Taxonomy and phenotypic relationships of the *Anastrepha fraterculus* complex in the Mesoamerican and Pacific Neotropical dominions (Diptera, Tephritidae)

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Abstract

Previous morphometric studies based on linear measurements of female structures of the aculeus, mesonotum, and wing revealed the existence of seven morphotypes within the *Anastrepha fraterculus* cryptic species complex along the Neotropical Region. The current research followed linear and geometric morphometric approaches in 40 population samples of the nominal species *A. fraterculus* (Wiedemann) spread throughout the Meso-American and Pacific Neotropical dominions (including Mexico, Central America, Venezuela, Colombia, Ecuador, and Peru). The goals were to explore the phenotypic relationships of the morphotypes in these biogeographical areas; evaluate the reliability of procedures used for delimitation of morphotypes; and describe their current distribution. Findings determined that morphotypes previously recognized via the linear morphometrics were also supported by geometric morphometrics of the wing shape. In addition, we found an eighth morphotype inhabiting the highlands of Ecuador and Peru. Morphotypes are related into three natural phenotypic groups nominated as Mesoamerican-Caribbean lineage,
Andean lineage, and Brazilian lineage. The hypothesis that lineages are not directly related to each other is discussed, supported by their large morphological divergence and endemicity in these three well-defined biogeographic areas. In addition, this hypothesis of the non-monophyly of the *A. fraterculus* complex is also supported by evidence from other authors based on molecular studies and the strong reproductive isolation between morphs from different lineages.

**Keywords**
Cryptic species complex, linear morphometrics, geometric morphometrics, distribution

**Introduction**

Phylogenetic relationships stated that infrageneric classification based on morphology of the genus *Anastrepha* Schiner recognizes nearly 21 species groups (Norrbom et al. 1999, 2012). One of them is the "fraterculus species group" consisting of 34 species, with some widely distributed in the Neotropics (e.g., *A. ludens* (Loew), *A. obliqua* (Macquart), *A. suspensa* (Loew), and *A. fraterculus*). One of these nominal species, the “South American fruit fly” *Anastrepha fraterculus* (Wiedemann) occurs from south of the USA (Texas) through Mexico, Central America to Argentina and represents a cryptic species complex (hereafter denoted as the *Af* complex).

First evidence of the *Af* cryptic species complex appeared in the comprehensive taxonomic revision of the genus *Anastrepha* made by Stone (1942). Since then, other findings from distinct populations along its distributional range were reported supporting this hypothesis, such as differences of karyotypes (Mendes 1958, Bush 1962, Solferini and Morgante 1987, Selivon et al. 2005b); isozyme divergence (Morgante et al. 1980, Steck 1991); DNA sequences (Steck and Sheppard 1993, Smith-Caldas et al. 2001); or studies with multiple approaches including karyotype, isozymes, and morphology (Selivon and Perondini 1998, Selivon et al. 2004, 2005a). Moreover, differences in host range and pest status (Baker 1945, Aluja et al. 2003, Hernández-Ortiz and Morales-Valles 2004, Zucchi 2007); reproductive isolation and sexual incompatibilities (Selivon et al. 1999, 2005a, Vera et al. 2006, Cáceres et al. 2009, Rull et al. 2013, Devescovi et al. 2014); or data on pheromone composition and cuticular hydrocarbon profiles (Břízová et al. 2013, Vaníčková et al. 2015). Morphometric analyses have been a useful technique in detecting morphological differences among organisms to distinguish closely related species of fruit flies (Adsavakulchai et al. 1999, Khamis et al. 2012, Schutze et al. 2012). Based on adult morphology of the *Af* complex, Hernández-Ortiz et al. (2004) developed a morphometric technique using linear measurements of the aculeus, wing, and mesonotum for the full recognition of the Mexican morphotype, separating it from other South American samples from Colombia, Brazil and Argentina. Further linear morphometric studies applied to 32 populations from Mexico, Central America, and South America (including Venezuela, Colombia, Ecuador, Peru, Brazil and Argentina) confirmed previous findings, and added the fact that seven morphotypes could be distinguished within the *Af* complex throughout the Neotropical region (Hernández-Ortiz et al. 2012).
Despite all evidence gathered by different sources, it is still difficult to set out the taxonomic status of the morphotypes mainly due to two reasons. The first one is that other methodological approaches, such as DNA sequences or sexual compatibility have shown large interpopulation divergences, without allowing full identification of interspecific boundaries; and the second one is that information about the overall distribution of the cryptic species still remains uncertain. This is especially true for morphotypes occurring in the North and Central Andes, and for the Brazilian morphotypes.

According to Daly (1985), multivariate methods of morphometric analysis (e.g., DFA, PCA) can be widely applied in biology. However, two general kinds of problems may be encountered in canonical variate analysis of morphometric data: a) linear dependence when two or more variables are highly correlated; and b) heteroscedasticity of the covariance matrices (inequality of dispersion matrices). In this sense, ratios have been used for scaling morphometric variables to remove variation in general body size; to express shape by finding the proportion of one dimension of a structure to another; and to express growth in the size of some structure from one instar to the next. Additionally, because linear distance measurements usually are highly correlated with size, much effort was spent in developing methods for size correction, so that size-free shape variables could be extracted and patterns of shape variation elucidated (Bookstein et al. 1985, Sundberg 1989). The most widespread approach of the geometric morphometrics, is to represent each specimen by the relative positions of morphological landmarks, that can be located precisely and establish a one-to-one correspondence among all specimens included in the analysis (Klingenberg 2010). Shape is defined as all the geometric information about a configuration of landmarks and it is extracted by a procedure called Procrustes superimposition, which removes variation in size, position and orientation from the data on landmark coordinates, and which is at the core of geometric morphometrics (Goodall 1991, Dryden and Mardia 1998, Zelditch et al. 2012).

Another crucial issue for the resolution of this cryptic species complex is understanding the distributional patterns of their morphotypes. Morrone (2014) recently revised the biogeographic regionalization of the Neotropical region. The Mesoamerican dominion comprises lowlands of central and southern Mexico, and most of Central America (Guatemala, Belize, Honduras, El Salvador and northern Nicaragua). The Pacific dominion encompasses southern Central America (southeastern Nicaragua to Panama) and northwestern South America (including western Colombia, Ecuador, Peru, northwestern Venezuela, Trinidad and Tobago, and the Galapagos Islands). Contiguous to these dominions, the Mexican Transition Zone (MTZ) occupies an area where the Neotropical and Nearctic regions overlap, corresponding basically to the mountainous areas of central and southern Mexico and northern Central America; and the South American Transition Zone (SATZ) represented by highlands of the Andes between western Venezuela and northern Chile, and central western Argentina (*sensu* Morrone 2006). In this sense, correlating the occurrence of the different morphotypes to biogeography will add valuable information to delimit the distribution of the species involved.
Given this scenario, systematic studies that identify the incidence areas of the different Af morphotypes throughout the Neotropical region are needed. Increasing the number of samples from Colombia, Ecuador and Peru will confirm previous evidence that suggests that biogeographical and ecological factors in these countries, contribute to the understanding of the distributional patterns of the morphotypes. As such, the goals of this study were to explore phenotypic relationships among different morphs of the Af complex in the Mesoamerican and Pacific biogeographical dominions; to make comparisons of the usefulness of the linear morphometrics and geometric morphometry of the wing shape for delimitation of the morphotypes; and to describe their distributional patterns throughout the biogeographical provinces currently recognized.

Methods

Biological material

We used samples from forty populations obtained from different sources. Most of them were collected from nature directly on their hosts and afterwards reared to adult specimens in the laboratory. Others were collected in McPhail traps baited with hydrolyzed protein, and in few cases, we analyzed samples from laboratory strains established for long time at the Seibersdorf facilities of the FAO/IAEA Agriculture and Biotechnology Laboratories (Austria). Specific data of collection, country, location, and specimens examined are listed in Table 1.

Permanent mounting slides were made prior to observations. Female aculeus was cleaned in a boiling solution, consisting of 10% sodium hydroxide, for approximately 15–20 min; in addition the right wing of each specimen was cut from its base. After that, structures were washed with distilled water and further dehydrated by gradual alcohol series (50, 70, 100% by holding them for 20 min at each step), placed in xylene 2–3 min, and immediately mounted with Canada balsam. Digital images of the mesonotum and wing were made with a digital camera (Olympus C5050) adapted to stereomicroscope (Olympus SZX7); and images of the aculeus were performed using an optical microscope (Olympus BX41) with objective 40X. Permanent slides and pinned voucher specimens of the studied samples were deposited at the Entomological Collections of the Instituto de Ecología AC (Xalapa, Mexico), Universidad del Tolima (Ibagué, Colombia), and the Universidad de las Fuerzas Armadas – ESPE (Quito, Ecuador).

Linear Morphometrics

In total, 612 female specimens were examined, considering 27 morphometric traits of structures such as mesonotum, aculeus, and wing. Variables as linear distances between two points, as ratios of two variables, and qualitative features of wing pattern were assessed following methods described by Hernández-Ortiz et al. (2004, 2012):
Figures 1–4. Morphological structures and variables used for morphometric assessment of the *Anastrepha fraterculus* complex: 1 Thorax in dorsal view 2 Aculeus tip in ventral view 3 Measurements for the linear morphometrics of wing 4 Landmarks used for the geometric analysis of the wing shape.
Table 1. List of samples examined of the *Anastrepha fraterculus* along the Neotropics, showing data of location, georeferentiation, and source of sample

| Sample-Key | Country  | Locality        | Altitude (m) | Coordinates                | Source                  | N Linear | N Geometrics |
|------------|----------|-----------------|--------------|----------------------------|-------------------------|----------|--------------|
| MEX-Jica   | Mexico   | La Jicayana     | 400          | 19°21′44″N, 96°39′23″W     | McPhail trap            | 10       | 10           |
| MEX-Teoc   | Mexico   | Tejería         | 980          | 19°23′14″N, 96°36′59″W     | Psidium guineense       | 10       | 10           |
| MEX-Apaz   | Mexico   | Apazapan        | 250          | 19°17′00″N, 96°39′23″W     | McPhail trap            | 10       | 10           |
| MEX-Coatec | Mexico   | Coatepec        | 1200         | 19°27′25″N, 96°57′29″W     | Syzygium jambos         | 10       | 10           |
| MEX-Tuxt   | Mexico   | Los Tuxtlas     | 160          | 18°35′06″N, 95°04′12″W     | Psidium guajava         | 10       | 10           |
| MEX-Chis   | Mexico   | San Vicente     | 1400         | 16°11′50″N, 92°02′57″W     | Psidium guajava         | 10       | 10           |
| MEX-Tap    | Mexico   | Tapachula       | 150          | ND                         | Seibersdorf Lab-strain  | 6        | 6            |
| MEX-QRoo   | Mexico   | Chunhuahub      | 30           | 19°37′39″N, 88°38′56″W     | McPhail trap            | 10       | 10           |
| GUA-City   | Guatemala | Guatemala City  | 1500         | 14°36′51″N, 90°32′22″W     | Psidium guajava         | 15       | 15           |
| PAN-LCarn  | Panama   | La Campana      | 61           | 08°44′16″N, 79°51′29″W     | Psidium guajava         | 15       | 15           |
| PAN-BCol   | Panama   | Barro Colorado Is. | 125    | 09°09′08″N, 79°50′47″W     | Eugenia uniflora        | 17       | 17           |
| VEN-Corr   | Venezuela | Corrales       | 40           | 10°44′35″N, 71°21′10″W     | McPhail trap            | 15       | 15           |
| VEN-LMitt  | Venezuela | Loma Mitimbis   | 1570         | 09°16′57″N, 70°14′59″W     | Rubus glaucus           | 15       | 15           |
| VEN-DDiaz  | Venezuela | Diego Díaz      | 1640         | ND                         | Eriobotrya japonica     | 15       | 15           |
| VEN-SDom   | Venezuela | Santo Domingo   | 2500         | 08°57′37″N, 71°02′54″W     | Coffea arabica          | 15       | 15           |
| VEN-Tig    | Venezuela | Tiguánín        | 1900         | ND                         | Psidium caudatum        | 15       | 15           |
| COL-Cund   | Colombia  | La Mesa         | 1350         | 04°38′09″N, 74°27′21″W     | McPhail trap            | 10       | 10           |
| COL-Tol    | Colombia  | Vereda Gamboa   | 1600         | 04°26′11″N, 75°11′29″W     | Seibersdorf Lab-strain  | 15       | 15           |
| COL-Bar    | Colombia  | Barbosa         | 1880         | 05°55′57″N, 73°37′16″W     | Psidium guajava         | 13       | 16           |
| COL-Cach   | Colombia  | Tocarema alto   | 1850         | 04°45′01″N, 74°23′01″W     | Coffea arabica          | 20       | 20           |
| COL-Duit   | Colombia  | Duitama         | 2569         | 05°49′29″N, 73°04′29″W     | Acacia sellowiana       | 20       | 20           |
| COL-Rold   | Colombia  | La Aguada       | 1764         | 04°23′05″N, 76°13′20″W     | Coffea arabica          | 20       | 20           |
| COL-Lun    | Colombia  | El Guabo        | 1704         | 01°36′53″N, 77°07′53″W     | Coffea arabica          | 20       | 20           |
| COL-Pen    | Colombia  | Pensilvania     | 2091         | 05°22′03″N, 75°09′29″W     | Acacia sellowiana       | 20       | 20           |
| COL-Sev    | Colombia  | Sevilla         | 1556         | 04°17′19″N, 75°54′23″W     | Coffea arabica          | 20       | 20           |
| COL-Sibu   | Colombia  | Fatima          | 2136         | 01°12′05″N, 76°54′48″W     | Ps. acutangulum         | 20       | 20           |
| COL-Ibag   | Colombia  | Ibagué          | 1433         | 04°24′53″N, 75°18′50″W     | Lab colony-U Tolima     | 20       | 20           |
| Sample-Key | Country    | Locality          | Altitude (m) | Coordinates                        | Source                | N Linear | N Geometrics |
|------------|------------|-------------------|--------------|------------------------------------|-----------------------|----------|--------------|
| ECU-Agro   | Ecuador    | Km39 via la Costa | 7            | 01°57’15”S, 79°55’17”W             | McPhail trap          | 17       | 20           |
| ECU-Guay   | Ecuador    | Guayaquil          | 80           | 02°12’13”S, 79°53’50”W             | Ps. guajava           | 14       | 15           |
| ECU-Bab    | Ecuador    | Recinto Tauín      | 91           | 01°45’29”S, 79°26’50”W             | McPhail trap          | 20       | 20           |
| ECU-Chac   | Ecuador    | Chacras            | 370          | 03°26’51”S, 79°49’53”W             | McPhail trap          | 20       | 20           |
| ECU-Chot   | Ecuador    | Ambuquí            | 1550         | 00°26’50”N, 78°00’18”W             | McPhail trap          | 20       | 20           |
| ECU-Per    | Ecuador    | Perucho            | 1861         | 00°06’48”N, 78°25’33”W             | McPhail trap          | 20       | 20           |
| ECU-Pich   | Ecuador    | Guayllabamba       | 2176         | 00°03’47”S, 78°20’56”W             | McPhail trap          | 20       | 20           |
| ECU-Pat    | Ecuador    | Patate             | 2034         | 01°19’04”S, 78°30’44”W             | McPhail trap          | 20       | 20           |
| PER-Piu    | Peru       | Piura              | 35           | 05°12’00”S, 80°37’00”W             | Seibersdorf Lab-strain | 15     | 15           |
| PER-LMol   | Peru       | La Molina          | 300          | 12°05’21”S, 76°55’41”W             | Seibersdorf Lab-strain | 15     | 15           |
| PER-Chon   | Peru       | Chongona           | 1502         | 12°45’49”S, 72°36’15”W             | McPhail trap          | 7        | 10           |
| PER-Echa   | Peru       | Puente Echarate    | 941          | 12°46’10”S, 72°34’37”W             | McPhail trap          | 11       | 14           |
| PER-VSag   | Peru       | Valle Sagrado      | 2859         | 13°19’00”S, 72°05’21”W             | McPhail trap          | 17       | 17           |
Mesonotum (Figure 1). M1) mesonotal length; M2) mesonotal width at level of postsutural supra-alar seta; M3) length from the apex of scutellum to the left postsutural supra-alar seta.

Aculeus (Figure 2). A1) total aculeus length; A2) basal width of the aculeus tip; A3) width at beginning of serrated section; A4) basal tip length of non-serrated section; A5) apical tip length of serrated section; A6) length from basal left side to aculeus apex; A7) mean number of lateral teeth; A8) aculeus tip length (A4+A5); A9) ratio of the length of nonserrated section/length of serrated section (A4/A5); A10) ratio of aculeus tip length/aculeus length (A8/A1); A11) ratio of length of non-serrated section/aculeus tip length (A4/A8).

Wing (Figure 3). W1) wing length; W2) wing width at R\textsubscript{1} apex; W3) width of apical section of S-band (from juncture of S-band and vein R\textsubscript{4+5} perpendicular to Costal vein); W4) distance from proximal end of proximal arm of V-band on posterior wing margin to apex of vein Cu\textsubscript{1}; W5) S- and V-band connection between R\textsubscript{2+3} and R\textsubscript{4+5} (1 = present; 2 = absent); W6) V-band anterior connection of proximal and distal arms between R\textsubscript{4+5} and M (1 = present; 2 = absent); W7) ratio of wing width/wing length (W2/W1).

Additional variables of ratios between two measurements were assessed as follows: X1) aculeus length/mesonotum length (A1/M1); X2) aculeus length/wing length (A1/W1); X3) mesonotum length/wing length (M1/W1); X4) mesonotum length/mesonotum width (M1/M2); X5) width at beginning of serrated section/length of serrated section (A3/A5); X6) mesonotum width/wing length (M2/W1).

Geometric morphometrics

Eighteen homologous landmark coordinates were digitized on the wings. A total as 626 females belonging to 40 populations distributed from Mexico through Central America, Venezuela, Colombia, Ecuador and Peru were examined.

Landmarks were determined by the intersection or termination of wing veins as follows:

1) junction of humeral and costal veins; 2) subcostal break along costal vein; 3) apex of vein R\textsubscript{1}; 4) apex of vein R\textsubscript{2+3}; 5) apex of vein R\textsubscript{4+5}; 6) apex of vein M; 7) apex of vein CuA\textsubscript{1} on posterior margin; 8) apex of vein CuA\textsubscript{2} on posterior margin; 9) basal bifurcation of R\textsubscript{2+3} and R\textsubscript{4+5}; 10) junction of R\textsubscript{4+5} and cross vein r-m; 11) basal angle of cell bm; 12) junction of M and cross-vein dm-bm; 13) junction of M and cross vein r-m; 14) junction of M and cross-vein dm-cu; 15) junction of CuA\textsubscript{1} and Cu\textsubscript{5}; 16) junction of CuA\textsubscript{1} and cross vein bm-cu; 17) junction of CuA\textsubscript{1} and dm-cu; 18) junction of A and Cu\textsubscript{2} (= apex of cell bcu) (Figure 4).

Data analyses

Linear measurements and the landmark coordinates were acquired from digitized images of wing, aculeus and mesonotum using the TPS DIG software package (Rohlf
The exploratory canonical variate analysis (CVA) of linear morphometrics, applied to 40 populations along the Mesoamerican and Pacific Neotropical dominions showed significant differences among them ($F = 9.40; \text{Wilk's lambda} < 0.0001; \text{DF} = 39/547; p < 0.0001$). The tree of similarities computed from the Squared Mahalanobis distance matrix, supported the presence of six well-differentiated morphotype clusters: the Mexican, Venezuelan, Andean, and Peruvian (previously established by Hernández-Ortiz et al. 2004, 2012), a new cluster designated as Ecuadorian morphotype, and a single population from East-Peru (Figure 5).

The predictive model of linear morphometrics showed that centroid means for the Andean, Peruvian and Ecuadorian morphotypes were mainly differentiated by the CV-1 scores, which contributed with 61.5% of the differentiation. The CV-2 accounted for
Figure 5. Phenogram showing the linear morphometric relationships among 40 population samples of the *Af* complex, based on Mahalanobis distances computed from the CVA. Clustering method UPGMA.

19.6%, distinguishing the East-Peru sample. The CV-3 scores accounted for only 8.4% of the variation among groups (Table 2, Figure 6). Variables with greatest statistical significance were represented by characters of the aculeus (A1, A2, A7, A9), mesonotum (M1, M3), wings (W3, W4, W5), and ratios between two variables (X2, X3, X5) (Table 3).

MANOVA tests showed significant overall differences among morphotypes based on linear morphometry (*F* = 366.73; Wilk’s lambda = 0.0024; DF = 25/2237; *p* < 0.0001), and all Hotelling’s pairwise comparisons (*post hoc*) also led to significant differences (*p* < 0.0001). Furthermore, reliability of morphotypes based on the wing shape also proved to be statistically significant (*F* = 159.93; Wilk’s lambda = 0.0235; DF = 25/2289; *p* < 0.0001), as well as all Hotelling’s pairwise comparisons between each other (*p* < 0.0001).

Morphological similarities through the Squared Mahalanobis Distance matrix (SMD) were assessed by pairwise comparisons among morphotypes. For example, closer distances were noted between morphs such as Ecuadorian vs. Andean (SMD = 15.2), and Peruvian vs. Andean (SMD = 19.7); or moderate distances between Peruvian vs. Ecuadorian (SMD = 37.9), and Mexican vs. Venezuelan (SMD = 37.7) (Table 4). The overall rate of reliability to identify individuals within expected morphotypes was very
Table 2. Chi-Square tests with successive roots removed. Results of five significant variates produced by two morphometric models.

| Model          | Function | Eigenvalue | Canonical R | % Variance | % Cumulative |
|----------------|----------|------------|-------------|------------|--------------|
| Linear morphometrics |          |            |             |            |              |
| 1              | 10.451   | 0.955      | 61.5        | 61.5       |              |
| 2              | 3.333    | 0.877      | 19.6        | 81.1       |              |
| 3              | 1.442    | 0.768      | 8.4         | 89.5       |              |
| 4              | 1.156    | 0.732      | 6.8         | 96.3       |              |
| 5              | 0.624    | 0.620      | 3.7         | 100        |              |
| Geometric morphometrics |          |            |             |            |              |
| 6              | 2.996    | 0.866      | 43.5        | 43.5       |              |
| 7              | 3.333    | 0.877      | 19.6        | 75.0       |              |
| 8              | 1.442    | 0.768      | 8.4         | 89.5       |              |
| 9              | 1.156    | 0.732      | 6.8         | 96.3       |              |
| 10             | 0.624    | 0.620      | 3.7         | 100        |              |

Table 3. Means and Standard Deviations for all measurements of the morphotypes encountered. Linear measures are in mm, except qualitative traits (W5, W6, A7), and ratios (A9, A10, A11, W7, X1, X2, X3, X4, X5, X6). See methods for explanations.

|        | Mexican   | Venezuelan | Andean   | Peruvian | Ecuadorian | East-Peru |
|--------|-----------|------------|----------|----------|------------|-----------|
| A1     | 1.773 ± 0.10 | 1.945 ± 0.05 | 1.801 ± 0.10 | 1.68 ± 0.07 | 1.905 ± 0.12 | 1.727 ± 0.06 |
| A2     | 0.123 ± 0.01 | 0.131 ± 0.01 | 0.123 ± 0.01 | 0.120 ± 0.01 | 0.136 ± 0.01 | 0.116 ± 0.01 |
| A3     | 0.087 ± 0.01 | 0.093 ± 0.01 | 0.080 ± 0.01 | 0.079 ± 0.01 | 0.083 ± 0.01 | 0.077 ± 0.01 |
| A4     | 0.117 ± 0.01 | 0.142 ± 0.01 | 0.120 ± 0.01 | 0.114 ± 0.01 | 0.128 ± 0.01 | 0.122 ± 0.01 |
| A5     | 0.161 ± 0.01 | 0.178 ± 0.01 | 0.126 ± 0.01 | 0.132 ± 0.01 | 0.132 ± 0.01 | 0.143 ± 0.01 |
| A6     | 0.284 ± 0.02 | 0.328 ± 0.02 | 0.253 ± 0.02 | 0.252 ± 0.01 | 0.268 ± 0.02 | 0.271 ± 0.02 |
| A7     | 11.83 ± 1.52 | 14.13 ± 0.77 | 10.97 ± 1.16 | 13.11 ± 1.12 | 10.80 ± 0.97 | 9.65 ± 0.63 |
| A8     | 0.277 ± 0.02 | 0.32 ± 0.01  | 0.250 ± 0.08 | 0.246 ± 0.01 | 0.260 ± 0.02 | 0.265 ± 0.02 |
| A9     | 0.730 ± 0.10 | 0.803 ± 0.06 | 0.954 ± 0.14 | 0.866 ± 0.10 | 0.974 ± 0.14 | 0.856 ± 0.11 |
| A10    | 0.157 ± 0.01 | 0.165 ± 0.01 | 0.139 ± 0.04 | 0.146 ± 0.01 | 0.137 ± 0.01 | 0.153 ± 0.01 |
| A11    | 0.420 ± 0.03 | 0.445 ± 0.02 | 0.485 ± 0.04 | 0.463 ± 0.03 | 0.491 ± 0.04 | 0.460 ± 0.03 |
| W1     | 6.287 ± 0.51 | 7.032 ± 0.26 | 6.653 ± 0.50 | 6.383 ± 0.31 | 7.089 ± 0.35 | 7.521 ± 0.41 |
| W2     | 2.681 ± 0.24 | 2.903 ± 0.12 | 2.837 ± 0.23 | 2.785 ± 0.17 | 3.002 ± 0.15 | 3.126 ± 0.20 |
| W3     | 0.441 ± 0.04 | 0.411 ± 0.03 | 0.314 ± 0.04 | 0.366 ± 0.03 | 0.300 ± 0.03 | 0.454 ± 0.03 |
| W4     | 1.401 ± 0.13 | 1.459 ± 0.10 | 1.317 ± 0.17 | 1.429 ± 0.18 | 1.749 ± 0.11 | 1.936 ± 0.13 |
| W5     | 1.16 ± 0.37  | 1.93 ± 0.26  | 1.98 ± 0.12  | 2.00 ± 0.00  | 2.00 ± 0.00  | 1.59 ± 0.51  |
| W6     | 1.00 ± 0.00  | 1.00 ± 0.00  | 1.70 ± 0.46  | 1.77 ± 0.42  | 1.62 ± 0.49  | 1.00 ± 0.00  |
| W7     | 0.426 ± 0.01 | 0.413 ± 0.01 | 0.427 ± 0.02 | 0.436 ± 0.01 | 0.424 ± 0.01 | 0.415 ± 0.01 |
| M1     | 2.884 ± 0.24 | 3.159 ± 0.12 | 2.879 ± 0.26 | 3.061 ± 0.17 | 3.083 ± 0.20 | 3.005 ± 0.22 |
| M2     | 1.900 ± 0.16 | 2.103 ± 0.09 | 1.856 ± 0.19 | 1.987 ± 0.11 | 2.036 ± 0.13 | 1.984 ± 0.14 |
| M3     | 1.815 ± 0.15 | 2.007 ± 0.08 | 1.792 ± 0.19 | 1.925 ± 0.12 | 1.992 ± 0.12 | 1.910 ± 0.15 |
| X1     | 0.617 ± 0.04 | 0.616 ± 0.02 | 0.628 ± 0.04 | 0.550 ± 0.03 | 0.621 ± 0.06 | 0.576 ± 0.04 |
| X2     | 0.283 ± 0.02 | 0.277 ± 0.01 | 0.271 ± 0.01 | 0.263 ± 0.01 | 0.269 ± 0.02 | 0.231 ± 0.02 |
| X3     | 0.459 ± 0.02 | 0.449 ± 0.01 | 0.433 ± 0.02 | 0.479 ± 0.02 | 0.434 ± 0.02 | 0.399 ± 0.02 |
| X4     | 1.520 ± 0.09 | 1.503 ± 0.04 | 1.555 ± 0.06 | 1.541 ± 0.06 | 1.518 ± 0.08 | 1.515 ± 0.05 |
| X5     | 0.540 ± 0.04 | 0.523 ± 0.04 | 0.635 ± 0.05 | 0.598 ± 0.04 | 0.630 ± 0.07 | 0.537 ± 0.04 |
| X6     | 0.303 ± 0.02 | 0.299 ± 0.01 | 0.279 ± 0.01 | 0.311 ± 0.01 | 0.287 ± 0.01 | 0.264 ± 0.01 |

Valid N 123  15  258  101  98  17
Figure 6. Scatter plot of means of centroids morphotypes from first three Canonical Variates resulted from the CVA applied to linear morphometric model. Percentage in parenthesis indicates the contribution to the differentiation of the groups.

Table 4. Squared Mahalanobis Distances from linear morphometric data produced by pairwise comparisons among morphotypes from Mesoamerica and Pacific Neotropical dominions.

|          | Mexican | Venezuelan | Andean | Peruvian | Ecuadorian | East-Peru |
|----------|---------|------------|--------|----------|------------|-----------|
| Mexican  | 0       | 37.7       | 62.8   | 54.6     | 87.0       | 84.3      |
| Venezuelan| 0       | 0          | 50.8   | 48.1     | 67.0       | 100.7     |
| Andean   | 0       | 0          | 19.7   | 15.2     | 81.2       |           |
| Peruvian | 0       | 0          | 0      | 37.9     | 108.0      |           |
| Ecuadorian| 0      | 0          | 0      | 80.1     |            |           |
| East-Peru|         |            |        |          | 0          |           |

high (96.1%). The correct classification of the specimens according to cross-validation of the CVA was 93.5 and 100% for Mexican and Venezuelan morphotypes, respectively; a range from 96.1–97% for the Andean, Peruvian and Ecuadorian morphotypes; while 100% of individuals from the East-Peru location (Per- Valle Sagrado) were correctly classified (Table 5).

Moreover, the predictive model based on the CVA of the wing shape showed that 43.5% of the variability can be explained by the first canonical variable (CV-1), which
recognizes the closely linked Andean and Ecuadorian morphotypes, and in turn, is clearly divergent from others. The second canonical variable (CV-2) described 31.5% of differences, recognizing the Mexican and Venezuelan morphotypes near each other, but differing from the Peruvian morphotype. The third canonical variable (CV-3) accounted for only 18.8% of the variability among groups. These wing shape variations are represented by the wireframes of morphotypes encountered, showing the change of the shape expected along the first two canonical variables (Table 2, Figure 7).

The allometric variation of the wing shape assessed by multiple regression of log-centroid size vs. shape scores, revealed significant differences ($p < 0.0001$), proving that wing size predicted for only 2.26% of the total shape variation. However though this test proved to be significant it is considered relatively minor given the low percentage shown (Figure 8). *A priori* allocation of individuals into each of the morphotypes resulted in an overall rate of 89.6% with some differences respect to linear model; the Andean and Ecuadorian morphotypes exhibited identification rates of 87.4% and 88.5%, respectively; the Mexican 87.8%, the Peruvian 95.2%, while in the Venezuelan and the East-Peru samples 100% of the specimens were correctly classified (Table 5).

**Phenotypic relationships**

In accordance with the results from previous cluster analysis of the 40 populations examined, morphotypes were linked at higher distance forming three different phenotypical groups herein called the Meso-Caribbean, Andean and Brazilian phenotypic

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**Table 5.** Classification matrix of individuals by morphotypes according to tested models: Above line: Linear morphometrics. Below line: Geometric morphometrics. Rows: observed classifications; Columns: Predicted classifications.

|        | % Correct | Mexican | Venezuelan | Andean | Peruvian | Ecuadorian | East-Peru | N  |
|--------|-----------|---------|------------|--------|----------|------------|-----------|----|
| Mexican| 93.5      | 115     | 7          | 1      | 0        | 0          | 0         | 123|
| Venezuelan| 100.0  | 0       | 15         | 0      | 0        | 0          | 0         | 15 |
| Andean | 96.1      | 0       | 0          | 248    | 1        | 9          | 0         | 258|
| Peruvian| 97.0      | 0       | 0          | 3      | 98       | 0          | 0         | 101|
| Ecuadorian| 96.9   | 0       | 0          | 3      | 0        | 95         | 0         | 98 |
| East-Peru| 100.0  | 0       | 0          | 0      | 0        | 0          | 17        | 17 |
| Linear model| 96.1 | 115     | 22         | 255    | 99       | 104        | 17        | 612|
| Mexican| 87.8      | 108     | 9          | 4      | 0        | 0          | 0         | 2  |
| Venezuelan| 100.0  | 0       | 15         | 0      | 0        | 0          | 0         | 15 |
| Andean | 87.4      | 6       | 5          | 229    | 4        | 18         | 0         | 262|
| Peruvian| 95.2      | 0       | 0          | 1      | 100      | 4          | 0         | 105|
| Ecuadorian| 88.5   | 0       | 0          | 11     | 1        | 92         | 0         | 104|
| East-Peru| 100.0  | 0       | 0          | 0      | 0        | 17         | 17        | 17 |
| Geometric model| 89.6 | 114     | 29         | 241    | 109      | 114        | 19        | 626|

In accordance with the results from previous cluster analysis of the 40 populations examined, morphotypes were linked at higher distance forming three different phenotypical groups herein called the Meso-Caribbean, Andean and Brazilian phenotypic
Figure 7. Average scores for the first two canonical variates (CV1 and CV2) derived from CVA for the total variation of wing shape between morphotypes of the *Af* complex. Wireframes showing the shape changes (red lines) from the consensus configuration of landmarks (blue lines) to each extreme negative and positive of CV scores.

lineages. Multivariate regression analysis (MANOVA) applied to scores obtained from the CVA, proved that accuracy of lineages was highly significant. The linear model showed highly significant differences between lineages ($F = 1150.8; \text{Wilk’s lambda} = 0.0437; \text{DF} = 4/1216; p < 0.0001$), and among all pairwise comparisons (Hotelling’s test, $p < 0.0001$). The predictive model, using the geometric method, also demonstrated highly significant differentiation between lineages ($F = 433.3; \text{Wilk’s lambda} = 0.1746; \text{DF} = 4/1244; p < 0.0001$) and all paired comparisons among them as well (Hotelling’s test, $p < 0.0001$). Mahalanobis distances exhibited remarkable divergence when contrasting morphotypes from distinct lineages; for instance, pairwise comparisons between East-Peru (Brazilian lineage) with all other morphotypes (SMD = 80.1–108), or distances among samples from the Andean lineage vs. the Meso-Caribbean lineage (SMD = 48.1–87.0) (Table 4).

Mesoamerican-Caribbean lineage (shortly named *Meso-Caribbean*). It clustered all samples from Mexico, Central America, and the Caribbean coast of Venezuela. This lineage consisted of the two vicariant Mexican and Venezuelan morphotypes (*sensu* Hernández-Ortiz et al. 2012). The former occurs in the territories of Mexico and Central America, and a single population from the Caribbean coast of Venezuela (Zulia state) distinguished the latter. The linear model showed close similarities among all
samples from Mexico, Guatemala and Panama, and a clear segregation of the Caribbean population (Ven-Corrales). The wing shape model also exhibited similar results, nevertheless keeping separated the Panamanian populations (Figure 9a–b).

This lineage exhibited distinctive morphological features such as the aculeus length (A1 = 1.77–1.95 mm); wider aculeus tip at beginning of serrated section (A3 = 0.087–0.093 mm); longer serrated section (A5 = 0.161–0.178 mm); ratio of non-serrated section/aculeus tip (A11 = 0.420–0.445); and lowest ratio of width/length of serrated section (X5 = 0.523–0.540), like specimens of the Brazilian lineage. Remarkable qualitative features in the wing pattern were also recorded: the typical Costal, S- and V- bands are broad and heavily colored; the upper connection between arms of V- band (W6) in nearly 100% of specimens examined; and wider apical section of S- band (W3 = 0.411–0.441 mm). In the Mexican morphotype, aculeus tip constriction at beginning of serrated section is almost unnoticeable, and connection between S- and V- bands is always present; whereas in the Venezuelan morphotype S- and V- band connection is typically absent in most specimens, and the aculeus tip wider with numerous marginal teeth (A7 = 14.1 teeth per side) (Figures 14–17, 26–29).

Andean lineage. It comprises three clusters of samples: a) the Andean morphotype grouped all 15 populations coming from high mountains of Venezuela and Colombia;
Figures 9–11. Scatter plots of individuals tested by CVA grouping samples by distributional areas: 9a–b Mesoamerican-Caribbean lineage represented by 12 populations from Mexico, Guatemala, and Panama, including the single Venezuela lowland for comparisons 10a–b Five populations from Venezuela 11a–b Eleven populations from Colombia a linear morphometrics b geometric morphometrics of wing-shape. Confidence ellipses 95%.

b) the Peruvian morphotype clustered six lowland populations along the Pacific coast of Ecuador and Peru; and c) the Ecuadorian morphotype, here recognized for the first time, including six highland populations from Ecuador and Peru. We highlight some variables, which may distinguish the morphs of this lineage from others: the apical section of S-band extremely narrow (W3 = 0.300–0.366 mm); S- and V-band con-
Taxonomy and phenotypic relationships of the Anastrepha fraterculus complex...

Figures 12–13. Scatter plots of individuals tested by CVA grouping samples by distributional areas: 12a–b Eight populations from Ecuador 13a–b Five populations from Peru. a linear morphometrics b geometric morphometrics of wing-shape. Confidence ellipses 95%.

ection (W5) missing in near 97% of the specimens examined; V- band arms upper connection (W6) absent in nearly one half of the specimens; and higher ratio between width/length of serrated section (X5 = 0.598–0.635). The Peruvian morph exhibited higher average teeth on the aculeus tip (A7 = 13.1 teeth per side) when compared to Ecuadorian and Andean morphotypes (A7 = 10.8, 10.9 teeth per side, respectively). The Andean morph showed a strong narrowing of apical section of S- band, in addition to distal arm of V- band diffuse and reduced (Figures 18–23, 30–35).

Brazilian lineage. It was recognized by a single population from the high mountains of the East-Andean region in Peru, which showed a clear differentiation from all other samples studied, and a preliminary analysis placed it closely related to the Brazilian morphs (sensu Hernández-Ortiz et al. 2012). We have not fully characterized this lineage, since other Brazilian morphotypes were not reviewed under this perspective. However, we made some comparative inferences from other lineages here described. The sample from East-Peru (Per-Valle Sagrado) exhibited particular morphological traits as follows: shorter aculeus length; aculeus tip narrow and longer in proportion to total length; lowest number of marginal teeth (A7 = 9.6 teeth per side), this last one probably a common feature in populations inhabiting southern Brazil and Argentina (see Hernández-Ortiz et al. 2012). Ratios of the aculeus/wing length (X2 = 0.231), and
Figures 14–25. Typical shape of the aculus tip in morphotypes from the Meso-American and Pacific dominions. Mexican morphotype: 14 Mexico-Apazapan 15 Guatemala-City 16 Panama-La Campana. Venezuelan morphotype: 17 Venezuela-Corrales. Andean morphotype: 18 Venezuela-Loma Mitimbis 19 Colombia-Barbosa. Peruvian morphotype: 20 Ecuador-Agroficial 21 Peru-La Molina. Ecuadorian morphotype: 22 Ecuador-Chota 23 Peru-Echarate. Brazilian lineage: 24 Peru-Valle Sagrado 25 Argentina-Tucuman. Scale bar 0.05 mm.
Figures 26–37. Typical wing patterns in morphotypes from the Mesoamerican and Pacific dominions. Mexican morphotype: 26 Mexico-Apazapan 27 Guatemala-City 28 Panama-La Campana. Venezuelan morphotype: 29 Venezuela-Corrales. Andean morphotype: 30 Venezuela-Loma Mitimbis 31 Colombia-Barbosa. Peruvian morphotype: 32 Ecuador-Agroficial 33 Peru-La Molina. Ecuadorian morphotype: 34 Ecuador-Chota 35 Peru-Echarate. Brazilian lineage: 36 Peru-Valle Sagrado, 37 Argentina-Tucuman.
mesonotum/wing length ($X_3 = 0.399$) showed the lowest values among all samples. This means that wing length is larger than aculeus and mesonotum respect to other morphotypes examined (Figures 24–25, 36–37).

Distribution

The dendrogram of morphometric similarities also provided evidence that more than one morphotype could occur in some South American countries located in the Pacific dominion. Therefore, further discriminant analyses were performed separately.

**Venezuela.** Samples from five locations were considered for the analyses and the results from both linear and geometric morphometry were almost identical. The single population examined of the Caribbean coast (Ven-Corrales) belonged to the Venezuelan morphotype (Meso-Caribbean lineage), and it was distinguished from a second group comprising all four populations coming from the highlands, identified as the Andean morphotype (Andean lineage) (Figure 10a–b).

**Colombia.** Linear morphometric analysis grouped all 11 Colombian populations under the Andean morphotype. Nevertheless, the wing shape analysis revealed three partially differentiated groups: one cluster with individuals from 9 populations, a second cluster with individuals from the laboratory strain of the Vienna facilities (Col-Tolima), and the other one from Ibagué (Col-Ibagué) (Figure 11a–b).

**Ecuador.** The linear morphometrics and wing shape analyses applied to eight populations from Ecuador yielded identical results, forming two distinct morphological clusters inhabiting this country. The four lowland samples were closely related to each other within the Peruvian morphotype (*sensu* Hernández-Ortiz et al. 2012), while the four other samples from the highlands were classified under a new Ecuadorian morphotype, here described for the first time (Figure 12a-b).

**Peru.** Both morphometric techniques applied to five populations analyzed of this country revealed the presence of three different morphological clusters. The first one comprised two lowland samples classified into the Peruvian morphotype (Per-Piura, Per-La Molina). The second cluster was represented by two samples from the highlands (Per-Echarate, Per-Chongona) and belonged to the Ecuadorian morphotype. The third morphological entity, consisting of a single population from the East-region of the Andes (Per-Valle Sagrado), proved to be distinct from all other samples examined, tentatively related to the Brazilian-1 morphotype within the Brazilian lineage (Figure 13a–b).

Distributional patterns based on the current classification of the Neotropical biogeographic provinces (*sensu* Morrone 2014), showed that the Mexican morphotype occurs in areas from 30–1400 m altitude in the Veracruzan, the Pacific lowlands, and the Yucatan peninsula provinces (Mesoamerican dominion); also in the Chiapas highlands province (Mexican Transition Zone), and in Central America recorded from the Guatuso-Talamanca province (north of the Pacific dominion), and probably spread to the Puntarenas-Chiriquí province (Table 6). The Venezuelan morphotype was recorded
Table 6. Distribution of the morphotypes through biogeographical provinces of the Mesoamerican and Pacific dominions (sensu Morrone 2014).

| Morphotype      | Biogeographical Sub-region | Biogeographical Province | Country       | Sample-Key |
|-----------------|-----------------------------|----------------------------|---------------|------------|
| Mexican         | Mesoamerica                 | Veracruz                   | Mexico        | MEX-Jica   |
|                 |                              |                            | Mexico        | MEX-Teoc   |
|                 |                              |                            | Mexico        | MEX-Apaz   |
|                 |                              |                            | Mexico        | MEX-Coat   |
|                 |                              |                            | Mexico        | MEX-Tuxt   |
|                 | Pacific Lowlands             | Mexico                     | MEX-Tap       |
|                 | Yucatan Peninsula           | Mexico                     | MEX-QRoo      |
| Mex Tran Zone   | Chiapas Highlands           | Mexico                     | MEX-Chis      |
|                 |                             | Guatemala                  | GUA-City      |
| Pacific         | Guatuso-Talamanca           | Panama                     | PAN-Lcam      |
|                 |                              | Panama                     | PAN-Bcol      |
| Venezuelan      | Pacific                     | Guajira                    | Venezuela     | VEN-Corr   |
|                 |                              |                            | Venezuela     | VEN-Lmit   |
|                 |                              |                            | Venezuela     | VEN-DDiaz  |
|                 |                              |                            | Venezuela     | VEN-Sdom   |
|                 |                              |                            | Venezuela     | VEN-Tig    |
| Andean          | Pacific                     | Magdalena                  | Colombia      | COL-Cund   |
|                 |                              |                            | Colombia      | COL-Tol    |
|                 |                              |                            | Colombia      | COL-Bar    |
|                 |                              |                            | Colombia      | COL-Cach   |
|                 |                              |                            | Colombia      | COL-Duit   |
|                 |                              |                            | Colombia      | COL-Pen    |
|                 |                              |                            | Colombia      | COL-Ibag   |
|                 | Cauca (north)               | Colombia                  | COL-Rold      |
|                 |                              |                            | Colombia      | COL-Lun    |
|                 |                              |                            | Colombia      | COL-Sev    |
|                 |                              |                            | Colombia      | COL-Sibu   |
| Ecuadorian      | Pacific                     | Cauca (south)              | Ecuador       | ECU-Chot   |
|                 |                              |                            | Ecuador       | ECU-Per    |
|                 |                              |                            | Ecuador       | ECU-Pich   |
|                 |                              |                            | Ecuador       | ECU-Pat    |
| South Brazilian | Yungas                      | Peru                      | PER-Chon      |
|                 |                              |                            | Peru          | PER-Echa   |
| Peruvian        | Pacific                     | Western Ecuador           | Ecuador       | ECU-Guay   |
|                 |                              |                            | Ecuador       | ECU-Agro   |
|                 |                              |                            | Ecuador       | ECU-Baba   |
|                 |                              |                            | Ecuador       | ECU-Chac   |
|                 |                              |                            | Ecuadorian    | PER-Piu    |
|                 |                              |                            | Peru          | PER-LMol   |
| Brazilian complex | S-Am Tran Zone             | Desert                    | Peru          | PER-VSag   |
from the Guajira province (40 m); however, it could reach out other nearby lowlands along the Caribbean coast into the Venezuelan province, as represented by lowland samples near Caracas examined by Steck (1991), and Steck and Sheppard (1993).

The Andean morphotype only occurs in the Pacific dominion along the Magdalena province, occupying the highlands of Venezuela (from 1570–2500 m altitude) and Colombia (from 1350–2569 m); it was also found in several Colombian locations in the north of the Cauca province (Roldanillo, La Union, Sevilla, and Sibundoy). However, in the Colombian Pacific lowlands represented by the Chocó-Darién province, we did not record any sample of the \( \text{Af} \) complex so far.

The Peruvian morphotype was distributed throughout the Pacific Coastal lowlands from Ecuador (7–370 m) and Peru (35–300 m), into the Western-Ecuador and Ecuadorian provinces (Pacific dominion), and the Desert province of the South American Transition Zone. The Ecuadorian morphotype exhibited a distribution along the mountains of the south of Cauca province in the inter-Andean valleys from Ecuador (1550–2176 m), together with two other Peruvian highland samples (Per-Chongona, Per-Echarate) located at 941–1502 m, respectively, in the East-side of the Andes within the Yungas province (South Brazilian dominion). A single population sample was characterized as belonging to the Brazilian lineage, and it was collected in Cusco at the Inca region called Sacred Valley (2859 m), located in the East-side of the Andes into the Puna province of the South American Transition Zone.

**Discussion**

Results showed that the nominal species *Anastrepha fraterculus* (Wiedemann) includes several cryptic species in concordance with previous morphometric findings (Hernández-Ortiz et al. 2004, 2012). Specifically, the \( \text{Af} \) complex consists of eight morphotypes throughout the Neotropics, and in turn, these are related to each other within at least three phenotypic lineages. Major similarities were seen between morphotypes belonging to the same lineage; for example, closer distances were observed between the Mexican and Venezuelan morphotypes (Meso-Caribbean lineage), or between the Andean and the Ecuadorian morphotypes (Andean lineage). The phenotypic proximity between the Ecuadorian and the Andean morphotypes, together with the fact that individuals were partially classified within each other, means that they could have a partial and incomplete isolation.

Linear and geometric morphometric analyses showed similar results, both demonstrating to be useful for diagnosis and recognition of morphotypes presumably representing the cryptic species of the \( \text{Af} \) complex. However, we should also mention that some differences were noted. For instance, differences between samples reared from laboratory colonies, originally stemmed from the same area in Colombia (Col-Tolima, Col-Ibagué) proved to be divergent in wing shape between each other. This is probably due to laboratory strains facing phenotypic selection under artificial conditions over many generations. Therefore, it is advisable to use wild samples for identification of natural morphs, especially if geometric morphometrics is applied. Wing shape analysis
also differentiated two Panamanian samples (Pan-La Campana, Pan-B Colorado) from other populations belonging to the Meso-Caribbean lineage, even though they belonged to field collections. This highlights the need to further investigate other samples from that region to assess natural variation.

It could be argued however, that other factors may have influenced the ultimate morphological phenotype of the wing shape of flies. In particular, altitude has been found to have an impact on the wing shape of the potato moth (Hernández et al. 2010); wing shape differences between the fruit flies *Rhagoletis pomonella* and *R. zephyria*, were hypothesized to have changed in relation to host associations (Yee et al. 2009). However, we consider this might not be the case within the *Af* complex for several reasons. Firstly, the nominal species *A. fraterculus* is highly polyphagous, and host usage, albeit not the same, is highly overlapping among morphotypes (Norrbom 2004, Hernández-Ortiz and Morales-Valles 2004, Zucchi 2007). For example, *Psidium guajava* L. is a host widespread along its geographic distribution and altitudinal range in Mexico, Central America, the Andean countries, Brazil and Argentina. Secondly, the morphometric analysis performed on *A. fraterculus* in Mexico distinguished a single Mexican morphotype, even though eight populations from a wide altitudinal range, and belonging to three distinct host species were examined (Hernandez-Ortiz et al. 2004). Thirdly, in the Brazilian territory there are proofs of the occurrence of three morphotypes (Hernández-Ortiz et al. 2012), and evidence of karyotype differentiation and reproductive isolation supporting the existence of distinct species (Selivon et al. 2004, 2005a, 2005b); however all of them feed on guava, among other hosts.

Species boundaries are related with the extent and limits of gene flow, the selection intensities on ecologically or reproductively functional phenotypes across the species range, and their genetic architecture, all indispensable pieces of information for predicting the course of early lineage divergence and the origins of new species (Shaw 1998). In the biological species concept defined as “groups of interbreeding natural populations that are reproductively isolated from other such groups” (*sensu* Mayr 1969), it is not clear that in all sexually reproducing species, reproductive ties such as gene flow between demes provide the major cohesive force. The concept of interbreeding is a rather complex idea, because hybridization can be discussed in terms of reproductive modes but also in terms of speciation, that is, hybridization as “*prima facie*” evidence for incomplete speciation (Wiley 1981).

By contrast, in the evolutionary species concept defined as “a single lineage of ancestor – descendant populations, which maintains its identity from other such lineages and which has its own evolutionary tendencies and historical fate” (*sensu* Wiley 1978, modified from Simpson 1961), all terminal lineages are evolutionary species or descendant of higher taxa represented by their ancestral evolutionary species. Each branch therefore is the result of a speciation event; however, the concept does not preclude a particular ancestral species from surviving a speciation event. In this regard, De Queiroz (1998) noted that the term lineage is used for a single line of direct ancestry and descent, while a clade is a unit consisting of an ancestral species and its descendants, and are monophyletic in terms of their component species, however, lineages can be paraphyletic or even polyphyletic in terms of their lower level components.
In the broad sense, the monophyly of the \textit{fraterculus} species group has been accepted based on morphology (Norrbom et al. 1999, Norrbom et al. 2012). In several papers it is assumed that reproductive isolation between cryptic species of the \textit{Af} complex has recently evolved, leaving implicit the idea that those morphospecies had direct relationships, and at some time of its evolutionary history there was an interpopulation divergence among them (Cáceres et al. 2009, Segura et al. 2011, Rull et al. 2013, Devescovi et al. 2014). This is probably true among morphospecies related within each phenotypic lineage that could have a common origin and most likely a more recent evolution. However, from a theoretical perspective, the monophyly of the \textit{Af} complex has never been tested so far. Conversely, other studies from various methodological sources stated that this species complex is not monophyletic. This assumption is supported by extreme allele differences found between highland and lowland Venezuelan samples (presumably from distinct lineages), being the largest genetic divergence found among samples studied by Steck (1991). The phylogenetic relationships inferred from mtDNA sequences of COI supported the presence of multiple gene pools and the non-monophyly among samples of the nominal species \textit{A. fraterculus} (Smith-Caldas et al. 2001). In the same way, a phylogenetic relationship analysis based on the nuclear gene period of \textit{Anastrepha} (Barr et al. 2005) with samples from Venezuela (Mérida and Caracas), Mexico, and Brazil (Sao Paulo) found them to be related in different clades.

The occurrence of strong sexual incompatibility between distinct phenotypic lineages also supports the non-monophyly hypothesis. For instance, high levels of pre- and post-zygotic isolation, karyotypic and polytene chromosome differences, and qualitative and quantitative differences in male pheromones were found in two laboratory strains from Argentina and Peru (Cáceres et al. 2009, Segura et al. 2011) which belong to the Brazilian and Andean lineages, respectively. In addition, pre-zygotic reproductive isolation resulted in strong assortative mating to gene flow among the Mexican morphotype and other populations classified in the Brazilian-1 and Peruvian morphotypes (Rull et al. 2013), all of them belonging to three distinct phenotypic lineages described herein. Moreover, there is strong pre-zygotic isolation through temporal partitioning of mating activity of a Colombian population (Andean morphotype) compared with four other morphotypes spanning from Mexico to Argentina (Devescovi et al. 2014).

In fact, the current study reveals that the \textit{Af} complex is integrated by eight morphotypes, which are related into three phenotypic lineages that are virtually endemic, as they are restricted to certain regions, and there is no evidence of contact zones among them so far. The Meso-Caribbean lineage is restricted to the Mesoamerican dominion, to part of the Mexican Transition Zone, and also to the northern of Pacific dominion in Central America and the Caribbean coast of Venezuela. The Andean lineage essentially occupies most of provinces in the Pacific dominion and some parts of the South American Transition Zone; while the Brazilian lineage would be distributed along the Parana dominion in the eastern part of Brazil, and the Chacoan dominion in southern Brazil and northern Argentina.

In this regard, there are also historical processes associated to each biogeographical dominion that cannot be neglected, since they explain the own history of the
biota they inhabit. According to Hoorn et al. (2010) plate subduction along the Pacific margin caused uplift in the Central Andes (Peruvian and Bolivian Andes) during the Paleogene (65 to 34 Ma). The posterior plate breakup in the Pacific, and subsequent collision with the South American and Caribbean plates, resulted in intensified mountain building in the Northern Andes (Venezuelan, Colombian and Ecuadorian Andes) by the late Oligocene to early Miocene (~23 Ma); while plate reorganization ultimately resulted in closing of the Panama Isthmus during the Pliocene (at ~3.5 Ma). These data sustain that the origin of the Northern and Central Andes, and their current connection with Mesoamerica, occurred in remarkable different times. This would mean prolonged periods of isolation between morphotypes inhabiting those geographical areas. In this sense, Drew (2004) stated that high levels of endemism in an area would indicate that speciation has occurred in relative isolation over a considerable time.

The relationship between morphological structure and genotype is complex and poorly understood for most characters, since we need to know if there is a relationship between the morphological characterizations and the real units of evolution (Shubin and Marshall 2000). This idea is particularly relevant when large numbers of sibling species occur. Therefore, from a practical point of view, it is necessary to understand the mechanisms of reproductive isolation between morphotypes, also as an essential precondition for applying control methods such as the sterile insect technique (SIT). However, from an evolutionary perspective, implications of the non-monophyly of the Af complex prevent making direct inferences about mechanisms of genetic or reproductive divergence among populations, since morphotypes belonging to distinct phenotypic lineages might have evolved independently in different clades.

**Conclusions**

In this research, the presence of eight morphotypes is established within the *Anastrepha fraterculus* (Wiedemann) complex, including the first characterization of the Ecuadorian morphotype with samples coming from the mountains of Ecuador and Peru. The morphotypes clustered into three phenotypic lineages we called Meso-Caribbean, Andean, and Brazilian. Based upon their morphological divergence and the current distributional areas, we suggest that these lineages would not have a direct connection with each other and might have evolved separately in these biogeographical regions. In terms of distributional areas or countries, the Mesoamerican dominion was only occupied by the Mexican morphotype. In other countries from the Pacific dominion such as Colombia and Venezuela, two morphotypes were encountered, the Venezuelan inhabiting the Caribbean lowlands of Venezuela, and the Andean in the highlands of both countries. In the territories from Ecuador and Peru, the Peruvian morphotype was found in the lowlands, and the Ecuadorian morphotype in the highlands. Furthermore, in the Eastern side of the Andes in Peru, another morphotype was detected that appears closely related to the morphotypes of the Brazilian lineage.
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