Anatomical Characteristics of Petiole and Pinna Cross-Sections in Plagiogyria and Their Taxonomic Significance: Postprint

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Abstract

The genus Plagiogyria is isolated in the taxonomic system, and to date no systematic comparative anatomical studies of its structural organization have been reported. This study employed optical microscopy for the first time to conduct a comparative anatomical investigation of petiole and pinna cross-sections in nine Plagiogyria species. The nine species exhibit similar structural features in the epidermis, ground tissue, and stele of the mid-petiole cross-section, as well as in the epidermis, mesophyll, and midrib of vegetative pinnae. For instance, the epidermis of the mid-petiole cross-section lacks trichomes or scales, with epidermal cells being subrounded, possesses sclerenchyma tissue, and the stele consists of amphicribral vascular bundles. In vegetative pinna cross-sections, stomata are restricted to the lower epidermis, epidermal cells are flattened, and the midrib exhibits an amphicribral vascular bundle structure. In systematic evolution, Plagiogyria shows certain phylogenetic affinities with Cyatheaceae, sharing both similar characteristics and distinct differences, which supports the view that Plagiogyria represents a natural taxon. Among the nine species, mid-petiole cross-sectional shapes are trapezoidal, elliptical, or triangular; vascular bundles in the mid-petiole cross-section assume U-shaped, V-shaped, or tripartite configurations; vascular bundle number is either one or three; xylem within the vascular bundle structure appears "八"-shaped, U-shaped, or linear; the lower side of the pinna midrib forms triangular, arc-shaped, or trapezoidal protrusions; petiolar xylem morphology comprises two types: typical seahorse-shaped and atypical seahorse-shaped. Typical seahorse-shaped xylem is characterized by both sides of the petiole xylem exhibiting hooked curvature, whereas atypical seahorse-shaped xylem features either both xylem bundles lacking hooked curvatures or one end of a xylem bundle without hooked curvature. These morpho-anatomical characters are stable and taxon-specific, providing novel evidence for the taxonomy and systematics of Plagiogyria. Finally, a species identification

key for Plagiogyria was constructed based on anatomical characters of petiole and pinna cross-sections.

Full Text

Preamble

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Title: Anatomical Characteristics and Taxonomic Significance of Transverse Sections of Petiole and Pinnule in *Plagiogyria*

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Abstract

The fern genus *Plagiogyria* occupies an isolated position in classification systems, yet no systematic comparative anatomical studies have been reported to date. This study presents the first comprehensive anatomical comparison of petiole and pinnule transverse sections across nine *Plagiogyria* species using light microscopy. The nine species share fundamental structural characteristics in the epidermis, ground tissue, and stele of mid-petiole cross-sections, as well as in the epidermis, mesophyll, and main vein of vegetative pinnules. Specifically, mid-petiole epidermis lacks trichomes or scales, consists of circular cells with thickened walls, and contains amphicribral vascular bundles. Pinnule transverse sections exhibit stomata exclusively on the lower epidermis, flattened epidermal cells, and amphicribral vascular bundles in the main vein. These features support the classification of *Plagiogyria* as a natural taxonomic group. Phylogenetically, *Plagiogyria* shows affinities with Cyatheaceae, sharing certain characteristics while displaying distinct differences, particularly in epidermal trichome presence.

Key diagnostic features include: mid-petiole cross-section shapes (trapezoidal, elliptical, or triangular); vascular bundle configurations (“U” -shaped, “V” -shaped, or triangular); vascular bundle number (one or three); xylem arrangement patterns (“八” -shaped, “U” -shaped, or linear); and pinnule main vein protuberances (triangular, arc-shaped, or trapezoidal). Notably, petiolar xylem morphology occurs in two forms: typical hippocampiform (with hooked bends at both xylem ends) and atypical hippocampiform (lacking hooks at one or both ends). These stable, taxon-specific anatomical characters provide novel

evidence for *Plagiogyria* taxonomy and systematics. A dichotomous key to the nine species based on petiole and pinnule anatomical features is presented.

Keywords: *Plagiogyria*; petiole; pinnule; anatomy; classification

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Introduction

Plagiogyria exhibits a disjunct distribution across tropical and subtropical Asia and subtropical America, with its highest concentration in the mountainous regions of southwestern China. Ching (1958) recognized the genus as isolated within classification systems, yet constituting a natural group. Its systematic position has remained controversial, with historical classifications placing it variously among tree ferns, *Pteris* allies, or *Blechnum* allies. The genus shares several plesiomorphic traits with Osmundaceae, including the absence of scales or true hairs, free venation, and lack of true indusia. However, it also exhibits derived characteristics, such as mixed-type soral origin, oblique and complete annuli, and 64 spores per sporangium. Consequently, Gao (2011) considered *Plagiogyria* a representative of primitive leptosporangiate ferns, consistently positioned at the base of leptosporangiates and potentially allied with Osmundaceae. Conversely, Pryer (2004), based on molecular phylogenetic and reproductive biological evidence, suggested affinities with tree ferns.

Taxonomic delimitation at the species level remains similarly contentious. Copeland (1929) recognized 33 species, with 23 from Southeast Asia and 10 from tropical America. Ching (1958) estimated approximately 50 morphologically similar species globally, including about 32 in China, nine in tropical America, one in northern Australia, and the remainder in Southeast Asia with a few extending to northern India (Himalayas). Zhang & Nooteboom (1998) recognized only 11 species, with one in the New World and ten in the Old World. Despite these divergent taxonomic treatments, no systematic comparative anatomical studies have been conducted. This study investigates nine *Plagiogyria* species to provide anatomical evidence for future taxonomic and systematic research, and to elucidate the diagnostic value of petiole and pinnule anatomical characters.

1.1 Materials

Nine *Plagiogyria* species were examined: *P. adnata*, *P. stenoptera*, *P. falcata*, *P. assurgens*, *P. pycnophylla*, *P. glauca*, *P. euphlebia*, *P. japonica*, and *P. matsumureana* (Table 1). Voucher specimens are deposited in the Biodiversity Herbarium of Guangxi Normal University.

Table 1 Sources and voucher specimens of nine sampled *Plagiogyria* species

Taxonomic Group	Locality	Voucher
P. adnata	Jinxiu, Guangxi, China	P1410
P. stenoptera (Ear-shaped *Plagiogyria*)	Longsheng, Guangxi, China	P1501
P. falcata (Sickle-pinnuled *Plagiogyria*)	Jinxiu, Guangxi, China	P1520
P. assurgens (Emei *Plagiogyria*)	Chongqing, China	P1601
P. pycnophylla (Dense-leaved *Plagiogyria*)	Jingdong, Yunnan, China	P1401
P. glauca (Glaucous-backed *Plagiogyria*)	Dali, Yunnan, China	P1510
P. euphlebia (Central China *Plagiogyria*)	Nanchuan, Chongqing, China	P1620
P. japonica (East Asian *Plagiogyria*)	Wuming, Guangxi, China	P1530
P. matsumureana (Japanese *Plagiogyria*)	Japan	P1701

1.2 Methods

Mid-petiole and vegetative pinnule segments were processed using paraffin sectioning. Materials were fixed in FAA solution, dehydrated through an ethanol concentration series, and embedded in paraffin. Mid-petiole sections were cut at approximately 10 m thickness, while pinnule sections were cut at approximately 20 m. Following standard processing, samples were stained with safranin-fast green, mounted in neutral balsam, and examined under light microscopy.

2 Results

Comparative anatomical structures of mid-petiole and pinnule transverse sections are summarized in Table 2 and Plate I .

Table 2 Comparison of anatomical structures of transverse sections of middle petioles and pinnules

Taxon	Mid-petiole Cross-section Shape	Vascular Bundle Number	Xylem Arrangement	Main Vein Shape
P. stenoptera	Trapezoidal	1	“U” -shaped	Arc-shaped
P. falcata	Triangular	1	“V” -shaped	Triangular

Taxon	Mid-petiole Cross-section Shape	Vascular Bundle Number	Xylem Arrangement	Main Vein Shape
P. assurgens	Triangular	1	"U" -shaped	Arc-shaped
P. pycnophylla	Trapezoidal	1	"V" -shaped	Trapezoidal
P. glauca	Elliptical	1	"U" -shaped	Trapezoidal
P. euphlebia	Elliptical	1	"U" -shaped	Arc-shaped
P. japonica	Elliptical	1	"V" -shaped	Arc-shaped
P. matsumureana	Triangular	3	"Eight" -shaped and linear	Curved

3 Discussion and Conclusion

3.1 Common Anatomical Features Across Nine *Plagiogyria* Species

As shown in Plate I, nine *Plagiogyria* species share consistent mid-petiole anatomical architecture: (1) **Epidermis**: Cells are compactly arranged, non-glandular, and circular with slightly thickened outer walls. (2) **Ground tissue**: Abundant sclerenchyma cells form a hypodermal layer, showing uniform distribution across all nine species. (3) **Stele**: Amphicribral vascular bundles feature "V" - or "U" -shaped phloem surrounding the xylem, enclosed by several layers of sclerenchyma cells.

Vegetative pinnule transverse sections exhibit: (1) **Epidermis**: Stomata restricted to the lower surface; upper and lower epidermal cells are flattened and regularly arranged. (2) **Mesophyll**: Spongy tissue cells are loosely arranged with large intercellular spaces, while palisade tissue cells are compactly arranged; neither shows pronounced differentiation. (3) **Main vein**: Amphicribral vascular bundle with endodermis; xylem arranged in a linear pattern; abundant sclerenchyma cells located above and below the epidermis.

These shared anatomical features support the classification of *Plagiogyria* as a natural monophyletic group. Phylogenetically, *Plagiogyria* shows affinities with Cyatheaceae, though with notable differences. Liu et al. (2015) reported that

Alsophila spinulosa possesses stomata exclusively on the lower epidermis, similar to *Plagiogyria*, but differs in having epidermal trichomes. These comparative data reinforce the natural taxonomic status of *Plagiogyria*.

3.2 Diagnostic Anatomical Differences and Taxonomic Significance

Petiole anatomy provides reliable characters for generic and specific delimitation in ferns. Wang (2001) demonstrated that vascular bundle number in petiole cross-sections serves as a stable taxonomic character for Dryopteridaceae in Northeast China. Zhou et al. (2006) similarly utilized petiole morphology to distinguish between *Athyrium* and *Athyriopsis* in Shandong province.

As shown in Table 2 and Plate I, nine *Plagiogyria* species exhibit distinct petiole cross-sectional shapes: trapezoidal in *P. adnata*, *P. pycnophylla*, and *P. stenoptera*; elliptical in *P. euphlebia*, *P. glauca*, and *P. japonica*; and triangular in *P. assurgens*, *P. falcata*, and *P. matsumureana*. Vascular bundle number varies, with all species except *P. matsumureana* possessing a single bundle; *P. matsumureana* has three. Vascular bundle shape differs markedly: "V" -shaped in *P. adnata*, *P. falcata*, *P. pycnophylla*, and *P. japonica*; "U" -shaped in *P. stenoptera*, *P. assurgens*, *P. glauca*, and *P. euphlebia*; and triangular in *P. matsumureana*. Xylem arrangement patterns show three variants: "八" -shaped in *P. adnata*, *P. falcata*, *P. pycnophylla*, and *P. japonica*; "U" -shaped in *P. stenoptera*, *P. assurgens*, *P. glauca*, and *P. euphlebia*; and both "八" -shaped and linear in *P. matsumureana*.

Petiolar xylem morphology occurs in two forms: typical hippocampiform (hooked bends at both xylem ends) and atypical hippocampiform (absence of hooks at one or both ends). All species except *P. matsumureana* display typical hippocampiform xylem, while *P. matsumureana* exhibits the atypical form. Pinnule main vein protuberances also vary: lower epidermal protuberances are triangular in *P. falcata* and *P. stenoptera*, arc-shaped in *P. adnata*, *P. assurgens*, *P. euphlebia*, and *P. japonica*, and trapezoidal in *P. glauca*, *P. matsumureana*, and *P. pycnophylla*.

Zhang & Nooteboom (1998) categorized petiole vascular bundle shapes as "V" - or "U" -shaped, suggesting possible transitional forms related to petiole size. Our comparative analysis of differently sized petioles demonstrates that vascular bundle shape remains stable regardless of size, confirming "V" - and "U" -shaped configurations as reliable diagnostic characters.

These stable, taxon-specific variations in mid-petiole cross-sectional shape, vascular bundle number and shape, xylem arrangement, and pinnule main vein protuberances provide novel diagnostic criteria for *Plagiogyria* taxonomy and systematics.

Key to Nine *Plagiogyria* Species Based on Anatomical Characters

1. Mid-petiole cross-section with three vascular bundles ····· *P. matsumureana*
2. Mid-petiole cross-section with one vascular bundle ····· .. 3
3. Vascular bundle "U"-shaped ····· .. 4
4. Petiole cross-section elliptical; pinnule main vein protuberance arc-shaped ····· *P. euphlebia*
5. Petiole cross-section trapezoidal; pinnule main vein protuberance triangular ····· *P. stenoptera*
6. Vascular bundle "V"-shaped ····· .. 5
7. Petiole cross-section triangular ····· . 6
8. Pinnule main vein protuberance triangular ····· . *P. falcata*
9. Pinnule main vein protuberance arc-shaped ····· . *P. assurgens*
10. Petiole cross-section trapezoidal ····· *P. pycnophylla*
11. Petiole cross-section elliptical ····· . 7
12. Pinnule main vein protuberance arc-shaped ····· . *P. japonica*
13. Pinnule main vein protuberance trapezoidal ····· . *P. glauca*

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Note: 1-2. *P. adnata*; 3-4. *P. stenoptera*; 5-6. *P. falcata*; 7-8. *P. assurgens*; 9-11. *P. pycnophylla*; 12-14. *P. glauca*; 15-16. *P. euphlebia*; 17-18. *P. japonica*; 19-20. *P. matsumureana*.

Plate I Anatomical structure of the middle part of the petiole and the main vein of the pinnule in nine *Plagiogyria* species

Note: Figure translations are in progress. See original paper for figures.