VARIATIONS IN LEAF SIZE AND LEAF SHAPE IN FOUR SPECIES OF *EUGENIA* (MYRTACEAE) USING GEOMETRIC MORPHOMETRICS APPROACH

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ABSTRACT

Eugenia (Myrtaceae) is one of the most diverse groups in Neotropical flora. This study evaluated leaf size and shape in four species of *Eugenia* as a model to test geometric morphometrics in species with distinct geographic distribution in Atlantic Forest biome. The species *E. hiemalis* Cambess*, E. pyriformis* Cambess*, E. subterminalis* DC and *E. uniflora* L. were selected and the leaves used were analysed from herbariums and scientific collections. Leaves were photographed from a standardized distance and anatomical landmarks and semi-landmarks were placed on the contour of each leaf. The leaf centroid size, leaf shape and the phylogenetic signal were analysed. The phylogenetic signal was obtained from tree available in literature for *Eugenia* genus. Leaf size differed amongst species. *E. pyriformis* and *E. hiemalis* displayed a larger leaf centroid size when compared to *E. uniflora* and *E. subterminalis.* For the leaf shape was observed that *E. pyriformis* and *E. subterminalis* present narrower leaves, different from *E. hiemalis* and *E. uniflora* with wider leaves. There is no phylogenetic signal in leaf morphology among the four species. The results indicate that species with broad geographical distribution or associated with areas of high environmental heterogeneity resulting in high leaf form amplitude. Phenotypic plasticity could be detected by geometric morphometrics, indicating this tool as a useful approach to quantify leaf shape variation in *Eugenia*, at least in these studied species.
Key words: acclimation, Atlantic Forest Biome, leaf shape.

RESUMO

Eugenia (Myrtaceae) é um dos grupos mais diversos da flora Neotropical. Este estudo avaliou o tamanho e a forma das folhas de quatro espécies de *Eugenia*, como modelo para testar a morfometria geométrica em espécies com distribuição geográfica distinta no bioma Mata Atlântica. As espécies *E. hiemalis* Cambess*., E. pyriformis* Cambess*., E. subterminalis* DC*.* e *E. uniflora* L. foram selecionadas e as folhas utilizadas foram analisadas em herbários e coleções científicas. As folhas foram fotografadas a uma distância padronizada e marcos anatômicos e semi-marcos foram colocados no contorno de cada folha. O tamanho do centroide da folha, a forma da folha e o sinal filogenético foram analisados. O sinal filogenético foi avaliado por meio de árvore filogenética disponível na literatura para o gênero *Eugenia*. O tamanho da folha diferiu entre as espécies. *E. pyriformis* e *E. hiemalis* apresentaram maior tamanho do centroide da folha quando comparados a *E. uniflora* e *E. subterminalis*. Para o formato da folha observou-se que *E.*

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pyriformis e *E. subterminalis* apresentam folhas mais estreitas, diferentemente de *E. hiemalis* e *E. uniflora* com folhas mais largas. Não há sinal filogenético na morfologia foliar entre as quatro espécies. Os resultados indicam que são espécies com ampla distribuição geográfica ou associadas a áreas de alta heterogeneidade ambiental resultando em alta amplitude de forma foliar. A plasticidade fenotípica pode ser detectada por morfometria geométrica, indicando esta ferramenta como uma abordagem útil para quantificar a variação do formato da folha nas espécies estudadas do gênero *Eugenia*.
Palavras-chave: aclimatação, bioma Mata Atlântica, formato da folha.

INTRODUCTION

In the last few years, the use of geometric morphometrics to quantify leaf shape, previously restricted to traditional morphometrics, has received increasing interest (Viscosi and Cardini, 2011). Variations in leaf shape, using analysis with landmarks and semi-landmarks, provide important information in order to successfully differentiate species from their hybrids (Klingenberg *et al*., 2011; Viscosi, 2015). Furthermore, geometric morphometrics can be used as a complementary tool to understand the origin of phenotypic differences, thereby contributing to studies in taxonomy and plant genetics (Klingenberg and Gidaszewski, 2010; Gallaher *et al.,* 2019). Despite works such as Viscosi *et al.* (2009), which had successfully differentiated hybrid species of *Quercus* L., the use of the geometric morphometrics approach for plant species is still scarce. Furthermore, the only morphometric study of *Eugenia* L. was made by Bünger *et al.* (2015), using Traditional Morphometrics to separate species from the *Eugenia involucrata* DC. complex.

Leaf morphology may vary not only between species, but also in the same individual (Royer *et al.,* 2009). Leaves are the main organs associated with environmental perception, plastically responding to a variety of environmental conditions, both biotic and abiotic (Gurevitch *et al*., 2006). Species with higher plasticity potential in surviving characteristics show adaptive advantages in heterogeneous or transitional environments. These modifications may facilitate the exploration of new niches, resulting in increased environmental tolerance (Via *et al.,* 1995).

Myrtaceae is one of the most representative families of the Neotropical tree flora, both in terms of diversity and abundance in different vegetation formations (Sobral, 2003; Flora do Brasil 2020, in construction). The family comprises about 140 genera and 3,500 species of trees and shrubs, distributed throughout tropical and subtropical regions of Australia, Asia and the Americas (APG IV, 2016). In Brazil, the species are restricted to the tribe Myrteae (Myrtoideae), represented by approximately 1,000 species (Sobral *et al*., 2016). The genus *Eugenia* L. is one of the largest genera in the Myrtaceae, with approximately 1,100 species (Snow, 2011; Govaerts *et al*., 2014).

The genus (alike its family) is considered a "taxonomic nightmare" due to the higher number of species and their high morphologic similarity, mainly in vegetative material. This becomes evident by the number of indeterminate taxa in herbaria and floristic or phytosociological works, combined with the uncountable changes in the group's internal circumscription over the years (De Candolle, 1828; Berg, 1857-1859; McVaugh, 1968; Landrum and Kawasaki, 1997; Lucas *et al.,* 2007; Mazine *et al.,* 2014; Bünger *et al.,* 2015).

In this context, the present work configures a first approach using Geometric Morphometrics to evaluate leaf morphology within *Eugenia*. We selected four species of *Eugenia* collected in different locations throughout the southern boundary of the Atlantic Forest biome as a model for this study. The main goal of this study was to evaluate leaf size and shape variation in four species of *Eugenia* to test geometric morphometrics as a tool in order to detect phenotypic plasticity in plants.

MATERIAL AND METHODS

Study species

The species were selected by their geographic distribution in the southern boundary of the Atlantic Forest biome (Sobral, 2003; INCT, 2016; Flora do Brasil, 2020, in construction). Four species were selected: *Eugenia uniflora* L., widely distributed in the southern boundary of the Atlantic Forest and occurring in all forest formations, *Eugenia hiemalis* Cambess., *Eugenia pyriformis* Cambess. and *Eugenia subterminalis* DC., with occurrence restricted to a few phytophysiognomies (Sobral, 2003) (Figure 1).

Data collection

The studied exsiccates were from three different herbaria: ICN, HAS and HPBR (Thiers, 2020, continuously updated).

The selection criteria for the exsiccates were as follows: (i) herborized material quality, with a sufficient number of adult leaves (completely expanded) and (ii) specimens collected in natural environments (not including forest plantations) at the southern boundary of the Atlantic Forest biome. Ten individuals were selected for each species, using three fully expanded adult leaves per individual, totalling 30 leaves per species and 120 samples. Based on the exsiccate collection points, the sampled individuals were grouped according to their geographic distribution in the phytophysiognomies of the Atlantic Forest biome (Figure 1).

The coordinates of each landmark and semi-landmark of all specimens were superimposed by Generalized Procrustes Analysis (GPA). The GPA removes de effects of position, orientation, and scale, producing a new set of shape variables (Rohlf and Slice, 1990; Adams *et al.,* 2013). As an estimate of leaf size, we used the log-transformed centroid size (Figure 2). The centroid size is the square root of the sum of the squares of the distances of each landmark from the centroid of the configuration (Bookstein, 1991).

Data analysis— Normality of the data was evaluated via the Shapiro-Wilk normality test. In order to explore leaf size among species, an ANOVA, followed by Tukey's test, was used to correct the degree of significance for multiple comparisons among species. Leaf shape among species was explored through the Principal Components Analysis (PCA). The Multivariate Analysis of Variance (MANOVA) was used for the statistical analysis of the shape, while for multiple comparisons, the pairwise MANOVA between each pair of species was used, followed by a Bonferroni correction. For all statistical analyses, we assume an alfa of 0.05 in significance level. We combine PCA with the preliminary phylogenetic tree proposed by Mazine *et al.* (2014) to evaluate the phylogenetic signal for leaf shape.

For the statistical analyses and to generate graphs, we used the R language version 2.14.1 (R Developed Core Team, 2011), as well as MASS libraries (Venables and Ripley, 2002), ape version 1.8-2 (Paradis *et al.,* 2004), stats (R Development Core Team, 2009) and ade4 (Dray and Dufour, 2007). In addition, the geometric morphometric procedures were performed with two packages, Rmorph (Baylac, 2008) and Geomorph (Adams, 2014).

RESULTS

Leaf size differed amongst species (Wilks'λ = 0.0218; $F_{(3;\ 3.62)}$ = 20.51; P < 0.001). Two species, *E. hiemalis* and *E. pyriformis* did not differ in centroid size and *E. subterminalis* and *E. uniflora* did not differ between each other. However, *E. pyriformis* and *E. hiemalis* displayed a larger leaf centroid size when compared to *E. uniflora* and *E. subterminalis* (Figure 3).

Concerning leaf shape, based on the PCA results, we could partition the four species in terms of differences in leaf morphospace (Figure 4). The PCA demonstrates that, in average, the four species are different in shape (Figure 4). In general, *E. pyriformis* and *E. subterminalis* occupy positive scores in PC 1, with narrower leaves, different from *E. hiemalis* and *E. uniflora*, which occupy negative scores in PC 1, with wider leaves (Figure 4). The four species statistically differed in leaf shape (Wilks'λ = 0.0277; $F_{(3;\ 3.82)}$ = 25.09; P < 0.001).

In the phylomorphospace, there is no congruence between phylogeny and leaf morphology (Figure 5).

DISCUSSION

The geometric morphometrics approach demonstrates to be very useful to quantify shape variation in *Eugenia*, successfully separating the species by their leaf shape and detecting size variation. Leaf size divided the species into two distinct groups, with *Eugenia hiemalis* and *E. pyriformis* showing a higher leaf centroid size when compared to *E. subterminalis* and *E. uniflora*. However, centroid size variation was not related with the species occurrence pattern in Atlantic Forest phytophysiognomies. But, a higher leaf size amplitude was detected for species with broad geographic distribution and distribution in heterogeneous environments, (*E. uniflora* and *E. hiemalis*, respectively).

On the other hand, leaf shape was associated with species distribution, indicating the influence of habitat heterogeneity (Gallaher *et al.,* 2019). Species with broad geographic distribution in heterogeneous environments, *Eugenia uniflora* and *E. hiemalis,* displayed wider leaves, whereas *E. subterminalis* and *E. pyriformis*, which are more restricted, presented narrower leaves. In fact, a high phenotypic plasticity had already been detected in *E. hiemalis*, both at the individual and population levels (Nascimento *et al.,* 2015). According to Gates (1980), larger and wider leaves (*E. hiemalis* and *E. uniflora*) absorb more sunlight compared to smaller and narrower leaves (*E. subterminalis* and *E. pyriformis*). Thus, the observed differences in leaf shape may indicate tolerance to lighted environments, which has already been observed for *Eugenia* by Cardoso and Lomônaco (2003) and associated with phenotypic plasticity (Nascimento *et al.,* 2015).

According to Gratani (2014), species with a wide geographic distribution present high phenotypic plasticity, since they occur in more different environments within their distribution areas. Such species are thus more likely to endure a variety of environmental conditions than species with restricted distribution (Hopkins and Thurman, 2010). This feature has already been detected in *Eugenia uniflora* (Turchetto-Zolet *et al*., 2016) at the southern boundary of the Atlantic Forest Biome and in this study was associates with larger and wider leaves.

Plant species respond differently to the environment, according to their functional traits facilitated by phenotypic plasticity (Violle *et al.,* 2007), thereby generating different patterns in variation of phenotypic characteristics within one species (Bastida *et al.,* 2015). This plasticity is extremely advantageous considering its role in the increase of the ecological niche of the species and, consequently, higher survival rates under new conditions (Lloret *et al.,* 2012). This adaptive plasticity to different environments positively influences the fitness of the species and is higher in heterogeneous environments compared to more constant or homogeneous habitats (Gianoli and Valladares, 2012). The result is a pattern of divergence in characteristics which, combined with the response to species interactions, can mediate the coexistence of similar species (Burns and Strauss, 2012). This can be observed in *Eugenia hiemalis,* which, although its geographical distribution is not as broad as *E. uniflora*, occurs in an area with high environmental heterogeneity, also resulting in leaf form amplitude.

Natural environments are heterogeneous at extremely small spatial scales, with different environments conferring different degrees of plasticity. This may enable the development of specific individual phenotypes and act as a factor that approximates species, irrespective of kinship or distribution (Burns and Strauss, 2012). Therefore, geometric morphometrics can be used as a complementary tool in taxonomy, evolution and plant ecology studies, assisting the detection of morphological variations between different levels of kinship and interaction (Gallaher *et al.,* 2019).

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EXAMINED MATERIAL

Eugenia hiemalis Cambess.

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Eugenia pyriformis Cambess.

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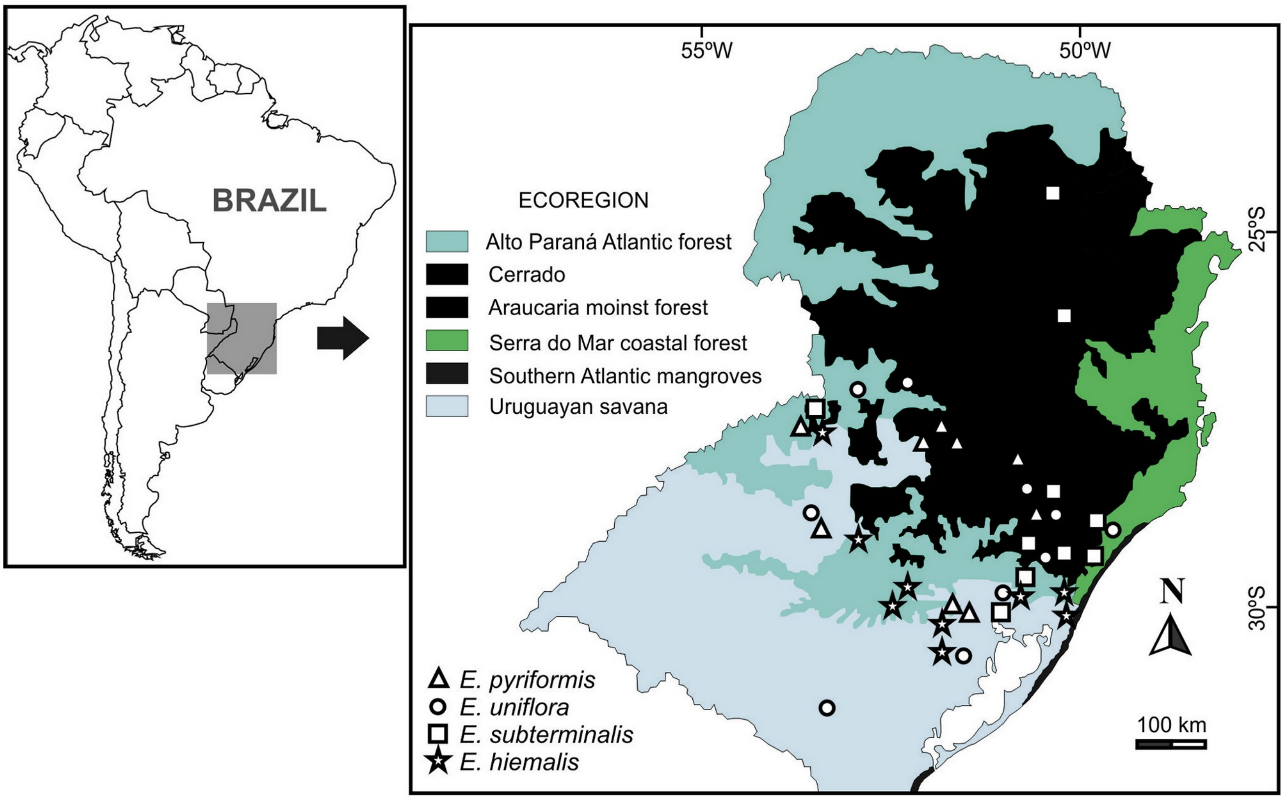

Figure 1 - Distribution of the specimens of *Eugenia* used in this work within the southern boundary of the Atlantic Forest biome in Southern Brazil.

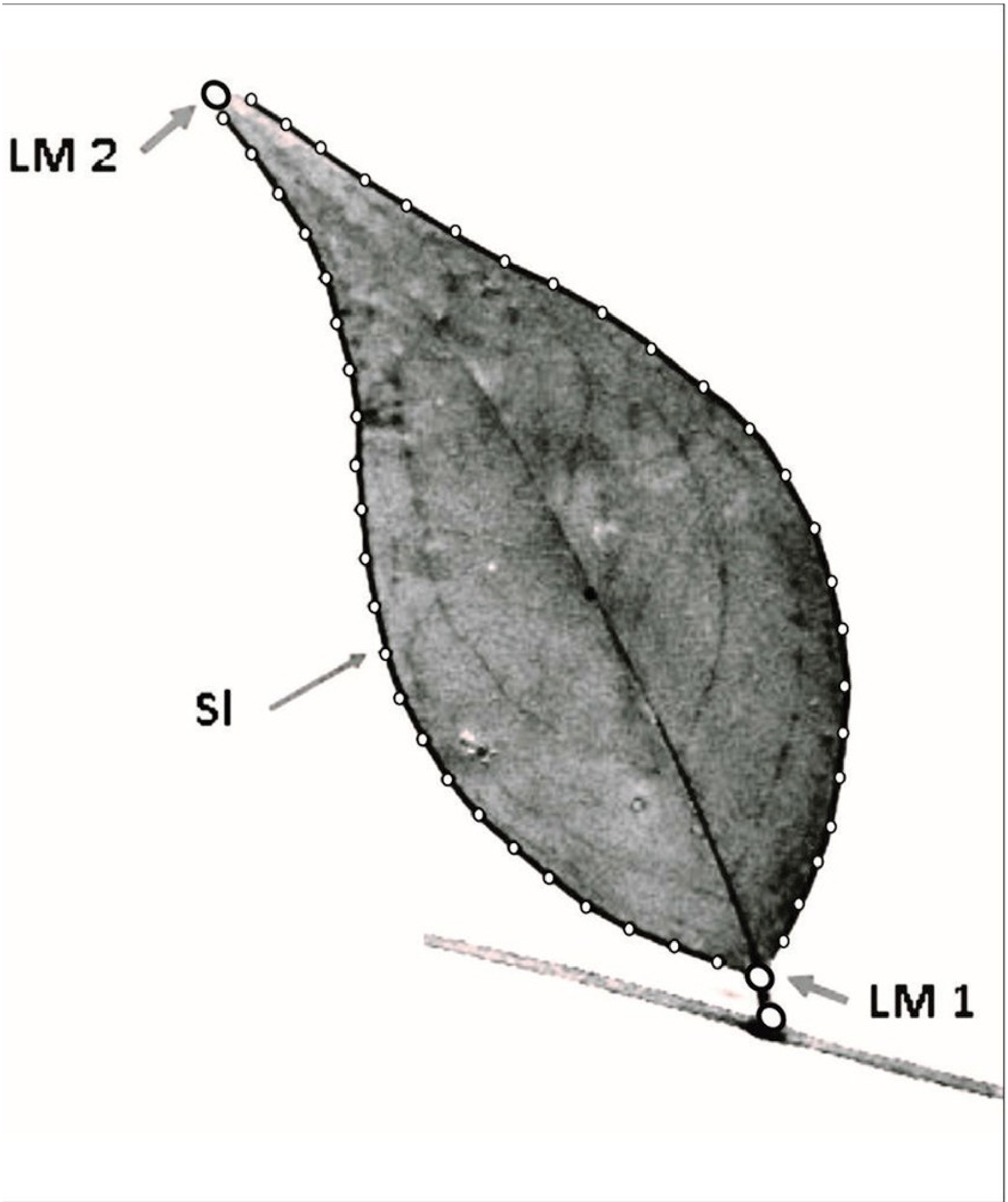

Figure 2 - Location of anatomical landmarks in leaf samples of *Eugenia*. “LM 1” represents the landmark at the intersection of the blade with the petiole, “LM 2” the landmark at the apex of the leaf and “SI” the 46 semi-landmarks arranged on both sides of leaf borders.

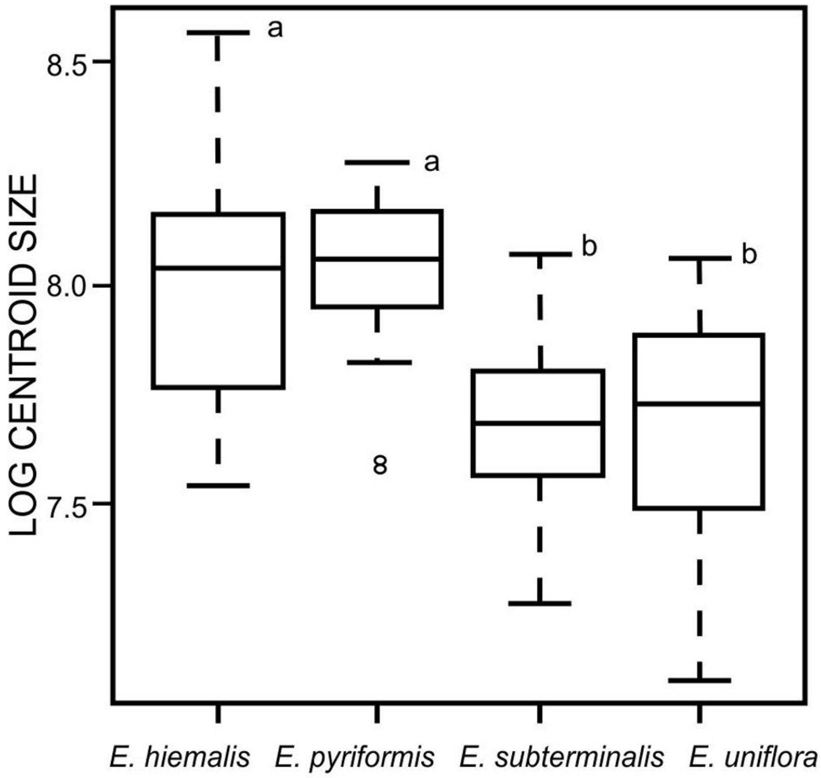

Figure 3 - Boxplot of centroid size for a leaf of *Eugenia hiemalis*, *E. pyriformis*, *E. subterminalis*, and *E. uniflora* in southern Brazil. Different letters on the boxes indicate significant differences for the Tukey test.

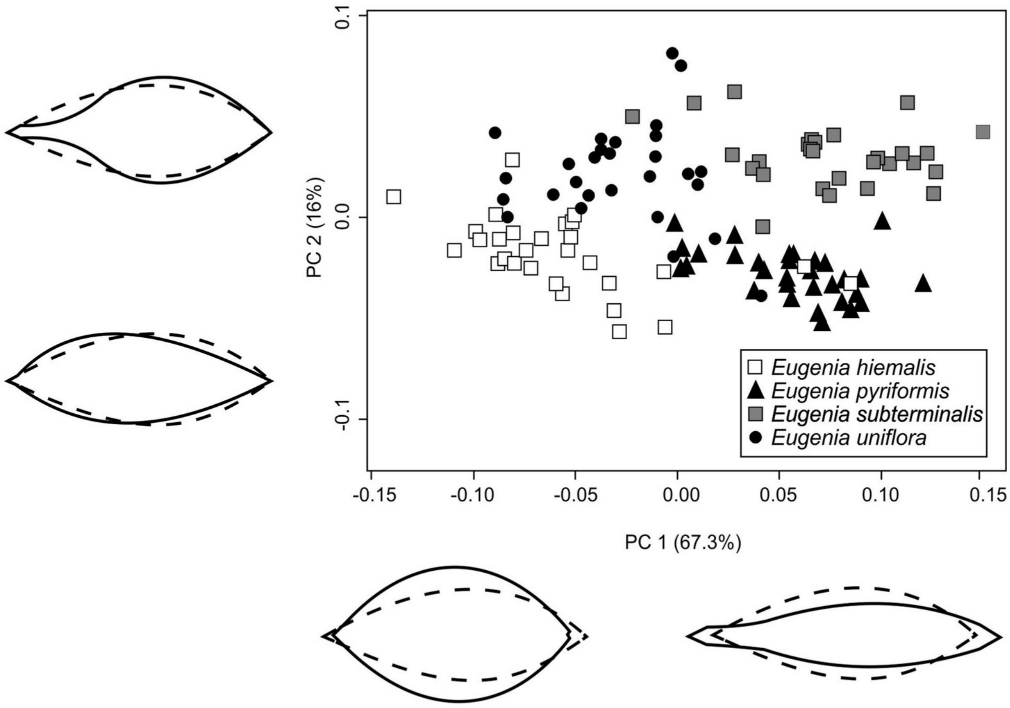

Figure 4 - Scatterplot of two first Principal Components Analysis for leaf shape among *Eugenia hiemalis*, *E. pyriformis*, *E. subterminalis*, and *E. uniflora* in southern Brazil. The shape projections represent the PC1 and PC2 scores at their positive and negative extremes, with the continuous line being leaf morphology of the extreme scores and the dashed line being the average shape.

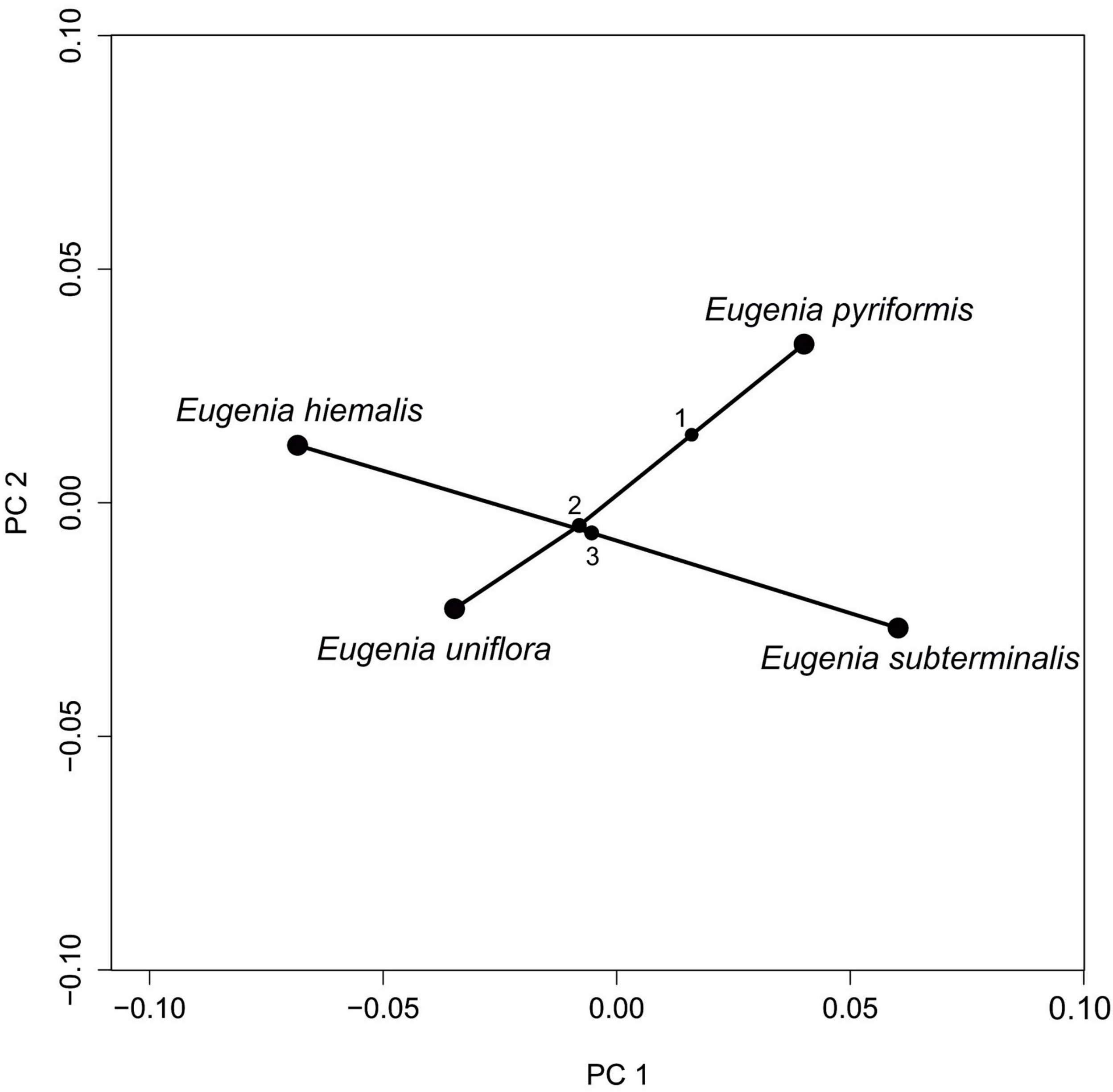

Figure 5 - Phylomorphospace for four species of *Eugenia* concerning leaf shape. The average scores of each species were displayed in two PC axes with the phylogeny projected onto the shape tangent space.