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Original Article

Cluster and Ordination Analyses of Leaf Architectural Characters in Classifying Polypodiales *sensu* PPG

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ABSTRACT: Polypodiales is the largest order in pteridophytes. It has been the subject of scrutiny particularly in taxonomic classification. Recently, the Pteridophyte Phylogeny Group (PPG I) split the eupolypods clade into eupolypods I and II, while families outside eupolypods were classified as cathetogyrates. The taxonomic position of these clades is still unstable. Thus, an alternative tool that is easy and genetically determined such as leaf architectural characters, were used to investigate the details. This study examined 28 representative species from the three groups of polypodiales to determine the leaf architectural characters making up the 3 groups of polypodiales. Similarities and differences in general morphological characters and venation patterns were observed among the groups of polypodiales. The results correspond to the current classification of PPG I for eupolypods I and II except with the species under cathetogyrates. Moreover, the dendrogram and principal component analysis of the 28 species highly supported the three clusters generated. It proved that leaf architecture characters are good taxonomic tool in delineating polypodiales.

KEY WORDS: Polypodiales, Leaf architecture, Principal Coordinate Analysis, Cluster Analysis

INTRODUCTION

Fern identification and classification has been problematic since the time of Linnaeus. There is no clear and established family concept because of numerous shared morphological characteristics of recognized families. Numerous approaches were used in pursuit of creating a monophyletic classification system (Christenhusz and Chase, 2014). Molecular characters have been the focus in plant classification to elucidate the phylogeny of species; yet, Takhtajan (1996) pointed out that it was not the ultimate solution in illuminating the evolutionary history of a certain taxa. It was because, molecular characters are also imperiled to random changes in DNA sequences, convergent evolution and, parallelism. Regardless of the great number of genetic data collected, splitting and lumping are still a huge dilemma in taxonomy (Christenhusz and Chase 2014). Lumping has gained criticism as families were subsumed into larger families without obvious shared characters. It is then important to make sure that families have distinct characters to ease identification and classification (Christenhusz *et al.*, 2015).

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Leaf architecture paved its way in systematics as the Leaf Architecture Working Group (LAWG) of the Smithsonian Institution in 1999 was able to develop a manual on leaf architecture for angiosperms. The manual was further improved by Ellis *et al.* (2009) with several modifications and additions such as comparative description of both modern and fossil leaves with detailed guidelines in the characterization of general leaf morphology.

Systematists often neglect the use of vegetative characters such as leaf venations because of their perception that these are not stable and so are phenotypically plastic characters (Laraño and Buot, 2010). Despite the controversies that leaf architecture is facing, several studies have proved its significance in taxonomy. In fact, numerous species of angiosperms have been effectively identified, described and delineated, generating phylogenetic relationships such as species of Rosaceae (Merrill, 1978), and Hoya species (Villareal and Buot, 2015; Jumawan and Buot, 2016; Torrefiel and Buot, 2017). Furthermore, leaf architecture has not only proved successful in identifying and delineating angiosperms but also in some pteridophytes. This includes studies on *Diplazium* species (Conda *et al.*, 2017), Lygodium (Shinta *et al.*, 2012) and, *Ophioglossum* species (Magrini and Scoppola, 2010). Most of the studies revealed that leaf venation was genetically fixed and closely related to development and their evolution (Roth-Nebelsick *et al.*, 2001). Hence, it is a good tool for systematics, especially for plants without reproductive parts present (Dilcher, 1974).

In PPG I (2016), Polypodiales was separated into three groups namely eupolypods I (Polypodiineae), eupolypods II (Aspleniineae) and cathetogyrates through molecular data and petiole anatomy. Among the ferns, the eupolypods appeared confusing and overwhelming due to lumping of several families. These families lack common characters due to its vast morphological differences, species richness and insufficient historical data (Rothfels *et al.*, 2012).

This study aimed to determine the leaf architecture characters of each representative species of eupolypods I, eupolypods II, and cathetogyrates; and to evaluate the taxonomic relevance of leaf architecture traits of eupolypods I, eupolypods II, and cathetogyrates.

MATERIALS AND METHODS

Selection of Herbarium Specimens

A total of twenty-eight specimens were selected from the identified representative species of polypodiales *sensu* Pteridophyte Phylogeny Group (PPG); ten species from eupolypods I and eupolypods II; and eight species from cathetogyrates. The number of species selected were based on the number of species present in each group. Two to three herbarium specimens of each representative species were examined depending on the availability of well-conditioned specimens in the herbarium. The representative species of each group were selected based on the type genus or the nearest type genus of each family available. The specimens examined were loaned from the Plant Biology Division Herbarium (PBDH), Institute of Biological Sciences, College of Arts and Sciences, University of the Philippines Los Baños. Exsiccatae of species were noted.

Examination of Leaf Architectural Characters

With the use of a stereomicroscope, twenty mature pinna or pinnules of two to three herbarium specimens were considered for examination in this study. The specimens

were described using leaf architecture terminologies of Hickey (1973), Leaf Architecture Working Group (1999); and that of Ellis *et al.* (2009). A typical fern structure is illustrated to aid in the understanding of morphology and fern leaf architecture description (Fig. 1). Measurements were determined using a ruler, protractor and vernier caliper.

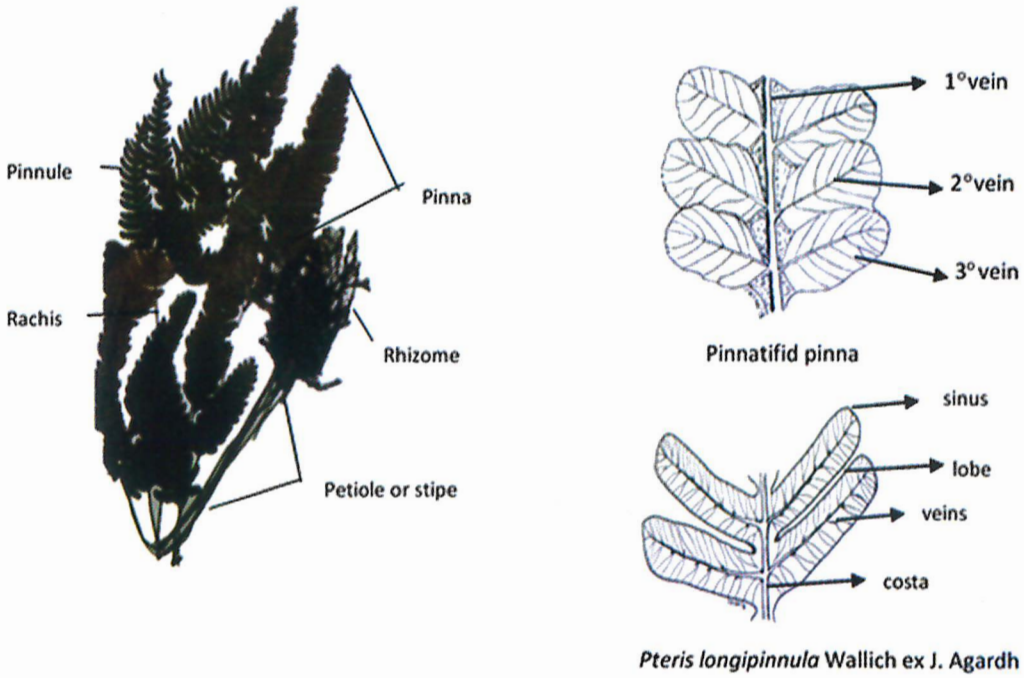


Figure 1. Illustration of a typical fern parts.

Analysis

The leaf characters obtained were used to determine the similarities of species between each group and to determine the differences of the three groups using Cluster and Principal Component Analysis of PAleontological Statistics (PAST) (Hammer *et al.*, 2001). Gower and Unweighted Pair-Group (UPGMA) were used to measure the distance and generate clusters, respectively.

RESULTS AND DISCUSSION

General Characteristics of Polypodiales

The general morphological characters and venation patterns of polypodiales were described based on the common leaf architecture characters of 28 species studied (Table 1). The former included leaf organization, shape, apex shape, length and width ratio, blade class, base shape, base angle, base symmetry, margin, and lobation in which this type of classification was parallel to Copeland (1958) and Price (1975). On the other hand, the latter included 1° vein, 2° vein, 3° vein, 4° vein and, areolation which was parallel with Conda (2017).

Leaf Architectural Characteristics of Eupolypods I, II and Cathetogyrates

The eupolypods I exhibited a varied

morphological character. Almost all species showed leptophyll to macrophyll blade class with mostly an acute base angle and linear to oblong leaf shapes. Most species have cladodromous with a few weak brochidodromous venation pattern with areoles (Table 2). Areolation were observed on *Aglaomorpha meyeniana* Schott., *Microsorum heterocarpum* Ching and, *Tectaria grandifolia* Copel. In contrast, all representative species under eupolypods II showed microphyll type of blade class with an acute to obtuse base angle and lanceolate to oblong leaf shape. This was supported by the study of Sato (1987) in which he showed that leaf shape indices were not only species-specific, but also determined during ontogenetic plant development. Furthermore, species in this group have tertiary vein category. Differences were observed in the 2° and 3° vein categories, angle of divergence variation in angle of divergence and spacing (Table 3-5). Species under cathetogyrates have leptophyll to notophyll blade class with acute to obtuse base angle and linear to lanceolate and rhomboid.

Table 1 General leaf architecture characters of selected Polypodiales species.

General Morphological Characters	
Leaf organization	Pinnate-deeply pinnatifid
Shape (pinna/pinnule)	Mostly lanceolate, few linear and elliptic, rare oblong
Apex shape (pinna/pinnule)	Mostly acute-acuminate, few round, rare obtuse
Base (pinna/pinnule)	
Shape	Usually truncate, few acute and attenuate, rare convex and rounded
Angle	Acute to obtuse
Symmetry	Mostly asymmetrical, few symmetrical
Blade class	Nanophyll to macrophyll
Margin type	Entire to serrate
Lobation	Mostly unlobed, few shallow to deep
Venation Pattern	
1° vein	
Category	Pinnate
Size	Weak to massive
2° vein	
Category	Mostly craspedodromous, few cladodromous and weak brochidodromous, rare reticulodromous and semicraspedodromous
Angle of divergence	Narrow to wide
Variation in angle of divergence	Mostly upper 2° vein more acute than lower, few nearly uniform, rare varies irregularly

Table 1 (Continued).

Spacing	Usually increasing towards the base, few irregular and uniform
3° vein	
Category	Free end in sinuses to forming commissural vein
Angle of divergence	Narrow to moderate
Variation in angle of divergence	Upper 3° vein more acute than lower
Spacing	Usually increasing towards the base, few irregular
4° vein	
Category	Mostly absent, few regular polygonal reticulate, rare alternate percurrent
Areoles	Generally absent, rare present

Dilcher (1974) pointed out that, "gross leaf form alone cannot produce reliable information and must be coupled with the finer venation to be useful in recognizing the taxa." With this, smaller veins were examined from the primary to quaternary veins and areoles. Interestingly, all the species showed pinnate primary vein but differentiated in the secondary, tertiary and quaternary venation. Fifteen species have craspedodromous 2° veins (Fig. 2) while eight species have cladodromous 2° veins (Fig. 3). Weak brochidodromous venation was observed in 3 species under eupolypods I (Fig. 4). Moreover, *Goniophlebium subauriculatum* C. Presl. exhibited semicraspedodromous 2° veins (Fig. 5) while *Histiopteris incisa* (Thunb.) J. Sm. exhibited reticulododromous veins (Fig. 6). This proved that high venation and areoles were key characters in delineating species (Salvaña and Buot, 2013). Moreover, this supports the findings of Fujita and Mochizuki (2006) that plants exhibit diverse and species-specific leaf venation pattern due to the leaf shape and expansion pattern with the association of auxin sink during leaf development. In fact, Nelson and. Dengler (1997), pointed out the association of genetics in leaf vascular pattern formation

Species Delineation using Leaf Architecture

The venation characters from the 28 selected species were used to determine the similarities and differences among species. These characters were loaded to PAST to generates the dendrogram (Fig. 7) and principal component analysis (PCA) (Fig. 8). The PCA with cophenetic coefficient value of 0.933 supported the dendrogram which grouped the polypodiales into three clusters namely Cluster 1: eupolypods II with some outliers from cathetogyrates (Craspedodromous, weak-massive 1° vein, areoles absent); Cluster 2: Eupolypods I with some outliers (Cladodromous, weak -stout 1° vein, areoles absent); Cluster 3: Eupolypods I (Weak brochidodromous, moderate and massive 1o vein, areoles present). The three clusters were separated at grower distance of 0.36. Interestingly, the dendrogram corresponds to the current classification of Pteridophyte Phylogeny Group (PPG I) except that some of the species under cathetogyrates were subsumed in eupolypods.

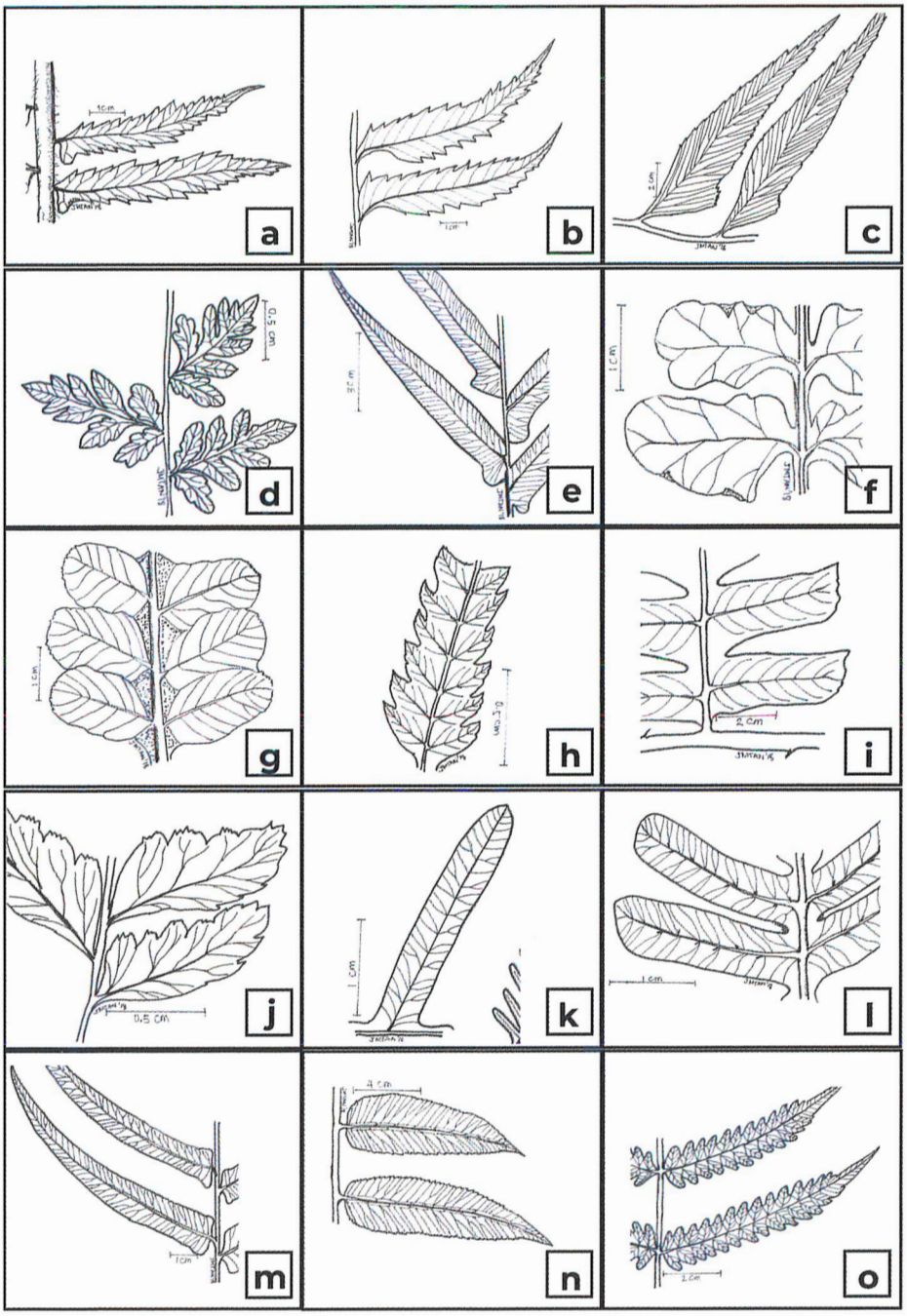


Figure 2. Species with craspedodromous venation, mostly belonging to Eupolypods II: (a) *Asplenium pellucidum* Lam.; (b) *Asplenium polyodon* G. Forster (c) *Asplenium vulcanicum* Bl.; (d) *Athyrium aristulatum* Copel.; (e) *Blechnum orientale* L.; (f) *Dennstaedtia philippinensis* Copel.; (g) *Diplazium esculentum* (Retz.) Sw.; (h) *Diplazium fructuosum* Copel; (i) *Diplazium polypodioides* Blume; (j) *Microlepia strigosa* (Thunb.) C. Presl; (k) *Pteris blumeana* J. Agardh; (l) *Pteris longipinnula* Wall.; (m) *Pteris vittata* L.; (n) *Stenochlaena areolaris* (Harr.) Copel (o) *Thelypteris normalis* Moxley.

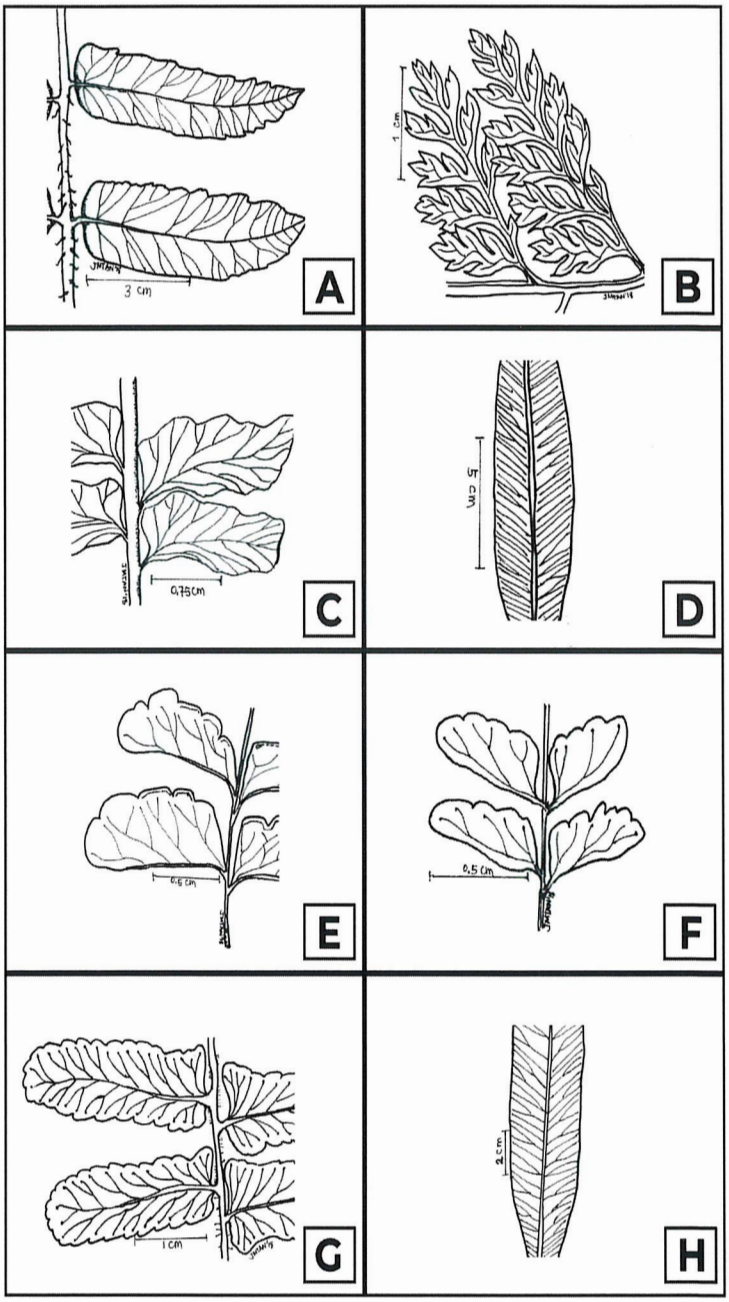


Figure 3. Species with cladodromous venation, mostly belonging to Eupolypods I: (A) *Bolbitis rhizophylla* (Kaulf.) Hennipman; (B) *Davallia hymenophylloides* (Blume) Kuhn; (C) *Didymochlaena truncatula* (Sw.) J. Sm.; (D) *Elaphoglossum blumeanum* J. Sm.; (F) *Lindsaea pulchella* (J. Sm.) Mett.ex Kuhn; (G) *Nephrolepis cordifolia* (L.); (H) *Oleandra maquilingensis* Copel.

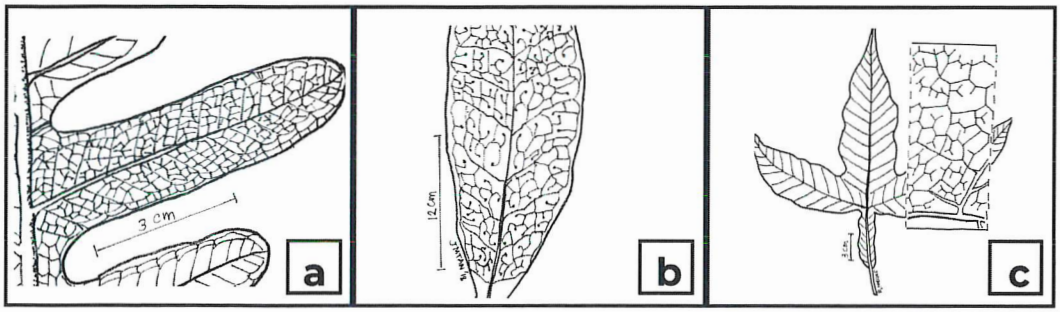


Figure 4. Species with weak brochidodromous venation belonging to Eupolypods I: (a) *Aglaomorpha meyeniana* Schott.: (b) *Microsorum heterocarpum* ching: (c) *Tectaria grandifolia* Copel.

Cluster1(EupolypodsII),Craspedodromous, weak-massive 1° vein, areoles absent, included all the representative species of eupolypods I namely *Asplenium pellucidum* Lam. (Hernaez & Cajano 154 PBDH), *Asplenium polyodon* G. Forster (Hernaez 1243 PBDH), *Asplenium vulcanicum* Bl. (Hernaez & Cajano 162 PBDH), *Athyrium aristulatum* Copel. (Hernaez & Cajano 164 PBDH), *Blechnum orientale* L. (Hernaez 1131 PBDH), *Diplazium esculentum* (Retz.) Sw. (delos Angeles 6193 PBDH), *Diplazium fructuosum* Copel (Hernaez & Cajano 168 PBDH), *Diplazium polypodioides* Blume (Banaticla 3284 PBDH), and *Thelypteris normalis* Moxley (Barrion 3480 PBDH) and *Stenochlaena areolaris* (Harr.) Copel. (Letana 1788 PBDH).

Five outliers were from the cathetogyrates clade namely *Dennstaedtia philippinensis* Copel. (Hernaez & Cajano 192 PBDH) from *Dennstaedtiaceae*, *Microlepia strigosa* (Thunb.) C. Presl. (Hernaez & Cajano 197 PBDH) from *Dennstaedtiaceae*, *Pteris blumeana* J. Agardh (Hernaez & Cajano 294 PBDH), *Pteris longipinnula* Wall. (Hernaez 1064 PBDH), *Pteris vittata* L. (Kumaunang 2410 PBDH) from Pteridaceae.

Common characters observed were mostly pinnate to rarely bipinnate; linear-lanceolate to rarely oblong leaf shape; acute-acuminate to rarely obtuse apex shape; mostly microphyll blade class to rarely notophyll; craspedodromous and weak-massive 1° vein; mostly upper 2° vein more acute than lower; and absence of areoles. Among the unlobed species, only Asplenium polyodon G. Forster have shallow lobation. Common characters observed were mostly pinnate to rarely bipinnate; linear-lanceolate to rarely oblong leaf shape; acute-acuminate to rarely obtuse apex shape; mostly microphyll blade class to rarely notophyll; craspedodromous and weak-massive 1° vein; mostly upper 2° vein more acute than lower; and absence of areoles. Among the unlobed species, only *Asplenium polyodon* G. Forster have shallow lobation.

Many genera of Pteridaceae were not monophyletic and based on Smith *et al.*, (2008) classification, it was included in Dennstaedtiaceae. In fact, this family appeared to be near the eupolypods II clade in PPG I (2016) and Smith *et al.*, (2006) which explains it being included. Furthermore, based in the rRNA sequencing, the genus *Dennstaedtia* under Dennstaedtiaceae is not monophyletic proved by Schuettpelz and Pryer (2007)

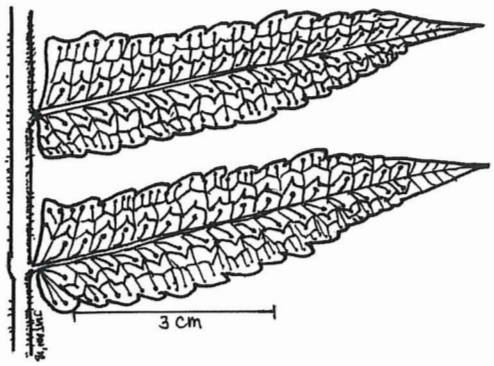


Figure 5. Species with semicraspedodromous venation: *Goniophlebium subauriculatum* C. Presl

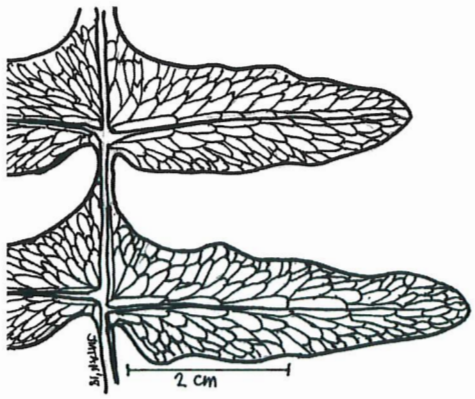


Figure 6. Species with reticulodromous venation: *Histiopteris incisa* (Thub.) J. Sm.

where *Microlepia* was derived from within Wolf (1995).

Cluster 2 (Eupolypods I), Cladodromous, weak -stout 1° vein, areoles absent, were observed in 7 representative species from eupolypods I including *Bolbitis rhizophylla* (Kaulf.) Hennipman (Hernaez & Cajano 243 PBDH), *Davallia hymenophylloides* (Blume) Kuhn (delos Angeles 6175 PBDH), *Didymochlaena truncatula* (Sw.) J. Sm. (Hernaez 5067 PBDH), *Elaphoglossum blumeanum* J. Sm. (Hernaez & Cajano 245 PBDH), *Nephrolepis cordifolia* (L.) (Banaticla 3288 PBDH), and *Oleandra maquilingensis* Copel. (Banaticla 3346 PBDH). Also, the three species from cathetogyrates including *Histiopteris incisa* (Thunb.) J. Sm. (Banaticla 3300 PBDH) from dennstaedtiaceae, *Lindsaea obtusa* J. Sm. (Hernaez 1147 PBDH) and, *Lindsaea pulchella* (J. Sm.) Mett. ex Kuhn (Hernaez & Cajano 238 PBDH) from Lindsaeaceae.

These species shared the following leaf architecture characters: acute-acuminate apex shape to rarely obtuse; mostly microphyll, rarely nanophyll; mostly acute base angle, rarely obtuse; cladodromous, weak-stout 1° vein and absence of areoles. The two outliers due to several changes in taxonomic status were *Goniophlebium subauriculatum* C. Presl. (Banaticla 3330 PBDH) (semicraspedodromous) and *Histiopteris incisa* (Thunb.) J. Sm. (Banaticla 3300 PBDH) (reticulododromous). Based on Lehtonen (2010), the generic delimitation of the family Lindsaeaceae based on morphology has been challenging. Previously, lindsaeoid ferns were considered part of Davalliaceae then later moved to Dennstaedtiaceae (Holttum, 1947; Kramer and Green, 1990) and was considered as a separate family as Lindsaeaceae (Christenhusz, 1938).

Cluster 3 (Eupolypods I), Weak brochidodromous, moderate and massive 1° vein, areoles present, includes *Aglaomorpha meyeniana* Schott (Hernaez 5322 PBDH), *Microsorum heterocarpum* (Bl.) Ching (Hernaez & Cajano 276 PBDH), and *Tectaria grandifolia* (Presl.) Copel. (Hernaez & Cajano 3804 PBDH). *Microsorum heterocarpum* (Bl.) Ching (Polypodiaceae) have entire margin and unlobed leaf while *Aglaomorpha meyeniana* Schott (Polypodiaceae) and *Tectaria grandifolia* (Presl.) Copel. (Tectariaceae) lobed margin and deep lobation. These three species from the family Polypodiaceae and Tectariaceae

Table 3. Venation pattern of 10 selected species of Eupolypods II.

TAXA		PRIMARY VEIN		SECONDARY VEIN				TERTIARY VEIN				QUATERNARY VEIN	AREOLATION
FAMILY	SPECIES	Category	Size	Category	AD	VAD	Spacing	Category	AD	VAD	Spacing	Category	Present/Absent
EUPOLYPODS II													
Aspleniaceae	*Asplenium pellucidum*	Pinnate	moderate	Craspedodromous	moderate	nearly uniform	Irregular	free end in sinuses	moderate	upper vein more acute than lower	Irregular	NA	Absent
	Asplenium polyodon	Pinnate	moderate	Craspedodromous	moderate	varies irregularly	Irregular	free end in sinuses	moderate	upper vein more acute than lower	Irregular	NA	Absent
	Asplenium vulcanicum	Pinnate	moderate	Craspedodromous	moderate	nearly uniform	increasing to wards base	free end in sinuses	moderate	upper vein more acute than lower	Irregular	NA	Absent
Athyriaceae	*Athyrium arstulatum*	Pinnate	Weak	Craspedodromous	Narrow	upper vein more acute than lower	increasing to wards base	free end in sinuses	narrow	upper 3 vein more acute than lower	Irregular	NA	Absent
	Diplazium esculentum	Pinnate	massive	Craspedodromous	wide	varies irregularly	increasing to wards base	forming commissural vein	narrow	upper 3 vein more acute than lower	increasing to wards the base	NA	Absent
	Diplazium fructuosum	Pinnate	massive	Craspedodromous	wide	upper vein more acute than lower	uniform	free end in sinuses	narrow	upper 3 vein more acute than lower	increasing to wards the base	NA	Absent
	Diplazium polypodiodes	Pinnate	stout	Craspedodromous	wide	upper vein more acute than lower	increasing to wards base	free end in sinuses	narrow	upper 3 vein more acute than lower	increasing to wards the base	NA	Absent
Blechnaceae	*Blechnum orientale*	Pinnate	massive	Craspedodromous	moderate	upper vein more acute than lower	increasing to wards base	free end in sinuses	narrow	upper 3 vein more acute than lower	increasing to wards the base	NA	Absent
	Stenochlaena areolaris	Pinnate	moderate	Craspedodromous	moderate	upper vein more acute than lower	increasing to wards base	free end in sinuses	narrow	upper 3 vein more acute than lower	increasing to wards the base	NA	Absent
Thelypteridaceae	*Thelypteris normalis*	Pinnate	moderate	Craspedodromous	moderate	varies irregularly	increasing to wards base	forming commissural vein	narrow	divergence angle varies regularly	increasing to wards the base	NA	Absent

Table 4. Venation pattern of 8 selected species of Cathetogyrates.

TAXA		PRIMARY VEIN		SECONDARY VEIN				TERTIARY VEIN				QUATERNARY VEIN	AREOLATION
FAMILY	SPECIES	Category	Size	Category	AD	VAD	Spacing	Category	AD	VAD	Spacing	Category	Present/Absent
EUPOLYPODS II													
Dennstaedtiaceae	*Dennstaedtia philippinensis*	Pinnate	moderate	Craspedodromous	moderate	upper vein more acute than lower	increasing to wards base	free end in sinuses	narrow	upper 3 vein more acute than lower	increasing to wards the base	NA	Absent
	Histiopteris incisa	Pinnate	moderate	Reticlodromous	Narrow	upper vein more acute than lower	increasing to wards base	NA	NA	NA	NA	NA	Absent
	Microlepia strigosa	Bipinnate	Massive	Craspedodromous	wide	upper vein more acute than lower	increasing to wards base	free end in sinuses	narrow	upper 3 vein more acute than lower	increasing to wards the base	NA	Absent
Lindsaeceae	*Lindsaea obtusa*	Pinnate	moderate	Cladodromous	moderate	upper vein more acute than lower	increasing to wards base	NA	NA	NA	NA	NA	Absent
	Lindsaea pulchella	Pinnate	moderate	Cladodromous	moderate	upper vein more acute than lower	increasing to wards base	NA	NA	NA	NA	NA	Absent
Pteridaceae	*Pteris blumeana*	Pinnate	moderate	Craspedodro mous	moderate	upper vein more acute than lower	increasing to wards base	free end in sinuses	narrow	upper 3 vein more acute than lower	increasing to wards the base	NA	Absent
	Pteris longipinnula	Pinnate	Massive	Craspedodro mous	wide	upper vein more acute than lower	increasing to wards base	free end in sinuses	narrow	upper 3 vein more acute than lower	increasing to wards the base	NA	Absent
	Pteris vittata	Pinnate	Massive	Craspedodro mous	moderate	upper vein more acute than lower	increasing to wards base	free end in sinuses	narrow	upper 3 vein more acute than lower	increasing to wards the base	NA	Absent

Table 5. Venation pattern of 10 selected species of Eupolypods I.

TAXA		PRIMARY VEIN		SECONDARY VEIN				TERTIARY VEIN				QUATERNARY VEIN	AREOLATION
FAMILY	SPECIES	Category	Size	Category	AD	VAD	Spacing	Category	AD	VAD	Spacing	Category	Present/Absent
EUPOLYPODS I													
Davalliacene	*Davallia hymenophylloides*	Pinnate	Massive	Cladodromous	Wide	Upper vein more acute than lower	Irregular	NA	NA	NA	NA	NA	Absent
Didymochlaenaceae	*Didymochlaena truncatula*	Pinnate	Weak	Cladodromous	Narrow	Upper vein more acute than lower	Incrasing toeard base	NA	NA	NA	NA	NA	NA
Dryopteridaceae	*Bolbitis rhisophylla*	Pinnate	Stout	Cladodromous	Moderate	Upper vein more acute than lower	Incrasing toeard base	NA	NA	NA	NA	NA	Absent
	Elaphoglossum blumeanum	Pinnate	Stout	Cladodromous	Moderate	Upper vein more acute than lower	Incrasing toeard base	NA	NA	NA	NA	NA	Absent
Nephrolepidaceae	*Nephrolepis cordifolia*	Pinnate	Moderate	Cladodromous	Wide	Upper vein more acute than lower	Irregular	NA	NA	NA	NA	NA	Absent
Oleandraceae	*Oleandra maquilngensis*	Pinnate	Moderate	Cladodromous	Narrow	Nearly uniform	Uniform	NA	NA	NA	NA	NA	Absent
Polypodiaceae	*Aglaomorpha meyeniana*	Pinnate	Massive	Weak bro chidodro mous	Moderarte	Nearly uniform	Irregular	Forming commissural vein	Moderarte	Upper vein more acute than lower	Irregular	NA	Present
	Microsorum heterocarpum	Pinnate	Moderate	Weak bro chidodro mous	Wide	Upper vein more acute than lower	Incrasing toeard base	Random reticulate	Moderarte	Upper vein more acute than lower	Irregular	Regular polygonal reteulate	Present
	Gonioplebium subauriculatum	Pinnate	Moderate	Semicrasped odromous	Moderarte	Upper vein more acute than lower	Uniform	NA	NA	NA	NA	NA	Absent
Tectariaceae	*Tectaria grandifolia*	Pinnate	Moderate	Weak bro chidodro mous	Wide	Upper vein more acute than lower	Incrasing toeard base	Random reticulate	Moderarte	Upper vein more acute than lower	Irregular	Regular polygonal reteulate	Present

were separated from the cluster 2 of eupolypods I by having weak brochidodromous venation (Fig. 8). The alienation of these species to another cluster can be explained by its taxonomic instability. The status of several genera in Microsoroideae is controversial including Microsorum which is not monophyletic (Kreier *et al.*, 2008). Furthermore, Tectariaceae consists of several general that were uncertain. Some genera were even placed to Dryopteridaceae because of the difficulty in separating these using morphological basis. Only a few molecular studies have been addressed, yet far from established (Hasebe *et al.*, 1994).

The results of this study coincide with the current classification of PPG I to eupolypods I and II except that some cathetogyrates were subsumed to eupolypods due to lack of established taxonomic status. Differences in species were observed in venation pattern, specifically on 2°, 3° and 4° vein categories, angle of divergence, variation in angle of divergence, vein spacing and areolation. The results were parallel from the previous studies conducted by Banaticla and Buot (2004), Baroga and Buot (2013), Torrefiel and Buot (2017). These characters were indeed relevant in delineating species and were found similar to the study of Conda (2017).

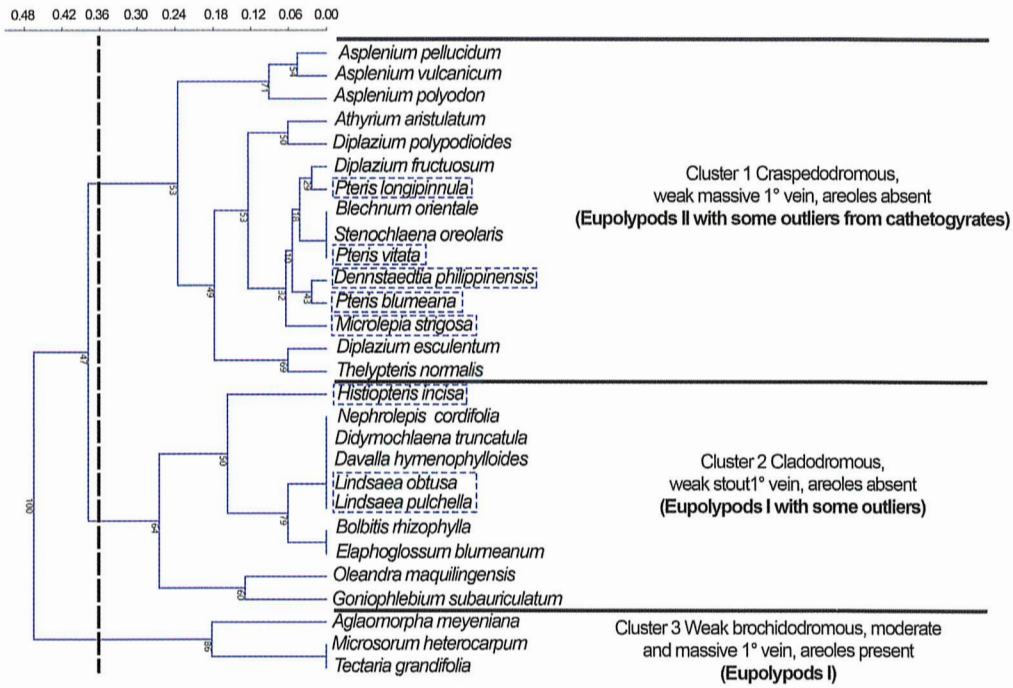


Figure 7. Dendrogram of the 28 species constructed by Unweighted Pair-Group (UPGMA) clustering and Gower using the Paleontological Statistcs software. With cophenetic correlation of 0.933 and bower distance of 0.36, three clusters were created: Eupolpods II: Cluster 1 (Craspedodromous, weak-massive 1^0 vein, areoles absent); Enpolypods I: Cluster2 (Cladodromous, weak-stout 1^0 vein, areoles absent); Eupolypods I: Cluster 3 (Weak brochidodomous, moderate and massive 1^0 vein, areoles present). Dotter lines indicate outliers.

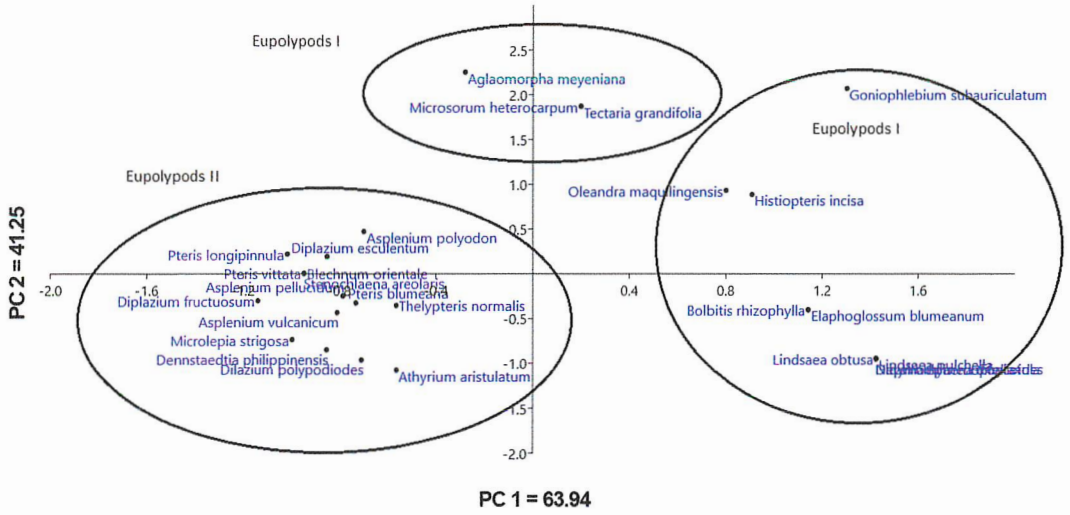


Figure 8. Principal Component Analysis of the 28 representative species using Paleontological Statistics (PAST) software. Three clusters were classified: Eupolypods II: Cluster I (Craspedodromous, weak-massive 1^0 vein, areoles absent); Eupolypods I: Cluster 2 (Cladodromous, weak-stout 1^0 vein, areoles absent); Eupolypods I: Cluster 3 (Weak brochidodromous, moderate 1^0 vein, areoles absent).

CONCLUSION

Based on the results, leaf architectural characters of eupolypods I, eupolypods II, and cathetogyrates have been recognized. General characters and venation patterns especially higher venations are useful characters in delineating species of Polyodiales. Thus, this study proved that leaf architecture, particularly leaf venation is genetically fixed and mainly linked to ontogeny and phylogeny. It is not only a good tool in identifying and describing but also in classifying fern taxa. To investigate more on the effectivity and practicality of leaf architecture in classification, it is recommended to explore its use on other taxa with numerous characters.

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