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Changes of leaf morphology of *Hoya amorosoae* from varying light exposure: Its implications to species description and taxonomy

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Abstract

In this paper, *H.amorosoae* (Green and Kloppenburg, 2014) leaves exposed to varing light intensities and its morphological changes have been documented using leaf morphometry and leaf morphological description. Morphometry of leaf samples provided evidence on the changes of measurements comparing leaf width, length, and thickness under shaded environment and in the natural habitat. In addition, anthocyanin has been proven to occur in the leaves in response to sunlight exposure. Using these data of the vegetative structures in describing *Hoya* is somewhat not very conclusive. It is therefore recommended to use both vegetative and reproducive structures when describing particular plant species specially *Hoya* as species of this plant group are very prone to exhibit phenoplasticity.

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Introduction

Since the beginning of taxonomy and systematics, morphological description has been the basis of naming and classifying plants. Following several species concept i.e. Biological species concept, Phylogenetic species concept and many others, still morphology is the core of description and naming. Although morphological description has served best for majority of taxonomists, there are a number of arousing doubts as to its credibility and accurateness.

In the Philippine context, morphological description is the practiced and well-accepted scheme of species description and naming particularly that the country is far from mastering the science of genetic and molecular data. Although many have attempted to use molecular data in species description but majority are still relying to morphological description. The use of molecular data also imposes several disadvantages such as inconclusiveness when only partial gene will be used to delineate species, the type of primer, and most especially it is expensive – at the moment.

Hoya amorosoae is a new species of Hoya discovered in Mt. Hamiguitan Range Wildlife Sanctuary, San Isidro Davao Oriental Philippines, described by Ted Green & Robert Dale Kloppenburg in 2014 and named in honor of the late Dr. Cecilia B. Amoroso who collected the plant materials (Green and Kloppenburg, 2014).

In this paper, leaf character of *Hoya amorosoae* will be tested its morphologic response to varying light exposure. This is very important in proving that vegetative structures such as leaves is not enough to use as main criterion in delineating new species to science.

This study aims to document the changes of leaf morphology of *Hoya amorosoae* from varying light exposure. Specifically, it aims to measure the mophometry (width, length, thickness) of the leaves exposed to shade and in the natural habitat. In addition, a note of the presence of anthocyanin was also recorded.

Material and methods

Plant material

H.amorosoae cuttings were collected from Mt. Hamiguitan Range Wildlife Sanctuary, San Isidro, Davao Oriental Philippines. Cuttings were grown in pots with the same growing material (3/4 coconut husk and ¼ loam soil).

Light exposure

Experimental pot was placed under shaded environment while the control is placed in the natural habitat - in this case *H. amorosoae* in-situ propagated by Mr. Larry Cahilog at Mt. Hamiguitan Range Wildlife Sanctuary, Davao Oriental with the same amount of water. Direct observation was made within the months of February and May 2016.

Morphometry

Using micrometer caliper, leaf length, width, and thickness were measured on 3 replicates each set-up. T-test was used to determine the significant difference between means. Results were tabulated in tables 1 and 2.

Photo-documentation

Photo-documentation was employed for further analysis and comparison.

Result and discussion

Leaf morphology was measured using micrometer caliper in centimeter. Two samples were tested: Pot 1 – under shaded environment and Pot 2 from the natural habitat (NH). Three (3) leaf replicates from each pot was used and measured on their width, length, and thickness. Results are tabulated in Table 1.

In terms of width, leaves of *H.amorosoae* placed under shaded environment exhibits slender leaves with 1.13 cm width compared to pots under natural habitat with 1.33 cm. However, T-test revealed no significant difference in terms of width with a t value of -1.604, df 4, and p-value of 0.184^{ns} (Table 2).

Table 1. Leaf morphometry of *H.amorosoae* under varying light exposure.

	Width (cm)		Length (cm)		Thickness (cm)	
	Pot 1	Pot 2 NH	Pot 1	Pot 2 NH	Pot 1	Pot 2 NH
Leaf 1	1.1	1.5	4.5	2.7	0.09	1.3
Leaf 2	1.3	1.2	4.7	2.4	0.1	1.4
Leaf 3	1.0	1.3	4.8	2.2	0.1	1.4
Mean	1.13	1.33	4.67	2.43	0.097	1.37

Legend: Pot 1 – under shaded environment; Pot 2 NH - Natural habitat.

In terms of leaf thickness, *H.amorosoae* under shaded environment has the thinner leaves with 0.097 cm compared to 1.37 cm leaves under natural habitat. Meanwhile, in terms of leaf length *H.amorosoae* under shaded environment have longer leaves with 4.67 cm compared to 2.43 cm leaves in the natural habitat. Statistical analysis revealed that there is a significant difference between the thickness and length of the leaves in the natural habitat and in the shaded environment (Table 2).

Table 2. T-test of leaf measurements in the natural and shaded environment.

Parameters	Groups	Means	Mean Difference	t	df	p-value
Width	Shaded environment	1.13	-.20000	-1.604	4	0.184ns
	Natural habitat	1.33				
Length	Shaded environment	4.67	2.23333	13.140	4	0.000**
	Natural habitat	2.33				
Thick	Shaded environment	0.10	-1.27000	-37.911	4	0.000**
	Natural habitat	1.37				

The elongated leaf of *H.amorosoae* under a shaded environment is the physiologic response of the leaf from a scarce light source. In the experiment, it is presumed that meristematic cells at the apex and base of the leaves are dividing faster than in the stem; hence the leaves under shaded environment develop an elongated structure. This is also evident in the formation of pointed apex and slender leaf base compared to leaves exposed to direct sunlight and in the natural habitat (Fig. 1).

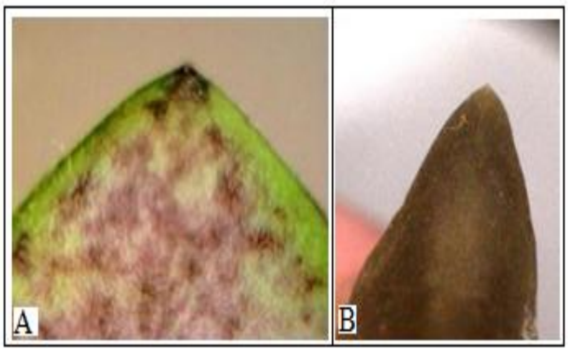

Fig. 1. Appearance of leaf apex of *H.amorosoae* under A. In the natural habitat (blunt); B. Under shaded environment (pointed).

Leaves of *H.amorosoae* exposed to varying lightintensity may have caused morphological differences. Experiment showed that when plant material exposed to enough sunlight there is a tendency to develop an ovoid formation resulting to stouter leaves but more succulent and blunt. While leaves placed in minimal light intensity will results to slender leaf base but more elongated leaf and more thinner (Fig. 2).

All these morphological changes from varying light intensity might affect species description especially when based on vegetative i.e. leaf morphological character.

Furthermore, thick succulent leaves of *H.amorosoae* under direct sunlight and in the natural habitat may suggest that photosynthetic rate of these materials is faster compared to leaves under shaded environment. Faster rate of photosynthetic activity would mean that more food has been manufactured by the plant and more water is needed in the process until equilibrium (Fig. 3) hence may explain the thick succulent leaf structure of leaves.

Light is the primary factor for plants (Kirk, 1996), and its effects on photosynthesis and growth have been repeatedly demonstrated in the field (Madsen and Maberly, 1991; Thomaz *et al.*, 1998; Schwarz *et al.*, 2000), as well as in the laboratory (Sand-Jensen, 1991). Shade tolerance and light-related morphological changes in some species may confer competitive advantages in light-limiting situations, thereby influencing community composition (Tanner *et al.*, 1986).

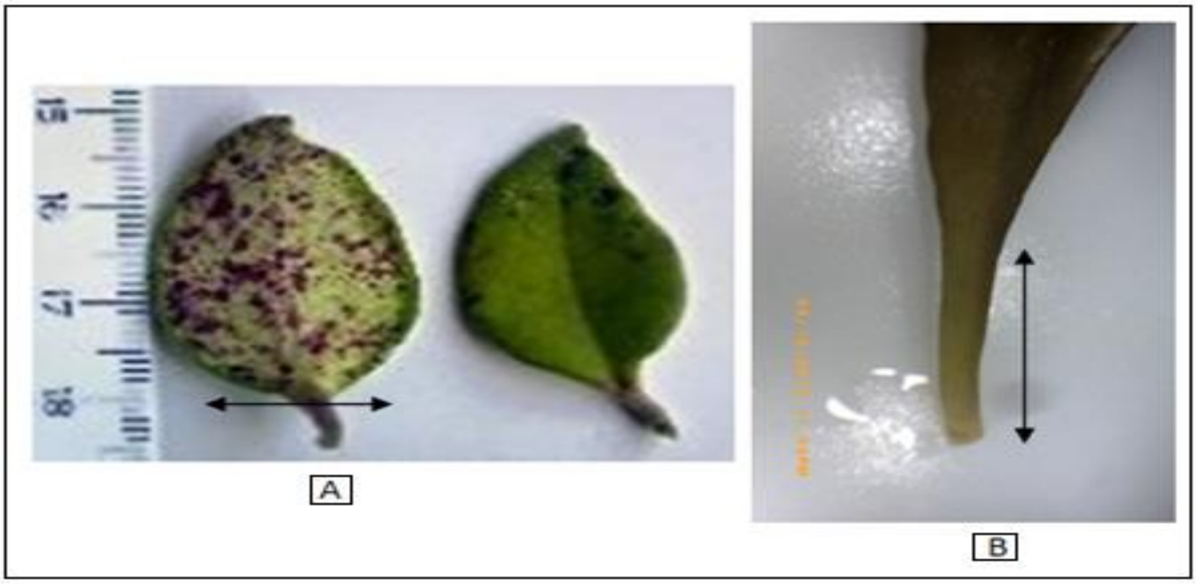

Fig. 2. Leaf base structure of *H.amorosoae*: A. ovoid (Natural habitat); B. Fusiform (Shaded environment).

As light intensity increases, the rate of the light-dependent reaction, and therefore photosynthesis generally, increases proportionately (straight line relationship) (Fig. 4). Tavechio *et al.* (2003) proved that Light stimulated shoot and root relative growth rates. The more photons of light that fall on a leaf, the greater the number of chlorophyll molecules that are ionised and the more Adenosine Triphosphate (ATP) and Nicotinamide Adenine Dinucleotide Phosphate (NADPH) are generated. Light dependent reactions use light energy and so are not affected by changes in temperature. As light intensity is increased further, however, the rate of photosynthesis is eventually limited by some other factor. So the rate plateaus. At very high light intensity, chlorophyll may be damaged and the rate drops steeply (not shown in the graph). Chlorophyll a is used in both photosystems. The wavelength of light is also important. PSI absorbs energy most efficiently at 700 nm and PSII at 680 nm. Light with a higher proportion of energy concentrated in these wavelengths will produce a higher rate of photosynthesis.

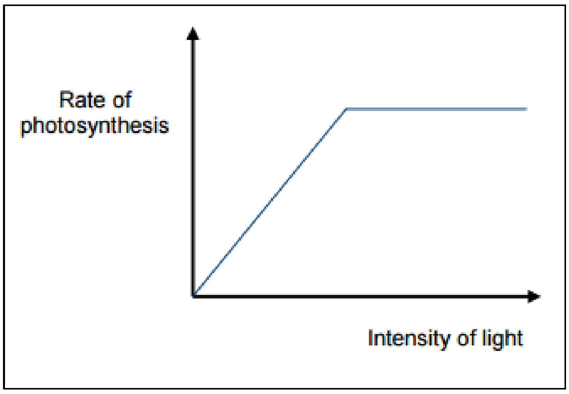

Fig. 3. Rate of photosynthesis and light intensity.

Presence/Absence of Anthocyanin

Anthocyanins are any of various soluble glycoside pigments producing blue to red coloring in flowers and plant (Goud *et al.*, 1995; Laleh *et al.* 2006). These are flavonoids that provide color to most flowers and fruits (Palamidis and Markakis, 1975).

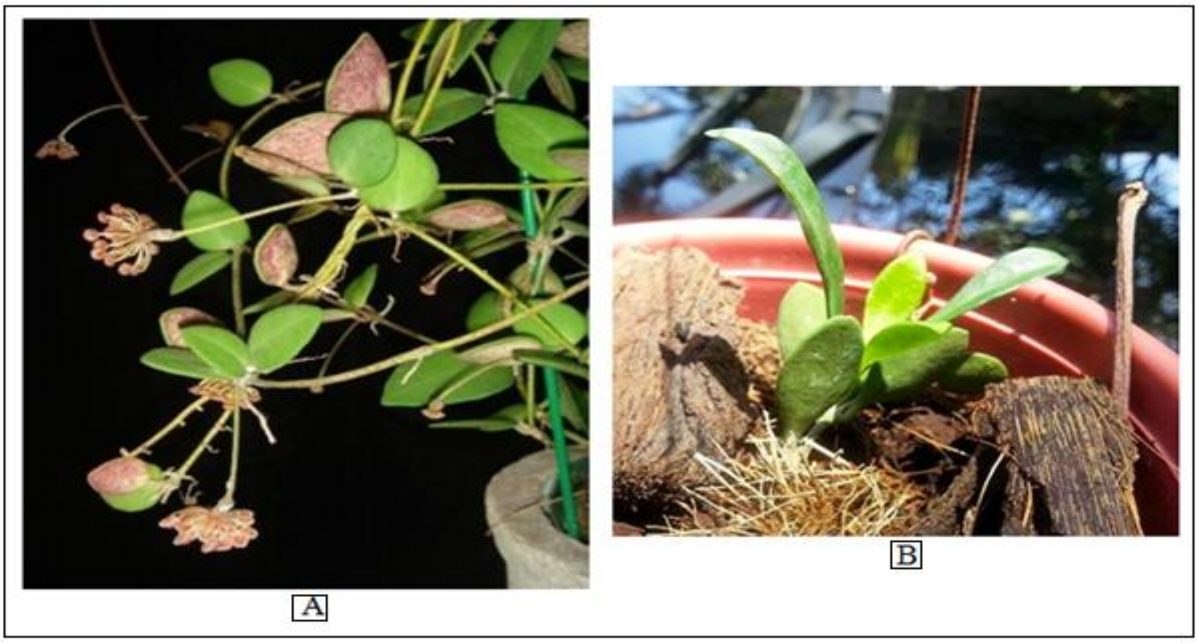

Fig. 4. A. *H.amorosoae* in natural habitat showing anthocyanins of the leaf dorsal surface (Green & Kloppenburg, 2014); B. Leaves of *H.amorosoae* under shaded environment – note the absence of anthocyanins and elongated-slender leaves.

It is said that this compound help attract pollinating animals to flowers and animals that will help disperse seeds (Angela and Little, 1997). In addition, anthocyanins are thought to deter herbivores in some species (Goud *et al*. 1995; 2000).

It is evident that *H.amorosoae* leaves secrete anthocyanin when properly exposed to sunlight, while no anthocyanin observed in leaves under shaded condition. This suggests that the presence of red pigments as mostly mentioned by some taxonomists is not a stable character and may depend on light intensity. Ultimately, anthocyanins occur in all tissues of higher plants, including leaves, stems, roots, flowers, and fruits. Hence one cannot tell that the presence of red pigment in certain plant a unique character of the plant being describe.

Fig. 4 showed the appearance of *H.amorosoae* in the natural habitat exhibiting red spots (anthocyani) under dorsal side of the leaves. When H.amorosoae leaves exposed to enough sunlight it will produce anthocyanin that causes red spots at the underside of the leaves. However, *H.amorosoae* leaves under shaded environment do not exhibit red spots since there is no enough sunlight to manufacture anthocyanin hence the leaves are green in both surfaces.

Results of this study also supported by Laleh *et al.*, 2006 temperature or exposure to light be able the production of anthocyanin molecule.

Conclusion

Leaf morphological changes occurred at varying light intensities. Anthocyanin has been proven to occur in the leaves in response to light. Hence, using these data on the vegetative structures in describing Hoya is somewhat not very conclusive. It is therefore recommended to use both vegetative and reproducive structures when describing particular plant species specially *Hoya* as these taxon are very prone to exhibit phenoplasticity.

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