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SPECIES DELINEATION OF THE GENUS *DIPLAZIUM* SWARTZ (ATHYRIACEAE) USING LEAF ARCHITECTURE CHARACTERS

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Keywords: Leaf architecture; Taxonomic marker; Cladodromous; Reticulodromous; Craspedodromous; Cophenetic correlation.

Abstract

The present study was conducted to delineate *Diplazium* Swartz species based on leaf architecture. Using PAleontological STatistics (PAST), a cluster and Principal Component Analysis of leaf architecture characters of 27 selected *Diplazium* species at the Philippine National Herbarium (PNH) was done. The dendogram (cophenetic correlation = 0.8436) and principal component analysis supported the four clusters of *Diplazium* using leaf architecture characters. At Gower distance of 0.25, *Diplazium* species were categorized as: Cluster 1 (Cladodromous – short stalked, stout and massive 1° vein); Cluster 2 (Reticulodromous – long stalked, moderate 1° vein); Cluster 3 (Craspedodromous – long stalked, stout to massive 1° vein); and Cluster 4 (Craspedodromous – short stalked, stout to massive 1° vein). The unifying characters were apex shape, base symmetry and 1° vein category, while the significant differentiating characters were 2^{o} vein angle of divergence and variation in the 2° vein angle of divergence, 3^{o} vein category, 3° vein angle of divergence, variation in 3° vein angle of divergence, 3° vein spacing and lobation. The successful delineation of *Diplazium* species proved that leaf architecture can be a good taxonomic marker and could be an alternative way of identifying species in the absence of sori.

Introduction

Diplazium Swartz consists of about 400 species distributed mainly in the tropics and sparingly in temperate forest (Kramer *et al.,* 1990). Copeland (1947) enumerated 62 *Diplazium* species in the Philippines. Meanwhile, 49 species of *Diplazium* were listed in Co Digital Flora of the Philippines (http://www. philippineplants.org/Families/Pteridophytes.html). Among genera under Athyriaceae, *Diplazium* species were always included in ethnobotanical studies (Rai *et al.*, 2005; Kumari *et al.*, 2011; Sujarwo *et al.*, 2014) as sources of food, medicine and decorative materials (Vasudeva, 1999). In Asian and Filipino dishes, *Diplazium esculentum* is served as salad, dietary staple, base for spicy condiments and vegetable (Kayang, 2007). As medicine, *Diplazium* species were noted for their antibacterial (Amit *et al.*, 2011), phytochemicals (Sivaraman *et al.*, 2011), antimicrobial and cytotoxic (Akler *et al.*, 2014), analgesic (Chawla *et al.*, 2015), and antioxidant properties (Pradhan *et al.*, 2015).

Despite of the well-studied uses of genus *Diplazium* their taxonomic classification and identification is still controversial among taxonomists and pteridologists. Some of the problems in accurate identification of the genus included insufficient data (Kramer *et al*., 1990) and continuous changes in taxonomic classification and morphological variations through apparently intermediate

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forms, which are commonly regarded as putative hybrids (Takamiya *et al*., 1999). The chance of misidentification is higher especially during field surveys and actual identification because *Diplazium* species are morphologically similar to their sisters *Athrium* and *Deparia* (Kato, 1977) and to some members of Woodsiaceae and Polypodiaceae to which *Diplazium* was formerly circumscribed (Smith *et al.*, 2006). Unconscious identification of *Diplazium* might lead to collection of wrong specimens, thus cannot satisfied the intent use and worst can be hazardous to human health or even cause death. The lack of knowledge or information when collecting for medicinal purposes, toxin-containing plants can result in misidentification with grave consequences (Voncina *et al.*, 2014).

Thus, several classification system and scholarly works were done to differentiate, delineate and investigate the phylogenetic relationship of *Diplazium* species. These include DNA sequencing (Wei *et al*., 2013), spore morphology (Praptosuwiryo *et al.*, 2007), stelar anatomy (Praptosuwiryo and Darnaedi, 2014), and cytology and reproduction (Takamiya *et al*., 1999). Takhtajan (1996) pointed out that molecular methods are not necessarily a universal remedy in elucidating the evolution of a certain taxon because molecular characters are also subjected to evolutionary convergence, parallelism and reversal besides random changes in DNA sequence. Further, molecular studies are expensive and not feasible in low cost-funded projects and inefficient in field surveys where actual identification is necessary.

One taxonomic tool useful in differentiating angiosperm taxa and also considered in ferns is leaf architecture, which is defined as the placement and form of elements constituting the outward expression of leaf structure, including venation pattern, marginal configuration, leaf shape, and gland position (Hickey, 1973). Pacheco and Moran (1999) resurrected *Callipteris* in their revision of the Neotropical species, and found diagnostic characters such as anastomosing veins and petiole/rachis scales with bifid-toothed margins. Recent studies on fern leaf architecture were done in the genus *Ophioglossum* (Magrini and Scoppola, 2010) and *Lygodium* (Shinta *et al*., 2012). Leaf architecture of fern species such as *Blechnum binervatum, Ctenitis falciculata, Magalastrum connexum, Microgramma squamulosa* and *Serpocaulon catharinae* were studied by Larcher *et al.*, (2013).

Though, leaf plasticity had been an issue on the use of leaf architecture as important taxonomic marker it proved its usefulness in differentiating angiosperm. As vascular plants with distinct venation pattern, ferns are expected to have similar stability in terms of venation pattern. In fact, fern stipes are reinforced by a very stiff sclerenchyma consisting of dead cells with non-extensible rigid cell walls (Leroux, 2012) providing support and preserving the leaf architecture (Larcher *et al*., 2013). In addition, ferns have persisted through their evolutionary history and represent highly successful forms in both past and present (Pittermann, 2010). Therefore, this study aims to delineate some *Diplazium* species of the Philippines using leaf architecture characters.

Materials and Methods

The leaf architecture characters of 27 *Diplazium* species at the Philippine National Herbarium (PNH) were summarized in Table 1 (leaf morphology) and Table 2 (venation pattern). The morphological leaf characters and venation pattern (Conda and Buot, 2017) were used to determine the species delineation of the genus *Diplazium* through Cluster and Principal Component Analysis of Paleontological Statistics (PAST). The distance measure and clustering method used were Gower and Unweighted Pair-Group Method of Arithmetic Mean (UPGMA), respectively.

For data analysis, 21 characters were selected for each species and each character was assigned to a corresponding legend as follows: LO1-6 for leaf organization, BlCl1-7 for blade class, Sh1-3 for shape, ApSh1-2 for apex shape, BaSh1-4 for base shape, BaAn1-3 for base angle, BaSy1-2 for base symmetry, Mar1-3 for margin, St1-2 for stalk, Lob1-4 for lobation, PVC1 for 1^o vein category, PVS1-4 for 1^o vein size, SVC1-3 for 2^o vein category, SAD1-6 for 2^o vein angle of divergence, SVAD1-4 for 2^o vein, variation in angle of divergence, SVS1-3 for 2^o vein spacing, TVC1-3 for 3^o vein category, TAD1-6 for 3^o vein angle of divergence, TVAD1-3 for 3^o vein, variation in angle of divergence, TVS1-3 for 3^o vein spacing, and AR1-2 for areole.

Results and Discussion

Leaf architecture characters of 27 *Diplazium* species are presented in Table 1. These characters varied especially in terms of L:W ratio, blade class, base angle and lobation. This interspecific variation illustrated that these characters could be good indicators of identification. The dendrogram (Fig. 1) with cophenetic correlation of 0.8436 and principal component analysis (Fig. 2) consistently separated *Diplazium* species into four clusters. At Gower distance of 0.25, *Diplazium* species were grouped into 4 clusters namely, Cluster 1 (Cladodromous - short stalked, stout and massive 1° vein); Cluster 2 (Reticulodromous - long stalked, moderate 1° vein); Cluster 3 (Craspedodromous - long stalked, stout and massive 1° vein) and Cluster 4 (Craspedodromous - short stalked, stout to massive 1° vein).

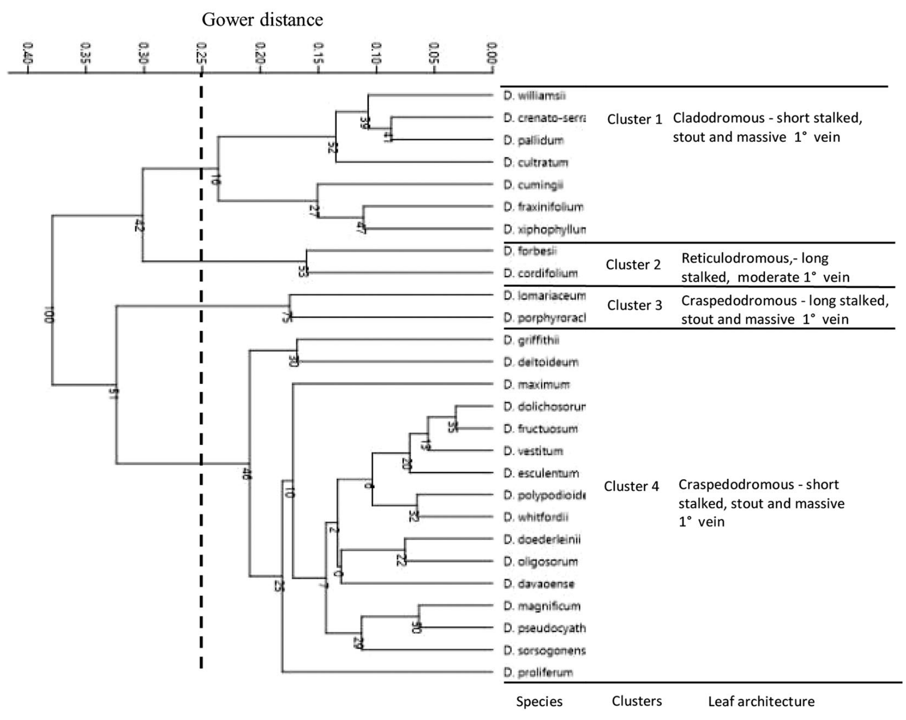

Fig. 1. Dendrogram of the 27 *Diplazium* species constructed by Unweighted Pair-Group of Arithmetic Mean (UPGMA) clustering and Bower using the Paleontological Statistics software. With cophenetic correlation of 0.8436 and gower distance of 0.25, four cluster were identified: Cluster 1 (Cladodromous - short-stalked, stout and massive 1° vein); Cluster 2 (Reticulodromous - long stalked, moderate 1° vein); Cluster 3 (Craspedodromous - long stalked, stout and massive 1° vein) and Cluster 4 (Craspedodromous - short stalked, stout to massive 1° vein).

Table 1. Leaf architecture characters of 27 selected *Diplazium* Swartz species (Athyriaceae): General leaf morphology.

Species	Leaf orga-nization	Shape	Apex shape	L : W ratio	Blade class	Base shape	Base angle	Base symmetry	Margin	Stalk	Lobation
Diplazium cordifolium Bl.	simple-pinnate	lanceolate	acute	2.7-3.4:1	mesophyll	cordate	WO	asymmetrical	entire	LS	unlobed
D. crenato-serratum T. Moore	pinnate	lanceolate	acute	3.4-4.6:1	microphyll	trruncate	WO	asymmetrical	serrate	SS	shallow
D. cultratum C. Presl.	pinnate	lanceolate	acute	1.4-5:1	microphyll	truncate	WO	asymmetrical	entire	SS	unlobed
D. cumingii C. Chr.	Pinnate	lanceolate	acute	3.6-9:1	Mesophyll	cuneate	A	asymmetrical	entire	SS	unlobed
D. davaoense Copel	tripinnate	lanceolate	acute	5.2-5.5:1	notophyll	truncate	O	asymmetrical	crenate	SS	shallow
D. deltoideum C. Presl	bipinnatifid	oblong	acute	3.3-5:1	notophyll	truncate	O	asymmetrical	serrate	SS	deep
D. dolichosorum Copel	tripinnate	lanceolate	acute	3.2-3.9:1	microphyll	truncate	O	asymmetrical	serrate	SS	shallow
D. doederleinii (Luerss.) Makino	tripinnatifid	lanceolate	acute	3.7-3.9:1	microphyll	truncate	O	asymmetrical	entire	SS	moderate
D. esculentum (Retz). Sw.	bipinnate-tripinnate	lanceolate	acute	4.1-5:1	microphyll	truncate	O	asymmetrical	serrate	SS	shallow
D. forbesii C. Chr.	pinnate	lanceolate	acute	3.4-7:1	mesophyll	rounded	O	asymmetrical	entire	LS	unlobed
D. fraxinifolium C. Presl.	pinnate	elliptic	acute	4.6-6.1:1	mesophyll	cuneate	A	asymmetrical	entire	SS	unlobed
D. fructuosum Copel	tripinnate	lanceolate	acute	4.7-7.8:1	microphyll	truncate	O	asymmetrical	serrate	SS	moderate
D. griffithii T. Moore	tripinnatifid	oblong	acute	3.1-4.1:1	microphyll	truncate	WO	asymmetrical	serrate	SS	deep
D. lomariaceum (C. Chr.) M.G. Price	pinnatifid	elliptic	acute	10.5-12.4:1	mesophyll	cuneate	A	asymmetrical	entire	LS	deep
D. magnificum (Copel) M.G. Price	tripinnatifid	lanceolate	acute	4-4.9:1	microphyll	truncate	O	asymmetrical	serrate	SS	deep
D. maximum (D. Don) C. Chr.	tripinnate	lanceolate	acute	3.9-5:1	mesophyll	truncate	O	asymmetrical	crenate	SS	shallow
D. oligosorum Copel	tripinnatifid	lanceolate	acute	3.9-4.7:1	microphyll	truncate	WO	asymmetrical	entire	SS	moderate
D. pallidum T. Moore	pinnate	lanceolate	acute	6.3-10.9:1	microphyll	rounded	O	asymmetrical	serrate	SS	shallow
D. polypodioides Blume	tripinnatifid	lanceolate	acute	3.9-5.1:1	microphyll	truncate	O	asymmetrical	serrate	SS	deep
D. porphyrorachis Diers.	pinnatifid	elliptic	acute	5.3-6.9:1	mesophyll	cuneate	O	asymmetrical	entire	LS	deep
D. proliferum (Lam.) Thou.	pinnate	lanceolate	acute	2.8-4.4:1	notophyll	truncate	O	asymmetrical	serrate	SS	shallow
D. pseudocyatheifoleum Rosent	tripinnatifid	lanceolate	acute	3.6-4.9:1	microphyll	rounded	O	asymmetrical	entire	SS	deep
D. sorsogonense C. Presl	bipinnatifid	lanceolate	acute	4.6-7:1	microphyll	truncate	O	asymmetrical	serrate	SS	deep
D. vestitum C. Presl.	tripinnate	lanceolate	acute	3.3-6:1	microphyll	truncate	O	asymmetrical	serrate	SS	shallow
D. whitfordii Copel	bipinnatifid	lanceolate	acute	2.7-3:1	nanophyll	truncate	O	asymmetrical	serrate	SS	deep
D. williamsii Copel	pinnate	lanceolate	acute	2.8-3.3:1	nanophyll	truncate	O	asymmetrical	serrate	SS	shallow
D. xiphophyllum C. Chr.	pinnate	lanceolate	acute	4.8-6.3:1	mesophyll	rounded	O	asymmetrical	entire	SS	unlobed

WO = Wide obtuse, A = Acute, O = Obtuse; LS = Long stalked, SS = Short stalked.

Table 2. Leaf architecture characters of 27 selected *Diplazium* Swartz species (Athyriaceae): Venation Characters.

Species	Primary Vein		Secondary Vein				Tertiary Vein			
	Category	Size	Category	AD	VAD	Spacing	Category	AD	VAD	Spacing
D. cordifolium	Pinnate	moderate	Reticulodromous	moderate	regular	irregular	none	none	none	none
D. crenato-serratum	pinnate	massive	Cladodromous	narrow	upper vein more acute than lower	uniform	none	none	none	none
D. cultratum	pinnate	massive	Cladodromous	wide	upper vein more acute than lower	increasing toward the base	none	none	none	none
D. cumingii	pinnate	stout	Cladodromous	moderate	nearly uniform	uniform	none	none	none	none
D. davaoense	pinnate	massive	Craspedodromous	moderate	upper vein more acute than lower	increasing toward the base	Free end in sinuses	narrow	upper vein more acute than lower	increasing toward the base
D. deltoideum	pinnate	stout	Craspedodromous	right	nearly uniform	irregular	free and forked touching margin	wide	upper 3° vein more acute than lower	upper 3° vein more acute than lower
D. dolichosorum	pinnate	massive	Craspedodromous	moderate	upper 2° vein more acute than lower	increasing toward the base	free end in sinuses	narrow	upper 3° vein more acute than lower	increasing toward the base
D. doederleinii	pinnate	massive	Craspedodromous	wide	upper 2° vein more acute than lower	increasing toward the base	free end in sinuses	narrow	upper 3° vein more acute than lower	increasing toward the base
D. esculentum	pinnate	massive	Craspedodromous	wide	varies irregularly	increasing toward the base	forming commiss ural vein	narrow	upper 3° vein more acute than lower	increasing toward the base
D. forbesii	pinnate	moderate	Reticulodromous	moderate	varies irregularly	irregular	none	none	none	none
D. fraxinifolium	pinnate	stout	Cladodromous	moderate	upper 2° vein more obtuse than lower	uniform	none	none	none	none
D. fructuosum	pinnate	massive	Craspedodromous	wide	upper 2° vein more acute than lower	increasing toward the base	free end in sinuses	narrow	upper 3° vein more acute than lower	increasing toward the base
D. griffithii	pinnate	massive	Craspedodromous	wide	upper 2° vein more obtuse than lower	increasing toward the base	free and forked touching margin	narrow	upper 3° vein more acute than lower	increasing toward the base
D. lomariaceum	pinnate	massive	Craspedodromous	right	nearly uniform	irregular	free and forked touching margin	moderate	varies irregularly	irregular

Table 2 Contd.

Species	Primary Vein		Secondary Vein				Tertiary Vein			
	Category	Size	Category	AD	VAD	Spacing	Category	AD	VAD	Spacing
D. magnificum	pinnate	massive	Craspedodromous	right	upper 2° vein more acute than lower	increasing toward the base	free end in sinuses	moderate	uniform	increasing toward the base
D. maximum	pinnate	stout	Craspedodromous	wide	upper 2° vein more acute than lower	irregular	free end in sinuses	moderate	uniform	increasing toward the base
D. oligosorum	pinnate	stout	Craspedodromous	moderate	upper 2° vein more acute than lower	increasing toward the base	free end in sinuses	moderate	upper 3° vein more acute than lower	increasing toward the base
D. pallidum	pinnate	massive	Cladodromous	wide	nearly uniform	uniform	none	none	none	none
D. polypodioides	pinnate	stout	Craspedodromous	wide	upper 2° vein more acute than lower	uniform	free end in sinuses	narrow	upper 3° vein more acute than lower	increasing toward the base
D.porphyrorachis	pinnate	stout	Craspedodromous	wide	upper 2° vein more obtuse than lower	uniform	free and forked touching margin	moderate	upper 3° vein more acute than lower	uniform
D. proliferum	pinnate	stout	Craspedodromous	moderate	upper 2° vein more obtuse than lower	increasing toward the base	forming commissural vein	narrow	upper 3° vein more acute than lower	uniform
D. pseudo-cyatheifoleum	pinnate	massive	Craspedodromous	right	upper 2° vein more acute than lower	increasing toward the base	free end in sinuses	moderate	uniform	increasing toward the base
D. sorsogonense	pinnate	massive	Craspedodromous	right	upper 2° vein more acute than lower	increasing toward the base	free end in sinuses	moderate	uniform	increasing toward the base
D. vestitum	pinnate	massive	Craspedodromous	wide	nearly uniform	uniform	free end in sinuses	narrow	upper 3° vein more acute than lower	increasing toward the base
D. whitfordii	pinnate	massive	Craspedodromous	moderate	upper 2° vein more acute than lower	uniform	free end in sinuses	narrow	upper 3° vein more acute than lower	increasing toward the base
D. williamsii	pinnate	massive	Cladodromous	moderate	upper 2° vein more acute than lower	irregular	none	none	none	none
D. xiphophyllum	pinnate	stout	Cladodromous	moderate	nearly uniform	uniform	none	none	none	none

AD = Angle of Divergence, VAD = Variation in Angle of Divergence

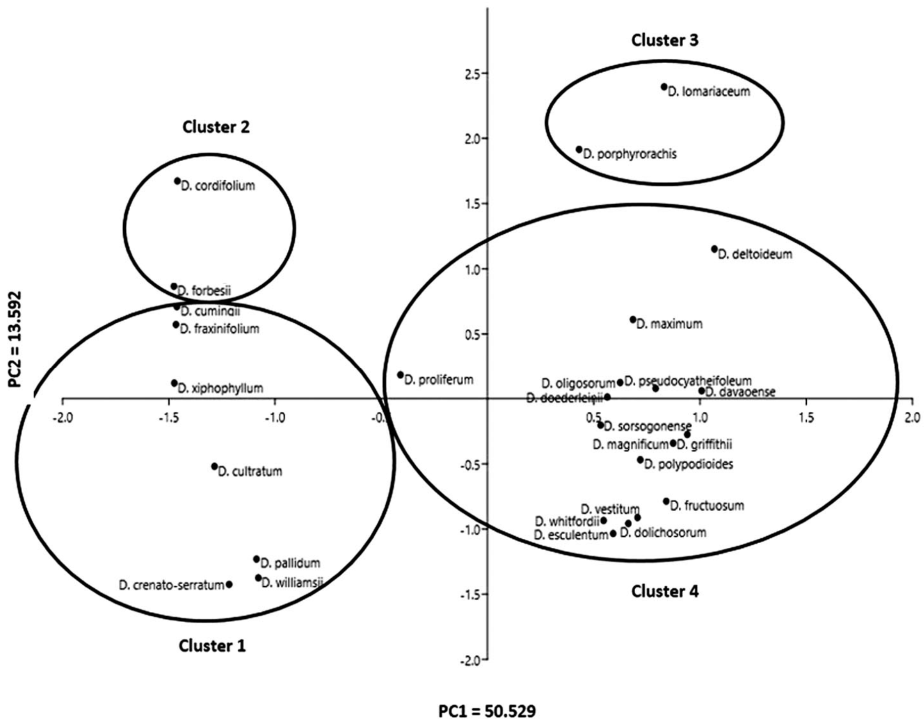

Fig. 2. Principal Component Analysis of 27 *Diplazium* species using PAleotological STatistics (PAST) software. Four clusters were classified: Cluster 1 (Cladodromous – short stalked, stout and massive 1° vein); Cluster 2 (Reticulodromous - long stalked, moderate 1° vein); Cluster 3 (Craspedodromous - long stalked, stout and massive 1° vein) and Cluster 4 (Craspedodromous - short stalked, stout to massive 1° vein).

Cluster 1, the Cladodromous - short stalked, stout to massive 1° vein: It includes *D. williamsii* Copel, *D. crenato-serratum* T. Moore, *D. pallidum* T. Moore, *D. cultratum* C. Presl., *D. cumingii* C. Chr. and *D. xiphophyllum* C. Chr. Sample line drawings of species under Cluster 1 (Figs. 3a-3c) were lifted from Conda and Buot (2017). Based on the illustrations, common leaf architecture characters were: pinnate leaf arrangement, lanceolate shape, acute apex, symmetrical base, entire and serrate margin, unlobed to shallow lobation, short stalked, pinnate 1° vein, stout to massive 1° vein size, cladodromous 2° vein category and absence of areole. In this cluster there is one outlier, *D. fraxinifolium*, which is reticulodromous.

Cluster 2, the Reticulodromous, long stalked - moderate 1° vein: Cluster 2 includes *D. cordifolium* Bl. (Fig. 3d) and *D. forbesii* C. Chr. (Fig. 3e). These species showed pinnate leaf arrangement, lanceolate leaf shape, acute apex, asymmetrical base, entire margin, long-stalked, unlobed blade, mesophyllous blade class, pinnate 1° vein, reticulodromous 2° vein, moderate 2° vein angle of divergence and presence of areole. The two species differed in variation in 2° vein angle of divergence. The former exhibits nearly uniform 2° vein angle of divergence while irregular in the latter. This cluster was found consistent with the classification of *Diplazium* species using stelar anatomy of stipe (Praptosuwiryo and Darnaedi, 2014) and spore morphology specifically perine ornamentation (Praptosuwiryo *et al.*, 2007).

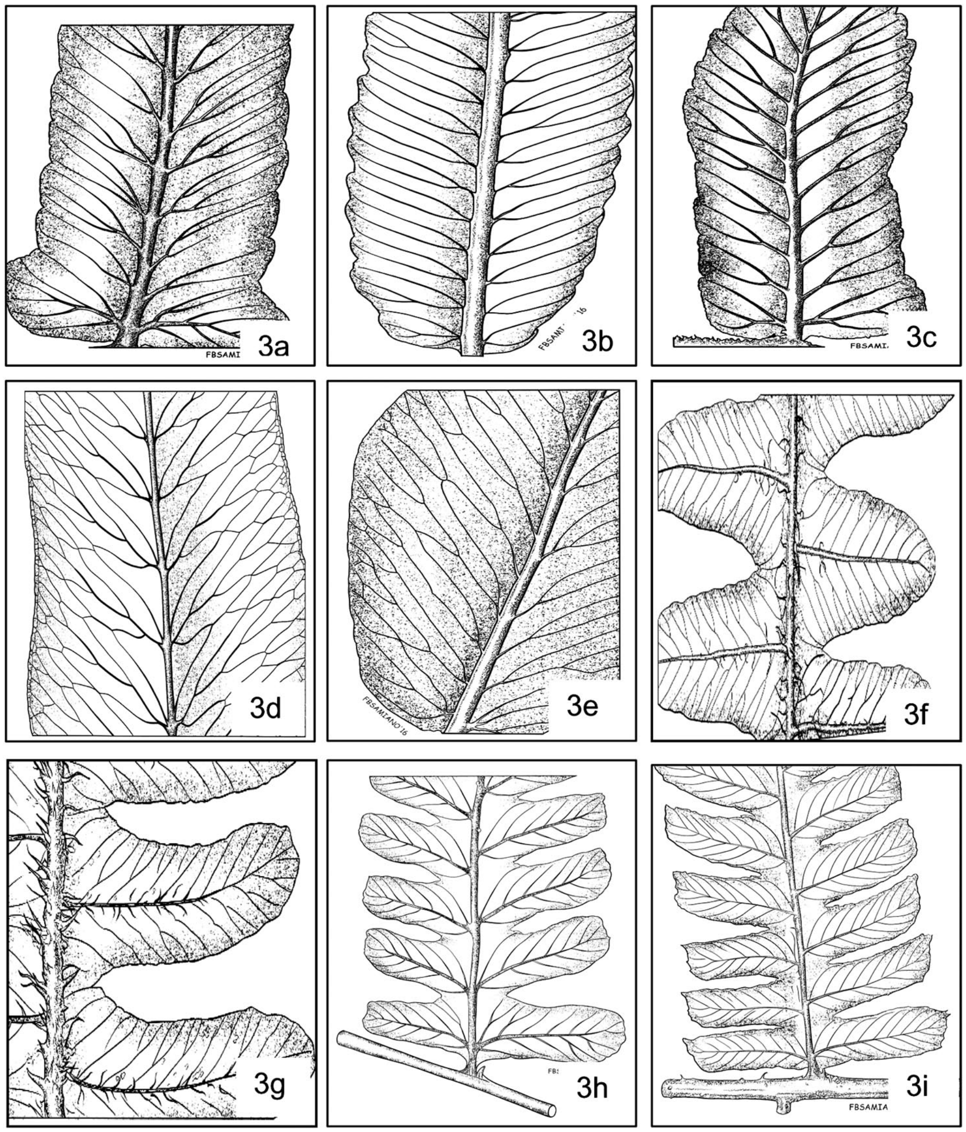

Fig. 3. Line drawings of *Diplazium* species with different venation pattern. Cladodromous - short stalked, stout to massive 1° vein venation pattern: *D. crenato-serratum* T. Moore (3a), *D. pallidum* T. Moore (3b) and *D. cutratum* C. Presl (3c). Reticulodromous - short stalked, moderate 1° vein: *D. cordifolium* Blume (3d) and *D. forbesii* C. Chr. (3e). Craspedodromous - long stalked, stout and massive 1° vein: *D. lomariaceum* (C. Chr.) M.G. Price (3f) and *D. porphyrorachis* Diers. (3g). Craspedodromous - short stalked, stout to massive 1° vein: *D.oligosorum* Copel (3h) and *D. polypodioides* Blume (3i).

Cluster 3, the Craspedodromous - long stalked, stout and massive 1° vein: This cluster consists of *D. lomariaceum* (C. Chr.) M.G. Price (Fig. 3f) and *D. porphyrorachis* Diers (Fig. 3g). They exhibit pinnatifid lamina, elliptic shape, acute apex, cuneate and asymmetrical base, entire margin, long stalked, deeply lobed, mesophyllous blade class, pinnate 1° vein, craspedodromous 2° vein, right 2° vein angle of divergence, free and forked touching margin 3° vein, moderate 3°

vein angle of divergence and absence of areoles. *D. porphyrorachis* differs by having stout 1° vein size, uniform 2° and 3° vein spacing and upper 3° vein more acute than lower variation in 3° vein angle of divergence. While *D. lomariaceum* showed a massive 1° vein size, irregular 2° and 3° vein spacing and irregular variation in 3° vein angle of divergence. This group was strongly supported using spore morphology (Praptosuwiryo *et al.*, 2007).

Cluster 4, the Craspedodromous - *short stalked, stout to massive 1° vein:* It includes majority of *Diplazium* species (16 individuals) namely, *D. griffithii* T. Moore, *D. deltoideum* (C. Presl.), *D. maximum* (D. Don) C. Chr., *D. proliferum* (Lam.) Thours., *D. esculentum* (Retz.) Sw., *D. oligosorum* (Copel), *D. sorsogonense* (C. Presl.) C. Presl., *D. magnifium* (Copel) M.G. Price, *D. pseudocyatheifolium* Rosent., *D. doederleinii* (Luerss.) Makino, *D. whitfordii* Copel, *D. polypodioides* (Blume), *D. vestitum* C. Presl., *D. fructuosum* (Copel), *D. dolichosorum* (Copel) and *D. davaoense* (Copel). Sample drawings (Figs. 3h & 3i) from Conda and Buot (2017) were incorported to emphasize the common leaf architecture characters namely, lanceolate to rarely oblong leaf shape, acute apex, truncate base, obtuse to wide obtuse base angle, asymmetrical base, pinnate 1° vein, stout to massive 1° vein size, upper 2° vein more acute than lower variation in angle of divergence and absence of areoles. At Gower distance of 0.19, *D. griffithii* and *D. deltoideum,* having oblong pinnule, was separated from the lanceolate group. Among the lanceolate group, only *D. esculentum* and *D. proliferum* possessed 3° vein forming commissural vein, while the rest have free end in sinuses 3° vein. This cluster coincides mostly with the work of Wei *et al.* (2013) using DNA sequencing of *Diplazium* from different geographical areas. Most species in this study fell under clade IV, subclade E (*Diplazium* species with short branches connecting deeper nodes and long branches leading to tip – occuring in Southeast Asia and adjoining regions) of Wei *et al.,* (2013) phylogram. The analysis of the leaf architecture characters of *D. davaoense*, *D. esculentum* and *D. doederlenii* (cluster 4) revealed similarities with subclade H (Wei *et al.,* 2013) possibly because these species are Asiatic in nature.

Leaf architecture, particularly the venation pattern, is a good taxonomic tool in delineating *Diplazium* species. Consistency in groupings with spore morphology, stelar anatomy and DNA sequencing proved leaf architecture's usefulness in the classification system for *Diplazium* species. The dendrogram (cophenetic coefficient = 0.8436) and principal component analyses highly supported the four clusters of *Diplazium* using leaf architecture characters, *viz.* Cluster 1 (Cladodromous - short stalked, stout and massive 1° vein); Cluster 2 (Reticulodromous - long stalked, moderate 1° vein); Cluster 3 (Craspedodromous - long stalked, stout and massive 1° vein) and Cluster 4 (Craspedodromous - short stalked, stout to massive 1° vein). The unifying characters in the genus are apex shape, base symmetry and 1° vein category, whereas 2^{o} vein angle of divergence and variation in 2° vein angle of divergence, 3^{o} vein category, 3° vein angle of divergence, variation in 3° vein angle of divergence, 3° vein spacing and lobation are the differentiating features. This study has proved that identification of sterile specimen is now feasible with leaf architecture.

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