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Morphological and foliar epidermal studies of fronds of *Platycerium bifurcatum* and *P. superbum* in Rivers State, Nigeria

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Abstract. *Ajuru MG, Joshua JA, Chikere LC, Ibiye A. 2024. Morphological and foliar epidermal studies of fronds of* Platycerium bifurcatum *and* P. superbum *in Rivers State, Nigeria. Cell Biol Dev 8: 28-35.* This work investigated the morphological and foliar epidermal characters of the sterile and fertile fronds of *Platycerium bifurcatum* (Cav.) C. Chr. and *Platycerium superbum* de Jonch. & Hennipman in Rivers State University, Nigeria. The two species of ferns, *P. bifurcatum* and *P. superbum*, belong to the family Polypodiaceae. They are unique epiphytes and have intrigued botanists, horticulturists, and plant enthusiasts; investigating plants' morphological and foliar anatomical structures has been crucial for plant classification. Qualitative and quantitative analytical methods were used for morphological study, while light microscopy was used for foliar anatomical study. Results showed that *P. bifurcatum* is a perennial, evergreen, broadly terrestrial, epiphytic fern. The rhizome is clingy, brown, or copper-colored, short, coarse, hidden, and enclosed in more than two peltate scales, and the frond is dimorphic (sterile and fertile), while *P. superbum* is a perennial, broad, and multi-branching terrestrial and epiphytic fern. The rhizome is a short-creeping, hidden, brown, or copper-colored structure, covered with chaffy, lanceolate scales and embedded in a mass of fronds and roots, and the frond is dimorphic. Epidermal cell shapes were found to be irregular, with thick and straight to wavy anticlinal walls in the fertile fronds, and irregular, with wavy to sinuous, thick anticlinal walls in the sterile fronds. The stomata types observed were diacytic, amphidiacytic, and anomocytic, except for the abaxial and adaxial surfaces of the sterile fronds of *P. bifurcatum*, which were paracytic and hemiparasitic. This study focuses on recognizing morpho-anatomical characters of the fronds of these species, which will help in taxonomy, species identification, future comparisons, and quality control of the botanicals.

Keywords: Dimorphic frond, epiphytic ferns, *Platycerium*, Polypodiaceae, stomata

INTRODUCTION

In tropical and subtropical regions of the world, *Platycerium* ferns primarily inhabit the canopies of trees, mostly growing as epiphytes. They thrive very well in the restricted space of tree branches, due to their unique growth form, and this helps them make efficient use of available light and nutrients. The lifestyle of this epiphytes play a vital role in forest ecosystems, and this contributes to canopy structure, thereby providing microhabitats for various organisms, and it enables them to participate in nutrient cycling (Koller 2005).

Horticulturists and gardeners are awed and captivated by the species of *Platycerium* because of their aesthetic beauty and ability to adapt to any environmental conditions during cultivation (Koller 2005). Several species of *Platycerium*, including *P. bifurcatum* and *P. superbum*, are popularly used for ornamental purposes in gardens; they are also used in indoor spaces and as greenhouse plants. They are seen as unique plants due to their intriguing frond shapes, addition to landscape environments, and growth habits that beautify the environment (Hennion et al. 2019). *Platycerium* serves as microhabitats for different organisms and helps recycle nutrients (Koller 2005).

Platycerium bifurcatum (Cav.) C. Chr., belonging to the family Polypodiaceae, is commonly called Elkhorn fern. It is an attractive and distinctive epiphyte, and it is known and recognized for its unique frond appearance. The fertile fronds resemble the antlers of a stag, while the sterile fronds form a shield-like basal structure. The shield-like structure of the sterile frond protects and supports the fern, while the antler-like structure of the fertile fronds is important for reproduction and spore production (Hennion et al. 2019). The *P. bifurcatum* is economically important for horticultural and ornamental purposes. The uniqueness of its and its adaptability to cultivation make it a very desirable ornamental houseplant. It is usually placed on wooden plaques or bark to facilitate its natural epiphytic growth habit, giving a touch of tropical elegance to indoor greenery and gardens (Hennion et al. 2019).

Platycerium superbum de Jonch. & Hennipman, commonly called Staghorn, is an epiphytic fern or sometimes lithophytic. The fern resembles a tangle of antlers, but on closer look, it possesses an impressive 'nest' frond, 1m wide when it matures. Falling leaves and insects are easily collected inside the 'nest' frond, serving as a valuable source of potassium and calcium. These are nutrients required for the production of their large fronds, and this frond enables the fern to attach itself to the host tree. The 'nest' frond wraps around the rhizomes and short roots to protect them. It is clasped using its root-like features onto the furrows in the bark (Kreier and Schneider 2006). The fern produces a mass of spores during the summer, which coupled with its general size, is a

distinguishing feature for the species (Hennipman and Roos 1982).

Young leaves of *P. superbum* are used to treat ulcers in Nigeria (Pemberton 2003). The leaf extract is also taken orally, two months after conception, to prevent miscarriage in women (Flora and Ubah 2006). It is also used to treat the following ailments: edema, coughs, and hypertension (Okoli et al. 2007). It has been reported that polysaccharides have been isolated and characterized in this species (Omeje et al. 2007). Morphological and anatomical plant features are seen as valuable tools for classification, and this has enabled plant scientists to organize and categorize plants into distinct groups based on shared characteristics (Kissinger 2015).

Moreover, little attention has been paid to species of *Platycerium*, including *P. bifurcatum* and *P. superbum*, especially regarding morphological and anatomical studies. Also, owing to the importance of morpho-anatomical characters as an additional tool to taxonomic and phylogenetic studies, the present study aimed at investigating the morphological and foliar anatomical characters of the leaves of *P. bifurcatum* and *P. superbum* in Rivers State University, Nigeria.

MATERIALS AND METHODS

Study area

The study area was Rivers State University (RSU) campus within Port Harcourt metropolis, 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 4°31'-4°40' N and longitudes 70°'-7°10'E (Figure 1). It has an elevation of about 10-15 m above sea level (Ubong et al. 2015).

Sample collection, identification, and preparation

The research study took place from July to September 2023. The samples of the two fern species were freshly collected from Pine trees found in Rivers State University, Nkpolu-Oroworukwo, Rivers State (Table 1). They were taken to the Department of Plant Science and Biotechnology Laboratory in polythene bags with tags. They were identified and authenticated by Dr. M. G. Ajuru, a Plant Taxonomist in the Department. The samples were rinsed in distilled water several times and used for various taxonomic studies.

Table 1. Sources of collection of plant materials used for the study

Plant Samples	Accession Number	Date of Collection	Location of Collection (Lat 4°31'-4°40' N, Lon 70°'-7°10' E)
Platycerium bifurcatum	RSUPb015	7-8-2023, 8-8-2023, 9-8-2023	EF, FSCP, RSC
Platycerium superbum	RSUPb016	7-8-2023, 8-8-2023, 9-8-2023	RMG, RCC, RRA

Note: RCC: RSU Catholic Church, RMG: RSU Main Gate, RRA: RSU Roundabout, EF: Engineering Faculty, FSCP: Faculty of Science Carpark, RSC: RSU Staff Club

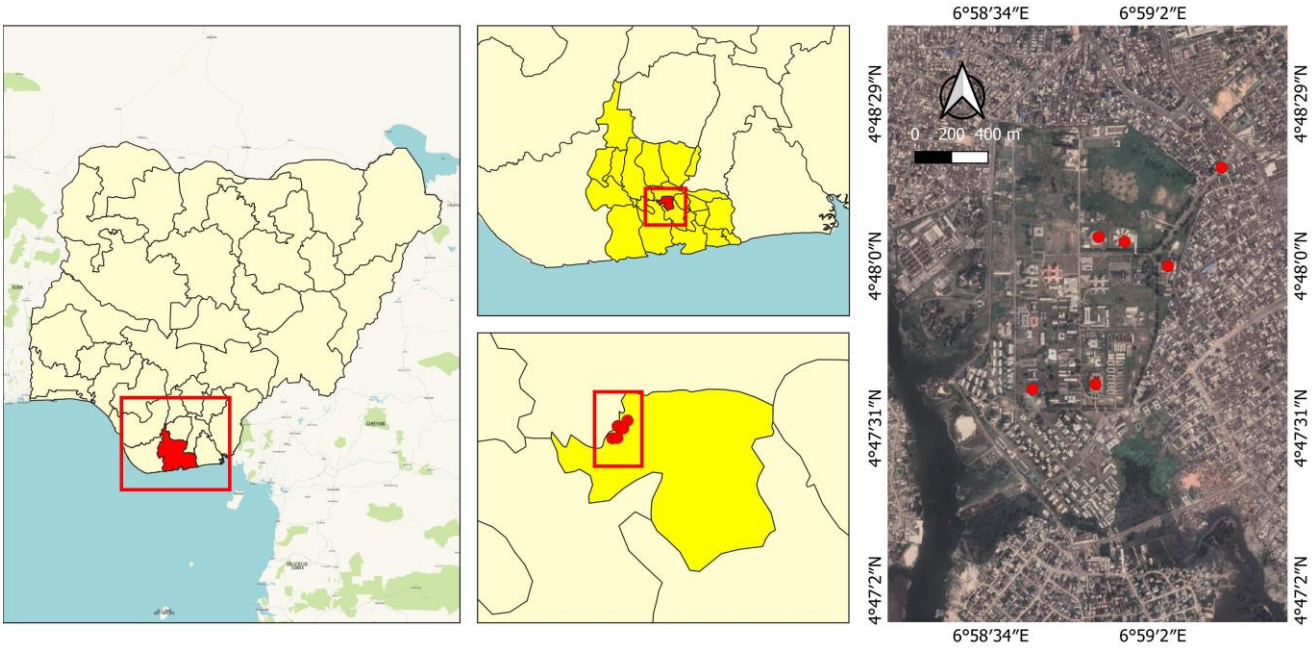

Figure 1. The map of Rivers State University (RSU), Nigeria shows the sampling stations

Procedures

Morphological study

The morphological features of the selected fern species, were recorded on matured living plant samples. References were also made to Cryptogrammic Gallery: A Reference Field Guide (Edwin-Wosu 2019). Quantitative morphological studies included measuring the frond length, width, petiole length, and plant height using a meter rule and measuring tape. Qualitative morphological plant features studied included the frond nature, color, texture, shape, surface, presence or absence of spore, plant habit, etc.

Foliar epidermal study

Fresh matured fronds were prepared according to the simplified method described by Okoli (1992). The fresh fronds were soaked in sodium hypochlorite (5%) for 3-5 minutes to soften the tissues and make it easy to scrap. The fronds were placed on a flat surface, and the adaxial surface was scraped off gently with a 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, which were stained with 1% safranin for 3 minutes and rinsed again in distilled water. The specimens were mounted with 3 drops of glycerine and a cover slip placed correctly. Slides of both abaxial and adaxial surfaces of fertile and sterile fronds were prepared. These were examined using a light microscope, and photographs were taken.

The qualitative characteristics of the foliar epidermis were observed and taken with a light microscope. The number of epidermal cells and stomatal per field view were noted and recorded.

The Stomatal Index for both species was calculated using the formula below:

The calculation for the Stomatal Index (I)

$$I = \frac{S}{E+S} \times 100\%$$

Where:

I : Stomatal Index

S : Number of stomata per unit area

E : Number of epidermal cells plus the subsidiary cells in the unit area.

RESULTS AND DISCUSSION

Morphological features of *Platycerium bifurcatum*

The *P. bifurcatum*, commonly called staghorn or elkhorn fern, belongs to the family Polypodiaceae. It is a perennial, evergreen, broadly terrestrial, epiphytic fern growing up to 1 m tall and 90 cm wide (Figure 2). It grows on trees, trunks, and branches. The stem or rhizome is clingy, brown, copper-colored, short, coarse, hidden, and enclosed in more than two peltate scales. The roots are tufted solely for anchoring the plant to the tree trunk. The fronds are evergreen, gray or silver colored, leathery or velvety, pinnately compound, complex, oblong, lobed, and glabrous.

The frond is dimorphic in two rows: The sterile fronds are few, persistent, appressed to substrate, sessile, heart-shaped, shield-like, papery, and grow close or cover the root crown. The sterile frond is 34 cm long and 24 cm wide. The fertile fronds are deciduous, short-stipitate, erect to arching or pendant, dichotomously branched, antler-like, and covered with a grayish-white felt. The fertile frond is 50 cm long and 24 cm wide. The spores are borne in sori on the lower part of the frond at the tips of the fertile fronds, as shown in Figure 2 and Table 2.

Morphological features of *Platycerium superbum*

The *P. superbum*, commonly called giant staghorn fern and of the family Polypodiaceae, is a perennial, broad, multi-branching terrestrial and epiphytic fern, growing up to 1.1 m tall and 95 cm wide (Figure 3). It has short roots that are not prolific and solely for anchoring the fern to the host tree trunk. The rhizomes are short-creeping, hidden, brown, or copper-colored structures, covered with chaffy, lanceolate scales and embedded in a mass of fronds and roots. The fronds are bright greenish, evergreen, leathery, simple pinnate, rosettely arranged, wedge-shaped, lobed, and entire, glabrous, though sometimes covered with stellate hairs.

The frond is dimorphic in two rows: The nest frond or sterile frond is rounded or reniform, appressed to the substrate, and densely covered with fawn stellate hairs, irregularly divided into elongated dichotomous spreading lobes, measuring 34 cm long and 25 cm wide. The fertile or foliage fronds are paired, pendulous, up to 5 times forked, base broadly cuneate, measuring up to 60 cm long and 30 cm wide. The sporangia are in a single patch, up to 20-40 cm wide, occurring at the sinus of a first fork of foliage frond on the lower surface of the frond, as shown in Table 2 and Figure 3.

Foliar epidermal features of *Platycerium bifurcatum* and *P. superbum*.

Foliar epidermal characteristics of *P. bifurcatum* and *P. superbum* are summarized in Figures 4-7:

Foliar epidermal features of fertile fronds of *Platycerium bifurcatum*

Abaxial surface or lower epidermal features

The epidermal cell is irregular, with thick and straight to wavy anticlinal walls, 85-102 per field. The stomata are diacytic and anomocytic with elliptical guard cells. Trichomes were absent. There were numerous crystal sands. Stomata were 56-63 per field.

Adaxial surface or upper epidermal features

Epidermal cells are irregular or polygonal in shape with thick and straight to waxy anticlinal walls, 116-126 per field. There are crystal sands numerous on the epidermal cell. There are no trichomes but very few stomata, 12-16 per field. They are diacytic and anomocytic. The guard cells are elliptical, surrounded by subsidiary cells.

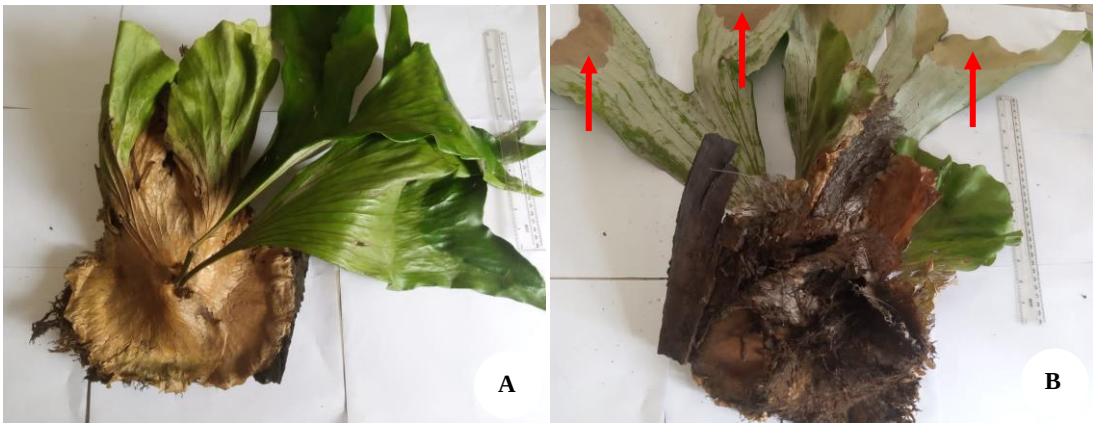

Figure 2. *Platycerium bifurcatum*: A. Habit, B. Sori (*arrow*)

Figure 3. *Platycerium superbum*: A. Habit, B. Sori (*arrow*)

Foliar epidermal features of sterile fronds of *Platycerium bifurcatum*

Abaxial surface or lower epidermal features

The epidermal cells are irregular, with wavy to sinuous, thick anticlinal walls. There are from 136 to 147 epidermal cells per field. The stomata are paracytic and hemiparasitic and ranging 121 to 134 per field. The guard cells are widely elliptic. There are no trichomes. There are numerous crystal sands present in the epidermal and subsidiary cells.

Adaxial surface or upper epidermal features

The epidermal cells are also irregular, with wavy to sinuous anticlinal walls that are thick. There are 111-122 epidermal cells per field, few stomata, and no trichomes. There are numerous crystals of sand in the epidermal cells. The stomatal type is paracytic and hemiparasitic, ranging between 52-67.

Foliar epidermal features of fertile fronds of *Platycerium superbum*

Abaxial surface or lower epidermal features

The epidermal cell is irregular, with thick, straight-to-wavy anticlinal walls. There are between 79-101 per field. The stomata were diacytic and amphidiacytic, with elliptical guard cells. Trichomes were present, stellate and non-glandular trichomes. Stomata were 75-84 per field with numerous crystal sands.

Adaxial surface or upper epidermal features

Epidermal cells are irregular, with thick and straight to wavy anticlinal walls. They are pentagonal, numerous, between 79-88 per field. There are no stomata and trichomes, with numerous crystal sands.

Foliar epidermal features of sterile fronds of *Platycerium superbum*

Abaxial surface or lower epidermal features

The epidermal cells were irregular, with thick, straight-to-wavy-to-sinuous anticlinal walls. They were hexagonal in shape, numerous, between 82-95 per field. The stomata were diacytic and amphidiacytic, with elliptical to oblong guard cells. Stomata were between 29-34 per field, with numerous crystal sand and no trichomes.

Adaxial surface or upper epidermal features

The epidermal cells were irregularly shaped, specifically pentagonal, with thick, straight-to-wavy anticlinal walls. The epidermal cells are between 76-84 per field. The stomata were diacytic and amphidiacytic, with elliptical to oblong guard cells. Stomata were between 39-48 per field with numerous crystal sands and no trichomes.

Table 2. Morphological features of *Platycerium bifurcatum* and *P. superbum*

Parameters	***Platycerium bifurcatum***	***P. superbum***
Plant lifespan	Perennial	Perennial
Habit/form	Cascading/Mounding	Broad and multi-branching
Habitat	Terrestrial	Terrestrial
Plant type	Epiphytic fern	Epiphytic fern
Root system	Tufted for anchoring	Short roots, not prolific
Plant height	1 m tall and 90cm wide	1.1m tall and 95cm wide
Climbing method	Clinging	Drooping
Stem/rhizomes color	Brown/Copper	Brown/copper
Texture	Coarse	Coarse
Stem bud scales	Enclosed in more than two scales.	Covered with chaffy lanceolate scales.
Frond nature	Evergreen broad-leaf plant	Greenish and evergreen
Frond colour	Gray/Silver	Bright green
Frond texture	Leathery and velvety	Leathery
Leaf type	Compound (Pinnate compound)	Simple pinnate
Frond arrangement	Complex	Complex (rosette)
Frond shape	Oblong	Wedge-shaped
Frond margin	Lobed	Lobed and entire
Frond surface	Glabrous	Glabrous, sometimes covered with stellate hairs
Frond length and width	Sterile frond = 34 cm long and 24 cm wide. Fertile frond = 50 cm long and 28 cm wide	Sterile frond = 34 cm long by 25 cm wide. Fertile frond = 60 cm long by 30 cm wide.
Frond appearance	Dimorphic in two rows 1. Sterile fronds, few persistent, appressed to the substrate, sessile, heart-shaped, shield-like, and papery. Grow close to the crown. 2. Fertile fronds are deciduous, short-stipitate, erect to arching or pendant, dichotomously branched (antler-like) covered with a grayish-white felt.	Dimorphic in two rows 1. Sterile or nest fronds rounded or reniform, appressed to the substrate, and densely covered with fawn stellate hairs, irregularly divided into elongated dichotomous spreading lobes. 2. Fertile or foliage fronds paired, pendulous, up to 5 times forked, base broadly cuneate.
Stipe length and width	11 cm long and 4.7 cm wide	15 cm long by 5.7 cm wide
spores		
Rhizomes	In sori on the lower part of the frond, at the tips of the fertile fronds Short, hidden, with peltate scales	Sporangia were in a single patch, 20-40 cm wide, occurring at the sinus of a first fork of foliage frond on the lower surface of the frond. Short-creeping, hidden, covered with chaffy, lanceolate scales, embedded in a mass of fronds and roots.

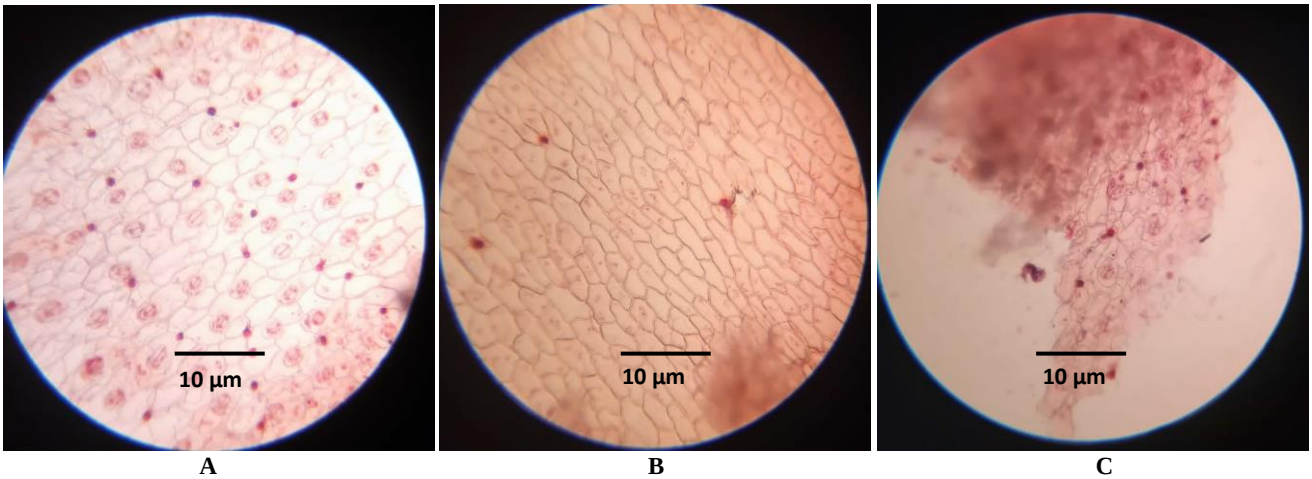

Figure 4. Foliar epidermal features of fertile frond of *Platycerium bifurcatum.* A. Abaxial Surface, B. Adaxial Surface without stomata, C. Adaxial Surface with stomata (x100)

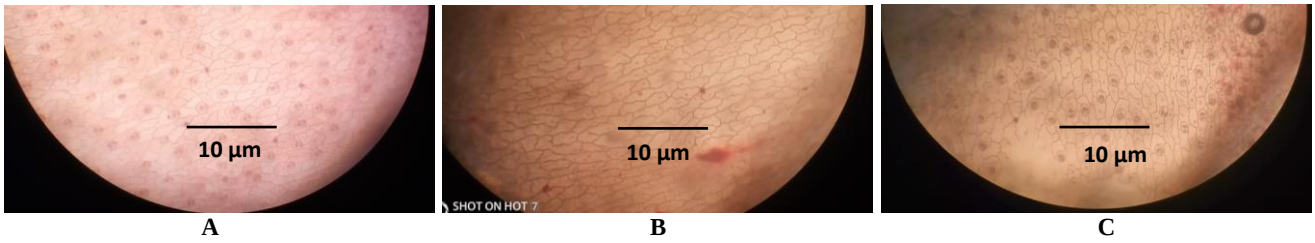

Figure 5. Foliar epidermal features of sterile frond of *P. bifurcatum.* A. Abaxial Surface, B. Adaxial Surface without stomata, C. Adaxial Surface with stomata (x100)

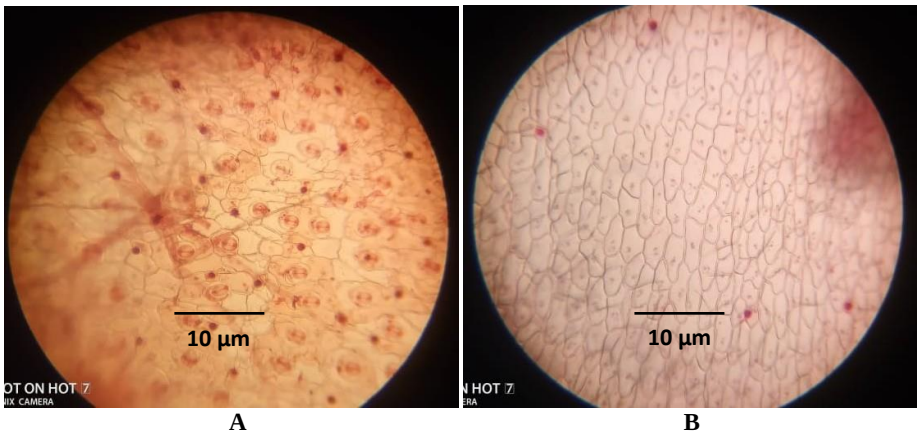

Figure 6. Foliar epidermal features of a fertile frond of *Platycerium superbum.* A. Abaxial Surface, B. Adaxial Surface (x100)

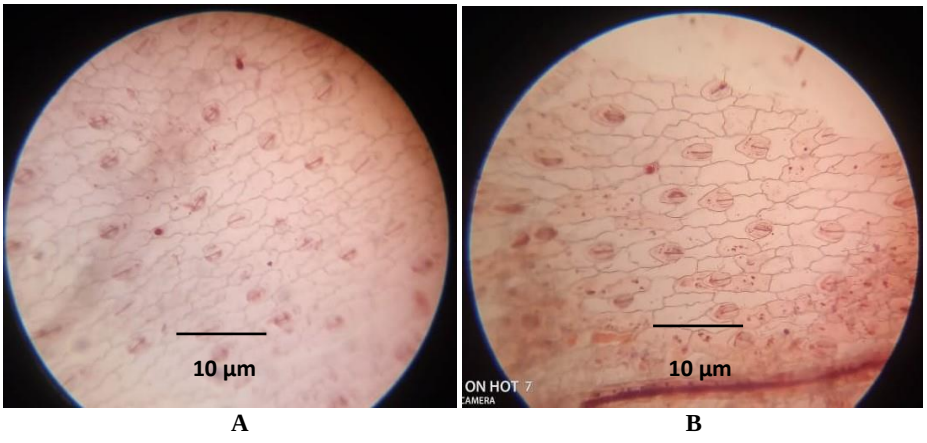

Figure 7. Foliar epidermal features of a sterile frond of *P. superbum.* A. Abaxial Surface, B. Adaxial Surface (x100)

Discussion

Morphological features are very diagnostic at the species level because they are usually employed in delimitation (Pryer et al. 2004); a mature *P. bifurcatum* can be as big as 3 feet across. Spores are produced in sporangia in the dark brownish masses (sori) on the underside of the tips of these fertile fronds, as Hoshizaki (1972) reported. Although *P. superbum* can look like a tangle of antlers at first, one can see the impressive 'nest' frond (sterile frond) on closer inspection. They are broad and multi-branching inhabit, and have short roots that are not prolific, as reported by Hoshizaki (1972) and Schneider et al. (2004). The *P. bifurcatum* and *P. superbum* have creeping rhizomes covered with scales; this is also similar with some species belonging to the family Polypodiaceae, mainly characterized by creeping rhizomes covered with varying scales (Smith et al. 2008). The leave or frond type of *P. bifurcatum* and *P. superbum* have the pinnate type of primary venation. On examination of the leaf venation traits, it has been observed that the 27 representative species of the family Polypodiaceae possess the pinnate type of primary venations (Tan and Buot 2020). The leaf nature of *P. bifurcatum* and *P. superbum* are evergreen, the leaf margins are lobed, and the sori are found on the lower

surfaces of the frond. This is similar to some species belonging to the family Polypodiaceae, which has evergreen leaves; the leaves are once-divided, with lobes or leaflets on opposite sides of the central axis, and the round sori are found on the underside of the frond (Tan and Buot 2020).

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. Foliar epidermal features are essential in taxonomy and species delimitation (Uphof 1962; Scatena et al. 2005; Pryer et al. 2016). This study provides a comprehensive micro-morphology of the two ferns studied. The epidermal cell shape of the two species was irregularly shaped with thick, wavy anticlinal walls, as reported by Oloyede et al. (2011).

The stomatal type in the two species studied was a useful diagnostic characteristics for the delimitation of the species, as stated by Oloyede et al. (2011). 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). The stomata consist of two elongated guard cells surrounding a stomatal pore and are often surrounded by one or more subsidiary cells (Barclay et al. (2007). According to Adegbite (2008), most plants have numerous stomata on the abaxial surfaces than on the adaxial surfaces; this curtails excessive water loss through transpiration. Different stomatal types were observed and studied; these are anomocytic, diacytic, and amphidiacytic. The stomatal type on the abaxial surface of *P. bifurcatum* was diacytic and anomocytic, as reported by Oloyede et al. (2011), Khan et al. (2013), and Shabeena et al. (2014); the Stomatal type in *P. superbum* was diacytic amphidiacytic. On the adaxial surface, *P. bifurcatum* had anomocytic and diacytic stomata, which can be used to delimit the species for taxonomic purposes, whereas *P. superbum* had no stomata but Oloyede et al. (2011) reported diacytic and anomocytic stomata in this same plant in Osun State, Nigeria; the variation in this report maybe as a result of environmental condition.

The stomatal index varied from one species to another. The stomatal index for the abaxial surfaces of *P. bifurcatum* was twenty-nine (29), followed by *P. superbum* twenty-two (22). 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 *P. bifurcatum* was forty-three (43), and the highest for *P. superbum* was thirty-seven (37). 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 identification tool.

Trichomes were absent on the abaxial and adaxial surfaces of *P. bifurcatum* while present on the abaxial and absent on the adaxial of *P. superbum*; 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 where they occur. They are also used for protection against insect infestation. The epidermal cell shape of the two species was irregularly shaped with thick, wavy, sinuous anticlinal walls, as Oloyede et al. (2011) reported.

Different stomatal types were observed and studied. These are paracytic, hemiparasitic, diacytic, and amphidiacytic. The stomatal type on the abaxial and adaxial surface of *P. bifurcatum* was paracytic and hemiparasitic, as reported by Oloyede et al. (2011), which can be used to delimit the species for taxonomic purposes, while *P. superbum* has diacytic and amphidiacytic stomata, as Oloyede et al. (2011) reported. The stomatal index varied from one species to another. The stomatal index for the abaxial surfaces of *P. bifurcatum* was twenty-nine (29), followed by *P. superbum* twenty-two (22). 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.

Trichomes were absent both on the abaxial and adaxial surfaces of *P. bifurcatum* and *P. superbum.* 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 where they occur. Additionally, they are also used for protection against insect infestation.

In conclusion, studies on plants' morphological and foliar epidermal features have helped us understand the diversity of plant forms and how plants evolved over time. The two species of ferns *P. bifurcatum* and *P. superbum*, though they may look similar, the studies on their morphological and foliar epidermal features have proven that there is variation in the Root system, Climbing method, Habit/Form, Rhizomes, Frond appearance, the shape of the frond, stem bud scales, adaxial and abaxial surface, etc. Platycerium's morphological and foliar epidermal features should be investigated in other parts of Rivers State to provide more reviews for researchers and students worldwide.

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