

The first record of grass-like fern, *Schizaea dichotoma* (L.) J. Sm. (Schizaeaceae) from Lingga Island, Indonesia: Its morphological, anatomical and palynological study

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Manuscript received: 4 July 2019. Revision accepted: 25 August 2019.

Abstract. Sofiyanti N, Iriani D, Fitmawati. 2019. The first record of grass-like fern, *Schizaea dichotoma* (L.) J. Sm. (Schizaeaceae) from Lingga Island, Indonesia: Its morphological, anatomical and palynological study. *Biodiversitas* 20: 2651-2660. *Schizaea dichotoma* (L.) J.Sm. is one of fern species from Family Schizaeaceae. This species is grass-like, dimorphic with fan-shaped fronds, and well-known with local name *Rumput Merak*. The first record of this species is reported from Lingga Island, Riau Islands Province, Indonesia. The aim of this study was to examine the morphology, anatomy and spore characteristics of this fern. This study had been carried from June to December 2017. Samples were collected using exploration method, documented, and prepared for herbarium specimen. In this study, we examined the morphological characters, anatomical characters of rhizome, stipes, and pinnae of both fertile and sterile fronds, as well as spore feature. The anatomical preparations were carried out using paraffin method, while spore specimens using acetolysis method. The anatomy of rhizome, stipes, and pinnae, as well as spore characters, were then observed and documented using digital microscope Celeron. *S. dichotoma* is dimorphic fern with grass-like habit, fan-shaped laminae that repeatedly forked end in sorospore in fertile frond. The rhizome has vascular bundle surrounded by star-shaped sclerenchymatous layer. The cross-section of stipes of both fertile and sterile fronds are "M" shaped with a vascular bundle located in the dorsal side, while the leaves have a vascular bundle in the middle of midrib. The type of vascular bundles and stele of those organs are similar, i.e. collateral closed and protostele. We observed The Casparian strip in the endodermis layer of rhizome, stipes, and leaves, as well as at cortex and among the xylem. The spore of *S. dichotoma* is monolete and large spore class (ca. $59.5 \pm 2.4 \times 35.86 \pm 2.01 \mu\text{m}$).

Keywords: Fern taxonomy, Riau Islands, rumput merak, spore

INTRODUCTION

Schizaea is one of the eldest genera in Schizaeaceae, together with *Actinostachys*, *Anemia*, *Lygodium*, and *Mohria*. However, the genera within Schizaeaceae had been changed many times, with the addition of published genera as follow, *Amenia*, *Lygodium*, *Klukia*, *Paralygodium*, *Pelletixia*, *Ruffordia*, *Schizaeangium*, *Schizaeopsis*, *Schizaeopteris*, *Stachypteris*, (Balme 1995; Avramenko and Svetlana 2013; Zhang and Mickel 2013). In the recent classification of this family, the number of genera from Schizaeaceae reduce to only two genera, i.e. *Schizaea* and *Actinostachys* that differed by their frond (Pynee et al. 2011) and gametophyte characteristic (Zhang and Mickel 2013). All of the members of Schizaeaceae are primitive fern family (Davidson et al. 2008) within Class Polypodiopsida.

The genus *Schizaea* comprises ca. 68 published species (Zhang and Mickel 2013; Iwashina and Matsumoto 2013). However, only 13 out of 68 species are accepted name, while the rest are unresolved (40 species) and synonym (13 species) (based on theplantlist.org). Morphologically, *Schizaea* is most distinctive and grasslike. Therefore, its members look different from other fern species. They are dimorphic and characterized by having two different frond types, i.e. barren / sterile frond called trophophylls and the

fertile frond (sporophyll). Both fronds are fan-shaped frond with dichotomous free veins. The sporophylls bear marginal sporangia that form a sorospore (Holttum 1939; Verdcourt 2000).

Schizaea species are distributed in mainly pantropically (Winter and Amaroso 2003), and only few records were reported from subtropical regions. This genus is recorded from India, Malaysia, Myanmar, Indonesia, China, Philippines, Japan, Australasia, Polynesia, Mascarene Islands and South America (Hartini, 2007; Murtaza et al., 2008; Amoroso et al. 2009; Fraser-Jenkins, 2012; Zang and Mickel, 2013). Based on The International Union for Conservation of Nature (IUCN), most of the *Schizaea* species have rare occurrence (Fraser-Jenkins, 2012; Amoroso et al. 2009), endangered (EN) or vulnerable (VU) (Fraser-Jenkins, 2012). In Indonesia, a total of four species of *Schizaea* had been recorded from some islands, i.e. *Schizaea dichotoma* (L.) J.SM. (Verdcourt 2000; Davidson et al. 2008; Hartini 2007), *Schizaea digitata* (L.) Sw. (Backer and Posth, 1939), *Schizaea inopinata* Selling and *Schizaea spirophylla* Troll. (Holttum 1955). The IUCN status of *Schizaea* species from Indonesia are not-evaluated (NE) (*S. inopinata*) (Amaroso et al. 2016), endangered (EN) (*S. digitata*) and vulnerable (VU) for *S. dichotoma* (Fraser-Jenkins, 2012). Only limited record of *S. dichotoma* had been reported from Indonesia such as in East

Kalimantan (Hartini, 2007), Bangka Belitung, western coast Sumatera (Davidson et al. 2003) and Jambi Province (Beukema and van Noordwijk 2004). There is no report on *S. dichotoma* from Riau Islands Province had been published (summarized from Sofiyanti 2013; Sofiyanti et al. 2015; Marpaung et al. 2016).

Riau Islands is one of province in Sumatera Region, that located between Sumatera Island and Peninsular Malaysia. This province consists of many islands and Lingga Island is one of main and the most populated island in this province. Lingga Island is located south of Singapore, off the eastern coast of Riau Province. We had carried out fern explorations in some islands in coastal area of Riau as well as Riau Islands Province such as Halang, Rupatt, Karimun, Batam, Bengkalis, Rangsang, and Selat Panjang Island from 2014 to 2016. A total of 126 fern species are recorded during our study (Summarized from Sofiyanti 2013; Sofiyanti et al 2015a, 2015b; Sofiyanti et al. 2016, 2017, 2018, 2019; Sofiyanti and Isda 2018, 2019). However, we didn't find the occurrence of *S. dichotoma* from our study sites in Riau and Riau Islands Province. During our first exploration in another island of Riau Islands Province, we discovered some populations of *S. dichotoma* from Lingga Island. This species was found near the Lingga beach. This finding is important record of this fern from the archipelago area. Therefore, it is necessary to investigate the morphology, anatomy and palynology of this fern. Those characters are important to provide basic data of a plant species. In taxonomic study, morphology is the most common data used by taxonomists to identify a species (Stesovic and Berg 2015; Sofiyanti et al. 2015, 2016). Anatomical data also plays important rule in plant identification and classification (Talib et al. 2016, 2017; Murtaza et al. 2008), while the palynological data

support the taxonomic status of many taxa (Moran et al. 2017). This study aimed to examine the morphology, anatomy and spore characteristics of this rare fern, *S. dichotoma*.

MATERIALS AND METHODS

Study area

This study had been carried from June to December 2017. Samples were collected from Lingga Island, Riau Islands Province, Indonesia (Figure 1). This island is a hilly island with red-yellow podzolic, litosol and organosol soil.

Procedures

Samples collection

All of the samples were collected from the field using exploration method based on Kamau (1992). The specimens were photographed and prepared for herbarium following the method by Seshagirao et al. (2016). The synonym was checked using www.theplantlist.org.

Morphological study and identification key

We observed detail morphological characters of *S. dichotoma*, photographed and described each part of root, rhizome, stipe and lamina. For micromorphology observations (spine of stem, part of sorophore and sporangium), were carried out under stereo Microscope (Celeron). For identification key, we also used the description of other *Schizaea* species (*S. digitata*, *S. inopinata*, and *S. spirophylla*) that had been recorded in Indonesia, based on the description prepared by Holttum (1959) and Zhang and Mickel (2013).



Figure 1. Location of Lingga Island, Riau Islands Province, Indonesia (black circle indicates study site).

Anatomical study

Samples were collected from three individuals. We observed the anatomy of rhizome, stipe, and leaves of both fertile and sterile fronds by using paraffin method (Johansen 1940). Samples of rhizome were cut in 0.5 cm long before treatment. Stipe specimens were taken 1 cm below the first dichotomous branch, while pinna specimens were cut 1 cm below the pinna tips for sterile pinnae, and 1 cm below the base of sporangiophore for fertile pinnae. The specimens were fixed for 24 hr in Formalin-acetic acid-alcohol (FAA), discard the solution after fixation and soak ethanol dehydration series. The specimens were then prepared for dealcoholisation in ethanol: xylol solution series; two times in xylol for 30 min each, and put in xylol: paraffin (1: 9) with incubation for 24 h at 58° C. The paraffin infiltration was carried out for 24 h at 58° C twice. Microtome Microm was used to cut the specimens 7 µm thick, put on object glass and stain in 1% safranin, Xylol I, Xylol II, alcohol-xylol series, alcohol series for 3 min each treatment. Cover the specimen using cover glass before sealed with paraffin on bunsen. Specimen documentation was done using digital microscope Celeron.

Palynological study

In this study, we collected spores from three different individuals. Spore specimens were prepared using acetolysis method (Erdtman 1960). Fixation was carried out for 24 h using Glacial Acetic Acid (GAA), before 5 min centrifugation. The specimens were then put in GAA: H₂SO₄ solution with the ratio of 9 : 1. Before second centrifugation, the specimens were heated in water bath for 15 min. The solution was then replaced with the solution of 2 ml GAA, Na Chlorate and HCl (3 drops each). The third centrifugation was carried for 5 min. dH₂O was used in washing process, discard dH₂O before staining process (glycerin + 1 % safranin). The spore specimens were put on object glass and covered by cover glass before being shield using transparent hand nail. The spore features were observed using digital microscope (Celeron). A total of 20 spore grains were used to make statistical measurement. The spore characteristic was based on Erdtman (1957).

Data analysis

The morphological, anatomical and palynological data were described, photographed, tabulated and presented in figure or table. All of these data were then descriptively analyzed.

RESULTS AND DISCUSSION

Taxonomic treatment

Schizaea dichotoma (L.) Sm., Mém. Acad. Roy. Sci. (Turin) 5: 422, t. 9. 1793. (Figure 2.A-G); Bl. En. Pi. Jav. 1828. 255; Hook. & Grev. Ic. Fil. 1827 T. 17; Bedd. Ferns S. India. 1863. T. 65; Handb. (1883) 452; Prantl, Unters. Morph. Gefasskr. 2. 1881. 138; Racib. Pterid. Buit. 1898. 6; V. A. V. R. Mai. Ferns (1908) 116; Merr. Int. Rumph. 1917. 69; Backer & Posth. Varenfl. Java. 1939. 256, Hg-66; Holtt. Ferns Mal 1955. 50, Figure 6. *Acrostichum*

dichotomum Linne. Sp. Pi. 1753. 1068. *Osmunda Dichotoma* Spr. In Schrader, J. Bot. 1799. Pt. 2, 268. *Ripidium dichotomum* Bernh. In Schrader, J. Bot. 1800, Pt 2. 1801. 127, T. 2, S. Forsteri Spr. Anleit. 3 (1804) 57. *S. Cristata* Willd. Sp. Pi. 5 1810. 88.—S. Biroi Richter, Math. Termeszt. Ertesito 29. 1911. 1074; Troll, Flora 128. 1933. 339. Boonkerd & Pollawatn, Pterid. Thailand: 35, 83. 2000. Murtaza, G., S. Majid, R. Asghar, Z.H. Malik. M. Pak. JBot. 40 (1): 59-63. 2008.; Jaman & Umi Kalsom, Fl. Pen. Malaysia, Ser. I, Ferns & Lycoph. 1: 112. 2010;

Description: Terrestrial fern. Rhizome shortly creeping, densely covered by hairs. Hairs pale brown to brown, multicellular, 2-3 mm long. Fronds appear from rhizome, erect, *dimorphic, fan-shaped on both sterile frond (trophophyll) and fertile frond (sporophyll)*. Stipes of sterile fronds ca. 10-15 cm long, ca. 1.5 mm wide, light green, scally, narrowly winged except at the base. Stipes of fertile frond up to 20 cm or more, ca. 1.5 mm wide, light green, scally, narrowly winged except at the base. *Laminae repeatedly forked, end in sorospore in fertile frond*, ca. 10 cm in length or more, ca. 2 mm in width, up to 8 times dichotomously divided, short trichome, in fertile fronds bear sorospore. Sorospore terminal, up to 34 in single stipe, bearing 7 pairs of lobes. Lobes pinnate, alternate, dark browns at maturity, forming sporangia. Sporangia reniform, brown. Spore monolete, prolate, ca. $60.5 \pm 2.3 \times 36.6 \pm 1.7 \mu\text{m}$.

Synonym: *Acrostichum dichotomum* L. Sp. Pl. 2: 1068 (1753); *Osmunda dichotoma* Spreng. J. Bot. (Schrader) 268 (1799); *Ripidium dichotomum* Bernh. J. Bot. (Schrader) 1800 (2): 127, t. 2, f. 3 (1801); *Schizaea cristata* Willd. Sp. Pl. 88 (1810); *Schizaea forsteri* Spreng. Anleit. Kenntn. Gew. 157 (1804).

Vernacular name: Paku Cakar Ayam (Bangka), Rumput Bulu merak (Belitung), Silaju (Sumatra western coast) (Davidson et al. 2008), Rumput Merak (Lingga Island)

Habitat: *Schizaea dichotoma* occurs at the wet coastal area, open space on sandy black soil. The altitude of its habitat is 0 m asl. We observed common plant species in this area, e.g. *Pandanus* sp., *Rhizophora* sp., and *Bruguiera* sp.

Specimens examined: SD01, SD02, SD04, SD04, Pasir Panjang Beach, Mepar, Daik Subdistrict, Lingga District, Kepulauan Riau, Indonesia. 0 m asl, Elevation 43m, S 00° 15' 46.4" E 104° 32' 12.2". Collector Fitmawati. 2017. We also observed *Schizaea dichotoma* specimens from virtual herbarium i.e. BM001048362 (Natural History Museum (BM)); K001109003 and K001109004 (Royal Botanic Gardens, Kew (K)); CGI12727 (the Cambridge University Herbarium); AK355669 (Auckland WMM).

Morphological characters

Schizaea is distinguished from its related genus, *Actinostachys*, by its dimorphic frond type (Wagner 2019). The members of genus *Schizaea* are terrestrial fern with simple (linear) or fan-shaped fronds (Zhang and Mickel. 2013) and characterized by having two different fronds (sterile and fertile fronds) in the same individuals. A total of four species from *Schizaea* are reported from Indonesia i.e. *S. dichotoma*, *S. digitata*, *S. inopinata* and *S.*

spirophylla (summarized from Backer & Posth, 1939; Davidson et al. 2008; Hartini 2007; Verdcourt 2000). In this study, we observed the detail morphological character of *S. dichotoma* collected from Lingga Island, Riau Islands Province (Figure 1). The comparison of this species with three other *Schizaea* species reported from Indonesia is also presented (Table 1).

S. dichotoma has short and creeping rhizome that covered by hairs. The hairy rhizome is also observed in three other *Schizaea* species from Sumatra (Table 1). The diameter of rhizome ca. 0.8-1.1 mm. The fronds of *S. dichotoma* are light green, erect and dimorphic (Wagner 2019) with ca. 35 cm height (Figure 2.B). Holttum (1959) and Zhang and Mickel (2013) recorded higher frond of this species, up to 50 cm. Among four *Schizaea* species occur in Indonesia. *S. spirophylla* has the smallest habit, ca. 8 cm height (Table 1). Fern with dimorphic frond type has two different fronts, i.e. sterile frond (tropophyll) and fertile frond (sporophyll) in an individual. These fronds have different function, tropophyll (Figure 2.C) plays a role in photosynthesis and will not produce spore at maturity, while sporophyll (Figure 2.F) functions in both photosynthesis and producing spore. In *S. dichotoma*, both sterile frond (tropophyll) and fertile frond (sporophyll) have fan-shaped frond, with linear stripes. This character distinguished *S. dichotoma* from *S. digitata*, *S. inopinata* and *S. spirophylla* that have undistinct stipe. The laminae of *S. dichotoma* is repeatedly forked and will end in sorospore (Figure 2.G) in fertile frond. The dichotomous laminae (Figure 2.D) becomes the characteristic of this species that differ from other *Schizaea* species that have linear laminae. Even though the sterile and fertile frond in *S. dichotoma* has similar shape, but sterile fronds have smaller size than fertile fronds. The tips of pinulae on sterile frond curved downward (Figure 2.C) while fertile fronds have erect tip of pinulae (Figure 2.F).

The other morphological characteristic of *S. dichotoma* is sorospore characters. Sorospore is terminal and consisted of up to 34 lobes (Figure 2.G). Zhang and Mickel (2013) reported smaller number of lobe in this species. The terminal sorospore is also found in *S. digitata*, *S. inopinata* and *S. spirophylla*. However, they have different type of sorospore. *S. dichotoma* has the higher number of sorospore (up to 34 in each stipe) than *S. digitata* (5-15) and *S. inopinata* (a. 4). The number of sporangia row in a lobe is similar (2 rows) among *S. dichotoma* and *S. digitata*, *S. inopinata* and *S. spirophylla*. Each lobe bears numerous reniform sporangia (Figure 2.F).

Identification key of *Schizaea* from Indonesia

- 1a. Stipe undistinct, winged; sterile and fertile fronds have similar size; laminae linear, grasslike, sterile laminae unbranched, fertile laminae unbranched except at the tip; sorospore < 20 per 2
- b. Stipe clearly present, not winged; fertile frond is higher than sterile frond; laminae of both sterile and fertile fronds fan-shaped, branching dichotomously 6-8 times; sorospore up to 34; lobe pinnate, alternate *S. dichotoma*
- 2a. Height > 20 cm; wing of stipe rest winged or thick and rigid; lobe of sorospore 2 rows 3

- b. Height < 10 cm; wing of stipe flat; lobe of sorospore 2 rows and apparently 4 rows near the middle..... *S. spirophylla*

- 3a. Stipe rest winged; laminae linear and slightly wavy; 15-20 x 2-3.5 mm; sorospore 5-18 *S. digitata*
- b. Stipe with thick and rigid wing; laminae linear, erect, Ca. 21 x 2 mm; sorospore 4 *S. inopinata*

Anatomical study

The anatomical study of *Schizaea* members is very limited. Murtaza et al. (2008) had reported the anatomical study of *S. dichotoma* from Kashmir. Their study examined the anatomy of rachis as well as macropalinology of *S. dichotoma*. In this study, we observed the anatomical character of rhizome, stipes, and pinnae of both fertile and sterile fronds, as well as spore feature of *S. dichotoma* from Lingga Island, Riau Island Province. Figure 3 shows the anatomical features of *S. dichotoma* rhizome in cross-section. The rhizome has single layer epidermis and multilayer cortex consists of polygonal cells that gradually bigger towards inner side (cross-section of rhizome presented in Figure 3.A). Only one vascular bundle was found and located in the middle of rhizome. The bundle is surrounded by thick star-shaped sclerenchymatous layers, and beneath this layer we observed a layer of endodermis with Casparian strip arranged in irregular shape (Figure 3.B).

The Casparian strip is deposited material made of suberin, lignin and some structural protein on the anticlinal cell wall and commonly found in endo-and exodermis of plant (Chen et al. 2011). This strip is found in vascular plant, and commonly occurs in root (Lersten 1997; Chen et al. 2011). The occurrence of Casparian strips was also reported in stem of some Angiospermae (Lersten 1997) and leaves of Pterodophyte (Sperry 1983). According to Chen et al. (2011), the Casparian strips function under salt stress. In ferns, the Casparian strips were previously reported in some species such as i.g. *Blechnum lehmanii* leaves (Sperry 1983) and *Achrosticum aureum* L. (Lobo and Krishnakumar 2014). The Casparian strips in *Blechnum lehmanii* located on adaxial side of leaves, while in *Achrosticum aureum* located on rachis.

In this study, the type of vascular bundle of rhizome is collateral closed, in which xylem and phloem located side by side without the presence of cambium. The thick-walled xylem located towards inner side. We observed 4 metaxylem in the center surrounded by some smaller xylem. The phloem towards outer side. Figure 3.B. shows that the stele type of this species is protostele, in which the vascular tissue is solid and without pith (Beck et al. 1982), this is the most primitive stele. The protostelic vascular bundle was also reported on other fern species such as *Adenophorus sarmentosus* (Wilson and Rickson 1966) and members of Lycophyta such as *Phlegmariurus squarrosus* (Pittermann et al. 2015). However, the vascular bundle type of older *Adenophorus sarmentosus* is different from *S. dichotoma*, because it becomes solenostelic when reach maturity.

The cross-section of both sterile and fertile stipe shows a pair of "M" shaped structure towards ventral/abaxial surface (Figure 4.A-B). this structure forms a narrow

groove along the stipe. The arrangement of epidermis, cortex and vascular bundle of sterile and fertile stipe is similar. Cross-section of stipes show that epidermis consists of one layer of thick-walled cells, with various cell shapes. The cortex is multilayer (Figure 4.C-D) consists of many cell that varies in shape. We observed many

sclerenchymatous cells in this layer. This structure was also reported in other fern species, *Parahemionitis cordata*, *Pteris confusa*, *Pteris pellucida*. In *Parahemionitis cordata*, sclerenchymatous cells are arranged in 4-5 layers, while *Pteris confusa* and *Pteris* showed 2-3 layers of sclerenchymatous cells (Resmi et al. 2016)..

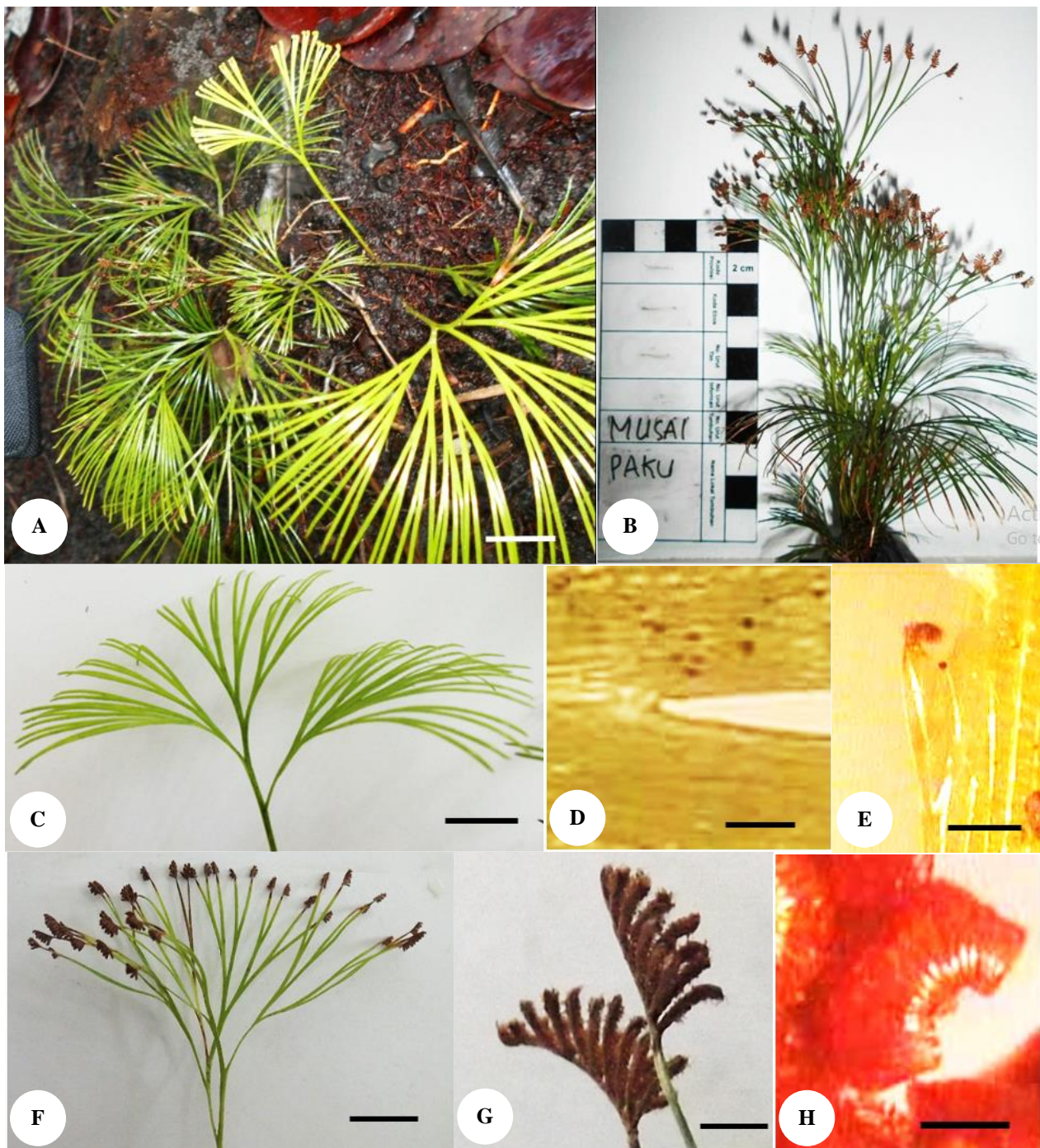


Figure 2. Morphology of *Schizaea dichotoma*. A and B. Habitus (A at the natural habitat), C. Sterile frond, D. Dichotomous branch of sterile frond, E. Scale of stipe, F. Fertile frond, G. Sorospore, H. Sporangia (scale bar; A, C, F = 3 cm, D = 1 mm, E = 1 mm, F = 0,1 mm, G = 0,5 mm).

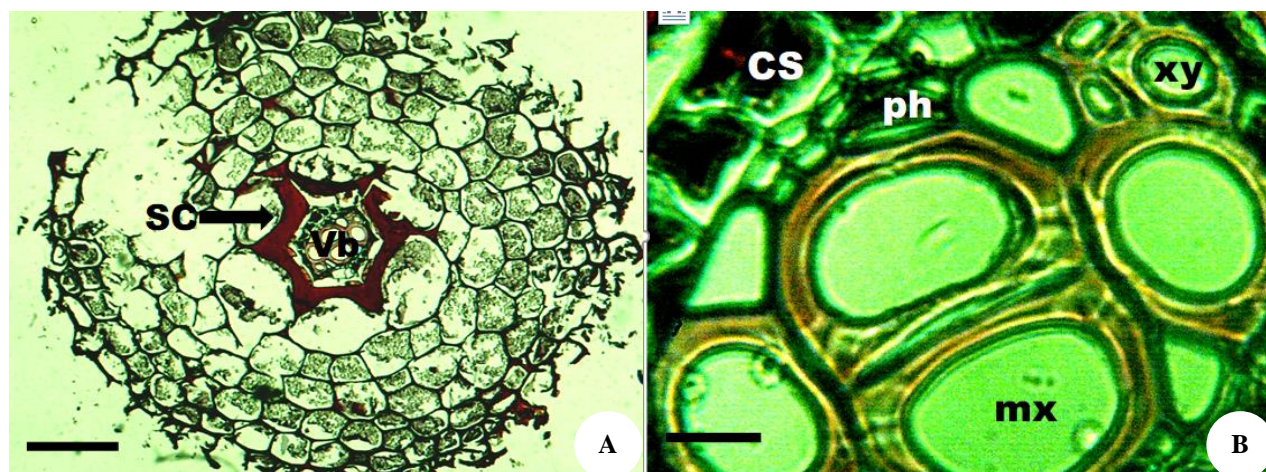


Figure 3. Rhizome cross-section of *Schizaea dichotoma*. A. cross-section showing protostele vascular bundle surrounded by star-shaped sclerenchymatous layer, B. thick-walled xylem and Casparian strips) (cs = Casparian strip, sc = sclerenchymatous layer, ph = phloem, xy = xylem) (scale bar s: a. 150 μ m, b. 30 μ m).

Table 1. Morphological comparison of *Schizaea dichotoma*, *S. digitata*, *S. inopinata* and *S. spirophylla*

Characters	<i>S. dichotoma</i> (This study)	<i>S. digitata</i> Zhang and Mickel (2013)	<i>S. inopinata</i> Holtum (1955)	<i>S. spirophylla</i> Holtum (1955)
Rhizome	Short, hairy	Short, hairy	Short, hairy	Short, apex hairy
Height (cm)	Ca. 35-45	20-35	Ca. 21	Ca. 8
Front type	Dimorphic	Dimorphic	Dimorphic	Dimorphic
Sterile frond				
Stipe	Clearly present	Undistinct	Undistinct	Undistinct
Length of stipes	Ca. 15-20 cm	-	-	-
Wing of stipes	Not winged	Rest winged	Thick and rigid	Flat
Shaped of laminae	Fan-shaped	Linear, slightly wavy	Linear, erect	Linear
Size of laminae	Ca. 15 cm long x 17 cm wide	15-20 x 2-3.5 mm	Ca. 21 x 2 mm	4-8 x 1mm
Branch of laminae	Branching dichotomously 6-8 times	Unbranched	Unbranched	Unbranched, sometime twisted
Fertile frond				
Length of stipes	Up to 20 cm	Undistinct	Undistinct	Undistinct
Winged stipes	Narrowly present	Rest winged	Thick and rigid	Flat
Shaped of laminae	Fan-shaped	Linear, slightly wavy	Linear, grass-like,	Linear
Size of laminae	Up to 35 cm or more	15-20 x 2-3.5 mm	Ca. 21 x 2 mm	4-8 x 1mm
Branch of laminae	Branching dichotomously 6-8 times	Unbranched, except at the tip	Unbranched, except at the tip	Unbranched, sometimes twisted
Sorosphore	Up to 34 per stipes, bearing 7 pairs of lobes	5-15	4*	NA
Lobes of sorosphore	2 rows, pinnate, alternate	2 rows; digitatus	2 rows, digitatus	2 rows, digitatus, (apparently 4 rows near the middle)
Sporangia	Reniform	NA	NA	NA

Note: * based on picture at phytoimages.siu.edu

A layer of endodermis surrounds vascular bundle on both steril and fertile fronds. The endodermis cells show reniform Casparian strips (Figure 4.E-F) located on the dorsal side. The position vascular bundle is also similar, only one vascular bundle located in the middle of dorsal side. The type is the same as vascular bundle in rhizome, i.e. collateral closed but different in xylem arrangement. Xylem in stipe forms "T" shaped structure towards ventral

side (Figure 4.E-F), and the tip of arms curve downward. The xylem is bigger in the middle and gradually narrower toward outer side. The phloem is commonly located toward dorsal side, surrounds both xylem arms. The stele is protostele and oval-shaped. This stele shape is almost similar to stele of *Shizaea dichotoma* from Khasmir that has "Y" shaped stele (Murtaza et al. 2008).

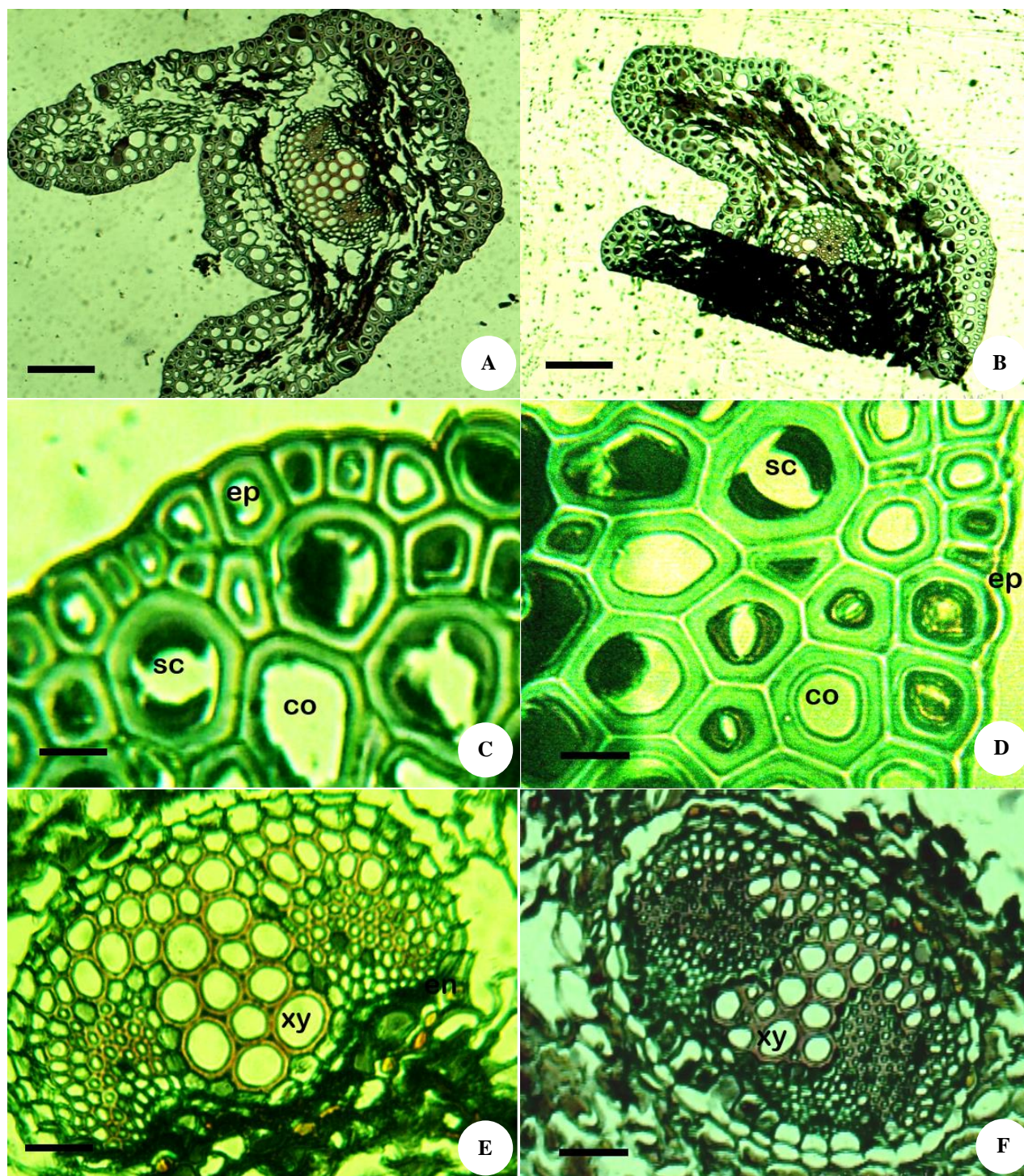


Figure 4. Stipe anatomy of *Schizaea dichotoma*. A-B. cross section of stipes (A. sterile frond, B. fertile frond), C-D. close up on epidermis and cortex, showing a pair of casparian strips in some cells (C. sterile frond, D. fertile frond), E-F. close up on vascular bundle showing T-shaped xylem (E. sterile frond, F. fertile frond) (co = cortex, cs = casparian strip, ep = epidermis, en = endodermis, sc = schlerencymatous layer, ph : phloem, xy = xylem) (Scale bars : A-B = 0.5 mm, C-D = 0.1 mm, E-F = 50 µm)

The sterile and fertile pinnae show the flying bird-shaped in cross-section (Figure 5.A-B present the cross-section of sterile and fertile leaves). The margin of both sides curves toward ventral side. one epidermis layer consists of polygonal thick-walled cells were observed.

Only two stomata are found at the ventral surface located at the left and right side midrib, the type of stomata is hypostomatic (stomata only located at foliar abaxial surface). Based on the position of guard cells, the type of stomata is cryptophore in which the guard cells are lower

than epidermis outer surface (Figure 5.G). The shape of guard cells is reniform. The mesophyll with 1-4 layers elongated palisade, and beneath the palisade, we observed loosely-arranged spongy cells with irregular shape (Figure 5.C-D). A vascular bundle was found in the middle of midrib in both sterile and fertile leaves (Figure 5.E-F). The type of these bundle is similar, i.e. collateral closed. A

layer of endodermis surrounds the vascular bundle, and beneath the endodermis, a layer of pericycle observed, consists of almost tubular cells. In this study, we also observed the Casparian strip in endodermis, with the bean-shaped structure. This structure is also found sparsely among the xylem.

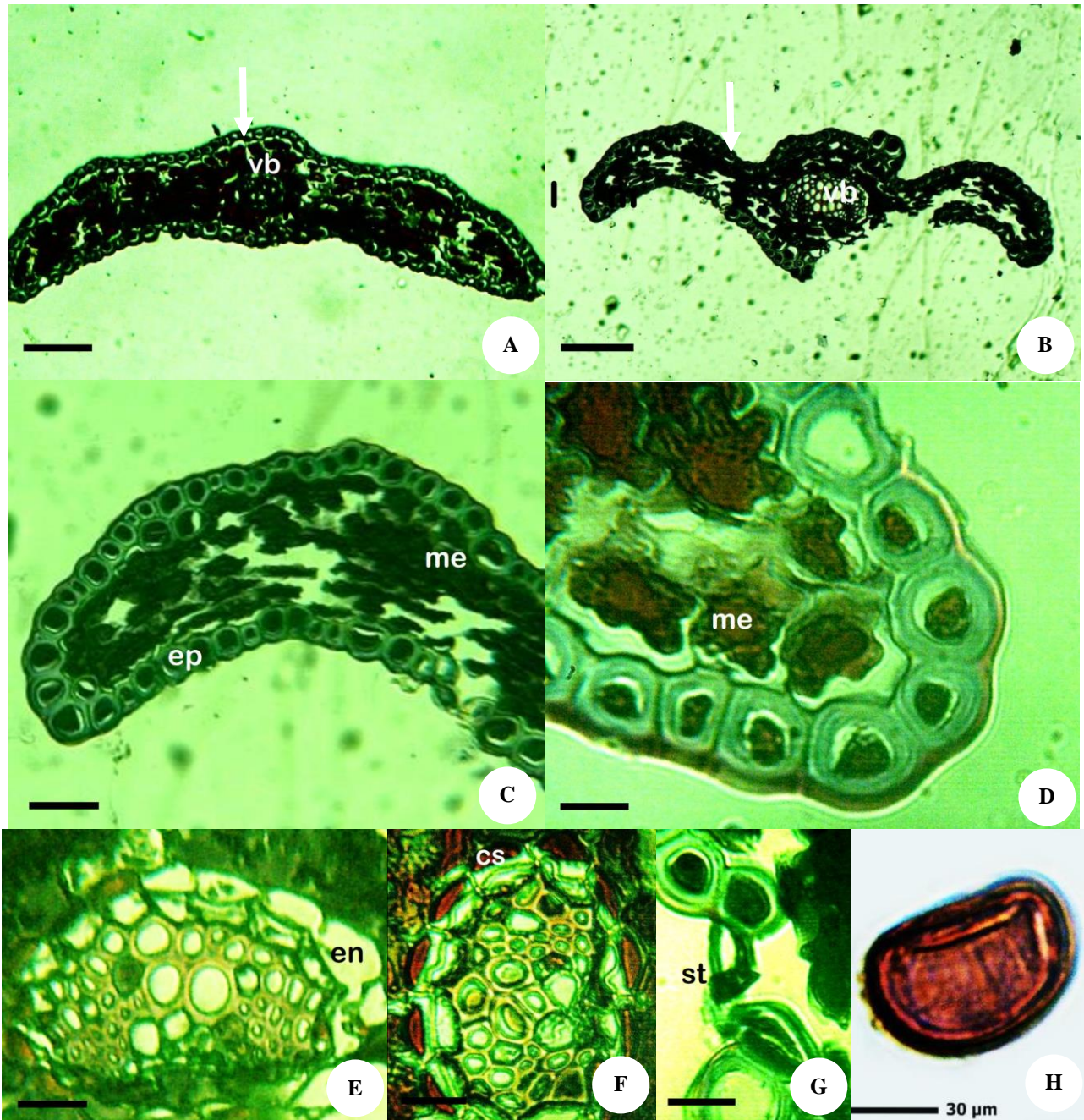


Figure 5. Cross section of pinnae and spore morphology of *S. dichotoma*. A-B. Cross section (A. sterile pinna, B. fertile pinnae), C-D. tip cross section of pinna showing epidermis and spongy layer (C. sterile pinna, D. fertile pinna), E-F. vascular bundle of pinnae (E. sterile pinna, F. sterile pinna), G. cryptophore stoma, H. spore grain. (ep = epidermis, cs = casparian strip, en = endodermis, me = mesophyll, xy = xylem, ph = phloem, vb = vascular bundle, st = stoma) (scale bars: A-B = 250 μ m, C = 150 μ m, D = 50 μ m, E-F = 10 μ m, G = 100 μ m, H = 25 μ m)

Palynological study

The spore grains of *S. dichotoma* is monolete. Monolete spore is bean-shaped (Figure 5.H) with a laesura along the ventral side. The size of spore grains ca. $59.5 \pm 2.4 \times 35.86 \pm 2.01$ μm . This size is bigger than the spore size of *S. dichotoma* from Khasmir reported by Murtaza et al. (2008). However, the spore class is the same. The class of spore-based on the longest size proposed by Erdtman (1957) divided spore into 6 classes i.e. gigantic (> 200 μm), very large (100-200 μm), large (50-100 μm), medium (25-50 μm), small (10-25 μm) and very small (< 10 μm). Therefore, the spore class of *S. dichotoma* is large spore. The monolete spore was also reported from other fern species such as *Anisocampium cuminganum*, *Asplenium apogamus* dan *Drynaria quercifolia*. (Makgomol 2006) However, their spores have different surface pattern from *S. dichotoma*.

ACKNOWLEDGEMENTS

This research was conducted by the support of Research Grant from Ministry of Research, Technology and Higher Education of the Republic of Indonesia 2017, led by main author.

REFERENCES

- Amoroso VB, Obsioma LD, Arlalejo DJ, Aspiras RA, Capili DP, Polizon JJA, Sumile EB. 2009. Inventory and conservation of endangered, endemic and economically important flora of Hamiguitan Range, Southern Philippines. *Blumea* 5: 71-76.
- Backer CA, Posthumus O. 1939. Varenflora voor Java. 66
- Beck CB, Schmid R, Rothwell GW. 1982. Stelar morphology and the primary vascular system of seed plants. *Bot Rev* 48 (4): 691-750. DOI: 10.1007/BF02860874
- Beukema H, van Noordwijk M. 2004. Terrestrial pteridophytes as indicators of a forest-like environment in rubber production systems in the lowlands of Jambi, Sumatra. *Agric Ecosyst Environ* 104: 63-73.
- Chen T, Cai X, Wu X, Karahara I, Schreiber L, Lin J. 2011. Casparian strip development and its potential function in salt tolerance. *Plant Signal Behavior* 6 : 1499-1502 DOI: 10.4161/psb.6.10.17054.
- Davidson C, Prusinkiewicz P, von Aderkas P. 2008. Description of a novel organ in the gametophyte of the fern *Schizaea pusilla* and its contribution overall plant architecture. *Botany* 86: 1217-1223.
- Erdtman G. 1957. Pollen and spore morphology plant taxonomy: Gymnospermae, Pteridophyta, Bryophyta, Illustrations. Almqvist and Wiksell. Stockholm.
- Erdtman G. 1960. The Acetolysis Method—A Revised Description. *Svensk Botanisk Tidskrift* 54: 561-564.
- Fraser-Jenkins CR. 2012. Rare and Threatened Pteridophytes of Asia 2. Endangered Species of India — the Higher IUCN Categories. *Bull Natl Mus Nat Sci Ser B*. 38 (4): 153-181.
- Hartini S. 2007. Keragaman Flora dari Monumen Alam Kersik Luway, Kalimantan Timur Flora Diversity from Kersik Luway Nature Monument, East Kalimantan. *Biodiversitas* 8 (1): 67-72.
- Holtum RE. 1939. Flora Malesiana. Sect. II. Vol. 1. p 41.
- Holtum RE. 1955. A Revised Flora of Malaya Vol. 2: Ferns of Malaya. Government Printing Office, Singapore.
- Iwashina T, Matsumoto S. 2013. Flavonoid glycosides from the fern, *Schizaea* (Schizaeaceae) in South Pacific Region, and their distribution pattern. *Bull Natl Mus Nat Sci Ser B* 39 (4): 195-201.
- Johansen AD. 1940. Plant Microtechnique. McGraw-Hill Book Company, Inc. London.
- Kamau HN. 1992. Germplasm exploration and collection. *Dinteria* 23: 50-54.
- Lersten NR. 1997. Occurrence of endodermis with a Casparian strip in stem and leaf. *Bot Rev* 63 (3): 265-272. DOI: 10.1007/BF02857952
- Lobo SM, Krishnakuma G. 2014. Studies on ecological anatomy of the mangrove fern *Acrostichum aureum* L. *Intl J Plant Anim Environ Sci* 4 (1): 195-200.
- Makgomol K. 2006. Morphology of fern spores from Phu Phan National Park. *Kasetsart J (Nat Sci)* 40: 116-122.
- Marpaung AA., Sofiyanti N., Iriani D., Fitmawati. 2016. Morfologi spora paku Pteridaceae di Hutan PT. CPI Rumbai Riau. *Jurnal Riau Biologia* 1 (2): 148-154. [Indonesian]
- Murtaza G, Majid S, Asghar R, Malik ZH. 2008. Morphopalynological and anatomical studies on fan fern *Schizaea dichotoma* (L.) Smith from Neelum Valley, Azad Kashmir. *Pakistan J Bot* 40 (1): 59-63.
- Pittermann J, James W, Katharine LC, Eric S, Craig B, Alan RS, Alex B. 2015. The structure and function of xylem in seed-free vascular plants: An evolutionary perspective. *Functional and Ecological Xylem Anatomy*. DOI: 10.1007/978-3-319-15783-2_1.
- Pynee K, Grangaud E, Rouhan G. 2011. *Actinostachys confusa*: An additional native fern species for the Mascarenes (Schizaeales: Schizaeaceae). *Cahiers scientifiques de l'océan Indien occidental*. 29-33.
- Resmi S, Thomas VP, Sreenivas AV. 2016. Stipe anatomical studies on selected pteridophytes of South India. *Acta Botanica Hungarica* 58 (1-2): 167-176. Doi: 10.1556/034.58.2016.1-2.7
- Seshagirirao K, Harikrishnanaik L, Venumadhav K, Nanibabu B, Jamir K, Ratnamma BK, Jena R, Babarao. DK. 2016. Preparation of herbarium specimen for plant identification and voucher number. *Roxburghia*. 6: 111-119.
- Sofiyanti N, Iriani D, Fitmawati, Marpaung AA. 2019. Morphology, palynology, and stipe anatomy of four common ferns from Pekanbaru, Riau Province, Indonesia. *Biodiversitas* 20 (1): 327-336. DOI: 10.13057/biodiv/d200138.
- Sofiyanti N, Iriani D, Fitmawati, Roza AA. 2015a. Morfologi Tumbuhan Paku di TAHURA Sultan Syarif Hasyim Riau. UR Press. Pekanbaru. [Indonesian]
- Sofiyanti N, Iriani D, Fitmawati, Roza AA. 2015b. *Stenochlaena riauensis* (Blechnaceae), A new fern species from Riau, Indonesia. *Bangladesh J Plant Taxon* 22 (2): 137-140. DOI: 10.3329/bjpt.v22i2.26075.
- Sofiyanti N, Iriani D, Fitmawati. 2016. Potensi Tumbuhan Paku di Daerah Gambut di Riau sebagai Agen Terapeutik. Universitas Riau, Pekanbaru. [Indonesian]
- Sofiyanti N, Iriani D, Fitmawati. 2017. Karakteristik dan Metode Pembuatan Preparat Spora Pteridoflora. UNRI Press, Pekanbaru. [Indonesian]
- Sofiyanti N, Isda, MN. 2018. Kajian morfologi dan mikromorfologi (sisik serta trikoma) 4 jenis *Pyrrosia* Mirb. (Polypodiaceae) di Provinsi Riau. *Jurnal Biologi Tropis* 18 (2): 174-181. DOI: 10.29303/jbt.v18i2.857. [Indonesian]
- Sofiyanti N, Isda, MN. 2019. Jenis-jenis tumbuhan paku (Pteridofita) dari Hutan Universitas Riau, Provinsi Riau dan kajian kekerabatannya. *Jurnal Biospecies* 12 (1): 24-32. [Indonesian]
- Sofiyanti N, Mat-Salleh K, Mahmud K, Mazlan NZ, Hasein MRA, Burslem DFRP. 2016. *Rafflesia parvimaclulata* (Rafflesiaceae), A New Species Of Rafflesia From Peninsular Malaysia. *Phytotaxa* 253 (3): 207-213. DOI: 10.11646/Phytotaxa.253.3.4.
- Sofiyanti N. 2013. The diversity of epiphytic fern on the oil palm tree (*Elaeis guineensis* Jacq.) in Pekanbaru, Riau. *Jurnal Biologi* 17 (2): 51-55.
- Sofiyanti N. 2019. Keanekaragaman jenis flora epifit di hutan Kota Pekanbaru, Provinsi Riau dan kajian kekerabatannya. *Jurnal Biologi Universitas Andalas* 7 (1): 34-42. [Indonesian]
- Sperry JS. 1983. Observations on the Structure and Function of Hydathodes in *Blechnum lehmannii*. *Am Fern J* 73 (3): 65-72.
- Stešević D, Berg C. 2015. *Botrychium matricariifolium*, a new fern species for the flora of Montenegro. *Acta Botanica Croatica* 74 (1): 181-186. Doi: 10.1515/botcro-2015-001
- Talib N, Cutler DF, Ahmad Puad AS, Ismail BS, Ruzi AR, Ahmad Juhari AA. 2017. Diagnostic and systematic significance of petiole anatomy in the identification of *Hopea* species (Dipterocarpaceae). *South African J Bot* 111: 111-125. DOI: 10.1016/j.sajb.2017.03.008
- Talib N, Ruzi AR, Ismail BS, Ummu Hani B, Salwa S, Azeyanty JA. 2016. Petiole vascular bundles and its taxonomic value in the Tribe Dipterocarpeae (Dipterocarpaceae) (Berkas vaskular petiol dan nilai

- taksonominya dalam Tribus Dipterocarpeae (Dipterocarpaceae) Sains Malaysiana 45 (2): 247-253.[Malays]
- Verdcourt B. 2000. Flora of Tropical East Africa: Schizaeaceae. AA. Brakelma, Rotterdam.
- Wagner WH. 2019. Schizaeaceae. Flora of Africa 2. http://www.efloras.org/florataxon.aspx?flora_id=1&taxon_id=10806
- Wilson K. Rickson FR. 1966. An anatomical study of the Hawaiian fern *Adenophorus sarmentosus*. Pacific Sci 21: 114-118.
- Winter WP. Amoroso, VB. 2003. Plant Resources of South-East Asia No 15 (2) Cryptogams: Ferns and Fern Allies. Backhuys Publishers, Leiden.
- Zhang X, Mickel, JT 2013. 2013. *Schizaeaceae*. In: Wu ZY, Raven PH, Hong DY (eds.) Flora of China, Vol. 2-3 (Pteridophytes). Science Press, Beijing & Missouri Botanical Garden Press, St. Louis, USA.