



Integrating field inventory and *in silico* phylogenetic analysis of pteridophytes at Gandul Mount, Indonesia

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Article info	Abstract
<p>Article History: Received: 28 January 2026, Revised: 19 March 2026, Available Online: 31 March 2026</p> <p>Keywords: Barcoding, Diagnostic, Ethnobotany, Fern, Lygodium</p> <p>©2026 Bioeksperimen. This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 (CC-BY-NC) International (https://creativecommons.org/licenses/by-nc/4.0/).</p>	<p>Pteridophytes are vascular plants that are considered the most primitive plants because they only use spores to reproduce. Several major fern families exhibit distinctive morphological and ecological characteristics that support their taxonomic recognition. Pteridophytes represent an important component of tropical biodiversity, yet their diversity and evolutionary relationships in karst ecosystems remain poorly documented. This study aimed to integrate field inventory and in silico phylogenetic analysis to assess pteridophyte diversity in Gandul Mount, Wonogiri, Central Java. Field data were collected using the cruise method, followed by morphological identification and habitat characterization. Phylogenetic relationships were inferred using matK gene sequences retrieved from the NCBI database and analyzed with the Maximum Likelihood method. The inventory recorded 17 species belonging to eight families, with Pteridaceae as the most dominant. Most species were lithophytic, indicating strong adaptation to rocky substrates. Two species were categorized as Least Concern, while the remaining species have not been evaluated by the IUCN. Eleven species showed ethnobotanical potential as medicinal plants, ornamentals, and phytoremediation agents. Phylogenetic analysis revealed clustering patterns consistent with taxonomic classification. These results demonstrate that Gandul Mount harbors considerable pteridophyte diversity and confirm that combining field-based inventory with in silico phylogenetic analysis provides a robust approach for biodiversity assessment and evolutionary interpretation.</p>

Introduction

Pteridophytes are vascular plants with differentiated organs, including roots, stems, and leaves, forming a complete tuber structure (Prasani et al., 2021). This group of plants is widely distributed worldwide and comprises approximately 10,000 species, with over 3,000 recorded in Indonesia (Yolla et al., 2022). In addition to their high species richness, pteridophytes provide various ecological and socio-economic benefits, including their use as sources of traditional medicine, ornamental plants, and food (Wahyudi et al., 2018). Ecologically, they function as ground cover vegetation, contribute to litter accumulation and soil formation, act as primary producers, and represent valuable genetic resources for ecosystem sustainability (Haribowo et al., 2023).

The distribution and diversity of pteridophytes are strongly influenced by environmental conditions. Most species prefer humid habitats, although some can survive in relatively dry environments (Adlini et al., 2021). Terrestrial ferns grow on soil substrates, and their species composition varies significantly across habitats and ecosystem types (Yolla et al., 2022). Pteridophytes generally exhibit macroscopic morphology and cosmopolitan distribution, with life forms ranging from saprophytes and terrestrials to epiphytes and aquatics (Milenia et al., 2022). Environmental heterogeneity often results in substantial morphological



variation within and between species (Pradipta et al., 2023), highlighting the importance of field-based morphological observations for accurate identification and ecological interpretation.

Several major fern families exhibit distinctive morphological and ecological characteristics that support their taxonomic recognition. The Aspleniaceae are characterized by compound leaves with a narrow elliptical shape and serrated margins (Prasani et al., 2021), with representative species such as *Deparia japonica*, *Asplenium nidus*, and *Diplazium esculentum* (Isa et al., 2023). Dennstaedtiaceae have branching, creeping rhizomes and sporangia clustered at the base (Pradipta et al., 2020) and are widespread in tropical regions (Isa et al., 2023), including species such as *Microlepia speluncae* (Wahyuningsih et al., 2023). Dryopteridaceae have fibrous roots, hairy stems, bipinnate leaves, and rounded sori along the leaf veins (Prasani et al., 2021) and are found in tropical to temperate regions, particularly on the forest floor (Wahyuningsih et al., 2023).

Hypodematiaceae are distinguished by their thick, scaly rhizomes, dictyostele vascular structure, hairy leaves, and diverse spore ornamentation (Fan et al., 2021), and are commonly found as epiphytes in limestone habitats throughout Asia, including Southeast Asia (Lee et al., 2018). Polypodiaceae, one of the largest fern families, is characterized by simple leaves, short stems, and widespread sori (Pradipta et al., 2020; Ulfa et al., 2023), inhabiting terrestrial and epiphytic environments throughout tropical and subtropical forests (Lindasari et al., 2015) and comprising over 60 genera and 1,000 species (Wahyuningsih et al., 2023). Pteridaceae is the largest true fern lineage, with diverse growth forms, scaly rhizomes, marginal sori, and a wide ecological tolerance from humid to dry regions (Andries et al., 2022; Arini et al., 2025; Wahyuningsih et al., 2023; Khairunisa et al., 2023; Ulfa et al., 2023). Schizaeaceae have fan-shaped leaves with dichotomous venation and marginal sporangia (Arini et al., 2025; Pradipta et al., 2020) and are distributed in open, humid habitats in tropical and subtropical regions (Hasan et al., 2018; Krisnawati et al., 2020). Selaginellaceae are characterized by small leaves, dimorphic sporophylls, and heterosporous reproduction (Pradipta et al., 2020). They are largely terrestrial and widespread in the humid highlands of Indonesia and elsewhere (Suryani et al., 2023).

Comprehensive documentation of this diversity requires a systematic biodiversity inventory. Inventory activities are the initial stage of taxonomic research, involving structured field exploration and species identification to assess species richness and distribution patterns (Adhia et al., 2022). Inventory data contribute to biodiversity databases, support ecosystem management, and provide essential information for the conservation of endemic, rare, and endangered plant species. Furthermore, these data serve as a basis for species description, classification, and the detection of new taxa or local variants (Adhia et al., 2022). Previous studies using cruise methods have successfully recorded several fern families, including Pteridaceae, Polypodiaceae, and Dennstaedtiaceae (Wahyuningsih et al., 2023).

Despite its unique ecological characteristics, Gandul Mount has never been the subject of a comprehensive pteridophyte inventory. Furthermore, a more comprehensive inventory is needed. Conventional morphological inventories alone are often insufficient to resolve evolutionary relationships among closely related taxa. Advances in molecular biology and bioinformatics now allow phylogenetic inference using DNA sequence data stored in public databases such as the National Center for Biotechnology Information, enabling efficient evolutionary analysis through *in silico* approaches. Therefore, this study aims to integrate field-based inventories and morphological characterization with *in silico* phylogenetic analysis to document pteridophyte diversity on Gandul Mount, Wonogiri, and to elucidate their evolutionary relationships using publicly available molecular sequence data.

Materials and methods

1. Research Subject

The subjects of this study were pteridophyte species found along observation trails in the Gandul Mount area, Wonogiri District, Wonogiri Regency, Central Java, Indonesia. Field sampling was conducted from July to October 2025 at altitudes between 195 and 355 m above sea level. The total length of the survey trail was approximately 1.21 km (Figure 1), covering representative habitats within the study area, including terrestrial, epiphytic, and lithophytic environments.



Figure 1. The observation route in the Gandul Mount area, Wonogiri District, Wonogiri Regency, Central Java, Indonesia

2. Materials

The materials and instruments used in this study included writing instruments (notebook and pencil), a digital Global Positioning System (GPS) device to record sampling locations, a digital camera for specimen documentation, newspaper sheets for temporary specimen preservation, label paper for sample coding, and a standard fern identification guidebook. Additional taxonomic references were obtained from online databases, including Plants of the World Online (POWO), Plantamor, and the Global Biodiversity Information Facility (GBIF).

3. Method and Research Design

This study employed a descriptive exploratory design using the cruise method for field inventory survey. The cruise method in field survey was chosen to allow comprehensive exploration of heterogeneous habitats across the karst landscape of Gandul Mount and to effectively document fern diversity. Field surveys are widely applied in botanical surveys due to their effectiveness in heterogeneous landscapes. This approach involves the systematic exploration of representative habitat types and allows detailed observation of morphological characteristics and habitat preferences (U'un et al., 2021). Gandul Mount, Wonogiri, is dominated by karst limestone formations, resulting in shallow soils, low rainfall, and uneven vegetation distribution. These conditions limit the effectiveness of plot-based sampling techniques and make field surveys particularly suitable for documenting pteridophyte diversity, including species occupying specific microhabitats such as epiphytic and lithophytic niches (U'un et al., 2021).

4. Field Inventory Research Procedure

The research procedure consisted of four main stages such as preparation, sampling, identification, and inventory. In the preparation stage, the observation route was determined based on topography, accessibility, and habitat representation, followed by preliminary field observations and a literature review. During the sampling stage, pteridophyte specimens found along the observation route were selectively collected, ensuring the presence of key identification characteristics such as spores and other parts such as leaves, stems, and roots, that are complete and undamaged. The specimens were then placed on newspaper sheets and labeled with the sampling code and location data for further examination.

The identification and inventory stages were conducted based on morphological characteristics, including stem structure, leaf shape and arrangement, root type, and sori position and shape. Species identification followed the reference journals "Identification of Ferns Based on Sorus Position and Altitude Differences in Bondowoso Regency" (Pradipta et al., 2023), "Field Guide for Ferns (Pteridophyta) at Ragunan Zoo" (Agatha et al., 2019), and verified using online taxonomic databases (POWO, Plantamor, and GBIF). Habitat classification is done by recording the type of substrate for each species, namely terrestrial (soil), epiphytic (tree surface), and lithophytic (rock surface).



5. Data Analysis Techniques

Data analysis was conducted using a qualitative descriptive approach. Each collected specimen was examined to document morphological characteristics, including leaf structure, spore characteristics, stem and root system characteristics, and habitat substrate type. Species composition and distribution patterns along the observation route were summarized to provide an overview of pteridophyte diversity in the study area. The resulting inventory data served as the basis for subsequent *in silico* phylogenetic analyses using molecular sequence data obtained from public genetic databases.

6. *In silico* Phylogenetic Analysis

Species identified from the field inventory were then analyzed using molecular data obtained from the National Center for Biotechnology Information (NCBI) database. The DNA sequence of the chloroplast *matK* gene was chosen as a molecular marker due to its high discriminatory power and widespread use in plant DNA sequencing and phylogenetic studies. For each identified species, available *matK* sequences were retrieved in FASTA format from the NCBI GenBank database.

Sequence alignment was performed using the alignment function implemented in MEGA (Molecular Evolutionary Genetics Analysis) software to ensure homologous nucleotide positions among all taxa. The aligned sequences were then evaluated to determine the most appropriate nucleotide substitution model using MEGA's built-in model selection tool based on statistical criteria such as the lowest Bayesian Information Criterion (BIC) or Akaike Information Criterion (AIC) values.

Phylogenetic tree reconstruction was performed using the Maximum Likelihood (ML) method under the best-fitting evolutionary model obtained from model testing. Bootstrap analysis with multiple replications was applied to assess the reliability and robustness of the inferred clades. The resulting phylogenetic tree was visualized and interpreted to elucidate the evolutionary relationships among the recorded pteridophyte species. Haplotype data were obtained from DnaSP v.6.

Results and discussion

1. Pteridophyte Diversity in Gandul Mount

Field inventory conducted in the Gandul Mount area recorded a total of 17 pteridophyte species belonging to eight families (Table 1). Among these families, the Pteridaceae family showed the highest species richness with seven species, followed by the Polypodiaceae with three species, the Dryopteridaceae with two species, and the other families each represented by one species.

Table 1. Diversity of Pteridophytes Recorded in Gandul Mount

Family	Species	Indonesian Local name	Habitat type	Conservation Status*
Aspleniaceae	<i>Deparia japonica</i>	<i>Lumut limpa palsu</i>	Terrestrial	NE
Dennstaedtiaceae	<i>Microlepia speluncae</i>	<i>Paku goa</i>	Lithophytic	NE
Dryopteridaceae	<i>Dryopteris nodosa</i>	-	Terrestrial	NE
	<i>Elaphoglossum</i> sp.	-	Lithophytic	NE
Hypodematiaceae	<i>Leucostegia immersa</i>	-	Lithophytic	NE
Polypodiaceae	<i>Drynaria quercifolia</i>	<i>Paku daun kepala tupai</i>	Lithophytic	NE
	<i>Microsorium commutatum</i>	-	Lithophytic	NE
	<i>Tectaria aurita</i>	-	Lithophytic	NE
Pteridaceae	<i>Adiantum capillus-veneris</i>	<i>Paku suplir</i>	Lithophytic	LC
	<i>Adiantum lunulatum</i>	<i>Kamuding</i>	Lithophytic	NE
	<i>Cheilanthes tenuifolia</i>	<i>Paku resam lumut</i>	Lithophytic	NE
	<i>Pityrogramma calomelanos</i>	<i>Paku perak</i>	Terrestrial	NE
	<i>Pteris biaurita</i>	<i>Paku cawan</i>	Terrestrial	NE
Schizaeaceae	<i>Pteris ensiformis</i>	<i>Paku pedang</i>	Lithophytic	NE
	<i>Pteris vittata</i>	<i>Pakis rem cina</i>	Lithophytic	LC
	<i>Lygodium flexuosum</i>	<i>Paku kembang</i>	Lithophytic	NE
Selaginellaceae	<i>Selaginella aristata</i>	-	Lithophytic	NE

Note: *Conservation status follows the IUCN Red List categories; NE = Not Evaluated, LC = Least Concern.



Habitat analysis showed that lithophytic species dominated the study area, with 13 species growing on damp moss-covered rocks, rock crevices, and vertical rock surfaces near drainage channels. The other four species were terrestrial, growing on flat ground surfaces in open areas. This distribution pattern is closely related to the karst-dominated geology of Gandul Mount, where limestone outcrops and rocky substrates predominate. Such environmental conditions favor lithophytic growth forms and shape the structure of fern communities. These results demonstrate that habitat-based inventories complement morphological analyses in providing a comprehensive understanding of species diversity and ecological adaptations in heterogeneous landscapes.

The dominance of Pteridaceae indicates high ecological adaptability to diverse environmental conditions, including habitats with low moisture availability and rocky substrates. Members of this family have small, segmented leaves that reduce evaporation and enable survival in both shaded and open environments. This finding is consistent with previous research by [Khairunisa et al. \(2023\)](#), which reported that Pteridaceae species are widespread due to their ability to grow in moist to dry habitats under varying light conditions. Globally, this family is recognized as one of the largest within the Pteridophyta, comprising approximately 950 species, further explaining its frequent occurrence in diverse ecosystems.

Based on conservation assessments using the IUCN Red List, two species, *Adiantum capillus-veneris* and *Pteris vittata*, are categorized as Least Concern, indicating stable populations and a low risk of extinction. The remaining 15 species have not yet been evaluated (status Unevaluated). Although most species lack formal conservation assessments, their presence highlights the ecological importance of Gandul Mount as a habitat for fern diversity.

2. Morphological Characteristics of Pteridophytes

Morphological analysis of the 17 recorded species revealed substantial variation in leaf, stem, root, and reproductive structures (Table 2), reflecting adaptation to heterogeneous environmental conditions.

Table 2. Morphological Characteristics of Pteridophytes Based on Field Observations

Species	Character Diagnostics			
	Leaves	Stem	Roots	Sorus
<i>Deparia japonica</i>	<i>Bipinnatilobate</i> , leaf lamina scaly, lightly hairy on rachis.	Creeping and scaly rhizomes	Growing from rhizomes	Linear along the leaf veins, the indusium pale brown.
<i>Microlepia speluncae</i>	Light green color, smooth surface, jagged edges, blunt ends, interspersed	Brown-scaly upright rhizome, forked venation	Fibrous roots	Brown color, submarginal placement of the saplings
<i>Leucostegia immersa</i>	Large and widely divided, the overall shape is like a triangle, smooth surface without feathers. The edges of the segments are serrated, the tips of the leaves are pointed.	Creeping, branched, finely scaly rhizomes	Fibrous roots emerge from the rhizome	Small rounded, at the end of the leaf bones small, indusium white when young and brown when old.
<i>Drynaria quercifolia</i>	True leaves are green, single, fingered, jagged edges, tapered tip, blunt base, smooth surface, leather-like texture. The leaves support the ovaries, covering the roots and rhizomes.	Thick, round, covered with brown to blackish hair	Fibrous roots	Brown color, rounded in shape, found in the abaxial of fertile leaves, irregularly spread



Species	Character Diagnostics			
	Leaves	Stem	Roots	Sorus
<i>Dryopteris nodosa</i>	Pinnate up to 2 (divided into 2 saplings), monomorphic, <i>rachis</i> covered with brown scales, extending to oval, saplings <i>lanceolate</i> , pointed tip, finely serrated edges.	Creeping, scaly rhizomes	Fibrous roots emerge from the rhizome	Near the middle side of the leaf, small rounded, pale indusium, yellowish-brown spores when ripe.
<i>Elaphoglossum</i> sp.	Dark green color, pointed tip and base, flat edges, smooth surface.	Creeping rhizomes, brown, short branches	Fibrous roots with a smoother texture	Open without inducium, in the abaxial leaves, elongated parallel to the bones of the leaves, brown in color.
<i>Microsorium commutatum</i>	Single and deep split sheets	Creeping rhizomes	Fibrous roots emerge from the rhizome	Sori scattered, yellow spores
<i>Tectaria aurita</i>	Monomorphic, slightly oblong shape, concave, tapered tip, slightly rough surface.	Brown scaly upright rhizome	Fibrous roots emerge from the rhizome	Rounded, abaxial leaves at terminal veinlet, indusium thin.
<i>Adiantum capillus-veneris</i>	Pale green color when young and green when old, spaced, pinnate, spade shape	Long round, smooth surface, brown	Fibrous roots	White color when young, brown when old, margins of leaves abaxial.
<i>Adiantum lunulatum</i>	Fan-like shape, smooth surface	The rhizomes creep long with brown scales, black stalks, smooth, shiny, black stem. Rhizomes creep upwards,	Fibrous roots	Abaxial leaves, brown in color, rounded.
<i>Cheilanthes tenuifolia</i>	Dimorphic, triple double compound, pointed tip, flat base, hairy rough surface.	dark brown in color, direction growing sideways, light brown scaly	Brown fibrous roots	Sori under the leaflet, the sorus spreads along the edges of the leaves, there is an indusium.



Species	Character Diagnostics			
	Leaves	Stem	Roots	Sorus
<i>Pityrogramma calomelanos</i>	Light green color, smooth surface, concave edges, pointed tips and bases, alternate, abaxial white scaly leaves. The petioles are round-blackish-brown.	Erect scaly brown, forked venation	Fibrous roots	Sorus on underleaves
<i>Pteris biaurita</i>	Light green color, smooth surface, split edges, pointed tips and bases, opposite. Light green and rounded petioles.	Creeping short hair	Fibrous roots	Form a line on the edge of the lower leaf
<i>Pteris ensiformis</i>	Triple double pinnate compound, green color, smooth surface, serrated edges, rounded tip, blunt base, facing.	Erect rhizome, brownish-green round stalks	Fibrous roots	Line shape, brown in color, along the edges of the sporophylic leaves, in the abaxial of the leaves.
<i>Pteris vittata</i>	Monomorphic, elongated, compound leaves, tapered tip, light green and dark green color, base of spear leaf, smooth surface, serrated edges, bibranchied. Dark green, smiling, alternating, each side of the branch 3-4 leaves, pointed tip, base rounded, glossy smooth surface, divided 2-5 lobes deep.	The rhizome is erect, dark brown in color, scaly.	Fibrous, dark brown color, brown scaly	Leaf abaxis, along the leaf edge, white when young brown when ripe, indusium open.
<i>Lygodium flexuosum</i>	Lateral leaves oblong and lanceolate, pointed tip, base rounded, edges serrated, smooth on one side. The median leaves are oval in shape, the tip is pointed, the base protrudes to the sides, the serrated edges of both sides. Axillary leaves lanceolate, tip pointed, base rounded.	The stem is round, smooth, green in color.	Brown fibrous roots	Leaf margins fertile, long, two rows on the edges of the leaves, green color.
<i>Selaginella aristata</i>	Lateral leaves oblong and lanceolate, pointed tip, base rounded, edges serrated, smooth on one side. The median leaves are oval in shape, the tip is pointed, the base protrudes to the sides, the serrated edges of both sides. Axillary leaves lanceolate, tip pointed, base rounded.	Creeping, branching out like a fan, thin forming an expanse	Small fibers, adventitious roots of the stem, grow in each segment	Strobilus with tetragenous type



Leaf morphology exhibits the highest degree of variation in surface texture, arrangement, margin type, shape, and complexity. Most species exhibit smooth, hairless anterior surfaces; however, *Cheilanthes tenuifolia*, *Tectaria aurita*, and *Deparia japonica* have rough, hairy surfaces, which likely enhance water retention and protection against environmental stress. Alternating phyllotaxy predominates among species, while *Selaginella aristata* uniquely displays a tetraphyllous leaf arrangement. The anterior margin varies from serrated to lobed and entire. Pinnate compound leaves are common, as observed in *Pteris vittata*, while bipinnate and tripinnate forms are found in *Tectaria aurita* and *Dryopteris nodosa*. In contrast, *Selaginella aristata* has microphyllous leaves arranged in four rows. This observation supports the findings of Rizky et al. (2018), who described *Selaginella* species as having small, scale-like microphyllous leaves without petioles.



Figure 2. Morphological appearance of Pteridophyte species recorded during field observations; a. *Deparia japonica*; b. *Microlepia speluncae*; c. *Leucostegia immersa*; d. *Drynaria quercifolia*; e. *Elaphoglossum* sp.; f. *Dryopteris nodosa*; g. *Microsorium commutatum*; h. *Tectaria aurita*; i. *Adiantum capillus-veneris*; j. *Adiantum lunulatum*; k. *Pityrogramma calomelanos*; l. *Cheilanthes tenuifolia*; m. *Pteris biaurita*; n. *Pteris ensiformis*; o. *Pteris vittata*; p. *Lygodium flexuosum*; q. *Selaginella aristata*.

Stem morphology also varies significantly between species. Eight species have creeping rhizomes, including *Dryopteris nodosa*; four species have erect rhizomes, such as *Pteris vittata*; three species exhibit erect stems, such as *Pityrogramma calomelanos*; and two species have creeping stems (*Selaginella aristata* and *Pteris*



biaurita). Interestingly, *Drynaria quercifolia* exhibits distinctive rhizome characteristics: thick, fleshy, creeping, and densely covered with brown scales. These characteristics align with the description provided by [Lindasari et al. \(2015\)](#), who reported that this species has thick, rounded rhizomes covered with fine brown scales. Root morphology is relatively uniform, with 16 species having fibrous roots emerging from the rhizome. Only *Selaginella aristata* exhibits adventitious roots emerging from each stem node, a characteristic also reported by [Sartika et al. \(2021\)](#). Root color is consistently brown across all species.

Reproductive structures further differentiate the species. Sori vary in shape, position, presence of an indus, and coloration. Rounded abaxial sori are observed in *Dryopteris nodosa*, while linear marginal sori occur in *Pteris ensiformis*. Most species exhibit white sori when immature, changing to yellowish-brown as they mature, while *Lygodium flexuosum* uniquely retains green sori. Indus types range from open (*Pteris vittata*), closed (*Dryopteris nodosa*), to absent (*Elaphoglossum* sp.). *Selaginella aristata* does not form sori but produces terminal strobili with a tetragenous arrangement, in which sporangia are arranged in four rows. This reproductive strategy aligns with the findings of [Elsifa et al. \(2019\)](#), who reported that *Selaginella* species form strobili rather than sori.

3. Ethnobotanical Potential of Pteridophytes

Ethnobotanical assessment revealed that 11 of the 17 recorded species have potential uses as medicinal plants, ornamental plants, decorative materials, craft resources, and phytoremediation agents (Table 3). Medicinal use was the most frequently *documented* application, indicating that Gandul Mount harbors biologically valuable plant resources for traditional healthcare practices.

Table 3. Ethnobotanical Potential of Pteridophytes in Gandul Mount

Species	Plant Parts	Ethnobotanical Potential
<i>Microlepia speluncae</i>	Whole section	Houseplants
<i>Drynaria quercifolia</i>		Folk remedies to lower fever, fractures, gastric disorders, dizziness, swelling, skin disorders, and improve urinary tract function.
<i>Adiantum capillus-veneris</i>		Houseplants; hair growth, fever medicine, chickenpox, canker sores.
<i>Adiantum lunulatum</i>		Houseplants; Medicine for stomach cramps, flu, fever, diarrhea, pain.
<i>Cheilanthes tenuifolia</i>		Cough, fever, immune-boosting medicine.
<i>Pityrogramma calomelanos</i>		Ornamental and decorative plants; dysentery, malaria, and kidney disease medications.
<i>Pteris biaurita</i>		Heavy metal hyperaccumulators (Hg)
<i>Pteris ensiformis</i>		Houseplants
<i>Pteris vittata</i>		Mercury (Hg) heavy metal hyperaccumulators, barrier plants, ornamental plants.
<i>Selaginella aristata</i>		Houseplants, handicrafts.
<i>Lygodium flexuosum</i>	Stem	Handicrafts in the form of handbags and hats

Several *Pteris* species have demonstrated phytoremediation potential, particularly for the uptake of heavy metals such as mercury (Hg). These findings suggest a relationship between the fern community and the mineral-rich sedimentary rocks of Gandul Mount, which contribute to its distinctive soil chemistry. Pteridophytes with high tolerance to heavy metals appear to be well-adapted to such environments. Overall, the research results indicate that pteridophytes on Gandul Mount have significant ecological, taxonomic, and ethnobotanical value. However, sustainable use and habitat conservation are crucial to ensure the long-term survival of these species in their natural ecosystems.

4. *In silico* Phylogenetic Analysis

A total of 14 matK sequences were included in the analysis after the alignment process. The selected region encompassed 227 nucleotide positions, with no sites excluded due to gaps or missing data. Of these, 189 sites varied, indicating a high level of nucleotide polymorphism among the sampled taxa. This proportion of varying sites reflects the considerable genetic divergence among the analyzed pteridophyte species. Haplotype analysis revealed the presence of 14 distinct haplotypes ($h = 14$), which corresponds exactly to the number of sequences analyzed, with each species represented by a unique haplotype.



Consequently, haplotype diversity was maximal ($H_d = 1.0000$), indicating complete haplotype differentiation among the taxa. Each haplotype was associated with a single species accession from GenBank. This pattern confirms that no shared haplotypes were detected among the sampled species, supporting a clear genetic separation between the samples.

Table 4. NCBI Sequence Data and Molecular Markers of Recorded Pteridophyte Species

Accession number	Species	Voucher	Sequence length (bp)	Haplotype
JN673831.1	<i>Deparia japonica</i>	TNS 763869 (TNS)	1306	Hap1
MK675435.1	<i>Microlepia speluncae</i>	Isolate M17	848	Hap2
KU500256.1	<i>Dryopteris nodosa</i>	Kuo2703 (TAIF)	1326	Hap3
JF303949.1	<i>Elaphoglossum</i> sp.	-	1264	Hap4
MW534113.1	<i>Leucostegia immersa</i>	Isolate Leuco07IM	1566	Hap5
NF	<i>Drynaria quercifolia</i>	NF	NF	NF
MH113603.1	<i>Microsorium commutatum</i>	Wade 3768 (TAIF)	837	Hap6
KJ196548.1	<i>Tectaria aurita</i>	S.Y. Dong 3386 rps16-matK	1795	Hap7
KX758471.1	<i>Adiantum capillus-veneris</i>	M6	450	Hap8
NF	<i>Adiantum lunulatum</i>	NF	NF	NF
	<i>Cheilanthes tenuifolia</i>			
MK629533.1	(syn. <i>Hemionitis tenuifolia</i>)	M51	771	Hap9
KF289551.1	<i>Pityrogramma calomelanos</i>	<i>Pteris</i> -F. Lu 19492	888	Hap10
MF972731.1	<i>Pteris biaurita</i>	A. Larsen 44455 (MO)	902	Hap11
MF972745.1	<i>Pteris ensiformis</i>	ARF3539 (cult.)	900	Hap12
		C. J. Rothfels 4016 (DUKE)		
MF972799.1	<i>Pteris vittata</i>		902	Hap13
MK690399.1	<i>Lygodium flexuosum</i>	Isolate M04	860	Hap14
NF	<i>Selaginella aristata</i>	NF	NF	NF

Note: NF = no sequence data available in NCBI

A Maximum Likelihood phylogenetic tree, generated using General Time Reversible has invariant sites (GTR+I) based on the chloroplast marker matK (1,000 bootstrap replicates) revealed several major clades and was largely consistent with the modern fern classification system proposed by Pteridophyte Phylogeny Group I (PPG I, 2016). Species belonging to the Dryopteridaceae (*Dryopteris nodosa* and *Elaphoglossum callifolium*) were more closely related to the Hypodematiaceae (*Leucostegia immersa*), followed by the Polypodiaceae (*Microsorium commutatum* and *Tectaria aurita*), all clustering into a strongly supported eupolypod clade (70–100 bootstraps), reflecting their common evolutionary ancestry despite morphological differences. Similarly, members of the Pteridaceae (*Hemionitis tenuifolia*, *Pityrogramma calomelanos*, *Pteris vittata*, *P. biaurita*, and *P. ensiformis*) form a distinct monophyletic group with high bootstrap support (99–100), corroborating their taxonomic placement and shared morphological features such as marginal or linear sori and adaptation to relatively dry habitats. *Microlepia speluncae* (Dennstaedtiaceae) and *Deparia japonica* (Aspleniaceae) occupy an intermediate position consistent with their early divergence among leptosporangiate ferns, whereas *Lygodium flexuosum* (Schizaeaceae) is placed in a basal lineage, in keeping with its status as an early-diverging fern family in the PPG I system.

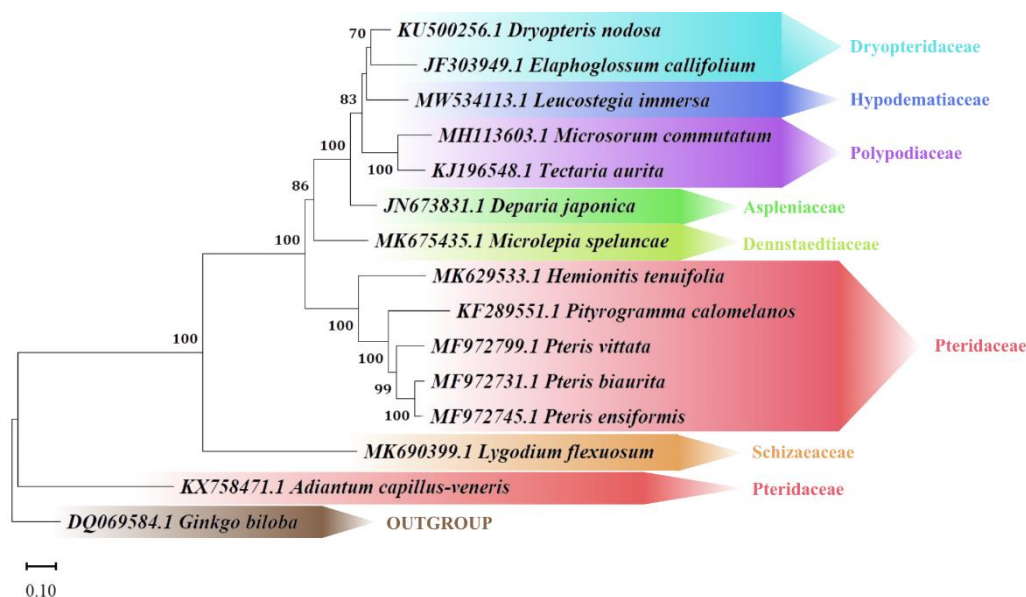


Figure 3. Phylogenetic relationships of Pteridophyte species recorded in Gandul Mount inferred using the Maximum Likelihood method based on chloroplast *matK* sequences with 1,000 bootstrap replications. Bootstrap values (>70%) are shown at the nodes. Scale bar indicates genetic distance.

However, there are some deviations from the expected family-level grouping, particularly in the placement of *Adiantum capillus-veneris* outside the main Pteridaceae clade. These differences are likely due to methodological constraints rather than genuine evolutionary inconsistencies, including reliance on a single molecular marker (*matK*), limited availability of sequence data in GenBank (often represented by only one accession per species), and the use of partial coding sequences for *Microlepia speluncae*, *Microsorium commutatum*, and *Adiantum capillus-veneris*. Furthermore, this study also used concatenated *rps16*–*matK* intergenic space sequences for *Tectaria aurita*, while other taxa are represented by only *matK*, resulting in alignment heterogeneity that affects branch topology. In line with the statement by [Gu et al. \(2024\)](#) that cpDNA analyses of Pteridaceae encountered alignment difficulties in non-coding regions (such as intergenic spacers), similar to the *rps16*–*matK* fusion in *Tectaria*, leading to genomic size variation and potential phylogenetic bias. Overall, the phylogenetic reconstructions show similar results to the PPG I (2016) higher-level taxonomic framework, while highlighting the need for multi-locus analyses and locally generated sequences to improve the resolution and stability of species-level relationships among Pteridophyta from Gandul Mount. In line with the findings of [Olsson et al. \(2021\)](#), PPG I (2016) is generally supported in DNA barcoding analyses of Pteridophyta, however, single markers such as *matK*/*psbA*-*trnH* are insufficient for identifying closely related species, so multi-locus analysis is recommended for greater reliability.

Conclusion

This study shows that Gandul Mount supports significant pteridophyte diversity, with 17 species belonging to eight families, dominated by *Pteridaceae*, demonstrating high ecological adaptability to heterogeneous and rocky environments. Morphological analysis revealed substantial variation in leaf, stem, root, and sorus characters, confirming their taxonomic value for species identification and supporting the reliability of field-based inventories. Habitat assessments indicated that most species are lithophytic, reflecting the strong influence of local geological conditions on community structure and species distribution. From a policy perspective, these findings emphasize the need to incorporate fern diversity into local biodiversity conservation planning, particularly in areas with high lithophytic habitats. Sustainable use strategies and regular monitoring are recommended to prevent overexploitation and habitat degradation while maintaining the ecological and cultural value of pteridophytes on Gandul Mount. Future studies should prioritize direct DNA extraction and sequencing of pteridophyte specimens from Gandul Mount to



generate species-specific and population-level molecular data. The use of newly collected material will allow the acquisition of complete gene sequences and multiple genetic markers, thereby increasing phylogenetic resolution, reducing reliance on limited public database records, and allowing a more accurate assessment of genetic diversity and evolutionary relationships within local populations.

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Generative AI: It has been used to correct grammatical errors.

Data availability: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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