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Spores Morphological Characteristics of Several Ophioglossaceae and Psilotaceae Fern in "Eka Karya" Botanical Garden - Bali

Ni Kadek Rika Pramesti[1], Wenni Setyo Lestari[2], Eniek Kriswiyanti[3], I Made Saka Wijaya*[4]

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[1,3,4]Biology Program, Faculty of Mathematics and Natural Sciences, Universitas Udayana. Jl Raya Kampus Unud Jimbaran, Kuta Selatan, Badung-Bali, Indonesia, 80361, [2]Research Center for Biosystematics and Evolution - National Research and Innovation Agency, Jl. Raya Jakarta-Bogor Km. 46, Cibinong, Bogor, West Java, Indonesia, 16911

e-mail:
[1]*rika.pramesti01@gmail.com*
[2]*wenn002@brin.go.id*
[3]*eniek@unud.ac.id*
[4]**sakawijaya@unud.ac.id*

**Corresponding author*

Abstract. *Ferns of Ophioglossaceae and Psilotaceae families can be found in the "Eka Karya" Botanical Garden, Bali as a collection or naturally growing in the botanical garden and those families are taxonomically classified as primitive ferns. The morphological characteristics of the family of Ophioglossaceae and Psilotaceae or even other types of ferns can be known through the morphological characteristics of the spores. This study aimed to characterize the spore morphology of the Ophioglossaceae and Psilotaceae fern of The "Eka Karya" Botanical Garden. Observation of the morphological characteristics of spores in the "Eka Karya" Botanical Garden was carried out by a simple method without acetolysis. The result of six types of fern plants from the family of Ophioglossaceae and Psilotaceae showed similarities to the spore unit, monads. Each family has unique characteristics that can be used to differentiate each other. The family Ophioglossaceae has a variety of spore colors, trilete spore types, mostly globose-shaped (except for one species the trilobate), the average polar diameter is 28.59±2.19 – 31.00±2.49 µm and the equatorial diameter is 32.85±1.74 – 37.55± 1.71 µm, the P/E ratio is 0.79-0.87, the shape based on the P/E ratio is suboblate, the spore size category is medium and has radial symmetry. For the family Psilotaceae, has a pale yellow-green spore color, monolete type, elongate-ellipsoidal shape, average polar diameter of 26.20±2.76 –29.81±2.96 µm and equatorial diameter of 52.98±2.55 – 63.31±4.65 µm, P/E ratio of 0.47-0.49, shape based on P/E ratio i.e. peroblate, large spore size category and has bilateral symmetry. Those characteristics are valuable taxonomic data that can be used as diagnostic characters for the identification of the families Ophioglossaceae and Psilotaceae.*

Keywords: *fern spore, monolete spore, palynology, primitive fern, trilete spore*

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INTRODUCTION

Ferns are primitive plants that already have a vascular system, have a wide distribution on earth, and have various life forms such as terrestrial, epiphytes, and aquatic forms (Nitta & Ebihara, 2019; Sofiyanti et al., 2019a). These plants can reproduce through spores formed inside sporangia (Sharma, 2012). The germination of spore involving

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the imbibition (water uptake) triggers the phytohormonal reaction and initiates the metabolism by mobilization of reserved energetic compound (Romanenko et al., 2020; Pedrero-López et al., 2023). The spore will develop to be fern gametophytes, a free-living photosynthetic thalloid that is responsible for forming gamets in its archaegonia and antheridium (Romanenko et al., 2020). The zygote is formed from the fertilization of ovum from archaegonia and sperm from antheridium, and then the zygote will develop to be a fern sporophyte. The sporophyte is responsible for formingspores inside the sporangia, and then the lifecycle isrepeated.

In general, ferns can be divided into two groups based on their sporangia characteristics, the leptosporangiate and eusporangiate (Martinetto et al., 2020). Leptosporangiate is sporangia that arise from the development of one initial cell, have a smaller size, and contain fewer spores (Yang et al., 2022; Nowak et al., 2022). Those characteristics in contrast with sporangia from eusporangiate group that developed from several initial cells are larger in size, and contain many spores (Sofiyanti et al., 2019b). Ferns that belong to the eusporangiate group, for example, are ferns from the Ophioglossaceae and Psilotaceae families (Zhang et al., 2020). Ophioglossaceae and Psilotaceae are also classified as primitive ferns (Patil et al., 2020; Chopparapu & Sabina, 2019). The collection of ferns from the Ophioglossaceae and Psilotaceae families can be found in the "Eka Karya" Botanical Garden located in Tabanan Regency, Province of Bali, Indonesia.

Species identification of ferns and relationship determination with other species is commonly based on their morphological characteristics. However, several characters of these plants have polymorphism, so robust characters are needed and can be used as a reference in classification. One of the robust characteristics that can be used is the morphology of the spore (Marpaung et al., 2016; Chen et al., 2021). The spores morphology in ferns have their own traits, so these characteristics would play an important role in the assessment of taxonomical rank in fern (Sofiyanti et al., 2019b; Adeonipekun et al., 2021; Chen et al., 2021). In addition, these morphological characters can be used as taxonomic evidence that can support a classification system (Hapsari et al., 2012). However, spores from ferns of the Ophioglossaceae and Psilotaceae families in the collection of "Eka Karya" Botanical Garden have insufficient data. So, this study aimed to characterize the spore morphology of Ophioglossaceae and Psilotaceae fern of "Eka Karya" Botanical Garden, Bali.

MATERIALS AND METHODS

Time and Location of Research

This research was carried out in January-February 2022 at the "Eka Karya" Botanical Garden, Bali. The samples were collected from Cyathea Park and Fern House, while the microscopic observation was done at The Conservation Genetics laboratory in The Plant Conservation Laboratory Building.

Research Methods and Data Collection

The fresh samples of six fern species of the family Ophioglossaceae and Psilotaceae is presented in Table 1. There are *Helminthostachys zeylanica*, *Botrychium daucifolium, Ophioglossum reticulatum*, *Ophioglossum pendulum*, *Psilotum nudum* and *Psilotum complanatum*. The spore samples were collected using the modified protocol of spore collection by Nebot et al. (2021). The harvesting of sporangia was done by cutting fertile leaves, then stored on collection paper or envelopes and labeled for each species obtained. The harvested sporangia were then stored for two days at room temperature so that the spo-

rangia broke and release the spores. After two days, the spores were separated from the sporangia fragments using a brush. The spores obtained were then collected and put inside the centrifuge tubes, and then stored in the refrigerator at 2-5°C before observation. Spore observations were carried out on 20 spores for each species.

The spores obtained were then observed macroscopically and microscopically. Macroscopically, spore color was observed by following the color standard guidelines of the Royal Horticultural Society (RHS) Colour Chart. Microscopically, spores were observed by a simple method without acetolysis through the following stages: the object glass was dripped first with water then a small amount of spores was taken with tweezers and sprinkled on the object glass. The object glass was then covered with cover glass. Microscopic characteristics were observed through Olympus CX-31 microscope that has been modified with DP2-SAL at 40x magnification. Especially for the measurement of the polar diameter and equatorial diameter of spores was carried out through software in DP2-SA.

Table 1 Ferns of family Ophioglossaceae and Psilotaceae observed

Species	Family	Source and Origin	Collection Date	Collection Number
Helminthostachys zeylanica (L.) Hook.	Ophioglossaceae	Kayan Mentarang National Park of North Kalimantan. The species planted in Fern House	14 April 2020	DL 272/ E2016060296
Botrychium daucifolium Wall. ex Hook. & Grev.	Ophioglossaceae	Tabanan Regency, Bali	15 October 2021	BA 933/ E2013010001
Ophioglossum reticulatum L.	Ophioglossaceae	"Eka Karya" Botanical Garden Bali. This species grows naturally in Cyathea Park at 1,381 m above sea level	7 January 2022	WN 623/ E2021120064
Ophioglossum pendulum L.	Ophioglossaceae	Grow naturally in "Eka Karya" Botanical Garden Bali. This species planted in Fern House, but not a collection	7 January 2022	-
Psilotum nudum (L.) P.Beauv.	Psilotaceae	"Eka Karya" Botanical Garden Bali. This species was an epiphyte on a large *Dacrycarpus imbricatus* tree, occupying habitat at 1,330 m above sea level	7 January 2022	WN 619/ E2021120062
Psilotum complanatum Sw.	Psilotaceae	Batukaru Nature Reserve, Tabanan Regency, Bali. The species grows as an epiphytes on palm trees at 740 m above sea level	7 January 2022	BA 748/ E201106208

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Data Analysis

The data were analyzed descriptively as a spore morphological description, table for each parameter, and microscopic visualization of spores. The spore parameter used comprised of the spore color, spore unit, spore type, spore shape, the average of polar (P) and equatorial (E) diameters, P/E ratio, spore shape based on P/E ratio, spore size category, and spore symmetry. The spore shape based on P/E ratio refers to the category from Erdtman (1952) in Table 2, while the spore size category refers to Kremp (1965) as shown in Table 3.

Table 2. The category of spore shape based on P/E ratio (Erdtman, 1952)

Shape	P/E ratio
Peroblate	< 0.50
Oblate	0.50-0.75
Suboblate	0.75-0.88
Oblate spheroidal	0.88-1.00
Prolate spheroidal	1.00-1.14
Subprolate	1.14-1.33
Prolate	1.33-2.00
Perprolate	> 2.00

Table 3. The category of spore size based on the longest side of spore (Kremp, 1965)

Length (µm)	Category
< 10	Very small
10-25	Small
25-50	Medium
50-100	Large
100-200	Very large
> 200	Gigantic

RESULTS AND DISCUSSION

Spore Morphological Characters

The spore morphological characters of several species of ferns of Ophioglossaceae and Psilotaceae families are shown in Table 4. The spore characteristics are shown as a description and spore microscopic visualization in Figure 1.

Helminthostachys zeylanica (L.) Hook.

Helminthostachys zeylanica spores macroscopically have a yellowish white color RHS2015(155B) and microscopically have: monad spore units with trilete type, globose shape, average polar (P) diameter 28.59±2.19 µm and equatorial (E) 32.85±1.74 µm, P/E ratio 0.87, spore shape based on P/E ratio is suboblate, medium spore size, and has radial symmetry.

Botrychium daucifolium Wall. ex Hook. & Grev.

Botrychium daucifolium spores macroscopically have a pale yellow green RHS2015(155A) color and microscopically have: monad spore units with trilete type, mostly globose or somewhat trilobate, aver-

age polar (P) diameter 31.00±2.49 µm and equatorial (E) 36.60±2.91 µm, P/E ratio 0.85, spore shape based on P/E ratio is suboblate, medium spore size, and radial symmetry.

***Ophioglossum reticulatum* L.**

Ophioglossum reticulatum spores macroscopically have a light yellow color RHS2015(160B) and microscopically have: monad spore units with trilete type, globose shape, average polar (P) diameter 29.82±1.49 µm and equatorial (E) 34.62±1.85 µm, P/E ratio 0.86, spore shape based on P/E ratio is suboblate, medium spore size, and has radial symmetry.

***Ophioglossum pendulum* L.**

Ophioglossum pendulum spores macroscopically have a pale greenish yellow RHS2015(160C) color and microscopically have: monad spore units with trilete type, globose shape, average polar (P) diameter 29.68±1.52 µm and equatorial (E) 37.55±1.71 µm, P/E ratio 0.79, spore shape based on P/E ratio is suboblate, medium spore size, and has radial symmetry.

***Psilotum nudum* (L.) P. Beauv.**

Psilotum nudum spores macroscopically have a pale yellow green color RHS2015(157C) and microscopically have: monad spore units with monolete type, elongate-ellipsoidal shape, average polar (P) diameter 29.81±2.96 µm and equatorial (E) 63.31±4.65 µm, P/E ratio 0.47, spore shape based on P/E ratio is peroblate, large spore size, and has bilateral symmetry.

***Psilotum complanatum* Sw.**

Psilotum complatum spores macroscopically have a pale yellow green color RHS2015(157C) and microscopically have: monad spore units with monolete type, elongate-ellipsoidal shape, average polar (P) diameter 26.20±2.76 µm and equatorial (E) 52.98±2.55 µm, P/E ratio 0.49, spore shape based on P/E ratio is peroblate P/E ratio, large spore size, and has bilateral symmetry.

Most spores of each species in Ophioglossaceae and Psilotaceae families have different macroscopic colors. According to the research conducted by Mellado-Mansilla et al. (2021), ferns of the family Ophioglossaceae and Psilotaceae are classified as a non-green spores or non-chlorophyllous spores. In general, the color of spores can be classified into three: non-chlorophyllous, chlorophyllous, and cryptochlorophyllous (Pedrero-López et al., 2023). Sundue et al. (2011) stated that the green color of spores for the chlorophyllous spore group arises due to the chlorophyll content, while cryptochlorophyllous spores actually contain chlorophyll when detected by epifluorescence, but have a non-green appearance when observed by naked eye or under a microscope. The presence of chlorophyll in the spore affects the spore viability. The chlorophyllous spores tend to have a high metabolic activity due to the presence of chlorophyll, resulting in a short-lived period of spore, while the non-chlorophyllous spores tend to be a long-lived spore (López-Pozo et al., 2019; Pedrero-López et al., 2021). The non-chlorophyllous and cryptochlorophyllous ferns also contain fatty acid in large quantities, making the spores have longer viability than the chlorophyllous fern (Pedrero-López et al., 2023).

Morphological characters that show similarities in these two families are spore units. The spore units for both of the family are monads. According to Sofiyanti et al. (2019b), monad spores (single) are spore units that commonly found in ferns than other types of spore units such as dyads, tetrads, or polyads. Spore type shows a unique separation among fami-

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lies. The spore type of all Ophioglossaceae is trilete, while for the Psilotaceae has a monolete-type spore. This finding in concordance with Olejnik et al. (2018) that found all the spore in Ophioglossaceae (*Botrychium lunaria*, *Botrychium matricariifolium*, *Botrychium multifidum*, *Botrychium simplex*, *Botrychium virgianum*, and *Ophioglossum vulgatum*) are trilete, while in Psilotaceae (*Psilotum nudum*) are monolete. According to Traverse (2007), trilete spores often referred to as spores with a 'Y sign' are spores that have laesura with three elongated arms and will later join in the middle. For the monolete, Sofiyanti et al. (2020) explained as spores with one laesura that extends in the proximal area. There is also a discrepancy among those spores according to the shape or form of the spore. Among these trilete and monolete spores, the trilete spores were considered primitive because their characteristics appeared earlier in the fossil record (Tryon & Lugardon, 1991). The trilete spore also has smooth non-perine exine patterns that are considered as a more primitive trait than monolete with ornamented perine exine patterns (Mondal et al., 2023).

Based on the shape and symmetry of the spores observed, it was found that almost all types of the Ophioglossaceae family have globose spores and radial symmetry. However, specifically for the type of *B. daucifolium*, the spores are globose to slightly trilobate. Meanwhile, all spores in Psilotaceae are elongate-ellipsoidal and have bilateral symmetry as shown in Figure 1. Some characteristics such as shape, symmetry, and type of spores are actually related. This explanation is supported by prior research which stated that the radial symmetry spores are usually globose with a trilete type, while the bilateral symmetry spores are usually ellipsoidal with a monolete type (Adeonipekun et al., 2021; Mondal et al., 2023). Research that has been done by Morajkar et al. (2021) also supports that the most spore shapes from a total of 45 types of ferns that have been studied are globose for trilete type and ellipsoidal for monolete type.

Spore shapes also can be classified into several types based on the P/E ratio. Determination of the P/E ratio is obtained by determining the average polar diameter and equatorial diameter. The average measurement of polar diameter in Ophioglossaceae ranges from 28.59±2.19 – 31.00±2.49 µm, while in the Psilotaceae ranges from 26.20±2.76 – 29.81±2.96 µm. Meanwhile, the average measurement of equatorial diameter in Ophioglossaceae ranged from 32.85±1.74 – 37.55±1.71 µm, while in Psilotaceae ranged from 52.98±2.55 – 63.31±4.65 µm.

Based on the results of the P/E ratio of the average polar diameter (P) and equatorial diameter (E) referring to Erdtman (1952), the spore shape based on the P/E ratio of all species in Ophioglossaceae is suboblate, which is indicated by the P/E ratio ranging from 0.79-0.87. However, for the species in Psilotaceae have peroblate shape, which is indicated by the P/E ratio ranging from 0.47-0.49. The results of the P/E ratio of the Psilotaceae are also in concordance with the research conducted by Vijayakanth & Sathish (2016) regarding the characteristics of Psilotaceae spores for *P. nudum*. In the study, it was obtained that *P. nudum* has a polar diameter of 35 µm and an equatorial diameter of 76 µm so the P/E ratio obtained was 0.46 (peroblate). The use of spore characteristics based on the P/E ratio can act as a major diagnostic taxonomic characteristic, especially in the Pteridophyta group (Adeonipekun et al., 2021).

Another characteristic that can characterize a spore of a particular species is its spore size. Spore sizes can be divided into several categories based on the longest axis (Tryon & Lugardon, 1991). Based on the re-

search, spores of the Ophioglossaceae belong to the medium category, while the Psilotaceae has spores that belong to a large category. According to Barrington et al. (2020), variations in spore size between closely related species can be caused by the influence of environmental factors such as related to geographical distribution which ultimately causes the evolution of spore size variations. Although the spore characteristics can be used as species delimitation (Sofyanti et al., 2019b; Adeonipekun et al., 2021; Mondal et al., 2023), the use of a single character in spore, such as the spore size, the only diagnostic character of a species should be considered wisely because the same species can have different spore size and ornamentation regarding the state of spore maturity, ploidy, and environment (Olejnik et al., 2018; Barrington et al., 2020; Nowak et al., 2022).

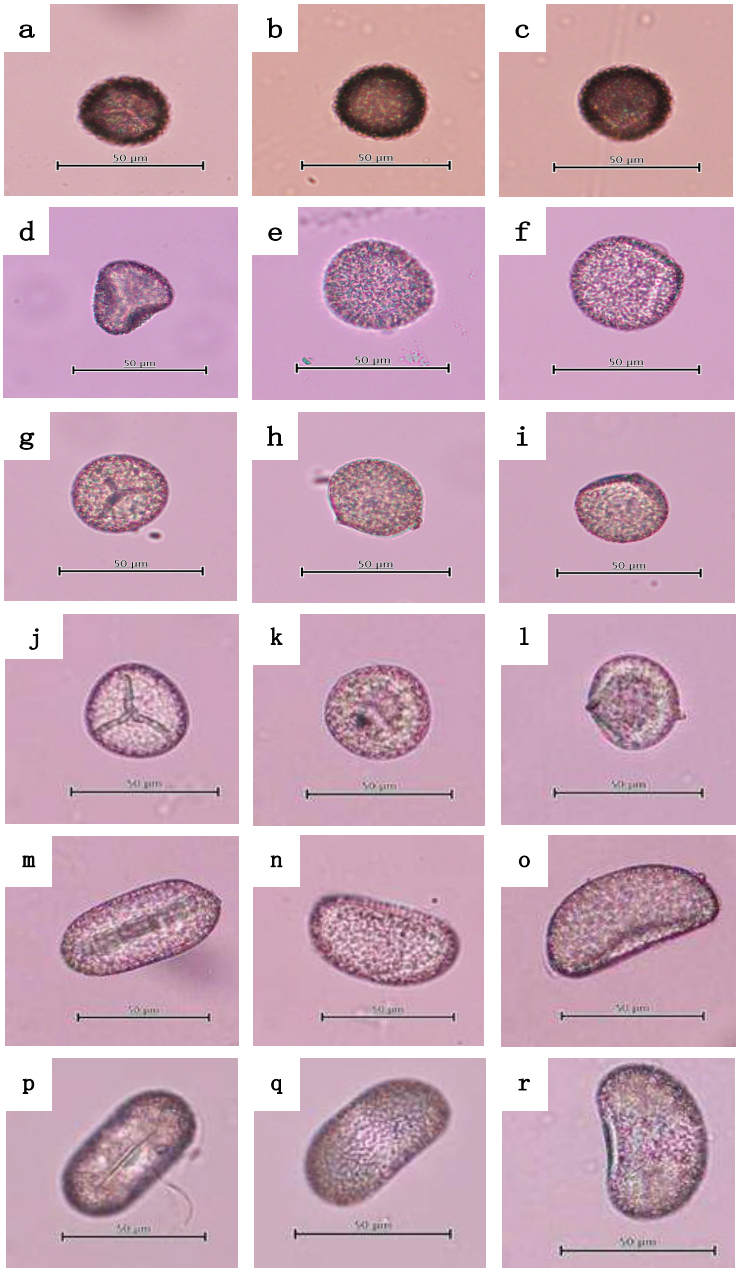

Figure 1. Spores of several species from family Ophioglossaceae and Psilotaceae: (a-c) *Helminthostachys zeylanica*; (d-f) *Botrychium daucifolium*; (g-i) *Ophioglossum reticulatum*; (j-l) *Ophioglossum pendulum*; (m-o) *Psilotum nudum*; (p-r) *Psilotum complanatum.*

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Table 4. Spore morphological characteristics of family Ophioglossaceae and Psilotaceae

Species	Spore Color*	Spore Unit	Type	Shape	Polar Diameter Average (P) (µm)	Equatorial Diameter Average (E) (µm)	P/E Ratio	Spore Shaped Based on P/E ratio	Spore Category	Spore Symetry
Helminthostachys zeylanica	*Yellowish white* RHS2015(155B)	Monad	Trilete	Globose	28.59±2.19	32.85±1.74	0.87	Suboblate	Medium	Radial
Botrychium daucifolium	*Pale yellow green* RHS2015(155A)	Monad	Trilete	Globose, Trilobate	31.00±2.49	36.60±2.91	0.85	Suboblate	Medium	Radial
Ophioglossum reticulatum	*Light yellow* RHS2015(160B)	Monad	Trilete	Globose	29.82±1.49	34.62±1.85	0.86	Suboblate	Medium	Radial
Ophioglossum pendulum	*Pale greenish yellow* RHS2015(160C)	Monad	Trilete	Globose	29.68±1.52	37.55±1.71	0.79	Suboblate	Medium	Radial
Psilotum nudum	*Pale yellow green* RHS2015(157C)	Monad	Monolete	Elongate-Ellipsoidal	29.81±2.96	63.31±4.65	0.47	Peroblate	Large	Bilateral
Psilotum complanatum	*Pale yellow green* RHS2015(157C)	Monad	Monolete	Elongate-Ellipsoidal	26.20±2.76	52.98±2.55	0.49	Peroblate	Large	Bilateral

CONCLUSION

The morphological characteristics of Ophioglossaceae ferns at the Bali "Eka Karya" Botanical Garden are various in spore colors for each species, monad spore units, trilete types, mostly have globose shape, the average polar diameter is 28.59±2.19 – 31.00±2.49µm and the equatorial diameter is 32.85±1.74 – 37.55±1.71 µm, P/E ratio 0.79-0.87, the shape based on the P/E ratio is suboblate, medium spore size, and has radial symmetry. The species in Psilotaceae has a pale yellow green spore color, monad spore unit, monolete type, elongate-ellipsoidal shape, average polar diameter 26.20±2.76 – 29.81±2.96 µm and equatorial diameter 52.98±2.55 – 63.31±4.65 µm, P/E ratio 0.47-0.49, the shape based on P/E ratio is peroblate, large spore size, and has bilateral symmetry.

AUTHOR CONTRIBUTION

N.K.R.P. collected the data, analyzed, and wrote the manuscript. W.S.L. supervised the data collection and proofread the manuscript. E.K. proofread the manuscript. I.M.S.W. supervised the process, ana-

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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