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Elliptic fourier analysis of leaf shape variation of mangroves found in Camiguin Island, Philippines

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Abstract

Mangrove forests are integral part of the ecosystem providing important services, including protecting coastlines from the impacts of extreme weather events, such as storm surge and erosion. In the Philippines, 40 species out of 54 in the world are present and 6 species are found in Camiguin. Delineating between species is important for ecosystem management and conservation. In this study, Elliptic Fourier Analysis (EFA) was used to look into leaf shape variations between six mangrove species: *Avicennia marina*, *Bruguiera cylindrica*, *Ceriops tagal*, *Rhizophora apiculata, Rhizophora mucronata* and *Sonneratia alba*. This was done to test whether significant yet subtle differences in leaf shape variation can be species-specific, habitat-influenced and provide evidence of sexual dimorphism. Results of PCA showed that the cumulative contribution of the first three significant principal components was about 97.78% of variance and the main source of shape differences was the variability in leaf laminar shape with respect to length-width ratio. Kruskal-Wallis test showed that the percentage proportion of each PC's significantly contributed to the overall variance (p-value <0.05). Also, results from MANOVA and CVA revealed significant interspecific variations in the leaf shape between groups examined with significant p-values <0.05. Moreover, species distinctiveness was manifested albeit overlapped from each other, implying that leaf shape variations is species-specific, habitat-influenced and provided evidence of sexual dimorphism. In addition, results from discriminant analysis provided further support for species-specific traits and sexual dimorphism among the six mangrove species. Herewith, EFA proved to be efficient in examining subtle leaf shape variations among mangrove species.

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Introduction

Mangroves are considered as the true rainforests of the Philippine coastline. They provide important services, including protecting coastlines from the impacts of extreme weather conditions. There are also among the most biologically-diverse coastal ecosystems in the country.

The Philippines is endowed with at least 40 species out of the 54 species worldwide. Six (6) of these species are found in the Island of Camiguin. There are current efforts to expand knowledge, coverage and strengthen the protection of mangrove areas in the country. This may suggest improvement of habitats for mangroves and for species that are mangrove dependent and consequently contribute to increase in fisheries stock and livelihoods (Primavera, 2000).

However, there is still lack of knowledge for mangroves, owing to similar morphology especially in leaf shapes. Delineating between species is important for ecosystem management and conservation. Hence, the importance of this study.

Herewith, the discrimination of one taxa to another based on well-defined diagnostic characters is one of the fundamental goals of systematic biology with a view to precisely classify and identify organisms.

This is normally accomplished by procuring morphological, ecological and/or molecular data (Adebowale *et al.* 2012). Generally, plant reproductive organs such as fruits, flowers and other vegetative plant structure are more robust characters to discriminate species distinctiveness and variations (Sheue *et al.* 2003; Kidyue *et al.* 2006).

In morphological analysis, quantitative evaluation of contour shape is often imperative. To evaluate contour shape, there have been several methods suggested. Length of a contour, moments of a contour region and Fourier descriptors are the examples. Among those methods, a series of Fourier descriptors has taken important roles (Ehrlich and Weinberg, 1970; Bookstein *et al.* 1982; Ehrlich *et al.* 1983; Rohlf and Archie; 1984, Ferson *et al.* 1985; Beirbaum and Ferson, 1986; White *et al.* 1988; Diaz *et al.* 1989), because such methods are good at directly representing contour shape itself.

The first modern studies of leaf morphometrics was made by Ray (1990, 1992) in the Araceae and more recently elliptic Fourier analysis (EFA) was used to compare leaf outline shape in aroids by Andrade (2006) and Andrade *et al.* (2008, 2010).

The later papers give further background on the use of EFA in previous botanical work.

In this study, Elliptic Fourier Analysis (EFA) was used to quantitatively measure leaf shape variation in six mangrove species, namely: *Avicennia marina*, *Bruguiera cylindrica*, *Ceriops tagal*, *Rhizophora apiculata*, *Rhizophora mucronata* and *Sonneratia alba* found in Camiguin Island.

This is done to effectively delineate species based on leaf shape variation. Understanding the nature of species and populations contribute to management and conservation.

Materials and methods

Sampling and Taxonomic Identification

The leaf samples were collected from the mangrove Forest of Camiguin Island specifically at Cantaan Giant Clams Sanctuary and White Beach Resort, Guinsiliban with a coordinates of 906'20.283"N, 124048'10.655"E (Fig. 1).

The Field Guide to Philippine Mangroves by Primavera *et al.* (2004) was used as a guide to identify the morphological descriptions of the leaves, flowers, fruits, and roots of the different kind of mangrove species used in this study. Ten (10) individual trees in every mangrove species were selected and a total of 90 mature leaves were collected in each species.

The leaves gathered in every tree were placed in properly labelled re-sealable plastic bags.

Fig. 1. The Sampling site: Camiguin, Island, Philippines.

The plastic bags were then placed properly in a box and transported back to Mindanao State University-Iligan Institute of Technology, Iligan City, for analysis.

Image acquisition, processing, chaincoding, elliptic fourier analysis

The collected leaf samples were carefully cleaned to remove dirt, soil particles, and salt crystals. The digital images from the cleaned leaves were made using Epson L220 scanner and saved in jpg format. A total of ninety (90) leaves per species were processed, removing the petiole and placed in one (1) canvas using Photoshop CS6 v. 13.0.

The image was then converted and saved into 24 bitmap format using Microsoft Paint 3D v. 4.18. A chain code was assigned to the binary images (black background and white object) of the leaf samples to construct the outline-based morphology of the leaves (Neto *et al.* 2006). The chain codes that describe the leaf shape geometry information was determined by the default setting of software SHAPE version 1.3. To reconstruct the leaf shape outline, the assigned chain codes were converted into shape variables in the form of Elliptic Fourier Coefficients (EFC) using the first 20 harmonics and then normalized using 80 Elliptic Fourier descriptors (Kuhl and Giardana, 1982).

Principal Component Analysis (PCA)

The variations derived after EFA of the chain code data was summarized using the Principal Component Analysis (PCA) (Iwata and Ukai, 2002). To characterize the leaf morphology that may contribute to the observed leaf shape interspecific variations of the mangroves species used in this study, only the significant principal components derived from the analysis were included. PCA was performed from the normalized Elliptic Fourier coefficients, wherein the scores in each component were used to describe the leaf shape characters. The leaf shape variations were reported as the mean leaf shape, the -2 standard deviation (-2SD) and the +2 standard deviation (+2SD).

Statistical Analyses

In addition, to test the significance of species distinctiveness of the six mangrove species, Multivariate Analysis of Variance (MANOVA) and Canonical Variate Analysis (CVA) were performed. Moreover, Kruskall-Wallis test was performed to further delineate variations in leaf shape among mangrove species. Wilks' lambda, Pillai trace values and *p*-values were also obtained. Box plots were generated using the significant principal component scores to visualize the distributions of leaf shape variations (Tabugo *et al.* 2012, 2014) and to determine the degree of similarity or distinctiveness among mangrove species, discriminant analysis was performed.

The method used in this study in determining the patterns of leaf shape variations was adapted from Futura *et al.* (1995). A summary of the various analyses done to determine the pattern of leaf shape variations is presented in Fig. 2. All statistical analysis was done using the Paleontological Statistics (PAST) v. 1.19 at statistical significance <0.05.

Results and discussion

Elliptic Fourier Analysis

Looking into the importance of mangroves as primary service providers to coastal populations and island dwellers in Camiguin, it is of high importance to assess the biodiversity. Understanding the nature of species and populations contribute to management and conservation. Leaf shape variation is one of the fundamental characteristics in assessing diversity. Using leaf specimen images from the field, shape variables were extracted [i.e. principal components (PCs)] from the Fourier coefficients and these variables describe leaf outline among the species examined. Figure 2 shows the outline of the Elliptic Fourier Analysis in determining leaf shape variations of the six mangrove species found in Camiguin Island.

Elliptic Fourier Coefficients (EFC) were generated using the first 20 harmonics. Elliptic Fourier harmonic functions were generated based on leaf boundary and yield a complexity index of the leaf shape as described by Neto *et al.*, 2006.

It described the overall leaf shape variation. Herewith, leaf shape variation was based on leaf length-width ratio, overall shape of the leaf outline and shape of basal and apical regions. Herewith, leaf shape proved as key feature for plant species identification.

Table 1. Percentage of variance with overall shape variation and Kruskal-Wallis Test (Non-parametric ANOVA) for the significant principal components (PC) of the group coefficients derived from the leaf outlines of six mangrove species found.

Principal Components and % Proportion	Variation	Kruskal-Wallis test	
		p-value	Remarks
PC 1 (91.5009%)	Captures anisotropy along the major axis of the all leaf, which appears to be a function of the leaf length/width ratio.	<0.05	Significant
PC 2 (4.8483%)	Describes variation in the extent of asymmetry of leaf laminar shape (e.g. elliptical-obovate variation).	<0.05	Significant
PC 3 (1.4273%)	Subtle variations along the leaf lamina/outline and the fine-scale variations around the basal and apical region.	<0.05	Significant

Principal Component Analysis

Principal component analysis was done by covariance matrix of the standardized elliptic Fourier coefficients to summarize independent body shape characteristics. Table 1 presents the relative contributions of the first three significant principal components and accounted 97.78% of overall variance. It further shows the detailed descriptions of leaf shape variation accounted by each principal component for mangroves found in Camiguin Island.

Table 2. Multivariate Analysis of Variance (MANOVA), p-values are Hotelling's pairwise Comparisons (Bonferroni corrected**)** showing significant difference in leaf shape variation of six mangrove species between groups.

Mangrove Species	*A. marina*	*B. cylindrica*	*C. tagal*	*R. apiculata*	*R. mucronata*	*S. alba*
A. marina	-					
B. cylindrical	2.71E-43*	-				
C. tagal	1.36E-35*	7.84E-72*	-			
R. apiculata	1.47E-51*	4.35E-28*	2.45E-96*	-		
R. mucronata	1.09E-35*	6.55E-82*	4.13E-82*	8.22E-59*	-	
S. alba	9.84E-77*	1.32E-10*	4.58E-80*	1.09E-11*	8.75E-93*	-

*Significant, *p*-value <0.05.

The major trend of leaf shape variation reflected by PC1 is anisotropy, i.e. the variability in length-width ratio. PC 2 describes variation in the extent of asymmetry of leaf laminar shape (e.g. elliptical-obovate variation) and PC 3 captures subtle variations along the leaf lamina/outline and the fine-scale variations around the basal and apical regions. Kruskal-Wallis test shows that the percentage proportion of each PC's significantly contributed to the overall variance (*p*-value <0.05). The effect of each principal component on the leaf shape was also clarified by redrawing the contours of leaflets using the Fourier coefficients inversely estimated under some typical values of principal component scores, including the positive (+) and negative (-) deviations from the mean leaf shape of mangroves. This is an exploratory process in order to determine comparison between shapes and elucidate possible biological significance. Considerable differences in the leaf shape of mangroves were noted.

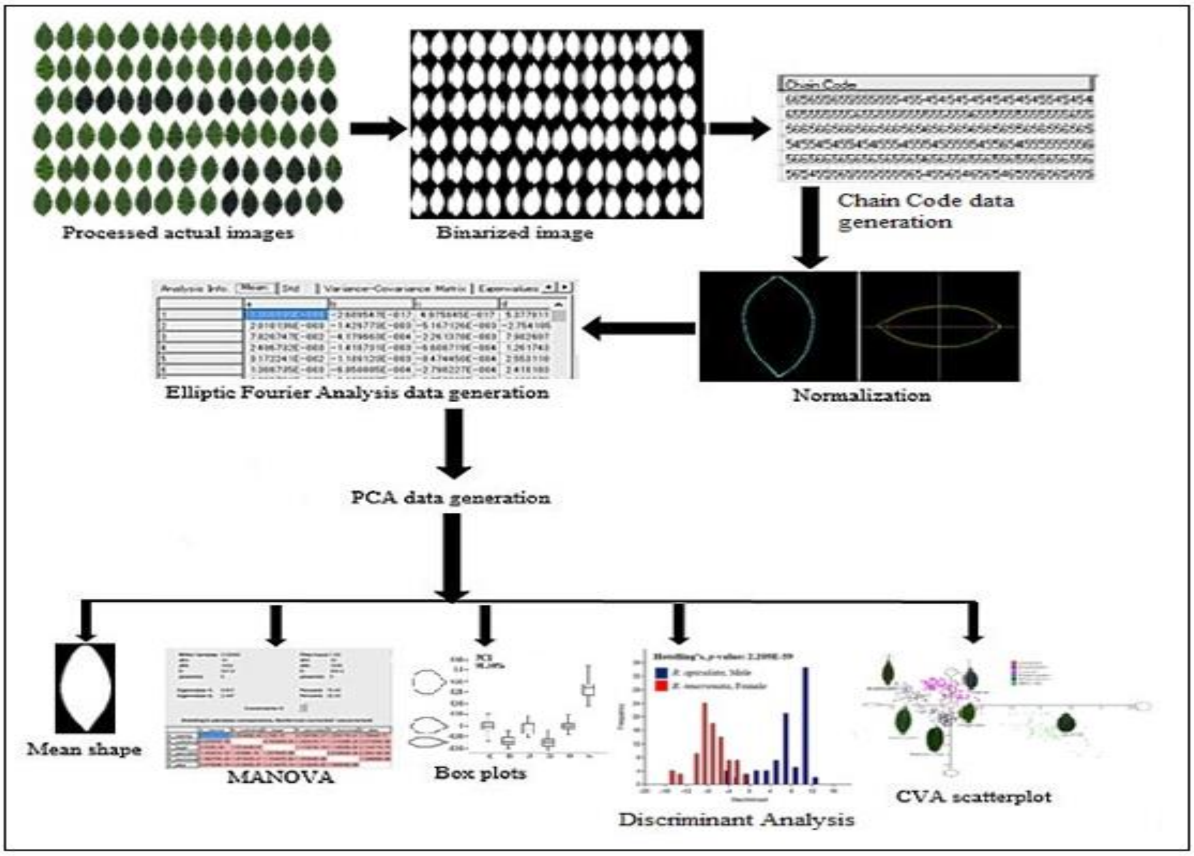

Fig. 2. Outline of the Elliptic Fourier Analysis to determine leaf shape variations of the six mangrove species found in Camiguin Island.

Canonical Variate Analysis (CVA) and Multivariate Analysis of Variance (MANOVA)

Results yield significant differences in the leaf shape of the mangroves for the six populations examined based on the distribution of the individuals along the first two canonical variate axes (Wilk's lambda: 0.02547; p-value: 0.0; Pillai trace: 1.617; p-value: 0.0). Each of the mangrove species occupies a shape space determined by projecting the scores of each specimen onto the first two Canonical Variate (CV) axes. Here, CV1 accounts for 81.82% and CV2 explains 18.18% of the overall variation, respectively. Individuals in the CV1 axis described variations were based on variability of leaf length/width ratio while those individuals in the CV2 axis vary in leaf laminar shape, and subtle variations in leaf basal and apical region (Fig. 3).

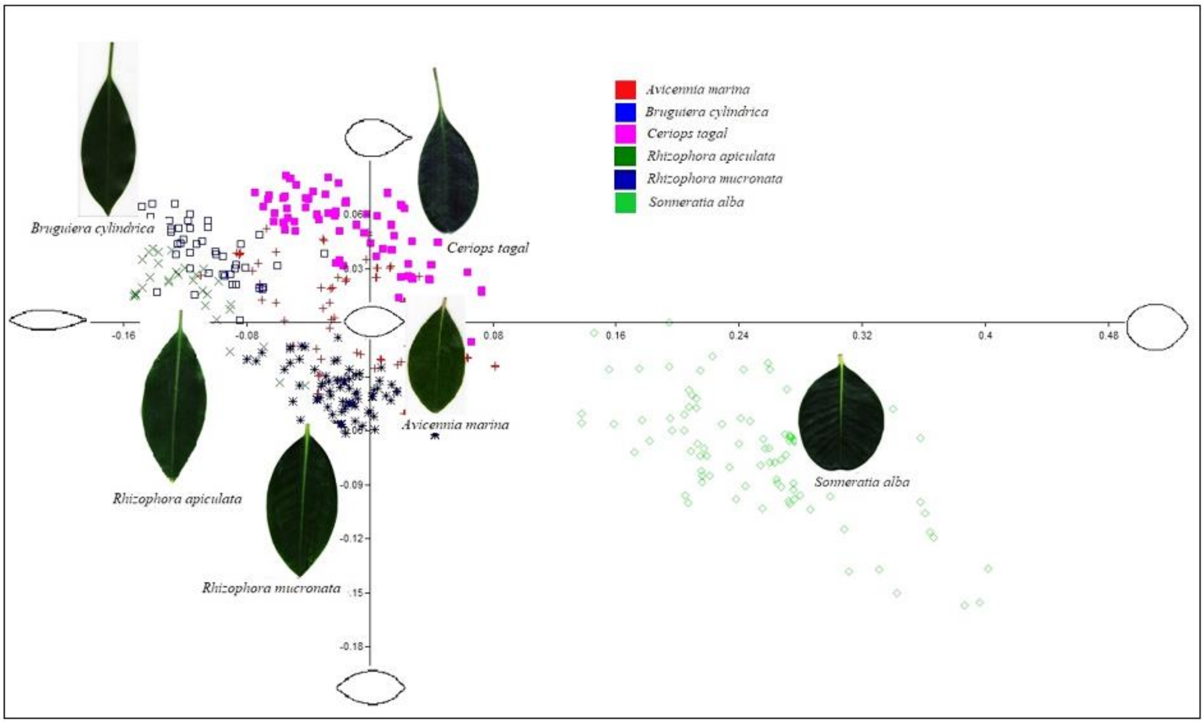

Fig. 3. Scatterplot of the first two Canonical Variate (CV) axes of the six mangrove species from Camigiun Island, showing significant difference in leaf shape variations.

Moreover, based on the distribution of points on the compromise space of the CVA plot (Fig. 3), the six mangrove species were significantly separated albeit overlapped at some point thus illustrates interspecific variations. These findings suggest that the leaf shape of all six mangrove species can be identified with high degree of accuracy, and according to Verdoorn (1963) this is further supported by their vegetative differences (e.g. flowers and/or fruits). Both *R. apiculata* male and *B. cylindrica* appeared close and overlapping to each other, probably due to their relative similarity in leaf shape outline, i.e. elliptical. *R. apiculata* male and *R. mucronata* female species appeared also close to each other but remained relatively distinct populations perhaps, due to shared habitats and sex differences.

The observed closeness and overlapping in leaf shape between *A. marina*, *B. cylindrica*, *C. tagal* and with two *Rhizophora spp*. could be perhaps explained with respect to their environmental and genetic variations. The aforementioned species was observed to occupy similarly dry open habitats whether during low tide or not in the sampling area, and according to Adebowale and colleagues (2012), this similarity in leaf shape outline could possibly reflect evolutionary adaptations to changes in environmental conditions, i.e., evolutionary convergence. Furthermore, the overlapping observed in the CVA plot between genus

Bruguiera, Rhizophora and Avicennia is due to their observable elliptical laminar shape, acute base and apex angle, and cuneate base shape. Genus *Ceriops* was separated, due to its rounded apical region but overlapped with *A. marina* due to their relative equal length-width ratio and acute basal angle while genus *Sonneratia* was due to its obovate laminar shape and rounded to retuse apical region.

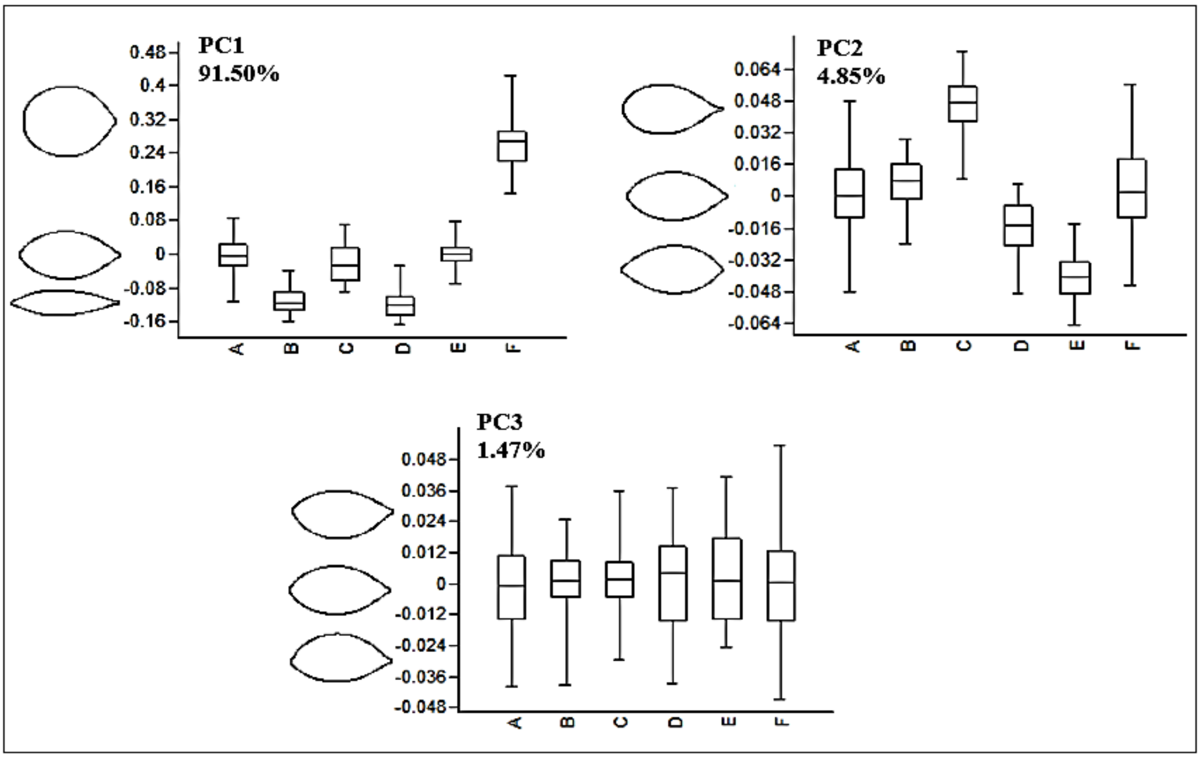

Fig. 4. Boxplots showing significant difference in leaf shapes among mangrove species: (A) *Avicennia marina;* (B) *Bruguiera cylindrica;* (C) *Ceriops tagal;* (D) *Rhizophora apiculata,* Male*;* (E) *Rhizophora mucronata,* Female*;* (F) *Sonneratia alba* found in Camiguin Island.

In addition, the relative similarity of leaf shape outline between genus *Rhizohopora, Bruguiera, Ceriops* is due to the fact that they are under the same family, Rhizophoraceae (Primavera, 2004) and are considered as true mangroves because of their fidelity to mangrove ecosystems and adaptive mechanism such as aerial roots and mechanism for salt exclusion (Tomlinson, 1986). As concluded by Adebowale *et al.* (2012), similarity of leaf shape outline may therefore reflect the retention of some characteristics from most common recent ancestor.

Table 2 shows the Multivariate Analysis of Variance (MANOVA), *p*-values are Hotelling's pairwise comparisons (Bonferroni corrected), showing significant difference in leaf shape variation of mangroves between groups. Notably, significant observed variation suggest that leaf shape variation can be species-specific, sex-influenced and habitat-influenced.

To visualize significant difference in leaf shapes among species of mangroves: *A. marina, B. cylindrica, C. tagal, R. apiculata* (M), *R. mucronata* (F) and *S. alba* found in Camiguin Island boxplot analysis was done (Fig. 4). Herewith, species tend to have a consensus shape which is common for all populations examined. However, notable differences in leaf shape between species and sex were also evident. But, generally variations observed were based on leaf laminar shape with respect to length-width ratio.

Moreover, the first three significant PC scores were also subjected to Discriminant Function Analysis (DFA) to test for sexual dimorphism and species distinctiveness. Herewith, this analysis was used as a standard method to visually confirm or reject the hypothesis that two groups are morphologically distinct. Results yield significant result for DFA thus, confirming the hypothesis that groups are morphologically distinct. Hence, implies sexual dimorphism and species distinctiveness based on leaf shapes (Fig. 5).

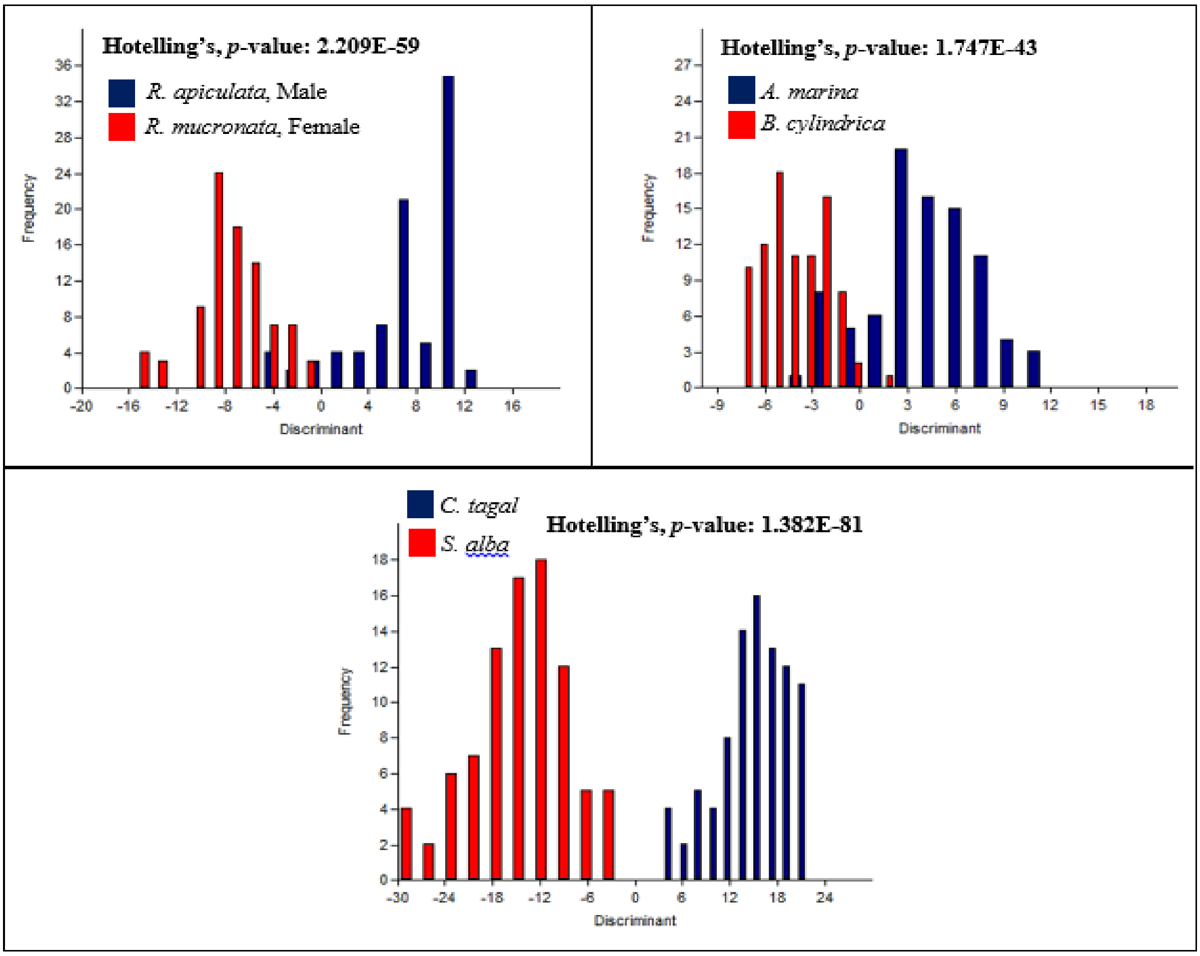

Fig. 5. Discriminant Analysis showing significant difference in leaf shape variation among six mangrove species found in Camiguin Island ($p<0.05$ is significant).

Conclusion

In this study, Elliptic Fourier analysis proved to be a good tool to increase efficiency in quantifying and understanding the nature of leaf shape variation in mangroves from Camiguin Island. The results showed that the mean shapes of all six mangrove species were significantly different albeit, shape variation within each species overlapped with others. Based on multivariate analysis of variance (MANOVA) and canonical variate analysis (CVA), significant differences in leaf shapes of the six mangrove species were observed with *p*-values <0.05. Herewith, the mangrove species were found to be very distinct to each other suggesting that leaf shape variation is species-specific, habitat-influenced and provided evidence for sexual dimorphism. The results of discriminant analysis further provide support for sexual dimorphism and species-specific traits. Moreover, this study has highlighted the importance of leaf shape in plant systematic studies, by applying the techniques of geometric morphometrics. Leaf characters, as employed in this study, proved to be an effective diagnostic key for identifying mangroves in the species level.

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References

Adebowale A, Nicholas A, Lamb J, Naidoo Y. 2012. Elliptic Fourier analysis of leaf shape in southern African Strychnos section Densiflorae (Loganiaceae). Botanical Journal of the Linnean Society **170,** 542–553.
https://doi.org/10.1111/j.1095-8339.2012.01308.x

Andrade IM. 2006. Estudos morfométricos e genêticos em populaces de duas espécies de Araceae em areas florestais do Brasil, especialmente no Ceará. Unpublished PhD thesis, Universidade Estadual de Feira de Santana (UEFS), Feira de Santana, Bahia.

Andrade IM, Mayo SJ, Kirkup D, Van den Berg C. 2008**.** Comparative morphology of populations of Monstera (Araceae) from natural forest fragments in Northeast Brazil using elliptic Fourier analysis of leaf outlines. Kew Bulletin **63,** 193 – 211.
https://doi.org/10.1007/s12225-008-9032-z

Andrade IM, Mayo SJ, Kirkup D, Van den Berg C. 2010**.** Elliptic Fourier analysis of leaf outline shape in forest fragment populations of Anturium sinuatum and A. pentaphyllum (Araceae) from Northeast Brazil. Kew Bulletin **65,** 3 – 20.
https://doi.org/10.1007/s12225-010-9188-1

Bookstein FL, Strauss RE, Humphries JM, Cheronoff B, Elder RL, Smith GR. 1982**.** A comment upon the uses of Fourier methods in systematics. Systemic Zoology **31,** 85-92.
https://doi.org/10.2307/2413416

Diaz G, Zuccarelli A, Pelligra I, Ghiani A. 1989**.** Elliptic Fourier analysis of cell and nuclear shapes. Computer Biomedical Research **22**, 405-414.
https://doi.org/10.1016/0010-4809(89)90034-7

Ehrlich R, Weinberg B. 1970. An exact method for characterization of grain shape. Journal Sedimentary Petrology **40,** 205-212.

Ferson S, Rohlf FJ, Koehn RK. 1985**.** Measuring shape variation of two-dimensional outlines. Systematic Zoology **34,** 59-68.
https://doi.org/10.1093/sysbio/34.1.59

Futura N, Ninomiya S, Takahashi N, Ohmori H, Ukai Y. 1995. Quantitative evaluation of soybean (G. max L. Merr.) leaflet shape by principal component scores based on elliptic Fourier descriptor. Breeding Science **45,** 315 – 320.
https://doi.org/10.1270/jsbbs1951.45.315

Iwata H, Ukai Y. 2002**.** SHAPE: A computer program package for quantitative evaluation of biological shapes based on elliptic Fourier descriptors. Journal of Heredity **93,** 384 – 385.
https://doi.org/10.1093/jhered/93.5.384

Kidyue M, Boonkerd T, Thaithong O, Seelanan T. 2006. Variation within the Hoya parasitica (Asclepiadaceae) complex in Thailand. BRT. **2549,** 149 – 158.

Kuhl FP, Giardina CR. 1982. Elliptic Fourier features of a closed contour. Computer Graphics and Image Processing **18,** 236 – 258.
https://doi.org/10.1016/0146-664X(82)90034-X

Neto JC, Meyer GE, Jones DD, Samal AK. 2006**.** Plant species identification using elliptic Fourier leaf shape analysis. Computers and Electronics in Agriculture **50,** 121-134.
https://doi.org/10.1016/j.compag.2005.09.004

Primavera JH. 2000. Development and Conservation of Philippine Mangroves: Institutional issues. Ecological Economics **35,** 91-106
https://doi.org/10.1016/S0921-8009(00)00170-1

Primavera JH, Sadaba RB, Lebata MJHL, Altamirano JP. 2004. Handbook of mangroves in the Philippines – Panay. SEAFDEC Aquaculture Department, Iloilo, Philippines. p 106.

Ray TS. 1990**.** Application of eigenshape analysis to second order leaf shape ontogeny in Syngonium podophyllum (Araceae). In: F. J. Rohlf & F. L. Bookstein (eds), Proceedings of the Michigan Morphometrics Workshop. Special Publication No. 2, pp. 201 – 213. The University of Michigan Museum of Zoology, Ann Arbor, Michigan.

Ray TS. 1992. Landmark eigenshape analysis: homologous contours: leaf shape in Syngonium (Araceae). American Journal of Botany **79,** 69 – 76. https://doi.org/10.1002/j.1537-2197.1992.tb12625.x

Sheue CR, Liu HY, Yang YP. 2003. Morphology on stipules and leaves of the mangrove genus Kandelia (Rhizophoraceae). Taiwania **48,** 248 – 258. https://doi.org/10.6165/tai

Tabugo SRM, Torres MAJ, Olowa LF, Sabaduquia MAB, Macapil RM, Acevedo AM and Demayo CG. 2012. Elliptic Fourier analysis in describing Shape of the Mandible of the Larvae of the Coconut Leaf beetle Brontispa longissima Gestro, 1885 (Chrysomelidae: Hispinae) collected from Plants with varying Degrees of Damage. International Research Journal of Biological Sciences **1,** 19-26.

Tabugo SRM, Demayo CG, Torres MAJ. 2014. Elliptic Fourier analysis in describing shape of male appendages in Neurothemis species found in Iligan City, Philippines. Natural Journal **18,** 2-18.

Tomlinson PB. 1986**.** The Botany of Mangroves. Cambridge University Press.

Verdoorn IC. 1963**.** Loganiaceae. In: Dyer RA, Codd LE, Rycroft HB, eds. Flora of Southern Africa **26,** 134–149.

White R, Rentice HC, Verwist T. 1988**.** Automated image acquisition and morphometric description. Canadian Journal of Botany **66,** 450-459.