RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
58 (1) 324-337, 2026
http://doi.org/10.54910/sabrao2026.58.1.30
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

INTEGRATIVE TAXONOMY AND MOLECULAR DATA CONFIRM THE OCCURRENCE OF *PYRROSIA STIGMOSA* (SW.) CHING IN WEST SUMATRA, INDONESIA

M. MILDAWATI*, A. ASHRIFURRAHMAN, S. SOLFIYENI, and Z. AUDINA

Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Padang, Indonesia
Corresponding author's email: mildawati@sci.unand.ac.id
Email addresses of co-authors: ashrifurrahman@gmail.com, solfiyeni@sci.unand.ac.id, zikkraaudina06@gmail.com

SUMMARY

The identity of the epiphytic fern *Pyrrosia stigmosa* (Sw.) Ching succeeded in its confirmation through integrative morphological and molecular analyses in West Sumatra, Indonesia. This finding expands the known geographic distribution of the species and contributes novel data to the global systematics of the genus *Pyrrosia*. Specimens collected from Silokek Geopark and Mount Padang, Indonesia, underwent identification based on key diagnostic traits, including narrowly grooved costae, dentate rhizome scales, and scattered sori. Species identity verification used the chloroplast trnL-F intergenic spacer marker (IGS) sequencing, showing 99.55% similarity to *P. stigmosa* (GenBank accession JX103800.1). For phylogenetic reconstruction, a dataset of 50 accessions representing various *Pyrrosia* species as retrieved from the GenBank database attained alignment with the newly generated sequence. Maximum likelihood analysis placed the West Sumatran samples within a strongly supported clade together with *P. costata*, indicating a close sister-species relationship. The combined morphological and molecular evidence provides a robust basis for delimiting *P. stigmosa* from morphologically similar taxa. Ecologically, the discovery highlights the adaptability of *P. stigmosa* to both lithophytic and epiphytic habitats in limestone-rich forest systems. This study represents the first documented record of *Pyrrosia stigmosa* in West Sumatra, extending its known distribution range and offering valuable insights into the biogeography and systematics of *Pyrrosia* in Southeast Asia.

Keywords: *Pyrrosia stigmosa*, *Pyrrosia*, morphological and molecular studies, taxonomy, trnL-F IGS, similarity, adaptability

Key findings: The investigation on *Pyrrosia stigmosa* (Sw.) Ching obtained a successful recording in West Sumatra, Indonesia.

Communicating Editor: Dr. Sajjad Hussain Qureshi

Manuscript received: August 07, 2025; Accepted: October 29, 2025.

Citation: Mildawati M, Ashrifurrahman A, Solfiyeni S, Audina Z (2026). Integrative taxonomy and molecular data confirm the occurrence of *Pyrrosia stigmosa* (Sw.) Ching in West Sumatra, Indonesia. *SABRAO J. Breed. Genet. 58 (1) 324-337. http://doi.org/10.54910/sabrao2026.58.1.30*.

INTRODUCTION

UNESCO Global Geoparks (UGGp) are single, unified geographical sites and landscapes of international geological significance, managed with a comprehensive protection and sustainable development concept. Geoparks represent an integration of biodiversity conservation, geological heritage, sustainable development, and local community engagement. Globally, geoparks are UNESCO-recognized and serve as centers for sustainable development and repositories of endemic biological resources (Fang and Guo, 2010; Fauzi *et al.,* 2018). Silokek Geopark, located in West Sumatra, Indonesia, is one of the highlighted geoparks, characterized by its karst or limestone topography, high ecological heterogeneity, and a reservoir for unique flora, including endemic pteridophytes. Being a tropical region, West Sumatra supports an exceptionally diverse assemblage of pteridophytes, non-flowering vascular plants that thrive in humid environments, and most of them have not yet undergone taxonomic studies (Mildawati *et al.,* 2020, 2024). These plants have achieved authentication for their ecological, evolutionary, and ethnobotanical significance, prompting a multidisciplinary approach involving phylogenetic systematics (Mildawati *et al.,* 2022a, 2023), micromorphology (Mukhopadhyay, 2021), and phytochemical and metabolomic analyses (Fan *et al.,* 2020; Mildawati *et al.,* 2022b; Alamsjah *et al.,* 2024).

The genus *Pyrrosia* of the family Polypodiaceae comprises approximately 52 to 69 species distributed across Asia, Oceania, and some parts of the African region (PPG I, 2016; Wei *et al.,* 2017; Brownsey *et al.,* 2021). The genus members are typically epiphytic and lithophytic, often found on forest trees and rocks in moist tropical habitats. *Pyrrosia stigmosa* is an endemic species belonging to this genus; however, it is morphologically distinct due to its thickly sclerified rhizome, elongated stipes, and characteristic reddish-green lamina. Its habitat preference is typically an exposure on rocky cliffs and vertical limestone outcrops, contributing to its ecological specificity and rarity. Although *P. stigmosa* has a distribution across Southeast Asia, its occurrence in Sumatra, particularly within biodiversity-rich regions such as Silokek Geopark and Mount Padang (Padang City), has remained poorly documented in formal biological studies. This study provides the first confirmed record of *P. stigmosa* in West Sumatra, extending the species' known distribution and contributing new data to the systematics and biogeography of the genus *Pyrrosia*.

Based on integrated morphological traits and molecular studies using the chloroplast trnL-F intergenic spacer (IGS) marker (Mildawati *et al*., 2023), this study confirmed the first occurrence of *Pyrrosia stigmosa* in West Sumatra, Indonesia. Field surveys conducted along the Batang Kuantan River (Silokek Geopark) and the limestone formations of Mount Padang, with elevations ranging from 200 to 400 meters above sea level (masl), revealed undocumented populations of this species. Herbarium comparison and DNA sequence analysis further supported the morphological identification. This study aimed to contribute taxonomic and biogeographic insights into the genus *Pyrrosia* by documenting *P. stigmosa* in West Sumatra and demonstrating the role of geoparks as reservoirs of unexplored biodiversity. Furthermore, it emphasized the importance of integrating molecular-assisted taxonomy to refine tropical ferns' systematics and inform effective conservation strategies in Southeast Asia.

MATERIALS AND METHODS

Study area and specimen collection

Field surveys carried out in West Sumatra, Indonesia, included Silokek Geopark, Sijunjung Regency, and Mount Padang, Padang City. Both sites are dominant with karst limestone formations at elevations ranging between 200 and 400 masl. Populations of *Pyrrosia* grew on shaded to semi-exposed rocky substrates along the Batang Kuantan River (Silokek) and on vertical limestone cliffs within the forested zones of Mount Padang (Figure 1).

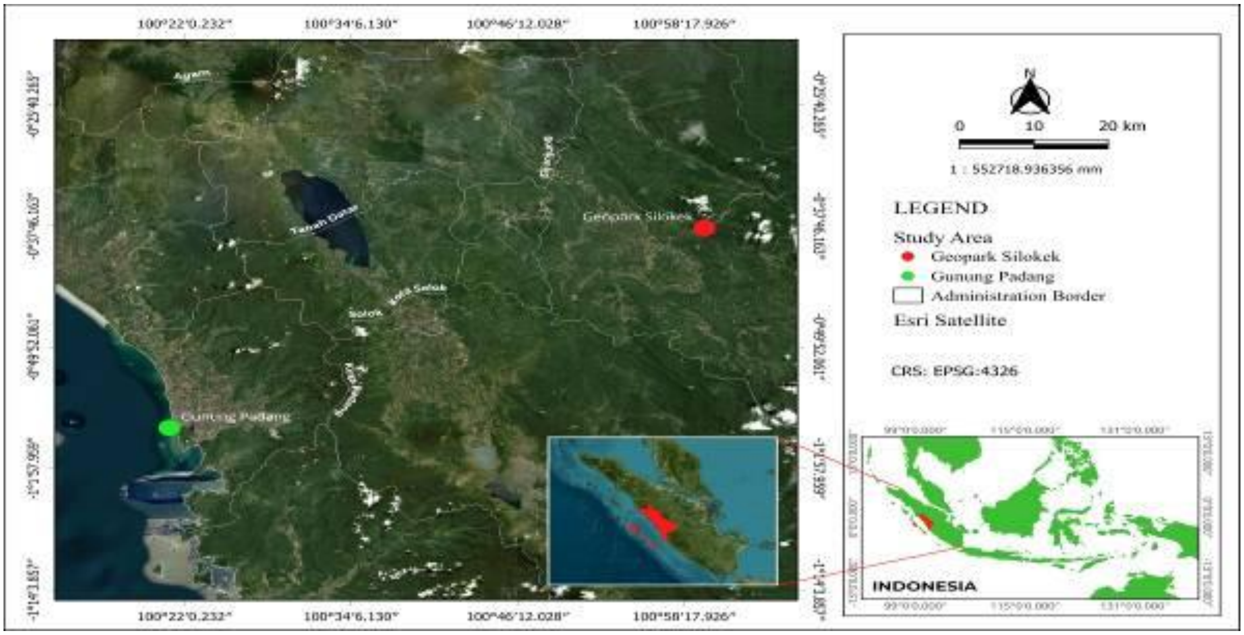

Figure 1. Study area and specimen collection of *Pyrrosia stigmosa* in West Sumatra, Indonesia, from Silokek Geopark (Sijunjung) and Mount Padang (Padang City).

The systematic collection of specimens during field surveys involved taking photographs in situ to document their natural habitat and substrate associations and processing them as herbarium vouchers following standard botanical protocols. Each specimen received a unique accession code to facilitate downstream morphological and molecular analyses. For example, accession Pyr-1, as collected from the Silokek Geopark, entailed depositing in the Herbarium of Andalas University (ANDA). Voucher specimens underwent careful pressing, drying, and mounting on herbarium sheets, with associated metadata (locality, habitat, elevation, and collector information) recorded to ensure reproducibility and traceability. All vouchers succeeded their curation within the ANDA, providing long-term preservation, accessibility for taxonomic verification, and future comparative research.

Morphological characterization

Morphological identification of genus *Pyrrosia* specimens proceeded through integrative comparison with the established regional floras, taxonomic monographs, and curated herbarium materials (Hovenkamp, 1986). Key diagnostic traits assessed include rhizome morphology, scale characteristics, stipe dimensions and vascular anatomy, lamina shape and apex structure, sori distribution patterns, and surface indumentum. Micro-anatomical observations ensued using a stereomicroscope, particularly with collenchyma strands, venation architecture, and sporangial features. The study obtained quantitative measurements using a calibrated ocular micrometer. Consulting high-resolution herbarium specimen images from the JSTOR Global Plants database helped enhance the taxonomic resolution and ensure broader comparative accuracy. Comparative emphasis depended on frond architecture, rhizome configuration, marginal scale morphology, and sori arrangement. All the morphological assessments reached validation against type descriptions and taxonomic keys specific to the genus *Pyrrosia* within the family Polypodiaceae (Mondal and Moktan, 2023).

Molecular analysis and phylogenetics

DNA extraction

Total genomic DNA extraction from silica gel-dried leaf tissues used the modified cetyl trimethyl ammonium bromide (CTAB) protocol (Doyle, 1991). DNA concentration and purity quantification utilized a nano-photometer NP80 (IMPLEN, Germany), while DNA integrity received verification on 1% agarose gel electrophoresis.

DNA amplification

The chloroplast trnL–F intergenic spacer region as targeted for amplification used the universal primer set (Taberlet *et al*., 1991):

- Forward (trnL-F): 5′-ATTTGAACTGGTGACACGAG-3′
-
- Reverse (trnF): 5′-GGTTCAGTCCCTCTATCCC-3′

Polymerase chain reaction (PCR) proceeded in a 25 µL reaction volume, comprising three µL of template DNA (100 ng/µL), 11 µL of MyTaq Extract-PCR Kit (Bioline), nine µL of nuclease-free water, and one µL each of forward and reverse primers. The cycling conditions followed Taberlet *et al*. (1991), with modifications after Li *et al*. (2017): initial denaturation at 94 °C for 3 min; 35–40 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 60 s, and extension at 72 °C for 30 s. The final extension was at 72 °C for 5 min.

DNA sequencing

PCR amplicons' purification and sequencing bi-directionally continued at First BASE Laboratories Sdn Bhd (Malaysia), using BigDye® Terminator v3.1 chemistry and an applied biosystems capillary electrophoresis platform. Raw sequence chromatograms underwent assembly, manual editing, and alignment using DNASTAR Lasergene software (Burland, 1999). Consensus sequences attained further validation via NCBI BLAST (https://www.ncbi.nlm.nih.gov/) to confirm species identity and retrieve homologous sequences.

Phylogenetic analysis

The inclusion of a dataset of 50 reference sequences (retrieved from GenBank; Table 1) served for phylogenetic reconstruction. Performing multiple sequence alignment used ClustalW in MEGA version 12 (Kumar *et al*., 2024). Sequence divergence calculation had the p-distance model as its basis. Phylogenetic tree extrapolation employed the maximum likelihood (ML) method with the Tamura-Nei substitution model (Tamura and Nei, 1993). Node support assessment used 1000 bootstrap replicates to evaluate topological robustness.

RESULTS

Taxonomic treatments

Pyrrosia stigmosa Ching is a lithophytic to epiphytic fern belonging to the family Polypodiaceae, having its first description in 1935. The species has a complex nomenclatural history and has been placed under several genera, including *Polypodium*, *Niphobolus*, and *Cyclophorus* (Zhou *et al*., 2017). Morphologically, the said species often becomes mistaken for *Pyrrosia costata*; however, one can reliably distinguish it by numerous diagnostic properties. These are narrowly grooved costae and secondary veins (especially visible in dried specimens), dentate rhizome scales, and widely spaced sori distributed across the entire lamina rather than concentrated apically (Figure 2). Anatomically, *P. stigmosa* has the characteristic of a central strand of collenchyma in the stipe as another valid feature for differentiation from the species *P. costata*.

Plants with epiphytic to epilithic nature have fertile fronds (sporophylls) ranging from 30 to 90 cm tall. Rhizomes were short-creeping to occasionally shortly elongate with a 2–4 mm diameter, dorsally bearing two alternate rows of phyllopodia. Rhizome scales were

Table 1. List of sequences with comparison.

No.	Accession	Species	Author
1	*KY931356.1*	*Pyrrosia costata*	(Zhang *et al.,* 2017)
2	*KY931423.1*	*Pyrrosia similis*	(Zhang *et al.,* 2017)
3	*KY931420.1*	*Pyrrosia similis*	(Zhang *et al.,* 2017)
4	*KY931359.1*	*Pyrrosia drakeana*	(Zhang *et al.,* 2017)
5	*KY931418.1*	*Pyrrosia sheareri*	(Zhang *et al.,* 2017)
6	*KY931419.1*	*Pyrrosia sheareri*	(Zhang *et al.,* 2017)
7	*KY931370.1*	*Pyrrosia flocculosa*	(Zhang *et al.,* 2017)
8	*KY931426.1*	*Pyrrosia sp*	(Zhang *et al.,* 2017)
9	*KY931351.1*	*Pyrrosia cf*	(Zhang *et al.,* 2017)
10	*KY931360.1*	*Pyrrosia drakeana*	(Zhang *et al.,* 2017)
11	*KY931422.1*	*Pyrrosia similis*	(Zhang *et al.,* 2017)
12	*KY931421.1*	*Pyrrosia similis*	(Zhang *et al.,* 2017)
13	*KY931349.1*	*Pyrrosia subfurfuracea*	(Zhang *et al.,* 2017)
14	*KY931434.1*	*Pyrrosia subfurfuracea*	(Zhang *et al.,* 2017)
15	*KY931435.1*	*Pyrrosia subfurfuracea*	(Zhang *et al.,* 2017)
16	*KY931342.1*	*Pyrrosia angustissima*	(Zhang *et al.,* 2017)
17	*KY931343.1*	*Pyrrosia angustissima*	(Zhang *et al.,* 2017)
18	*DQ164534.1*	*Pyrrosia polydactyla*	(Schneider, 2006)
19	*DQ164532.1*	*Pyrrosia linearifolia*	(Schneider, 2006)
20	*DQ164530.1*	*Pyrrosia porosa*	(Schneider, 2006)
21	*MT210539.1*	*Pyrrosia lingua*	Yao and Zhou, unpublished
22	*MN617019.1*	*Pyrrosia assimilis*	Yao and Zhou, unpublished
23	*MT210540.1*	*Pyrrosia lingua*	Yao and Zhou, unpublished
24	*MN885668.1*	*Pyrrosia lingua*	Yao and Zhou, unpublished
25	*MT210541.1*	*Pyrrosia petiolosa*	Yao and Zhou, unpublished
26	*MN885667.1*	*Pyrrosia petiolosa*	Yao and Zhou, unpublished
27	*MT210543.1*	*Pyrrosia angustissima*	Yao and Zhou, unpublished
28	*MN885669.1*	*Pyrrosia sheareri*	Yao and Zhou, unpublished
29	*MT210542.1*	*Pyrrosia drakeana*	Yao and Zhou, unpublished
30	*EU128519.1*	*Selliguea lateritia*	(Schneider *et al.,* 2008)
31	*DQ164529.1*	*Pyrrosia christii*	(Schneider, 2006)
32	*DQ164535.1*	*Pyrrosia serpens*	(Schneider, 2006)
33	*DQ164531.1*	*Pyrrosia lanceolata*	(Schneider, 2006)
34	*AY529463.1*	*Aglaomorpha coronans*	Janssen and Schneider, unpublished
35	*AY529474.1*	*Aglaomorpha splendens*	Janssen and Schneider, unpublished
36	*AY529471.1*	*Aglaomorpha novoguineensis*	Janssen and Schneider, unpublished
37	*AY529476.1*	*Drynaria descensa*	Janssen and Schneider, unpublished
38	*AY529483.1*	*Drynaria willdenowii*	Janssen and Schneider, unpublished
39	*DQ642252.1*	*Pyrrosia foveolata*	Jansen, unpublished
40	*DQ642254.1*	*Pyrrosia niphoboloides*	Jansen, unpublished
41	*DQ642256.1*	*Pyrrosia samarensis*	Jansen, unpublished
42	*DQ642250.1*	*Pyrrosia angustata*	Jansen, unpublished
43	*EU571221.1*	*Drynaria fortune*	Wang, unpublished
44	*EU571226.1*	*Aglaomorpha coronans*	Wang, unpublished
45	*EF551141.1*	*Serpocaulon levigatum*	(Kreier *et al.,* 2008)
46	*NC 040226.1*	*Pyrrosia bonii*	(Cai *et al.,* 2018)
47	*NC 047436.1*	*Pyrrosia subfurfuracea*	(Min *et al.,* 2019)
48	*KP136832.1*	*Polypodium glycyrrhiza*	Marchant *et al.,* unpublished
49	*NC 054156.1*	*Drynaria acuminata*	Liu and Wang, unpublished
50	*JX103800.1*	*Pyrrosia stigmosa*	Kim *et al.,* unpublished

Figure 2. Macromorphological characteristics of *Pyrrosia stigmosa*.
A) Frond, adaxial surface; B) Frond, abaxial surface; C) Lamina without sori; D) Lamina with sori; and Illustrative reference of *P. stigmosa* (adapted from GBIF occurrence no. 4858964934).

pseudopeltate, measuring 7–13 × 0.7–1.5 mm, brownish, and distinctly dentate at the base. The fronds were monomorphic. Stipes were 8–28 cm long, brown, and covered with fine hairs, with a central green groove. Laminae were lanceolate, 25–60 cm long and 2–5 cm wide at the broadest point, with an acute base and long, acuminate apex. The adaxial surface was dark green with scattered white hydathodes, while the abaxial surface was light brown. The frond margins were entire and glabrous, with free and reticulate venation. Stellate hairs with aciculate rays were evident on the abaxial surface. Sori were superficial, forming yellow-green patches at the younger stage and turning brown at maturity. The sori, densely scattered from the middle to the apex of the lamina, have arrangements either in 1–2 rays or sometimes irregularly dispersed across the soriferous areoles. The sori were 1–2 mm in diameter, with sporangia exerted from the indument and borne on stalks approximately equal in length to the capsule.

Distribution: *Pyrrosia stigmosa* is a widely distributed plant across tropical and subtropical Asia, including Cambodia, China (South-Central and Tibet), Laos, Vietnam, Thailand, Myanmar, Peninsular Malaysia, Sumatra, Sulawesi, the Lesser Sunda Islands, and New Guinea. In Indonesia, *P. stigmosa* is a newly recorded fern from West Sumatra, specifically in the Silokek Geopark (Sijunjung District) at coordinates 00° 37′ 58.9″ S and 100° 59′ 47.3″ E, and the forested cliffs of Mount Padang (Padang City) at the coordinates 06° 59′ 39.7″ S and 107° 03′ 23.1″ E.

Habitat: *Pyrrosia stigmosa* predominantly occurs in low- to mid-elevation forests (200–400 masl) and typically grows on limestone outcrops and rocky cliff faces. It is prevalent in shaded, moist habitats but persists in more exposed environments. The species, primarily lithophytic, exhibited facultative epiphytism in forested ravines and along the riverine corridors.

Note: *Pyrrosia stigmosa* (Swartz) Ching, first discovered in West Sumatra, appeared considerably related to the species *P. costata*, particularly in specimens with incomplete

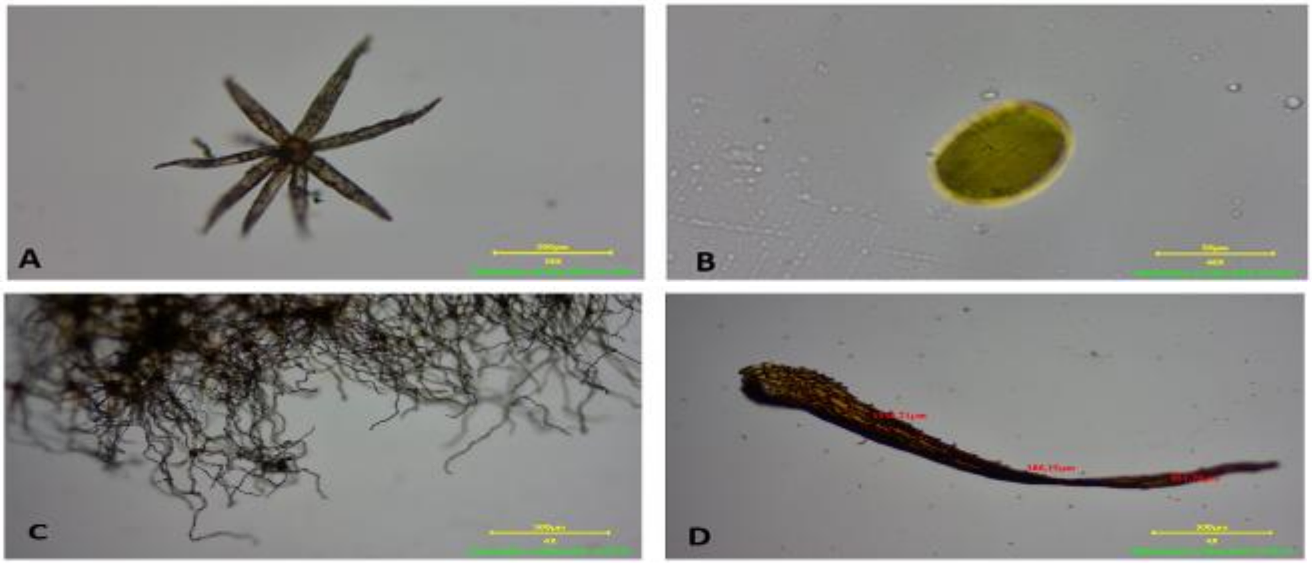

Figure 3. Micromorphological features of *Pyrrosia stigmosa***.** A) Stellate hair on the lamina surface; B) Monolete spore; C) Combination of stellate hairs and lamina scale; D) Pseudo-peltate scale on the rhizome.

rhizome collection and closely inrolled fronds, which resemble more or less stipitate forms of the species *P. costata*. However, several key morphological differences allow for their distinction. In species *P. stigmosa*, the fronds were distinctly stipitate, and the costa and the secondary veins exhibited a narrowly grooved upper surface, which was more distinct in dried specimens (Figure 3). In contrast, *P. costata* typically has a shorter stipe or no stipe, and its costa and veins are flat, shallowly, and broadly grooved. Anatomically, *P. stigmosa* possesses a central strand of collenchyma in the stipe, which was not evident in the species *P. costata*. The soral distribution also differed, and the species *P. stigmosa* had sori of short spacing and even distribution across the entire lamina. However, in the species *P. costata*, the sori were more closely packed and usually restricted to the apical region. Additionally, the rhizome scales of the species *P. stigmosa* were more distinctly dentate at the base, whereas those of *P. costata* tend to be less prominently toothed. By considering integration, these diagnostic features were compelling for distinguishing between the two species even in incomplete herbarium specimens.

Identification key for genus *Pyrrosia* in West Sumatra

a) *Pyrrosia stigmosa*—Rhizome scales distinctly dentate at the base, long stipe (8–28 cm) with a central green groove, fronds lanceolate with long acuminate apex, costa and secondary veins narrowly grooved on adaxial surface, sori dispersed across the entire lamina, stipe with central collenchyma strand
b) *Pyrrosia costata*—Rhizome scales entire or slightly dentate at the base, stipe short or subsessile, lacking a central groove; fronds were more variable in shape, often blunt-tipped; costa and veins flat or shallowly and broadly grooved on the adaxial surface; sori confined to the apical portion of the lamina, and costa lacking a central collenchyma strand.

Specimens examined: INDONESIA—West Sumatra, Silokek Geopark, February 12, 2024, Milda 005ML Silokek (ANDA), and West Sumatra, the forested cliffs of Mount Padang

Table 2. Sequences producing significant alignments with Pyr-1.

No.	Scientific Name	Max Score	Total Score	Query Cover	E value	Per.Ident (%)	Accessions
1	*Pyrrosia stigmosa*	403	403	66%	7.00E-108	99.55	JX103800.1
2	*Pyrrosia costata*	424	424	92%	6.00E-114	91.37	KY931356.1
3	*Pyrrosia drakeana*	398	398	99%	3.00E-106	88.27	MT210542.1
4	*Pyrrosia bonii*	398	398	99%	3.00E-106	88.2	NC_040226.1
5	*Pyrrosia serpens*	390	390	100%	6.00E-104	88.01	DQ164535.1
6	*Pyrrosia sheareri*	392	392	99%	2.00E-104	87.98	MN885669.1
7	*Pyrrosia samarensis*	388	388	99%	2.00E-103	87.98	DQ642256.1
8	*Pyrrosia subfurfuracea*	392	392	99%	2.00E-104	87.91	NC_047436.1
9	*Pyrrosia angustata*	377	377	97%	4.00E-100	87.76	DQ642250.1
10	*Pyrrosia polydactylos*	390	390	100%	6.00E-104	87.68	DQ164534.1
11	*Pyrrosia similis*	357	357	92%	6.00E-94	87.66	KY931423.1
12	*Pyrrosia sheareri*	357	357	92%	6.00E-94	87.66	KY931418.1
13	*Pyrrosia drakeana*	357	357	92%	6.00E-94	87.66	KY931359.1
14	*Pyrrosia subfurfuracea*	357	357	92%	6.00E-94	87.62	KY931349.1
15	*Pyrrosia similis*	355	355	92%	2.00E-93	87.62	KY931420.1

(Padang City), September 12, 2024, Milda 001 ML Gunung Padang (ANDA). Observations using online specimens also took place based on the Carnegie Museum of Natural History Herbarium (CM), Royal Botanic Gardens, Kew (K), Herbarium of the Arnold Arboretum (A), and the University of California (UC). These images include CM0163, K001044179, A00022018, and UC1490580, with earlier identifications as an isotype of *Pyrrosia pseudocalvata* Ching, Boufford, and K.H. Shing (family Polypodiaceae).

Type: ***Pyrrosia stigmosa* (Sw.) Ching** Unknown Indonesia. Botany L 0052112, Thunberg s.n., Java (holotype S, not seen). **Synonyms**: *Polypodium stigmosum* Sw. (1801); *Niphobolus stigmosus* T. Moore (1861), *Cyclophorus stigmosus* Desv. (1811), *Pyrrosia chinensis* Mirbel (1803), nom. Misapplied. Published in: Ching (1935). In: Bull. Chin. Bot. Soc. 1: 67.

DNA band profile based on trnL-F IGS

The DNA isolation results obtained had a concentration value of 444.05 ng/μL and a purity ratio of A260/A280, with a value of 1.9. After DNA isolation, the PCR process also continued. The formed PCR product was approximately 300 bp in size, as determined by comparison with a DNA ladder. The results confirmed the target DNA reached amplification using the employed primers. Subsequently, sequencing ensued to obtain the DNA sequence.

Identification with similarity

After obtaining the sequence with Sanger sequencing, the forward and reverse sequences' assembly was successful. This resulted in a consensus sequence of 336 nucleotides, which bore subsequent analysis. The sample sequence assessment for sequence similarity used the BLAST program from NCBI's GenBank. The presented 15 sequences have the closest similarity values to the sample sequence (Table 2).

Sequence similarity will be crucial for determining and confirming the tested samples' sequence. In the presented study, the analyzed samples showed a 99.55% similarity to the species *Pyrrosia stigmosa*, with a match of 221 nucleotide bases and a query cover of 66% (Table 2). The species *Pyrrosia costata* boasts the highest maximum score, total score, and query coverage, and its overall similarity was convincingly lower than that of the species *Pyrrosia stigmosa.* With reduced uncertainty in species confirmation, 221 nucleotide bases of the Pyr-1 sequence, on observation, aligned with JX103800.1 *Pyrrosia stigmosa* and KY931356.1 *Pyrrosia costata*. A comparison between Pyr-1 and JX103800.1 *Pyrrosia stigmosa* revealed one differing nucleotide with no deletions. However, comparing Pyr-1 and KY931356.1 *Pyrrosia costata,* it showed 15 differing nucleotides, three deletions in Pyr-1, and one deletion in *Pyrrosia costata*. These results further confirmed that Pyr-1 was the species *Pyrrosia stigmosa*. A phylogenetic analysis also continued for further confirmation.

Genetic distance and phylogenetic analysis

Genetic distance (Table 3) and phylogenetic tree (Figure 4), when analyzed, engaged the MEGA 12 software. In the 40 analyzed sequences, one can conclude that the interspecific genetic distance within the genus *Pyrrosia*, based on the chloroplast trnL–F IGS regions, ranged from 6.9% to 21.9%. These results were in line with past findings of De-Groot *et al.* (2011), who reported that interspecific distances were larger than intraspecific distances using the rbcL and trnL-F IGS regions in several fern taxa. Therefore, based on the genetic distance values, the trnL-F IGS region can also distinguish the species within the genus *Pyrrosia.*

The phylogenetic tree of Pyr-1 and comparative species in several fern genera is available in Figure 4; the phylogenetic tree comprises two major clades that separate the genus *Pyrrosia* from other fern genera. Clade 1 includes species of the *Pyrrosia*, which forms a monophyletic group, while clade 2 comprises the species from other fern genera. Within clade 1, three subclades further appeared distinctly, separating numerous *Pyrrosia* species. Pyr-1 position was within subclade 3, with the species *Pyrrosia stigmosa* and *Pyrrosia costata*. Therefore, one could establish that Pyr-1 has the closest relation to these two fern species. In subclade 3, identifying the Pyr-1 emerged as the species *Pyrrosia stigmosa*, showing the closest phylogenetic relationship (sister taxon) with the species *Pyrrosia costata*. This subclade branches off earliest within the phylogenetic tree of *Pyrrosia*, suggesting that it occupies the most basal position and represents an early diverging lineage associated with other fern genera. However, this clade acquired support from a high bootstrap value (91%).

DISCUSSION

The latest study provides a comprehensive integrative taxonomic framework, combining morphological and molecular evidence for *Pyrrosia stigmosa*, confirmed in West Sumatra, Indonesia. The methodology incorporates morphological, anatomical, and molecular datasets, offering a multi-evidence approach widely endorsed in contemporary biosystematics (Sundue *et al.,* 2015; Wei *et al.,* 2017). Integrative taxonomy has become indispensable for resolving the complex species boundaries, particularly in taxa like the genus *Pyrrosia*, where subtle morphological differences can lead to taxonomic ambiguities.

Morphological observations from this study demonstrate that *Pyrrosia stigmosa* has long been prone to misidentification, particularly with *P. costata*, when herbarium materials are fragmentary or lack fertile fronds (Hovenkamp, 1986; Cheng *et al*., 2014). This issue underscores the importance of comprehensive morphological assessments beyond superficial frond similarities. Our findings show a consistent suite of diagnostic characters reliably separates *P. stigmosa* from *P. costata*. Key features include the presence of narrowly grooved costae and well-defined secondary venation, distinctly dentate margins on rhizome scales, and the dispersed sori pattern across the lamina rather than clustered or linear groupings. These traits correspond with earlier reports highlighting scale morphology and sori distribution as critical taxonomic markers in *Pyrrosia* (Abotsi *et al*., 2015).

Anatomical examinations revealed *P. stigmosa* possesses a collenchyma central strand within the stipe, a character absent in *P. costata*. This feature provides an additional, non-overlapping line of evidence that enhances species delimitation. The reliance on internal anatomical traits for fern taxonomy has had

Table 3. Genetic distance of species of *Pyrrosia*.

No.	Sequences	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
1	*Pyr_1*																															
2	*JX103800.1_Pyrrosia_stigmosa*	0.01																														
3	*KY931356.1_Pyrrosia_costata*	0.07	0.06																													
4	*KY931423.1_Pyrrosia_similis*	0.16	0.17	0.22																												
5	*KY931420.1_Pyrrosia_similis*	0.16	0.17	0.22	0.00																											
6	*KY931359.1_Pyrrosia_drakeana*	0.16	0.17	0.22	0.00	0.00																										
7	*KY931418.1_Pyrrosia_sheareri*	0.16	0.17	0.22	0.00	0.00	0.00																									
8	*MN885669.1_Pyrrosia_sheareri*	0.16	0.17	0.22	0.00	0.00	0.00	0.00																								
9	*KY931419.1_Pyrrosia_sheareri*	0.16	0.17	0.22	0.00	0.00	0.00	0.00	0.00																							
10	*MT210542.1_Pyrrosia_drakeana*	0.16	0.17	0.22	0.00	0.00	0.00	0.00	0.00	0.00																						
11	*KY931360.1_Pyrrosia_drakeana*	0.16	0.17	0.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00																					
12	*KY931422.1_Pyrrosia_similis*	0.15	0.16	0.21	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01																				
13	*KY931421.1_Pyrrosia_similis*	0.15	0.16	0.21	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00																			
14	*KY931349.1_Pyrrosia_subfurfuracea*	0.15	0.16	0.21	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00																		
15	*KY931434.1_Pyrrosia_subfurfuracea*	0.16	0.17	0.22	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01																	
16	*KY931435.1_Pyrrosia_subfurfuracea*	0.16	0.17	0.22	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01																
17	*NC_040226.1_Pyrrosia_bonii*	0.15	0.16	0.21	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.01	0.01															
18	*NC_047436.1_Pyrrosia_subfurfuracea*	0.16	0.17	0.22	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01														
19	*DQ164534.1_Pyrrosia_polydactyla*	0.17	0.17	0.22	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.064													
20	*DQ164532.1_Pyrrosia_linearifolia*	0.15	0.16	0.20	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.02												
21	*DQ164530.1_Pyrrosia_porosa*	0.15	0.16	0.19	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.07	0.07	0.07	0.08	0.08	0.07	0.08	0.03	0.02											
22	*KY931370.1_Pyrrosia_flocculosa*	0.14	0.15	0.19	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.02	0.01	0.02										
23	*MN617019.1_Pyrrosia_assimilis*	0.15	0.16	0.19	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.07	0.07	0.07	0.08	0.08	0.07	0.08	0.03	0.02	0.00	0.02									
24	*DQ642256.1_Pyrrosia_samarensis*	0.12	0.12	0.16	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.06	0.06	0.06	0.07	0.07	0.06	0.07	0.08	0.08	0.08	0.06	0.08								
25	*DQ642250.1_Pyrrosia_angustata*	0.12	0.12	0.16	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.06	0.06	0.06	0.07	0.07	0.06	0.07	0.08	0.08	0.08	0.06	0.08	0.00							
26	*KY931426.1_Pyrrosia_sp*	0.12	0.12	0.16	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.06	0.06	0.06	0.07	0.07	0.06	0.07	0.08	0.08	0.08	0.06	0.08	0.00	0.00						
27	*MT210539.1_Pyrrosia_lingua*	0.15	0.15	0.20	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.10	0.10	0.10	0.11	0.11	0.10	0.11	0.12	0.12	0.12	0.10	0.12	0.06	0.06	0.06					
28	*MT210540.1_Pyrrosia_lingua*	0.15	0.15	0.20	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.10	0.10	0.10	0.11	0.11	0.10	0.11	0.12	0.12	0.12	0.10	0.12	0.06	0.06	0.06	0.00				
29	*MN885668.1_Pyrrosia_lingua*	0.15	0.15	0.20	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.10	0.10	0.10	0.11	0.11	0.10	0.11	0.12	0.12	0.12	0.10	0.12	0.06	0.06	0.06	0.00	0.00			
30	*KY931351.1_Pyrrosia_cf*	0.14	0.15	0.19	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.11	0.11	0.12	0.10	0.12	0.05	0.05	0.05	0.02	0.02	0.02		
31	*MT210541.1_Pyrrosia_petiolosa*	0.13	0.14	0.18	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.08	0.08	0.08	0.09	0.09	0.08	0.09	0.10	0.10	0.10	0.09	0.10	0.04	0.04	0.04	0.08	0.08	0.08	0.06	
32	*MN885667.1_Pyrrosia_petiolosa*	0.13	0.14	0.18	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.08	0.08	0.08	0.09	0.09	0.08	0.09	0.10	0.10	0.10	0.09	0.10	0.04	0.04	0.04	0.08	0.08	0.08	0.06	0.00

increasing emphasis in previous years to resolve species complexes and clarify evolutionary relationships within Polypodiaceae (Sundue *et al*., 2015). By integrating both external morphology and internal anatomy, this study reinforces the taxonomic boundaries of *P. stigmosa* and contributes to refining species circumscription within the genus.

These results confirm misidentification between *P. stigmosa* and *P. costata* is avoidable when considering both morphological and anatomical markers carefully. Our findings further support the argument that fern taxonomy benefits from a multidisciplinary approach, combining morphology, anatomy, and molecular tools to provide a more comprehensive framework for species delimitation in the genus *Pyrrosia*.

Molecular analysis using the trnL–F intergenic spacer region reinforces the morphological diagnosis. BLAST analysis showed Pyr-1 has a sequence identity of 99.55% for the species *P. stigmosa* (JX103800.1), while the identity of species *P. costata* was significantly lower at 91.37%. The molecular divergence involved further underscoring with 15 nucleotide substitutions and multiple insertion-deletion events. These results, coupled with phylogenetic reconstruction, considerably support the placement of the West Sumatran specimen within a monophyletic clade that includes the species *P. stigmosa* and confirms its taxonomic validity. The highest bootstrap support (91%) revealed robust phylogenetic confidence, corroborating findings by Zhou *et al.* (2017) that positioned the species *P. stigmosa* and *P. costata* as sister taxa.

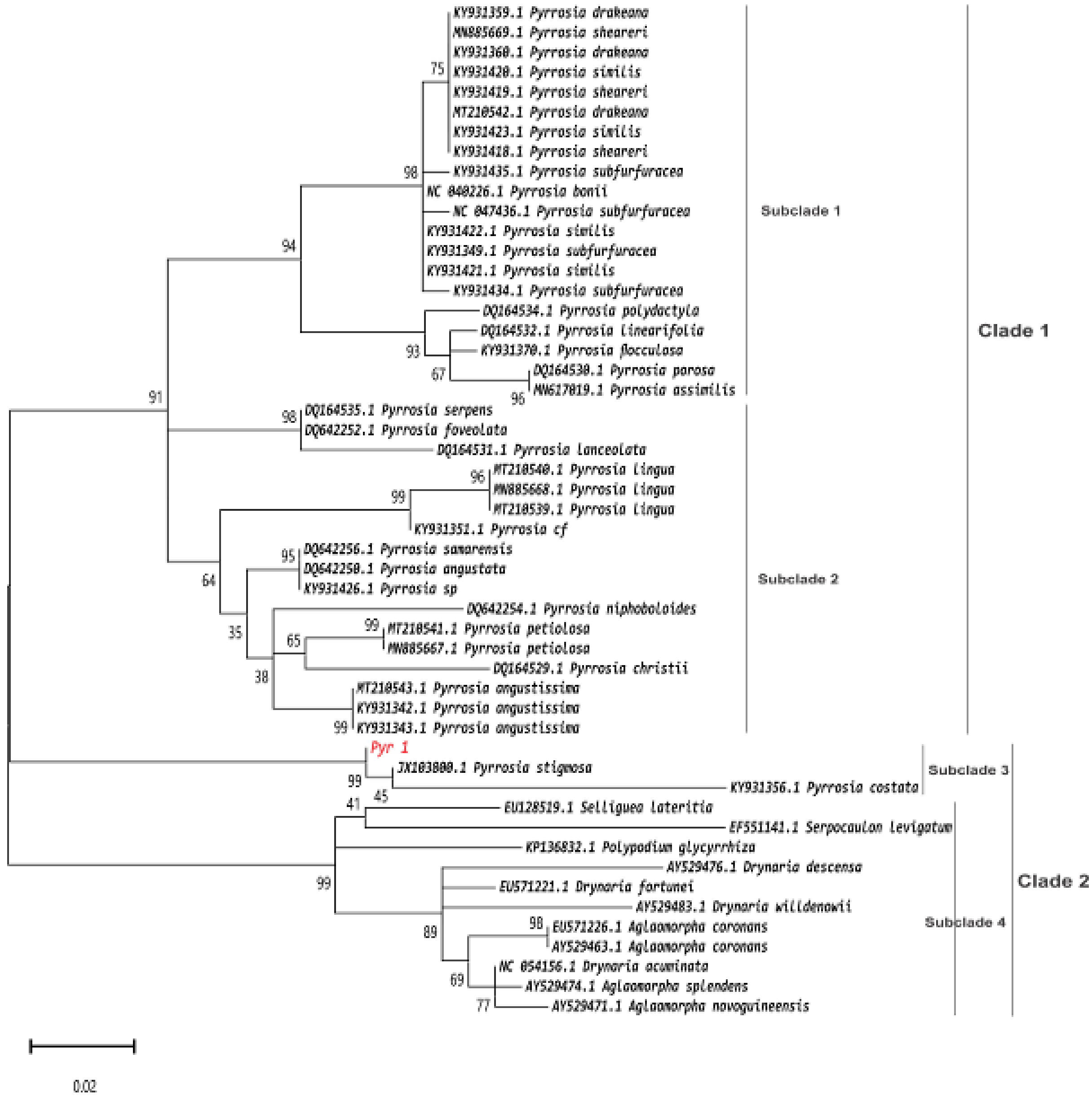

Figure 4. Phylogenetic tree constructions using the Maximum Likelihood method with 1000 bootstrap replicates. The heuristic search's initial tree(s) resulted automatically from applying Maximum Likelihood. All positions containing gaps and missing data entailed elimination (complete deletion option).

Biogeographically, species *P. stigmosa* in Silokek Geopark and Mount Padang represent a significant range extension in Western Indonesia. These species demonstrate ecological versatility by thriving in lithophytic and epiphytic habitats, especially on limestone substrates and shaded forested cliffs at mid-elevation. This ecological plasticity is a hallmark of the genus *Pyrrosia* species and suggests local adaptation to microhabitat heterogeneity, a factor increasingly recognized as a driver of speciation in pteridophytes.

In ecological terms, epiphytic ferns, such as species *P. stigmosa,* play multifunctional roles in tropical ecosystems. They enhance the canopy microclimates, mediate hydrological cycles, and enhance the habitat heterogeneity for invertebrates and microbial communities (Ellis, 2020; Mahata *et al.,* 2023). Their ecological sensitivity and dependency on stable microclimates render them valuable bioindicators for forest health assessment. Consequently, preserving limestone forests and associated epiphytic microhabitats is vital for the species *P. stigmosa* and the broader epiphytic flora support (Diliarosta *et al.,* 2020; Mildawati *et al.,* 2020).

These investigations also carry implications for conservation planning. West Sumatra's karst landscapes face increasing threats from deforestation, agricultural exploitation, and limestone quarrying. In line with global biodiversity targets, integrating micro-endemic taxa like *P. stigmosa* into site-based conservation priorities could elevate the ecological value of underprotected areas (Mahata *et al.,* 2023). From a pharmacological standpoint, the genus *Pyrrosia* is an increasingly recognized plant for its medicinal potential. Previous studies have also reported the presence of mangiferin, oleanolic acid, stigmasterol, quercetin, chlorogenic acid, and other bioactive compounds in related species (Bandyopadhyay and Dey, 2022; Cheng *et al.,* 2014). These metabolites exhibited antimicrobial, antioxidant, hepatoprotective, and anti-inflammatory properties, with a recognition as valuable candidates for phytotherapeutic applications (Putra and Mustaqim, 2021; Chen *et al.,* 2023). The phytochemical profile of *P. stigmosa* remains unexplored; however, based on its taxonomic affinity, targeted screening was justified and yielded compounds with biomedical relevance.

CONCLUSIONS

The integration of morphological and molecular evidence confirms the identity of the collected specimens as *Pyrrosia stigmosa*. This finding validates the species as a distinct taxon within the family Polypodiaceae and extends its confirmed distribution range to West Sumatra, Indonesia. The study underscores the importance of combining classical morphology with modern molecular tools to achieve robust species delimitation. Such a multidisciplinary taxonomic framework provides critical insights into the diversity, biogeography, and conservation of *Pyrrosia* and related ferns.

ACKNOWLEDGMENTS

The author thanks the Directorate General of Higher Education, Research, and Technology, Ministry of Education, Culture, Research, and Technology, with Andalas University, Indonesia, for supporting and publishing this manuscript.

REFERENCES

Abotsi K, Radji A, Rouhan G, Dubuisson J-Y, Kokou K (2015). The Pteridaceae family diversity in Togo. *Biodivers. Data J.* 3, e5078. https://doi.org/10.3897/BDJ.3.e5078.

Alamsjah F, Fandini S, Mildawati M (2024). Short communication: Evaluation of antibacterial activities and phytochemical composition of ethanolic extract of *Diplazium esculentum*. *Biodiversitas*, 25(3): 937–941. https://doi.org/10.13057/biodiv/d250304.

Bandyopadhyay A, Dey A (2022). Medicinal pteridophytes: Ethnopharmacological, phytochemical, and clinical attributes. *Beni Suef Univ. J. Basic Appl. Sci*. 11(1): 113. https://doi.org/10.1186/s43088-022-00283-3.

Brownsey P, Shepherd L, De Lange P, Perrie L (2021). *Pyrrosia serpens* (G. Forst.) Ching, a new record for the fern flora of the Kermadec Islands. *N.Z.J. Bot*. 59(2): 229–243. https://doi.org/10.1080/0028825X.2020.1796716.

Burland TG (1999). DNASTAR's Lasergene Sequence Analysis Software. In S. Misener and S.A. Krawetz, Bio. Methods Protoc. 132: 71–91. Humana Press. https://doi.org/10.1385/1-59259-192-2:71.

Cai S, Cai X, Li S, Liu S, Wang Z, Wang T, Su Y (2018). The complete chloroplast genome of *Pyrrosia bonii* (Polypodiaceae), an important ornamental and medical fern. *Mitochondrial DNA Part B*. 3(2): 801–802. https://doi.org/10.1080/23802359.2018.1491347.

Chen J, Ning S, Lu X, Xiang W, Zhou X, Bu Y, Li L, Huang R (2023). Variation in flavonoid and antioxidant activities of *Pyrrosia petiolosa* (Christ) Ching from different geographic origins. *Front. Plant. Sci*. 14, 1173489. https://doi.org/10.3389/fpls.2023.1173489.

Cheng D, Zhang Y, Xin X, Gao D (2014). Comparative pharmacognosy of *Pyrrosia petiolosa* and *Pyrrosia davidii. Rev. Bras. Farmacogn*. 24(4): 368–380. https://doi.org/10.1016/j.bjp.2014.07.017.

De-Groot GA, During HJ, Maas JW, Schneider H, Vogel JC, Erkens RHJ (2011). Use of rbcL and trnL-F as a two-locus DNA barcode for identification of NW-European ferns: An ecological perspective. *PLoS ONE* 6(1), e16371. https://doi.org/10.1371/journal.pone.0016371.

Diliarosta K, Ramadhani R, Indriani D (2020). Diversity of Pteridophyta in Lubuak Mato Kuciang Padang Panjang, Sumatera Barat. *Phcog J.* 12(1): 180–185. https://doi.org/10.5530/pj.2020.12.27.

Doyle J (1991). DNA protocols for plants. pp. 283–293. In: G. Hewitt, A.W.B. Johnson, J.P.W. Young (Eds.). Molecular Techniques in Taxonomy. *NATO ASI Series H. Cell Biol*. 57: 104–115.

Ellis CJ (2020). Microclimatic refugia in riparian woodland: A climate change adaptation strategy. *For. Ecol. Manag*. 462, 118006. https://doi.org/10.1016/j.foreco.2020.118006.

Fan Y, Xin X, Liu L, Feng H, Wang P, Zhang Y, Gao D (2020). Diversity analysis and associated antimicrobial activity of essential oil in *Pyrrosia petiolosa*. *Chem. Biodivers*. 17(12), e2000666. https://doi.org/10.1002/cbdv.202000666.

Fang S, Guo S (2010). Chinese Geoparks' Construction and the Sustainable Utilization of Geological Heritage Resources. pp. 1–4. https://doi.org/10.1109/ICIECS.2010.5678383.

Fauzi NSM, Misni A, Kamaruddin SM, Ahmad P (2018). Geo-heritage conservation: The content analysis study of Kilim Geoforest Park, Langkawi. *AjQoL* 3(12): 87–97. https://doi.org/10.21834/ajqol.v3i12.145.

Hovenkamp P (1986). A Monograph of the Fern Genus *Pyrrosia* (Polypodiaceae). Brill. https://doi.org/10.1163/9789004628090.

Kreier HP, Rex M, Weising K, Kessler M, Smith AR, Schneider H (2008). Inferring the diversification of the epiphytic fern genus *Serpocaulon* (Polypodiaceae) in South America using chloroplast sequences and amplified fragment length polymorphisms. *Plant Syst. Evol*. 274(1–2): 1–16. https://doi.org/10.1007/s00606-008-0021-3.

Kumar S, Stecher G, Suleski M, Sanderford M, Sharma S, Tamura K (2024). MEGA12: Molecular Evolutionary Genetic Analysis version 12 for adaptive and green computing. *Mol. Biol. Evol*. 41: 1–9.

Li Y, Zhao H, Yan X, Li M, Chen P, Zhang S (2017). A universal method for direct PCR amplification of plant tissues. *Anal. Methods* 9(11): 1800–1805. https://doi.org/10.1039/C6AY03156K.

Mahata A, Panda RM, Dash P, Naik A, Naik AK, Palita SK (2023). Microclimate and vegetation structure significantly affect butterfly assemblages in a tropical dry forest. *Climate* 11(11): 220–236. https://doi.org/10.3390/cli11110220.

Mildawati M, Sobir S, Sulistijorini S, Chikmawati T (2020). The diversity of pteridophytes in Siberut National Park, Mentawai Islands, West Sumatra, Indonesia. *Biodiversitas* 21(7): 3200–3208. https://doi.org/10.13057/biodiv/d210742.

Mildawati M, Sobir S, Sulistijorini S, Chikmawati T (2022a). Diversity, distribution, and ecology of *Davallia* in Sumatra (Indonesia) and the nearby small Islands. *Biodiversitas* 23(1):33–42. https://doi.org/10.13057/biodiv/d230105.

Mildawati M, Sobir S, Sulistijorini S, Chikmawati T (2022b). Metabolite profiling of *Davallia* in the Mentawai Islands, West Sumatra, Indonesia: 7th International Conference on Biological Science (ICBS 2021), Yogyakarta, Indonesia. https://doi.org/10.2991/absr.k.220406.010.

Mildawati M, Sobir S, Sulistijorini S, Chikmawati T (2023). Phylogeny of *Davallia* (Davalliaceae) from Sumatra and Mentawai Islands, Indonesia: Evidence from trnL-F Intergenic Spacer. *Biodiversitas* 24(8): 4589–4596. https://doi.org/10.13057/biodiv/d240841.

Mildawati, Azka SA, Azirda MF, Solfiyeni (2024). Diversity and bioprospecting of pteridophytes in the indigenous Talang Mamak Tribe, Riau, Indonesia. *SABRAO J. Breed. Genet.* 56(5): 1845–1857. http://doi.org/10.54910/sabrao2024.56.5.9.

Min Y, Cai S, Xiao H, Zhang M, Hong Y, He Z, Wang Z, Wang T, Su Y (2019). The complete chloroplast genome of *Pyrrosia calvata* (Polypodiaceae), a traditional Chinese medicinal fern, is restricted to Guangxi, China. *Mitochondrial DNA Part B*. 4(1): 1757–1758. https://doi.org/10.1080/23802359.2019.1610095.

Mondal KC, Moktan M. (2023). Detailed morphological and anatomical characters in *Pyrrosia costata*, *P. heteractis*, *P. lanceolata*, *P. mannii,* and *P. glabra* (Polypodiaceae) with artificial key, anatomy of rhizome, stipe, rhizome scales, venation, and sori. *Thailand Natural History Museum Journal*, 17(1), 45–65.

Mukhopadhyay R (2021). Stomatal structure, ontogeny and inter-relationships in pteridophytes: An overview. *Indian Fern J*. 38: 81–93.

Palacios-Rios M, Gabriel Y, Galán JM, Prada C, Rico-Gray V (2019). Structure of the petioles and costae of Mexican and Central American species of *Pteris* (Polypodiopsida, Pteridaceae). *Phytotaxa* 401(2): 101. https://doi.org/10.11646/phytotaxa.401.2.2 NO citation within the paper. Please indicate in page/s where cited.

PPG I (2016). A community-derived classification for extant lycophytes and ferns. *J. Syst. Evol*. 54(6): 563–603. https://doi.org/10.1111/jse.12229.

Putra HF, Mustaqim WA (2021). Diversity, conservation, potential uses, and alien species of lowland plants of Batang Toru, Sumatra, Indonesia. *Biodiversitas* 22(3): 1580–1591. https://doi.org/10.13057/biodiv/d220365.

Schneider H (2006). Phylogeny and biogeography of the staghorn fern genus *Platycerium* (Polypodiaceae, Polypodiidae). *Am. J. Bot*. 93(2): 217–225. https://doi.org/10.3732/ajb.93.2.217.

Schneider H, Kreier HP, Hovenkamp P, Janssen T (2008). Phylogenetic relationships of the fern genus *Christiopteris* shed new light onto the classification and biogeography of drynarioid ferns. *Bot. J. Linn. Soc*. 157(4): 645–656. https://doi.org/10.1111/j.1095-8339.2008.00825.x.

Sundue MA, Testo WL, Ranker TA (2015). Morphological innovation, ecological opportunity, and the radiation of a major vascular epiphyte lineage: Radiation of major vascular epiphyte lineage. *Evolution* 69(9): 2482–2495. https://doi.org/10.1111/evo.12749.

Taberlet P, Gielly L, Pautou G, Bouvet J (1991). Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Mol. Biol.* 17(5): 1105–1109. https://doi.org/10.1007/BF00037152.

Tamura K, Nei M (1993). Estimation of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol. Biol. Evol*. 10: 512–526. https://doi.org/10.1093/oxfordjournals.molbev.a040023.

Wei X, Qi Y, Zhang X, Luo L, Shang H, Wei R, Liu H, Zhang B (2017). Phylogeny, historical biogeography, and character evolution of the drought-resistant fern *Pyrrosia Mirbel* (Polypodiaceae) inferred from plastid and nuclear markers. *Sci. Rep.* 7(1): 12757. https://doi.org/10.1038/s41598-017-12839-w.

Zhang L, Zhou XM, Chen DK, Schuettpelz E, Knapp R, Lu NT, Luong TT, Dang MT, Duan YF, He H, Gao XF, Zhang LB (2017). A global phylogeny of the fern genus *Tectaria* (Tectariaceae: Polypodiales) based on plastid and nuclear markers identifies major evolutionary lineages and suggests repeated evolution of free venation from anastomosing venation. *Mol. Phylogenet. Evol*. 114: 295–333. https://doi.org/10.1016/j.ympev.2017.05.020.

Zhou XM, Zhang L, Chen CW, Li CX, Huang YM, Chen DK, Lu NT, Cicuzza D, Knapp R, Luong TT, Nitta JH, Gao XF, Zhang LB (2017). A plastid phylogeny and character evolution of the Old World fern genus *Pyrrosia* (Polypodiaceae) with the description of a new genus: *Hovenkampia* (Polypodiaceae). *Mol. Phylogenet. Evol*. 114: 271–294. https://doi.org/10.1016/j.ympev.2017.06.020.