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Short Communication: Comparative foliar epidermal study of some species of pteridophytes in Rivers State University, Nigeria

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Abstract. *Ibiye AI, Green BO, Ajuru MG. 2023. Comparative foliar epidermal study of some species of pteridophytes in Rivers State University, Nigeria. Cell Biol Dev 7: 51-55.* Pteridophytes show many forms and are cosmopolitan in distribution, from sea level to high mountains. They are about 13,500 species of fern and allies distributed throughout the world. Some are edible, while some are ornamental. They are economically important to humans in food, medicine, ornaments, fibers, and cultural usages. Ferns generally are an understudied group of plants, and no work on the foliar epidermal study of the three species in the South-South part of Nigeria has been done. The study aimed to investigating the foliar anatomy of three species of pteridophytes at Rivers State University, Nigeria, to elucidate their taxonomic knowledge using this line of evidence. The standard method for foliar epidermal analysis was employed. Fresh mature leaves were soaked in sodium hypochlorite (5%) for 3-5 minutes to soften the tissues, and the adaxial surface was scraped off with a sharp razor blade until the abaxial surface was reached; equally, the abaxial surface was scraped off to reveal the adaxial surface. The species include: *Nephrolepis biserrata* (Sw.) Schott, *Phymatosorus scolopendria* (Burm.F.) Pic. Serm., and *Microgramma mauritiana* (Wild.) Tardieu. Results of the foliar epidermal anatomical study revealed that the presence of uniseriate non-glandular trichomes on both the abaxial and adaxial surfaces of *N. biserrata* and absent in *P. scolopendria* and *M. mauritiana* is diagnostic of this species. The presence of diacytic stomata in addition to anomocytic type in the abaxial and adaxial surfaces of *N. biserrata* also separates it from *P. scolopendria* which had amphidiacytic stomata on the abaxial surface and *M. mauritiana* lacked stomata on this surface. All three species possessed numerous crystal sand on both surfaces, which is also diagnostic. These characters have been reported to be diagnostic and considered highly significant for solving taxonomic disputes through identification and delimiting among the species, thus broadening the scope of their taxonomic knowledge.

Keywords: Foliar anatomy, *Microgramma mauritiana, Nephrolepis biserrata, Phymatosorus scolopendria,* pteridophytes

INTRODUCTION

Pteridophytes have a great range of forms and are cosmopolitan in their distribution. Altogether, there are about 13,500 species of pteridophytes found throughout the world (Moran 2006). Some are edible, and some are used for ornamental purposes. Economically, they are important to humans in food, medicine, fiber, and cultural utilization (Camus et al. 1991). There is a high intake of *Nephrolepis biserrata* (Sw.) Schott by domestic animals in the tropics. Babayemi et al. (2006) reported that *N. biserrata* can be used as animal feed due to its high nutritional value.

The *N. biserrata* is used for ornamental purposes (Oloyede et al. 2011). Also, the rhizomes are used for treating sores, boils, abscesses and blisters of the skin in Sarawak but in India, it is used for treating respiratory diseases (Christensen (1997). Due to its high nutritive content, It is used as fodder for feeding the African dwarf goats, sheep, and other small ruminants in Nigeria (Nwosu 2002; Babayemi et al. 2006; Oloyede et al. 2008; 2013). In Sabah and Malaysia, the locals use the tip of the young shoots of *N. biserrata* as a vegetable (Kulip et al. 2010). In traditional medicine, *N. biserrata* is recommended for the treatment of various diseases, such as for prevention of miscarriage, fetus development, and different microbial infections, including boils, blisters, sore, and abscesses; it is employed in the treatment of wounds, bleeding, and stomach ache (Piggott 1996: Jiofack et al. 2008; Malan and Neuba 2011).

It is also prescribed as a ground cover on wooded edges and borders and is available in local nurseries (Florida Native Plant Society 2020). In Java and New Guinea, very young, soft, curled-up fronds of *N. biserrata* are eaten either cooked or steamed as a vegetable. The rhizomes are sometimes dried, pounded, prepared, and eaten like *sago* (Darnaedi and Praptosuwiryo 2003). In Micronesia, the fronds are used as cockroach repellent (David 1987). In traditional medicine, *N. biserrata* treats dropsy, swellings, diarrhea, venereal diseases, dysentery, gout, and edema, even as a pain-killer (Burkill 1985).

Fronds of *Phymatosorus scolopendria* (Burm.F.) Pic. Serm. are used in Indo-China to treat boils and filariasis, chronic diarrhea, while the whole fronds are kept on beds to ward off bed bugs (Mannan et al. 2008). In Polynesia, the fronds are grounded and mixed with *Atuna racemosa* Raf. scraps to make perfume. Sometimes, the mashed fronds are wrapped with *Morinda citradolia* L., then cooked and used as a medical bandage for medicinal

treatment. Juice from leaves is used in Fiji to treat stomach pain, boils, and swollen breasts (Snogan et al. 2007). When crushed, the fern issues a scent similar to maile; sometimes, pieces of the fern are interlaced in leis made of the pandanus fruit (Ukui and Elbert 1986).

Whole plants of *Microgramma mauritiana* (Wild.) Tardieu are used for the treatment of pubic lice in humans and the prevention of the lice from being transferred from one person to another. The transfer of these lice is believed to be inflicted by witchcraft (Hutchings 1996; Roux 2003).

The foliar epidermal features of the epidermis have played an important role in taxonomy and for a plant systematist. The epidermis possesses several important diagnostic characteristics that offer valuable clues for taxonomic identification, such as size, shape, distribution of stomata (Oznur and Tugha 2006; Ajuru and Okoli 2012; Bassey et al. 2016), guard and subsidiary cells, as well as presence or absence of trichome and their different types and lengths (Metcalfe and Chalk 1979). The presence or absence of foliar appendages is significant in the delimitation of taxa, especially at the generic and specific levels.

Anatomical characters are highly valued in taxonomy, as supported by several researchers (Metcalf and Chalk 1979; Shaheen et al. 2009; Oloyede et al. 2011). The anatomical features are popularly used for identifying and classifying plant species and indicating relationship patterns and phylogeny (Essiet and Iwok 2014). Ajuru and Okoli (2012) used anatomical characters as one of the parameters in delimiting the different types of melons in the family Cucurbitaceae.

Generally, ferns have been classified into a different taxonomic hierarchy, though there is very poor or little information about the relationships or differences between several ferns in their taxonomic classification. Also, most species of ferns are difficult to identify easily using only the traditional morphological characteristics, which often leads to wrong identification and classification. The ripple effect of these actions could lead to wrong results and under-usage of these plants. Also, it can lead to adulteration in using these plants for medicinal purposes since they are highly sought after for medicinal and pharmaceutical purposes. This study is therefore carried out to provide more features for delimiting these species using foliar epidermal characteristics.

MATERIALS AND METHODS

Study area

The study area is the Rivers State University (RSU) campus within the Port Harcourt metropolis, Port Harcourt Local Government area in Rivers State, Nigeria. Port Harcourt is an industrialized cosmopolitan city located in the heart of the Niger Delta. The study area, RSU, lies South-South of the Niger Delta within Latitudes 4º31' - 4º 40'N and Longitudes 70º0'-7º10' E. It has an elevation of about 10-15 m above sea level.

Plant collection and identification

The plant materials used for this study were collected from Rivers State University in polythene bags and taken to the Biosystematics laboratory for proper identification and authentication; Prof. B.O. Green, a Plant Taxonomist, identified them. The plants were dried, pressed, and properly mounted to be deposited in the University Herbarium. Voucher specimens were deposited in the Rivers State University Herbarium with the following Herbarium number assigned to them: RSUPb0102, RSUPb0103, and RSUPb0104 for *N. biserrata*, *P. scolopendria*, and *M. mauritiana*, respectively.

Foliar epidermal study

Fresh matured leaves were prepared according to the simplified method described by Okoli (1992). The fresh leaves were soaked in sodium hypochlorite (5%) for 3-5 minutes to soften the tissues and make them easy to scrap. The leaves were placed on a flat surface (tile), and the adaxial surface was scraped off gently with the razor blade until the abaxial surface was reached. Equally, the abaxial surface was scraped off to reveal the adaxial surface.

The transparent epidermal peels were soaked in distilled water to rehydrate the cells, after which they were stained with 1% safranin for 3 minutes and later rinsed again in distilled water. The specimens were mounted with 3 drops of glycerin and a cover slip placed correctly. Slides of both abaxial and adaxial surfaces were prepared. These were examined using a light microscope, and photographs were taken with a micrograph unit.

The qualitative characteristics of the foliar epidermis were observed and taken with a light microscope. Measurements of epidermal cells, trichomes, and stomatal lengths and widths were taken. All measurements were taken at x10 objective. Stomata Index (SI) was calculated using the formula:

$$SI = \frac{S}{E+S} \times 100$$

Where: S = Number of stomata per view and E = Number of epidermal cells per view (Metcalf and Chalk 1979). Basic terminologies used in the description of stomata were those of Metcalf and Chalk (1979).

RESULTS AND DISCUSSION

The foliar epidermal characteristics of the three ferns studied are presented in Figure 1 and Table 1.

Abaxial surfaces

Nephrolepis biserrata

Epidermal cells were irregularly shaped with thick and wavy anticlinal walls, epidermal cells were 63-66 per field view. The stomatal type was diacytic and anomocytic, with elliptical to oblong guard cells filled with chloroplasts. Stomata were about 18 per field view. Trichome types were non-glandular, multicellular, uniseriate trichomes present, and numerous.

Phymatosorus scolopendria

Epidermal cells are irregularly shaped with thick wavy to sinuous anticlinal walls. Epidermal cells were 205-210 number per field view. Crystal sand was present. The stomatal type was aphidiacytic with elliptic to oblong guard cells filled with chloroplasts. The stomata were 84-87 per field view. Trichomes were not present.

Microgramma mauritiana

Epidermal cells were irregularly shaped with thick wavy to sinuous anticlinal walls. Epidermal cells were 183-185 in number per field view. There was numerous crystal sand. The stomatal type was anomocytic and diacytic with elliptical to oblong guard cells filled with chloroplasts; the stomata were 23-25 per field view. There were no trichomes.

Adaxial surfaces

Nephrolepis biserrata

Epidermal cells were irregularly shaped with thick, wavy, sinuous anticlinal walls. Epidermal cells per field view were 47-53. The stomatal type was anomocytic and diacytic, bigger but fewer than those in the abaxial surface. No stomata per field view = 38. Trichomes, non-glandular, multicellular, uniseriate, and numerous guard cells were filled with chloroplasts. There was crystal sand in the foliar epidermal layer.

Phymatosorus scolopendria

Epidermal cells were irregularly shaped with less wavy anticlinal walls. Epidermal cells per field view = 146-153. The stomatal type was anomocytic and diacytic, with oblong to elliptic-shaped guard cells filled with chloroplasts. Stomata were 88-90 per field view. There were no trichomes. There were numerous crystals in the form of crystal sand.

Microgramma mauritiana

Epidermal cells were highly irregularly shaped with thick, wavy, and highly sinuous anticlinal walls. Epidermal cells were 188-193 in number per field view. There was numerous crystal sand. There were no stomata; also, trichomes were not found.

Discussion

Epidermal anatomical characters have been regarded as important in the classification of vascular plants, and these characteristics are known to provide additional features, which along with other characters, are usually of taxonomic value in the classification and identification of plants.

This study provides a comprehensive micro-morphology of the three ferns studied. The epidermal cell shape of the three species was irregularly shaped with thick wavy anticlinal walls, as reported by Oloyede et al. (2011). The stomatal type patterns of the studied species represented useful diagnostic characteristics, as Oloyede et al. (2011) reported. Variations in types, arrangements, and distribution of stomata are characters that are taxonomically important at the generic level of classification, as reported by Oloyede et al. (2011).

Table 1. Foliar epidermal characters of three fern species studied

Abaxial surface	***Nephrolepis biserrata***	***Phymatosorus scolopendria***	***Microgramma mauritiana***
Epidermal	63-66 per field view	205-210 per field view	188-193 per field view
Cell shape	Irregularly shaped	Irregularly shaped	Irregularly shaped
Epidermal cell wall	Thick and wavy anticlinal wall	Thick, wavy, and highly sinuous anticlinal walls.	Thick, wavy, and highly sinuous anticlinal walls
Stomatal type	Diacytic and anomocytic	Amphidiacytic	Stomata absent
Stomatal distance	18 per field view	84-87 per field view	Stomata absent
Stomatal index	22	29	Stomata absent
Shape of guard cells	Elliptical to oblong	Elliptic to oblong	Guard cells absent
Trichome	Non-glandular	Trichomes absent	Trichomes absent
Trichome type	Uniseriate trichomes	Trichomes absent	Trichomes absent
Adaxial	*N. biserrata*	*P. scolopendria*	*M. mauritiana*
Epidermal	47-53 per field view	146-153 per field view	183-185 per field view
Cell shape	Irregularly shaped	Irregularly shaped	Irregularly shaped
Epidermal Cell wall	Thick and wavy to sinuous anticlinal walls	Less wavy anticlinal walls	Thick wavy to sinuous anticlinal walls
Stomatal type	Anomocytic and diacytic and bigger size than the abaxial	Anomocytic and diacytic	Anomocytic and diacytic
Stomatal distance	38 per field view	88-90 per field view	23-25 per field view
Stomatal index	43	37	12
Shape of guard cells	Elliptical to oblong	Oblong to elliptical	Elliptical to oblong
Trichome	Non-glandular	No trichomes	No trichomes
Trichome type	Uniseriate trichomes	Not present	Not present
Crystals	Crystal sands found in foliar epidermal layer	Presence of numerous crystal sands in form of crystal sand	Presence of numerous crystal sand

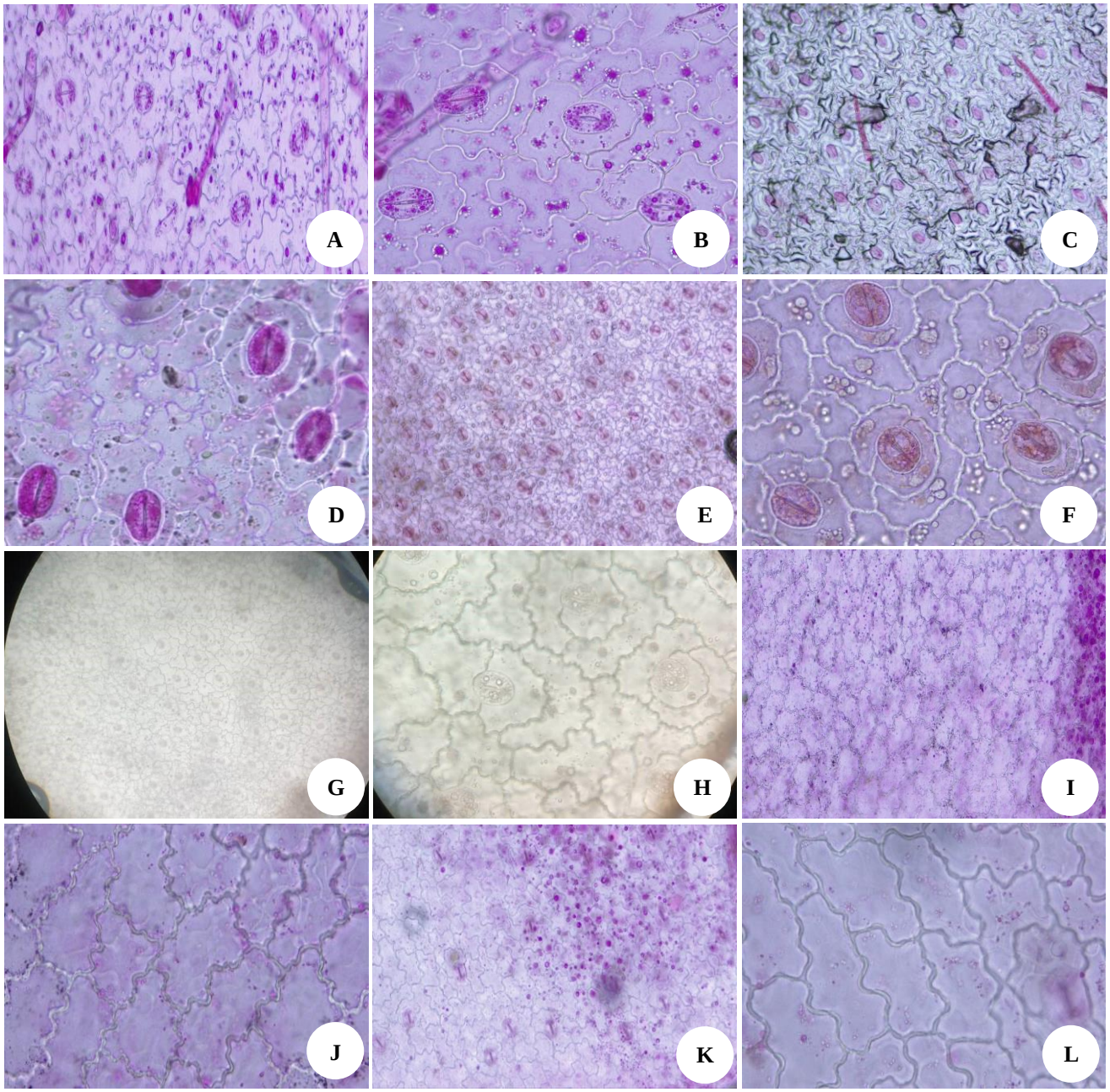

Figure 1. A-L. Epidermal characters of the ferns studied. A. Abaxial of *Nephrolepis biserrata* x100, B. Abaxial of *N. biserrata* x400, C. Adaxial of *N. biserrata* x100, D. Adaxial of *N. biserrata* x400, E. Adaxial of *Phymatosorus scolopendria* x100, F. Adaxial of *P. scolopendria* x400 G. Abaxial of *P. scolopendria* x100, H. Abaxial of *P. scolopendria* x400, I. Adaxial of *Microgramma mauritiana* x100, J. Adaxial of *M. mauritiana* x400, K. Abaxial of *M. mauritiana* x100, L. Abaxial of *M. mauritiana* x400

Stomata are microscopic openings on plant leaves surfaces that allow for easy passage of water vapor, carbon dioxide, and oxygen. Different stomatal types were observed and studied. These are anomocytic, Diacytic, and amphidiacytic. The stomatal type on the abaxial surface of *N. biserrata* was diacytic and anomocytic, but Oloyede et al. (2011) reported diacytic and anisocytic stomata in this same plant in Osun State, Nigeria; the variation in this report maybe as a result of environmental condition. The Stomatal type in *P. scolopendria* was amphidiacytic, while *M. mauritiana* had no stomata on the abaxial surface. On the adaxial surface, the three fern species all had anomocytic and diacytic stomata, which can be used to delimit the species for taxonomic purposes.

The stomatal index varied from one species to another. The stomatal index for the abaxial surfaces of *P. scolopendria* was twenty-nine (29), followed by *N. biserrata* twenty-two (22), while *M. mauritiana* had no stomatal index. Adedeji and Jewoola (2008) reported that the stomatal index is constant for any given, and the value is more uniform on the abaxial surface than the abaxial surface except in an isobilateral leaf. The adaxial stomatal

index for *N. biserrata* was forty-three (43) as the highest, *P. scolopendria* thirty-seven (37), and *M. mauritiana* twelve (12) as the lowest (Table 1). This result conforms to the findings by Essiet and Iwok (2014) that the stomatal index is independent of the environment or size of the leaf surface and, thus, serves as a reliable tool for identification.

Trichomes were found both on the abaxial and adaxial surfaces of *N. biserrata*, and it conforms to the findings of Oloyede et al. (2011) that the trichomes of *N. biserrata* are multicellular non-glandular, uniseriate, and numerous while absent on the abaxial and adaxial of *P. scolopendria* and *M. mauritiana.* This is due to the nature of the leaf surface of the fern species. Trichome types in plants are very useful for delimitating and identifying plants, even in the present study. Trichomes function in the reduction of the rate of transpiration in plants they occur. They are also used for protection against insect infestation.

In conclusion, the three species studied show close interrelationships in their foliar anatomical features, which can be used to identify, delimit and classify them. The foliar epidermal characters of significance in the delimitation of the studied species include type, shape, and number of stomata, epidermal cell shape, number, presence and absence of trichomes and trichome type on both abaxial and adaxial surfaces as well as the epidermal cell wall pattern. It is, therefore, indicated that the foliar anatomical similarities displayed among these three species are why they are grouped into the same family, while their differences are also why they are separated into different genera. Further evaluation using other taxonomic markers, such as phylogenetic and molecular properties, is recommended to provide more diagnostic characteristics.

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