

Short Communication: Morphometric variations in two populations of *Limnophora marginata* Stein, 1904 (Diptera, Muscidae) in Ecuador

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Abstract. Ruiz MA, Acosta-López C, Soto-Vivas A. 2021. Short Communication: Morphometric variations in two populations of *Limnophora marginata* Stein, 1904 (Diptera, Muscidae) in Ecuador. *Biodiversitas* 22: 2654-2657. *Limnophora* includes 230 species, eleven of which are present in Ecuador. There are few studies on the bioecology of these species in the country. However, recent studies indicate that *Limnophora marginata* Stein, 1904 as the most abundant species in the Evergreen High Montane Forest (EHMF) located at 3400 masl in Tocachi parish, in contrast to a low abundance in the Dry Forest (DF) at 2500 masl in Malchingui parish, Pedro Moncayo Canton. This study was described the wing morphogeometric variation of *L. marginata* associated with these two habitats. Thirty-two wings were photographed: 16 specimens in EHMF and 16 in DF. The coordinates configurations (x,y) were registered and aligned through Generalized Procrustes Analysis. The centroid size between habitats was significantly different (Kruskal-Wallis; $p < 0.005$); EHMF specimens were larger than DF. Reclassification *a posteriori* was perfect in DF specimens (100%), and almost perfect in EHMF (93.75%). The landmarks that most contributed to the variation between habitats were: Interception between M and wing border, interception dm-cu, and interception between Cu and dm-cu. The wing morphometric variations observed in *L. marginata* could be associated with phenotypic plasticity.

Keywords: Bergmann's rule, geometric morphometrics, *Limnophora*, phenotypic variation

INTRODUCTION

Muscidae is one of the families of Order Diptera with higher diversity; including eight subfamilies: Acanthipterinae, Atherigoninae, Muscinae, Azeliinae, Phaoniinae, Mydaeinae, Coenosiinae, and Cyrtoneurinae; in Ecuador, 77 species are reported. The members of the family occupy tropical and high mountains as habitats. The species of the group have different habits and provide ecosystemic services as organic matter decomposers (Loewenberg-Neto and Carvalho 2013; Courtney et al. 2017).

In the Neotropical region, Coenosiinae is the most diverse subfamily, this group is characterized by aquatic larvae. The subfamily included *Limnophoriini* and *Coenosiini*, with cosmopolitan distributions. Most species of *Limnophoriini*, associated with humid environments such as flooded soils and moss, whilst some grow in dry environments. To this date, 12 genera are reported for the neotropics (Loewenberg-Neto and Carvalho 2013).

The *Limnophora* genus is formed by 230 species, known for using different habitats. They are found from high mountains to valleys, rivers, and streams; inhabiting both high forests and dry forests (Xue et al. 2012). In Ecuador had been reported: *Limnophora bifasciata* Fogaca and Carvalho 2015, *L. longivittata* Fogaca and Carvalho 2015, *L. polleti* Fogaca and Carvalho 2015, *L. equatoriensis* Fogaca and Carvalho 2015, *L. femurosetalis* Fogaca and Carvalho 2015, *L. lamasi* Fogaca and Carvalho 2015, *L. penai* Fogaca and Carvalho 2015, *L. marginata* Stein 1904,

L. pica (Macquart, 1851) and *L. saeva* (Wiedemann, 1830) (Loewenberg-Neto and Carvalho 2013; Fogaça and Carvalho 2015).

The ecology and biology of the *Limnophora* species are limited. Recently, Moreira (2019) studied the diversity and composition of Muscidae in two altitudinal gradients in Pedro Moncayo Canton, Pichincha – Ecuador; found *L. marginata* as the most abundant species associated with the Evergreen High Montane Forest (EHMF), in contrast to a lower abundance in Dry Forest (DF). Later, Blacio et al. (2020) investigated the diversity and synanthropy in sarcosaprophagous Diptera was associated with EHMF; they found a higher positive synanthropic index for *L. marginata*. Species such as this one, give good candidates for analyzing the influence of environmental conditions in phenotypic variation.

The variation of the shape of the wing in Diptera, comes forward as a good morphological marker to study variations in populations. Studies such as the one presented by Alves et al. (2016) refer that some dipteran species exhibit variations in morphological traits correlated with selective pressures such as temperature. In the same way, the effects of the altitude in morphological characters in Diptera have been reported. In this sense, as a contribution to the knowledge of Diptera in Ecuador, this study describes the morphogeometric variations of the wings in two populations of *L. marginata*. The CIFV3-CV-FCB3 Project/Research Direction and Faculty of Biological Sciences. Project developed with research permit No. 007-

2018-RIC-FLO-FAU-DPAP-MA and collection permit No. 007-2019 -DPAP-MA.

MATERIALS AND METHODS

Biological samples and collection site

The *L. marginata* specimens were collected between May 2017 and November 2018, in the Tocachi parish, in EHMF (-78.290500 Longitude W 0.048400 Latitude N) and the Malchingui parish, in DF (-78.358324 Longitude W, -0.000693 Latitude S); located at Pedro Moncayo Canton of the Pichincha Province, Ecuador Ecuador (Cuesta et al. 2017; Moreira 2019).

The EHMF appertains the High Montane Forest classification, it extends from an altitude of 3000 to 3400 masl, and it displays tempered cold weather with a temperature between 6 and 14 °C with annual precipitation of 922 mm. The forest exhibits vegetation of a transition zone between Paramo and Montane Forests of zones with a higher altitude, with trees having ramifications starting from the base of the moss coating). The wildlife of the forest includes well-adapted species with specific feeding habits, while the flora features trees with a canopy of 15 to 20 m with epiphytes. The DF appertains Espinar's Montane Dry Forest and Montane Dry Shrub classifications, it is located between 1400 and 2500 masl, exhibits annual mean precipitation that varies between the 200 to 575 mm, its temperature ranges between 5 and 18 °C. The species of flora and wildlife in this forest are perfectly adapted to drought, its forests exhibit bushes up to 2 m high and are present in the montane slopes of dry streams in Ecuador (Cuesta et al. 2017; Moreira 2019).

Data acquisition

This study was following the criteria of Cardini et al. (2015) and De Luna (2020) who indicate that geometric morphometry is an informative tool for the detection of

anatomical variations. Where the total size of the sample must be greater than the variables analyzed to obtain a reliable estimate of the variance-covariance structure in the data. These authors mentioned that the size of the centroid is very precise; even when the sample size is small and the number of samples required to achieve a reasonable degree of precision may be between 15-20 samples. In this sense, after identifying the biological material, a total of 32 wings of female specimens were dissected and slide mounting: 16 collected in the EHMF, and 16 DF. In each wing, eight landmarks (LM 1-8) were located, corresponding with the type I *sensu*: LM 1, rupture of the subcosta; LM 2, a junction of R1 with the border of the wing; LM 3, a junction of R2+3 with the border of the wing; LM 4, a junction of R4+5 with the border of the wing; LM 5, a junction of M with the border of the wing; LM 6, a junction dm-cu; LM 7, junction of Cu with dm-cu and LM 8, a junction of M with r-m. The configuration of the eight pairs of coordinates (x,y) of each wing (Figure 1) was captured with the help of the TPSDig program (De Luna 2020).

Geometric morphometrics analysis

From the matrix of the 32 configurations of geometrical coordinates of the eight landmarks (LM1-8), the Generalized Procrustes Analysis was conducted, with the CoordGen software (Sheets 2005a) to obtain a matrix with the conformational variables (partial warps = Pw) and the centroid size (CS). This Pw matrix was used to perform a principal component analysis and analysis of Canonical Variables with the PCAGen and CVAGen software, through which the proportion of reclassified groups (EHMF and DF) was found, and a MANOVA analysis was performed for each canonical axis (De Luna 2020). The difference in CS values between habitats was analyzed with a Kruskal-Wallis test (P 0.05). Finally, we calculate the relative deformations compared to the average shape of the studied populations (relative deformations or Rw) (Klingenberg 2013).

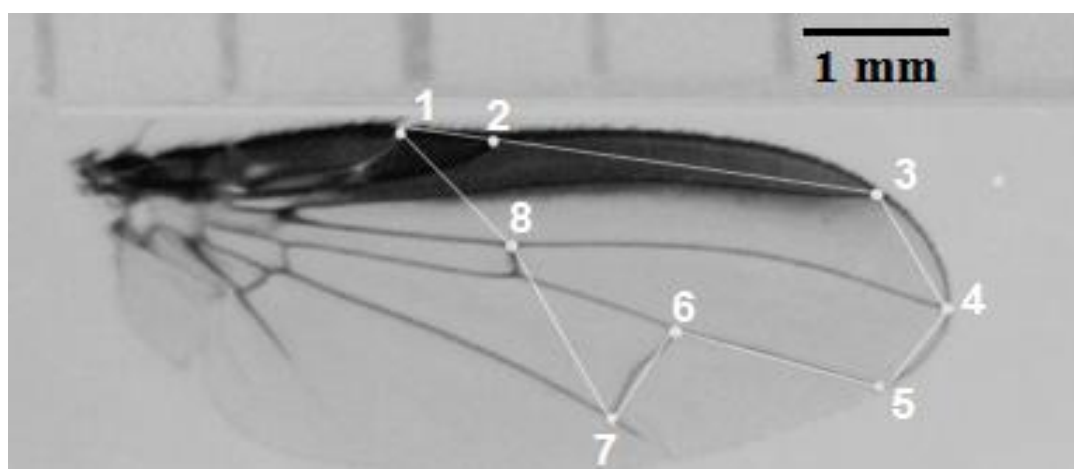


Figure 1. Photograph of the right-wing of *L. marginata*, showing the disposition of the anatomical reference landmarks (LM). LM 1, rupture of the subcosta; LM 2, a junction of R1 with the border of the wing; LM 3, a junction of R2+3 with the border of the wing; LM 4, a junction of R4+5 with the border of the wing; LM 5, a junction of M with the border of the wing; LM 6, a junction dm-cu; LM 7, a junction of Cu with dm-cu and LM 8, a junction of M with r-m.

RESULTS AND DISCUSSION

Statistically, significant difference was found in the centroid size of *L. marginata* between habitats (Kruskal-Wallis $X^2 = 8.42$; $p < 0.0037$); the specimens from EHMF were larger ($2.004\text{mm} \pm 0.171$), in comparison with the ones from the DF ($1.805\text{mm} \pm 0.134$).

Principal component analysis (PCA) indicates that PCA1 and PCA2 explain 42% and 22% of the sample variance, respectively. The anatomical points that improve the total variance in the sample are PAR 2, 6, and 7. The PCA of *L. marginata* for each forest is observed in Figure 2.

The CVA shows a total variation of 66 % (CVA1= 47%, CVA2= 19%). The lambda Wilks values, X^2 , degrees of freedom, and probabilities in the reclassification test in the discriminant axe from the CVA were respectively: $\Delta 1 = 0.2718$, $\chi^2 = 31.26$, $df = 12$, and $p < 0.001$. The percentage of reclassification *a posteriori* analyzed through CVA indicates 100% of reclassification (16/16) for *L. marginata* belonging to DF and 93.75% of correct reclassification (15/16) for *L. marginata* collected in EHMF.

The interpolation function of the thin plate spline, allowed to describe conformational differences as deformations of the eight analyzed landmarks. In the data plot of the intraspecific analysis of *L. marginata* amongst the two habitats, the crosses correspond to the *L. marginata* specimens from EHMF and the black dots to the DF specimens. The deformation grill A displays the wing changes that are present in *L. marginata* both in the EHMF and DF (Figure 2.A). Changes were determined in *L. marginata*, collected in the EHMF and the DF. The greatest deformations are observed at the junction of R1 with the

edge of the wing (LM 2), it moves diagonally to the right towards R2 + 3 with the edge of the wing (LM 3); the dm-cu junction and the Cu-dm-cu junction (LM 6 and LM 7 respectively) move diagonally to the left towards the lower edge of the wing and the M-rm junction (LM 8) moves anteriorly part of the wing, causing the cells of the warp grid to widen.

The shape and size variation detected in this study in *L. marginata*, may be due to phenotypic plasticity, in response to environmental conditions such as temperature and altitudinal gradient. This was confirmed by Alves et al. (2016), who mentions that in Muscidae, the environmental factors influence directly the shape and size variability of the wings. Specimens that showed the smaller size and thinner shape of the wing were registered in the DF, which inhabit between 1400 to 2500 masl, where temperature varies between 12 and 18 °C. In the dry season, the temperature might be between 18 to 22 °C during the day and -2°C at dawn (Moreira 2019). Whereas the specimens of *L. marginata* that showed the bigger size and wider wings, are distributed in the EHMF, inhabiting between 3000 and 3400 masl with an annual mean temperature that varies between 6 and 14°C.

Carl Bergmann in 1847, proposed that individuals of a species or clade in higher latitudes, are bigger than those in lower latitudes (Shelomi 2012). Bergmann, indicates that thermal regulation would be the factor that explains the larger size in animals that inhabit higher altitudes, given that the relation surface/volume, allows reducing the heat loss in colder environments. Some insects like Diptera, thermoregulate their temperature, the bigger the body the slower the heat loss, enabling them to tolerate lower temperatures (Azócar et al. 2016).

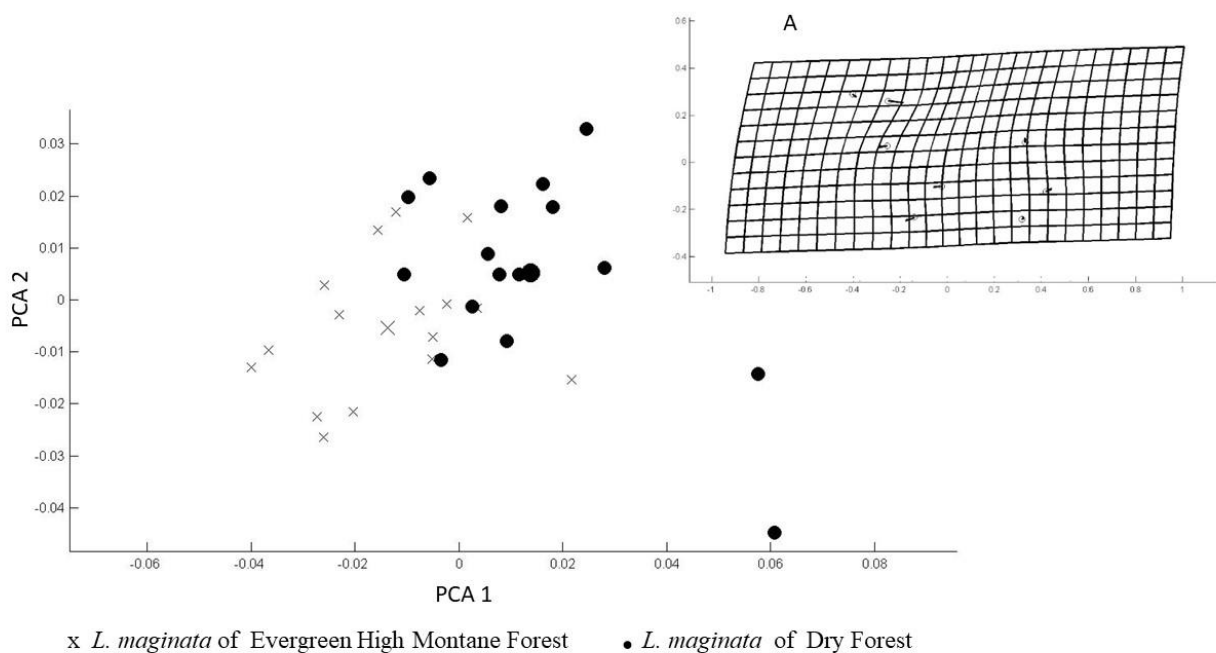


Figure 2. Diagram of the two first components principals PCA for the 32 specimens of *L. marginata*, showing the deformation grid in the conformations. A. Deformation grid, shows the changes between mean wing configuration of the two populations studied.

However, little is known of the bio-ecology of *L. marginata*, recently Moreira (2019), mentions the species, as the most abundant in the EHEMF and with higher synanthropic value in the Tocachi parish, Pedro Moncayo Canton (Blacio et al. 2020). Krivosheina et al. (2014), mentions that most of the *Limnophora* species, in their immature phase develop in aquatic environments, in streams with flowing water and rapid fluxes, some of them submerged in algae or moss. The same authors indicate that larvae and adults are predators, of other invertebrates. In this study larvae were not collected, for this reason, the natural breeders of the species are not known. Nevertheless, the variation in shape and size of the wing could be related to the availability of nourishment during the development of the larvae stage, causing intra and interspecific competition for food (Sasmita et al. 2019).

The metric changes are associated with the environmental pressures are the first changes that can be detected in populations. Changes in altitude consider indirectly some variables, amongst them temperature and rainfall, that affect the ecology of the species. Previous studies like the one reported by Demirci et al. (2012) and Gómez et al. (2014), have found phenotypic differences in populations of Culicidae located at different altitudes, both studies support the adaptative explication previously described, suggested by Bergmann. The results obtained in this work agree with the ones reported by Courtney et al. (2017), who indicate that Diptera found in high and cold environments are larger. Sasmita et al. (2019), compare in their study, diets and temperature, concluding that temperature is a factor that affects the size of individuals. However, the results of this study coincide with those obtained by Demirci et al. (2012) and Motoki et al. (2012), which suggests that there is a direct correlation between wing shape and size with the altitudinal gradient.

In conclusion, morphogeometric patterns detected in the wing in *L. marginata*, are related to the fact that phenotypic plasticity is adaptive, in this case, it could be regulated by the temperature and altitude that the studied populations occupy, with the habitat and possibly with the elevation. This is the first study that seeks to explain the morphogeometric variations in *L. marginata*. However, it is necessary to continue studies of the biology and distribution of the species in the country to corroborate whether the patterns observed in the populations studied are associated with the habitat.

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