Determining relevant petiole anatomy characters to delimit Eupolypods I families

JEANETTE MARA P. TAN*, INOCENCIO E. BUOT, JR.

Institute of Biological Sciences, College of Arts and Sciences, University of the Philippines Los Baños, College, Laguna 4031, Philippines.

*email: jptan7@up.edu.ph

Abstract. Tan JMP, Buot Jr. IE. 2020. Determining relevant petiole anatomy characters to delimit Eupolypods I families. Biodiversitas 21: 2721-2726. The delineation of the families under eupolypods I has been quite challenging. With that, characters that are stable and taxonomically significant are necessary. The study attempted to use petiole anatomy characters to study the taxonomy of the eupolypods I families. Fresh eupolypods I representatives were collected from the field and were fixed with formalin-acetic acid alcohol (FAA). The petioles were cut and processed into permanent mounts using alcohol dehydration series and following standard microtechnique protocols. The petiole anatomy characters of the 21 representative species of eupolypods I were first studied of their stabil...
MATERIALS AND METHODS

Selection and preparation of the fresh Eupolypods representative specimens

A total of 21 representatives of eupolypods I were studied. Twenty specimens were fresh samples from the field (Mt. Makiling Forest Reserve, Los Baños, Laguna; Quezon-Laguna UP Land Grant; and Mt. Mantalingahan Protected Landscape, Palawan, Philippines). These were as follows: 

- Davallia hymenophylloides, Davallia solida, Davallia repens (Davalliaceae);
- Bolbitis heteroclita, Polystichum horizontal, Arachniodes amabilis (Dryopteridaceae);
- Hypodematum crenatum (Hypodematiaceae);
- Oleandra maquilingensis (Oleandraceae), Lomariopsis lineata, Lomariopsis kiiing, Cyclopetelis crenata (Lomariopsidaceae);
- Nephrolepis cordifolia, Nephrolepis biserrata, Nephrolepis falcat (Nephrolepidaceae);
- Microsorum heterocarpum, Microsorum punctatum, Phymatotors flabellata (Polypodiaceae); Tectaria angulata, Tectaria dissecta, Tectaria hilocarpa (Tectariaceae). One was not spotted though during fieldwork (Didymochlaena truncatula (Didymochlaenaceae), hence, we used the description of Zhang and Zhang (2015) for the purpose of this study.

The petioles of each specimen were cut into three parts: basal region (next to the rhizome), middle region (half of the petiole), and distal region (next to the blade) and were fixed and preserved in formalin-acetic acid alcohol (FAA) (Johansen 1940). Free-hand sectioning technique was used, and the specimens underwent the alcohol dehydration series and permanently mounted using Canada Balsam. Permanent slides were deposited in the Plant Biology Division Herbarium, Institute of Biological Sciences, University of the Philippines Los Baños, Laguna, Philippines. Cross-section of the petioles were observed using Olympus light compound microscope. Images were documented, and scales of each section were obtained through ImageJ software, generating scales through pixels of the photo.

Determining delimiting taxonomic characters of Eupolypods I

The data obtained from petiole anatomy were analyzed. Stable characters were determined by examining each species collected from different environments, particularly, substrates, gradients, and altitude. Those characters that remained constant were chosen and further analyzed using Multiple Correspondence Analysis, FactoMineR package (MCA) (Sebastien et al. 2008). MCA is equivalent to Principal Component Analysis for nominal data and can be used for qualitative variables (Le Roux and Rouanet 2010).

RESULTS AND DISCUSSION

Stable and distinct petiole anatomy characters of the nine families of Eupolypods I

Eupolypods I families exhibited unique petiole anatomy characters such as diverse stelar shape, xylem shape, vascular bundle shape, vascular bundle number, circumendodermal band, and adaxial grooves (Figure 1). Each family showed similarities and differences in terms of the presence circumendodermal band, number of vascular bundles, and xylem strand shape. For instance, Davalliaceae species showed the presence of circumendodermal band, U-shaped vascular bundles, and arc-shaped xylem strand shape (except D. hymenophylloides with V-shaped xylem) (Figures 1.A-C). Also, Dryopteridaceae species showed similar arc-shaped xylem but out of the three species examined, Bolbitis heteroclita and Polystichum horizontal showed the presence of circumendodermal band except for Arachniodes amabilis which does not have circumendodermal band (Figures 1.D-F). Moreover, the species under Hypodematiaceae (Figure 1.G) and Oleandraceae (Figure 1.H) showed similar arc-shaped xylem strand and has circumendodermal band. However, one distinct character of Hypodematiaceae is the presence of only two vascular bundles unlike Oleandraceae having three vascular bundles. Furthermore, the other four families have the presence of circumendodermal band in various thicknesses but have distinct characteristics. Lomariopsidaceae species (Figures 1.I-K) shared the same S-shaped xylem strand while species of Tectariaceae (Figure 1R-T) have hook-shaped xylem strand. On the other hand, the species under Nephrolepidaceae (Figures 1.L-N) and Polypodiaceae (Figures 1.O-Q) have similar C-shaped xylem strands.

These characters were screened based on stability across varying environments. It was observed that xylem shape, vascular bundle shape, and number of vascular bundles of species from different habitat, substrate, and elevation, remained constant across varying environmental conditions. Table 1 presented petiole characters of the same species collected from different elevation, collection sites, and habitats so as to clearly illustrate the stable and unstable petiole anatomy characters. Despite differences in microenvironmental states, species showed similar petiole anatomy characters such as xylem shape, vascular bundle shape, and number of vascular bundles (Table 1).

This indicated that these character states are more or less stable. Transverse sections of Microsorum heterocarpum collected from 352 m asl and 1230 m asl, showed similar C-shaped xylem, U-shaped vascular bundle, and 3 to 4 vascular bundles (Table 1). If this petiole anatomy character were unstable, the one collected from 352 m asl should have possessed a different petiole anatomy character from the one collected from 1230m asl. Further, the transverse sections of Lomariopsis lineata which were collected from Mt. Makiling (lithophyte) and Mt. Mantalingahan (epiphyte), showed similar S-shaped xylem, U-shaped vascular bundle, and 2-5 vascular bundles (Table 1). These remarkable observations illustrated that vascular bundles are constant and stable characters (Table 1), and hence, can be useful taxonomic and systematic characters.

After analysis of Table 1, the following petiole anatomy characters were found constant even if collected from varying environmental conditions: stellar type, xylem strand shape, shape of vascular bundles, and number of
vascular bundles. Based on the studies of Ogura (1972), Lin and De Vol (1977), Hernandez-Hernandez et al. (2012), and Noraini et al. (2012), these characters are taxonomically and systematically significant. Also, the architecture of vascular bundles was conserved for a long time starting from the Upper Jurassic to the Cretaceous period (Hacke and Sperry 2001), making it a very stable character.

**Delimiting petiole anatomy characters of the Eupolypods I families**

The four petiole anatomy characters, namely, type of stele, xylem shape, vascular bundle shape, and number of vascular bundles were subjected to MCA, in order to determine which were the delineating characters. Based on the MCA biplot, the distinct petiole anatomy characters delimiting eupolypods I families were grouped together. Meanwhile, those characters such as dictyosteole, V-shaped vascular bundles, and U-shaped vascular bundles that were common to two or more families were separated from other families (Figure 2). Those species under one family were also grouped together. This implied that characters that were clumped nearest to the families were those distinct and unique characters that can be used to identify and distinguish the specific families (Figure 2).

It is very clear that three petiole anatomy characters, including xylem shape, vascular bundle shape, and number of vascular bundles were found to delimit each family. The following characters were the petiole anatomy characters found to be useful in distinguishing families of eupolypods I based on the Multiple Analysis Biplot (MCA): Davalliaceae-arc-shaped xylem and one to five vascular bundles; Didymochlaenaceae-arc-shaped xylem, semi-circle, and three vascular bundles; Dryopteridaceae-arc-shaped xylem, U-shaped and three vascular bundles; Hypodematiaceae-two vascular bundles; Lomariopsidaceae-S-shaped xylem, two to five vascular bundles; Nephrolepidaceae-C-shaped xylem and three to five vascular bundles; Oleandraceae-arc-shaped xylem, V-shaped and two to three vascular bundles; Polypodiaceae-C-shaped xylem, U-shaped and three to four vascular bundles; and Tectariaceae-hook-shaped xylem and three to seventeen vascular bundles.

The number and the shape of the vascular bundle and xylem strands distinguished the family. The three distinct characters observed for Polyodiaceae (U-shaped vascular bundles with C-shaped xylem strand, and 3-4 vascular bundles) corresponded to the findings of Henningman (1990) (Figure 3.E). This study also illustrated that Nephrolepidaceae (Figure 3.G) has C-shaped xylem and 3-5 vascular bundle characters while Lomariopsidaceae (Figure 3.H) has S-shaped xylem strand and 2-5 vascular bundles in U-shaped. Until now, there is an ongoing debate about the relationship of Nephrolepidaceae and Lomariopsidaceae. PPG I (2016) considered both families as sister families, however, Liu et al. (2013), Kuo et al. (2011), and Testo and Sundue (2016) disagree. Through molecular data, they considered Nephrolepidaceae as sister to a larger clade including Polyodiaceae, Tectariaceae, Davalliaceae, and Oleandraceae congruent to the results of the study of Kramer (1990). Results of this study using petiole anatomy characters or evidence proved that Nephrolepidaceae and Lomariopsidaceae are not sister families supporting Liu et al. (2013), Kuo et al. (2011), and Testo and Sundue (2016) but contradicting the claims of PPG I (2016).

![Figure 1](image.png)

**Figure 1.** Hand illustrations of the cross-sections of eupolypods I families petiole cross-sections showing the vascular bundle arrangement of the middle petiole. (A) Davallia bymenophylloides, (B) Davallia solida, (C) Davallia repens, (D) Bolbitis heteroclita, (E) Polystichum horizontale, (F) Arachniodes amabilis, (G) Hypodematum crenatum, (H) Oleandra maquilingensis, (I) Lomariopsis lineata, (J) Lomariopsis kingii, (K) Cyclopetelis crenata, (L) Nephrolepis cordifolia, (M) Nephrolepis biserrata, (N) Nephrolepis falcata, (O) Microsorum heterocarpum, (P) Microsorum punctatum, (Q) Phymatosorus scolopendria, (R) Tectaria angulata, (S) Tectaria dissecta, and (T) Tectaria hilocarpa. grid = sclerenchyma, thick line = endodermis + pericycle, dotted = phloem, parallel lines = xylem. Bars, A-I 500 µm (40x).
Figure 2. Multiple correspondence analysis biplots of the nine families of eupolypods I using four petiole anatomy characters. (DAVA-Davalliaceae, DODY-Didymochlaenaceae, DRYO-Dryopteridaceae, HYPO-Hypodematiaceae, LOMA-Lomariopsidaceae, NEPH-Nephrolepidaceae, OLEA-Oleandraceae, POLY-Polypodiaceae, TECT-Tectariaceae)

Figure 3. Transverse sections of eupolypods I petiole specimens obtained from the middle region of the petiole (A) Davallia solida (Davalliaceae), (B) Bolbitis heteroclita (Dryopteridaceae), (C) Hypodematum crenatum (Hypodematiaceae), (D) Oleandra maquilingensis (Oleandraceae), (E) Microsorum punctatum (Polypodiaceae), (F) Tectaria angulata (Tectariaceae), (G) Nephrolepis cordifolia (Nephrolepidaceae), and (H) Cyclopeltis crenata (Lomariopsidaceae) (x-xylem, yellow arrow-endodermis, blue arrow-circumendodermal band). Bars. a-c 100 µm (100x).
Davalliaceae can be identified using the following characters: protostele and dictyostele, arc-shaped xylem, and 1–5 vascular bundles (Figure 3A). This exactly agrees with the result of Noraini et al. (2012), Kramer (1990), and Kato and Mitsuta (1980). Meanwhile, Hypodemataceae sets apart from the rest of the families under eupolypods I by having only two vascular bundles (Figure 3C) which eventually fused as it reached the distal region of the petiole supporting the findings of Khare and Shankar (1987). This character as seen in Figure 3 is nearest and closely related to Hypodemataceae making it a unique and distinct character. Currently, the taxonomic position of Hypodematiaceae is still uncertain. It was moved from one family to another until PPG I moved it to its own family Hypodemataceae (Tan et al. 2020).

Based on petiole anatomy characters, both Didymochlaenaceae and Dryopteridaceae shared the same arc-shaped xylem, but Didymochlaenaceae differs by having three semi-circle shaped vascular bundles. On the other hand, Dryopteridaceae showed three U-shaped vascular bundles that delimit the family Dryopteridaceae from other families under eupolypods I similar to the findings of Kramer (1990) (Figure 3B).

It can be observed that Didymochlaenaceae is related to Dryopteridaceae. Previously, molecular data considered Didymochlaena as a member of Dryopteridaceae (Ching 1965; Tryon and Tryon 1982; Kramer et al. 1990). However, recent molecular studies confirmed that it merits its own family Didymochlaenaceae and a member of eupolypods I (Zhang and Zhang 2015). The petiole anatomy results showed that Didymochlaenaceae, whose taxonomic placement has always been a subject of controversy, merits its own family and not part of Dryopteridaceae which conforms to the findings of PPG I.

Finally, the petiole anatomy of family Oleandraceae with its representative genus, Oleandra is illustrated in Figure 3D. Results showed that Oleandraceae can be uniquely characterized as having 2–3 U-shaped vascular bundles which conform with the study of Kramer (1990). The results indicated support to the current circumscription of Oleandra in its own family Oleandraceae.

The distinctiveness and species delineation of Oleandra and Oleandraceae are still uncertain and difficult (Smith et al. 2006). In fact, this family has been previously linked to other families such as Davalliaceae, Nephrolepidaceae, and Dryopteridaceae (Tan et al. 2020). The circumscription of this family has been challenging as many of the species are similar in appearance (Kramer 1990).

In conclusion, the multiple correspondence analysis generated the results for the identification of eupolypods I families and species. Similar anatomical
features may have high potentials in characterizing and delineating other groups of ferns too.

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