NUMERICAL TAXONOMIC ANALYSIS IN LEAF ARCHITECTURAL TRAITS OF SOME *HOYA* R. BR. SPECIES (APOCYNACEAE) FROM PHILIPPINES

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Abstract

The present study examines the leaf variations in leaf traits of four *Hoya* R. Br. species from Philippines namely: (1) *H. buotii* Kloppenburg, (2) *H. halconensis* Kloppenburg, (3) *H. mindorensis* Schlechter red bearing flowers; and (4) *H. mindorensis* Schlechter yellow bearing flowers. Leaf samples (n= 30 leaves) were collected from each plant group and measured with nine architectural traits. The results showed variability in the leaves using univariate and multivariate analysis. Data ordination depicted variations in leaf morphology. The two plant groups *H. mindorensis* red bearing flowers and *H. mindorensis* yellow bearing flowers were consistently variable as supported by principal coordinate analysis, cluster analysis and two way Anova (P<0.001). The variability of the two plant groups could be due to developmental instability, plasticity or taxonomic identity, one being the subspecies of the other. Hence, a closer study to investigate the significant variability of the two plant groups was recommended. Distinct separation of *H. buotii* and *H. halconensis* was detected being regularly mistaken as one species. The study demonstrated the applicability of multivariate analysis as effective tool in numerical taxonomy. Multivariate analysis can be employed to demonstrate likelihood of relationship among various *Hoya* species.

Introduction

Most *Hoya* species of Philippines were considered endemic to the country with several new discoveries for the past decade. The genus *Hoya*, is commonly known as wax plant belong to family Apocynaceae, was considered to be taxonomically complex (Wanntorp *et al*., 2006). The estimated number of *Hoya* species in the country ranges from 80 – 104 (Kloppenburg *et al*., 2012 and Aurigue *et al*., 2013). The Philippines was considered as one of the richest and most diverse range of *Hoya* species which are located all throughout the archipelago (Kloppenburg and Siar, 2008).

Identification of *Hoya* species largely depend on traditional taxonomy that put emphasis on reproductive characters. Descriptions on qualitative and quantitative characteristics of inflorescence, corolla, corona and pollinarium were very significant in identification of *Hoya* species (Kleijn and Van Donkelaar, 2001; Omlor, 1996; Forster and Little, 1996). Nomenclature issues were still largely unresolved for various taxa (Rodda and Juhoneweb, 2013). Many species were documented to exhibit phenotypic plasticity in morphological characters (Tungmunnithum *et al*., 2011). The structure of many *Hoya* species was described to possess complex corona morphology (Kunze, 2008). Many of these problems in *Hoya* taxonomy had risen due to dependence on reproductive parts. Reproductive features are not present all the time and makes difficulty in identification.

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DNA barcode was the method suggested to properly identify the endemic Philippine *Hoya* species (Maranan and Diaz, 2013). The technique was regarded as an effective tool for species identification but considerably weak attempt to discovery and description of species (Wheeler, 2004). Aside from being an expensive method for species identification, DNA barcoding is insufficient in terms of theoretical basis of traditional taxonomy (Lipscomb *et al.*, 2003).

Leaves of Philippine *Hoya* species are present throughout the year and can be used extensively for detecting variations. Leaf characters were proven to be valuable in taxonomic studies of tropical plants which seldom produce flowers and angiosperm remains as fossils (Hickey and Taylor, 1991; Dilcher, 1974). Leaf morphological characters of Gunneraceae were subjected to multivariate analysis to support genus monophyly (Fuller, 2005). Leaf morphometric data are important and the variation displayed by morphological traits reflects the evolutionary arrangement manifested as morphological changes (Otte and Endler, 1989). Multivariate analysis is a tool in the examination of leaf morphometric traits, an important component in the field of numerical taxonomy. The main objective of the study was to examine the variations of the leaf morphometric traits of the selected *Hoya* species namely: *Hoya buotii, Hoya halconensis,* and *Hoya mindorensis*.

Materials and Methods

Plant materials

The *Hoya* species were acquired from the propagated plant collections of Dr. I. E. Buot Jr., Professor and curator of IBS Herbarium, PBD in UPLB. There were 3 species of *Hoya* included in the study which were *Hoya buotii, Hoya halconensis,* and *Hoya mindorensis*. However, it was noticed that *H. mindorensis* bears two flower types: the red bearing plants and the yellow bearing plant. For the purpose of this examination, the analyses were conducted in four plant groups: (1) *H. buotii,* (2) *H. halconensis* (3) *H. mindorensis* red bearing flowers; and (4) *H. mindorensis* yellow bearing flowers. The selected *Hoya* species usually encountered confusion in proper taxonomic identification.

Leaf character selection and measurements

The selection of leaf morphometric characters were based from manual of leaf architecture with modifications (Leaf Architecture Working Group, 1999). A total of nine morphometric traits were chosen in the study. The description and illustration of the parameters considered for leaf morphometric measurements were shown in Table 1 and Fig. 1.

Table 1. Parameters of leaf morphometric measurements used in the analysis.

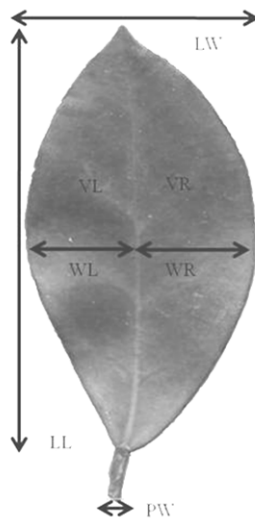| Code	Description of characters
LL	Lamina length
LW	Lamina width
PW	Petiole width
WL	Width in left side of lamina
WR	Width in right side of lamina
VL	Number of secondary veins in left side of the lamina
VR	Number of secondary veins in right side of the lamina
LR	Leaf ratio (LL/LW)
LA	Leaf area (LL X LW X 2/3)

Fig. 1. The illustration of the parameters taken for leaf morphometric measurements.

Data analysis

There were four plant groups, nine leaf morphometric data, and thirty leaf sample replicates which has a total of 1,080 data sets. The data generated from the leaf morphometric traits were subjected to univariate and multivariate statistical analyses. The univariate data comprised the minimum value, maximum value, mean and standard deviation. The univariate data sets were plotted in a box and whisker to evaluate the distribution of data. The multivariate data matrix was subjected to similarity matrix using Morisita index of similarity. The similarity matrix was explored using data ordination technique. Ordination refers to projection of multivariate data sets in a two dimensional space to detect patterns upon visual inspection (Pielou, 1984). Principal coordinate analysis (PCOa) or also known as metric multidimensional scaling was implemented as data ordination (Gower, 1966). PCOa reduces the dimensionality of the data similar to principal component analysis but the advantage of PCoA is that it may be used with all types of variables (Legendre and Legendre, 1998). Cluster analysis was performed combining quantitative data into clusters in constructing a dendrogram. The resulting pattern generated from multivariate analysis detected variations in leaf morphometric traits. An inference on the sources of leaf morphometric variation was tested using two way analysis of variance (Anova). It was investigated if the significant variation could be attributed by the leaf characters, the species, and the interaction of leaf characters and species. Post hoc analysis was conducted when $P<0.05$ using Tukey's test. The PAST (Paleontological Statistical Software) software (Hammer *et al*., 2009) was used in analyzing univariate and multivariate analysis.

Results

The univariate statistics of the morphometric traits of the plant groups are shown in Table 2 and Fig. 2. The results indicated that the leaf area and leaf length were the most variable among the measured leaf characters. The least variable traits were petiole width and leaf ratio. The rest of the leaf traits were relatively similar to the four plant groups.

Table 2. Univariate statistics of the nine morphometric traits of the four plant groups.

|	Parameter	LL	LW	PW	WL	WR	VL	VR	LR	LA
H. halconensis,	Min	81	30	1	15	15	3	3	1.62319	1800.01
(n=30)	Max	156	75	4	37	38	6	7	3	7336.7
	Mean	115.367	53.7667	2.43333	26.6333	27.3	4.43333	4.5	2.18327	4249.33
	St. dev.	19.2703	11.4189	0.727932	5.70833	5.57797	0.773854	0.937715	0.292643	1520.62
H. buotii,	Min	63	22	1	10	11	4	4	1.90909	953.338
(n=30)	Max	119	47	3	27	23	9	9	3.47826	3332.02
	Mean	97	35.4667	1.83333	16.4667	16.7667	6.23333	6.5	2.77783	2328.99
	St. dev.	13.5341	6.27383	0.592093	4.40793	3.81181	1.0063	1.27982	0.385205	638.016
H. mindorensis (red),	Min	63	24	2	12	12	4	4	2.17241	1218.01
(n=30)	Max	174	58	4	27	31	9	8	3.83333	6573.37
	Mean	108.9	39.0667	3.03333	19.2333	19.8333	5.8	5.46667	2.77893	2979.37
	St. dev.	30.0899	8.31672	0.718395	4.02307	4.36351	1.15669	1.04166	0.387058	1490.31
H. indorensis (yellow),	Min	56	32	3	15	15	4	4	1.55769	1194.67
(n=30)	Max	157	67	5	33	34	8	7	2.74419	7012.7
	Mean	100.4	48.3	3.86667	24.1667	24.1333	5.7	5.73333	2.05773	3381.31
	St. dev.	27.7148	9.3593	0.62881	4.41848	5.07008	0.987857	0.868345	0.288655	1505.66
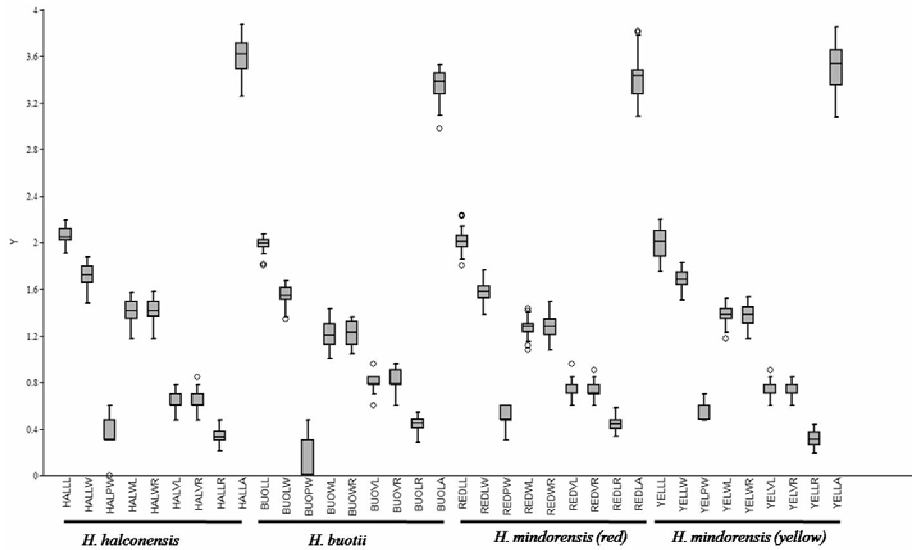
Fig. 2. Box and whisker plot on the univariate metrics of leaf morphometric traits of the four plant groups.

The results in PCOa accounted 4 effective coordinate axis with a total of 68.09% of the cumulative variance (Table 3). Axis 1 and axis 2 contributed 36.30% and 59.02% of variances respectively. The components in axis 1 and axis 2 were analyzed and used to project into a two dimensional plane (Table 4 and Fig. 3). The highest values generated in axis 1 and axis 2 was largely attributed to the leaf morphometric traits of *H. mindorensis* red bearing plant. The lowest values on the other hand were attributed to leaf traits of *H. mindorensis* yellow bearing plant. It can be viewed that leaf traits of the two *H. mindorensis* plants were highly variable. The leaf traits from *H. buotii* and *H. halconensis* were less variable.

Table 3. The eigenvalue of the principal coordinate axis and the respective accounted variance in the PCOa.

Axis	Eigenvalue	Percent variance	Cumulative variance
1	0.033984	36.301	36.301
2	0.021271	22.721	59.022
3	0.0047677	5.0928	64.1148
4	0.003722	3.9759	68.0907

The data ordination of PCOa clearly displayed the variation of the leaf morphometric traits between the two *H. mindorensis* plant groups (Fig. 3). The red bearing flower *H. mindorensis* occupied quadrant 1 and quadrant four of the orthogonal plane. The yellow bearing flower *H. mindorensis* largely occupied quadrant 2. The rest of the values were distributed closely in quadrants 3 and 4.

Another technique employed in the exploratory analysis of the morphometric data was cluster analysis. The dendrogram also revealed a similar pattern observed in PCOa. Again, the two *H. mindorensis* plant groups were located on the opposite ends of the dendrogram. It indicated that the two plant groups were highly variable. The *H. mindorensis* yellow bearing flower was more

morphometrically similar *H. buotii* but their Euclidean distance was still far. This indicated variability of leaf morphometric traits. The *H. mindorensis* red bearing flower was very similar to *H. halconensis* in terms of the measured leaf traits. In general, the four plant groups revealed distinct leaf characteristics as indicated by the clusters in the dendrogram (Fig. 4).

Table 4. The principal coordinate scores of the two highest accounted variances in axis1 and axis 2 on the nine morphometric traits derived from the four plant groups. (Legend: The first three letters comprise the plant groups as HAL = *H. halconensis* ; BUO = *H. buotii*; RED = *H. mindorensis* (red); and YEL = *H. mindorensis* (yellow). The last two letters comprise the code characters of the leaf in Table 1).

Leaf traits	axis 1	axis 2	Leaf traits	axis 1	axis 2
REDLA	0.1130	0.0470	BUOLL	-0.0025	-0.0007
REDLL	0.0374	0.0153	HALWL	-0.0059	-0.0333
BUOPW	0.0336	0.0051	YELVR	-0.0068	0.0120
REDWR	0.0296	0.0097	HALWR	-0.0083	-0.0357
REDPW	0.0294	-0.0007	HALLR	-0.0085	0.0114
REDLW	0.0287	0.0102	HALLW	-0.0092	-0.0361
REDWL	0.0281	0.0107	HALLL	-0.0101	-0.0223
BUOWR	0.0167	-0.0050	YELVL	-0.0112	0.0137
HALPW	0.0166	-0.0258	YELLR	-0.0141	0.0086
BUOWL	0.0138	-0.0076	BUOLR	-0.0177	-0.0064
BUOLA	0.0101	-0.0027	HALLA	-0.0206	-0.0796
REDVR	0.0082	-0.0100	YELWL	-0.0244	0.0156
REDVL	0.0077	-0.0126	HALVR	-0.0248	-0.0034
BUOLW	0.0065	0.0001	YELPW	-0.0276	0.0078
REDLR	0.0020	0.0025	YELLW	-0.0279	0.0154
BUOVL	0.0001	-0.0040	YELWR	-0.0321	0.0154
HALVL	-0.0018	-0.0019	YELLL	-0.0385	0.0268
BUOVR	-0.0024	-0.0087	YELLA	-0.0869	0.0691

The emerging pattern generated in data exploration using multivariate analysis suggested variability and resemblances on the leaf morphometric traits. To detect if the variation was significant or not, two way Anova was conducted. The sources of variation were generated from the leaf characters, four plant groups and the interaction between leaf characters and four plant groups. The two way Anova detected a highly significant differences among the mentioned sources of variation. Post hoc analysis was conducted using Tukey's test in the four plant groups only. It was not conducted to the leaf characters as it may give irrelevant output (e.g. leaf area is obviously significantly different to petiole width). The Tukey's test revealed that *H. halconensis* and *H. mindorensis* red bearing flower were more similar compared to other comparison. The *H. mindorensis* red bearing flower and *H. mindorensis* yellow bearing flower was highly significantly different. Other combinations of plant group comparisons showed highly significant differences in their leaf morphometric traits. The summary on the two way Anova table was shown in Table 5 and 6.
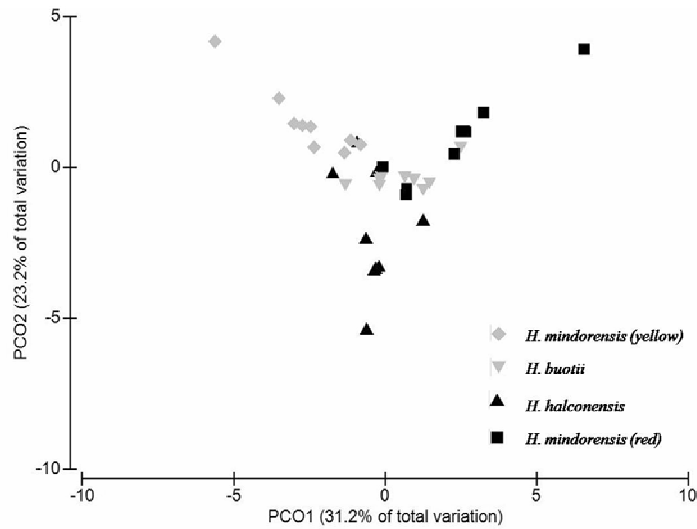
Fig. 3. The data ordination on the nine morphometric traits contributed by the four plant groups using PCOa.
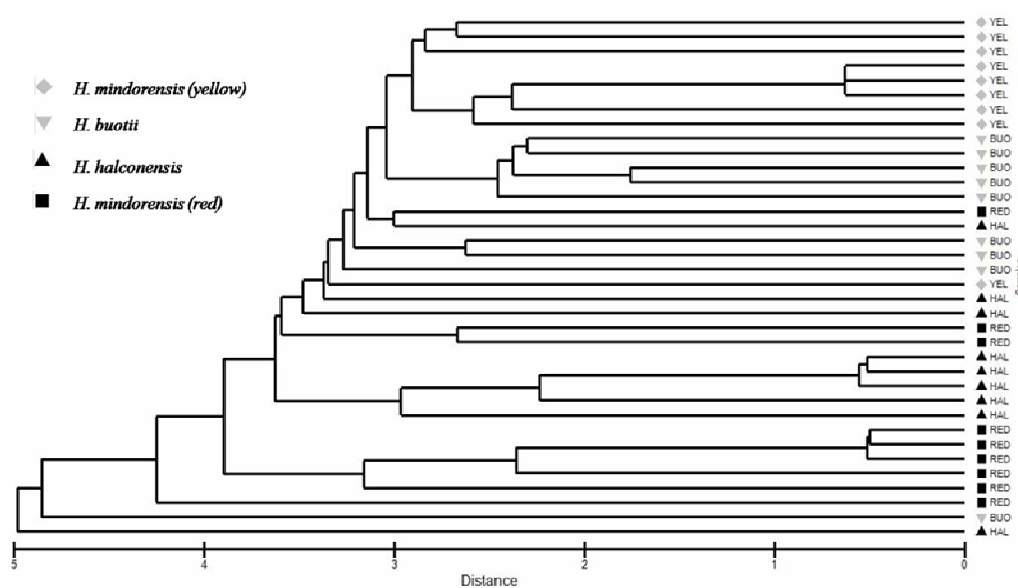

Fig. 4. Single linkage or nearest neighbor cluster dendrogram on the nine morphmetric traits from the leaves of the four plant groups.

Table 5. Two way Anova table on the sources of variation in leaf morphometric traits.

Source	Sum of squares	df	Mean squares	F ratio	P value
Leaf characters (A)	985.8	8	123.2	F (8, 232) = 2530	P < 0.0001
Species (B)	9.887	3	3.296	F (3, 87) = 25.86	P < 0.0001
Interaction: A x B	62.51	24	2.605	F (24, 696) = 52.17	P < 0.0001
Residual	34.75	696	0.04993		

Table 6. Pair wise comparison on the different plant groups using Tukey's test.

Multiple Comparisons Test	Mean diff.	95% CI of diff.	Summary
H. halconensis vs. *H. buotii*	0.1244	0.04389 to 0.2048	***
H. halconensis vs. *H. mindorensis* (red)	-0.0312	-0.1116 to 0.04931	ns
H. halconensis vs. *H. mindorensis* (yellow)	-0.1443	-0.2248 to -0.06386	****
H. buotii vs. *H. mindorensis* (red)	-0.1555	-0.2360 to -0.07506	****
H. buotii vs. *H. mindorensis* (yellow)	-0.2687	-0.3492 to -0.1882	****
H. mindorensis (red) vs. *H. mindorensis* (yellow)	-0.1132	-0.1936 to -0.03269	**

Discussion

The study was conducted to examine the variations of the leaf morphometric traits of the four plant groups consisting *Hoya* species. The univariate statistical analysis showed pronounced variability in leaf area and laminar length. On the other hand, less variability was observed in petiole width and leaf ratio. Leaf variation was more conspicuous to parts of the leaf with bigger morphometric values. The multivariate analysis generated a pattern that the *H. mindorensis* red bearing flower and *H. mindorensis* yellow bearing flower were highly variable. This was supported by results on cluster analysis and the comparison using Tukey's test as post hoc analysis to two way Anova. The detected variability of the two plant groups, although belonging to the same species could be attributed to environmental stress (Van Valen, 1962). The effects of environmental stress can eventually lead to developmental instability manifested in variability of leaf traits (Valentine and Soule, 1973). *Hoya* species were known also to exhibit phenotypic plasticity (Tungmunnithum *et al.*, 2011). Leaf variations of the two plant groups could be phenotypic variation attributed to plasticity. On the other hand, a closer investigation should be conducted to consider other leaf parameters or another vegetative part of the plant. The variation of leaf traits between the two plant groups was highly significant, as also indicated by difference in the colour of the flowers. The possibility that they were taxonomically different, as a subspecies probably is at large. Hence, it is recommended that a separate study should be conducted that would incorporate many characters in the analysis.

In general, the multivariate analysis was able to detect variations of the morphometric traits on the leaves of selected Philippine *Hoya* species. The detected variation was statistically tested to discriminate one plant group to another. This study confirms the distinct separation of *Hoya buotii* and *Hoya halconensis* that had been always mistaken to be one species (Aurigue, 2013). This study demonstrated the applicability of multivariate analysis as a tool in numerical taxonomy. The technique detected variations in leaf morphometric traits and can further be employed to demonstrate likelihood of relationship among the *Hoya* species.

Acknowledgement

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