Original Article

Wing Shape in the Taxonomic Identification of Genera and Species of the Subfamily Dolichopodinae (Dolichopodidae, Diptera)

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Abstract

Characters of the wing morphology have significant importance in the systematics and taxonomy of the family Dolichopodidae, but there are only a few studies concerning the variation in wing shape of dolichopodid flies. The detailed analysis of interspecific and generic wing shape variation can provide data for the taxonomic studies, and understanding of the selective forces shaping wing morphometric characters is important for studying of their pattern of evolutionary change. A geometric morphometric analysis was carried out on 72 species belonging to 5 genera of the subfamily Dolichopodinae in order to determine whether wing shape can be successfully used as a character for taxonomic discrimination of morphologically similar genera and species. Canonical variate analysis based on wing shape data showed significant differences among the studied genera and species. Discriminant analysis allowed for the correct genera identification from 74.50% to 91.58% specimens. The overall success for the reassignment of specimens to their a priori species group was on average 84.04%. The detailed analysis of the variation in wing shape in the subfamily and outgroup taxa revealed evolutionary trends, the functional significance of which is discussed.

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Introduction

The Dolichopodinae species have a wide geographical distribution; they particularly abundant in humid forest, shores of water bodies and others wet habitats. The largest genera of the subfamily are *Dolichopus* Latreille, 1796 (about 600 species), *Hercostomus* Loew, 1857 (about 500 species) and *Gymnopternus* Loew, 1857 (about 100 species) (Grichanov, 2018).

Although reliable information on the phylogeny of the subfamily Dolichopodinae is limited, recent studies confirmed monophyly of a clade, consisting of *Dolichopus* and *Gymnopternus*, and their separate systematic position were supported (Brooks, 2005; Germann *et al*., 2011; Pollet, 2003). The genera *Hercostomus*, *Poecilobothrus* Mik, 1878 and *Sybistroma* Meigen, 1824 have been placed in a sister clade. However, before that was shown that *Gymnopternus* is an evolutionary independent entity, European and Russian dipterologists considered the genus *Gymnopternus* as a subgenus of *Hercostomus* (Stackelberg, 1933). A strong dorsal seta on the first segment of hind tarsi can be used as discriminator between *Hercostomus* and *Dolichopus* species; however *Poecilobothrus* and *Gymnopternus* species are not clearly different from *Hercostomus*.

There is a considerable interspecific variation within subfamily in terms of wing morphology, namely in the relative position of distal parts of *R4+5* and *M1+2* (Fig. 1). The terminus of these veins may be subparallel beyond bending *M1+2* (many species of *Hercostomus*) or convergent apically in species of *Dolichopus* и *Gymnopternus* (Pollet, 2003). Diagnostic character of *Dolichopus* species is vein *M1+2* beyond crossvein *dm-m* with obtuse to angular S-shape bend and sometimes with stub vein, while species of *Gymnopternus* are characterized by straight vein *M1+2* and *R4+5* with slight posterior curve in distal section. But several representatives of *Gymnopternus* have straight distal parts of vein *M1+2* and *R4+5*, for example, *Gymnopternus metallicus* (Stannius, 1831).

Such kind of a situation had arisen with the genus *Poecilobothrus*. This genus is a group of relatively large flies with distinct dark spot on notopleuron, triangular cercus and well-developed epandrial lobe of hypopygium (Khaghaninia *et al.*, 2013). Negrobov (1991) proposed a classification in which he downgraded *Poecilobothrus* to subgeneric rank within *Hercostomus*. The recent studies confirmed that *Hercostomus* is polyphyletic group (Bernasconi *et al.*, 2007), and presently *Poecilobothrus* is known as a separate genus, characterized by distinct dark spot above the notopleuron, triangular cercus and medium or large size.

In recent years three species previously assigned to the genus *Hercostomus* were transferred into *Poecilobothrus*: *P. caucasicus* (Stackelberg, 1933), *P. varicoloris* (Becker, 1917) and *P. chrysozygos* (Wiedemann, 1817) (Grichanov, 2018). This fact had made the determination of the diagnostic characters of the genus *Poecilobothrus* much more complicated, because *P. chrysozygos* has trapezoidal cercus, and *P. caucasicus* and *P. varicoloris* do not have
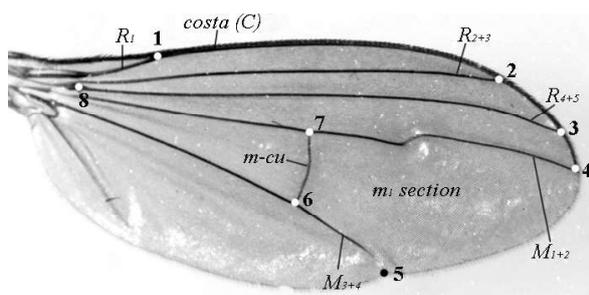
notopleural dark spot. Several species of the genus *Hercostomus* are characterized by the presence of similar spot, for example, *H. convergens* and *H. daubichensis*, and some species (*H. appolo*, *H. phoebus*) are relatively large.

This confusion is not unexpected because the genus *Hercostomus* already long time been a "basket" for species that do not fit into the other dolichopodine genera (Brooks, 2005). The main morphological characters distinguishing between Dolichopodinae species are the morphology of male termilalia and male secondary sexual characters. As a rule, these characters are often used as diagnostic at least some cases (Bickel, 2009), while females of closely-related species are often inseparable morphologically. There are also difficulties in using taxonomic keys hard-to-detect diagnostic characters or characters, the intraspecific variation of which has not been quantified. These difficulties can complicate taxonomic identification and classification.

Because dolichopodid flies play an important role in forest and agro-ecosystems as natural enemies of pests and have considerable potential as bioindicators (Gelbič & Olejníček, 2011), it requires the development of new alternative approaches.

The results of recent studies indicated that morphometric exploring wing shape variation represents an effective approach for finding differences between taxa. Wing morphometric analysis has been conducted in taxonomic studies of flies from the following families: Tabanidae (Torres & Miranda-Esquivel, 2015), Tephritidae (Schutze *et al.*, 2012), Psychodidae (Dvorak *et al.*, 2006) and Caliphoridae (Sontigun *et al.*, 2017). The data resulting of geometric method in conjunction with traditional morphological characters, molecular and ecological data has utility for describing evolutionarily transformation within a character system (Pepinelli *et al.*, 2013; Chazot *et al.*, 2015).

In the present study we used geometric morphometric wing shape analysis to examine the differences among genera and species of Dolichopodinae. This information is expected to allow for the determination of the diagnostic characters of the genera and species. The resulting data of shape variation has the potential to contribute toward a better understanding of trends of evolutionary transformations in the subfamily.

Figure 1. Wing of *Dolichopus cilifemoratus* male and landmarks used in the study.

Materials and methods

In total, 3606 specimens (1513 females and 2093 males) of 70 species of the subfamily Dolichopodinae were examined, representing five the most common dolichopodid genera in Palaearctic region.

Material examined (number of wings): *Dolichopus acuticornis* Wiedemann, 1817 (11 ♀, 5 ♂), *D. argyrotarsis* Wahlberg, 1850 (11 ♂), *D. austriacus* Parent, 1927 (5 ♀, 13 ♂), *D. campestris* Meigen, 1824 (12 ♀, 6 ♂), *D. claviger* Stannius, 1831 (6 ♀, 8 ♂), *D. jaxarticus* Stackelberg, 1927 (12 ♀, 15 ♂), *D. latelimbatus* Macquart, 1827 (60 ♀, 14 ♂), *D. lepidus* Staeger, 1842 (7 ♀, 16 ♂), *D. linearis* Meigen, 1824 (14 ♀, 18 ♂), *D. longicornis* Stannius, 1831 (38 ♀, 50 ♂), *D. longitarsis* Stannius, 1831 (50 ♀, 46 ♂), *D. meigeni* Loew, 1857 (4 ♀, 6 ♂), *D. migrans* Zetterstedt, 1843 (6 ♀, 10 ♂), *D. nataliae* Stackelberg, 1930 (7 ♀, 14 ♂), *D. nigricornis* Meigen, 1824 (4 ♀, 14 ♂), *D. pennatus* Meigen, 1824 (9 ♀, 28 ♂), *D. plumipes* Fallén, 1823 (26 ♀, 98 ♂), *D. plumitarsis* Fallén, 1823 (28 ♀, 62 ♂), *D. ptenopedilus* Meuffels, 1982 (14 ♀, 62 ♂), *D. remipes* Wahlberg, 1839 (18 ♀, 13 ♂), *D. rezvorum* Stackelberg, 1930 (11 ♀, 16 ♂), *D. ringdahli* Stackelberg, 1930 (69 ♀, 81 ♂), *D. rupestris* Haliday, 1833 (25 ♀, 41 ♂), *D. sabinus* Haliday, 1838 (13 ♀, 8 ♂), *D. simius* Parent, 1927 (19 ♀, 36 ♂), *D. simplex* Meigen, 1824 (16 ♀, 32 ♂), *D. trivialis Haliday, 1832* (113 ♀, 148 ♂), *D. ungulatus* (Linnaeus, 1758) (59 ♀, 97 ♂), *D. zernyi* Parent, 1927 (21 ♀, 21 ♂), *Gymnopternus aerosus* (Fallén, 1823) (114 ♀, 142 ♂), *G. angustifrons* (Staeger, 1842) (44 ♀, 16 ♂), *G. assimilis* (Staeger, 1842) (8 ♀, 6 ♂), *G. brevicornis* (Staeger, 1842) (8 ♀, 28 ♂), *G.celer* (Meigen, 1824) (62 ♀, 118 ♂), *G. congruens* (Becker, 1922) (6 ♀, 10 ♂), *G. metallicus* (Stannius, 1831) (81 ♀, 56 ♂), *G. pseudoceler* (Stackelberg, 1933) (5 ♀, 7 ♂), *G. ussurianus* (Stackelberg, 1933) (20 ♀, 19 ♂), *Hercostomus albibarbus* Negrobov, 1976 (10 ♀, 8 ♂), *H. apollo* (Loew, 1869) (6 ♀, 8 ♂), *H. chetifer* (Walker, 1849) (14 ♀, 11 ♂), *H. convergens* (Loew, 1857) (24 ♀, 22 ♂), *H. daubichensis* Stackelberg, 1933 (6 ♀, 6 ♂), *H. eugenii* Stackelberg, 1949 (4 ♀, 6 ♂), *H. excisilamellatus* Parent, 1944 (6 ♀, 9 ♂), *H. fulvicaudis* (Haliday, 1851) (10 ♀, 12 ♂), *H. fugax* (Loew, 1857) (11 ♀, 15 ♂), *H. fulvicaudis* (Haliday, 1851) (10 ♀, 12 ♂), *H. germanus* (Wiedemann, 1817) (24 ♀, 38 ♂), *H. kedrovicus* Negrobov, Logvinovskij, 1977 (10 ♀, 13 ♂), *H. longiventris* (Loew, 1857) (8 ♀, 10 ♂), *H. nigriplantis* (Stannius, 1831) (53 ♀, 70 ♂), *H. phoebus* Parent, 1927 (8 ♀, 11 ♂), *H. pterostichoides* Stackelberg, 1934 (9 ♀, 8 ♂), *H. rivulorum* Stackelberg, 1933 (6 ♀, 7 ♂), *H. rohdendorfi* Stackelberg, 1933 (6 ♀, 8 ♂), *H. rusticus* (Meigen, 1824) (13 ♀, 12 ♂), *H. udovenkovae* Negrobov, Logvinovskij, 1977 (6 ♀, 7 ♂), *H. vivax* (Loew, 1857) (12 ♀, 8 ♂), *Poecilobothrus caucasicus* (Stackelberg, 1933) (9 ♀, 27 ♂), *P. chrysozygos* (Wiedemann, 1817) (40 ♀, 91 ♂), *P. clarus* (Loew, 1871) (8 ♀, 5 ♂), *P. comitialis* (Kowarz, 1867) (6 ♀, 11 ♂), *P. nobilitatus* (Linnaeus, 1767) (24 ♀, 50 ♂), *P. principalis* (Loew, 1861) (11 ♀, 17 ♂), *P. regalis* (Meigen, 1824) (80 ♀, 221 ♂), *P. varicoloris* (Becker, 1917) (24 ♀, 25 ♂), *Sybistroma binodicornis* Stackelberg, 1941 (11 ♀, 15 ♂), *S. crinipes* Staeger, 1842 (2 ♀, 12 ♂), *S. obscurella* (Fallén, 1823) (22 ♀, 22 ♂), *Sympycnus pulicarius* (Fallen, 1823) (25 ♀, 12 ♂).

Sympycnus pulicarius (Fallen, 1823) (subfamily Sympycninae) served as outgroup taxon. Dolichopodinae specimens were taken from the collection of Voronezh State University (Voronezh, Russia). For the widespread species, we selected specimens from as many localities as possible in order to cover the range of intraspecific variation.

We used species of the subfamily Sympycninae as outgroup taxon for the following reasons. The morphological analysis revealed a close relationship between Dolichopodinae and Sympycninae (Ulrich, 1981). The most complete study of phylogenetic relationships of Dolichopodidae was provided by Bernasconi (2007) and Lim *et al.* (2010). As demonstrated by these analyses, sympycnine species made separated and well supported clade.

Wings of each fly were removed from body and mounted on a glass slide and covered with a cover glass. The slides were photographed by means of a Levenhuk C NG microscopic camera. Over each photo, a configuration of 8 type I landmarks (Fig. 1) was digitized using the software tpsDig.

Then geometric morphomertic analysis was performed. Firstly, a generalized Procrustes analysis was conducted in several steps: landmarks configurations were scaled to a unit of centroid size for eliminating the impact of variation in wing size, superimposed so that the centroid of each had coordinates (0, 0) and

rotated so that the distance between landmarks of all specimens become minimal. A new set of variables (Procrustes residuals) contained the shape information and were used as shape data (Zelditch & Swiderski, 2004).

The centroid size of each wing was calculated to characterize an overall measure of wing. All morphomertic and statistical analyses of these sets of variables were performed using MorpholJ software and Statistica 10 for Windows.

In order to prove evidence for the significant differences in wing centroid size and shape among the genera and species, one-way analysis of variance (ANOVA) with a Tukey post-hoc test and a multivariate analysis of variance (MANOVA) was undertaken. Principal component analysis (PCA) was performed as an ordination method to describe the patterns of wing shape variation and to calculate the positions of each taxon within the morphospace. The shape changes associated with principle axes were visualized through thin-plate spline technique (Bookstein, 1991). Comparative analyses of Dolichopodinae flies with outgroup taxa allowed for the determination of plesiomorphic and apomorphic characters states of wing shape.

We used Canonical Variate Analysis (CVA) combined with Discriminant Analysis (DA) to evaluate of diagnostic characters of the genera and to examine a probability of belonging of each specimen to its a priori group (genera). The percentages of correct classification were used to evaluate the discriminatory power of wing shape.

Then we quantified the intraspecific variation in wing shape to determine whether wing shape can be an effective tool to separate taxa on species level. CVA and DA allowed the estimation the differences in wing shape and the classification success like in the previous case.

The matrix of the morphometric data was analyzed heuristically with stepwise addition option using Mesquite software. The tree was reconstructed by squared-change parsimony. These methods has been used extensively for displaying evolutionary change of morphometric traits.

Results

Highly significant differences in wing centroid size were observed both among genera ($F = 16.3$, $P < 0.0001$, $df = 4$) and among species ($F = 27.0$, $P < 0.0001$, $df = 71$) (results of ANOVA). The Tukey post hoc test revealed no wing size differences between *Poecilobothrus* and *Dolichopus*, *Hercostomus* and *Gymnopternus*. The MANOVA results indicated that there are significant wing shape differences among genera (Wilks' Lambda = 0.057, $F = 701$, $P < 0.0001$, $df = 48, 30561$) and among species (Wilks' Lambda = 0.00002, $F = 168$, $P < 0.0001$, $df = 828, 93115$). The Tukey post hoc test revealed significant differences between all genera ($P < 0.001$).

Intergeneric variation and taxonomic discrimination. Detailed comparison of landmark configurations after Procrustes superimposition made it possible to determine wing shape features of studied genera. Landmarks 1, 5, 6 and 7 had
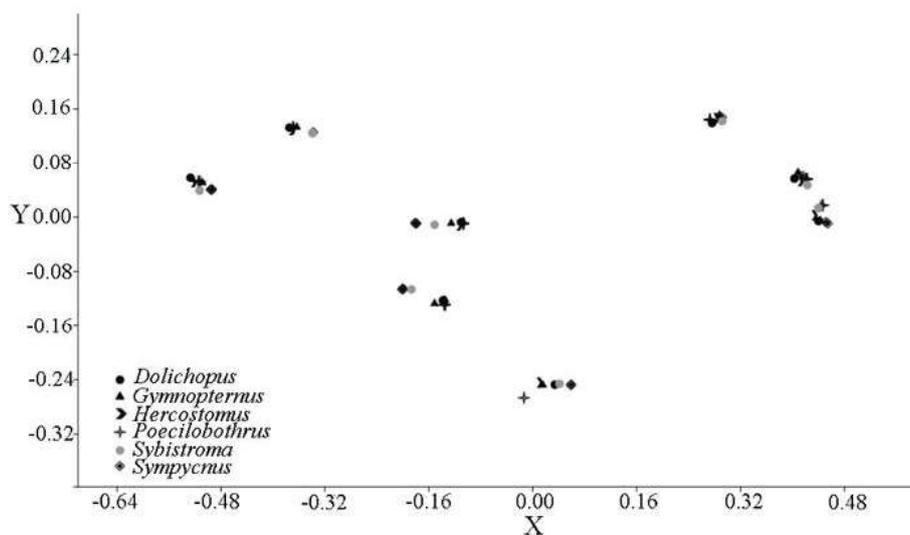

Figure 2. Landmark configurations of the five Dolichopodinae genera and one outgroup genus after Procrustes superimposition.
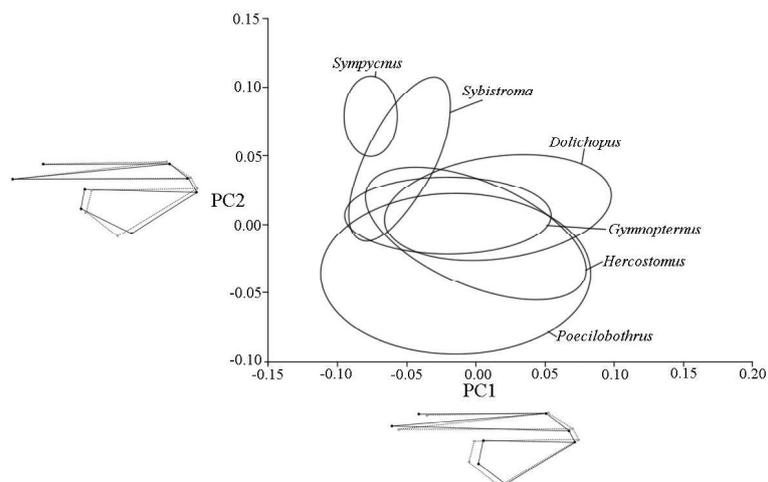
Figure 3. Scatter plot from the first two principle components for Dolichopodinae genera and one outgroup genus with the associated shape changes.

the largest displacements. The posterior crossvein (landmarks 6 and 7) had most proximal position in *Sympycnus* species (Fig. 2), followed *Sybistroma*. *Dolichopus*, *Hercostomus*, *Gymnopternus* and *Poecilobothrus* species had more distal crossvein. Besides that, *Sybistroma* and *Sympycnus* species exhibited the most distal insertion point of *R1* with costal vein.

Landmark and 5 (the insertion point of *M3+4* with wing edge) also exhibited high variation among studied genera. The most distal position of this point was observed in *Sympycnus* species, *Poecilobothrus* possessed the most proximal position of this point. The other landmarks did not vary widely among dolichopodine genera.

PCA results in two principle components accounting 70.02% of the overall wing shape variation. The first principle component (PC1) explained approximately 47.44% of total variability. As shown by thin-plate splines, PC1 reflects displacement of landmarks 5, 6 and 7 in such a way that relative area of *m1* section decreases along the axis (Fig. 3). In other words, the first axis (PC1) described a variation from a wing with relatively large *m1* section (*Sympycnus* species) to a wing with decreasing *m1* section (such species as *Poecilobothrus varicoloris*, *P. caucasicus*, *Dolichopus claviger*).

The second principle component (PC2) accounted for about 22.58% of the variance and showed displacement of landmarks 5 toward the distal part of the wing, in other words, PC2 described a variation from a wider wing with shorter *m1* (for example, *Hercostomus rivulorum*, *Poecilobothrus comitialis*) to an elongated wing

with extended *m1* (*Sympycnus*).

Two first canonical variates (CV1) accounted about 84% of the total variability. As shown on Fig. 4, CV1 reflects displacement of landmarks 4 and 5 toward each other. The CV1 axis (66.93% of total variability) exhibited a variation from a wider wing pointed apically (*Poecilobothrus* species) to an elongated wing with more obtuse apex (*Dolichopus* and *Gymnopternus* species).

The second canonical variate (CV2) accounted 17.15% of the variance, was associated with displacements of landmarks 2 and 3 toward the posterior margin of the wing and can be described as a variation from a wider wing (*Gymnopternus* species) to a wing pointed apically (*Sybistroma*). CV2 clearly separated *Sybistroma* and *Gymnopternus* species.

The scatter plot from CV1 and CV2 showed some overlap among studied genera (Fig. 4); this fact was confirmed by the results of discriminant analysis (Table 1). The percentage of correctly classified was only about 86%, which indicated wing shape is not a reliable predictor of intergeneric discrimination. *Dolichopus* was the best-assigned genus, while *Hercostomus* had the lowest accuracy.

Ten percent of *Hercostomus* specimens (namely, *H. apollo* and *H. phoebus*) resembled and were classified as *Poecilobothrus*. Overall 83.3% of *Poecilobothrus* specimens were correctly identified to genus, the rest of the specimens belonging mainly to *P. chrysozygos*, were classified as *Hercostomus*. Misclassifications of *Gymnopternus* caused mainly by cases of classification *Gymnopternus* specimens as
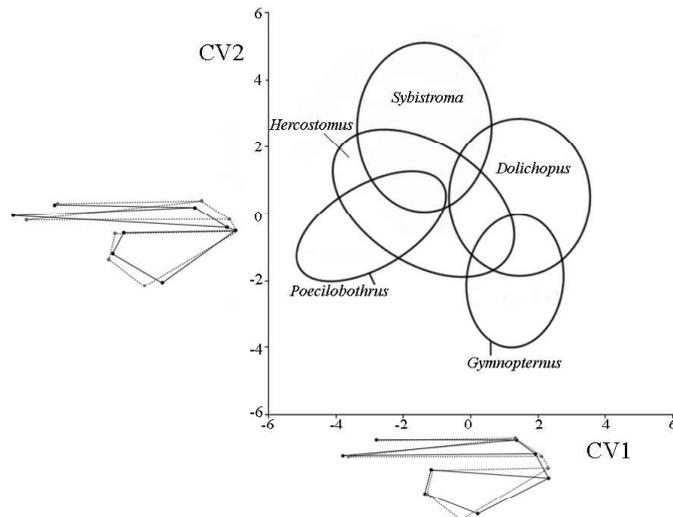
Figure 4. Scatter plot from the first two canonical variates of wing shape for the five Dolichopodinae genera with the associated shape changes.

Dolichopus. These cases of misclassification was not confined to certain species and probably caused by a measurement error.

Identification of species. Nineteen species (*Dolichopus meigeni*, *D. ungulatus*, *D. latelimbatus*, *D. lepidus*, *D. longitarsis*, *D. rezvorum*, *D. ringdahli*, *D. sabinus*, *Gymnopternus metallicus*, *Hercostomus* convergens, *H. germanicus*, *H. pterostichoides*, *H. rivulorum*, *Poecilobothrus chrysozygos*, *P. nobilitatus*, *P. regalis*, *P. varicoloris*, *Sybistroma crinipes* and *S. obscuripes*) were the best-assigned, reaching 95±5% of accuracy. *Gymnopternus assimilis* demonstrated the worst assignment (0.00%); 40% of its specimens were assigned as *Gymnopternus aerosus* and 60% as *G. celer*. Another case of the worst accuracy (0.00%) was showed by *Hercostomus daubichensis*, the specimens of which were identified as *Gymnopternus aerosus*, *G. celer*, *G. pseudoceler*, and *G. ussurianus*.

Hercostomus fulvicaudis, *H. vivax* and *P. clarus* also exhibit low percentages of correct assignments (from 23 to 35%). Otherwise the classification success on species level ranges from 42.85 to 89.58%.

The following species of the genus *Dolichopus* showed a trend to cluster in the shape space: *D. cilifemoratus*, *D. claviger*, *D. rezvorum*, *D. migrans*, *D. plumitarsis*, *D. ptenopedilus*, *D. simius*, *D. linearis* (PC1 from 0.075 to 0.010; PC2 from 0.02 to 0.05) (Fig. 3). These species showed a combination of both morphometric traits: displacement of *dm-m* toward the distal margin of the wing and widened wing. This combination stands out in the subfamily, because otherwise displacement of *dm-m* toward the distal margin of the wing was combined with narrowing of wing (*Poecilobothrus nobilitatus*, *Hercostomus appolo*).

The position of *Poecilobothrus* species within the morphospace should be also focus attention.

Table 1. Assignment of specimens to their *a prori* defined groups (genera).

	1	2	3	4	5	6	% correct
1. *Dolichopus*	3458	72	233	12	1	0	91.58
2. *Hercostomus*	73	888	77	123	31	0	74.50
3. *Gymnopternus*	161	4	1027	0	0	0	85.99
4. *Poecilobothrus*	16	256	0	1357	0	0	83.30
5. *Sybistroma*	0	32	0	0	154	0	82.80
6. *Sympycnus*	0	0	0	0	0	74	100,00
Total	3708	1251	1337	1492	187	74	86.32

Rows: observed classification; columns – predicted classification.

Although the genus was represented by relatively few species, its confidence ellipse occupied the widest space, because *Poecilobothrus* species were occupying an extreme position relative to the axis PC1. *P. varicoloris* were placed in the bottom right corner (PC1 = 0.07) and had a shorter *m1* section; and *Poecilobothrus comitialis* were placed in the bottom left corner (PC1 = –0.1) and had elongated *m1*.

Discussion

The first result of our study is the determination of taxonomic value of wing shape for dolichopodid flies. Analysis based on the shape data classified 86.32% of the specimens to the correct genera and about 84% of the specimens to the correct species. Therefore, our results show that geometric morphometric analysis has utility for discriminating taxa of dolichopodine flies, but wing shape as taxonomic character is of limited use, and must be complemented by traditional morphological traits.

Broad overlap zone between *Hercostomus* and other genera, which has been exhibited by canonical variate analysis, was a major issue. This suggests that there are important challenges in the finding of diagnostic characters that can be used to distinguish between *Hercostomus* and other dolichopodine genera or separation of *Hercostomus* by subgenera.

The results derived from wing shape analysis were not always consistent with standard Dolichopodinae taxonomy. Wing shape of *H. phoebus* and *H. appolo* is more similar to those presented in the genus *Poecilobothrus*, while they formally would not be include in this genus on the basis of cercus morphology (*Hercostomus phoebus* has strip-shaped cercus and *Hercostomus appolo* has oval ones) and the absence of dark spot above the notopleuron. In contrast, *Poecilobothrus chrysozygos* tended to cluster with *Hercostomus* species in the shape space.

With regard to these cases, two explanations are possible. On the one hand, such close similarity between the species with respect to wing shape may reflect close phylogenetic relationship between species. On the other hand, previous studies showed varying degrees of phylogenetic signal in wing shape. The data suggest that there were parallel trends indicating a presence of homoplasy. Therefore, distantly related species also may tend to cluster together in the shape space.

Natural selection acting on flight behavior may lead to similar wing shape in not closely related species. Such ecological drivers of wing evolution could include the following: microhabitat-, predator- or prey selection (Combes & Daniel, 2001). Suggesting that both closely related species and species that occupy the same microhabitat may tend to cluster in the shape space.

With respect to assign exemplars into their *a priori* species, some species were better distinguished than others. It could be assumed that the reason for low percentages of correct classification were the small sample size (*Gymnopternus assimilis*, *Hercostomus daubichensis*), but other species represented by small number of individuals were correctly reassigned to their respective species (*Dolichopus meigeni* – 100%, *Hercostomus eugenii* – 80%, *H. rivulorum* – 100%). It is likely that cases of environmental specialization cause result in clearly differ wing shape (for instance, *Dolichopus meigeni*, *Hercostomus eugenii*, *H. rivulorum*).

The second notable result of our study was the establishment of evolution trends in wing shape in the subfamily Dolichopodinae. Overall shape changes primarily occurred by the displacement of: (a) 5, 6 and 7 landmarks, which determine the position of posterior crossvein *dm-m* and apices of *M3+4*; (b) 3 and 4 landmarks, determining the position of the apices of *R4+5* and *M1+2*; and (c) 1 landmark – the insert point of *R1* with costa.

Comparison with outgroup taxa reveals that the proximal position of posterior crossvein *dm-m* and a longer *R1* are plesiomorphic in the subfamily Dolichopodinae, so the wing shape of *Sybistroma* can be construes as the most plesiomorphic in the subfamily. Other dolichopodine genera characterized by a more distal position of *dm-m*. This result is in good agreement with current phylogeny hypotheses.

The second trend within the subfamily is displacement of the apices of *R4+5* and *M1+2*, resulting in the apex of wing exhibiting a trend toward a more pointed shape. According to phylogenetic relationships of dolichopodid flies, wing pointed apically as a discrete character has evolved several times independently in the subfamily Dolichopodinae by the different ways: (a) *Sybistroma* exhibited a shift of landmark 4 toward a more anterior position, which correlated

with displacement of landmark 7 toward the distal margin of the wing; (b) wing shape of *Hercostomus* and *Poecilobothrus* was formed through displacements of landmark 3 toward the posterior margin of the wing; (c) several *Dolichopus* species showed a convergence of *R4+5* and *M1+2*, combined with the displacements of their apical sections toward the anterior wing margin.

Ennos (1989) identified three functional wing types. According to this classification, Dolichopodidae possess wings capable of ventral flexion. The wings of this type can be bent in the basal part, another flexion line occurs in distal part of the wing. A change in location of termius *R1* toward the base of the wing causes shifting of ventral flexion line, while changes in the placements of the crossvein, cubitus and media forming a "false margin" results in displacement of distal flexion line. Generally, the closer posterior crossvein is to the wing margin and the closer terminus of *R1* is to the wing base, the more accurately will they be able to control the wing shape.

Another trend that has been observed by the analysis is the change of the wing width through displacements of landmark 5 along the Y axis, which was discovered in *Poecilobothrus*, *Hercostomus* and *Dolichopus* species. This trend toward broadening of the wing blade suggests that these species are better adapted to behaviors requiring maneuverability during flight, for example, for greater predator escape ability (Combes & Daniel, 2001) than the more plesiomorphic species.

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