Application of elliptic Fourier analysis in understanding leaf shape characters of *Anthurium*

Jona C. B. Calacat, Mark A. J. Torres, and Cesar G. Demayo

Department of Biological Sciences, College of Science and Mathematics,
Mindanao State University - Iligan Institute of Technology,
9200 Iligan City, Lanao del Norte, Philippines.
Corresponding author: C. G. Demayo, cgdemayo@gmail.com

Abstract. The neotropical genus *Anthurium* is the largest and possibly the most complex genus of the Araceae. Conservative estimates suggest that a total of 600-800 species exist worldwide; and undoubtedly, many of these have not yet been described. The genus is taxonomically difficult, exhibiting considerable morphological plasticity in most structures and ordinary brief descriptions are without much value. Also, aroid systematists in general, and those applying numerical methods in particular, frequently use ratios to express the shape of the leaves and spathe in order to standardize for size. However, ratios may have undesirable statistical properties and do not accurately represent continuous shape differences in the leaves of these plants. In this study, however, advances in image analysis and geometric morphometric techniques were applied to discriminate between thirteen varieties of *Anthurium* and one outgroup, *Spathiphylum commutatum*; and to allow for complete and uniform quantitative descriptions for more accurate comparisons of the plants. To do this, full-color digital pictures of the leaves of the aroid plants were converted into binary images using an image processing and analysis software. Then, the contours of the leaves were summarized as chain-codes that were later converted into elliptic Fourier coefficients (equivalent to 20 harmonic modes). These coefficients were used as shape descriptors that were employed to automatically reconstruct and produce line drawings of the leaves. Principal component analysis of these elliptic Fourier descriptors revealed a total of 8 principal components that are associated with 8 independent shape characteristics. Results also showed that most of the variations described by the first principal component (PC1) could be attributed to differences in the shapes of the sinus between the posterior lobes on the base of the leaves (parabolic, hippocrepiform and spathulate). On the other hand, PC2 is associated with the leaf-aspect ratio and could be used to group varieties into those that have wide ovate (cordiform), narrow triangular-ovate, triangular-ovate, and ovate leaves. Principal components 3, 5, 6, 7 and 8 describe asymmetries in the shapes of the leaves while PC4 differentiates the varieties and species based on the extent and breadth of the posterior lobes. Further analysis using Kruskal-Wallis (non-parametric ANOVA) revealed that the variations defined by the first seven principal components are statistically significant (P<0.001). Decomposition of the symmetric and asymmetric components of leaf shape variation revealed that among the varieties and species studied, the leaves of the 'kaumana' variant are more asymmetrical when compared to the 'flamingo' variant. Cluster analysis was also employed on the elliptic Fourier coefficients to determine the systematic relationships of the ariod plants. The results of this study suggest that geometric morphometric analysis should be included among the tools used by taxonomists to objectively quantify and compare the shapes of two-dimensional structures such as the leaves of aroid plants.
Keywords: *Anthurium*, elliptic Fourier coefficients, Principal Component analysis, dendrogram.

Introduction. Leaf morphology has always played an important role in plant taxonomy, particularly for identifying taxa in which variation in floral structures is uninformative (e.g. *Anthurium spp.*) or in which flowering specimens are infrequent owing to, for example, a limited flowering season (Stace 1989). However, the relative paucity of informative leaf characters in many taxa means that often the most effective way to characterize taxonomic variation is to sample extensively a set of landmarks or other measures of leaf shape and so generate an accurate numerical representation of the leaf types in each sample (Dickinson et al 1987; Marcus 1990; Marcus et al 1993). Statistical analysis of this type of data has revealed that it can be a powerful key for taxonomic differentiation among species, populations and hybrids across a broad systematic and taxonomic spectrum, Araceae (Ray 1992), of which *Anthurium* belongs. Taxonomonic

information on *Anthurium* is important due to its economic value as commercial species for historical breeding processes and traditional consumer preferences.

Although the analysis of landmark data has become a popular choice for leaf morphology studies (Jensen et al 2002), analysis of leaf measurement data using multivariate techniques such as Principal Components Analysis (PCA; Sneath & Sokal 1973) and elliptic Fourier descriptors method is also employed successfully for the investigation of taxonomic relationships (Ehrlich et al 1983; Kuhl & Giardina 1982; Rohlf & Archie 1984; White et al 1988). These methods have been applied in studies of organs of several plant species, for example, *Betula* leaf (White et al 1988), soybean leaflet (Furuta et al 1995), Japanese radish root (Iwata et al 1998) and citrus leaf (Iwata et al 2002a, 2002b).

Elliptic Fourier method is an outline based morphometrics that relies on some advantages mentioned by Iwata et al (1998): it does not require neither landmarks or previous knowledge about the shape variation of the objects under study; it can visualize the contour of information and reconstruct the original shape; it can be mathematically normalized to size, rotation and starting point of the contour trace; and it can be conducted automatically using computer software. The present study evaluates the advantages when using the elliptic Fourier method in the numerical and descriptive analysis of *Anthurium* leaf shapes. This non-traditional taxonomic discrimination techniques (EF-PCA) was applied to: first, detect small variations in shape, and second, evaluate the shapes of the leaf shape independently of their size. Hence, the variation of *Anthurium* leaf shape could be decomposed into several mutually independent quantitative characteristics. In this manner, unacceptable errors based only on human visual judgment of shape which is frequently deceived and misled by size factors, can be effectively eliminated. Likewise, principal component analysis (PCA) of the coefficients can extract the independent shape characters, and make it possible to analyze the shape quantitatively by using the component scores as ordinary quantitative characters.

Materials and Methods. Leaf samples of thirteen commercial varieties of Anthurium comprising the species of *Anthurium andreanum*, one species of *Anthurium antioquense* and one cross hybrid from both Anthurium species were examined. These were randomly sampled and taken from different mini plantation areas in Mibolo, Brgy. Tipanoy Iligan City, Kauswagan, Lanao del Norte, MSU Campus, Marawi City, and Brgy. San Miguel, Iligan City. The aroid "peace plant" *Spathyphillum commutatum* which belongs to the same Araceae family with *Anthurium* was assigned as the Outgroup. These varieties provided representative variation of *Anthurium* leaf shapes (Table 1).

Table 1

List of *Anthurium* plant cultivars used in this study

SCIENTIFIC NAME	VARIETY/CULTIVAR
1. *Anthurium andreanum*	"Acropolis"
2. *Anthurium andreanum*	"Baguio red"
3. *Anthurium andreanum*	"CDO red"
4. *Anthurium andreanum*	"Dark red Ozaki"
5. *Anthurium andreanum*	"Ozaki"
6. *Anthurium andreanum*	"Flamingo"
7. *Anthurium andreanum*	"Kaumana"
8. *Anthurium andreanum*	"Midori"
9. *Anthurium andreanum*	"Nitta" or "Nitta orange"
10. *Anthurium andreanum*	"Red butterfly"
11. *Anthurium andreanum*	"Obake pink"
12. *Anthurium antioquiense*	"Pink"
13. *A. andreanum* X *A. antioquiense* hybrid	Nitta X antioquiense" / "NOAH"
14. *Spathiphyllum commutatum* (Outgroup)	

Image processing and quantitative measurement of the leaf shapes. Digitized images of *Anthurium* leaves were captured with an Olympus digital color image processor. Outlines

83

of leaf samples were traced digitally while maintaining proper orientation of leaf structures was important in for consistent data capture. Each image was saved as an RGB color image in BMP image format with 256 brightness levels per channel (blue). The binary image of each leaf was obtained by thresholding the original blue digital image of the leaf as it gave the clearest contrast between the leaves and the background. Undesirable marks also termed as "noise" were found on the transformed binarized images and consequently eliminated by erosion-dilation and dilation-erosion filter process. After noise reduction, the closed contour shape of each leaf was extracted by edging the binary image and was then described by chain-code matrix (Freeman 1974).

Analysis of Leaf Morphometric shape characterization. Leaf shape outlines as revealed from the chain-code derived method were further analyzed and were calculated into coefficients of EFDs (elliptic Fourier descriptors). Because these coefficients are not invariant in size, rotation, shift or starting point of chain-coding for contour traces, these cannot be used as indices in comparing the shape of objects like the leaf shape contours of plant species. For example, the Fourier coefficients differ even for exactly the same shape when the starting point of the chain-coding differs. Conversely, the Fourier coefficients were then subjected to normalized modes to avoid variation, as suggested by Kuhl & Giardina (1982). This normalizing formula is based on the ellipse of the first harmonic. By this procedure, the shape of each *Anthurium* leaf in this study was approximated by the first 20 harmonics. Thus, 80 coefficients could be calculated and classified into two groups related to symmetrical variation (40 coefficients) and asymmetrical variation (40 coefficients) (Iwata et al 1998; Yoshioka et al 2004).

Given the enormous number of normalized coefficients of EFDs ceded from the above technique, it is still impossible to openly interpret these coefficients as shape descriptors and that the morphological correlation for each coefficient is tough to explain discretely. Hence, Principal Components Analysis (PCA) based on a variance and co-variance matrix was carried out in order to summarize the information of the variations stored in the Fourier descriptor coefficients. The coefficients of the EFDs were recalculated to determine the effect of each principal component on leaf shape by allowing a particular principal component score be equal to the mean ± 2 SD (standard deviations) while keeping the scores of the remaining components as means. Conversely, the leaf contour shapes were reconstructed on the basis of the recalculated EFDs coefficients for each PC score condition. The mean scores of PCA is a useful statistical technique that has found application in fields such as face recognition and image compression, and is a common technique for finding patterns in data of high dimension. The PCA calculation scheme is executed based on a variance-covariance matrix, whereby the derived component scores were utilized in the subsequent analysis (Iwata & Ukai 2002).

In determining the differences of leaf shapes among the aroid species, a non-parametric ANOVA, using Kruskal-Wallis test, was performed on the principal component scores. Box-and-whisker plot visualization patterns were also illustrated as represented by each decomposed principal component scores pertaining to leaf shape differences. The elliptic Fourier descriptors and the PCA were calculated with SHAPE Ver. 1.3 (Iwata & Ukai 2002).

Systematic relationships on the phenotypic attributes among the plant varieties focused on the leaf shape similarities were evaluated. This is primed to detect the extent of compounding dissimilarities between two species or varieties by means of hierarchical cluster analysis, specifically, the Ward's method, which makes use of the Euclidean distance algorithm.

Results and Discussion. Among the first seven significant principal component scores of the coefficients of leaf shape, the major proportion of the total variation was found in the first and second principal component which accounted for the depth of the notch between the posterior lobes on the base of the leaves and the ratio of leaf length to width respectively (Table 2 and Fig. 2). Leaf elongation is the cumulative effects of cell elongation wherein several genes are know to be involved in the developmental process. Cell production and cell elongation in leaves are relatively independent (Green 1976).

Tsukaya (1995) suggested that the polar elongation of leaf cells in the lateral and longitudinal directions are influenced by two independently acting genetic systems in his study on *Arabidopsis* leaves. This may mean that the same genetic system may control leaf length and width in *Anthurium* species. A decade later, Tsukaya (2005) expound his findings on the genetic controls and environmental mechanisms regarding leaf shape determination. He stated that the lateral, two-dimensional expansion of leaf blades is highly dependent on the determination of the dorsoventrality of the primordia, a defining characteristic of leaves and that having a determinate fate is also a characteristic feature of leaves and is controlled by many factors. Furthermore, lateral expansion is not only controlled by general regulators of cell cycling, but also by the multi-level regulation of meristematic activities, e.g., specific control of cell proliferation in the leaf-length direction, in leaf margins and in parenchymatous cells. In collaboration with the polarized control of leaf cell elongation, these redundant and specialized regulating systems for cell cycling in leaf lamina may realize the elegantly smooth, flat structure of leaves. The unified, flat shape of leaves is also dependent on the fine integration of cell proliferation and cell enlargement. In addition, it has long been known that shape and form of plants are highly affected by their environmental conditions. Stressful growing environments typically cause deviation from regularity of form and shape of plant leaves. The variations in leaf shape of the varieties in this investigation also indicate that organs in the leaves of plants show a more or less quantitative variation in spite of being governed by genotype. This variation is considered to be due, in addition to local fluctuation in environmental conditions outside and/or inside the plant body, to mistakes in the developmental course of organs resulting probably from the intrinsic nature of the organisms.

Table 2

Eigenvalues and contributions of the first eight principal components of the elliptic Fourier descriptors estimated for the Symmetrical and Asymmetrical data

COMPONENT	EIGENVALUE (10^{-4})	PROPORTION (%)	CUMULATIVE (%)	TOTAL VARIANCE
Symmetrical				
1	124.82	69.25	69.25	185.64 (86.83%)
2	26.19	14.53	83.78	
3	17.22	9.56	93.34	
4	5.25	2.91	96.25	
Asymmetrical				
1	19.35	70.68	70.68	28.15 (13.17%)
2	3.46	12.65	83.34	
3	1.17	4.28	87.61	
4	1.04	2.99	91.41	

The same principal components were responsible for the large contribution of symmetrical variations to the total variance in the leaf shape (Fig. 3, Table 2). The degree of roundness and the extent and breadth of the posterior lobes which accounted for by the fourth principal component, approximated 8% of the total shape variation.

85

Figure 1. Thirteen varieties and species of *Anthurium* and one outgroup. 1, "Acropolis"; 2, "Baguio red"; 3,"CDO red"; 4, "Dark Ozaki"; 5 "Ozaki"; 6, "Flamingo"; 7, "Kaumana"; 8, "Midori"; 9, "Nitta orange"; 10, *Anthurium antioqueinse*; 11, "Red butterfly"; 12, "NOAH"; 13, "Obake pink"; 14, "Outgroup", *Spathiphyllum commutatum*.
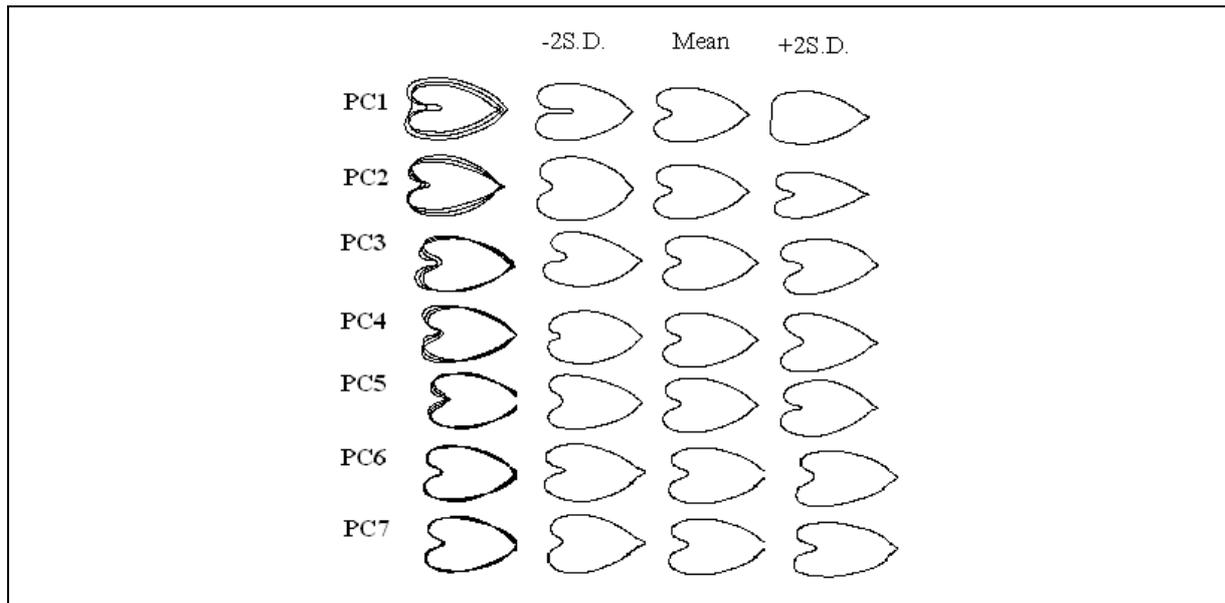
Figure 2. Contour shape of the leaf drawn using elliptic Fourier descriptors estimated under seven principal component scores (PC). Each column shows that the score takes either +2S.D., mean or -2S.D. The leftmost column illustrates the overlaid drawings of the three cases the asymmetrical variations among leaf samples with more than 60% contribution of the total variance.

Results suggest that there is a high heritability factor accompanying these leaf shape traits provided by the component scores due to its high genotypic effects based from the results. It also indicates that such leaf shape characters are suitable indices for comparing leaf shape variation among varieties and between species. Symmetry is a basic property of shapes and structures. In biological and physical systems, symmetry seems to imply stability and natural development. Accordingly, symmetric individuals have greater developmental stability and usually exhibit greater reproductive success, and better survival rates than asymmetric individuals (Freeman et al 1993). Based on the results of this study, knowledge on the symmetrical nature of *Anthurium* leaf shapes could correlate to the reproductive ability of the varieties. Reproductive success and better survival rate is highly essential in plant breeding industry since breeders would always choose and cultivate for varieties with such superior and heritable traits to gain maximum production and profit. According to Rettig et al (1997) measures of developmental instability such as fluctuating asymmetry may provide a more sensitive indicator of stress than traditional measures (e.g. growth, survival). It has also been suggested that the orientation and symmetry of a leaf can be referred to the action of growth-regulating substances moving basipetally from the apical cell and that these characteristic developments are mediated through the organization and physiological activity of the apex as a whole, the intact apical cell being a central and essential element of the system (Wardlaw 1955).
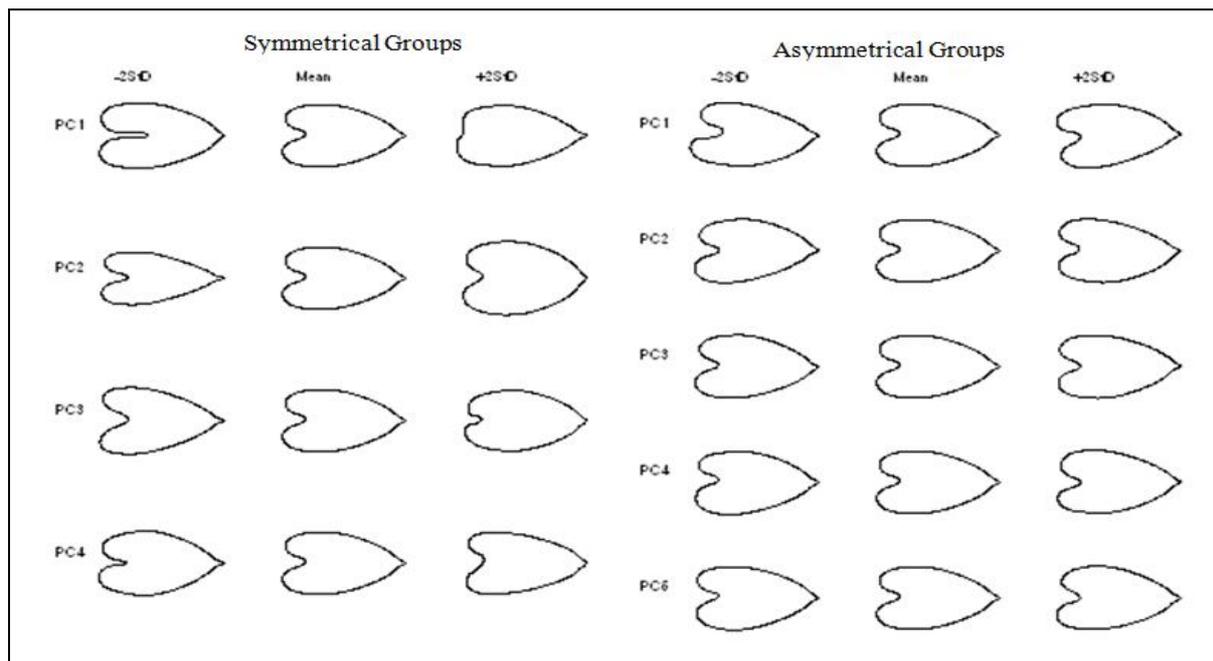
Figure 3. Aspects of Leaf shape variations expressed for by significant principal components reconstructed from the coefficients. Each column illustrates the score that assumes either +2S.D., mean and -2S.D.

In contrast to the components described above, the asymmetric group variations accounted for by the third, fifth, sixth and seventh principal components was associated primarily to environmental effects which explained a cumulative proportion to almost 24% (Table 2). These results suggest that the asymmetrical variance due to genotypic differences is comparatively low in the leaf shape. This indicates that the controlling influence of genotype on asymmetrical shape variation was not strong (Table 3). However, there were significant differences at ($P<0.001-<0.0001$) level in some principal components of the asymmetrical group except for the third principal component which is moderately significant at ($P: <0.0062$). Phenotypic differences between individuals for bilateral traits exhibiting fluctuating asymmetry (Van Valen 1962) are considered to reflect the degree to which development is affected by environmental stress. The asymmetrical variation of *Anthurium* leaf shapes implies that the observed curve forms or semi roundedness of one side of the leaf was different from the other side. Hence, these can be categorized as antisymmetry or fluctuating symmetry or both. Fluctuating symmetry is defined to occur when the difference between a character on the left and right side of individuals normally distributed about a mean of zero (Van Valen 1962). The experimental basis for using fluctuating asymmetry as a measure of developmental stability is well established (Valentine & Soulé 1973). Developmental stability or homeostasis refers to the ability of an individual to produce a consistent phenotype in a given environment (Graham et al 1993). Reduced developmental stability can result from a wide variety of environmentally (or genetically) induced perturbations (Valentine & Soulé 1973; Siegel et al 1992; Yablokov 1986; Clark & Mckenzie 1992). In plants, fluctuating asymmetry has also been used as a tool for monitoring levels of ecological stress. Several authors have cited the application of measuring fluctuating asymmetries in Holm oak (Hodar 2005); tree birches (Wisley et al 1998; Nagamitsu et al 2004) and sea grass (Ambo-Rappe et al 2007).

Additional analysis was also carried out in this investigation using Kruskal-Wallis (non-parametric ANOVA). Table 3 revealed the results of this test performed on each principal component scores of both symmetrical and asymmetrical groups. Significant differences on the principal component comparisons among the leaves of the varieties and species are remarkable. The variations defined by the first seven principal components created from the leaf shape characteristics are statistically significant

88

(P<0.001). While principal component three, gave a significant yet intermediate score value (Table 3), this indicates that to be less phenodeviant from the asymmetrical orientation viewpoint based on the pooled variation expressed by reconstructed shapes in the leaf samples compared to the rest of the principal component scores. According to Yoshioka et al (2004), asymmetrical variations of leaves can be related to environmental stress experienced by plants.

The effects of each principal component described separately into Symmetrical and Asymmetrical groups are visualized in Figure 3. The symmetrical groups of reconstructed leaf contours ascribed to the first four principal components while the first five principal components were produced to asymmetrical group. Based from the redrawn component scores in the symmetrical group, the first principal component in the symmetrical group reflects a good assessment of the leaf aspect ratio and it also characterizes ovate-cordate shape variations concerning the depth of the sinus in the leaf base (deep, shallow or absent notch). The symmetrical shape variation on the second principal component is associated with length to width ratio. The third principal component expresses bluntness of the base part of the leaf while the fourth significant component is related to the bluntness of the apical region of the leaf. In contrast, the first component in the asymmetrical group describes the inclination of the lobe curvature in leftmost or rightmost lateral aspect of the laminar direction proximal to the base part. The rest of the principal components (2, 3, 4 and 5) in the asymmetrical class generally present notable curvature dissimilarity differences and in the distal (base-portion) and proximal (apical-portion) parts of the leaf.

Leaf shape difference among the thirteen varieties of *Anthurium* shown in Figure 5 and 6 could be classified and identify variants with similar shape patterns using the principal component scores variations which are organized into box-and-whisker plot presentations. From these results, measures of variations, central tendency to mean shape and distribution form could be detected in specific varieties as leaf shape characters. Observable shape variations related to leaf aspect ratio is attributed by the second component (PC2). 'Nitta orange' (8) 'Ozaki' (13) gave the lowest first principal component scores because they assumed shallow or almost absent notch cordate bases while compared in contrast to 'Midori'(7) and Nitta-Orange X *A. antioqiuense* hybrid or 'Noah'(9) which have deep notch cordate base both attained highest component scores.

Table 3. Kruskal-Wallis Test (Nonparametric ANOVA) results for the significant principal components (PC) using the variation Fourier coefficients of the leaf shape contours

COMPONENT	KW	P-VALUE	REMARKS
Overall			
1	346.81	<0.0001	extremely significant
2	270.67	<0.0001	extremely significant
3	35.889	<0.0006	extremely significant
4	285.57	<0.0001	extremely significant
5	232.13	<0.0001	extremely significant
6	123.88	<0.0001	extremely significant
7	229.49	<0.0001	extremely significant
Symmetric			
1	345.17	<0.0001	extremely significant
2	263.24	<0.0001	extremely significant
3	283.89	<0.0001	extremely significant
4	308.03	<0.0001	extremely significant
Asymmetric			
1	66.280	<0.0001	extremely significant
2	93.607	<0.001	extremely significant
3	29.151	<0.0062	very significant
4	115.20	<0.0001	extremely significant
5	90.574	<0.0001	extremely significant
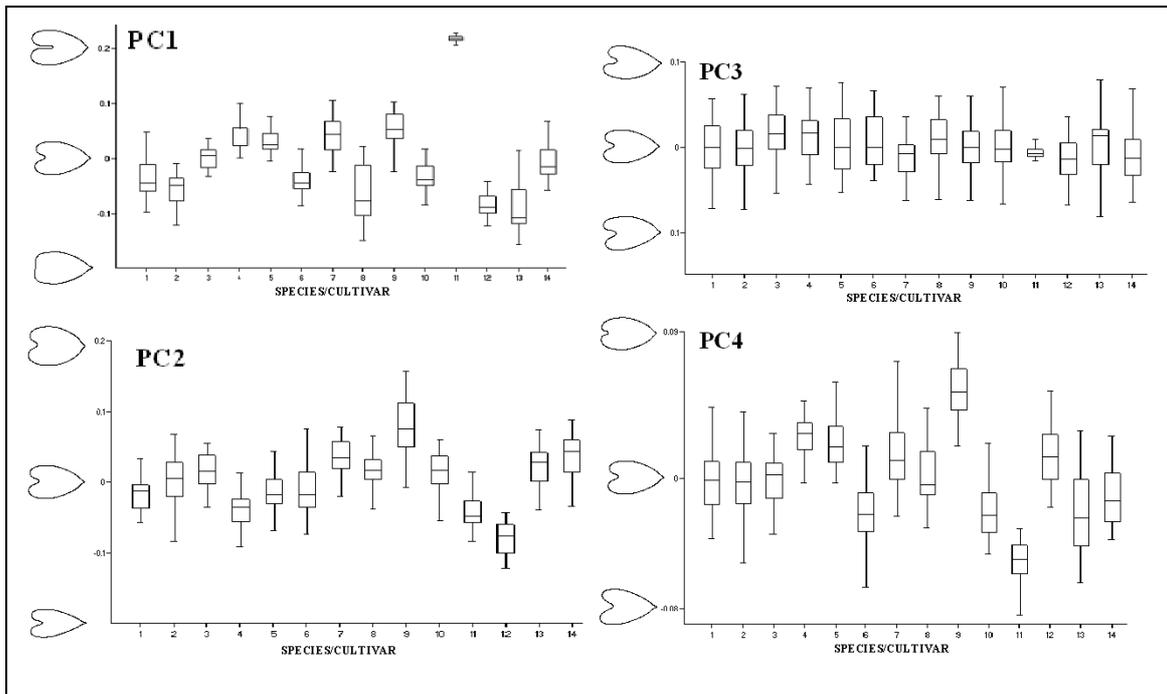
Figure 4. Redrawn Overall leaf shape among *Anthurium* varieties in Box-and-whisker plots visual representations exhibiting shape variance and distribution of the first 4 significant principal components scores based on variation coefficients. 1, "Acropolis"; 2, "Baguio red"; 3,"CDO red"; 4, "Kaumana"; 5, "Dark Ozaki"; 6, "Flamingo"; 7, "Midori"; 8, "Nitta orange"; 9, "Noah"; 10, "Obake pink"; 11,"Outgroup"; 12, *Anthurium antioqueinse*; 13, "Ozaki"; 14, "Red butterfly".
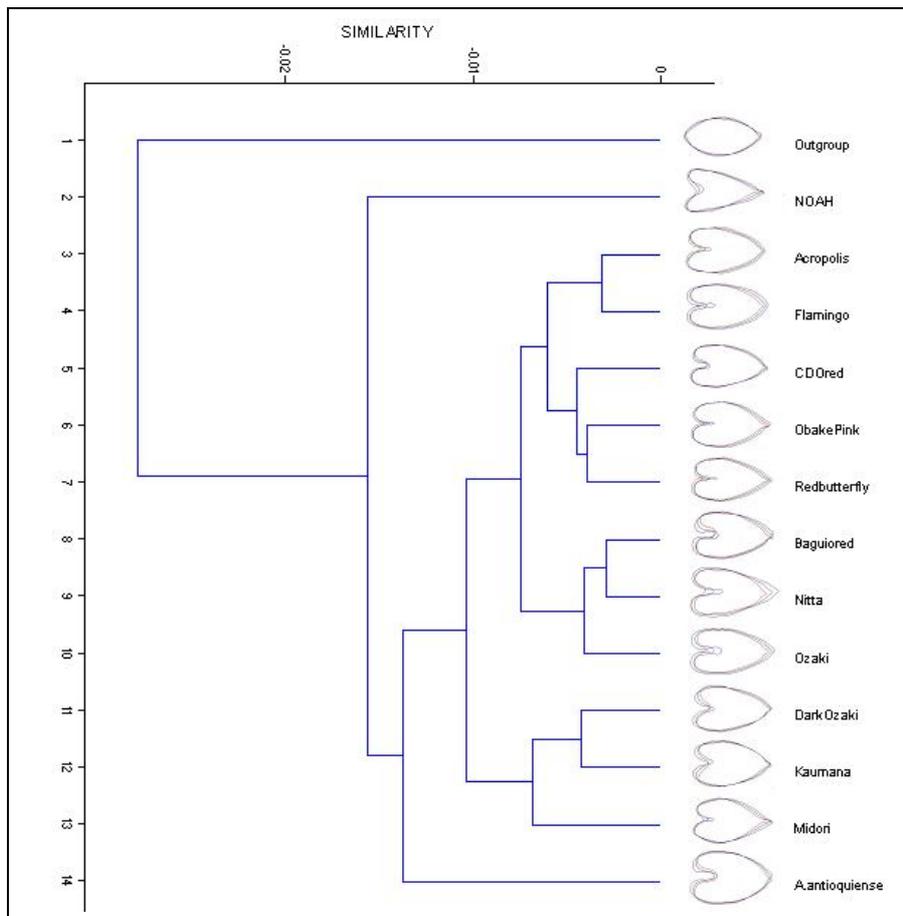
Figure 5. Cluster analysis based on the seven significant principal components of the leaf shape.

90

Conclusions. The elliptic Fourier method fixed with Principal Component Analysis used in this study provides a convenient and powerful tool for quantitatively measuring leaf shape variations of *Anthurium* species and varieties. The technique was also successful in dissociating the variation scores in the data sets into symmetrical and asymmetrical group categories. The significant component scores obtained for the hierarchical cluster analysis could be successfully used to represent differences among varieties. The success of this approach was attributed to the facts that it represents the relative closeness between varieties. Cluster analysis could be a good auxiliary tool in elucidating leaf shape relationship of the Aroid plants.

References

Ambo-Rappe R., Lajus D. L., Schreider M. J., 2007 Translational fluctuating asymmetry and leaf dimension in seagrass, *Zostera capricorni* Aschers in a gradient of heavy metals. Environmental Bioindicators **2**(2):99-116.

Clark G. M., Mckenzie J. A., 1992 Fluctuating asymmetry as a quality control indicator for insect mass rearing projects. Journal of Economic Entomology **85**:2045-2050

Dickinson T. A., Parker W. H., Strauss R. E., 1987 Another approach to leaf shape comparisons. Taxon **36**:1-20.

Ehrlich R., Baxter Pharr R., Healy-Willians N, 1983 Comments on the validity of Fourier descriptors in systematics: reply to Bookstein et al. Systematic Zoology **32**: 202–206.

Freeman H., 1974 Computer processing of line drawing images. Comp Surv. **6**:57-97.

Freeman D. C., Graham J. H., Emlen J. M., 1993 Developmental stability in plants: symmetries, stress, and epigenesis. Genetica **89**:97-119.

Furuta N., Ninomiya S., Takahashi N., Ohmori H., Ukai Y., 1995 Quantitative evaluation of soybean (*Glycine max* L. Merr.) leaflet shape by principal component scores based on elliptic Fourier descriptor. Breed Sci **45**:315–320.

Graham J. H., Freeman D. C., Emlen J. M., 1993 Developmental stability: a sensitive indicator of populations under stress. W. G. Landis, J. S. Hughes, M. A. Lewis (eds.), Environmental toxicology and risk assessment. American Society for Testing and Materials, Philadelphia. 136–158.

Green S., 1976 Accuracy of decoupling approximations for rotational excitation: Low-energy CO-He collisions. Chem Phys Lett **38**:293-296.

Hódar J. A., 2005 Leaf fluctuating asymmetry of holm oak in response to drought under contrasting climatic conditions. J Arid Environ **52**(11):233-243.

Iwata H., Niikura S., Matsuura S., Takano Y., Ukai Y., 1998 Evaluation of variation of root shape of Japanese radish (*Raphanus sativus* L.) based on image analysis using elliptic Fourier descriptors. Euphytica **102**:143–149.

Iwata H., Ukai Y., 2002 SHAPE: a computer program package for quantitative evaluation of biological shapes based on elliptic Fourierdescriptors. The Journal of Heredity **93**: 384–385.

Iwata H., Nesumi H., Ninomiya S., Takano Y., Ukai Y., 2002a The evaluation of genotype x environment interactions of citrus leaf morphology using image analysis and elliptic Fourier descriptors. Breeding Science **52**:243-251.

Iwata H., Nesumi H., Ninomiya S., Takano Y., Ukai Y., 2002b Diallel analysis of leaf shape variations of citrus varieties based on elliptic Fourier descriptors. Breeding Science **52**:89-94.

Jensen L., Gupta R., Blom N., Devos D., Tamames J., Kesmir C., Nielsen H., Stærfeld H., Rapacki K., Workman C., Andersen C., Knudsen S., Krogh A., Valencia A., Brunak S., 2002 Prediction of human protein function from post-translational modifications and localization features. J Mol Biol **319**:1257-1265.

Kuhl F. P., Giardina C. R., 1982 Elliptic Fourier features of a closed contour. Comp Graph Image Proc **18**:236–258.

Marcus L. F., 1990 Traditional morphometrics. F. J. Rohlf and F. L. Bookstein, eds. Proceedings of the Michigan morphometrics workshop. Special publication no. 2. Univ. of Michigan Museum of Zoology, Ann Arbor, MI. 77–122.

Marcus L. F., Bello E., García-Valdecasas, A., 1993 Contributions to Morphometrics. Madrid: Monografías del Museo Nacional de Ciencias Naturales de Madrid (C.S.I.C), 8.

Nagamitsu T., Kawahara T., Hotta M., 2004 Phenotypic variation and leaf fluctuating asymmetry in isolated populations of an endangered dwarf birch *Betula ovalifolia* in Hokkaido, Japan. Plant Species Biology **19**:13–22.

Ray T. S., 1992 Landmark eigenshape analysis: homologous contours: leaf shape in *Syngonium* (Araceae). American Journal of Botany **79**:69-76.

Rettig J. E., Fuller R. C., Corbett A. L., Getty T., 1997 Fluctuating asymmetry indicates levels of competition in an even-aged poplar clone. Oikos **80**:123-127.

Rohlf F. J., Archie J. W., 1984 A comparison of Fourier methods for the description of wing shape in mosquitoes (Riteraculicidae). Syst Zool **33**:302–317.

Siegel M. I., Mooney M. P., Taylor A. B., 1992 Dental and skeletal reduction as a consequence of environmental stress. Acta Zool Fennica **191**:145-149.

Sneath P. H. A., Sokal R. R., 1973 Numerical taxonomy - the principles and practice of numerical classification. W. H. Freeman: San Francisco.

Stace C. A., 1989 Plant taxonomy and biosystematics. 2nd edition. Edward Arnold, London.

Tsukaya H. 1995 Developmental genetics of leaf morphogenesis in dicotyledonous plants. J Plant Res **108**:407-416.

Tsukaya H., 2005 Molecular variation of *Spiranthes sinensis* (Orchidaceae) in Japan, with special reference to systematic treatment of seasonally differentiated groups and a dwarf form, f. gracilis, from Yakushima Island. J Plant Res **118**:13-18.

Valentine D. W., Soulé M., 1973 Effect of p,p'- DDT on developmental stability of pectoral fin rays in the grunion, Leuresthes tenuis. Fisheries Bulletin **71**:921–926.

Van Valen L., 1962 A study of fluctuating asymmetry. Evolution **16**:125–142.

Wardlaw C. W., 1955 Embryogenesis in plants. New York: John Wiley & Sons, Inc.

White R. J., Rentice H. C., Verwist T., 1988 Automated image acquisition and morphometric description. Can J Bot **66**:450–459.

Wisley B. J., Haukioja E., Koricheva J., Sulkinoja M., 1998 Leaf fluctuating assymetry increases with hybridization and elevation in tree-line birches. Ecology **97**:2092-2099.

Yablokov A. V., 1986 Population Biology: Progress and problems on natural populations. Adv Sci Tech USSR Biol Ser, MIR Publishers Moscow.

Yoshioka Y., Iwata H., Ohsawa R., Ninomiya S., 2004 Analysis of petal shape variation of *Primula sieboldii* E. Morren by elliptic Fourier descriptors and principal component analysis. Ann Bot **94**:657–664.

Authors:
Jona Christine B. Calacat, Department of Biological Sciences, College of Science and Mathematics, Mindanao State University - Iligan Institute of Technology, 9200 Iligan City, Lanao del Norte, Philippines, jonachristine@yahoo.com
Mark Anthony J. Torres, Department of Biological Sciences, College of Science and Mathematics, Mindanao State University - Iligan Institute of Technology, 9200 Iligan City, Lanao del Norte, Philippines, torres.markanthony@gmail.com
Cesar G. Demayo, Department of Biological Sciences, College of Science and Mathematics, Mindanao State University - Iligan Institute of Technology, 9200 Iligan City, Lanao del Norte, Philippines, cgdemayo@gmail.com