Advanced Journal of Plant Biology
Volume 4(1), pages 1-7, February 2023
Article Number: 7ED3339C1
ISSN: 2992-4928
https://doi.org/10.31248/AJPB2021.016
https://integrityresjournals.org/journal/AJPB

Full Length Research

Morphology and anatomy of rhizome and root structures in seven *Pteris* L. species (Pteridaceae) from Southwestern Nigeria

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Received 28th October, 2021; Accepted 1st December, 2021

ABSTRACT: Morphological and anatomical structures of seven *Pteris* species from various locations in Southwestern Nigeria were examined. This study aimed to affirm the affinities and relevance of rhizome and root structures within the genus *Pteris.* Mature healthy plants of each species were collected, and morphological observations of rhizomes and roots were documented for each species. The transverse sections of rhizomes were prepared by cutting at 10 µm thickness using Reichert Sliding Microtome while roots were embedded through standard procedures. The results showed that morphologically, rhizomes in *P. acanthoneura, P. atrovirens*, *P. mildbraedii*, *P. togoensis* and *P*. *similis* were erect to sub erect, but rhizomes were short creeping in *P. ensiformis* and *P. vittata*. The anatomy of the rhizomes showed a typical dictyostele pattern with variable number of leaf gaps, while roots of all the *Pteris* species were the diarch type with two xylem bundles alternating with two phloem bundles. The morphological and anatomical structures of the rhizomes and the roots provide useful specific distinctions of taxonomic importance for the genus *Pteris* and the family Pteridaceae.

Keywords: Fern systematics, Pteridaceae, *Pteris,* rhizome, root.

INTRODUCTION

Pteridaceae is a large family with worldwide distribution preferentially in the tropics. It belongs to the order Polypodiales and class Polypodiopsida, consisting of about 50 genera (PPGI, 2016). The family is well distributed in various habitats and occupies different ecological niches such as aquatic, epiphytic terrestrial, xeric and hot deserts (Sharpes et al., 2010). Ferns differ from other vascular plants in some features. They have three major parts – the rhizome, the fronds, and the reproductive structures called sporangia. The character-ristics of each of these three parts of the fern plant are used for classification and identification (Christenhusz and Chase, 2014). The rhizome is the stem of the fern plant and comes in three basic forms. The first form is an erect plant that is laying on its side which develops horizontally beneath the surface of the soil. In the second form, the rhizome may protrude from the soil to form a small trunk referred to as caudex. This is prominent in tree ferns such as Blechnum are essentially the stem of the fern plant. Third, the rhizomes may creep along or be completely underground. New fronds and roots grow from the rhizomes. These structures contain xylem and phloem tissues assisting in transporting water, minerals and nutrients throughout the plant (Rasheen,1999). The rhizome has a growing tip that produces new fronds. Rhizomes can be comprised of solid, hard tissue or of fleshy, soft skin. Within the rhizome is vascular tissue that transports water, minerals, and food. Ferns can have either a short, medium, or long creeping rhizome. The shorter rhizomes have less surface area for fronds, resulting in a cluster of fronds as in *Asplenium nidus.* Fern rhizomes**,** as in other plants, are capable of producing the shoot and root systems of a new plant. They are used to store starches and proteins and enable plants to survive during unfavorable seasons. When classifying fern species, vegetative characters such as the rhizome characters become important. Rhizomes can serve the same function on ferns as a root system in other types of plants. Ferns are naturally hardy and reproduce by division of the rhizomes or by spores that grow on the underside of the leaves. However, growing ferns from the rhizomes is the easier and faster (USDA, 2019). In nature, fern rhizomes serve a greater purpose to the environment. Fern rhizomes help aid in erosion control and soil stabilization. The rhizomes, because they are thin and long and grow horizontally beneath the surface of the ground, help to stabilize the soil (Xue et al., 2016). Sathiyaraj et al. (2015) and Maroyi (2017) compiled detailed medicinal benefits, including those of the fern roots and rhizomes. Therefore, the aim of this study is to examine the relevance of morphology and anatomical structures in the *Pteris* species in relation to their identification and classification.

MATERIALS AND METHODS

Transverse sections of rhizomes

The transverse section of rhizome was done at 15 µm using sledge microtome. Sections were preserved in 50% ethanol. Sections were stained in Safranin O for three minutes, rinsed in water and counterstained in Alcian blue for three minutes. The counterstained sections were rinsed again in water and then treated in serial grades of alcohol for dehydration and differentiation process. Sections were mounted on clean slides in 25% glycerol. Photomicrographs were taken with the aid of 3013ACCU-SCOPE Trinocular Microscope with Digital Camera.

Rhizome macerate

Matured rhizome of each of the *Pteris* species was macerated using Schulz`s fluid obtained by mixing equal volume of 10% chromic acid [by dissolving 1 gm Potassium Nitrate (KNO_3) in 50 ml concentrated nitric acid (HNO_3)] and 10% nitric acid. The maceration was carried out in beaker placed on a hot plate for 15 minutes. The macerated rhizome samples were washed in five changes of water and preserved in 50% ethanol prior to staining as described by Oladipo and Oyaniran (2013).

Embedment procedure of root samples

The roots of each species were cut into smaller pieces in glass vials. These were dehydrated by soaking the root samples for 12 hours in absolute alcohol. After dehydration, clearing of the samples was carried out in absolute xylene for 12 hours. Root samples were then infiltrated by keeping them in liquid paraffin wax, which was set in the oven at 60°C for suitable period of time. The infiltrated specimens were then poured into mounds and fixed into wooden blocks. Surfaces of blocks were perforated to aid gripping. The wax blocks were trimmed to a suitable size and shape.

Root sections were cut at 5 to 15 µm with a rotary microtone. With the aid of a knife, ribbons of the paraffin sections were detached from the microtome and fastened in a water bath to clean glass slides previously rubbed with an adhesive (egg albumen). The slides were allowed to stand for few days to harden. Afterwards, slides were de-waxed with absolute xylene stained with Safranin O for five minutes, rinsed in water and counter stained with Alcian blue for 5 minutes and rinsed in water. Slides were then passed through series of alcohol (60, 70, 80, and 100%). The sections were mounted on clean slides in DPX mountants. Photomicrographs of sections were taken.

RESULTS

Transverse sections of rhizomes

***P.acanthoneura* (Plate 1 A):** Rhizome has a thick walled, storage parenchyma cells whose shapes ranges from circular to, oval short rectangular or cylindrical to polygonal. Parenchyma cell house variously shaped starch granules.

***P. atrovirens* (Plate 1 B):** Rhizome has a thick wall, variously shaped storage parenchyma cells whose shape varies from circular or cylindrical to polygonal. Parenchyma cells housed variously shaped starch granules.

***P.ensiformis* (Plate 1C):** Rhizome is occupied by variously shaped parenchyma thick walled storage parenchyma cells whose shape varied from circular, oval, short rectangular or cylindrical to polygonal.

***P. mildbraedii* (Plate 1D):** The rhizome is occupied by thick walled and variously shaped storage parenchyma cells. The shapes range from circular, oval, short rectangular or cylindrical to polygonal. Parenchyma cell contains different shapes of starch granules.

***P. similis* (Plate 1E):** Rhizome is occupied by thick walled and variously shaped storage parenchyma cells whose shapes varies from circular, oval, short, rectangular to polygonal. Parenchyma contained different shapes of starch granules.

***P. togoensis* (Plate 1F):** Rhizome is occupied by thick walled and variously shaped storage parenchyma cells whose shapes varies from circular, oval, rectangular or cylindrical to polygonal. Parenchyma cells housed different shapes of starch granules.

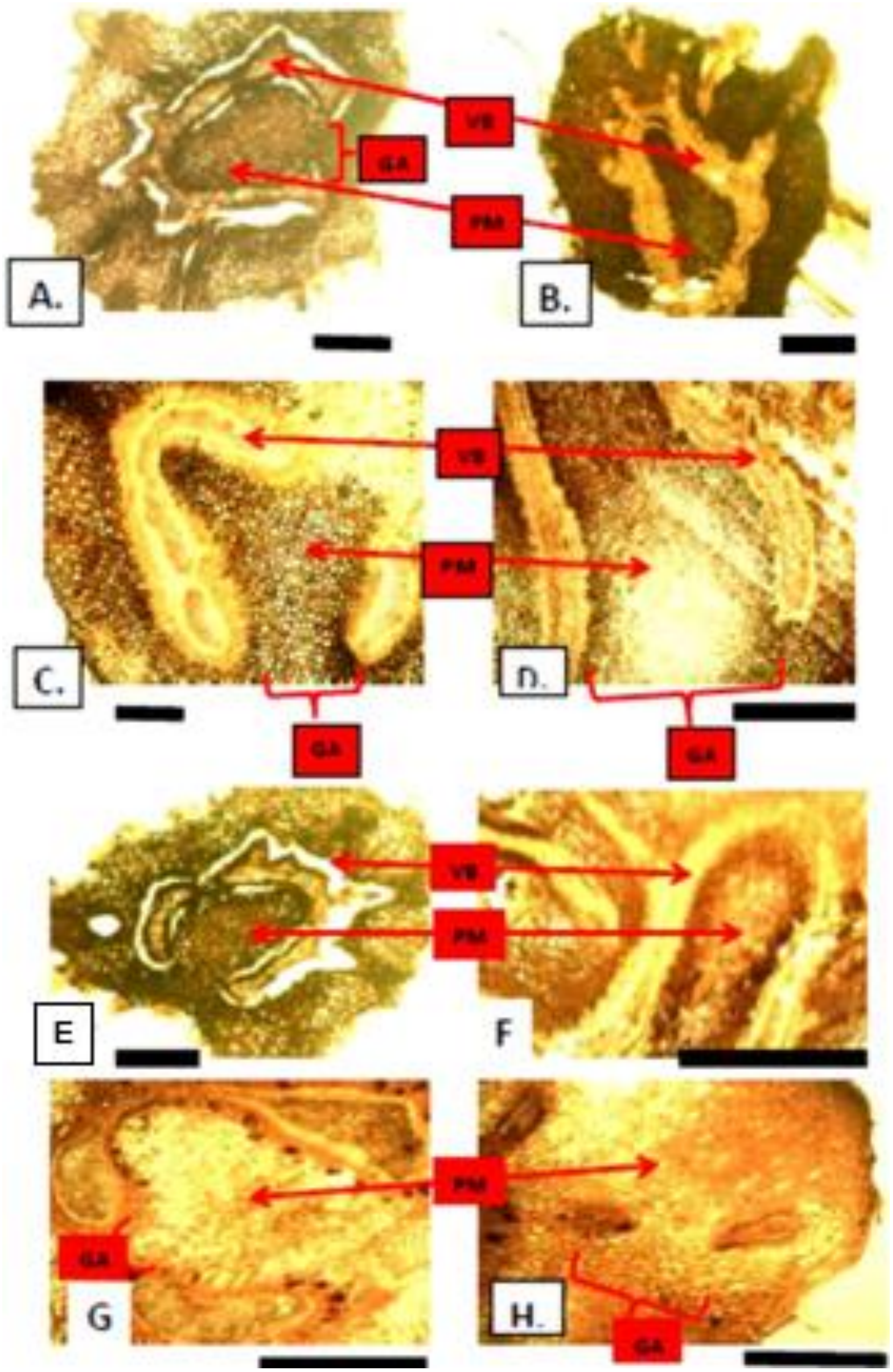

Plate 1. TS of rhizome in *Pteris* species (A - *P. acanthoneura*; B - *P. atrovirens*; C - *P. ensiformis*; D - *P. mildbraedii*; E - *P. similis*; F -*P. togoensis*; G & H - *P. vittate)*. **Legend:** VB - Vascular Bundle; PM - Parenchyma; GA - Gap. Bar = 1000 µm.

***P. vittata* (Plate 1G & H):** The rhizome is occupied by variously shaped, thick walled storage parenchyma cells. The shape ranged from circular, oval, short, rectangular or cylindrical to polygonal parenchyma. cells housed various shaped, starch granules.

Results of rhizome macerates (Plate 2A-H) from the seven *Pteris*s pecies showed scanty, thick walled, narrow lumen, non-pitted fibres that were septate only in *P. ensiformis*. In transverse sections, tracheids appear circular, oval, polygonal or polyhedral in all the species. Fibre size

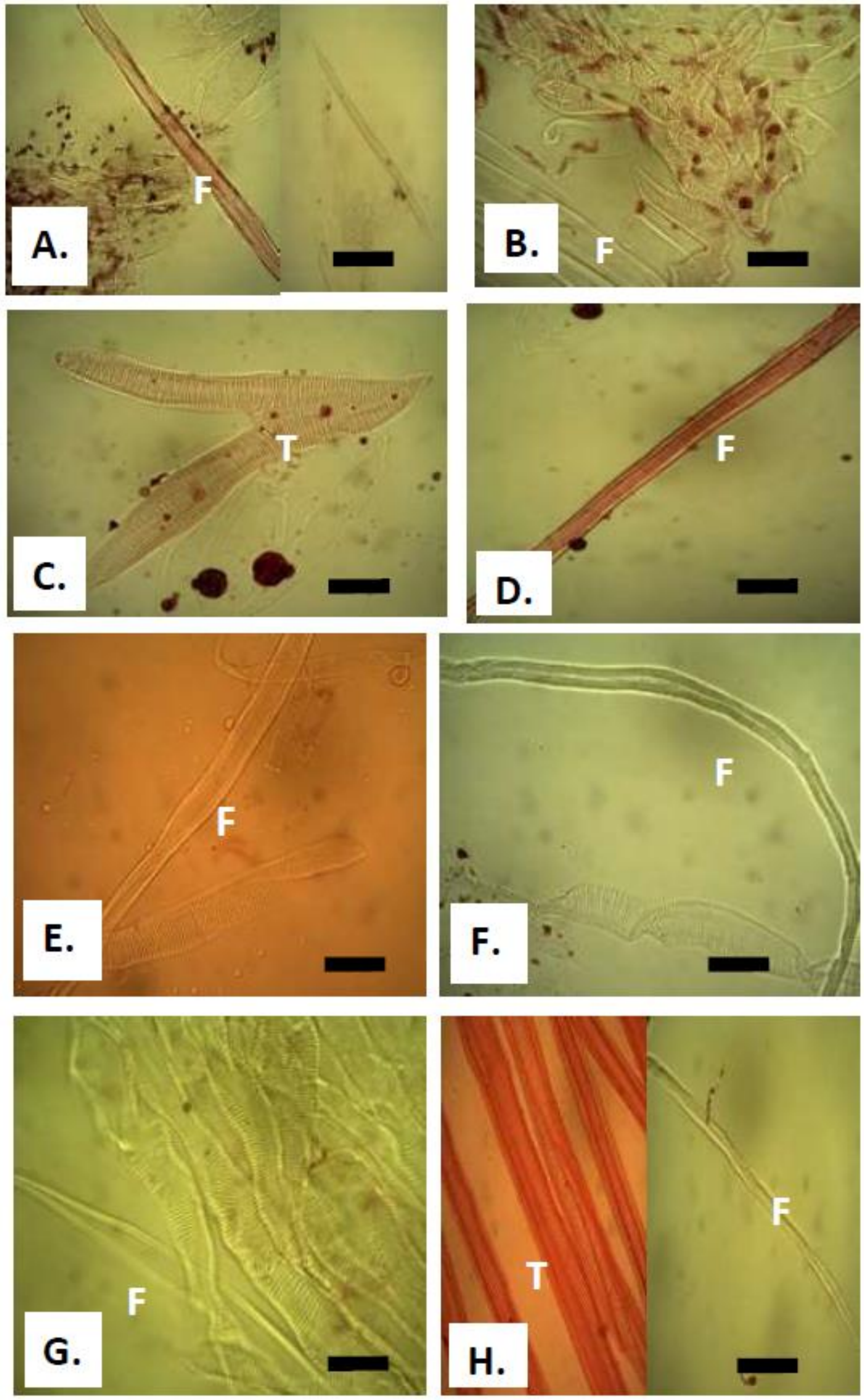

Plate 2. Rhizome macerates of *Pteris* species (A- *P. acanthoneura*; B-*P. atrovirens,* C & D - *P. ensiformis;* E - *P. mildbraedii*; F - *P. similis*; G - *P. togoensis*, H - *P. vittata*). **Legend:** F- Fibre; T- Tracheid. **Bar** A, B & H = 100 μm; C, D & G = 166 μm; E = 125 μm.

followed no specific trend but *P. acanthoneura* had a longer length, wider breadth, and wider lumen than the other species. However, size of wall thickness was constant for all the species.

Root anatomy

The cuticle was thick and striated. The epidermis was uniseriate, with the epidermal cells circular, rectangular,

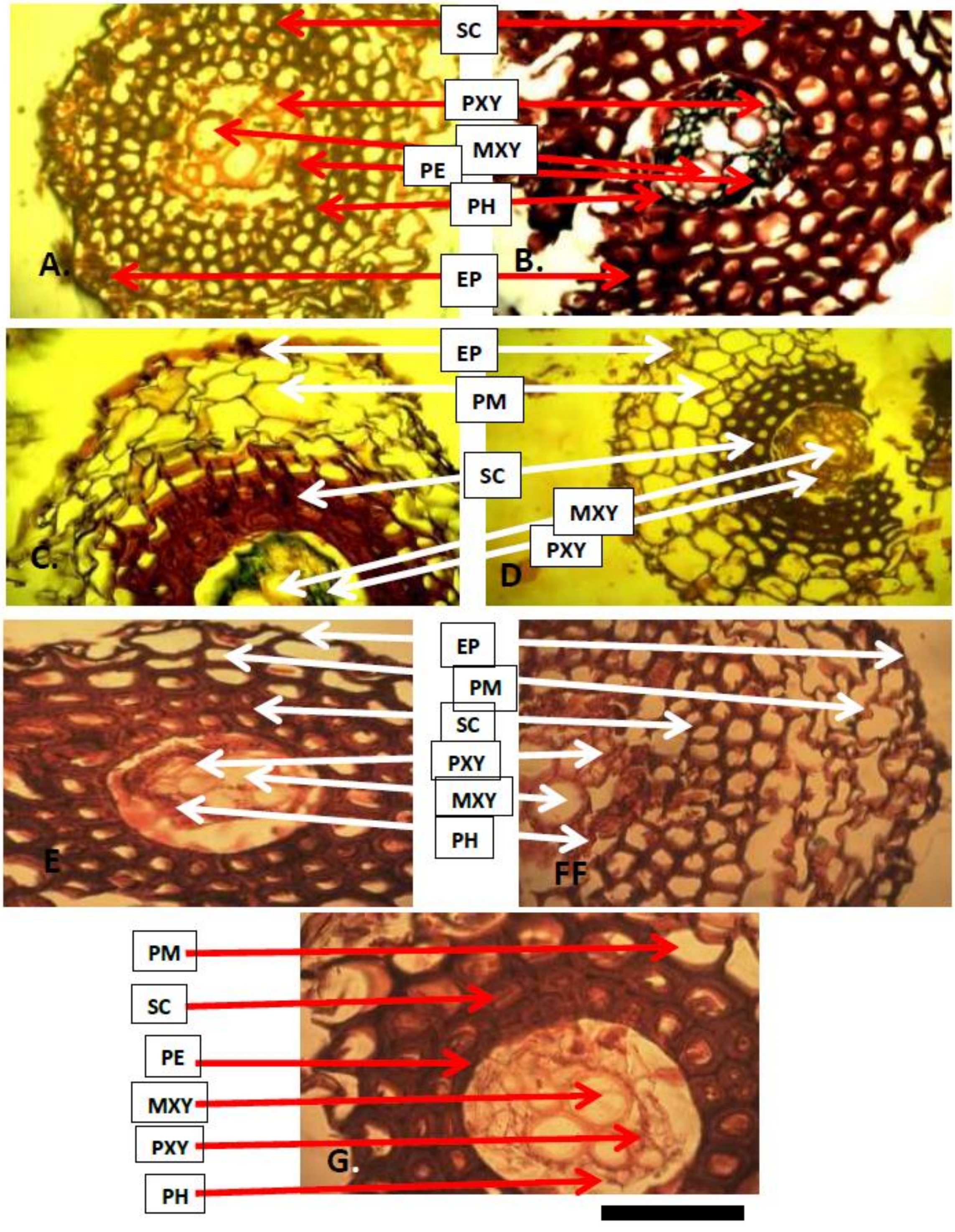

Plate 3. Transverse Sections of roots of *Pteris*species (A - *P. acacanthoneura,* B - *P. atrovirens;* C - *P. ensiformis,* D – *P. mildbraedii;* E - *P. similis*, F - *P. togoensis*, G - *P. vittata).* **Legend:** MXY- Metaxylem; PXY- Protoxylem; EP- Epidermis/ Epiblema; SC- Sclerenchyma; PH- Phloem; PM- Parenchyma; PE- Pericycle. **Bar** = 1000 µm.

and polygonal**.** The cortex was the widest zone of the root and consisted of thick-walled parenchyma cells whose sizes and shape varied from oval, cylindrical, rectangular, arc to polygonal. A narrow zone of sclerenchyma cells was found before the stele. The conducting tissue was surrounded by the pericycle, which was made up of thick-walled parenchyma cells. The xylem was in the centre with one or more metaxylem, larger in size than the two protoxylem in exarch position. Phloem surrounded the xylem. The vascular bundle was conjoint, concentric, and amphicribral.

DISCUSSION

The rhizome anatomy of all the taxa studied showed a dictyostele with variable number of meristeles due to the number of leaf gaps. The outermost layers were uniseriate, composed of thin walled, somewhat barrel shaped, compactly arranged, and cutinized parenchymal cells. The region below the epidermis was composed of multilayered, lignified, thick walled, compactly arranged sclerenchymatous cells which sometimes formed a complete cylinder. Each stele was surrounded by a uniseriate, barrel shaped, and compactly arranged parenchyma cell having casparian strips.

These findings are consistent with the description of Chang (1927) who reported that the vascular system of the rhizome in *Pteris* species is dorsiventral dictyostele of the polypodium type. He opined that the development of vascular system accessories is in connection with lateral elaboration of the leaf traces and is somewhat adaptative. Following the classification of Smith et al. (2006) and considering the leptosporangiate homosporous ferns: siphonostele (dictyostele and solenostele) stelar anatomy was reported in all observed families of polypodiales, solenostele in Osmundiales and Cyatheales, but haplostele in Hymenophyllales, Schizaeales and Gleicheniales (Nopun et al., 2016). Also, citing the heterosporous fern group, solenostele was found associated with Marsileaceae and haplostele with Salviniaceae. Protostele was regarded as more primitive than the siphonostele, while the solenostele was recognized as more advanced than the haplostele,

Xylem in all the studied taxa was composed of tracheids and libriformfibres. The scalariform and reticulate pitting were encountered. This observation agrees with that of Pittermann et al. (2015) who reported that the vast majority of ferns and lycopods possess homogenous pit membranes that span the length of the tracheid walls in a scalariform arrangement, appearing less variable than those of angiosperms. However, these workers ascertained that the presence of conifer-like pit membranes observed in *Botrychium* as well as bordered pit in *Psilotum* indicates fern genetic potential to evolve xylem that is functionally comparable to those of higher plants.

All the fibres in the taxa studied were non septate except for those of *P. ensiformis* which has septate fibres. Analysis of the fibre measurement (Table 1) revealed uniform wall thickness of 1 µm. *P. acanthoneura* with the highest length (277.5 µm), while *P. similis* has the least length (180 µm). In contrast to the observations in the petioles of the species in this study, the rhizome has more tracheids and very scanty fibres.

The roots of all the *Pteris* species studied were the diarch type with two xylem bundles alternating with two phloem bundles. Xylem meets in the centre and appears plate-like with two protoxylem vessels in exarch position. The xylem has tracheids and xylem parenchyma, but vessels are absent. Similar root patterns have been reported in *Polystichum* fern species (Roux and Wyk Van, 2000); *Asplenium trichomames ramosum* L. (Bercu, 2007); and *Ceradenia deltodon* (Baker) Parris (C.Chr.) Parris and *Zygophlebia humberti* (Deroin and Rakotundrainibe, 2015). In these studied species, root anatomy appears to be of little or no taxonomic value at the species level.

Conclusion

The anatomy of the rhizomes showed a typical dictyostele pattern with variable number of leaf gaps which is common to most ferns in the order Polypodiales. Roots of all the *Pteris* species were also the diarch type with two xylem bundles alternating with two phloem bundles typical of most ferns. These structures provided useful specific distinctions of taxonomic importance at the generic level.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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