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Spore-Based Palynological Study of Common *Pteridophyte* Species in *Cagraray* Island, Philippines: Grounds for Classification in Lower Taxa

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***Abstract*—**This mixed-design study aims to document the spore characteristics of common *pteridophyte* species in *Cagraray* Island, Philippines through palynology. This bridges the gap between spore-based palynological study and scheme for classifications in lower taxa of *pteridophyte* species. A total of 18 *pteridophytes* collected from three *barangays* in *Cagraray* Island were identified under eight distinct types of families. The spores from the fronds of *pteridophytes* were gathered and observed using compound microscope while its measurement was supervised by a micrometer. The study revealed three major spore shapes as to tetrahedral, tetrahedral-globose and ellipsoidal, whereas spore appendages were primarily spiny, longitudinally striate, globose, reticulate, angular, warted, and rugose/wrinkled. Sizes of spore vary from 32.67µm to 34.32µm showing parallelism from the majority of previously documented works with significant and minimal changes in the spore size of other species. Classification in lower taxa following the spore shape and appendage was proposed as an augmentation for future classification of *pteridophytes* particularly under sub-genera and sub-families. This study concluded the variation in the morphological characteristics of *pteridophyte* spores and the abundance of genetic variety resulting into multiple arrays of *pteridophyte* spores' characteristics. This study recommends the need to update the records on palynology research specifically in the characterization of spores of *pteridophyte* species. Future parallel investigations and studies are also recommended to other *pteridophyte* species not considered in the present research.

Keywords—Pteridophytes, Morphology, Taxonomy, Spore, Cagraray, Palynology

I. INTRODUCTION

For several decades, the taxonomical classification of fern species was grounded on morphological characteristics and previously documented studies. With the advent of cladistic manner and molecular sequencing techniques, a growing body of literature has been established to fully understand the mechanism of fern classification following evolutionary relationships [1]. Knowing that the Earth's surface experiences turbulent weather and climate changes, it is necessary to re-document changes in the morphological characteristics of plants to determine potential changes in their classification for higher or lower taxa.

Pteridophyte is a term excerpted from Greek word *pteron* which means feather, and *phyton* which means a plant. Hence, *pteridophytes* are a group of plants with feather-like leaf structures, called sporophylls (fronds). This is a group of plants characterized by Linnaeus in 1754 describing its method of propagation via spores, not seeds [2]. *Pteridophytes* are commonly referred to as ferns capable of living in moist and shady places. These heterotrophs produce spores through sporangia emerging either on the leaves or in axils between the leaves and the stem [3]. *Sori* is the term for the group of *Sporangia*. *Sporangium* has *sporocytes* that divide twice giving rise to a tetrad of 4 nonsexual spores [4]. Members of this group like *Azolla, Marsilea, Savinia* can live in water while other types like *Equisetum arvense* grow in *Xerophytic* habitats [5].

Studying the spore-based physiology of ferns, some species have spores encapsulated by *sporangium* which emerged from hypodermal cells. There are also some species with spore-based bearing *sporangia* from epidermal cells. Morphology of its leaf structure revealed that they usually have large compound leaves while others are simple. Some species have hollow, simple, and branched leaves with reduced sheaths and nodes while others have impregnated silica [6]. Documentation of the common shapes, size, and characteristics of *pteridophyte* spores includes monolete and trilete. Monolete spores have linear aperture and are bilaterally symmetrical and ellipsoidal. On the other hand, trilete spores are commonly tetrahedral with globular hemispherical distal face, and radially asymmetrical. For spores with trilete aperture, the shape was globose/spheroidal or tetrahedral. Moreover, when the polar view of the spore was ellipsoidal, the aperture was monolete. The trilete spores have radial symmetry and were flat or convex at the distal face, while monolete, ellipsoidal or reniform spores have bilateral symmetry [7]. These aforementioned physiological and morphological characteristics of *pteridophytes* were used for its classification especially in lower taxa under Kingdom Plantae.

Palynology is the science that deals with *palynomorphs*, an inclusive term describing palynological preparations for diatom, cysts, pollen, and spores [8]. A term coined by Hyde and Williams [9] as a credible instrument in documenting the history and dynamics of past plant communities, while present accounts used the method for studying dust-like particles for micro-based studies [10]. For the past years, palynology was primarily used to study and document the fossils from sedimentary deposits to understand the environmental trends both in terrestrial and aquatic habitats [11][12]. In this study, spore-based palynology of *pteridophyte* species was conducted at Cagraray Island, Philippines. This locale for the study is situated at the eastern coast of the Philippines facing the Pacific Ocean characterized by mountain ranges and comparatively varying temperatures across different areas. It is said that the biosphere status of an area has relative essential outcomes in shaping present organisms residing in the particular habitat [13][14][15]. It is therefore presumed that the isolation and biosphere variation of the locale brought significant impacts in the alteration, or mutation, of *pteridophyte* species in Cagraray island, which can be used as grounds for classification in lower taxa. The present morphological data of spores are beneficial for palynologists, allergic studies [16], and taxonomists.

II. RELATED WORK

Studies in molecular genetics have emitted an understanding of the evolution of the leaf and its development. *Pteridophyte* leaf, specifically, gained attention in the understanding of leaf development by studying its evolution. In a Palynology study of Reference [17], he mentioned that *Ceratopteris thalictroides (L.) Brongn.* has the largest spores. He further noted that the spores of *Pteridium aquilinum* have mutagenic and carcinogenic activities [18].

In a study of Reference [19], he found out that ellipsoidal, tetrahedral, and spherical/globoid are the common shapes of the spores, with monolete and trilete types as common spore apertures. Furthermore, spore pattern surfaces were predominantly cristate, granulate, reticulate, and tuberculate. The study of Reference [20] revealed the oblate-spheroidal shape of spores of *Asplenium aethiopicum, Asplenium trichomanes, Asplenium adiantum-nigrum, Asplenium sp.1, Asplenium sp.2,* and *Ceterach officinarum.* Previously documented studies on spore shape of *Asplenium aethiopicum, Asplenium trichomanes, Asplenium adiantum-nigrum, Asplenium sp.2.* revealed monolete spores while there are some studies indicating that trilete spores were observed among *Asplenium sp.1* and *Ceterach officinarum* species of *pteridophytes.* Studies on the *exine* surface of spores also revealed that *Asplenium aethiopicum* is creates-ridged, *Asplenium adiantumnigrum* is cristae-granulate, while *Asplenium trichomanes* is creates-scan rate. *Asplenium sp.1* and *Ceterach officinarum* have regulate-scabrates, while *Asplenium sp.2* has papillate or tuberculate sculpture [21].

Based on the study of Reference [22], advanced spores (monolete) and primitive spores (trilete) are two distinct types of spore classification. The largest one with the size of 289 × 300 µ is *Selaginella radicata* megaspore. Other observed spores were showing different surface patterns such as reticulate, verrucate, psilate, gemmate, regulate, cristate, echinate, and tuberculate. In terms of types, sizes, shape, colors, the *Asplenium* genus spores shared the same morphological characteristics however, the surface is different such as reticulate, regulate, ornate, and cristate. Among many species tested in the study, most of the species were primitive with trilete - non - perinous spores.

In the study of Reference [23], he documented that *Nephrolepidaceae, Hyminophyllaceae, Telypteridaceae,* and *Polypodiaceae* are some of the advanced non- perinous spores observed in these families of ferns whereas *Oleandraceae, Aspleniaceae, Athyriaceae, Tectariaceae, and Dryopteridaceae* are the most advanced spores of monolete - perinous found in these families. Spinulose spores were found in *Thelypteris xylodes* and *Pseudocyclosorus ochthodes*. Because of the presence of chlorophyll, some of the spores were greenish as observed on the spores in the fern species of *Crepidomanes minutum* and *Leptochilus thwaitesianus*. The chlorophyll occurring in spores was short viability and early germination indicators. Some other *pteridophyte* species were in brown, dark brown, reddish-brown, yellowish-brown, red, yellow, greenish-yellow, and white color [24].

Reference [25] mentioned that classification of ferns commonly shows a trend from highly artificial-based illumination and interpretation of a few supererogatory characters, via natural triage derived from a multitude of characters, towards more evolutionary circumscriptions of groups that do not, in general, align well with the distribution of these previously used characters. The placement of numerous genera was vague until the arrival of molecular phylogenetics, which has rapidly been improving our understanding of fern relationships. Moreover, there are various ways that *sporangia* can be organized. Sometimes they cover the entire lower lamina of the blade, especially towards its apex, which is called an *acrostichoid sorus*. The typical leptosporangiate *sorus* consists of a stalk to which *sporangia* are fixed. A *sorus* can be covered with a scale-like structure called the *indusium*. The shape and organization of the *indusium* have been an important character for the higher classification of leptosporangiate ferns and are still employed today, although usually in combination with others [26].

Reference [27] indicated that the characteristics of spores are very significant in classification and phylogenetic information, and they have also allowed calibration of DNA-based phylogenetic trees with the ages of fossil spores where heterosporous ferns are the most remarkable. The condition in which a single sporophyte produces a smaller number of large spores, megaspores are called *Heterospory*, that develop into female gametophytes and, more typically, numerous microspores that develop into male gametophytes. Spores in heterosporous ferns are formed in special structures called sporocarps. All other ferns and *Lycopodiaceae* are homosporous, the plesiomorphic condition.

Based from the consolidated review of related works, there are no documented studies about the palynology of fern species in *Cagraray* Island, Philippines. The attempt to document the spore-based morphological characteristics of the locale's ferns will contribute to the field of taxonomy for classification in lower taxa by updating the spore characteristics of the identified *pteridophyte* species in the locale through palynology. This identified goal of the study served as its state-of-the-art which primarily aims to bridge the gap between updated palynology research of *pteridophyte* spores vis-a-vis taxonomical classification particularly in the lower taxa. The attempt of developing a scheme for classification will supplement the body of literature as a future reference to taxonomists in review/update of *pteridophyte* classification.

Gleaned from the ongoing scenarios, the researchers established the specific research goals grounded from the main goal of the study as to; (1) bridge the literature gap by determining the morphological characteristics (spores) of selected *pteridophyte* species in *Cagraray* Island, Philippines; and (2) develop scheme for classifications in lower taxa through morphological characteristics of *pteridophyte* spores.

III. METHODOLOGY

This study involves observation of morphological characteristics of spores (spore shape and spore appendage) and calculation of spore size by mean of the longest and shortest diameter expressed in micrometer (µm). Hence, this study utilized mixed approach of qualitative and quantitative designs.

For research goal 1, on the morphological characteristics of spores, microscopy was applied using compound microscope to observe the characteristics of spores from the selected *pteridophyte* species. Verification of *pteridophyte* species was testified true by SPS Biological Testing-Biology Department and *Plantae* Database. Scalpel was used to scrape off the lower epidermis of fronds where collected spore dusts were transferred directly into slides. Then, it was hydrolyzed using 70% Isopropyl Alcohol (C_3H_8O) and stained using Merthiolate, and allowed to settle for 5 minutes. Micrometer 5X embedded in cover slip was used in the slides and magnified using a 10X objective lens. The shapes of spores were described using the matrix quotation of Reference [28]. The micrometer measured the spore size and was computed through descriptive statistics using mean of the shortest and longest spore diameter (µm) in 3 trials.

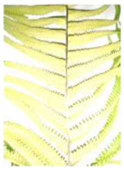
For research goal 2, on scheme for classification in lower taxa, findings were used to develop a representation for classifications using the morphological characteristics of *pteridophyte* spores. This was gleaned based from the results of research goal 1.

Materials and *Pteridophyte S*pecies

The use of compound microscope with 10X microscopic objective lens provides clear and sufficient transparency for being able to observe and identify the morphological characteristics of spores. Scalpel was used to scrape off spore dusts on the lower epidermis of fronds. This study also used Merthiolate as staining agent and 70% Isopropyl Alcohol as hydrolyzing agent. Micrometer 5X (for 10X objective lens) was utilized to measure the shortest and longest diameter of spores to obtain its mean expressed in micrometer (µm). Eighteen (18) *pteridophyte* species were collected from 3 *barangays* in *Cagraray* Island, Philippines and assessed to determine its morphological characteristics. Table 1 shows the summary of identified *pteridophyte* sample species.

Table 1: Common *Pteridophyte* Species Collected in *Cagraray* Island, Philippines

Pteridophyte Plate	Frond and Sori	Scientific Name and Family	Location at Cagraray island
		*Aglaomorpha quercifolia (Polypodiaceae)*	Purok 1 Cagraray, Bacacay, Albay, Philippines
		*Asplenium nidus (Aspleniaceae)*	Purok 2 Cagraray, Bacacay, Albay, Philippines
		*Dryopteris tokyoensis (Dryopteridaceae)*	Purok 4 Cagraray, Bacacay, Albay, Philippines
		*Nephrolepis cordifolia (Nephrolepidaceae)*	Purok 4 Cagraray, Bacacay, Albay, Philippines
		*Diplazium esculentum (Athyriaceae)*	Purok 2 Cawayan Bacacay Albay Philippines

		*Pteris vitta* *(Pteridaceae)*	Purok 1 Cagraray, Bacacay Albay Philippines
		*Pteris multifida* *(Pteridaceae)*	Purok 1 Cagraray Bacacay Albay Philippines
		*Dryopteris carthusiana* *(Dryopteridacea e)*	Purok 2 Cagraray Bacacay Albay Philippines
		*Pityrogramma calomelanos* *(Pteridaceae)*	Purok 1 Cagraray Bacaacay Albay
		*Polystichum munitum* *(Dryopteridacea e)*	Purok 1 Cagraray Bacacay Albay
		*Nephrolepis hirsutula* *(Nephrolepidac eae)*	Purok 6 Cagraray Bacacay Albay
		*Deparia acrostichoides* *(Athyriaceae)*	Purok 1 Cagraray Bacacay Albay
		*Taenitis blechnoides* *(Pteridaceae)*	Sitio Biga-Biga Cawayan Bacacay Albay
		*Pyrrosia elaeagnifolia* *(Polypodiaceae)*	Purok 1 Cagraray Bacacay Albay
		*Blechnum orientale l.* *(Blechnaceae)*	Cabasan, Bacacay Albay
		*Rumohra adiantiformis* *(Dryopteridacea e)*	Purok 1 Cagraray Bacacay Albay
		*Cibotium menziesii* *(Cibotiaceae)*	Purok 7 Cagraray Bacacay Albay Philippines
		*Athyrium filix-femina* *(Athyriaceae)*	Purok 7 Cagraray Bacacay Albay Philippines

Of 18 *Pteridophyte* species collected from three *barangays* in *Cagraray* Island, eight distinct types of families were identified. Two species belong to *Polypodiaceae* family, one species belongs to *Aspleniaceae* family, four species belong to *Dryopteridaceae* family, two species belong to *Nephrolepidaceae* family, three species from *Athyriaceae* family, then four species to *Pteridaceae* family, one species belongs to *Blechnaceae* family, and one from *Cibotiaceae* family.

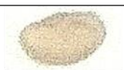
IV. Results and Discussion

The discussions of the results below were the salient findings of the study on palynological research of *Pteridophyte* species in *Cargraray* island, Philippines.

A. Morphological Characteristics of Spores of Pteridophyte Species in Cagraray Island

In this study, the morphological characteristics of spores from the different *Pteridophyte* species collected were identified and analyzed. Ellipsoidal, Tetrahedral, and Tetrahedral-globose are the primary spore shapes characterized by the collected *Pteridophyte* species in Cagraray Island, Philippines. Majority of the spore shape documented an ellipsoidal configuration. On the other hand, there are seven identified classifications of spores based on its appendage namely; spiny, rugose/wrinkled, longitudinally striate, globose, reticulate, angular, and warted. Longitudinally striate is the most dominant classification of spore appendage among all identified *Pteridophyte* spores.

Table 2. The Morphological Characteristics of *Pteridophyte* Species in Terms of Spore Shape and Appendage

Scientific name	Plate	Spore shape	Spore Appendage
Aglaomorpha quercifolia		Ellipsoidal	Spiny
Asplenium nidus		Ellipsoidal	Rugose
Dryopteris tokyoensis		Tetrahedral-glubose	Longitudinally Striate
Nephrolepis cordifolia		Ellipsoidal	Globose
Diplazium esculentum		Ellipsoidal	Spiny
Pteris vitta		Tetrahedral-globose	Reticulate
Pteris multifida		Tetrahedral-globose	Angular
Dryopteris carthusiana		Ellipsoidal	Globose
Pityrogramma calomelanos		Tetrahedral-globose	Longitudinally Striate
Polystichum munitum		Tetrahedral-globose	Longitudinally Striate
Nephrolepis hirsutula		Tetrahedral-globose	Longitudinally Striate
Deparia acrostichoides		Ellipsoidal	Rugose
Taenitis blechnoides		Tetrahedral	Rugose
Pyrrosia eleagnifolia		Ellipsoidal	Warted
Blechnum orientale l.		Ellipsoidal	Rugose
Rumohra adiantiformis		Ellipsoidal	Rugose
Cibotium menziesii		Ellipsoidal	Reticulate
Athyrium filix-femina		Ellipsoidal	Rugose

Aglaomorpha quercifolia and *Pyrrosia eleagnifolia*, both belong to *Polypodiaceae* family, had monolete ellipsoidal spore shape. The perispore of *Pyrrosia eleagnifolia* seemed to be double-layered and contains crumpled lines on its side. The *Aglaomorpha quercifolia* had dotted lines on the spore surface that seemed to be rough. Although these two species had same spore shape, they showed difference in terms of spore appendage. *Aglaomorpha quercifolia* has spiny spores while *Pyrrosia eleagnifolia* has warted spores. In the literature provided by Reference [29], majority of species in *Polypodiaceae* family have a single-layered perispore attached to an exospore, sometimes micro-ornamented, and perforated. Based on the study of Reference [30], all the spores of *Polypodiaceae* family are monolete, ellipsoidal and elliptic shapes. The findings revealed that the two species of *Polypodiaceae* family varied in spore appendage but its shapes were consistent with the findings in the literature body.

Asplenium nidus had ellipsoidal spore shape. Observation of its spore revealed lesions and rough lumps with wrinkled perispore. Therefore, its spore appendage is considered to be rugose. According to the given literature by Reference [31], cristates-ridged, cristates granulate, cristates scabrate, regulate-scabrates, and papillate or tuberculate surfaces are the morphological characteristics of *Aspleniaceae* family spore which could correspond to the observed forms on the spore features- the lesions and lumps. They further noted that *Aspleniaceae* perispore had single or double-layered microlacunouse structure.

Diplazium esculentum, *Deparia acrostichoides*, *Athyrium Filix-Femina*, members of *Athyriaceae* family, all exhibited ellipsoidal spore shape. However, only two species showed similar spore appendage; *Deparia acrostichoides*, and *Athyrium Filix-Femina* had both wrinkled/rugose spore surface structures. On the other hand, only *Diplazium esculentum* revealed spiny spore appendages in this family group. All species in this family seemed to be rough as shown in the furrows on its surface.

Blechnum orientale l. showed ellipsoidal spore shape exhibiting spore appendage associated with rugose. On the study of Reference [32], it indicated the perispore characteristics of this species having smooth, rugate, low reticulate, and with ornamental orbiculate. Similarly, *Cibotium menziesii* also showed ellipsoidal spore shape with spore-reticulated spore appendage. These species belong to *Cibotiaceae* which, according to Reference [33], spores of this family were primarily characterized with equatorial flange, and with distal face usually defined by thick, bold, parallel, and an *astomosing* ridges. The present study did not able to fully define the exhibited characteristics of spore appendage but it is presumed that the observed ridges on the surface of the spores by previously documented studies were attributed to the observed crude rugose and reticulation.

Compared to the previous *pteridophyte* species, the species from *Dryopteridaceae* family had different spore shapes. *Dryopteris tokyoensis*, and *Polystichum munitum* both exhibited tetrahedral-globose spore shape whereas *Dryopteris carthusiana*, and *Rumohra adiantiformis* had ellipsoidal spore shape. In terms of spore appendage, the species *Dryopteris tokyoensis*, and *Polystichum munitum* showed similar longitudinal striate appendage on spore surface. However, *Dryopteris carthusiana*, and *Rumohra adiantiformis* showed dissimilar spore appendages having globose and rugose spore features respectively. According to Reference [34], in Korea, some species of examined *Dryopteridaceae* indicated three perispore shape types categorized as to echinate, spinose, and rugate which are the most common perispore shapes among all selected examined species. This literature runs somewhat parallel with the present study indicating rugose as one of the characteristics of spore appendage of a certain species within this family.

Some of the species from *Nephrolepidaceae* family were included in this study. *Nephrolepis hirsutula* species had tetrahedral-globose spore shape while *Nephrolepis cordifolia* species had ellipsoidal spore shape. In terms of spore appendage, they also showed unlike spore surfaces having longitudinally striate, and globose spore appendage respectively. Literature from Reference [35] mentioned that these species were trilete when the shape was spheroidal or tetrahedral, and monolete when it was ellipsoidal in the polar view. This literature attested that the actual positioning of spores under observation for microscopy can result to varying spore shapes with respect to the orientation of spore position. Nonetheless, both observations in the literature run parallel with the findings of the current study.

Many species of *Pteridaceae* family were identified and collected in the research locale. In fact, *Pteridaceae* is the largest family among *Pteridophyte species* with spore shapes commonly described as trilete, tetrahedral, and globose [36]. The study revealed that *Pteris vitta*, *Pteris multifida*, and *Pityrogramma calomelanos* had tetrahedral-globose spore shape while *Taenitis blechnoides* had tetrahedral spores among all *Pteridaceae* family members identified. This implied a conformable finding in the literature body indicating that majority of spores in the family of *Pteridaceae* had tetrahedral shape. Though they were dominantly tetrahedral in shape, members of family *Pteridaceae* had different spore appendages. *Pteris vitta*, *Pteris multifida*, *Pityrogramma calomelanos*, and *Taenitis blechnoides* had reticulate, angular, longitudinally striate, and rugose appendages.

The consolidated findings showed similar and consistent results with respect to the previously documented works in *Pteridophyte* studies on palynology. Though there are some variations particularly in the spore appendages, this testified for the truthfulness that variation in spore appendage and features occurred in the locale brought by certain conditions which need to be studied and explored further. Table 2 shows the result of microscopy of identified *Pteridophyte* species and its spore-based palynological characteristics.

Other than identifying the morphological characteristics of the spores of *Pteridophyte* species in *Cagraray* Island such as shape and appendage, its size was also measured and calculated. To secure the assurance of the measured average spore size, the spores underwent 3 trials of measurement. Findings revealed that the mean of spores ranges from 32.67µm ± 0.14µm to 34.32µm ± 0.42µm. The *Dryopteris tokyoensis* from *Polypodiaceae* family showed the largest spore size whereas *Nephrolepis cordifolia* from *Nephrolepidaceae* family cast the smallest size of spore.

Table 3. Measurement of spore size based from the mean of shortest and longest diameter in 3 trials.

***Pteridophyte* Species**	**Mean of Shortest and Longest Spore Diameter in 3 trials**
Aglaomorpha quercifolia	33.7µm ± 0.31µm
Asplenium nidus	33.77 µm ± 0.28µm
Dryopteris tokyoensis	34.32µm ± 0.42µm
Nephrolepis cordifolia	32.67µm ± 0.14µm
Diplazium esculentum	33.7µm ± 0.24µm
Pteris vitta	34.27µm ± 0.19µm
Pteris multifida	33.17µm ± 0.31µm
Dryopteris carthusiana	33.41µm ± 0.09µm
Pityrogramma calomelanos	33.22µm ± 0.56µm
Polystichum munitum	33.25µm ± 0.12µm
Nephrolepis hirsutula	33.08µm ± 0.11µm
Deparia acrostichoides	34.03µm ± 0.22µm
Taenitis blechnoides	33.22µm ± 0.42µm
Pyrrosia eleagnifolia	33.78µm ± 0.44µm
Blechnum orientale l.	33.00µm ± 0.39µm
Rumohra adiantiformis	33.22µm ±0.12µm
Cibotium menziesii	32.74µm ±0.25µm
Athyrium filix-femina	33.45µm ± 0.51µm

Polypodiaceae family dominated the spore sizes. *Aglaomorpha quercifolia* has spore size of 33.7µm ± 0.31µm while *Pyrrosia eleagnifolia* has 33.78µm ± 0.44µm. The measurement of spores for both species is considered statistically the same (p>0.05). Based from the study provided by Reference [37], the spores of *polypodiaceae* family have 40-90µm in major equatorial diameter. The exospore ranges from 2-5µm thick whereas the perispore ranges from 0.3-1µm thick. This study implies that the sizes of spores of *Polypodiaceae* family species in this study were found to be smaller based from the data documented in the body of literature. This calls for further testing of the spore shape, and understanding of the genetic codes of the identified *Polypodiaceae* family species to determine whether mutation occurred resulting to smaller spore size.

The *Asplenium nidus,* which had ellipsoidal and rugose structure, gained a comparatively larger spore size similar to that of the species from *Polypodiaceae* family equivalent to 33.77 µm ± 0.28µm. The findings provided by Reference [38] was consonance in the present findings which indicated that this spore belongs to the *Pteridophyte* family average and diverse sizes from 32.5 - 35 µm in polar diameter and 35 - 37.5 - 40 µm in equatorial diameter. Furthermore, the exospore ranges from 0.5 - 3 µm thick, smooth and the perispore is 0.3 - 1 µm thick and ornamented. The literature and the present study showed similar results.

One of the species from *Dryopteridaceae* family has a comparatively large spore size among all examined and identified *Pteridophyte* species in *Cagraray* Island. This species is *Dryopteris tokyoensis* with 34.32µm ± 0.42µm spore size with tetrahedral-globose and longitudinally striate spore characteristics. Other species belonging from this family contain comparatively smaller spore size like *Dryopteris carthusiana*, *Polystichum munitum,* and *Rumohra adiantiformis* showing statistical similarity (p>0.05) in spore size. Reference [39] mentioned that the size of spores of *Dryopteridaceae* family has a mean estimation interval from 28.3µm to 58.3µm in equatorial diameter. This implied that the spore sizes of the identified *Dryopteridaceae* family species run parallel with the present study.

Diplazium esculentum, *Deparia acrostichoides*, *Athyrium Filix-Femina*, members of *Athyriaceae* family, all exhibited ellipsoidal spore shape and were characterized with statistical similarity in spore size (p>0.05). Reference [40] supported this assumption indicating that spores from this family contain large to medium spore size although the literature revealed no quantified measures of spore measurements.

Blechnum orientale l. showed a quite small spore size at 33.00µm ± 0.39µm. Reference [41] noted that the spore size media of equatorial diameters range from 31-89µm x 22-66µm for the family of this species with two perispores recognized namely, smooth and distinctly ornamented. The thickness of the perispore varies from 1 to 10µm. Similarly, *Cibotium menziesii* also contains small spore size at 32.74µm ±0.25µm.

Pteridaceae family is the largest family among all *Pteridophyte* species while its spore shape is primarily tetrahedral and globose. The species *Pteris vitta* had the biggest spore size among the identified species at 34.27µm ± 0.19µm. This size ranked 2nd among the biggest spore size in all identified *Pteridophyte* species, next to *Dryopteris tokyoensis.* Conversely, the rest of the species identified in this family contain comparatively smaller spore size like *Pteris multifida* (33.17µm ± 0.31µm), *Pityrogramma calomelanos* (33.22µm ± 0.56µm) and *Taenitis blechnoides* (33.22µm ± 0.42µm). These were the only species among all examined *Pteridophyte* species with statistically the same spore size (p>0.05). In the study of Reference [42], the spore size of *Pteris* species varies from 35 µm to 65 µm which connotes that the identified *Pteris* species in the present study showed comparatively smaller spore size. This could be a manifestation that alteration in genes for spore size occurred, or an error in measurement testing. Nevertheless, this calls for further studies to testify the trustfulness of this finding.

Some of the species of *Nephrolepidaceae* family were characterized with tetrahedral-globose and ellipsoidal shape of spore. Compared to other *Pteridophyte* species with almost the same size of spore, this family showed diversity in spore size. *Nephrolepis hirsutula* had spore size of 33.08µm ± 0.11µm whereas *Nephrolepis cordifolia* at 32.67µm ± 0.14µm. This indicates gene alteration in the spore size of the identified species in the family.

Among all examined *Pteridophyte* species, the spore size varies though some statistical findings revealed similarity. Two tetrahedral-globose spore shape dominated the largest spore size among all collected *Pteridophyte* species. Most of the spiny spores have comparatively bigger spores with measurements varying from from *Polypodiaceae* and *Athyriaceae* families. The two selected species from *Nephrolepidaceae* showed varying spore sizes though they both belong in the same family. Although majority of the documented spore sizes correlated to the existing records in the literature, there are some accounts of contradicting results indicating the need to do further investigation of the identified species. This implied the need to conduct genome analysis of those species to understand and determine DNA sequences resulting in different spore sizes, or repeat the procedure of the experimental research to validate the findings.

B. Scheme for Classifications in Lower Taxa through Morphological Characteristics using Pteridophyte Spores.

Reference [43] indicated that fern classification generally shows a trend from highly artificial, based on an interpretation of a few extrinsic characters, via natural classifications derived from a multitude of intrinsic characters. There are also some records indicating the criteria on classifying ferns as to molecular data [44], phyletic lineages [45], and the use of synopsis of included genera and emphasis on ordinal and familiar ranks [46]. Meanwhile, Reference [47] also mentioned that in classification of ferns, spore morphology features were also conversed and illustrated, however these were not used to group taxa. Hence, this study attempted to develop a classification scheme on lower taxa of ferns based on the morphological characteristics of spores in terms of shape and appendages. Figure 1 shows the proposed schematic diagram of the lower taxa for *pteridophyte* classification.

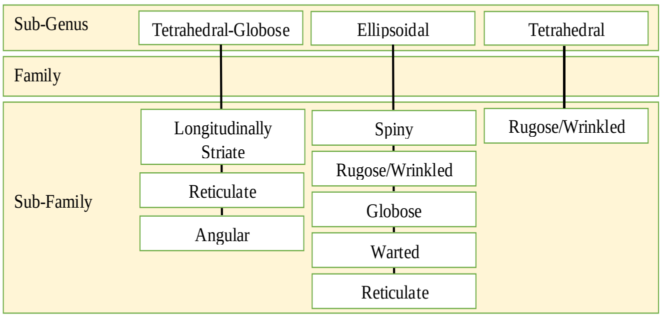


Figure 1. The proposed classification of *pteridophyte* species in lower taxa using palynology of spores.

In this study, the target classification was designed under sub-genus and sub-family. This is because there are no documented reports that these groups were already filled with criteria and grounds for classifying fern species. The sub-genera are proposed to consider the spore shape categorized into three distinct classifications namely; tetrahedral-globose, ellipsoidal, and tetrahedral. On the other hand, specific to each sub-genus, is the classification of fern species under sub-family using the spore appendages like longitudinally striate, reticulate and angular for sub-genus tetrahedral-globose; spiny, rugose/wrinkled, globose, reticulate, and warted for sub-genus ellipsoidal; and rugose/wrinkled for sub-genus tetrahedral. Family group was considered a mediator since it was previously classified into several *Pteridophyte* families. The development of the new lower taxa classification scheme was basically grounded on the identified palynological characteristics and traits of *Pteridophyte* spores.

The proposed scheme of fern classification is an augmentation to the traditional and existing models for fern taxonomy. These morphological characteristics of spores have significant function in ferns as it will give the taxonomists an idea on the similarity and discrepancy in spore structure. Nonetheless, this type of classification is appropriate only in the *Pteridophyte* species found and collected within the locale of the *Cagraray* Island, Philippines.

V. CONCLUSION AND FURTURE SCOPE

Pteridophyte species are vascular and asexual plants that produce spores to sustain their reproduction and evolution. After gathering and collecting various *pteridophyte* species in *Cagraray* Island, 18 distinct *pteridophytes* under eight different families were used for examination and classification. This study observed, described, and measured the *Pteridophyte* spores based on their morphological characteristics in terms of shape, spore appendage, and size. Ellipsoidal, Tetrahedral, and Tetrahedral-globose are the primary and fundamental spore shapes among all collected *pteridophyte* species in *Cagraray* Island, Philippines. Ellipsoidal spore shape was the common shape of spores. Moreover, seven novel spore appendage features were determined like spiny, longitudinally striate, globose, reticulate, angular, warted, and rugose/wrinkled. The findings showed similar connection in the morphological characteristics that exist in the certain family of *pteridophytes.* This implied the credibility of the result since species under the same family should obviously share the same exact characteristics. The size of spores was also measured and calculated revealing size variations from 32.67µm (*Dryopteris tokyoensis)* to 34.32µm (*Nephrolepis cordifolia)*. Despite the parallelism of the present study regarding the measurements of spores in consonance to the previously documented body of research, there are certain fern species showing significant and minimal changes in spore size. Since fern variation through palynology of spores is evident, this research proposed a classification in lower taxa following the spore shape and spore appendage of *pteridophyte* species based on the fern characteristics in *Cagraray* Island, specifically under sub-genera and sub-families.This study concluded the unique and distinct morphological characteristics laid in different species of *pteridophytes* further attesting to the abundance of genetic variety contributing to spore shape, appendage, and size. Some of these traits seem to exhibit relationship and connection with the family of other species. However further studies and investigations are needed to explore and determine the precision of results. For future scope, it is recommended to document other emerging fern species in the locale which were not considered in the present study. This can be done by doing parallel palynology research of its spore-based characteristics to add up in the findings of the current study. Furthermore, it is also recommended to update the records in palynology research particularly in spore characteristics of ferns.

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