently distributed in an area stretching from the eastern coastal slopes of Mexico, incorporating the Yucatan Peninsula, extending into Central America, and with its southern distribution limits going as far as Panama. Importantly, it appears that by Mesoamerican highland populations evidently have a preference for feeding on certain host plants including Psidium guajava L. and other myrtaceous fruits, and more rarely on Prunus persica (L.) Batsch (Baker 1945, Hernández-Ortiz et al. 2004), who described a distinct karyotype from the same Ecuadorian sample examined by us (ECU) compared with a single one from the lowland area near Caracas.

Likewise, the Peruvian morphotype was clearly defined, because populations from the Pacific coastal plains of Peru and Ecuador could be clearly defined as constituting a different single taxon. These differences were also previously observed by Selvón et al. (2004), who described a distinct karyotype from the same Ecuadorian sample examined by us (ECU-Guay), which they named as Anastrepha sp.4 aff. fraterculus. The northwest South American samples can be separated into three distinct morphotypes, where even though they come from nearby geographical locations, it is likely that the effect of altitude and particularly ecological conditions, as well as predominant host plants in each of these areas, all play a key role.

Table 4. Classification matrix of individuals grouped by morphotype

| Morphotypes | Percent correct | Mexican | Andean | Venezuelan | Peruvian | Brazilian-1 | Brazilian-2 | Brazilian-3 | Predicted |
|-------------|----------------|---------|--------|------------|----------|-------------|-------------|-------------|-----------|
| Mexican     | 92.3           | 108     | 0      | 0          | 0        | 3           | 0           | 1           | 117       |
| Andean      | 98.8           | 0       | 1      | 0          | 0        | 0           | 1           | 0           | 117       |
| Venezuelan  | 100.0          | 0       | 0      | 15         | 0        | 0           | 0           | 0           | 15        |
| Peruvian    | 97.8           | 0       | 0      | 0          | 44       | 0           | 1           | 0           | 45        |
| Brazilian-1 | 98.3           | 0       | 0      | 0          | 0        | 1           | 0           | 1           | 115       |
| Brazilian-2 | 96.7           | 0       | 0      | 0          | 0        | 0           | 0           | 29          | 115       |
| Brazilian-3 | 91.7           | 0       | 0      | 0          | 0        | 0           | 1           | 11           | 12        |
| Observed    | 96.4           | 108     | 0      | 0          | 16       | 44          | 118          | 35          | 14        |

Rows = predicted classifications; Columns = observed classifications.
role in their isolation. However, the extreme morphometric divergence among Venezuelan, Andean, and Peruvian morphotypes also suggests the disjunct origin of these groups.

Populations examined from the Chacoan and Paraense sub-regions revealed the presence of three further morphotypes. Additional evidence has indicated that three different taxonomic entities occur in Brazil (referred as *Anastrepha* sp.1 aff. *fraterculus* and *Anastrepha* sp.2 aff. *fraterculus*), and these have been identified with reference to their distinct egg-shell morphology (Selivon and Perondini 1998), reproductive isolation, genetics, as well as to their morphological differentiation (Selivon et al. 1999, 2005); whereas a third Brazilian taxon within the complex (named as *Anastrepha* sp.3 aff. *fraterculus*), was distinguished with reference to karyotypic and morphological traits (Selivon et al. 2004). Notably this differentiation into three biological entities in Brazil largely concurs with our results because a number of populations studied by those authors were also included in our investigation, being classified into the following morphotypes: Brazilian-1 (=A. sp.1, samples BRA-Uber, BRA-Botu), Brazilian-2 (=A. sp.2, samples BRA-Illha, BRA-SSeb), and Brazilian-3 (=A. sp.3, sample BRA-Ubat).

The morphotype Brazilian-1 showed the most widespread distribution, as it included most Brazilian samples and all those from Argentina. Also of note is the fact that Argentinian populations showed a close morphometric similarity, although clustering analysis revealed some dissimilarities concerning the ARG-Tucu sample when compared with others, however, this finding requires further investigation. Additional data have shown that Argentine populations belong to a single taxon (recognized by us as Brazilian-1), with evidence from studies of low genetic variability and full sexual compatibility (Alberti et al. 2002, Basso et al. 2003, Petit-Marty et al. 2004). Argentinian populations are associated with 22 different host plants (Oronío et al. 2008), whereas Brazilian populations feed on 76 host plant species (Zucchi 2007). The morphs Brazilian-1 and Brazilian-2 clearly indicate their mutual sexual incompatibility, although they coexist in sympathy for example in the valley of Paraíba (Sao Paulo state), where they infest different hosts; guavas and oranges, respectively (Selivon et al. 1999, 2005), likewise hybrids are rare (D. S., unpublished data). Moreover, the morph Brazilian-3 (=A. sp.3 aff. *fraterculus*) has been detected in the Paranaense coastal regions, where it occurs in sympathy with A. sp.2 infesting the same hosts (guava and tropical almond), however it has also been detected in southern and southeastern areas of the Brazilian Plateau, coexisting with the two other morphotypes (A. sp.1 and A. sp.2), but has never been found in oranges (Selivon and Perondini 2007).

We analyzed some samples used in the studies on sexual compatibility by Vera et al. (2006), which were
also in agreement with the morphotypes encountered by us. For instance, they found full mating compatibility in the case of samples from Tucuman and Concordia (ARG-Tucu versus ARG-Conc), and a low degree of isolation was evident in comparative mating tests of samples from Tucuman and Piracicaba (ARG-Tucu versus BRA-Pira), all of which classified within the morphotype Brazilian-1. In contrast, other tested combinations proved to be highly isolated, as occurred in the case of populations from Tolima (COL-Tol) and La Molina (PER-LMol), recognized by us as falling within the Andean and Peruvian morphotypes, respectively. Similarly, taking the findings of pre- and post-zygotic isolation, as well as the pheromones and chromosomal analyses performed by Cáceres et al. (2009), it was evident that two strains from Argentina (Tucuman) and Peru (La Molina) constitute distinct biological entities. In this regard, our populations from the Tucuman province (morphotype Brazilian-1) were also differentiated from La Molina (PER-LMol), which was the same population that these authors reviewed and subsequently recognized as falling within the Peruvian morphotype.

Based on the morphological similarities among 32 populations within the AF complex, distributed throughout the American tropics, we propose the use of morphometric multivariate analyses for recognizing these seven morphotypes for the first time. Also, with reference to differences of genetics, karyotypes, or sexual compatibility (e.g., Selivon et al. 1999, 2004, 2005; Vera et al. 2006; Cáceres et al. 2009) as observed in some of these populations, we argue that such morphotypes are in fact real taxonomic entities that will be described in the future as constituting valid species.

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