

LEAF MORPHOLOGY AND ANATOMY OF SELECTED PHILIPPINE *Dischidia* R. Br. (APOCYNACEAE: ASCLEPIADOIDEAE)

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Abstract: The epiphytic genus *Dischidia* R.Br. known for its close association to Hoya R.Br., remains poorly understood genus particularly in the Philippines. Though many are uniquely found in the country, many are still inadequately documented for conservation nor advance research purposes. As many discoveries are identified only by herbarium materials and out of date, particularly its morphology and anatomy aspects. So far in the Philippines, no studies had discussed specifically the leaf morphological and anatomical characteristics of these genera undermining any potential taxonomic usefulness of both attributes. This study seeks to determine more on the morphological and anatomical features of Dischidia leaves. For leaf stomata and epidermal structure an impression approach was done using nail polish. Leaf anatomy were studied by preparing transverse sections of the midrib by hand cut free sectioning method. The study shows that leaf morphology of the selected Philippine *Dischidia* agrees well to other literatures but to no avail for significant taxonomic characters. As for the anatomical area of the leaves, isopalisade and homogenous mesophyll type were determined, the first to report about the nature of *Dischidia* mesophyll type. For its systematic value, this mesophyll characterization is a gray feature due to its environmental susceptibility.

Key Words: Dischidia; Leaf; Philippines; Taxonomy

1. INTRODUCTION

The genus *Dischidia* R.Br. consists of c. 80 species of epiphytic climbers some with common association with ants, predominantly growing in Indochina, Malesia, Melanesia and the east Pacific. *Dischidia* has laticiferous opposite leaves as common in the milkweed family. It shares morphological characters with *Hoya* R.Br. as its closest sister genus. With both pseudo-umbelliform inflorescences, *Dischidia* differs by having urceolate or campanulate shape of corolla, while *Hoya* has usually salver or revolute shape of corolla lobes. This genus is further classified into three sections: (1) section *Dischidia* characterized by laminar leaves, (2) section *Conchophylla* with shell-shaped leaves, (3) section *Ascidifera* with pitcher-leaves. Inclusion of genus *Dischidiopsis, Oistonema, Conchophyllum* and *Leptostemma* to *Dischidia* is proposed. (Livshultz, 2003).

There are taxonomic studies done for this genus (e.g. Lu & Kao, 1980; Rintz, 1980; Forster et al., 1996; Jagtap & Singh, 1999; Livshultz et al., 2005) and these studies are confined to specific areas. Still there is a gap regarding the taxonomy and ecology of *Dischidia* at large (Livshultz et al., 2005) particularly in the Philippines. In the Philippines, significant contributions for this genus were made by Pearson (1902), Merrill (1904, 1923), Schlechter (1915), Elmer (1938) and the latest study was done by Livshultz (2003). To this date, 21 species are identified, 16 of which are considered endemic and 8 species remains uncertain due to



invalid publication. From hence, less attention has been given to *Dischidia* judging from the fact that recent comprehensive updates and revisions have not been made. The level of taxonomic knowledge for Philippines *Dischidia* are hardly elementary. Taxonomic knowledge, species distribution, taxonomic keys, diagnostic illustrations and ecology of Philippine *Dischidia* are lacking and most of the species needed to be "rediscovered" for clarification due to the fact that many remain uncertain and out of date.

Even though reproductive characters have greater role for familial up to genus delimitation still vegetative morphology can further strengthen the restriction particularly in Dischidia. In which, leaf morphology has been utilized to further classify the species into three sections. Leaf shape of the Dischidia plays a sectional taxonomic importance but still its morphological and anatomical attributes remain poorly understood. So far, no studies had discussed specifically the leaf morphological and anatomical characteristics of these genera particularly in the Philippines undermining any potential taxonomic usefulness of both attributes. This study seeks to determine more on the leaf morphological and anatomical features of Dischidia and to assess its taxonomic value in delimitation for Philippine Dischidia species.

2. METHODOLOGY

2.1 Plant Acquisition

Dischidia species were collected specifically from Kalinga Province, Nueva Vizcaya, Daraitan, Rizal, Mt. Banahaw de Majayjyay and Polillio Quezon. Collection of samples were done from August to November 2016. A total of twelve living Dischidia species were collected (Table 1). Moreover, herbarium specimen examination was conducted at the Philippine National Herbarium (PNH) of the National Museum in Manila (See Appendix, Table 5).

2.2 Leaf Morphological Studies

Mature leaves were measured and described in both dry and living conditions. Quantitative characters (e.g. petiole length, leaf length and width, number of nerves) were measured using a ruler (if necessary) while qualitative characters (e.g. leaf apex, margin, base, shape, leaf texture on both surface) were observe under the

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stereomicroscope (Nikon SMZ 745T) for observing minute qualitative characters (e.g. petiole and leaf indumentum). Botanical terminology was based from the Kew Plant Glossary (2010).

Table 1. Collected Dischidia Species

Species Collected	Location	Collection #
D. sp1	Kalinga	K-001
D. sp2	Mt. <u>Banahaw</u> de <u>Maiaviav</u>	Q-001
D. sp3	Polillio, Quezon	Q-002
D. sp4	Nueva Vizcaya	NV-001
D. sp5	Polillio, Quezon	Q-003
D. sp6	Daraitan, Rizal	DR-001
D. hirsuta (Blume) Decne.	Daraitan, Rizal	DR-002
D. lancifolia Merr-	Daraitan, Rizal	DR-003
D. nummularia R. Br.	Daraitan, Rizal	DR-004
D. giantha Schltr.	Manila	MNL-001
D. platyphylla Schitr.	Lucban, Quezon	Q-004
D. ruscifolia Decne ex Becc	Nueva Vizcaya	NV-002

2.3 Leaf Surface and Anatomical Studies

To examine and determine the leaf stomata and epidermal structure an impression approach was done using nail polish. Both sides of the leaf were covered and three fields per leaf side were randomly selected and photomicrographed. Leaf area was determined using ImageJ 1.50i (Rasband, USA). Stomatal patterns of the leaf epidermis were examined and stomatal index were calculated and estimated using:

$$[s/(e+s)] \ge 100$$
 (Eq. 1)

Where:

s = the number of stomata

e = the number of epidermal cells (Radoglou and Jarvis, 1990; Xu and Zhou, 2008).

Leaf anatomy was studied by preparing transverse sections of the midrib by hand cut free sectioning method. Light microscopic (Nikon DS-Fi2) observations were used to observe the specimens from both studies.

3. RESULTS AND DISCUSSION

3.1 Leaf Morphology

The leaf of twelve live *Dischidia* species were morphologically examined. The leaf characteristics presented (Table 2.1-2.2) were based from the usual leaf diagnostic analysis for this genus. Leaf external features typically includes the



leaf arrangement, leaf shape (e.g. apex, margin, base) and indumentum hair presence and types.

Table 2.1 Leaf Characteristics of Fresh Specimens

Lear	D. 5p 1	D. sp z	D. SP 3	D. sp 4	D. sp 5	D. sp в
Morphology						
Sectional	Sect.	Sect.	Sect.	Sect.	Sect.	Sect.
delimitation	Dischidia	Dischidia	Dischidia	Ascidifera	Dischidia	Dischidia
Leaf	opposite	opposite and	opposite	opposite and	opposite	opposite and
Arrangement	and	decussate	and	decussate	and	decussate
	decussate		decussate		decussate	
Leaf Shane	elliptic	elliptic to	elliptic to	obovate to	Ovate	ovate
cear shape	emptic	lanceolate	lanceolate	lanceolate	01012	01010
	-	share	anceoloce	anceorate		
Leal Apex	obtuse	obtuse		attenuate	acute to	acute to
					attenuate	attenuate
Leaf Base	subcordate	obtuse	cuneate	cuneate to	rounded	rounded
			to	attenuate		
			attenuate			
Leat Margin	entire	entire	entire	entire	entire	entire
Leaf Texture	thin and	thick and	thick and	thick and	thick and	thick and
	smooth	smooth	smooth	smooth	smooth	slightly
						smooth
Number of	5 pairs	inconspicuous	4pairs	inconspicuous	3-4 pairs	inconspicuous
Visible Nerves						
Leaf Width	12-17 cm	2 5-3 cm	2.6-3.cm	1-1.2 cm· (MI	1-1.9 cm	0.8-1 cm
			2.0 2 2	3-5 cm)		
LosfLongth	2-2.8 cm	5.7 cm	45.56	2-2 E cm. (MI	2-2 E cm	13-14 cm
Lear Length	2-2.0 cm	5-7 cm	4.5-5.0	2-2.5 cm, (Mc	2-2.5 cm	1.2-1.4 (11)
			cm	4-0 cm)		
Leaf thickness	0.26 X	0.54 X .001"	0.41 X	0.28 X .001"	0.51 X	1.27 X .001"
	.001"		.001"		.001"	
Adaxiai Lear	absent	absent	absent	absent	absent	present
Hair Presence						auto autoria
Adaxial Lear						BABELAIGHE
Hair Type	abreat	abcent	abcoat	shrant	abreat	
Abaxial Leal	absent	absent	ausent	auseni	auseni	present
Abayial Loaf						puberulous
Hair Type						83651313995
Petiole Length	0.5 cm	0.8-1 cm	1-2 cm	0.2-0.4 cm	03-06	0.1-0.3 cm
r choic tength	0.5 cm	0.0 1 011		0.2 0.4 Cm	cm	6.2 0.3 Cm
Petiole Hair	absent	absent	absent	absent	absent	present
Presence			advent		and and	present
Petiole Hair						puberulous
Types						
.,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,						

Leaves: From the collections made, all three sections were represented namely: Sect. Dischidia, Sect. Conchophylla, and Sect. Ascidifera. Leaves were all opposite, entire, with variable leaf shapes ranging from orbicular, ovate, obovate, elliptic to lanceolate types, leaf apices tend to have an obtuse, attenuate, acute to acuminate forms, leaf bases ranges from subcordate, attenuate, cuneate, rounded to obtuse types. Living specimen textures were mostly smooth due to the presence of cuticle unlike the preserved specimens that tends to loss in time. Generally, leaf width ranges from 0.4 - 7 cm, leaf height from 0.5 - 7 cm; all have petiole with sizes ranging from 0.1 - 1 cm. Leaf nerves seven of the live specimens have inconspicuous nerves, the nerves were hardly visible maybe due to the leaf thickness. For the remaining leaf samples, leaf nerves range from 3-5 nerve pairs.

Leaf surface and nerves: All of the species shades were green to dark green. Utmost leaves have glabrous surface both sides except for D. sp 6 with puberulous hairs, D. *hirsuta* with hirsute type, D. *nummularia* with pilose hair and subglabrous with few hirsute hairs on the adaxial and petiole area for D. *platyphylla*. Same remark was seen for the petiole hair presence and types. Leaf nerves seven of the live specimens have inconspicuous nerves, the nerves were hardly visible maybe due to the leaf thickness. For the remaining leaf samples, leaf nerves range from 3-5 nerve pairs.

Table 2.2 Leaf Characteristics of Fresh Specimens (cont.)

.0110./						
Leaf	D. hirsuta	D.	D.	D. oiantha	D.	D. ruscifolia
Morphology	(Blume) Recoe	lancifelia. Merr.	nymmylaria R. Br.	Schltr-	platyphylla Schitr.	Recne ex Becc
Sectional	Sect.	Sect.	Sect.	Sect.	Sect.	Sect.
delimitation	Rischidia	<u>Rischidia</u>	Rischidia	<u> Rischidia</u>	Cencheehvile,	Rischidia
Leaf Arrangement	opposite and decussate	opposite and decussate	opposite	opposite and decussate	opposite	opposite and decussate
Leaf Shape	ovate to lanceolate	lanceolate	orbicular	obovate	orbicular	ovate
Leaf Apex	acute	acute	acute to acuminate	obtuse	rounded	attenuate to acuminate
Leaf Base	rounded to obtuse	attenuate	rounded to obtuse	attenuate	rounded	rounded
Leaf Margin	entire	entire	Entire	entire	entire	entire
Leaf Texture	thick and slightly smooth	thick and smooth	thick and smooth	thick and smooth	thick and slightly smooth	thick and smooth
Number of Visible Nerves	inconspicuous	4-5 pairs	inconspicuous	inconspicuous	3pairs	inconspicuous
Leaf Width	0.9-1.1 cm	0.9-1.5 cm	0.4-0.6 cm	1.4-1.9	4-7 cm	0.6-0.8 cm
Leaf Length	1.9-2.2 cm	3.5-6 cm	0.5-0.7 cm	2.6-3.4	3-5 cm	0.8-1 cm
Leaf thickness	0.71 X .001"	0.70 X .001"	1.02 X .001"	0.65 X .001"	0.61 X .001"	0.88 X .001"
Adaxial Leaf Hair	present	absent	present	absent	subglabrous	glabrous
Presence Adaxial Leaf Hair Type	hirsute		Pilose		hirsute (few)	
Abaxial Leaf Hair	present	absent	present	absent	absent	absent
Presence Abaxial Leaf Hair Tyne	hirsute		Pilose			
Petiole	0.2-0.4 cm	0.9-1 cm	0.1-0.2 cm	0.5-1 cm	0.3-0.4 cm	0.2 cm
Petiole Hair Presence	present	absent	present	absent	semi glabrous	absent
Petiole Hair Types	hirsute		Pilose		hirsute (few)	

3.2 Stomatal Characteristics

Stomata: The stomatal diagnosis of the twelve fresh samples were microscopically observed. The specimen with the smallest area were as follows respectively, *D. ruscifolia* (0.48 cm²), *D. nummularia* (0.59 cm²) and *D. species* 6 (0.97 cm²) while the largest was *D. platyphylla* with 16.11 cm². Distribution of paracytic and anomocytic stomatal types were determined and presented in Table 3 and Fig. 1.

Stomatal outline: General outline was elliptic either narrow and broad. Narrowly elliptic in *Dischidia*



species 1, 2, 3, *D. lancifolia*, *D. nummularia*, *D. oiantha*, *D. platyphylla*; broadly elliptic in *Dischidia species* 4, 5, 6, *D. hirsute* and *D. ruscifolia* (Fig. 1). Also, stomatal size was generally decreased.

Table 3. Stomatal Characteristics of Fresh Specimens

Species	Leaf Area (cm2)	Epidermal cell count		f Area Epidermal cell Stomatal count cm2) count		Stomatal index		Stomatal type
		Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	
D. sp1	2.66	587	513	16	17	2.65	3.21	Paracytic
D. sp2	15.02	378	426	15	9	3.82	2.07	Paracytic.
D. sp3	14.08	400	358	11	13	2.68	3.50	Paracytic
D. sp4	2.7	421	483	5	10	1.17	2.03	Anomocytic
D. sp5	2.45	486	292	13	15	2.61	4.89	Paracytic.
D. sp6	0.97	340	477	12	13	3.41	2.65	Anomocytic
D. lancifolia	5.27	264	402	7	14			Paracytic.
Merr.		204	405			2.58	3.36	
D. hirsuta	1.99			8	14			Paracytic
(Blume)		362	323			2.16	4.15	
D nummularia	0.59			16	13	2.10	4.15	Anomoratic
R. Br.	0.00	462	396	10	10	3.35	3.18	08090039060606
D. oiantha	4.86	120	460	17	14			Anomocytic
Schltr.		450	402			3.80	2.94	
D. platyphylla	16.11	500	517	17	16			Paracytic
Scultr.	0.49			20	10	3.29	3.00	Anomoratic
Decne ex Becc	0.46	398	411	20	19	4.78	4.42	CHISTOSCIES

Stomatal index: For the leaf sides, highest for the epidermal and stomatal count were found in abaxial sides. As for the leaf area in relation to its stomatal index (adaxial, abaxial), the largest leaf area, D. *platyphylla* has 3.29 and 3.00 index respectively while D. ruscifolia, having the smallest leaf area, has 4.78, 4. 42 stomatal index respectively, this means this species have more compact stomata regardless of its small leaf area.

Epidermal cells: More or less epidermal cells of most species have rectangular to polygonal shapes (e.g. *Dischidia* species 1, 4, *D. lancifolia*, *D. hirsuta*, *D. ruscifolia*). While the rest have unclear epidermal cells (Fig.1).

From the collections of the Philippine Leaves: National Herbarium (PNH), three sections were also denoted namely: Sect. Dischidia, leaves laminar e.g. D. hirsute, D. luzonica, D. merrillii, D. nummularia, D. oiantha, D. ruscifolia; Sect. *Conchophylla*, shell-shaped leaves e.g. Dplatyphylla and D. purpurea and Sect. Ascidifera, pitcher leaves e.g. D. major. Leaves were all opposite, entire, with variable leaf shapes ranging from orbicular, obovate, ovate to lanceolate types, leaf apices tend to have an obtuse, attenuate, acute to acuminate forms, leaf bases ranges from obtuse, cuneate to attenuate, rounded to obtuse types. Preserved specimen textures were mostly

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coriaceous, few chartaceous. Commonly, leaf width ranges from 0.4 - 6 cm, leaf height from 0.4 - 7.5 cm; all have petiole with sizes extending from 0.1-1 cm (Tables 4.1-4.2).



Fig. 1. Stomata of the twelve collected *Dischidia* species. (A) *Dischidia* sp. 1. (B) *Dischidia* sp. 2. (C) *Dischidia* sp. 3. (D). *Dischidia* sp. 4. (E). *D. nummularia*. (F). *D. oiantha*. (G.) D. *Dischidia* sp. 5. (H). *Dischidia* sp. 6. (I). *D. lancifolia*. (J). *D. hirsuta*. (K). *D. platyphylla*. (L). *D. ruscifolia*.

Leaf surface and nerves: All of the species shades were light brown to dark brown. Most of the leaves have glabrous surface on both sides except for *D. hirsuta* with hirsute hairs, *D. nummularia* with pilose hair on the abaxial surface, *D. platyphylla* with adaxial few pilose hair, *D. purpurea*, minimal hirsute hair type, and *D. ruscifolia* with few hirsute hairs on the abaxial surface (Fig. 2). Petiole hair types were usually hirsute e.g. *D. hirsuta*, *D. platyphylla* and *D. ruscifolia*; for D. *nummularia*, pilose hair was seen. Leaf nerves seven of the live specimens have inconspicuous nerves, the nerves are



hardly visible maybe due to the leaf thickness. For the remaining leaf samples, leaf nerves range from 3-5 nerve pairs.

Table	4.1	Leaf	Characteristics	of	Philippine
Dischi	<i>dia</i> PN	VH Spe	cimens		

11	D bloots		B	0	D
Leaf Morphology	D. hirsuta Recne	D. <u>Iuzonica</u> (Schltr.) Livshultz	U.maior. (Yahl.) Merr.	D. merrillii Schltr.	D. nummularia R. Br.
Sectional	Sect.	Sect. Dischidia	Sect. Acidifera	Sect.	Sect. Dischidia
delimitation	Dischidia			Rischidia	
Leaf	opposite	opposite	opposite	opposite	opposite
arrangement					
Leaf Shape	ovate to	lanceolate	ovate, modified leaf	lanceolate	orbicular to
	lanceolate		(ML)- oblong-elliptical		ovate
Leaf Apex	acute	acute	acute	acuminate	acute to
Leaf Base	rounded to	cuneate to	rounded to obtuse	cuneate	rounded to
	obtuse	attenuate			obtuse
Leaf Margin	entire	entire	entire	entire	entire
Leaf Texture	coriaceous	chartaceous	coriaceous	chartaceous (drv)	chartaceous
Number of	lateral nerves	lateral nerves 4	lateral nerves	lateral	lateral nerves
Visible Nerves	2-4 pairs	pairs (opposite, hardly visible)	inconspicuous	nerves 4-5 pairs	inconspicuous
Leaf Width	0.5-1.3 cm	1- 2.1 cm	0.8-1.2 cm, ML- 1-3.7 cm	0.9-1.2 cm	0.4-0.8 cm
Leaf Length	1-2.6 cm	3.1-7.5 cm	1.2-2.3 cm, ML- 4.3- 12.5 cm	2-2.9 cm	0.4-1.2 cm
Leaf Thickness	0.90 X .001"	0.21 X .001"	0.32 X .001"	0.15 X .001"	0.30 X .001"
Adaxial Leaf	present	absent	absent	absent	absent
Hair Presence					
Adaxial Leaf	hirsute				
Hair Type					
Abaxial Leaf	present	absent	absent	absent	present
Hair Presence					
Abaxial Leaf	hirsute				pilose
Hair Type					
Petiole Length	0.1-0.3 cm	0.6- 1 cm	0.1-0.3 cm	0.2-0.4 cm	0.1- 0.2 cm
Petiole Hair	persisting	not seen	not seen	not seen	seen
Presence					
Petiole Hair	hirsute				pilose
Types					

Table 4.2 Leaf Characteristics of Philippine *Dischidia* PNH Specimens (cont.)

Leaf Morphology	D. <u>oiantha</u> Schltr.	D. platyphylla Schltr.	D. purpurea Merr.	D. <u>ruscifolia Decne</u> ex Becc
	Sect.	Sect. Conchophylla	Sect.	Sect. Dischidia
	Dischidia		Conchophylla	
Leaf arrangement	opposite	opposite,	opposite	opposite
Leaf Shape	obovate	orbicular	orbicular	ovate
Leaf Apex	obtuse	rounded	rounded	attenuate to acuminate
Leaf Base	attenuate	rounded	rounded	rounded
Leaf Margin	entire	entire	entire	entire
Leaf Texture	coriaceous	coriaceous	coriaceous	coriaceous
Number of Visible Nerves	lateral nerves inconspicuous	lateral nerves 3-4 pairs	hardly visible lateral nerves (3pairs)	lateral nerves 5 pairs
Leaf Width	0.8- 2.1 cm	2.8-6 cm	1.5-4 cm	0.5- 1 cm
Leaf Length	2-5 cm	2.5- 4.5 cm	1.7-3 cm	0.8- 1.7 cm
Leaf Thickness	0.37 X .001"	0.30 X .001"	0.4 X .001"	0.16 X .001"
Adaxial Leaf Hair Presence	absent	present	present	absent
Adaxial Leaf Hair Type	glabrous	semi-glabrous to hirsute (few)	hirsute (few)	glabrous
Abaxial Leaf Hair Presence	absent	absent	present	present
Abaxial Leaf Hair Type	glabrous	glabrous	hirsute (very few)	hirsute (few)
Petiole Length	0.5- 1 cm	0.3-0.4 cm	0.1cm	0.1-0.2 cm
Petiole Hair Presence	absent	present	not seen	seen
Petiole Hair Types		hirsute (few)		birsute



Fig. 2. Sample Leaf Hairs from PNH Herbarium Specimens. (A) *D. hirsuta*. (B) *D. nummularia*. (C) *D. platyphylla*. (D) *D. ruscifolia*

Epidermal surface: Common *Dischidia* epidermis were thick and compact. Prominent epidermal thickness was shown clearly in *D. species 1, D. species 6, D hirsute, D. lancifolia, D. nummularia, D. oiantha* and *D. ruscifolia* (Fig. 3-3.1).

Mesophyll: Two types of mesophyll had been identified; (1) isopalisade for *D. species* 1,2,5,6, *D. lancifolia*, *D. nummularia*, *D. oiantha*, and *D. ruscifolia*, (2) homogenous mesophyll symmetry *D. species* 3,4, *D. hirsute*, *D. platyphylla* (Fig. 3-3.1). While *D. species* 1 has bifacial symmetry. Both mesophyll types have undifferentiated cells but differs on the cell shape, for isopalisade it was characterized by having elongated to elliptical cells while homogenous types have rounded shape cells (P'yankov and Kondrachuk, 2001).

3.3 Philippine Dischidia Leaf Morphology

To our knowledge, there were no initial observation nor study regarding the leaf morphology and anatomical aspects of *Dischidia* particularly in the Philippines. This study was the first attempt for such endeavor. Morphological plasticity often occurs due to environmental adaptation and response particularly seen in its vegetative parts like the leaves. Some of this plasticity can be seen in external



and anatomical features of leaves. With regards to the morphological features stated in this paper (Tables 2-2.2 & 4-4.1) most of the characters were similar to that of Malayan species of *Dischidia* that recognizes that most leaves are opposite and glabrous (Rintz, 1980; Omino, 1996).



Fig. 3. *Dischidia* Leaves Cross Section Photomicrographs; Mesophyll type:(A-B, E-F, H) Isopalisade; (C,D,G) Homogenous type; e= epidermis; p,s= mesophyll; bs= bundle sheath. (A-F) *Dischidia* species 1-6 respectively. (G). *D. hirsuta*. (H) *D. lancifolia*.

The most frequent stomatal type is paracytic which corroborates on the study of Freire et al. (2005). For the mesophyll, there were no known study regarding the mesophyll characteristics of this genus making this study first to gather such information that has typical isopalisade and homogenous mesophyll symmetry.

Diagnostic Value: Anatomical parts like stomata and mesophyll features in relation to its identificatory value remains uncertain due to the fact that most of these parts are highly variable and susceptible to environmental changes like altitude and climatic events. Making this characters less useful when delimiting species identification. This features are not conserved and adapting easily as supported by the findings of P'yankov & Kondrachuk (2003), Xu & Zhou (2008), Pyakurel & Wang (2014).



3.1. Dischidia Leaves Fig. Section Cross (I,J,L) Photomicrographs Mesophyll type: e=Isopalisade; (K) Homogenous; epidermis; p,s=mesophyll; bs= bundle sheath. (I) D. nummularia. (J) D. oiantha. (K) D. platyphylla. (L). D. ruscifolia.

3.4 Leaf Physiological Aspect

External Morphology: Dischidia tends to have narrow and generally thick leaves compared to other plants. It gives reinforcement to endure hot climates and dry environment. Knowing that this genus is an epiphytic, this kind of plant structure is an adaptation. Moreover, most of the species collected have smaller leaf area (e.g. D. sp. 1-6, D. hirsuta, D. merrillii, D. nummularia, D. ruscifolia) that enables this aerial plant to track air temperature compare to broader leaves that can overheat easily. As for the leaf hairs, this feature enables less transpirational loss in which some of the *Dischidia* species possess but not at large (Tables 2-2.2; 4-4.1). Reduced leaf size and hair presence are common morphological modification for plants with water deficiency (Xu & Zhou, 2008; Pyakurel & Wang, 2014).



Stomata: Vital intercellular opening in the leaf surface that regulate gases between the atmosphere and the plant tissue are called stomata. This research revealed despite having less leaf area of the Dischidia studied it doesn't limit the opacity of the stomata in both leaf sides but highest stomatal count was found in abaxial sides (Table 3) but with prominent small stomatal sizes. With connection to the epiphytic state of the genus that leads to increases water loss it validates other studies about having water deficiency tends to increase stomatal density and reduced stomatal size (Xu & Zhou, 2008; Pyakurel & Wang, 2014). Also, large influence can be directed on the environment where it can affect the leaves developments such as stomatal density (Radoglou and Jarvis, 1990).

Mesophyll: Photosynthesis occurs mainly in these cells (palisade and spongy cells) containing chloroplast. The compactness of the mesophyll tissues allows the maximum effort to trap sunlight for photosynthesis as shown in Fig. 3-3.1. Isopalisade mesophyll are common from plants that are surviving in dry or water deficit conditions (Ivanova and Pyankov, 2000; P'yankov and Kondrachk, 2003) which includes Dischidia known for its epiphytic and water lacking environment. While some of the Dischidia species exhibit homogenous structure that are common in shady conditions (Ivanova and Pyankov, 2000). The differences between the mesophyll types between species can be due to altitude and environmental conditions (P'vankov and Kondrachuk, 2003) making these mesophylls structure a feature in characterizing a plants ecological condition (Ivanova and Pyankov, 2000). But in relation to its photosynthetic pathway there were no known direct correlation between the two. Though, referencing from other study can somehow classify some Dischidia as a Crassulacean Acid Metabolism (CAM) plant. This water-conserving pathway is usually observed in plants prone to dryness or drought. Primarily in epiphytes, which is largely represented throughout the tropical angiosperms. This highly links to the epiphytic traits to conserve water (Tsen and Holtum, 2012). Furthermore, there is a known obligate CAM plant from this genus, the D. formosa and its sister genus Hoya, H. carnosa (Martin et al., 2009).

5. CONCLUSION

Initial leaf morphology and anatomy of selected Philippine Dischidia were examined. External leaf characteristics agrees well with the other literatures but without any unique leaf features with identificatory value were observed. As for the anatomical parts, the present study identifies the presence of two types of mesophyll namely, isopalisade and homogeneous mesophyll tissues but this feature were susceptible to environmental and ecological adaptation making these parts a grim diagnostic feature for systematics of Dischidia. To extend the diagnostic of the comprehensive dimension taxon morphological observation must be made knowing that this genus remains understudied particularly in the Philippines.

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7. REFERENCES

- Elmer, A. D. E. (1938). *Leaflets of Philippine botany*. Oriental Printing Company, 10: 3551-3571.
- Ivanova, L.A., Pyankov, V.(2000). Physiological basis of leaf mesophyll types in C3-palnts. Congress: Plant Physiology & Biochemistry, Volume 38.
- Forster, P.I., Liddle, D.J. & Nicholas, A. (1996). Asclepiadaceae. In: R. Robertson et al. (eds). *Flora of Australia* 28: 197-283.
- Freire, S. E., Arambarri, A. M., Bayón, N. D., Sancho, Gisela, Urtubey, Estrella, Monti, Claudia A., ... & Colares, M. N. (2005).
 Epidermal characteristics of toxic plants for cattle from the Salado River basin (Buenos Aires, Argentina). Boletín de la Sociedad Argentina de Botánica, 40(3-4), 241-281.
- Jagtap, A. P., & Singh, N. P. (1999). Fascicles of Flora of India: fascicle 24. Asclepiadaceae and Periplocaceae. Calcutta: Botanical Survey of



India 332p.-illus., col. illus.. En Icones, Chromosome numbers, Anatomy and morphology, Keys. Geog, 6.

- Livshultz, T., Bounphanmy, S., & Schott, D. (2005). Dischidia (Apocynaceae, Asclepiadoideae) in Laos and Vietnam. Blumea-Biodiversity, Evolution and Biogeography of Plants, 50(1), 113-134.
- Livshultz, T. (2003). Systematics of Dischidia (Apocynaceae, Asclepiadoideae). Cornell University, Aug.
- Lu, F.Y. & Kao, M.T. (1980). Asclepiadaceae. In: H.L. Li et al. (eds.). *Flora of Taiwan* 4: 222-246.
- Martin, C. E., Hsu, R. C., & Lin, T. C. (2009). The relationship between CAM and leaf succulence in two epiphytic vines, Hoya carnosa and Dischidia formosana (Asclepiadaceae), in a subtropical rainforest in northeastern Taiwan. *Photosynthetica*, 47(3), 445-450.
- Merrill, E. D. (1923). An enumeration of Philippine flowering plants. *An enumeration* of *Philippine flowering plants.*
- Merrill, E. D.1904. New or Noteworthy Philippine Plants. Bureau of public printing.17:1, 39-40.
- Omino, E. (1996). A contribution to the leaf anatomy and taxonomy of Apocynaceae in Africa. *Belmontia*, 29, 1-178.
- Pearson, H.H.W.1902.On some species of Dischidia with Double Pitchers. *Journal of the Linnean Soiety Botany* 35, 377-389.
- Pyakurel, A. and Wang, J.R. (2014) Leaf Morphological and Stomatal Variations in Paper Birch Populations Along Environmental Gradients in Canada. *American Journal of Plant Sciences*, 5, 1508-1520.
- P'yankov, V. I., & Kondrachuk, A. V. (2003). Basic types of structural changes in the leaf mesophyll during adaptation of Eastern Pamir plants to mountain conditions. *Russian journal of plant physiology*, 50(1), 28-35.

- Radoglou, K. M., & Jarvis, P. G. (1990). Effects of CO2 enrichment on four poplar clones. II. Leaf surface properties. *Annals of Botany*, 65(6), 627-632.
- Rintz, R. E. (1980). The peninsular Malayan species of Dischidia. *Blumea*, 26(1), 81-126.
- Schlechter, R. (1915). Repertorium specierum novarum regni vegetabilis. *Berlin :Selbstverlag des Herausgebers*, 13: 554-561.
- Tsen, E. W., & Holtum, J. A. (2012). Crassulacean acid metabolism (CAM) in an epiphytic ant-plant, Myrmecodia beccarii Hook. f.(Rubiaceae). *Photosynthesis research*, 113(1-3), 311-320.
- Xu, Z., & Zhou, G. (2008). Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. *Journal of experimental botany*, 59(12), 3317-3325.

8. Appendix

Table 5. List of Specimen	Examined at the Philippine
National Herbarium	

Dischidia species	PNH Voucher Number	Location of Collection
D. hirsuta Decne	PNH55690	Mt. Pulgar, Palawan
	PNH3534	Mt. Halcon, Mindoro
	PNH119979	Baybay, Leyte
D. luzonica (Schltr.) Livshultz	PNH1685	Mt. Bulusan, Sorsogon
	PNH18542	Banao, Guinobatan, Albay
D.maior (Vahl.) Merr.	PNH17	Mt. Bacungan, PP, Palawan
	PNH35920	Mangrove swamp, Yagaw, Mindoro
	PNH42131	Tungao, Butuan, Agusan
D. merrillii Schltr.	PNH4093	Mt. Kuvapo, Bataan
D. nummularia R. Br.	PNH40768	Near Ondol. Bohol
	PNH10481	Mt. Apo, Davao
	PNH42385	Tungao, Butuan, Agusan
D. platyphylla Schltr.	PNH161732	Mangrove <u>wamp, Jawili, Tangalan,</u> Aklan
	PNH55291	Patag.Sorsogon
	PNH161288	Magdiwang, Sibuyan
D. giantha Schltr.	PNH8980	Polillo Isld
	PNH34112	Manila
	PNH8320	Zamboanga Mindanao
D. purpurea Merr	PNH165854	Palawan
	PNH34463	Mt. Malinao Albay
	PNH36491	Mona's Garden Pasay