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Variation on morphology and spore characters of *Dicranopteris* and *Sticherus* (Gleicheniaceae) from Rokan Hulu District, Riau, Indonesia

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Abstract. *Marpaung AA, Susandarini R. 2021. Variation on morphology and spore characters of* Dicranopteris *and* Sticherus *(Gleicheniaceae) from Rokan Hulu District, Riau. Biodiversitas 22: 4475-4486. Dicranopteris* and *Sticherus* are genera in the family Gleicheniaceae which have unique habits and distinctively specific growth with the presence of a bud on the forking branches. This study aims to reveal the species diversity of Gleicheniaceae members found in the forest area of Bukit Suligi Tandun, Rokan Hulu District, Riau and to document variations in their morphological characters and spores. Eleven collections were obtained from the exploration in the study area. The identification of plant specimens showed that these eleven taxa consisted of *Dicranopteris curranii*, *Dicranopteris speciosa,* seven varieties of *Dicranopteris linearis,* and two varieties of *Sticherus truncatus*. Seven varieties within *D. linearis* were *D. linearis* var. *linearis, D. linearis* var. *alternans, D. linearis* var. *altissima, D. linearis* var. *demota, D. linearis* var. *inaequalis, D. linearis* var. *subspeciosa,* and *D. linearis* var. *tetraphylla*. The only one species of *Sticherus* found was *S. truncatus* which consisted of two varieties, namely *S. truncatus* var. *truncata* and *S. truncatus* var. *involuta*. Morphological characters were observed from fertile specimens, while spore characters were observed from spore microscopic slides prepared using acetolysis method. There were 42 morphological characters and spores examined and used as the basis for determining the phenetic relationships using cluster analysis and principal component analysis. The results of cluster analysis showed the formation of two clusters that clearly separated the genus *Sticherus* from *Dicranopteris.* The three species of *Dicranopteris* were also clearly separated from each other in different sub-clusters. The result of principal component analysis indicated characters that contribute to the grouping of the eleven taxa and was able to explain the distinguishing characters in each group recognized from the cluster analysis. Overall, the results of this study contributed to documenting in detail the morphological variations in sporophyte and spore characters in eleven taxa of *Dicranopetris* and *Sticherus,* as well as proving the role of morphological and spore characters in the classification and defining phenetic relationships using numerical taxonomic approach.

Keywords: Forking ferns, palynology, phenetics, sporophyte, taxonomic relationship

INTRODUCTION

Gleicheniaceae is a family of primitive and ancient ferns with its existence on the Earth was marked by the first appearance of mega fossil evidence in the Permian (Skog 2001). The classification of Gleicheniaceae continues to develop with the integration of increasingly more taxonomic evidence, and currently, the classification widely accepted is the system developed by Smith et al. (2006) which recognized six genera within the family. Christenhusz and Chase (2014) had the same opinion in recognizing six genera in Gleicheniaceae. The six genera are *Dicranopteris, Gleichenella, Diplopterygium, Gleichenia, Sticherus,* and *Stromatopteris*. The recognition of these six genera was adopted in the current classification system by the Pteridophyte Phylogeny Group (PPG I 2016). Despite the agreement on the number of genera within the family, taxonomic revisions on the placement of some species into particular genus are still very dynamic. In this regard, Perrie (2018) reported a change in the status of *Gleichenia hooglandii* from Papua New Guinea to different genus, *Sticherus*. Similarly, recent study on *Gleichenia boryi* based on morphological and molecular data lead to the recognition of *Rouxopteris* as an endemic genus to the Madagascar region (Liu et al. 2020).

Some species in Gleicheniaceae have high morphological variations as indicated by the presence of intraspecific categories, such as *Dicranopteris linearis* with 13 varieties found in Southeast Asia (De Winter and Amoroso 2003). This high species variability is evident from its morphology which becomes the basis for identification and classification at below species level. Until now morphology is still used as the basis for identification, classification, and even for phylogeny reconstruction although various molecular techniques are available. This also applies to ferns, in that the results of phylogenetic analysis based on morphology and molecular data showed similar patterns of relationships (Schneider et al. 2009). The role of morphology as the basic taxonomic evidence for species determination is still relevant, and still has an important role in phylogenetic reconstruction (Pochynok 2012). Accordingly, the exploration of morphological characters in ferns and their role in determining taxonomic relationships reported in this study is relevant and provides important contribution in plant systematics.

The importance of exploratory research on the diversity of ferns was proven by a number of studies on this topic that have been carried out in the Sumatra region. The study of Beukema et al. (2013) on ferns diversity in relation to the quality of agroforest habitats in Sumatra showed there were two *Dicranopteris* species that can be used as indicators of forest disturbance and ecological restoration. In this regard, *Dicranopteris linearis* var. *linearis* was one of fern species identified as indicator of highly to moderately disturbed early succession habitat. Meanwhile, *Dicranopteris linearis* var. *subpectinata* was identified as fern species indicating moderate disturbance. A study on the diversity of ferns in Lubuak Mato Kuciang as an area developed for tourism in West Sumatra showed the presence of two species belonging to the Gleicheniaceae, namely *Dicranopteris linearis* and *Gleichenia linearis* (Diliarosta et al. 2020). Studies on the diversity of ferns in another region in Southeast Asia were also reported by a number of researchers, some of which also revealed the presence of species belonging to the Gleicheniaceae. Nazihah et al. (2018) in a study on the diversity and distribution of ferns in Kuantan Pahang, Malaysia reported the presence of *Dicranopteris linearis* as one of 14 species found in open areas in forest. Research on the diversity of ferns as the basis for producing a checklist in two tourism forests in Kedah, Malaysia by Rahmad and Akomolafe (2019) found two species of Gleicheniaceae, namely *Dicranopteris linearis* and *Gleichenia truncata*. A study to uncover ferns that were classified as threatened was carried out by Coritico and Amoroso (2020) which served as a basis for protection and conservation, as well as growing public awareness of the diversity of ferns that need to be protected. Recently, Saharizan et al. (2021) investigated the species composition, diversity, and species richness of ferns in oil palm plantation areas in Segamat, Johor. One species belonging to the Gleicheniaceae that was recorded at the study sites was *Dicranopteris linearis*.

Gleicheniaceae is unique in terms of its leaf morphology as characterized by a pseudodichotomous branching pattern that produces a forking architecture, and the presence of buds on older branches resulted in indeterminate leaf growth. This unique pattern of branching and leaf growth generated specific terminology applied to this family (Shaw and Ranker 2011; Gonzales and Kessler 2011). The uniqueness of Gleicheniaceae morphology is attractive by which some species were used as ornamental plants. *Dicranopteris linearis*, known as a scrambling fern, is one of species used as ornamental plants (De Winter and Amoroso 2003). *Dicranopteris* also has important ecological functions due to its rapid clonal growth ability to fill gaps after disturbance and to facilitate habitat succession, and able to reduce soil erosion due to rainfall (Yang et al. 2021). With these various biological roles, it is very interesting to study the species diversity in the Gleicheniaceae family.

Spore is part of gametophyte generation in ferns which will germinate to produce free-living prothallium. The morphology of fern spores shows specific features and thus might serve as a diagnostic tool for identification and classification (Adeonipekun et al. 2021). Several studies have proven the important role of spores in fern taxonomy. Regalado and Sánchez (2002) reported that spore morphology was proven to be useful in the identification and delimitation of three *Asplenium* species. A study by Passareli et al. (2010) on the genus *Blechnum* showed that spore characteristics combined with morphological characters of the sporophyte were complementary in defining specific taxonomic characters at species level. Similarly, Chao and Huang (2018) in their systematic study of the genus *Pteris* mentioned that combination of spore morphology and leaf characters was useful in infrageneric classification. Results of those studies become the basis for the use of spore morphology along with sporophyte morphological characters in determining taxonomic relationships between members of *Dicranopteris* and *Sticherus* in this study.

Until recently there was no comprehensive study on Gleicheniaceae in Indonesia. Publications on the existence of Gleicheniaceae species were only available from studies on fern diversity in general, and not only focused on this family. The records on small number of Gleicheniaceae species were found from exploration studies in Enggano Island (Wardani and Adjie 2017), in Siberut National Park, West Sumatra (Mildawati et al. 2020), in five mountains in West Java (Suryana et al. 2020), and in Mount Gede Pangrango National Park, West Java (Andriyani and Nurza 2021). The objective of this study was to reveal the species diversity of the members of Gleicheniaceae in the Rokan Hulu, Riau. This study also aimed to document variations in morphology and spore characters as well as their role in determining phenetic relationships of two genera within Gleicheniaceae namely *Dicranopteris* and *Sticherus.*

MATERIALS AND METHODS

Study area, collection of plant samples, and species identification

Plant materials for this study were obtained from the exploration of Bukit Suligi Tandun, Rokan Hulu District, Riau, Indonesia (Figure 1). The plant specimens were collected from their natural habitat by exploring along Jalan Raya Dayo as the only access road in the area (Figure 2). The collected specimens were fertile adult plants that were recognized based on their morphology as members of Gleicheniaceae. The fertile frond containing sori were kept separately in zip-lock plastic bag as materials for preparing spore microscopic slides. The methods of collecting plant samples and making herbarium specimens referred to the procedures of Bean (2010). Identification of specimens was carried out with reference to Holtum (1957; 1959). Verification of species names and their author was carried out by referring to the Plants of the World Online database (http://www.plantsoftheworldonline.org/).

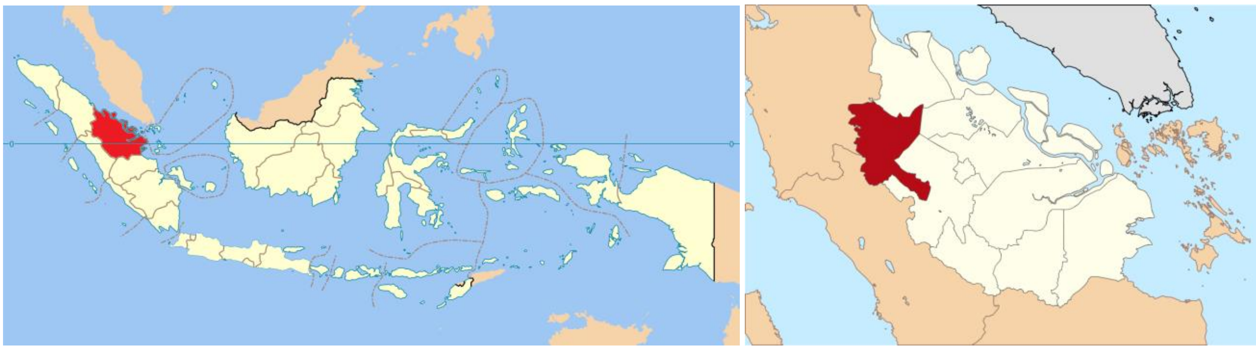

Figure 1. Location of Rokan Hulu District, Riau Province, Indonesia

Figure 2. Location of plant samples collection: A. Aerial map of Bukit Suligi Tandun, B. Jalan Raya Dayo

Preparation of spore microscopic slides

Preparation of spore microscopic slides was carried out using the acetolysis method as described by Jones (2014). The observations and documentation of spore morphological characters were performed using light microscope equipped with an OptiLab microscope camera at a magnification of 10×40. Six morphological characters of spores were examined namely spore type, spore shape, the length of polar axis (P), the diameter equatorial plane (E), spore size class based on P/E ratio, and exine ornamentation. Observations and measurements of spore morphological characters were done on 10 spores in each sample. The terminology used for defining spore morphological characters referred to Punt et al. (2007).

Analysis of taxonomic relationships

Analysis of taxonomic relationships of the taxa under study was performed based on morphological and spore characters. Cluster analysis to construct the dendrogram was done by calculating Euclidean distance and employing Unweighted Pair Group Method with the Arithmetic mean (UPGMA) clustering method. Principal component analysis was carried out to find hypothetical variables accounted for. for the variance in the data. The results of principal component analysis were presented as eigenvalues and eigenvectors. This two multivariate analyses were done using PAST 3.20 (Hammer et al. 2001).

RESULTS AND DISCUSSION

Species diversity of *Dicranopteris* and *Sticherus* from Rokan Hulu

Based on the exploration along the road on Bukit Suligi Tandun Forest, 11 fern specimens belong to Gleicheniaceae family were found. The species identification based on morphological characters showed that these specimens consisted of two genera namely *Dicranopteris* and *Sticherus* (Table 1). There were three species of *Dicranopteris* recognized in this study, *D. curranii, D. speciosa,* and the most variable one *D. linearis* with seven varieties. Meanwhile, two other specimens were identified as *Sticherus truncatus* var. *truncata* and *S. truncatus* var. *involuta.*

Table 1. List of *Dicranopteris* and *Sticherus* species and varieties found in Rokan Hulu District, Riau, Indonesia

Genus	Species and varieties
Dicranopteris	*Dicranopteris linearis* (Burm. f.) Underw. var. *altissima* Holttum
	D. linearis var. *inaequalis* (Rosenst.) Holttum
	D. linearis var. *linearis*
	D. linearis var. *demota* Holttum
	D. linearis var. *alternans* (Mett.) Holttum
	D. linearis var. *tertraphylla* (Rosenst.) Nakai
	D. linearis var. *subspeciosa* Holttum
	D. curranii Copel.
	D. speciosa (Presl) Holttum
Sticherus	*Sticherus truncatus* (Willd.) Nakai var. *involuta* Holttum
	S. truncatus var. *truncata*

Morphological Variability of *Dicranopteris* and *Sticherus*

The members of *Dicranopteris* and *Sticherus* found in this study showed notable variation in the pinna (plural: pinnae) morphology (Figure 3). Following the specific leaf terminology applied for Gleicheiaceae, pinna is the primary division of the leaf. *Sticherus* was characterized by pinnae that are indistinguishable from accessory costa, which is the major axis of the accessory leaflets. *Dicranopteris,* on the other hand, showed variations in the pinnae morphology, especially the size of the pinnae. The longest and widest pinnae were found in *D. curranii* (55 cm long and 11 cm width), while the smallest pinnae were found in *D. linearis* var. *subspeciosa* (14 cm long and 5 cm width). It was obvious, therefore, that pinnae morphology was one of the characters distinguishing *Sticherus* from *Dicranopteris,* and the characters differentiating between species of the latter genus.

The variation on pinna morphology identified in this study as distinguishing characters between species was in line with results of morphological studies in other fern genera. Jaman et al. (2018) showed that pinna size, pinna scale size, venation pattern, and sorus position on sporophylls were used as the basis for recognizing different *Asplenium* species. The characters of the pinna apex and hairs on the abaxial surface of the pinna and rachis were among the morphological characters used by Shepherd et al. (2019) to recognize differences between *Dicksonia fibros*a and *D. lanata*, and at the same time were used as the basis for affirming the identity and status of the hybrid between the two species.

Detailed observations on pinnules, that is the smallest or last order of division of pinna, showed variations on their veins. The veins showed differences in the number forking which varied from once, twice, three times, and four times (Figure 4). Once-forked veins were found only in *Sticherus,* whereas the members of *Dicranopteris* showed high variations on the forking patterns of veins. Twice-forked veins was found in *D. linearis* var. *inaequalis*, *D. linearis* var. *alternans*, *D. linearis* var. var *tertraphylla*, *D. linearis* var. var *subspeciosa*, and *D. speciosa*. Veins of three times-forked was found in *D. linearis* var. *altissima*, *D. linearis* var. *linearis*, and *D. linearis* var *demota*, while four times-forked vein pattern was found only in *D. curranii*. Previous studies have mentioned that the number of forking in veins of the pinnules or ultimate leaflet was the key characteristics that distinguishes the genus *Dicranopteris* from *Sticherus* (Chinnock and Bell 1998; Jin et al. 2013). Moreover, Perrie and Brownsey (2015) confirmed that one of the distinguishing characters between *Dicranopteris* and *Sticherus* was the branching pattern of venation located between the midvein and the margin on the ultimate segment or pinnules. Vein character was also reported for distinguishing species in the genus *Tectaria* (Dong et al. 2020).

Another noticeable morphological variation that distinguished *Stichers*s from *Dicranopteris* was the protective structure on rachis bud (Figure 5). The rachis bud is a bud borne at the apex of rachis flanked by two pinnae, and there were variations on the stipules found on rachis bud. Stipules are protective structures on rachis buds when they are dormant. Stipule morphology also varied in three species of *Dicranopteris,* and even showed variations between different varieties within *D. linearis.* Østergaard and Øllgaard (1996) noted that stipules in Gleicheniaceaeeae can be flat, forked, lobed, or pinnatifid leaf-like structures. Stipules found on the rachis bud of *Sticherus truncatus* have unique shapes that did not resemble leaflets (Figure 5.A, 5.B), whereas leaf-like stipules were found in *D. curranii* (Figure 5.C). The presence of two small lobe-shaped stipules characterized the rachis buds of *D. linearis* var. *altissima*, *D. linearis* var. *linearis*, *D. linearis* var. *demota*, *D. linearis* var *tertraphylla* and *D. speciosa*. Two large lobed stipules as protective structures on rachis bud were found in *D. linearis* var. *inaequalis*, *D. linearis* var. *alternans*, and *D. linearis* var. *subspeciosa* (Figure 5.E, 5.I, 5.K).

Variation of stipules on rachis buds was one of the morphological characters used for species identification of ferns, as mentioned by Lima and Salino (2018) that within the genus *Sticherus* there were species that have stipules and the others do not have stipules. The morphology of the rachis buds as a distinguishing character between fern genera was reported by Perrie and Brownsey (2015) in which the members of Gleicheniaceae have rachis buds protected by either pseudostipules or accessory leaflets. In addition to characteristics of the stipules, the rachis buds in *Dicranopteris* and *Stricherus* also showed differences in the presence of hairs and scales that protect the rachis buds.

A prominent morphological variation found in three species and seven varieties of *Dicranopteris* and two varieties of *Sticherus truncatus* was the number of sporangia composing the sorus (plural: sori) as shown in Figure 6. It was obvious that the number of sporangia per sorus was one of the most conspicuous characters that distinguished the genus *Dicranopteris* from *Sticherus.* The sorus in two varieties of *S. truncatus* consisted of four sporangia, while the three species of *Dicranopteris* showed variations in the number of sporangia per sorus ranging from 7 to 17. This result was similar to previous records on *Dicranopteris* and *Sticherus* in other countries as mentioned by Chinnock and Bell (1998), and Jin et al. (2013). In this regard, Perrie and Bownsey (2015) noted that in New Zealand the number of sporangia per sorus in *Dicranopteris* was 7-12, while *Sticherus* had 3-5 sporangia per sorus.

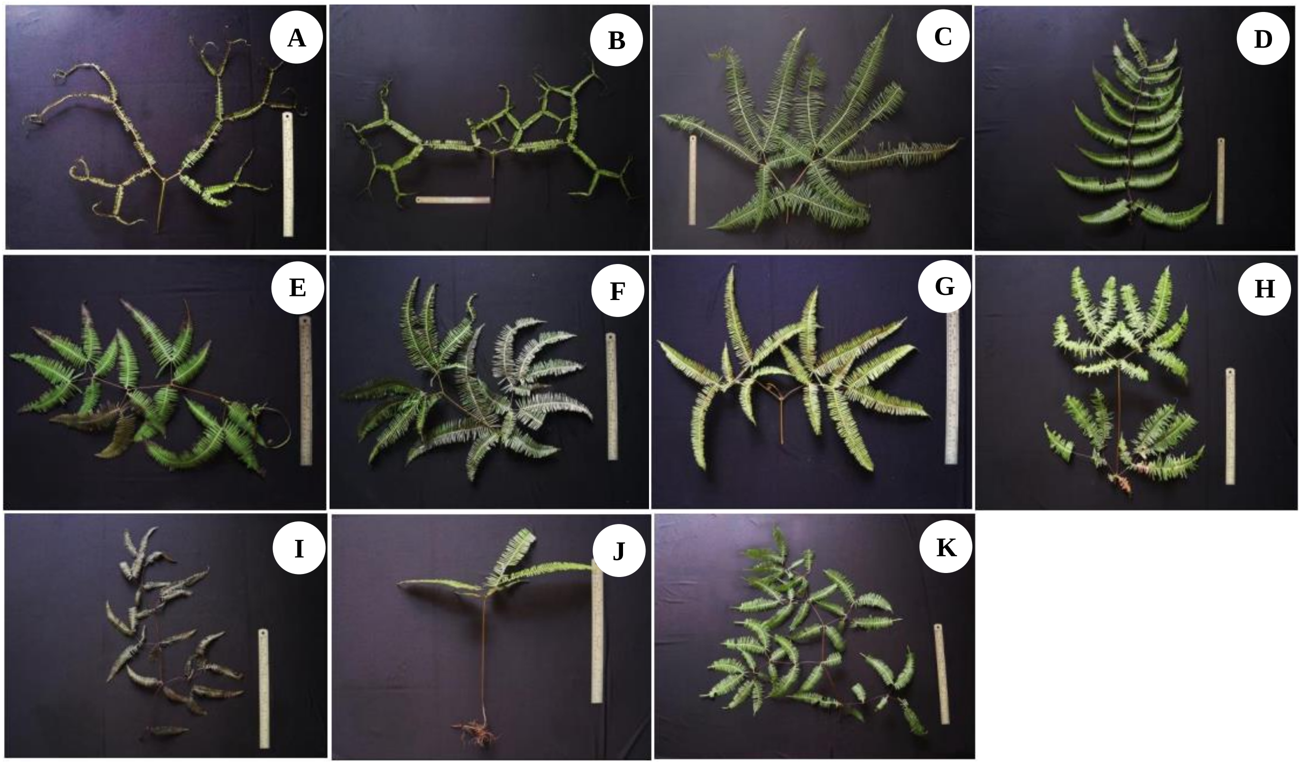

Figure 3. Variation on pinna morphology. A. *Sticherus truncatus* var. *involuta*, B. *S. truncatus* var. *truncata*, C. *Dicranopteris curranii*, D. *D. speciosa*, E. *D. linearis* var. *inaequalis*, F. *D. linearis* var. *altissima*, G. *D. linearis* var. *linearis*, H. *D. linearis* var. *demota*, I. *D. linearis* var. *alternans*, J. *D. linearis* var. *tetraphylla*, K. *D. linearis* var. *subspeciosa*

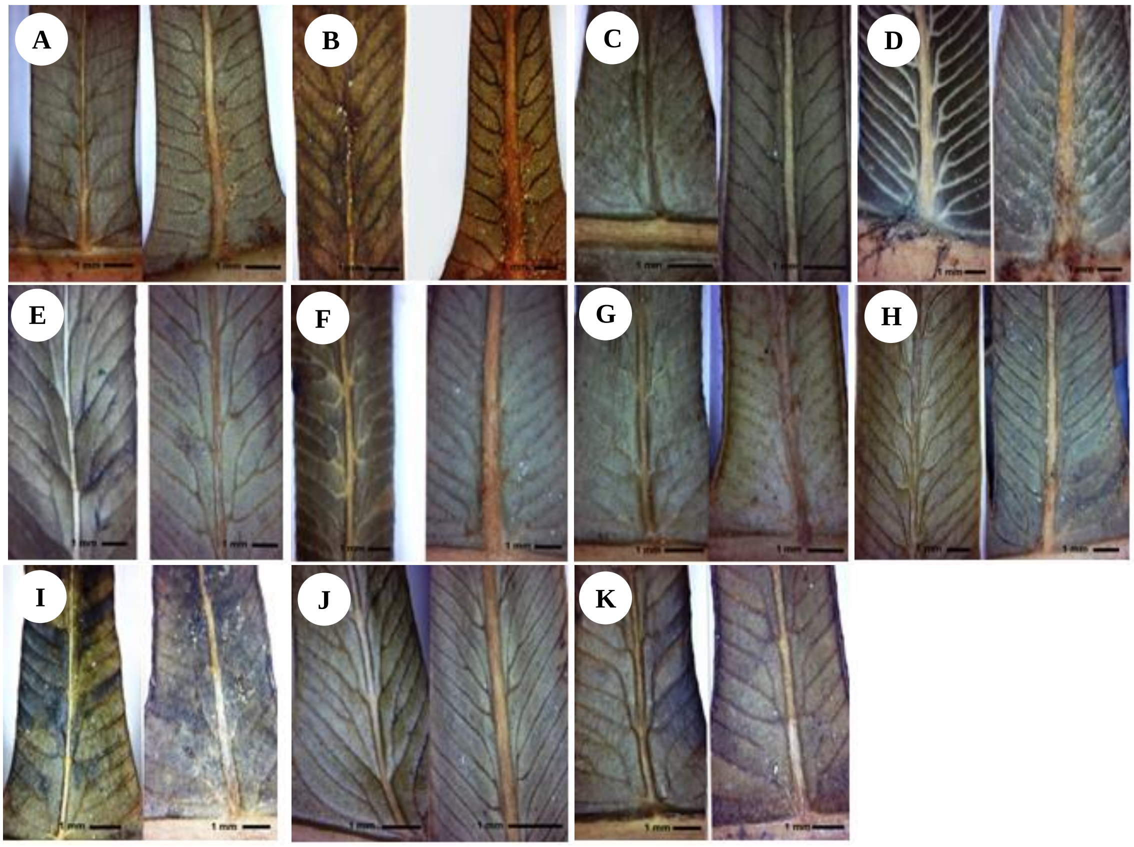

Figure 4. Variation on veins forking patterns of the pinnules. A. *Sticherus truncatus* var. *involuta*, B. *S. truncatus* var. *truncata*, C. *Dicranopteris curranii*, D. *D. speciosa*, E. *D. linearis* var. *inaequalis*, F. *D. linearis* var. *altissima*, G. *D. linearis* var. *linearis*, H. *D. linearis* var. *demota*, I. *D. linearis* var. *alternans*, J. *D. linearis* var. *tetraphylla*, K. *D. linearis* var. *Subspeciosa*

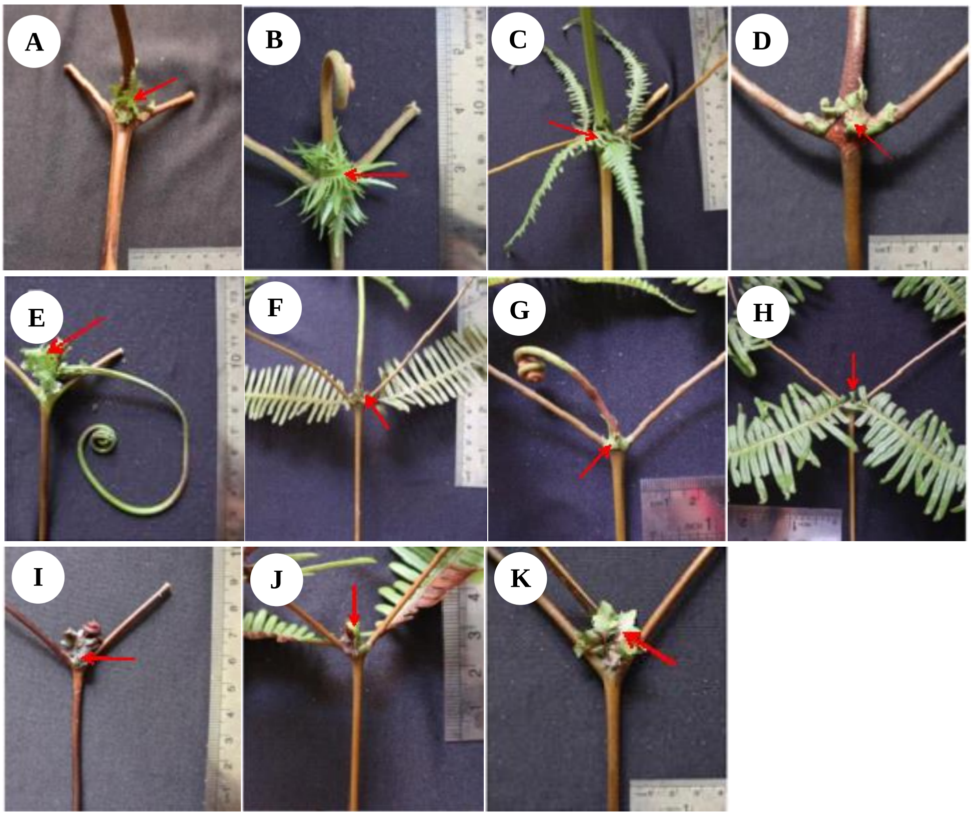

Figure 5. Variation on rachis bud protective structure (stipules). A. *Sticherus truncatus* var. *involuta*, B. *S. truncatus* var. *truncata*, C. *Dicranopteris curranii*, D. *D. speciosa*, E. *D. linearis* var. *inaequalis*, F. *D. linearis* var. *altissima*, G. *D. linearis* var. *linearis*, H. *D. linearis* var. *demota*, I. *D. linearis* var. *alternans*, J. *D. linearis* var. *tetraphylla*, K. *D. linearis* var. *Subspeciosa*

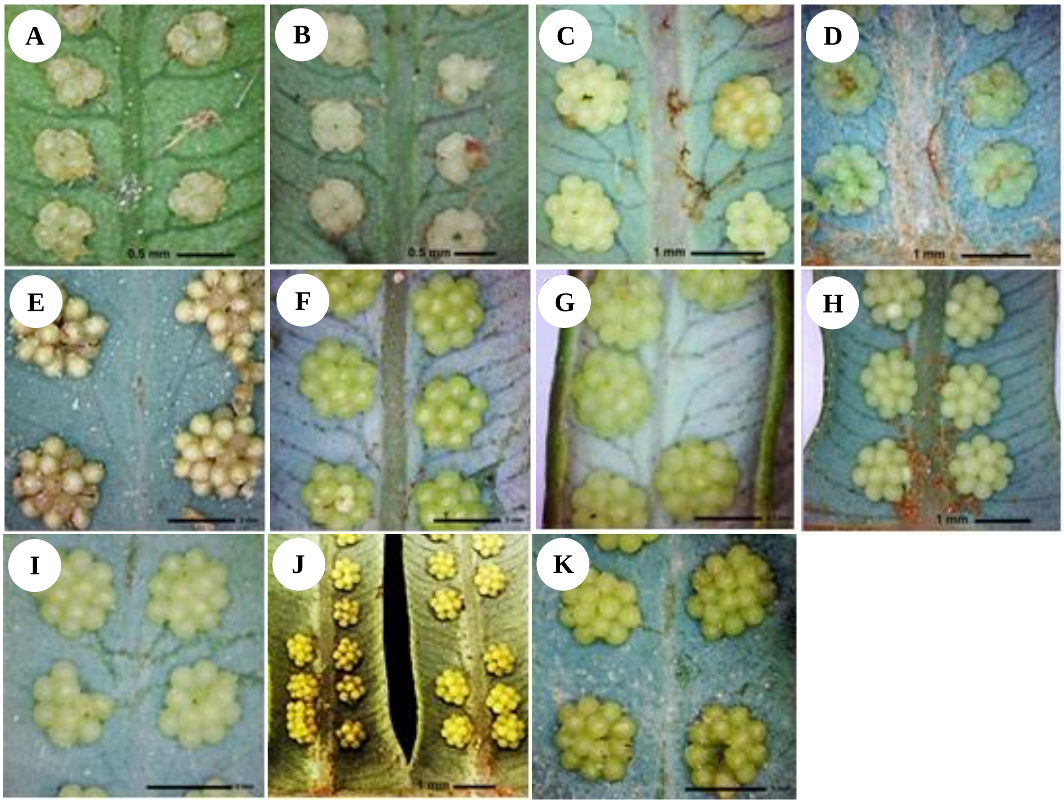

Figure 6. Variations in fern sori morphology. A. *Sticherus truncatus* var. *involuta*, B. *S. truncatus* var. *truncata*, C. *Dicranopteris curranii*, D. *D. speciosa*, E. *D. linearis* var. *inaequalis*, F. *D. linearis* var. *altissima*, G. *D. linearis* var. *linearis*, H. *D. linearis* var. *demota*, I. *D. linearis* var. *alternans*, J. *D. linearis* var. *tetraphylla*, K. *D. linearis* var. *Subspeciosa*

Spore morphology

Spores as part of gametophyte generation in fern life cycle are having important role in sexual reproduction. Spores on 11 taxa in this study showed considerable morphological variations. There were six morphological characters of spores examined, namely spore type, the length of polar axis, the diameter of equatorial plane, spore shape, spore class based on size, and exine ornamentation. The six spore morphological characteristics of species and varieties under study were shown in Table 2. Photomicrographs of spores observed under light microscope were displayed in Figure 7.

Spore type was determined based on the presence of laesura, in which spores without any laesura were called as alete, spores with one laesura were called as monolete, and those with three laesura were classified as trilete (Punt 2007). There were two types of spores found in specimens observed in this study, the monolete and trilete spores. *Sticherus truncatus* had monolete spores, while three species in *Dicranopteris* showed variations, with monolete spores were found in *D. curranii* and trilete spores were found in *D. speciosa* and *D. linearis*. Variations in spore types in various genera and species within the Gleicheniaceaeeae family were common, as reported by Farfán-Santillán (2017) that *Gleichenella pectinata* and *Stricherus bifidus* have monolete spores, while *Diplopterygium bancroftii* and *D. flexuosa* have trilete spores. Variations in spore types in the same genus were also found in *Aspenium* (Lashin 2012) in that *A. aethiopicum, A. trichomanes,* and *A. adiantum-nigrum* had monolete spores while *Asplenium* sp.1 had trilete spores.

The size of spores observed in this study was obtained based on measurements of the length of polar axis (P) and the diameter of equatorial plane (E). Determination of spore size classes referred to the classification of Halbritter et al. (2018) who divided spores into five classes of size based on the P/E ratio. The spores of the eleven taxa examined in this study were classified into two categories, namely small spores (10 – 24 µm) which were only owned by *D. curranii*, and spores in the medium size (25 – 49 µm) which belonged to the other ten taxa. In general, spores of members of *Dicranopteris* were smaller than those of *Sticherus*. Adeonipekun et al. (2021) who studied spore characteristics of various fern families noted that spore morphology was important in resolving classification of ferns and proposed the use of spore P/E ratio as diagnostic taxonomic tools in Pteridophyte.

Exine ornamentation is a spore morphological character that showed considerable variations, ranging from psilate, micro-scabrate, scabrate, to verrucate (Table 2). This study showed both intraspecies and interspecies variations on exine ornamentation. Intraspecies variation in exine ornamentation has been reported in two varieties of *Adiantum reniforme*, in which spores of *A. reniforme* var. *reniforme* had psilate ornamentation while spores of *A. reniforme* var. *sinense* had rugate ornamentation (Wang et al. 2015). Variations of exine ornamentation in species of the genus *Dicranopteris* have been reported in several studies such as those by Farfán-Santillán (2017) and Sofiyanti et al. (2019). These studies showed that the spores of *D. flexuosa* had scabrate ornamentation, while *D. bancroftii, D. linearis,* and *D. medusae* had psilate ornamentation. The important role of spore morphology in species delimitation has been proven by Mazumdar (2018), that based on a very notable difference in exine ornamentation confirmed that *Lygodium giganteum* was a distinct species and not a synonym for *L. yunnanense* as previously thought. Interspecies variation on spore ornamentation is also found in other fern genera such as those reported by Passarelli et al. (2010) in genus *Blechnum* and Li et al. (2020) in genus *Ceratopteris.*

Table 2. Spore morphological characters

Species and varieties	Characters					
	Spore type	Length of polar axis (µm)*	Length of equatorial axis (µm)*	Spore shape**	Class of spore size**	Exine ornamentation
Sticherus truncarus var. *involuta*	Monolete	34.14	18.11	Prolate	Medium	Psilate
Sticherus truncatus var. *truncata*	Monolete	36.73	18.64	Prolate	Medium	Micro-scabrate
Dicranopteris curranii	Monolete	24.12	14.00	Prolate	Small	Psilate
Dicranopteris speciosa	Trilete	25.72	24.39	Prolate-spheroidal	Medium	Scabrate
Dicranopteris linearis var. *inaequalis*	Trilete	33.22	33.06	Oblate-spheroidal	Medium	Verrucate
Dicranopteris linearis var. *altissima*	Trilete	32.21	25.69	Sub-prolate	Medium	Psilate
Dicranopteris linearis var. *linearis*	Trilete	31.43	-23.56	Prolate	Medium	Psilate
Dicranopteris linearis var. *demota*	Trilete	32.91	26.80	Sub-prolate	Medium	Psilate
Dicranopteris linearis var. *alternans*	Trilete	31.76	27.35	Sub-prolate	Medium	Micro-scabrate
Dicranopteris linearis var. *tetraphylla*	Trilete	30.72	28.90	Prolate-spheroidal	Medium	Psilate
Dicranopteris linearis var. *subspeciosa*	Trilete	30.46	25.34	Sub-prolate	Medium	Verrucate

Note: *based on average of 10 measurements; **determined based on Erdtman (1986)

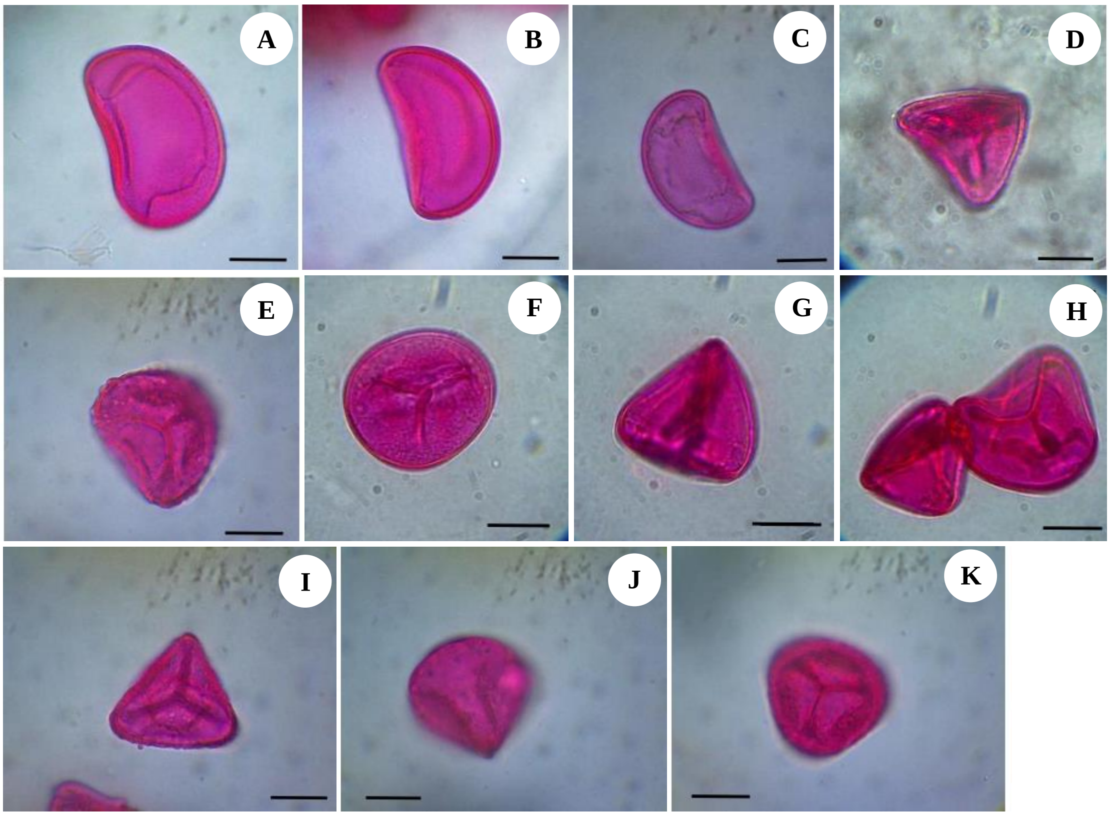

Figure 7. Light microscopic photomicrograph of spores (bar: 10 µm). A. *Sticherus truncatus* var. *involuta*, B. *S. truncatus* var. *truncata*, C. *Dicranopteris curranii*, D. *D. speciosa*, E. *D. linearis* var. *inaequalis*, F. *D. linearis* var. *altissima*, G. *D. linearis* var. *linearis*, H. *D. linearis* var. *demota*, I. *D. linearis* var. *alternans*, J. *D. linearis* var. *tetraphylla*, K. *D. linearis* var. *subspeciosa*

Phenetic Relationships of species and varieties of *Dicranopteris* and *Sticherus*

In order to determine the taxonomic relationships between three species of *Dicranopteris* along with its seven varieties and two varieties of *Sticherus truncatus*, a cluster analysis was performed based on 42 characters. The characters used in this analysis consisted of 26 morphological characters of sporophytes (plant specimens) and 6 spore characters (Table 3).

The result of cluster analysis was presented in a dendrogram (Figure 8) which showed the existence of two main clusters. The first cluster was consisted of two varieties of *S. truncatus*, whereas the second cluster consisted of nine taxa within the genus *Dicranopteris*. The dendrogram also showed a clear division of three species of *Dicranopteris*, in which *D. curranii* and *D. speciosa* were well separated from all seven varieties of *D. linearis* which formed a solid group in a distinct sub-cluster. The phenetic relationships as presented in the dendrogram confirmed the differences between two genera observed in this study and showed a clear separation between three *Dicranopteris* species. This result confirmed that cluster analysis was a useful tool for classification and for defining relationships of plant species (Ugwuanyim 2000; Manoko 2018). The cophenetic correlation coefficient of the dendrogram was 0.926. This coefficient was used to measure degree of fit of classification and a criterion for evaluating the efficiency of clustering techniques (Saraçli et al. 2013). The coefficient value of greater than 0.8 for the dendrogram in this study indicated that the clusters generated from the analysis was very good in terms of representing the original distance matrix or degree of resemblance between samples (Albuquerque et al. 2016).

In addition to cluster analysis, the principal component analysis was performed to identify characters that contributed to the grouping of species and varieties figured out from cluster analysis. The result of principal component analysis was presented in Table 4 which listed the character loadings and eigenvalues in the first two principal components. The eigenvalues indicated the variance accounted for by the corresponding principal components or eigenvectors. Principal component analysis has been known as complementary to cluster analysis (Mulima et al. 2018). Therefore, combination of these two methods was commonly applied in plant systematics studies in various plant taxa at any taxonomic level, from species (Fitriana and Susandarini 2019; Singh et al. 2020), genus (Alzahrani et al. 2021), to family (Arogundade and Adedeji 2019). Due to the effectiveness of these two multivariate analysis methods, cluster analysis and principal component analysis have also been applied in systematics studies of ferns such as for species delimitation in the genus *Lemmaphyllum* (Wei and Zhang 2013) and for determining the species relationships in *Selaginella* (Bautista et al. 2018).

Observations on the character loadings of the first component showed that there were seven characters most responsible for the variance as indicated by the absolute value of higher than 0.2. This cut-off value indicated that the characters having loadings of 0.2 were considered acceptable variables that explain at least 20% of variance (Santos et al. 2019). These seven characters were those that contributed to the separation of genus *Sticherus* from *Dicranopteris* as shown in the dendrogram generated by cluster analysis. These characters were the number of primary forks, distance between pinnules, pinnule's apex, position of sori on the pinnule, spore shape, and exine ornamentation. Observation on the second principal component showed there were six characters that had loadings higher than 0.2. These characters were identified as those that contributed to the separation of *D. speciosa* and *D. curranii* from all members of *D. linearis* as shown in the dendrogram. These six characters were rachis bud protective structure (stipules), branch surface, the number of branches at each forking, distance between pinnules, and hairs color on pinna abaxial surface. Results of principal component analysis showed that the number of characters distinguishing genera was greater than those separating species within a genus. This was in line with the general principle of hierarchical classification in which the higher the taxonomic ranks the greater the number of characters differing between taxa (Singh 2010; Simpson 2019).

Table 3. List of characters used in cluster analysis and principal component analysis

No	Characters	Character states or unit
1.	Plant habit	Erect; climbing
2.	Height of the first branch	Measurement (cm)
3.	The number of primary forks	Number (1-6)
4.	Stipule on the first branch	Present; absent
5.	Distance between branches	Measurement (cm)
6.	Angle of the primary branch	Degree
7.	Rachis bud's surface	Hairy; scaly
8.	Rachis bud's protective structure	Two or many stipules
9.	Branching pattern	Equal; unequal
10.	Branch surface	Glabrous/smooth, hairy
11.	Accessory branches	Present; absent
12.	Accessory leaflets	Present, absent
13.	Number of branches at each fork	Number
14.	Length of primary pinnae	Measurement (cm)
15.	Width of primary pinnae	Measurement (cm)
16.	Length of pinnules	Measurement (mm)
17.	Width of pinnules	Measurement (mm)
18.	Distance between pinnules	Measurement (mm)
19.	Costa surface	Glabrous; laminar
20.	Pinna's veins	Convex; flat
21.	Pinna's apex	Acute, acuminate; aristate
22.	Pinna's margin	Equal; unequal
23.	Color of pinna's abaxial surface	Whitish green; light green
24.	Hairs on pinna's abaxial surface	Hairy; almost glabrous
25.	Hairs color on pinna's abaxial surface	Reddish-brown; brown; whitish brown
26.	Position of first forking on pinnule's vein	At the bottom of pinnule; at the middle of pinnule
27.	Veins color on pinna's adaxial surface when dry	Brown; white
28.	The nature of veins on pinna's adaxial surface when dry	Convex; flat
29.	Curvature of the pinna veins on abaxial surface	Curved; flat
30.	Pinnule's apex	Retuse; truncate
31.	The number of forking on pinnule's veins	Once-forking; twice forking; three times-forking; Four times-forking; five times-forking
32.	Length of scales	Measurement (mm)
33.	Position of sori on pinnules	Third quarter from base; halfway from apex; Halfway from base; along the pinnule
34.	The number of sporangia per sorus	Number (4-17)
35.	The number of sporangial stacks	1; 2
36.	Sori color	Greenish white; yellowish-white
37.	Spore type	Monolete; trilete
38.	Length of spore's polar axis (P)	Measurement (µm)
39.	Diameter of spore's equatorial plane (E)	Measurement (µm)
40.	Spore shape based on P/E ratio	Sub-prolate; prolate; prolate-spheroidal; oblate-spheroidal
41.	Exine ornamentation	Psilate; micro-scabrate; scabrate; verrucate
42.	Class of spore size	Small; medium

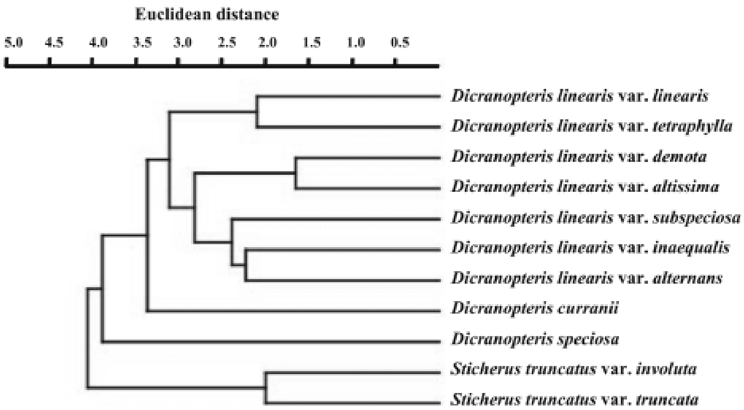

Figure 8. Dendrogram of taxonomic relationships of *Dicranopteris* and *Sticherus* species based on morphological and spore characters

Tabel 4. Character loadings on the first two principal components

Character	Principal component	
	PC1	PC2
Plant habit	-0.042	0.101
Height of the first branch	-0.057	0.038
The number of primary forks	-0.052	0.212
Stipule on the first branch	0.039	-0.037
Distance between branches	-0.035	0.118
Angle of the primary branch	-0.036	0.066
Rachis bud's surface	-0.159	-0.077
Rachis bud's protective structure	-0.280	-0.092
Branching pattern	0.044	0.055
Branch surface	-0.150	0.069
Accessory branches	-0.153	0.015
Accessory leaflets	0.107	-0.094
Number of branches at each fork	-0.030	-0.061
Length of primary pinnae	0.251	-0.057
Width of primary pinnae	0.465	-0.036
Length of pinnules	0.153	-0.026
Width of pinnules	0.034	0.360
Distance between pinnules	0.026	-0.312
Costa surface	-0.140	-0.027
Pinna's veins	0.076	0.150
Pinna‘s apex	0.121	0.121
Pinna's margin	0.110	-0.153
Color of pinna's abaxial surface	-0.159	-0.077
Hairs on pinna's abaxial surface	0.076	0.150
Hairs color on pinna's abaxial surface	-0.150	0.069
Position of first forking on pinnule's vein	-0.100	0.067
Veins color on pinna's adaxial surface when dry	0.044	0.055
The nature of veins on pinna's adaxial surface when dry	-0.134	-0.179
Curvature of the pinna veins on abaxial surface	-0.094	0.141
Pinnule's apex	-0.112	-0.240
The number of forking on pinnule's veins	0.324	-0.154
Length of scales	-0.216	-0.077
Position of sori on pinnules	-0.091	0.289
The number of sporangia per sorus	0.286	0.102
The number of sporangial stacks	0.236	0.003
Sori color	0.102	-0.091
Spore type	-0.134	-0.179
Length of spore polar axis (P)	0.064	-0.139
Diameter of spore equatorial plane (E)	-0.106	-0.148
Spore shape based on P/E ratio	-0.121	-0.210
Exine ornamentation	-0.073	0.421
Class of spore size	0.064	-0.139
Eigenvalue	1,873	1,227
% Variance	30,604	20,047
% Cumulative Variance	66,882	50,651

Overall, this study emphasized the use of morphology and spore characters in assessing taxonomic relationships of ferns analyzed using numerical taxonomic methods. The application of multivariate methods in analyzing phenetic relationships of ferns based on morphology and spore characters has been reported for many taxa. Petchsri et al. (2012) reported the use of cluster analysis and canonical discriminant analysis in resolving taxonomic status and species delimitation in *Microsorum punctatum* species complex. Wei and Zhang (2013) used cluster analysis, principal coordinate analysis, and principal component analysis based on morphological variation for species delimitation in the fern genus *Lemmaphyllum.* Sen and Mukhopadhyay (2016) reported the use of morphology and spore characters and the use of numerical taxonomy methods in resolving taxonomic relationships of Indian cheilanthoid fern. It could be concluded, therefore, that results of this study provided evidence on the efficiency of combining sporophyte morphology and spore characters in ferns systematics study. This study also supported the use of more than one numerical taxonomic method in defining relationships between closely related species and genera.

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