The Thailand Natural History Museum Journal 15(1), 21-30, 30 June 2021

http:doi.org/10.14456/thnhmj.2021.2

Leaf Outline Evaluation in Selected Philippine *Hoya* R. Br. Species (Apocynaceae) Using Elliptic Fourier Analysis

Jess H. Jumawan[1,2*] and Inocencio E. Buot, Jr.[2]

[1] *Biology Department, Caraga State University, Butuan City 8600 Philippines*
[2] *Plant Biology Division, Institute of Biological Sciences, University of the Philippines Los Baños (UPLB), College, Laguna 4030 Philippines*
*Corresponding Author: jhjumawan@up.edu.ph

ABSTRACT

Variations in leaf shape contours were analyzed using elliptic fourier descriptors (EFDs) of three *Hoya* species namely: *Hoya buotii*, *Hoya coriacea*, and *Hoya halconensis*. The species were grown under greenhouse conditions and exhibited resemblances which could become source of taxonomic confusion as reproductive parts were needed for species discrimination. Thirty leaf samples were photographed, converted to binary images, and subjected to elliptic fourier analysis (EFA). The extracted EFDs were quantitatively analyzed by PCA. The results utilized four effective principal component (PC) scores with 84.93% total variation. The largest variation contributed by PC1 (58.43% variation) was due to laminar size. Significant differences contributed by PC1 and PC3 (Anova P<0.01) could be genotypic heritability. The non-significant variations attributed by PC2 and PC4 (Anova P>0.05) were suggested to be environmentally influenced. The three species depicted similarity at roundness of base, tapering towards tip (PC2) and roundness along leaf margin (PC4) were possible sources of taxonomic confusion. The dendrogram from cluster analysis revealed leaf outlines similarity between *H. coriacea* and *H. buotii* at about 50% Euclidean index. The leaf outline variations could be intricate by human recognition and small quantitative variations detected by EFA provided an effective platform in characterizing leaf shapes.

Keywords: elliptic fourier analysis, principal component analysis, *Hoya* species, cluster analysis.

INTRODUCTION

Species of *Hoya* were commonly named as wax plants and considered as the most cultivated ornamental plants for its beautiful and fragrant flowers (Tran *et al*., 2011). The genus *Hoya* belonging to family Apocynaceae has approximately more than 200 recognized species (Kleijn and Van Donkelaar, 2001) but listed with more than 860 species names in The Plant Names Project (IPNI, 2015). Taxonomists working with *Hoya* species could not agree on its total number (Aurigue *et al*., 2013). Its distribution is widespread in the tropical forests of Southeast Asian region, China, India, New Guinea, and the Pacific Islands (Li *et al*., 1995; Forster and Little 1996; and Wanntorp *et al*., 2006a).

The Philippines is one of the biologically richest countries in the world with more than half of the biodiversity found nowhere else on earth (Ong *et al*., 2002). The country is also known as the center of diversity in the world for *Hoya* species along with Borneo and New Guinea (Cabactulan *et al*., 2017). The *Hoya* species in the country are one of the highest in terms of species richness with a high level of endemism. The total account for *Hoya* species ranges from 80 – 104 (Kloppenburg *et al*., 2012 and Aurigue *et al*., 2013). This number is considered as one of the richest and most diverse in the range of *Hoya* species (Kloppenburg and Siar, 2008). Many of the *Hoya* species were still discovered and the species counts are expected to increase. Most *Hoya* were endemic species and the growing number of enthusiasts in the country both increased the interests and demands for the market. This resulted to some endemic species being sold in the market as ornamental plants (Maranan and Diaz, 2013).

Traditional taxonomy required great importance to qualitative and quantitative features of flower parts and pollinarium to identify and delineate *Hoya* species (Kleijn and Van Donkelaar, 2001). However, not all plants in the wild might bear reproductive structures like flowers and fruits. This makes identification more challenging and often leads to confusion with limited taxonomic characters to consider. Aside from that, *Hoya* is considered as a taxonomically complex genus (Wanntorp *et al*., 2006b). Nomenclature issues were unresolved for various taxa (Rodda and Juhoneweb, 2013). The three *Hoya* species, due to similarity of its leaf morphological traits, were categorized to be controversial species (Salvańa and Buot, 2014). Both *Hoya buotii* and *Hoya halconensis* were argued to be morphologically distinct in leaf architectural traits

(Jumawan and Buot, 2016). The resolve to conduct DNA analyses for confirmation of species is a resolute promise. But this method is impractical as it is expensive which still needs initial identification using traditional taxonomy.

This study attempted to employ elliptic fourier leaf shape analysis in selected *Hoya* species in the Philippines namely *H. buotii* Kloppenburg, *H. coriacea* Blume and *H. halconensis* Kloppenburg. Elliptic fourier analysis (EFA) was recognized and accepted taxonomic tool using digital imaging. The technique was not used to its full potential, in that it allows comparison of phenotypic measurements of specimens and assigning the taxa to which they belong (Corney *et al*., 2012). The EFA was used to describe leaf variability of some *Hoya* species (Torres *et al*., 2008a) and provided insights for leaf, flower and seed variations (Torres *et al*., 2008abc; Dalayap *et al*., 2011; Calacat *et al.*, 2011; and Apuan *et. al*., 2011) of various plants. It also endeavors to evaluate the quantitative aspect about the potential source of confusion in the leaf outlines of the three *Hoya* species. The selected *Hoya* species were initially collected in the wild and later propagated under greenhouse conditions. The use of traditional taxonomy has caused confusion in the identification of the three *Hoya* species due to its resemblances in morphology. Reproductive parts of the plants were often discriminatory in species identification but absent most of the time. The objective of this study was to utilize EFA to analyze the leaf shape outlines of the selected *Hoya* species.

MATERIALS AND METHODS

Plant materials

The selected *Hoya* species were acquired from the propagated plant collections of Dr. I. Buot, Professor and curator of IBS herbarium in UPLB (Figure 1). The *Hoya* species were collected from field explorations and propagated for taxonomic studies. The *Hoya* plants grown under greenhouse conditions expressed resemblances in leaf traits (Figure 2). The morphological

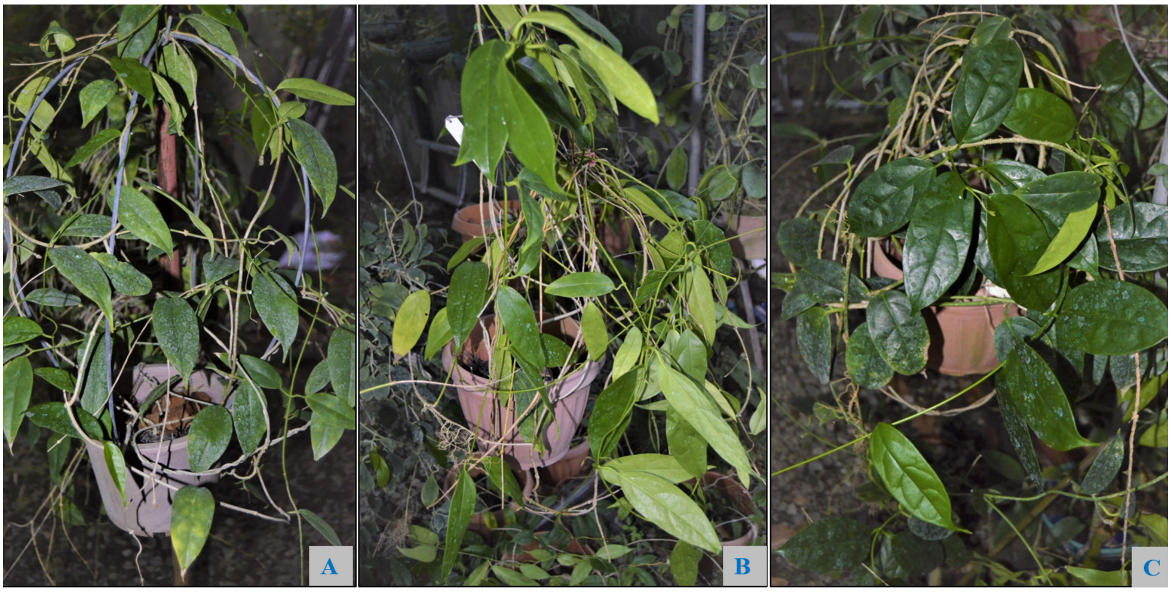

Figure 1. The plant materials consist of *Hoya buotii* Kloppenburg (A), *Hoya coriacea* Blume (B) and *Hoya halconensis* Kloppenburg (C) were grown under greenhouse conditions.

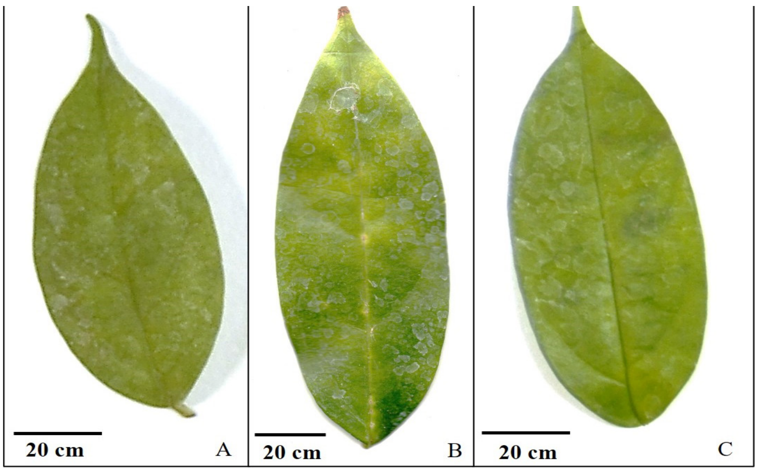

Figure 2. Leaf morphological traits of *Hoya buotii* (A), *Hoya coriacea* (B) and *Hoya halconensis* (C) exhibit resemblances when grown under greenhouse conditions.

similarities in leaves could potentially become sources of taxonomic confusion. At least 30 leaf samples were used for the study. Matured leaves were considered for analysis. Leaves at the first position from the tip were excluded for consistency.

Leaf image recording and processing

Leaf images were acquired using a DSLR camera with

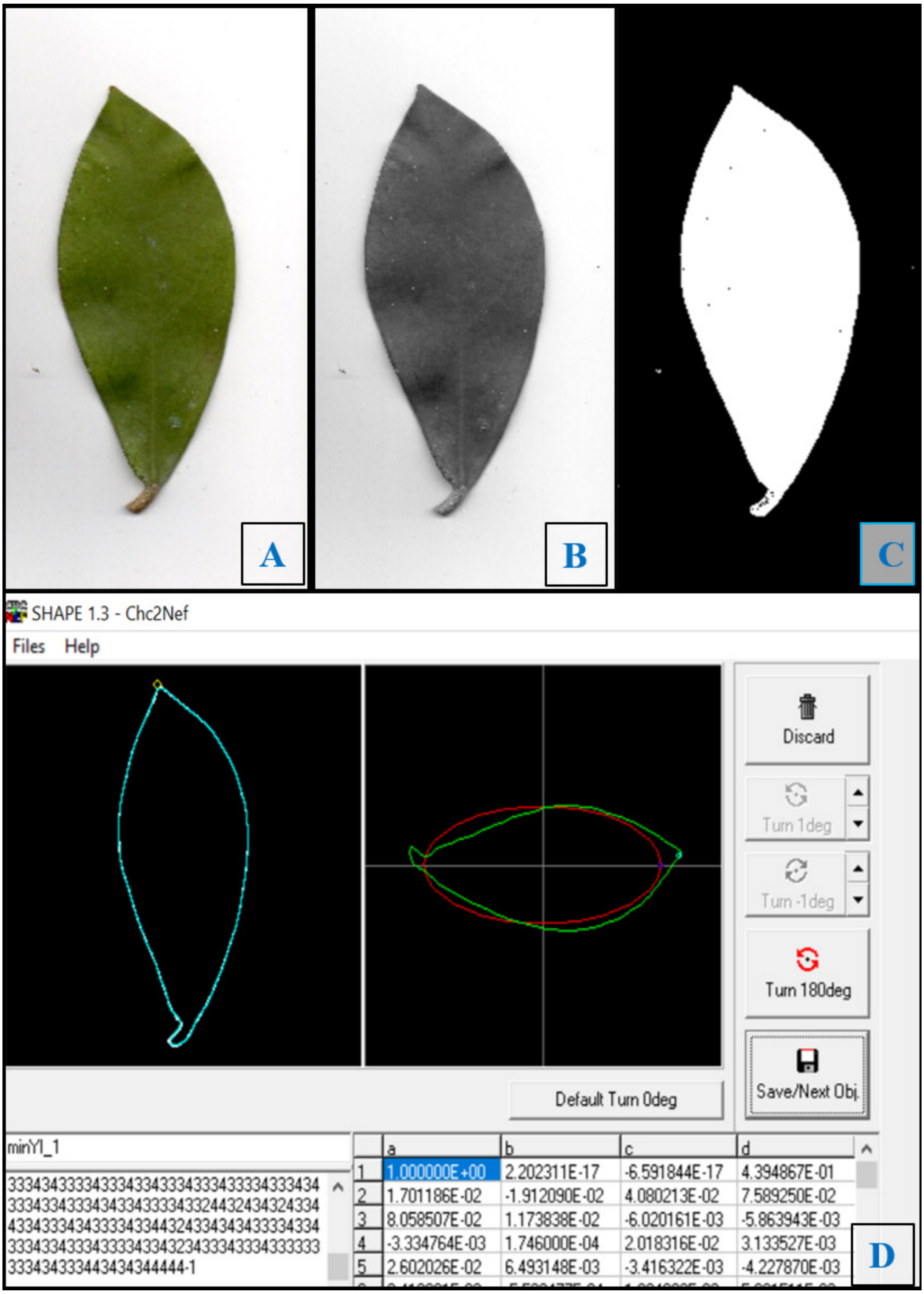

Figure 3. Leaf image processing include photograph of leaf samples (A) and conversion to bitmap image (B) and binary image (C). The processed images were subjected to leaf outline evaluation using elliptic Fourier analysis (D).

zoom lens. The camera was mounted on a tripod to take photograph of samples directly overhead (Figure 3). Leaf samples were placed on a board with contrasting background from leaf color (Yoshioka *et al*., 2006). Leaf outlines from photographs were obtained by converting the full color bitmap image to a binary image (black and white image). The software Chc2Nef was used to trace and record the outline of leaf images as chain codes (Freeman,1974). The software also reduced the noise, unnecessary markings from the image, and calculated the normalized elliptic fourier descriptors (EFDs) from the chain coded information (Kuhl and Giardina, 1982).

Leaf shape evaluation by elliptic fourier analysis

The procedure in calculating EFDs from the original digitized image reconstructs the outlines to approximate level and the decision to set the number of harmonics was arbitrary to some extent (Rohlf and Archie, 1984). The fourier analysis was implemented using 20 harmonics as suggested by Iwata (2006). The resulting data matrix of normalized EFDs was explored by principal component analysis (PCA) using a variance-covariance matrix described in the procedure of Yoshioka *et al*. (2004). The PCA of the coefficient matrices reduced the data dimensionality of uncorrelated shape descriptor variables to a smaller number (Andrade *et al*., 2008). The leaf shape variations were analyzed using one way analysis of variance (ANOVA) of the principal component (PC) scores at p=0.05. To determine the effect of each PC on leaf shape, the coefficients of EFDs were recalculated to let the scores on a particular PC equal to the mean ± 2 SD, while keeping the scores of the remaining components as means (Yoshioka *et al*., 2006).

Computer software for data analysis

The SHAPE computer software package (Iwata, 2006) was utilized for evaluating contour shapes based on EFDs. SHAPE software package contained 4 programs namely: ChainCoder, ChcsNef, PrinComp and PrinPrint for digital image processing, obtaining EFDs, perform PCA and visualizing shape variations by the PCs. The PAST (Paleontological Statistical Software) software (Hammer *et al*., 2009) was used in analyzing univariate one-way ANOVA, data ordination of PC scores, and descriptive analysis using box plots. Digital images were processed using ImageJ (Rueden *et al*., 2017) and Irfan View (Skiljan, 2016).

RESULTS

The calculated elliptic fourier coefficients from the leaves of three *Hoya* species was used to perform a principal component analysis (PCA) based on variance-covariance matrix. The resulting PCA summarizes the information derived from the coefficients of leaf contour shape. There were 4 effective principal components considered in the analysis composed of 84.93% cumulative variance. Principal component 1 (PC1) contributed 58.43% of the explained variance, this was followed by PC2 with 16 .06% variance, PC3 with 6.18% variance and PC4 with 4.26% variance accounted (Table 1).

The variation of the leaf shape was illustrated using the accounted variation of each principal component (Figure 4). In this aspect, the coefficients of the elliptic fourier descriptors were calculated such that the score on each principal component was equal to the mean with ±2 s.d. (standard deviation) and the scores of the remaining components were kept zero (Iwata, 1998; Yoshioka, 2004). The contour shape of the leaf can be reconstructed with the estimated coefficients for the corresponding principal components (Furuta, 1995).

Table 1. Eigenvalues and the accounted variances explained by the significant principal components

Principal Component	Eigenvalue	Proportion(%)	Cumulative(%)
PC 1	0.0080	58.4270	58.4270
PC 2	0.0022	16.0635	74.4905
PC 3	0.0008	6.1843	80.6747
PC 4	0.0006	4.2573	84.9321

The estimated contour shape of the leaves was illustrated in Figure 4 along with the variation of the first to fourth principal component scores. The influence depicted in PC1 was largely on the size of the lamina and this constituted the largest accounted variation. The PC2 represented roundness of the leaf base and some degree of tapering towards the tip. The effect accounted by PC3 was distortions at the lower leaf base and the right distal part of the leaf. The variation contributed by PC4 was roundness along the leaf margin.

The principal component scores from the 4 effective PCs of *Hoya* species were extracted for further data evaluation. The extracted principal component scores of each *Hoya* species were plotted in box and whisker plots (Figure 5). One-way ANOVA was performed

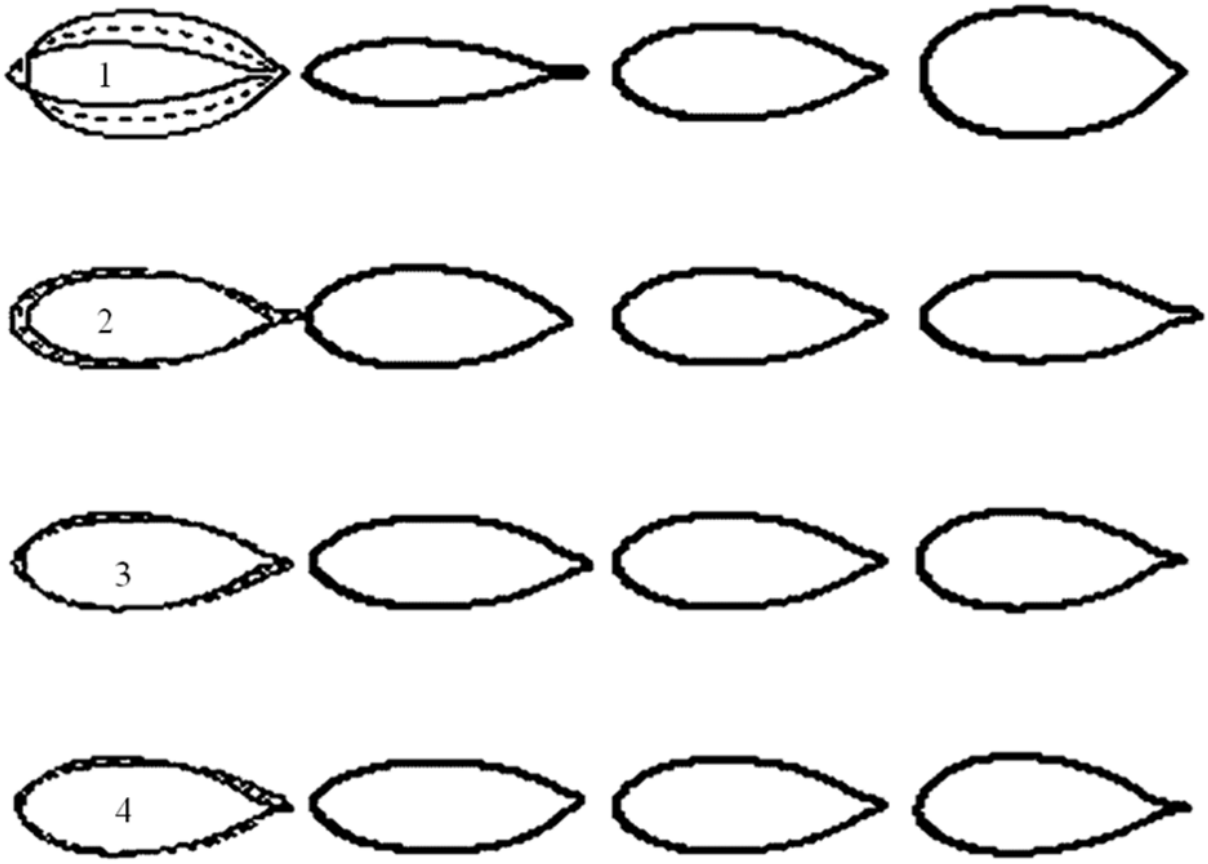

Figure 4. Reconstructed contour shape of the leaves using Fourier coefficients accounted by each principal component scores. Numbers 1-4 corresponds to PC1 – PC4 respectively followed by typical values of each principal component score (-2 s.d., mean and +2 s.d.). The overlaid drawing consisted dashed line (mean), thick solid line (-2 s.d.) and thin solid line (+2 s.d.).

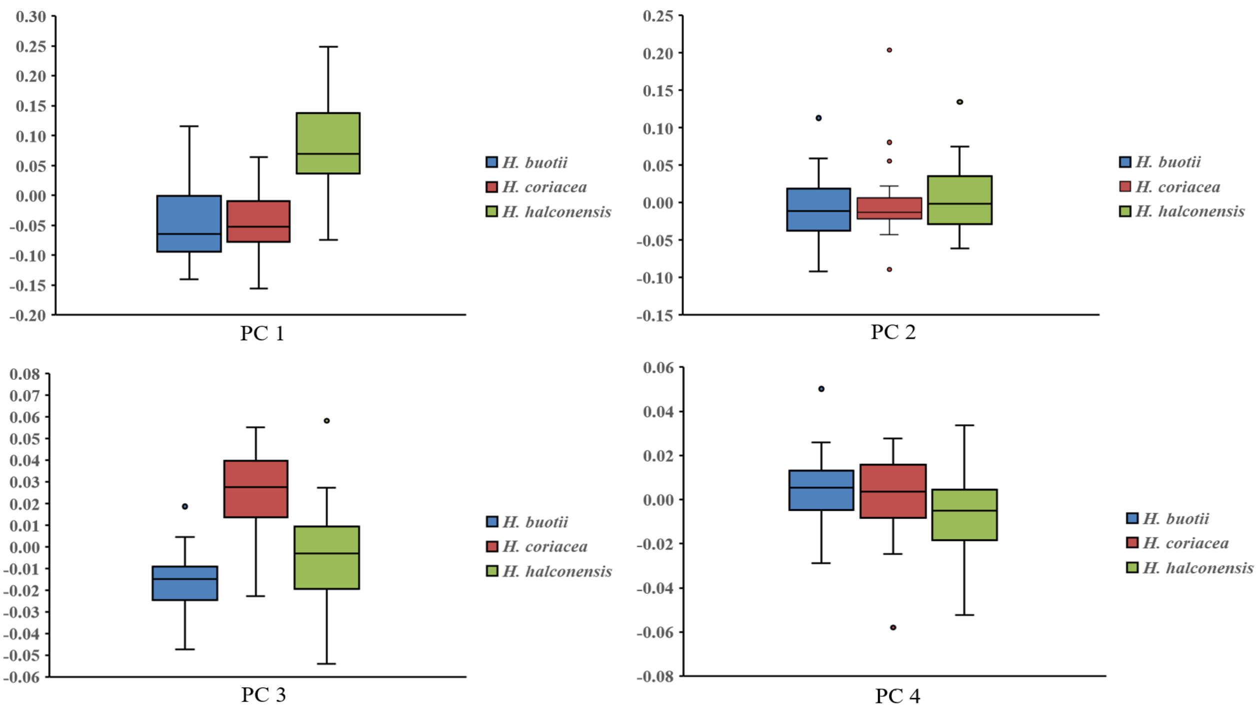

Figure 5. Variations of the first to fourth principal component scores on the leaf contour of three *Hoya* species. The vertical bars were specified by standard deviations and the indicated leaf variations on the left side of each graph. *H. buotii* (left); *H. coriacea* (center); *H. halconensis* (right) of each graph.

on the PC scores to discriminate the characteristics influenced by effective Principal components (Table 2). The results revealed that scores accounted in PC1 was highly significant among the three species detected using one-way ANOVA. This implied that effect on the laminar size on the three species were highly significant on the three species. In PC2 the effect was characterized by roundness of the base and the tapering towards the tip. Effects detected in PC2 were not significant among the three species. The effect contributed by PC3 was distortions at the lower leaf base and the right distal part of the leaf. One-way ANOVA revealed highly significant difference on this effect among the three Hoya species. A variation accounted in PC4 was roundness along the leaf margin and one-way ANOVA revealed no significant differences among the species.

PC Scores	df	SS	F value
PC 1	2	0.3157	0.38^{**}
PC 2	2	0.0041	0.96^{ns}
PC 3	2	0.0221	32.19^{**}
PC 4	2	0.0019	2.71^{ns}

Table 2. One-way ANOVA performed to extracted PC scores among leaf contour of the three *Hoya* species

Legend: df= degree of freedom; SS= sum of squares; **= highly significant (P<0.01); and ns= not significant (P>0.05).

The two accounted principal components consisting PC1 and PC3 were subjected into data ordination to reveal a two-dimensional image of the leaf contour variations on the three *Hoya* species (Figure 6). PC1 and PC3 were selected because the two principal components gave significant differences in accounted leaf variations (Table 2). The values of the species *H. halconensis* were largely distributed in quadrant 1 and 4 in the two-dimensional plot. This suggested that the leaves of *H. halconensis* have the variation of increasing leaf size compared to the mean leaf size. Also, it has the tendency to reduce on its distortions at the lower leaf base and the right distal part of the leaf. The species *H. coriacea* tends to be distributed in quadrants 1 and 2. The variation of *H. coriacea* was likely to increase in lamina, lower leaf base and the right distal part of the leaf. Majority of the distribution of PC scores in *H. buotii* was in quadrant 3. In comparison with the mean leaf contour shape,

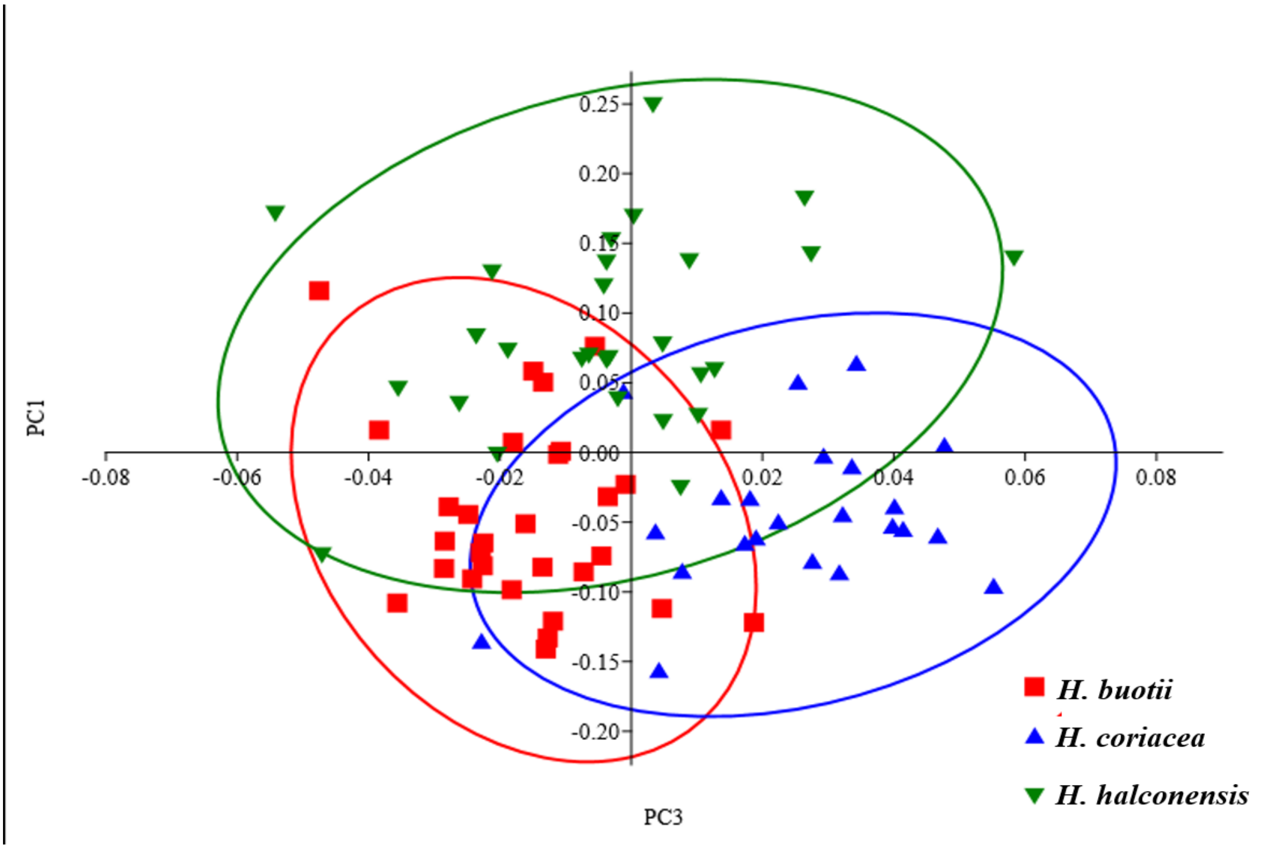

Figure 6. Data ordination of the principal components PC1 and PC3 indicating 95% data ellipsoid among three *Hoya* species.

the variation of *H. buotii* was expected to decrease in laminar size, lower leaf base and the right distal part of the leaf.

The PC scores extracted from the four effective principal components were utilized to perform cluster analysis (Figure 7). A similarity matrix using Euclidean index was utilized in the principal component scores. The resulting matrix was employed to create a cluster analysis using single linkage or nearest neighbor clustering method. The data was displayed in the form of dendrogram. The dendrogram revealed that the species *H. coriacea* and *H. buotii* were more similar in their leaf contour variations. At about 50% similarity index, the two species distinctively separated from *H. halconensis*. The similarity of leaf contour variations could lead to resemblances of appearance. As shown in box and whisker plot (Figure 5), PC1 being the highest accounted variation in laminar size revealed similarity between *H. coriacea* and *H. buotii*. The species *H. halconensis* on the other hand was expected to be bigger in terms of laminar size compared to the previous two species. In the accounted PC3, both *H. halconensis* and *H. buotii* were expected to have similar distortions at the lower leaf base and the right distal part of the leaf .

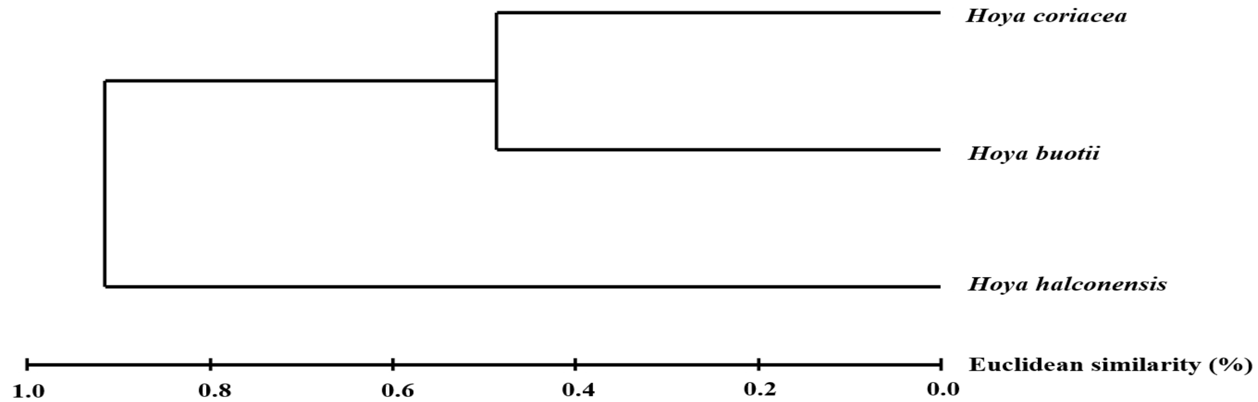

Figure 7. Cluster analysis indicated by Euclidean distance of the leaf contour variations of *H. coriacea*, *H. buotii*, and *H. halconensis*.

DISCUSSION

Elliptic fourier descriptors (EFDs) has been regularly applied technique in the leaf shape contour analysis (Kuhl, 1982). This analytical approach was described as superior method in characterizing leaf shape contours (White, 1988). Normalization of EFDs can represent shapes independent of orientation, size or location to facilitate simple comparison between shapes (Cope, 2012). Also, there have been many discussions in the application of EFDs to morphological analysis (Furuta, 1995). The application of EFD to analyze leaf shape outlines has been very well applied and utilized in various research. This study implemented the use of EFDs to study the variation in the leaf shape contours of three *Hoya* species.

The results indicated in the EFD-PCA detected variations of small shape contributed to the third and fourth principal components. The analysis also discriminated variations in the leaf outlines of the three species. Detections on these levels would certainly be difficult for humans to discern. In traditional taxonomy, the three species were confused with on another because of plasticity, being propagated together in similar greenhouse conditions. Discriminating descriptive characteristics for instance size, shape and color is very important in taxonomy. The standardized EFDs can evaluate size and shape and can discriminate independently one from the other (McLellan, 1998 and Furuta, 1995). EFDs were also one of the tools utilized in species identification of plants by means of digital morphometry (Cope, 2012). The boxplot on PC1 showed remarkable high values for *H. halconensis* while the boxplot on PC3 depicted high values for *H. coriacea* (Figure 5). The traits attributed to PC1 and PC3 could initially distinguish *H. halconensis* and *H. coriacea* leaf traits. These traits are not totally discriminatory but suggests correlation with leaf traits of *Hoya* species. While the boxplots of PC2 and PC4 gave similar values suggesting the similarity of leaf traits influenced by the two PCs. The leaf contour variation influenced by PC2 and PC4 were not significant as evaluated utilizing one way ANOVA. The leaf contour variations were clearly detected in this method as contributed by PC1 and PC3 ($P<0.01$). However, it was not clearly understood the explanation of PC2 and PC4 variations were not significant ($P>0.05$). There were difficulties in understanding fourier coefficients since many coefficients must be considered and the effect of coefficient on shape is almost impossible to understand (Furuta, 1995). The highly significant variations contributed by PC1 and PC3 were suggested to be highly heritable (Iwata, 1998). On the other hand, the non-significant variations in PC2 and PC4 could be related to environmental effects (Yoshioka, 2004).

The ordination plot of the first PC1 and PC3 revealed that leaf outline variations in the three species tend to be distributed in opposite quadrants of the orthogonal plane (Figure 6). This was displayed in the variability of *H. halconensis* distributed between quadrant 1 and 2; the *H. coriacea* in quadrant 1 and 4; and the *H. buotii* in quadrant 2 and 3. This could be an indication of fluctuating asymmetry, an asymmetrical shape variation. Leaves can vary significantly in heritable traits including leaf fluctuating asymmetry as random deviations in symmetry of leaf shape due to interactions with environment (Kozlov *et al*., 2018). Deviations in leaf shapes attributed to PC1 and PC3 among the three *Hoya* species were detected in opposite quadrants in orthogonal plane. Aside from inherent heritable variations, asymmetrical shape of leaves influenced by PC1 and PC3 was noted as indication of fluctuating asymmetry. Left and right sides of the leaves in PC1 and PC3 showed distortion as compared to the mean shape (Figure 4). Fluctuating asymmetry is the phenotypic difference between bilateral traits and occurs as an indicator of environmental stress (Van Valen, 1962). The evidence for fluctuating asymmetry as indicator of developmental instability were well known (Valentine, 1973).

The cluster analysis dendrogram showed similarity of the leaf contour variations between *H. buotii* and *H. coriacea*. This can also lead to similarity of appearance which was also similarly detected using leaf architecture (Salvańa, 2014). The resemblances can become a problem in proper taxonomic identification.

The study is limited only in the utilization of the leaf outlines subjected to elliptic fourier analysis on the selected *Hoya* species. The study has detected leaf shape traits which could be helpful in discriminating *Hoya* species. However, it is suggested that DNA barcode method as the recommended analysis to accurately identify endemic *Hoya* species in the Philippines (Maranan and Diaz, 2013). But this technique is an expensive method deemed insufficient for theoretical basis of traditional taxonomy (Lipscomb *et al*., 2003). An alternative is to explore practical detection methods in distinguishing *Hoya* species through leaf characters which is proven to be valuable in taxonomic studies (Jumawan and Buot, 2016; Hickey and Taylor, 1991).

CONCLUSION

The study has demonstrated the application of EFDs to analyze the leaf shape outline of *H. buotii*, *H. coriacea*, and *H. halconensis*. The EFDs provided an efficient method to quantitatively analyze the leaf shape of the selected *Hoya* species. The data utilized the variances of the leaf shape contours contributed by four principal components. This is the first study to analyze the three *Hoya* species propagated under greenhouse conditions. The confusion brought about by resemblances in leaf characteristics of the three species has become a taxonomic obstacle. The overall variation contributed by PC1 and PC3 could be attributed to genotypic heritability. The variation accounted by PC2 and PC4 were suggested to be related to environmental effects. The results indicated significant variations in leaf shape outlines of the three *Hoya* species. This detection could be proven difficult by human recognition. This detection of leaf shape variations is important component in taxonomic identification. The leaf shape variations were suggested to be an interaction of genetic and environmental effects acting on the contours of the leaves.

ACKNOWLEDGEMENTS

The authors would like to extend their appreciation to Philippine government agency DOST-ASTHRDP for all the support to accomplish this study.

REFERENCES

Andrade M, S.J. Mayo, D. Kirkup, and C. Van Den Berg. 2008. Comparative morphology of population of *Monstera adans* (Araceae) from natural forest fragments in northeast Brazil using elliptic fourier analysis of leaf outlines. *Kew Bulletin* 63: 193–211.

Apuan, D.A., M.A.G. Torres, J. Gorospe, R. Amparado and C.G. Demayo. 2011. Intra-panicle variation in seed shapes of weedy rice. *Australian Journal of Basic Applied Science* 5: 185–191.

Aurigue, F.B., J. R. Sahagun, and W. Suarez. 2013. *Hoya cutis-porcelana* (Apocynaceae): A new species from Samar and Biliran Islands, Philippines. J*ournal of Nature Studies* 12(1): 12–17.

Cabactulan, D.D., M. Rodda, and R. Pimentel. 2017. *Hoya* of the Philippines part I. *Hoya migueldavidii* (Apocynaceae, Asclepiadoideae), a new species from Northern Mindanao, Philippines. *PhytoKeys* 80: 105–112.

Calacat, J.C.B., M.A.J. Torres and C.G. Demayo. 2011. Application of elliptic fourier analysis in understanding leaf shape characters of *Anthurium*.

Advances in Agriculture & Botanics-International Journal of the Bioflux Society AAB Bioflux 3: 82–92.

Cope, J.S., D. Coeney, J.Y. Clark, P. Remagnino and P. Wilkin. 2012. Plant species identification using digital morphometrics. *Expert Systems with Applications* 39: 7562–7573.

Corney, D. P. A., J. Y. Clark, H. L. Tang, and P. Wilkin. 2012. Automatic extraction of leaf characters from herbarium specimens. *Taxon* 61 (1): 231–244.

Dalayap, R., M.A.J. Torres and C.G. Demayo. 2011. Landmark and outline methods in describing petal, sepal and labellum shapes of the flower of Mokara orchid varieties. *International Journal of Agriculture and Biology* 13: 652–658.

Forster, P.I. and D.J. Little. 1996. *Flora of Australia* 28: 231–237. CSIRO, Canberra.

Freeman, H. 1974. Computer processing of line drawing images. *ACM Computing Surveys* 6: 57–97.

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

Hammer, O., D. Harper and P. Ryan. 2001. Paleontological statistics software package for education and data analysis. *Paleontologia Electronica* 4:1–9.

Hickey, L.J. and D.W. Taylor. 1991. The leaf architecture of *Ticodendron* and application of foliar characters in discerning its relationships. *Ann. Missouri. Bot. Gard.* 78: 105–130.

International Plant Names Index (IPNI). 2016. http://www.ipni.org. on 26 February 2019.

Iwata, H. 2006. SHAPE Ver 1.3 A Software package for quantitative evaluation of biological shape based on elliptic fourier descriptors.

Iwata, H., S. Niikura, S. Matsuura, Y. Takano and Y. Ukai. 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.

Jumawan, J.H. and I.E. Buot Jr. 2016. Numerical taxonomic analysis in leaf architectural traits of some *Hoya* R. Br. species (Apocynaceae) from Philippines. *Bangladesh Journal of Plant Taxonomy*. https://doi.org/10.3329/bjpt.v23i2.30851

Kloppenburg, R.D. and S.V. Siar. 2008. Three new species of *Hoya* R.Br. (Apocynaceae) from the Philippines. *Asia Life Sciences* 17(1): 57–70.

Kloppenburg, R.D., M.L.D. Guevarra, J.M. Carandang and F.S. Maranan. 2012. New species of *Hoya* R. Br. (Apocynaceae) from the Philippines. *Journal of Nature Studies* 11(1&2): 34–48.

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

Li, P.T., M.G. Gilbert and W.D. Stevens. 1995. Asclepiadaceae. In: Z. Y. Wu, and P. H. Raven (eds.), *Flora of China*. 16: 189–270. Science Press, Beijing, China and Missouri Botanical Garden, St. Louis, USA.

Lipscomb, D., Platnick, N. and Q.D. Wheeler. 2003. The intellectual content of taxonomy: A comment on DNA Taxonomy. *Trends in Ecology and Evolution* 18(2): 65–66.

Maranan, F.S. and M.G.Q. Diaz. 2013. Molecular diversity and DNA barcode identification of selected Philippine endemic *Hoya* species (Apocynaceae). *The Philippine Agricultural Scientist* 96 (1): 86–92.

Ong, P.S., L.E. Afuang, and R.G. Rosell-Ambal. 2002. *The Philippine Biodiversity Conservation Priorities: A second iteration of the National Biodiversity Strategy and Action Plan*. Department of Environment and Natural Resources – Protected Areas and Wildlife Bureau, Conservation International Philippines, Biodiversity Conservation Program-University of the Philippines Center for Integrative and Development Studies, and Foundation for the Philippine Environment, Quezon City Philippines.

Maranan, F.S. and M.G.Q. Diaz. 2013. Molecular diversity and DNA barcode identification of selected Philippine endemic *Hoya* Species (Apocynaceae). *The Philippine Agricultural Scientist* 96 (1): 86–92.

McLellan, T. and J.A. Endler. 1998. The relative success of some methods for measuring and describing the shape of complex objects. *Systematic Biology* 47: 264–281.

Rodda, M. and N.S. Juhoneweb. 2013. The taxonomy of *Hoya micrantha* and *Hoya revoluta* (Apocynaceae, Asclepiadoideae). Webbia. *Journal of Plant Taxonomy and Geography* 68(1): 7–16.

Rohlf, F.J. and J.W. Archie. 1984. A comparison of fourier methods for the description of wing shape in mosquitoes (Diptera: Culicidae). *Systematic Zoology* 33(3): 302–317.

Salvańa, F.R.P. and I.E. Buot, Jr. 2014. Leaf architecture study of *Hoya coriacea*, *Hoya halconensis* and *Hoya buotii* (Apocynaceae). *International Research Journal of Biological Sciences* 3:(3) 37–44.

Torres, M.A.J., C.G. Demayo, and S.V. Siar. 2008a. Elliptic fourier analysis of leaf outline differences between and among sixteen Species of *Hoya*. *The Philippine Agricultural Scientist* 91(1): 18–28.

Torres, M.A.J., C.G. Demayo and S.V. Siar, 2008b. Corona shape variations among nine species of *Hoya*. *The Philippine Agricultural Scientist* 91: 7–17.

Torres, M.A.J., M.M.E. Manting and C.G. Demayo, 2008c. Elliptic fourier analysis (EFA) of leaflet outline differences in thirteen species of weed legumes. *J. Nat. Stud*. 7: 117–138.

Tran, T.B., M. Rodda, J.H. Kim, J. Lee, D.K. Kim, and B.T Ha. 2011. *Hoya sapaensis* (Apocynaceae, Asclepiadoideae), a new species from Vietnam. *Annales Botanici Finnici* 48:511–514.

Valentine, D.W. and M. Soule. 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, 1962. A study of fluctuating asymmetry. *Evolution* 16: 125–142.

Wanntorp, L., A. Kocyan, R. van Donkelaar and S. S. Renner. 2006a. Towards a monophyletic *Hoya* (Marsdenieae, Apocynaceae): Inferences from the chloroplast trnL region and the rbcL-atpB spacer. *Systematic Botany* 31: 586–596.

Wanntorp, L., A. Kocyan and S.S. Renner. 2006b. Wax plants disentangled: A phylogeny of *Hoya* (Marsdenieae, Apocynaceae) inferred from nuclear and chloroplast DNA sequences. *Molecular Phylogenetics and Evolution* 39: 722–733.

White, R., H.C. Prentice and T. Vwerwijst. 1988. Automated image acquisition and morphometric description. *Canadian Journal of Botany* 66: 450–459.

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

Yoshioka Y., R. Ohsawa, H. Iwata, S. Ninomiya and N. Fukuta. 2006. Quantitative evaluation of petal shape and picotee color in *Lisianthus* by image analysis. *Journal of American Society of Horticulture Science* 131(2): 261–266.

Received: 26 January 2021
Accepted: 15 February 2021
Published Online: 29 April 2021
Published in Print: 30 June 2021