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RESEARCH ARTICLE

Phenetic Analysis of Polypodiaceae Ferns in Nagarawangi Village, Sumedang District, Indonesia

Suryana[1]*, Asep Zaenal Mutaqien[1], Joko Kusmoro[1], Budi Irawan[1], Twista Davela Putri[1], Sukono[2], Astrid Sulistya Azahra[3]

[1]*Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Jatinangor, Sumedang, Jawa Barat 45363, Indonesia*
[2]*Department of Mathematics, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Jatinangor, Sumedang, Jawa Barat 45363, Indonesia*
[3]*Doctoral Program in Mathematics, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Jatinangor, Sumedang, Jawa Barat 45363, Indonesia*

*Corresponding Author: suryana.bio@unpad.ac.id

Abstract: Polypodiaceae is a family of ferns with the largest members and a wide distribution in forest and high-humidity areas. The availability suggests the need for Phenetic analysis of Polypodiaceae in Nagarawagi Village to facilitate the classification of ferns as well as create a database of flora and fauna diversity in Indonesia. Therefore, this study aimed to determine the species and phenetic relationships of Polypodiaceae, including differences in characteristics based on morphological and anatomical characters. The method used was a preliminary explorative at the study site, where samples were observed in morphological characters such as root, stem, leaf, and sorus, including anatomical characters, namely stomata type and petiole anatomy. Data obtained were analyzed using MVSP (MultiVariate Statistical Package) 3.22 program Percent Similarity method. The results showed that there were 7 species of Polypodiaceae family, namely *Pyrrosia lanceolata*, *Microsorum punctatum* var. grandiceps, *Drynaria quercifolia, Drynaria rigidula, Belvisia mucronata, Platycerium bifurcatum*, and *Pyrrosia piloselloides*. The species most closely related to others at a 48.22% similarity value were *Pyrrosia piloselloides* and *Pyrrosia lanceolata*. Meanwhile, the closest relatives were *Drynaria quercifolia* and *Drynaria rigidula* with a 87.89% similarity value. The most influential morphological and anatomical characters were stalk and leaf color, leaf tip, location and shape of sorus, as well as stomata type.

Keywords: Morphology, Petiole Anatomy, Polypodiaceae, Stomata Type

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Introduction

Indonesia is a country with very high biodiversity, formed by the combination of Asia and Australia (Australasia) [1]. The diversity of flora in this country is recorded at more than 110,000 species [1]. Among several species, only a few are cultivated, while others still grow wild in forests, including ferns [2, 3]. This group of plants is known as seedless vascular, with body structure differentiated into root, stem, leaf, and pinnae, similar to others [4]. Polypodiaceae is one of the largest ferns families [5, 6], distributed in forest and high humidity areas [7, 8].

The family Polypodiaceae is one of the largest fern families, comprising over 1,600 species widely distributed across tropical and subtropical regions (PPG I, 2016). In Indonesia, the diversity of Polypodiaceae is estimated to be very high, yet accurate and up-to-date inventory data remain limited. A recent inventory study by [9] in the Lesser Sunda Islands recorded 32 Polypodiaceae species out of 216 fern species found in Bali, indicating that this family is a major component of Indonesian fern diversity. Similarly, [10] documented 39 fern species from 19 families in Riau, with Polypodiaceae species such as Pyrrosia and Microsorum recorded as dominant groups.

Traditionally, the identification and classification of Polypodiaceae species have been based on morphological characters such as rhizome type, leaf shape, and sori type. However, these classic manuals are outdated and do not reflect modern taxonomic developments following the phylogenetic revolution initiated and the consensus classification of PPG I (2016). For instance, changes in the taxonomic status of many species within the genera Pyrrosia, Microsorum, and Drynaria are not covered in these older manuals.

In the last decade, integrative taxonomic approaches combining morphological and molecular data have become the gold standard. [11] specifically studied the genus Leptochilus (Polypodiaceae) using an integrative approach in the Indo-Burma region and successfully identified cryptic species that could not be distinguished morphologically. [12] also demonstrated that spore micromorphological analysis can serve as an effective identification tool for species within the genus Pyrrosia.

This study aims to inventory the Polypodiaceae species in Nagarawangi Village, Rancakalong District, Sumedang Regency, and to identify them using a phenetic approach based on morphological and anatomical characters. Although this approach has limitations compared to contemporary integrative studies, the results of this study are expected to serve as baseline data for more comprehensive taxonomic studies in the future.

A potential method to determine the kinship of plants is through numerical taxonomy or numerical taxometry [13]. This is a quantitative evaluation method for identifying similarities in characteristics between groups of organisms and their formation, known as cluster analysis [14]. In plant classification, the boundaries of Polypodiaceae family are unclear due to differing opinions among taxonomists, which is mainly caused by various classification bases and the selection of taxonomic traits [15, 16].

One characteristic that can be used as a basis for plant classification is morphology [17]. Specifically, plant morphology is the study of the physical form and structure. Anatomical studies, such as paradermal and petiole, can support morphological data and assist in solving taxonomic problems [18, 19]. Stomata are modified epidermal cells in the form of a pair of guard cells creating gaps that allow water vapor and gas exchange between the interior and the environment [20-22]. Various plant species have different types of stomata, particularly in the same family [23]. Therefore, petiole anatomy is considered suitable for systematic plant study due to the variety of stomata types and shapes, including the presence of sclerenchyma and other anatomical characteristics [24].

The Polypodiaceae family is the fern family with the most genera and distribution. However, research related to phenetic analysis has not been widely revealed. This phenetic analysis is very important research, but this research would be better if it was strengthened or further tested with a phylogenetic approach.

Based on the description above, this study aims to identify the species of ferns in the Polypodiaceae family, their phenetic relationships, and the influential characters (morphology and anatomy) that influence the grouping of each species.

Fern sampling was conducted in Nagarawangi Village due to the abundance of the Polypodiaceae family. This research is focused only on Nagarawangi Village with the consideration of an in-depth case study (intensive case study) to:

(1) Fill the gap in fern biodiversity data in the area

(2) Enable comprehensive sampling of various microhabitats within a single village landscape

(3) Optimize limited research resources

(4) Reduce environmental confounding variables so that the resulting phenetic relationships better reflect pure taxonomic closeness

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This location was chosen because it has never been studied before and represents a unique mosaic between remaining natural forest and cultivated land at the foot of Mount Manglayang.

The novelty of this research lies in the analysis of the kinship relationships of the Polypodiaceae family of ferns based on the integration of morphological and anatomical characters, which was carried out for the first time in Nagarawangi Village, Rancakalong, Sumedang, West Java, as an area that has never been studied before.

Thus, this research is a development of methods (character integration) and expansion of the study area which contributes new data to the science of taxonomy and Indonesian biodiversity.

Materials and Methods

The research was conducted from March to October 2024 in Nagarawangi Village, Sumedang Regency, West Java. The tools and materials used in this study were distilled water, writing instruments, an altimeter, specimen envelopes, ferns identification book, a GPS, a hygrometer, calipers, coverslips, and slides. Other materials included a digital camera, newsprint, nail polish, labels, a light microscope, a stereo microscope, a field knife, a soil tester, a razor blade, spirit lamp, ferns, and a worksheet. This experiment was divided into two stages, namely a preliminary survey and an intensive study. The preliminary survey was conducted to assess field conditions and determine sampling locations. The intensive study served as a follow-up activity conducted after determining the location to collect necessary data. This stage included data collection, environmental parameter measurements, plant identification, and recording of ferns morphological characteristics, observation of leaf stomata, herbarium preparation, and data analysis.

The data collection technique was carried out using an exploratory method, namely by exploring every corner of the location that could represent the types of ecosystems or vegetation in the studied area. Data collection techniques used exploratory methods, namely exploring every corner of the location that could represent the type of ecosystem or vegetation in the study area. Data collection was conducted during the same reproductive vegetation phase to minimize ontogenetic variation.

Measurements of environmental parameters were carried out in the research zone consisting of soil acidity, air humidity, temperature, light intensity, altitude, and soil moisture. For plant identification, using identification books:

1) Ferns of Malaysia in Colors

2) Ferns Growner's Manual [25]

3) Types of Indonesian Ferns

4) Fern of Malaysian Rain Forest

Subsequently, data analysis was performed using MVSP (MultiVariate Statistical Package) program with Percent Similarity method. Data collected were the names of ferns species (terrestrial, epiphytic, and lithophytic), substrate, presence, and environmental parameter data.

A total of 24 morphological and 13 anatomical characteristics were selected based on taxonomic relevance in Polypodiaceae family, as documented in a previous study. Morphological characteristics, such as rhizome shape, sorus position, and venation patterns, were selected, showing evolutionary differences among Polypodiaceae ferns. One of the studies that could support morphological data and helped resolve taxonomic problems was paradermal and petiole anatomy [26]. Anatomical characteristics such as stomatal type and vascular bundle configuration were included due to relative stability against environmental variation and provision of additional resolution attributed to insufficient morphological characteristics. Subsequently, all characteristics were given equal weight to avoid bias towards those with greater variation, according to practice in previous phenetic studies using Unweighted Pair Group with Arithmetic Mean (UPGMA) clustering and similarity percentage.

UPGMA algorithm combined with similarity percentage was selected because of the ability to produce reliable clustering for discrete characteristics datasets with moderate sample sizes. Similarity percentage served as a simple coefficient suitable for morphological and anatomical characteristics encoded in a binary manner, thereby allowing for direct comparison among Operational Taxonomic Units (OTUs). This method had been successfully applied to ferns systematics [14] and was considered appropriate, balancing computational simplicity and biological interpretation for non-molecular datasets.

Morphological Characteristics

In this study, 24 morphological characteristics were used to phenetics analysis. These included habitat, habitus, rhizome shape, rhizome surface, stalk shape, stalk color, stalk height, stalk surface, leaf type, leaf type, leaf shape, leaf base, leaf margin, leaf tip, leaf venation, leaf color, upper leaf surface, lower leaf surface, leaf flesh, nest leaf, nest leaf shape, sorus location and shape, and indusium.

Anatomical Characteristics

A total of 13 anatomical characters were used to phenetics analysis. These included stomata type (anomocytic, polycytic, diacytic, copolycytic, cyclocytic, and pericytic), epidermis shape, vascular bundles comprising stele type, vascular bundle shape, xylem strand shape, number of vascular bundles, and the presence or absence of circumendodermal bands.

Stomata observation on leaf lamina was performed by thinly slicing leaf surface using a razor blade to obtain a thin and transparent section. It was then placed on a glass slide and cleared by dropping a 5.25% hypochlorite solution (chlorox) for 3-5 minutes, cleaned with water, and stained using 2% safranin dye for 30 seconds. The section was placed on a glass slide, administered 30% glycerin, and covered with a cover glass glued with clear nail polish. On a thin leaf, stomata observation was conducted by applying nail polish to leaf under observation and allowed to dry. This was followed by attaching electrical tape to the part of leaf coated with nail polish and careful removal using tweezers. The tape was attached to the slide and observed under a light microscope. Petiole or stalk anatomy was performed by incising the stalk transversely with a razor blade until a thin, transparent section was obtained. The section was placed on a glass slide and treated with hypochlorite solution for 3-5 minutes. This was followed by cleaning with water, which was dripped with 30% glycerin and covered using a coverslip sealed with clear nail polish.

Data Analysis

The data analysis was conducted through the taxometry method using MVSP 3.22 program consisted of the following stages.

Association Coefficient

Association or similarity coefficient was used to determine the kinship relationships of each OTU based on the degree of similarity in characteristics. OTUs were ferns to be analyzed. After comparison, matching characteristics were coded as (1), and non-matching were (0). The data were entered into MVSP program by selecting cluster analysis and calculated using Percent Similarity to obtain a similarity coefficient matrix and dendrogram. The Similarity Coefficient was chosen because:

1) It provides a comprehensive picture of similarity by considering the similarities of "present" and "absent"
2) It is easy to calculate, interpret, and compare with other phenetic studies
3) It is suitable for the purpose of initial exploration of the morphological diversity of Polypodiaceae in a new location (Nagarawangi Village)

Principal Component Analysis

The results of association coefficient calculations were followed by cluster analysis to group OTUs pairs. The coefficient matrix obtained was used to generate cluster analysis data, which was performed by grouping and comparing the most closely related OTUs. These included pairs with the highest association coefficient values based on the association coefficient matrix. The clustering results were reconfirmed through Principal Component Analysis (PCA), which was used to determine the weighting of distinguishing characteristics values in OTUs separation.

Results and Discussion

Location Description

Nagarawangi Village covers an area of 463 hectares and is located in the capital of Rancakalong District, serving as the administrative center. Geographically, the village is located at coordinates 107°49'37.7" - 107°51'25.6" South Latitude and 6°49'50.2" - 6°48'58.6" South Latitude, along the provincial road that serves as the access road between Sumedang - Rancakalong - Tanjungsari. Nagarawangi Village is a hilly location with an elevation of over 800 m above sea level, a temperature of approximately 25-28°C, humidity ranging from 60-90% according to the Meteorology, Climatology, and Geophysics Agency (BMKG), and rainfall of around 2,570 mm.

The ecosystems include rice fields, settlements, dry fields, forests, or leuweung, as well as water bodies and ponds. Furthermore, Nagarawangi Village is suitable for ferns due to the high humidity and hilly location, which allows for the discovery of several species from various families.

These include Adiantaceae (*Adiantum concinum*; *Adiantum* sp.; *Adiantum pedatum*), Pteridaceae (*Pteris biaurita; Pteris ensiformis; Pteris vittata; Pityrogramma calomelanos*), Cyatheaceae (*Cyathea contaminans*), Nephrolepidaceae (*Nephrolepis biserrata; Nephrolepis* sp.), Selaginellaceae (*Selaginella wildenowii*), Blechnaceae (*Blechnum orientale*), Davaliaceae (*Davalia denticulata; Davalia* sp.), Lygodiaceae (*Lygodium flexuosum*), Gleicheniaceae (*Dicranopteris linearis* var. Linearis), and Polypodiaceae.

Ferns of Polypodiaceae Family

This study successfully identified seven Polypodiaceae species in Nagarawangi Village, Sumedang Regency: Pyrrosia lanceolata, Pyrrosia piloselloides, Microsorum punctatum var. grandiceps, Drynaria quercifolia, Drynaria rigidula, Belvisia mucronata, and Platycerium bifurcatum. This number is comparable to the findings of [27] in the Dieng Valley tourism area, Malang, which recorded several Polypodiaceae species such as Phlebodium aureum (syn. Polypodium aureum) as a dominant species. Similarly, [28] reported 18 epiphytic fern species in the Girimanik Natural Forest Area, Wonogiri, with Polypodiaceae as the family with the highest diversity.

The occurrence of the same species across multiple locations in Indonesia suggests that Polypodiaceae species have wide distributions, particularly in Java and surrounding areas. This is consistent with [9] who noted that many Polypodiaceae species are cosmopolitan or at least widely distributed throughout Malesia.

It must be acknowledged that this study has limitations due to its exclusive reliance on a morphology-based phenetic approach. As demonstrated by [11] in the genus Leptochilus, integrative approaches combining molecular (plastid and nuclear) and morphological data can reveal cryptic species that are morphologically indistinguishable. [12] also emphasized that morphology-based identification alone, while still useful, requires validation with other approaches such as spore analysis or molecular methods to avoid misidentification.

Contemporary studies on Polypodiaceae, such as on the genus Platycerium and at the family level, have demonstrated that plastid phylogenomics can resolve phylogenetic relationships that were previously unresolved. Therefore, the results of this study are preliminary and require further testing using molecular approaches.

The number of individuals collected can be seen in table 1.

Table 1: Number of specimens collected per species

No.	Spesies	Number of individuals collected
1	*Pyrrosia lanceolata*	12
2	*Pyrrosia piloselloides*	12
3	*Microsorum punctatum var. grandiceps*	13
4	*Drynaria quercifolia*	13
5	*Drynaria rigidula*	12
6	*Belvisia mucronata*	15
7	*Platycerium bifurcatum*	13

The morphological and anatomical characteristics were described as follows.

Morphological Characteristics

Drynaria rigidula (Sw.) Bedd

Epiphytic plant. Herbaceous. Short, creeping rhizome. Rhizome surface is scaly, with round stem that is dark brown, and a medium size of approximately 30–50 cm. Stem surface is hairy. Leaf is monomorphic and single. Leaf shape is pinnate, with an attenuate base and serrate margin Leaf tip is attenuate, venation is net-like, and the color is dark green. The upper surface of leaf is smooth and glabrous. Meanwhile, the underside is smooth and glabrous, with slightly hairy. Leaf is also parchment-like, and the nest is pinnatifid. Sorus is round and medial, without indusium but with stellate trichomes.

Drynaria quercifolia (L.) J. Sm

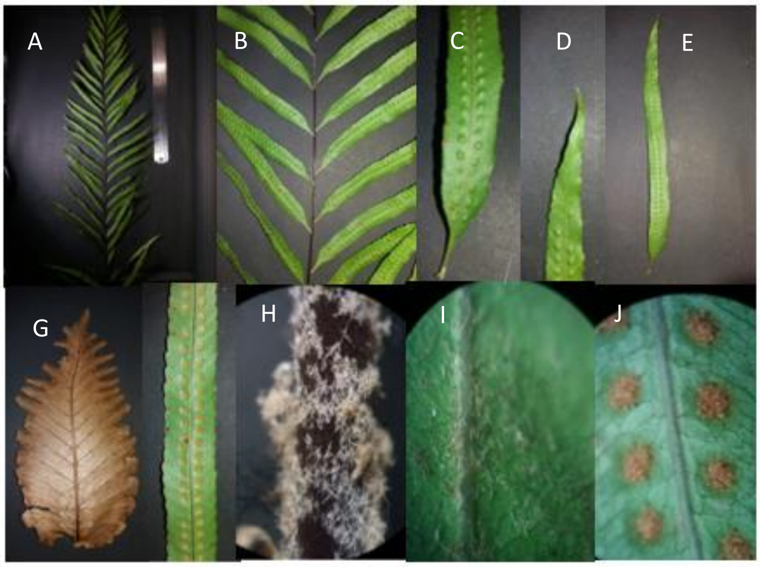

Fig. 1: Morphology of *Drynaria rigidula*: A.B. Frond, C. Leaf base, D. Leaf tip, E. Leaf blade, F. Nest leaf, G. Linear and medial sorus, H. Stem surface with stellate hairs (trichomes), I. Upper leaf surface, J. Lower leaf surface, and sorus

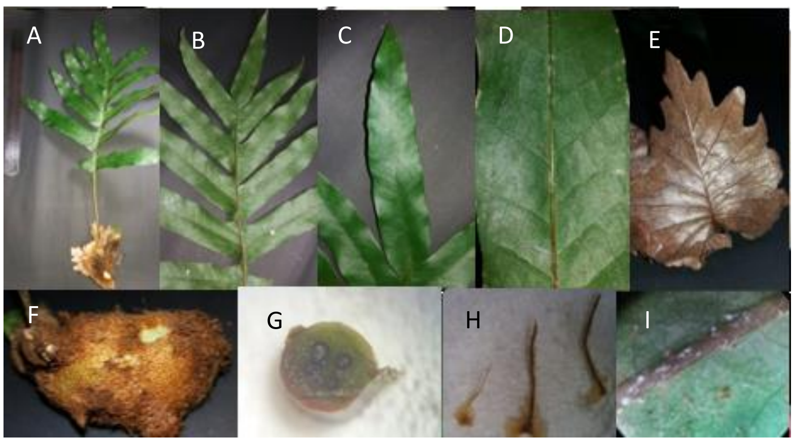

Fig. 2: Morphology of *Drynaria quercifolia*: A. Frond, B. Lower surface of leaf, C. Leaf tip, D. Upper surface of leaf, E. Nest leaf, G. Round leaf stalk, F. Rhizome scales, I. Stellate hairy leaf stalk surface (trichomes)

Epiphytic plant has herbaceous stature and short, creeping rhizome. The surface of rhizome is scaly, has round stalk that is dark brown stalk, and is medium-sized of approximately 30 – 50 cm. Stem surface has stellate trichomes. Leaf is monomorphic and single, with pinnatifid shape, attenuate basenad tip, sinuate margin, and net-like venation. Dark green leaf has parchment-like texture, with a smooth upper and lower, slightly hairy surface. Nest leaf is pinnatifid. Sorus is round and spread on the lower surface of leaf, without indusium. There are stellate trichomes.

Belvisia Mucronata Copel

Epiphytic plant is herbaceous, with short, creeping rhizome. Rhizome surface is scaly, with approximately 15-20 cm tall, round stalk that are dark brown. Stalk surface is smooth and slightly hairy (trichomes). Leaf is monomorphic and single, with lanceolate shape. The base is attenuate, and leaf margin is entire. Leaf tip is cuspidate, green, having upper and lower surfaces that are smooth and slightly hairy. Leaf is thin and soft (herbaceous), lacking nest. Sorus is round and medial, without indusium but with peltate trichomes.

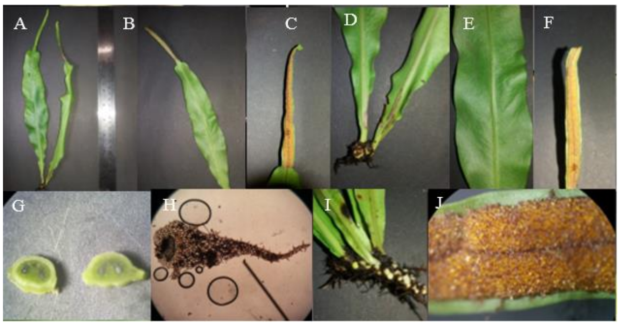

Fig. 3: Morphology of *Belvisia mucronata*: A. Frond; B. Lower leaf surface; C. Leaf tip; D. Leaf base; E. Upper leaf surface; F. Sorus; G. Leaf stalk shape; H. Rhizome scales; I. Leaf rhizome; J. sorus

Platycerium bifurcatum (Cav.) C. Chr

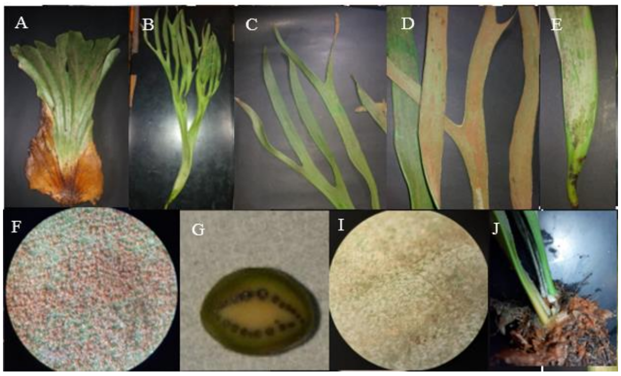

Fig. 4: Morphology of *Platycerium bifurcatum*: A. Sterile frond; B. Fertile frond; C. Leaf tip; D. Lower leaf surface and sorus; E. Leaf base; F. Sorus; G. Leaf stalk shape; I. Hairy leaf surface; J. Rhizome

Epiphytic plant has herbaceous status, with short, creeping rhizome and scaly surface. It has oval, green stalk of approximately 30–60 cm in height, covered with hairy stalk surface. Leaf is dimorphic, single, and bifurcate, with an attenuate base, entire margin, and obtuse tip. Green leaf is fleshy (cornous) and round, with stem hairs (trichomes) on the upper and lower surfaces [25]. Sorus is densely arranged at the lower leaf surface, lacking indusium, while stellate-shaped trichomes are also present.

Pyrrosia lanceolata (L.) Farw

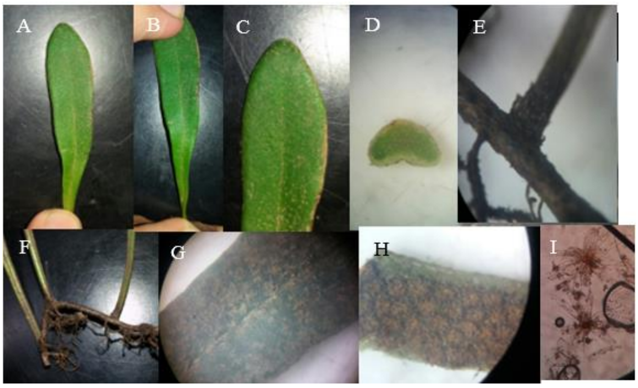

Fig. 5: Morphology of *Pyrrosia lanceolata*: A. Frond; B. Leaf base; C. Leaf tip; D. Stem shape; E. Stem and rhizome surface; F. Rhizome; G. Upper leaf surface; H. Lower leaf surface and sorus; I. Stellate hairs (trichomes) (P.100x)

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This epiphytic plant has herbaceous status with wide, creeping rhizome and branching. Rhizome surface is scaly, with green triangular stem of approximately 1–5 cm high. Stem surface is scaly, and leaf is monomorphic and single, having lanceolate shape. Leaf base is attenuate, margin is sinuate, and tip is obtuse. The upper and lower surfaces are hairy, characterized by thin and soft (herbaceous) leaf without nest. Sorus is densely arranged on the underside of leaf, lacking an indusium, while trichomes are stellate.

Pyrrosia piloselloides (L.) M.G. Price

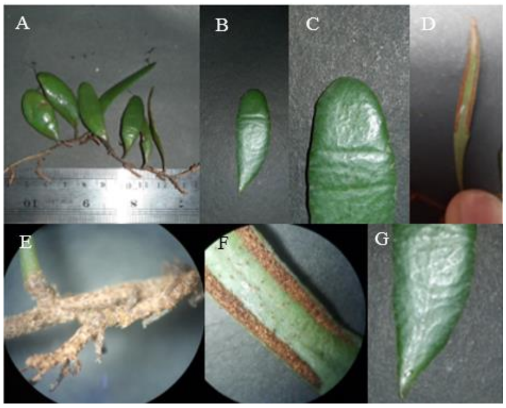

Fig. 6: Morphology of *Pyrrosia piloselloides*: A. Frond; B. Leaf blade; C. Leaf tip; D. Lower leaf surface and sorus; E. Rhizome; F. Sorus and stellate hairs (trichomes); G. Leaf base

Epiphytic plant has herbaceous status, alongside wide and creeping rhizome with branches. Rhizome surface is scaly, stalk is approximately 1-5 cm, triangular, and green, with scaly surface. Leaf is dimorphic [25] and single, characterized by lanceolate shape. Leaf base is attenuate, with sinuate margin, obtuse tip, and green. The upper and lower surfaces are hairy, having thin and soft (herbaceous) texture, without nest. Sorus is linear and marginal, lacking indusium, but there are stellate trichomes.

Microsorum punctatum var. grandiceps (L.) Copel

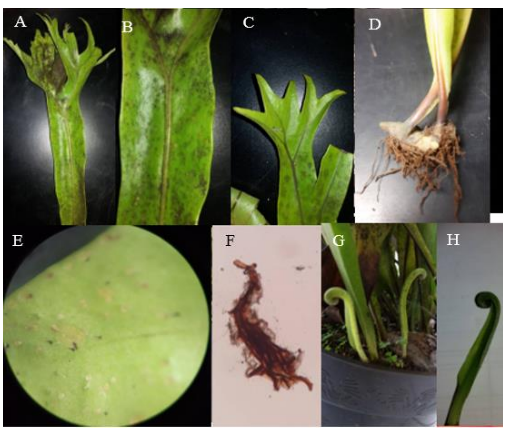

Fig. 7: Morphology of *Microsorum punctatum* var. grandiceps: A. Frond; B. Upper leaf surface; C. Leaf tip; D. Rhizome; E. Leaf surface; F. Rhizome scales; G. Leaf base and coil; H. Coil; I.J. Stellate hairs (trichomes)

Terrestrial plant has herbaceous status and short, creeping rhizome. Surface is scaly and slightly, with triangular, smooth, and bright green stalk of approximately 15–20 cm tall. Leaf is monomorphic, single, and bifurcate, having attenuate base and sinuate margin. Leaf has acute tip, light green color, smooth upper and lower surfaces, as well as leathery texture without nest. Sorus is scattered on the underside of leaf [25], lacks indusium, but stellate trichomes are present.

The morphological characters of the seven Polypodiaceae species found at the study location are presented in full as narrative descriptions and illustrations in the following sections. To facilitate comparison among species, the key diagnostic characters are summarized in Table 2.

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Table 2: Key diagnostic characters of seven Polypodiaceae species from [study location]

Species	Rhizome type	Leaf shape	Leaf margin	Sori type	Lower leaf surface	Distinctive character
Pyrrosia lanceolata	Widely creeping, branched	Lanceolate	Sinuate	Densely arranged	Hairy (stellate)	Leathery leaf, obtuse tip
Pyrrosia piloselloides	Shortly creeping	Bifurcate	Sinuate	Scattered	Glabrous	Very small leaf (<5 cm)
Microsorum punctatum var. grandiceps	Shortly creeping	Pinnatifid	Sinuate	Round, scattered	Glabrous	Crested leaf apex (antler-like)
Drynaria quercifolia	Shortly creeping	Pinnate	Serrate	Round, medial	Glabrous	Two leaf types (sterile nest leaves, fertile pinnate leaves)
Drynaria rigidula	Shortly creeping	Lanceolate	Entire	Densely arranged at apex	Glabrous	Rigid leaf, cuspidate apex
Belvisia mucronata	Shortly creeping	Bifurcate	Entire	Linear, marginal	Hairy	Sori forming lines along leaf margin/apex
Platycerium bifurcatum	Widely creeping, branched	Oblanceolate	Entire	Linear, marginal	Hairy (tomentum)	Two leaf types (sterile shield leaves, fertile antler-like leaves)

Anatomy of Ferns

Drynaria rigidula (Sw.) Bedd

A total of 4 stomata are found in this species, namely anomocytic, polycytic, diacytic, and coplocytic. However, only polycytic is found in some observations. This difference occurs because of adaptation to environmental changes that cause variation in morphology, anatomy, gene expression, and metabolism. The epidermis is irregular, stele is dictyosteleic, and xylem strands are C-shaped. The vascular bundles are U-shaped, with four vascular bundles and circumendodermal bands.

A total of 3 stomata are found in this species, namely anomocytic, polycytic, and coplocytic. Stomata characteristics observed in *D. quercifolia* were consistent with previous results. The epidermis is irregular in shape, stele is dictyosteleic, and xylem strands are C-shaped. There are four vascular U-shaped bundles and circumendodermal band.

Belvisia mucronata Copel

In this species, 5 stomata are found, namely anomocytic, polycytic, diacytic, coplocytic, and cyclocytic. Some observations have reported the presence of only two types of stomata in Belvisia mucronata, namely polycytic and coplocytic. This difference is because of adaptation to environmental changes that affect morphology, anatomy, gene expression, and metabolism. The epidermis is irregular in shape, stele is dictyosteleic, and xylem strands are C-shaped. There are four vascular U-shaped bundles and a circumendodermal band.

Drynaria quercifolia (L.) J. Sm

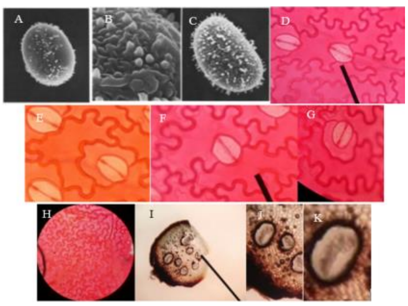

Fig. 8: Anatomy of D. rigidula: A. B. C. Spores; D. Anomocytic stomata; E. Polycytic stomata; F. Diacytic stomata; G. Coplocytic stomata; H. Irregular epidermis (P.100x); I. U-shaped vascular bundles on petioles; J. Vascular bundles; K. C-shaped xylem strands

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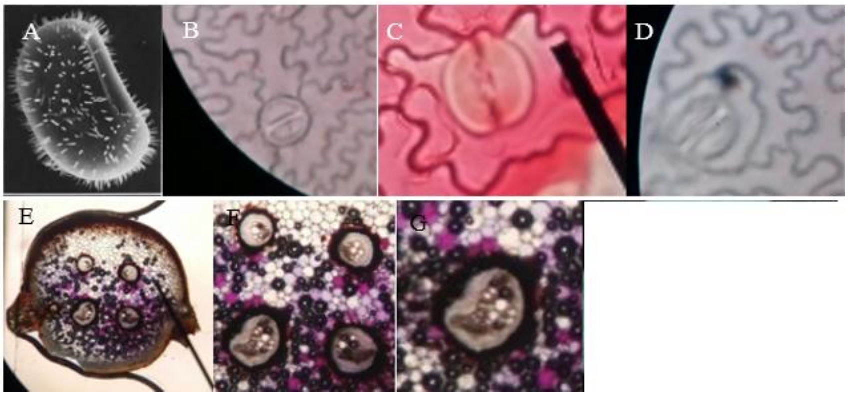

Fig. 9: Anatomy of D. quercifolia: A. Spores; B. Anomocytic stomata; C. Polycytic stomata; D. Coplocytic stomata; E. Paradermal section of petiole; F. U-shaped vascular bundles; G. C-shaped xylem strands

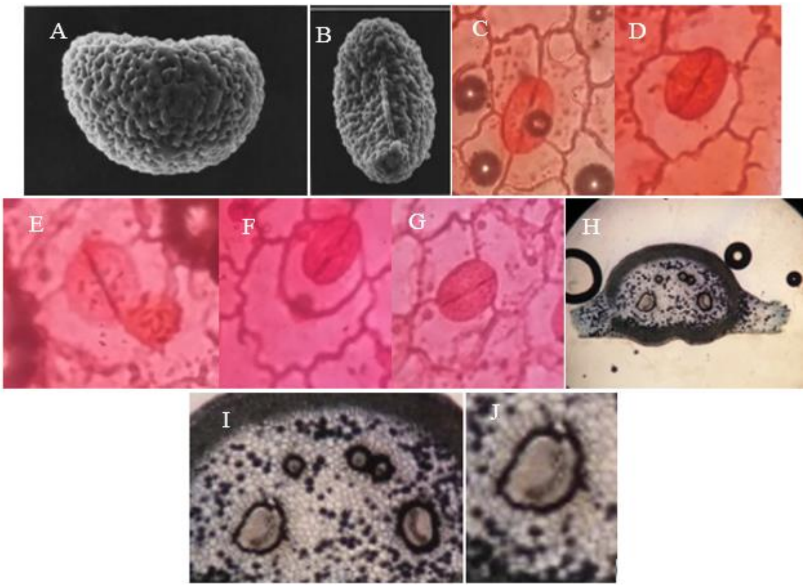

Fig. 10: Anatomy of Belvisia mucronata: A.B. Spores; C. Anomocytic stomata; D. Polycytic stomata; E. Diacytic stomata; F. Coplocytic stomata; G. Cyclocytic stomata; H. Transverse section of petiole; I. U-shaped vascular bundles; J. C-shaped xylem strands

Platycerium Bifurcatum (Cav.) C. Chr

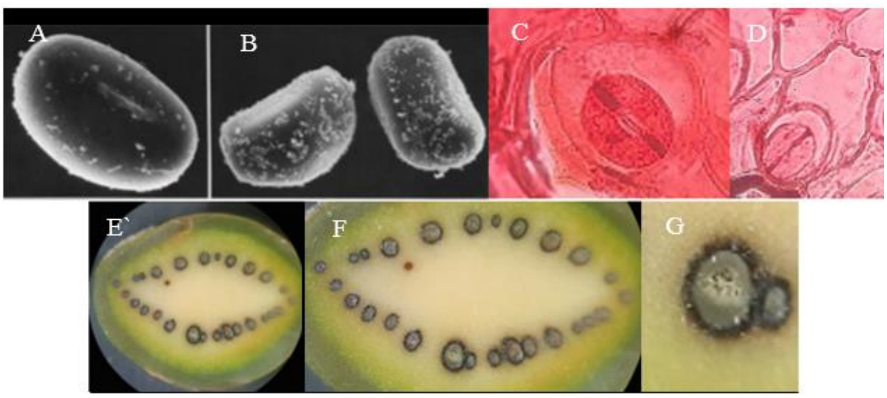

Fig. 11: Anatomy of Platycerium bifurcatum: A.B. Spores; C. Polycystic stomata; D. Coplocytic stomata; E. Transverse section of petiole; F. Semi-circular/oval-shaped vascular bundles; G. C-shaped xylem strands

The epidermis is irregular in shape, stele is dictyosteleic, and xylem strands are C-shaped. There are 26 vascular bundles with semi-circular/oval-shaped and circumendodermal bands.

Pyrrosia lanceolata (L.) Farw

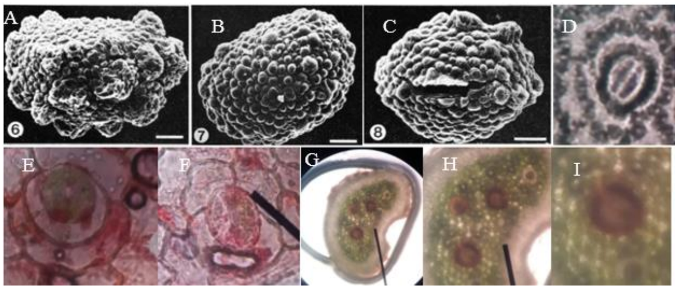

Fig. 12: Anatomy of P. lanceolata: A. B. C. Spores; D. Diacytic stomata; E. Coplocytic stomata; F. Cyclocytic stomata; G. Transverse section of petiole; H. U-shaped vascular bundles; I. C-shaped xylem strands

This species has three types of stomata, namely diacytic, cyclocytic, and coplocytic. The epidermis is irregular in shape, stele is dictyosteleic, and xylem strands are C-shaped. The five vascular bundles are U-shaped and have a circumendodermal band.

Pyrrosia piloselloides (L.) M.G. Price

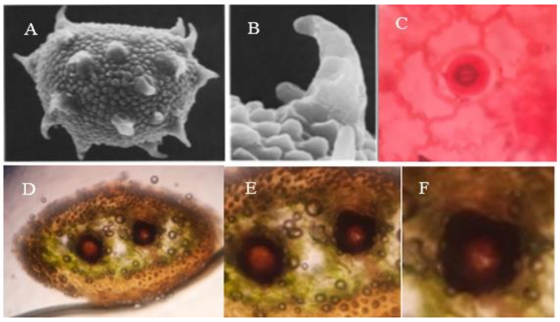

Fig. 13: Anatomy of P. piloselloides: A.B. Spores; C. Pericytic Stomata; D. Transverse Section of Petiole; E. V-shaped vascular bundles; F. C-shaped xylem strands

In this species, only one type of stomata is found, namely pericytes. The epidermis is irregularly shaped, stele is dictyosteleic, and xylem strands are C-shaped. The two vascular bundles are V-shaped, withcircumendodermal band.

Microsorum punctatum var. Grandiceps (L.) Copel

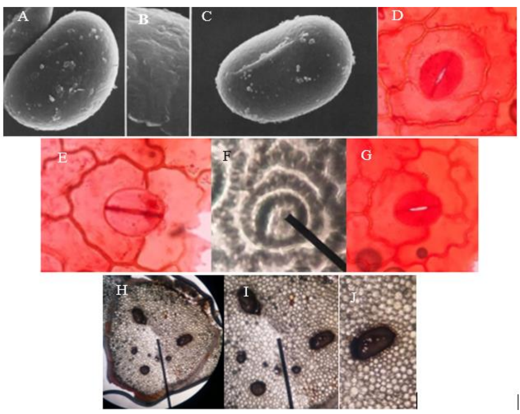

Fig. 14: Anatomy of M. punctatum var. grandiceps: A. B. C. Spores; D. Polycytic stomata; E. Diacytic stomata; F. Copolyctic stomata; G. Cyclocytic stomata; H. Transverse section of M. punctatum; I. U-shaped vascular bundles; J. C-shaped xylem strands

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Anatomical characters that distinguish among species are presented in Table 3. Characters that were uniform across all species (abaxial stomatal position, dictyostele stele type, C-shaped xylem strand, and presence of circum endodermal band) are not shown in the Table 3.

Table 3: Summary of key anatomical characters of seven Polypodiaceae species

Species	Stomatal type	Stomatal index (%)	Stomatal density (per mm^2)	Epidermal shape	Vascular tissue shape	Number of vascular bundles
Pyrrosia lanceolata	Anomocytic, Diacytic, Copolocytic, Cyclocytic	11	38.7	Irregular	U-shaped	5
Pyrrosia piloselloides	Diacytic, Copolocytic, Cyclocytic	6	15.28	Irregular	U-shaped	5
Microsorum punctatum var. grandiceps	Anomocytic, Copolocytic	18.72	41.70	Irregular	U-shaped	4
Drynaria quercifolia	Anomocytic, Diacytic, Copolocytic	26.23	54	Irregular	U-shaped	7
Drynaria rigidula	Anomocytic, Diacytic, Copolocytic, Cyclocytic	6.89	18.34	Irregular	U-shaped	5
Belvisia mucronata	Copolocytic	9.71	17.32	Polygonal	Semi circle/ oval	26
Platycerium bifurcatum	Pericytic	4.83	61.14	Irregular	V-shaped	2

In this species, four types of stomata are found, comprising polycytic, diacytic, coplocytic, and cyclocytic. The results differ from previous observations that identify only polycytic and coplocytic. This variation occurs because of the adaptation to environmental changes, which affect morphology, anatomy, gene expression, and metabolism. The epidermis is irregular in shape, stele is dictyostelic, xylem strands are C-shaped. The five vascular bundles are U-shaped, with a circumendodermal band. Compared to other species in the same genus, stele type, xylem strand shape, and vascular bundle shape are similar. However, the number of vascular bundles differs due to variation in the part of plant examined or by intraspecific differences.

Anatomical data (stomata types, vascular bundle shapes, and presence of circumendodermal bands) were directly integrated into the phenetic interpretation. For example, the shared U-shaped vascular bundles and cyclocytic stomata among Belvisia mucronata, Microsorum punctatum var. grandiceps, and Drynaria species support the grouping in both the dendrogram and PCA plot. This shows the contribution of anatomical traits to species relationships. The observations are consistent with the broader anatomical patterns reported in previous studies.

The variation in anatomical data observed among the seven species correlates with previous descriptions of Polypodiaceae anatomy [7, 16]. However, the broader diversity of stomatal and vascular bundle types identified in this study suggests possible regional adaptation and supports the phonetic grouping based on shared anatomical traits. This suggests the taxonomic significance of integrating both morphological and anatomical characteristics in the classification of Polypodiaceae.

Phenetics Analysis in Polypodiaceae Family Using MVSP 3.22

Phenetic analysis is based on the analysis of a number of phenotypic appearances of an organism. The relationship between two individuals or populations can be measured based on similarities in several characteristics. Plant relationships can be determined using phenetic or phylogenetic methods. The phenetic method is dependent on similarities in phenotypic characteristics such as morphology, anatomy, embryology, and phytochemistry. Meanwhile, the phylogenetic method is more focused on the evolutionary value of each character.

In this study, grouping analysis to determine the kinship relationship between species in Polypodiaceae family was carried out based on 24 morphological and 13 anatomical characteristics using MVSP 3.22 program. Morphological characteristics were used for grouping, including habitat, habitus, rhizome shape, rhizome surface, stalk shape, color, height, and surface.

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Others included leaf type, shape, base, edge, tip, venation, color, upper and lower leaf surface, flesh, nest leaf, nest leaf shape, location, and shape of sorus, as well as indusium. Meanwhile, anatomical characteristics observed were stomata type (anomocytic, polycytic, diacytic, copolycytic, cyclocytic, and pericytic), epidermis shape, vascular (*vascular bundle*) consisting of stele type, vascular bundle shape, xylem strand shape, number of vascular bundles, and the presence or absence of circumendodermal bands. Descriptive data were assessed numerically by assigning scores to the characteristics appearing in each species. Quantitative data were obtained through direct measurements and ranged according to the specified categories. The seven species were grouped using cluster analysis by measuring similarities between OTUs using UPGMA based on Percent Similarity. The calculation results were obtained from the scor

ed data and processed with MVSP 3.22.

Tables 4 and 5 show similarity matrix and hierarchical grouping of the seven Polypodiaceae species. Based on the results, similarity values ranged from 41.76% to 87.88%, indicating moderate to high phenetic affinity. The highest similarity (87.88%) between Drynaria quercifolia and *D. rigidula* showed shared pinnatifid fronds and stellate trichomes, while lower values (< 60%), including *Platycerium bifurcatum*, emphasized distinctive frond dimorphism and soral arrangement. The grouping hierarchy in Table 5 confirmed that *Pyrrosia lanceolata* and *P. piloselloides* clustered together at 68%. This was in line with genus-level relationship but separated from other taxa by sorus position and vascular bundle differences.

Table 4: Results of similarity calculations with percentage similarity

	Sp A	Sp B	Sp C	Sp D	Sp E	Sp F	Sp G
Sp A	100						
Sp B	65.52	100					
Sp C	47.27	73.02	100				
Sp D	49.18	72.46	87.88	100			
Sp E	62.50	69.44	66.67	72.00	100		
Sp F	41.76	46.47	50.00	58.86	53.33	100	
Sp G	68.00	44.83	40.00	39.34	50.00	41.76	100

Sp A: Pyrrosia lanceolata

Sp B: Microsorum punctatum var. grandiceps

Sp C: Drynaria quercifolia

Sp D: Drynaria rigidula

Sp E: Belvisia mucronata

Sp F: Platycerium bifurcatum

Sp G: Pyrrosia piloselloide

Value in Percent

Similarity matrix (Table 4) shows values ranging from 41.76% to 87.88%, indicating moderate to high phenetic affinity among species. The highest similarity (87.88%) occurs between *Drynaria quercifolia* and *D. rigidula*, which is in line with the placement in the same genus and shows shared morphological traits such as pinnatifid fronds and stellate trichomes. In comparison, *Platycerium bifurcatum* shows relatively low similarity scores (< 60%) with most species, supporting the distinctive frond dimorphism and soral arrangement.

Table 5 groups species hierarchically, with *Pyrrosia lanceolata* and *P. piloselloides* forming a subcluster at 68%. This suggests a closer affinity in the genus Pyrrosia despite the differences in sorus shape and vascular bundle configuration. The dendrogram generated by UPGMA (Fig. 15) replicates similarities, separating Pyrrosia species from the remaining taxa. The branching at 51.67% shows that *Platycerium bifurcatum* diverges earlier than the cluster containing Microsorum, Drynaria, and Belvisia. This pattern shows distinct reproductive strategies and frond morphologies observed in previous *Polypodiaceae* *phylogenies* [16]. The clustering of *Belvisia mucronata* with Microsorum and Drynaria species suggests shared anatomical characteristics such as U-shaped vascular bundles and cyclocytic stomata, corroborating similarity matrix results.

Table 5: Grouping species based on Percent Similarity value

Node	Group 1	Group 2	Similarity value (%)	Groups on the Dendogram
1	Sp C	Sp D	87.88	Group h
2	Node 1	Sp B	72.74	Group f
3	Node 2	Sp E	69.37	Group d
4	Sp A	Sp G	68.00	Group b
5	Node 3	Sp F	51.67	Group a
6	Node 5	Node 4	48.22	

Description: Nodes are a combination of species in group 1 and group 2 based on similarity values from highest to lowest.

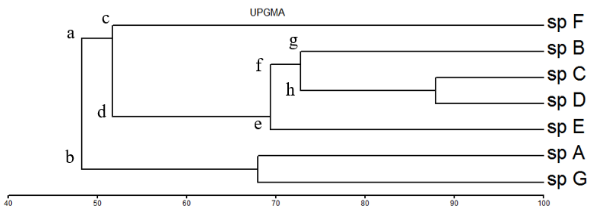

Fig. 15: Dendogram of the phylum between seven species of ferns in Polypodiaceae family, compared in terms of morphology and anatomy (stomata type and petioles)

Sp A: Pyrrosia lanceolata

Sp B: Microsorum punctatum var. grandiceps

Sp C: Drynaria quercifolia

Sp D: Drynaria rigidula

Sp E: Belvisia mucronata

Sp F: Platycerium bifurcatum

Sp G: Pyrrosia piloselloides

Based on the dendogram, with similarity value of 48.22%, two groups were identified, marked with the letters a and b. Group a consists of *Pyrrosia lanceolata, Microsorum punctatum* var. grandiceps, *Drynaria quercifolia, Drynaria rigidula, Belvisia mucronata*, and *Platycerium bifurcatum*. Meanwhile, group b consists of *Pyrrosia lanceolata* and *Pyrrosia piloselloides*. The results show that *Pyrrosia lanceolata* and *Pyrrosia piloselloides* have the lowest similarity among the other species. This is due to a few morphological similarities, including habitat, habitus, rhizome surface, leaf type, leaf base, indusium, and hairs (trichomes). Meanwhile, the anatomical characteristics shared include stomata position, stele-type epidermis, xylem strand shape, and circumendodermal band presence. Both species have a similarity value of 68%, which is significant considering the classification in the same genus, Pyrrosia.

At similarity value of 51.67%, group a is divided into c and d. Based on the classification, group c consists of only *Platycerium bifurcatum*, while group d includes *Microsorum punctatum* var. grandiceps, *Drynaria quercifolia, Drynaria rigidula*, and *Belvisia mucronata*. This group is divided due to differences in stem shape, color, height, and surface, including leaf type, shape, margin, tip, venation, color, surface, nest, and flesh.

With similarity value of 69.37%, group d is divided into e and f. In line with the classification, group e contains *Belvisia mucronata*, while group f consists of *Microsorum punctatum* var. grandiceps, *Drynaria quercifolia*, and *Drynaria rigidula*. Characteristics of *Belvisia mucronata* that differ from species in group f include stem color, leaf shape, tip, margin, venation, color, and flesh, sorus position and shape, number of vascular bundles, and trichome or hair shape.

Group f is further separated into g and h with similarity value of 72.74%. Based on the new classification, group g contains *Microsorum punctatum* var. Grandiceps, while h consists of *Drynaria quercifolia* and *Drynaria rigidula*. *Belvisia mucronata* and *Microsorum punctatum* var. grandiceps, with similarity score of 69%. According to PPG I (2016), both groups are in the same subfamily of Microsoroideae, allowing initial separation. The two species with the highest similarity score of 87.88% are *Drynaria quercifolia* and *Drynaria rigidula*. This is appropriate because the two species belong to the same genus, differing in leaf shape, leaf margins and color, sorus position and shape, as well as stomata type.

The dendrogram separates ferns in Polypodiaceae family based on similarities in morphological and anatomical characteristics until a species is found that truly separates or clusters with others. Groups between species are formed according to the level of similarity expressed in values, indicating ratio of shared characteristics to the total number. Therefore, higher shared characteristics correspond to a greater similarity value, indicating a closer relationship. This kinship relationship is indicated by shared similarities. When a group consists of several species, the first branch formed in the dendrogram has the lowest similarity value of 49.68%. The group formed with the highest at 87.88% represents species of very close kinship due to nearly identical morphology.

PCA shows the characteristics most responsible for group separation (Table 3). Based on the results, Component 1 (explaining the largest variance) was dominated by leaf color (0.434) and petiole color (0.383), indicating powerful discriminators. Component 2 emphasized leaf tip (0.321) and petiole shape (0.233). In the scatter plot (Fig. 16), *Pyrrosia lanceolata* and *P. piloselloides* are isolated in a separate quadrant, confirming a distant relationship compared to the green-cluster taxa (Microsorum, Drynaria, Belvisia, and Platycerium). PCA results support dendrogram's topology, showing that sorus morphology and stomatal types are critical for distinguishing taxa.

Morphological and Anatomical Characteristics Influencing Species Grouping in Polypodiaceae Family of Ferns

In this study, PCA was performed to identify the characteristics with a significant influence and separate OTUs. These results were presented by showing several key distinguishing components along with the values of each character. Table 6 shows the character components that influenced the grouping of OTUs in Polypodiaceae family of ferns.

Table 6: Main component values of the characteristics of ferns of Polypodiaceae family

Character	Component 1	Component 2
Habitat	0.006	0.09
Habitus	0	0
Rhizome shape	0.21	0.03
Rhizome surface	0	0
Petiole shape	0.062	0.233
Petiole color	0.383	0.127
Petiole height	0.314	0.029
Petiole surface	0.341	0.057
Leaf type	0.054	0.224
Leaf kind	0	0
Leaf shape	0.137	0.062
Leaf base	0	0
Leaf margin	0.027	0.257
Leaf tip	0.178	0.321
Leaf venation	0.122	0.181
Leaf color	0.434	0.145
Upper leaf surface	0.172	0.231
Lower leaf surface	0.24	0.284
Leaf texture	0.13	0.039

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Nest leaf	0.172	0.092
Nest leaf shape	0.205	0.198
Sorus position & shape	0.138	0.316
Indusium	0	0
Stomata position	0	0
Anomocytic	0.148	0.122
Polocytic	0.21	0.03
Diacytic	0.001	0.182
Copolyctic	0.11	0.041
Cyclocytic	0.062	0.133
Pericytic	0.11	0.041
Epidermis shape	0.056	0.183
Stele type	0	0
Xylem strand shape	0	0
Vascular bundle shape	0.022	0.331
Number of vascular bundles	0.201	0.379
Presence of circumendodermal band	0	0
Trichomes / hairs	0.032	0.031

In PCA matrix component, there are two main characteristics components that play a role in separating groups of ferns in Polypodiaceae family. Component 1 is the primary component, while component 2 acts as a supporting component. The bold value is the characteristics with the most influence in grouping because of higher value. In line with the results, the most influential morphological characteristics are stem color, leaf color, leaf tip, and the location and shape of sorus. Meanwhile, the most influential anatomical characteristics include the type of stomata. In component 1, the most influential characteristics include leaf color. According to Munsell color chart for Plant Tissue, leaf color varies widely based on environmental factors such as light intensity, nutrition, shade, and the process of photosynthesis and genetics. Exception occurs in *Pyrrosia lanceolata* and *Pyrrosia piloselloides,* showing the same leaf and stalk color, which varies in the genus Pyrrosia and Drynaria. In component 2, the most influential characteristics include leaf tip. In ferns, sorus is one of the main characteristics in determining the species.

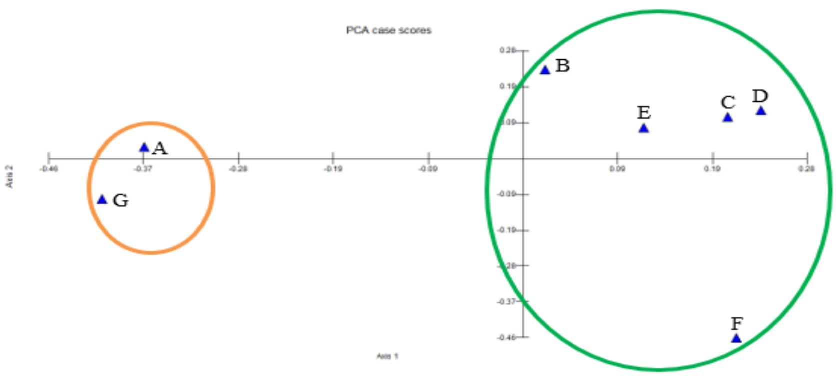

Fig. 16: Scatter Plot Graph of the kinship of Ferns of Polypodiaceae Family based on morphological and anatomical characters

As shown in Fig. 16, the results of PCA analysis interpreted into the Scatter Plot Graph form two groups in orange and green circle, respectively. The orange circle contains *Pyrrosia lanceolata* and *Pyrrosia piloselloides*, while green circle consists of *Platycerium bifurcatum, Belvisia mucronata, Microsorum punctatum* var. grandiceps, *Drynaria quercifolia*, and *Drynaria rigidula.* This shows that the kinship of species separates into two groups according to the dendrogram, where *Pyrrosia lanceolata* and *Pyrrosia piloselloides* are the most distant. Accessions in the same quadrant show a very close kinship relationship. However, when in different quadrants with an angle of > 90°, the accessions have a distant kinship relationship. The result suggests that the closest related species in green circle are in the same quadrant, including *Belvisia mucronata, Microsorum punctatum* var. grandiceps, *Drynaria quercifolia*, and *Drynaria rigidula.* Meanwhile, *Platycerium bifurcatum* is the furthest away in the circle. The graph correlates with the cluster analysis shown in the dendrogram, which is consistent with recent molecular studies. For example, found that the genus Pyrrosia in the traditional definition was paraphyletic to Platycerium, indicating the formation of several separate clades.

It should be acknowledged that this study has methodological limitations due to its sole use of a phenetic approach based on morphological characters. In the past five years, fern taxonomy has undergone a major revolution through plastid phylogenomics approaches. Analyzed 81 plastid genomes spanning the entire family Polypodiaceae and successfully resolved backbone-level relationships previously unsolved using morphological data alone. This study serves as a benchmark for future research at the family level.

At a lower genus level, used a similar approach in the genus Platycerium (including P. bifurcatum, one of the species in our study) and revealed biogeographic patterns and species relationships undetectable by morphology alone. Similarly, demonstrated that genomic approaches are essential for defining genus boundaries within Polypodioideae.

[11] provided an example of integrative taxonomy in the genus Leptochilus (a close relative of Microsorum) by combining molecular (plastid and nuclear) and morphological data, successfully identifying a number of cryptic species that are indistinguishable morphologically. This study serves as a model for how modern taxonomic research should be conducted.

Therefore, the phenetic grouping results we obtained are preliminary hypotheses that require further verification using phylogenomic approaches, as used in the studies mentioned above. Further research using plastid genome (plastome) sequencing or at least molecular markers such as rbcL, trnL-trnF, or matK is highly recommended to confirm the relationships among Polypodiaceae species at the study site.

Conclusion

In conclusion, 7 species of ferns in Polypodiaceae family are found in Nagarawangi Village. These include Pyrrosia lanceolata, Microsorum punctatum var. grandiceps, Drynaria quercifolia, Drynaria rigidula, Belvisia mucronata, Platycerium bifurcatum, and Pyrrosia piloselloides. The results of phenetic analysis show that the species most distantly related to others, with similarity value of 48.22% are Pyrrosia piloselloides and Pyrrosia lanceolata. Meanwhile, the most closely related species are Drynaria quercifolia and Drynaria rigidula, with similarity value of 87.88%. The most influential morphological and anatomical characteristics in grouping species are found to be stem color, leaf color, leaf tip, sorus position and shape, as well as stomata type.

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Author’s Contributions

Suryana: Contributed to the conceptualization of the study, the development of the theoretical framework, and provided general guidance on the methodology and analytical structure.
Asep Zaenal Mutaqien: Contributed to data processing and validation of analytical results.
Joko Kusmoro: Contributed to data analysis, interpretation of results, and preparation of the discussion section.

Budi Irawan: Participated in the design of the research methodology, data collection and ensured consistency between analytical results and research objectives.
Twista Davela Putri: Involved the preparation of the literature review, and drafting of the manuscript.
Sukono: Provided academic guidance, scientific supervision, and comprehensive evaluation.
Astrid Sulistya Azahra: Conducted data analysis, manuscript editing and formatting to ensure compliance with journal's submission guidelines.

Ethics

This study is original and contains unpublished material. The corresponding author confirms that all other authors have read and approved the manuscript, and there are no ethical issues.

Conflict of Interest

The authors declare no conflicts of interest.

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