The spores structure of ferns growing in mountain Gumitir coffee plantation area Jember Regency

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Abstract. Ferns spores diverse in their characteristics such as shapes, types and sizes. These characteristics are commonly used for species identification instead of the roots, stems and leaves. People can use the spores characteristics to identify the ferns species. Therefore, there is a need to characterize the structure of ferns spores growing in mountain Gumitir coffee plantation area. The whole mount method was used for spore preparations. Parameters observed were the type, size, shape and ornamentation of the exine of spores. The results showed the spore characters of ten ferns species. *Pityrogramma calomelanos* (L.) Link. *Nephrolepis falcata* (Cav.) C. Chr, *Pyrrosia nummularifolia* (Sw.) Ching and *Pyrrosia penangiana* (Hook.) Holtt had large spores size (51-100 µm), while the others had medium-sized spores (26-50 µm). Monolete spore type was found in nine species and trilete spore type was found only in *Pityrogramma calomelanos* (L.) Link. Subspheroidal spore forms was found in six species, while oblate spore form was found in four species. Exine ornamentation pattern varied from rugulate (3 species), baculate (2 species), scabrate, faveolate, gemmate, verrucate and echinate (the last five only in one species) with (P/E) value 0.64 - 0.97.

Keywords: coffee plantation, fern plants, mountain Gumitir, spores, structure

1. Introduction

Indonesia is a tropical country that has a variety of plant and fauna diversity. One of the plant richness is ferns estimated about 2197 species [1]. Fern is a group of pioneering plants that grows in many habitat in tropical area. Fern also plays important role in developing forest structure. Based on its structure, fern is grouped into Cormophyta, a division of comprising all plants that their root, stem, and leaves can be discriminate clearly [2].

Ferns reproduce via spores as vegetative reproduction, due to their lack of seeds. There are two types of ferns based on their spore shapes, i.e. homospory and heterospory [2]. Homospory ferns produce only a single size spore, but heterospory ferns produce dimorphic spore: microspore dan macropore [2].

According to Suntoro [3], spore has some unique characters. The characters are type, size, structure, and shape used as key character for ferns identification [4,5,6]. Ferns has 2 type of spores, i.e. monolete and trilete [7,8]. They are characterized by the presence of thin structure like aperture as patch that coming from the tetrad development. Spore monolete has a single row of elip form and radial chimetric coming from the braking part of spore mother cell division in the axis [6]. Spore
trilete, coming from megaspore tetrad that develop further but still have a connection in their tip. Then this part remains exist and create three line on the spore surface [9]. Spore trilete is the most common ferns spore and the monolete is more primitive [10].

Spore structure supported by two cell walls, exine (outside) and intine (inside). In some spore, their exine is covered by perispore. Ornamentation at perispore also varied in each species, i.e. spin, warf, or like a wing [11].

Exine ornamentation pattern of ferns spores is grouped in to 13 types i.e. Baculate, Clavate, Echinate, Foveolate, Gemmata, Perforate, Pilate, Psilate, Reticulate, Scabrate, Rugulate, Striata dan Verrucate [12,13].The Baculate have stick shape, 1 µm long. Clavate type have stalk shape, narrow at base and the length value is bigger than the wide. Echinate type has spine form. Foveolate type has has a 1 µm hole in exine. Gemmata type has isodiametric shape and size less than 1 µm. Perforate type has less than 1 µm. Pilate type with exine shape ornamentation with holes, stalk shape with bigger apical. Psilate type have smooth, flat ornamentation. Reticulate type has netted ornamentation. Rugulate type has irregular pattern ornamentation. Scabrate type has isodiametric shape and size less than 1 µm. Striata type has elongate horizontal ornamental. Verrucate type has isodiametric shape and longer than 1 µm [13]. This ornamentation on cell wall is used as main characterization in plant taxonomy [10].Based on the size, spore is grouped into six cluster, i.e. very small (<10 µm), small (10-25 µm), middle size (25-50 µm), big (50-100 µm), very big (100-200 µm) and mega size (>200 µm) [9].

Spores disperse by wind and need high moisture habitat for growing. The water presence is the main factor for supporting spore germination, gamete fertilization, and ferns development [14]. One area in East Java that has high humidity is in the Gumitir mountain coffee plantation area of Jember Regency. The Gumitir Mountain area is located on the border between Jember Regency and Banyuwangi Regency, more precisely between Silo District and Kalibaru District [15]. Mount Gumitir is geographically located at 8°15’01.37" S and 113°55’23.33" T with an altitude of 440-625 meters above sea level [15].

Exploration and research on fern plant spores in the Gumitir Mountain area have never been done before. Therefore, the aim of this study was to characterize the structure of ferns spores from mountain Gumitir coffee plantation area. Research on the characteristics of fern spores, including the type, size, structure and shape is important. This is because spores are a breeding tool in the Pteridophyta group of plants and spores also play an important role as a basis for identification of fern species.

2. Materials and Methods

2.1. Materials
The tools used in this study consisted of: herbarium press equipment, GPS (Global Positioning System) Garmin etrex 10, knives, metlin, eppendorf tubes, HERAEUS centrifugators, falkon tubes, vial tubes, beaker glass (50 mL, 100 mL and 500 mL), object glass, cover glass, pipette, waterbath, BINDER oven, needle, binocular microscope, stereo microscope, OLYMPUS CX-21 optic, gloves, tissue, lux meter IHOKI 3421, THM (Thermo Higro Meter) V&A VA8010, and soil tester DEMETRA.

The materials used in this study consisted of: aquadest, label paper, newsprint, acid free paper, 70% alcohol, fern polish, 45% glacial acetic acid, glycerin, a mixture of 45% glacial acetic acid and concentrated sulfuric acid in a ratio of 9:1 and safranin.

2.2 Sample Collection
Sample collection was from March to June 2018 at pine forest area in mountain Gumitir coffee plantation area, Jember Regency. Sample was collected through the area of 5 km tracking route. Ferns morphological character was recorded i.e. species name, growing form, root, stem, leaves, sorus size, and sorus position.
2.3 Fern Identification
Ferns identification based on their morphological characteristic. As reference we use some book for identification i.e. Van Steenis [16], Andrews [17], Holttum [18], de Winter and Amoroso [10], Cobb et al. [19]. All Ferns specimens was stored at Herbarium Jemberiense. We validate our Ferns identification to Pusat Penelitian Lembaga Ilmu Pengetahuan Indonesia (LIPI), Purwodadi Pasuruan.

2.4 Spore Analysis
Spores slide was prepared by Whole mount method [20], in four steps: acetalysis, cleaning, staining, and mounting. In acetalysis step, the structure of spore cell wall was lysed. Spores in 1 mL glacial acetic acid was incubated for 24 hours, then centrifugate 2000 rpm for 30 minutes. The remain pellet then diluted to glacial acetic acid and sulfuric acid (9:1) and heated in boiling water in waterbath for 10 minutes. Second centrifugation was performed at 2000 rpm for 30 minutes (3 times).

Washing collected spore samples is done using aquadest. Aquadest 1mL was added to the centrifuge tube containing spore samples and then centrifuged at 3000 rpm for 10 minutes. Washing the spore sample is done three times. Next step was staining by using safranin for 90 minutes and then washed by aquadest 2 times. The last step was mounting with glyserin. The spore slides were observed by microscope 400 times illumination. The main characters were observed: size, shape, exine ornamentation pattern, and the presence of aperture.

3. Result and Discussion

3.1. Fern Species
Total ten fern species were identified from Gumitir mountain coffee plantation area of Jember Regency, representing 7 families (Table 1). Of the seven families found, the Aspidiaceae, Nephrolepidaceae and Polypodiaceae each have two species members and four other families each with one Species.

Based on the habitat preference, almost ferns in Gumitir coffee plantation were terestrial ferns (6 species) and only 4 epiphyte species; Asplenium nidus, Nephrolepis falcata (Cav.) C. Chr., Pyrrosia nummularifolia (Sw.) Ching and Pyrrosia penangiana (Hook.) Holtt.

Table 1. List of Fern Species

| No | Family            | Species                                      |
|----|------------------|----------------------------------------------|
| 1  | Aspidiaceae      | 1. Tectaria devexa (Kze) Kopel.              |
|    |                  | 2. Tectaria variolosa (Wall.) C. Chr.        |
| 2  | Aspleniaceae     | 3. Asplenium nidus L.*                       |
| 3  | Dennstaedtiaceae | 4. Microlepia speluncae (L.) Moore           |
| 4  | Dryopteridaceae  | 5. Stenosemia aurita (Sw.) C. Presl         |
| 5  | Hemionitinaceae  | 6. Pityrogramma calomelanos (L.) Link        |
| 6  | Nephrolepidaceae | 7. Nephrolepis falcata (Cav.) C. Chr.*      |
|    |                  | 8. Nephrolepis radicans (Burm.) Kuhn         |
| 7  | Polypodiaceae    | 9. Pyrrosia nummularifolia (Sw.) Ching*      |
|    |                  | 10. Pyrrosia penangiana (Hook.) Holtt*       |

* Epiphyte Habitat
3.2. Spore Description

Two types of spores had been observed from all fern specimen i.e. trilete and monolete (Table 2). The trilete spore is characterized by three radial lines from the center pole that have in contact before. The monolete only has one line that indicating the axis came from the spiting of mother spore in to four along vertical axis [12]. The trilete spore only found at *P. calomelanos*.

| No | Species                  | Spore Type |
|----|--------------------------|------------|
| 1  | *Tectaria devexa* (Kze) Kopel. | Monolete   |
| 2  | *Tectaria variolosa* (Wall.) C. Chr. | Monolete   |
| 3  | *Asplenium nidus* L       | Monolete   |
| 4  | *Microlepia speluncae* (L.) Moore | Monolete   |
| 5  | *Stenosemia aurita* (Sw.) C. Presl. | Monolete   |
| 6  | *Pityrogramma calomelanos* (L.) Link | Trilete    |
| 7  | *Nephrolepis falcata* (Cav.) C. Chr | Monolete   |
| 8  | *Nephrolepis radicans* (Burm.) Kuhn | Monolete   |
| 9  | *Pyrrosia nummularifolia* (Sw.) Ching | Monolete   |
| 10 | *Pyrrosia penangiana* (Hook.) Holtt. | Monolete   |

The spores shape were *oblate* and subspheroidal (Table 3). These shape determination was based on the value of ration P/E (Polar/Equatorial) length. Punt et al. (2007), described spore shape in 5 type: perprolate, prolate, subspheroidal, oblate and peroblate. Perprolate spore has P/E >2.0 µm, prolate 1.34 µm – 2.0 µm, subspheroidal 0.76 – 1.33 µm, oblate 0.6 – 0.75 µm, and peroblate <0.5 µm.

The oblate were found from 6 species with P/E value varied from 0.64-0.97 and the smallest P/E was from *Nephrolepis radicans* (Burm.) Kuhn. The subspheroidal were found from 4 species with P/E 0.69-0.90 and the biggest P/E was from *Pityrogramma calomelanos* (L.) Link (Table 3).

| No | Species                  | Spore Shape | P/E  |
|----|--------------------------|-------------|------|
| 1  | *T. devexa* (Kze) Kopel. | Subspheroidal | 0.90 |
| 2  | *T. variolosa* (Wall.) C. Chr | Subspheroidal | 0.83 |
| 3  | *A. nidus* L             | Subspheroidal | 0.80 |
| 4  | *M. speluncae* (L.) Moore | Oblate      | 0.76 |
| 5  | *S. aurita* (Sw.) C. Presl. | Oblate      | 0.76 |
| 6  | *P. calomelanos* (L.) Link | Oblate      | 0.97 |
| 7  | *N. falcata* (Cav.) C. Chr | Oblate      | 0.76 |
| 8  | *N. radicans* (Burm.) Kuhn | Oblate      | 0.64 |
| 9  | *P. nummularifolia* (Sw.) Ching | Subspheroidal | 0.69 |
10. *P. penangiana* (Hook.) Holtt. | Oblate | 0.77

Exine ornamentation pattern is one character for fern identification [12]. Our result indicating that total 7 exine ornamentation pattern type were observed from 10 fern i.e. *rugulate*, *scabrate*, *faveolate*, *baculate*, *gemmate*, *verrucate* and *echinate* (Table 4). The most common exine ornamentation pattern *rugulate*, was found in three species. While the type i.e. *baculate* only in 2 species and *scabrate*, *faveolate*, *gemmate*, *verrucate* and *echinate* was only in one species. (Table 4). Ornamentation of the exine *rugulate* is usually the horizontal surface of the exin-shaped horizontally [13].Ornamentation of the type of exine *rugulate*, among others, was found in *Tectaria de vexa* (Kze) Kopel. and *Pityrogramma calomelanos* (L.) Link.

The *verrucate* (found at *P. nummularifolia* (Sw.) Ching) showed isodiametric pattern, length more than 1 µm, and had no shrink at the base. *Baculate* type is found in *Microlepia speluncae* and *Stenosemia aurita* with ornamentation on the rod-shaped exine surface, the height is more than 1 µm and larger than the width [13]. The *faveolate* was found only at *Asplenium nidus* has 1 µm hole size. *Scabrate* type is found in *Tectaria variolosa* while *gemmate* in *Nephrolepis falcata*. The *Verrucate* type is found in *Pyrrosia nummularifolia*.

| No | Species | Exine Ornamentation |
|----|---------|---------------------|
| 1  | *T. de vexa* (Kze) Kopel. | Rugulate |
| 2  | *T. variolosa* (Wall.) C. Chr | Scabrate |
| 3  | *A. nidus* L | Faveolate |
| 4  | *M. speluncae* (L.) Moore | Baculate |
| 5  | *S. aurita* (Sw.) C. Presl. | Baculate |
| 6  | *P. calomelanos* (L.) Link | Rugulate |
| 7  | *N. falcata* (Cav.) C. Chr | Gemmate |
| 8  | *N. radicans* (Burm.) Kuhn | Rugulate |
| 9  | *P. nummularifolia* (Sw.) Ching | Verrucate |
| 10 | *P. penangiana* (Hook.) Holtt | Echinate |

All spores characters were described below:

1. *Tectaria de vexa* (Kze) Kopel
Terrestrial fern, *monolete* spore type, *subspheroidal* shape, polar 41.5 µm, equatorial 46.1 µm, P/E 0.90, medium size spore, *rugulate* exine ornamentation (Figure 1).
2. *Tectaria variolosa* (Wall.) C. Chr.
Terrestrial fern, *monolete* spore type, *subspheroidal* shape, polar 46.5 µm, equatorial 38.9 µm, P/E 0.83, medium size spore, *scabrate* exine ornamentation (Figure 2).

3. *Asplenium nidus* L.
Epiphyte fern, spore *monolete* spore type, *subspheroidal* shape, polar 30.9 µm, equatorial 38.6 µm, P/E 0.80, medium size spore, *fâveolate* exine ornamentation (Figure 3).
Figure 3. *Asplenium nidus* L.: Habitus (a), leaf (b), root (c), sori (d) and exine shape (e)

4. *Microlepia speluncae* (L.) Moore
Terrestrial fern, spore *monolete* spore type, *oblate* shape, polar 36.0 µm, equatorial 27.5 µm, P/E 0.76 medium size spore, *baculate* exine ornamentation (Figure 4).

Figure 4. *Microlepia speluncae* (L.) Moore: Habitus (a), leaf (b), root (c), sori (d) and exine shape (e)

5. *Stenosemia aurita* (Sw.) C. Presl.
Terrestrial fern, *monolete* spore type, *oblate* shape, polar 33.1 µm, equatorial 43.2 µm, P/E 0.76 medium size spore, *baculate* exine ornamentation (Figure 5).
6. *Pityrogramma calomelanos* (L.) Link
Terrestrial ferns, *trilete* spore type, *oblate* shape, polar 50.8 µm, equatorial 51.9 µm, P/E 0.97 big size spore, *rugulate* exine ornamentation (Figure 6).

7. *Nephrolepis falcata* (Cav.) C. Chr
Epiphyte fern, *monolete* spore type, *oblate* shape, polar 62.4 µm, equatorial 47.8 µm, P/E 0.76 big size spore, *gemmate* exine ornamentation (Figure 7).
Figure 7. *Nephrolepis falcata* (Cav.) C. Chr: Habitus (a), leaf (b), root (c), sori (d) and exine shape (e)

8. *Nephrolepis radicans* (Burm.) Kuhn
Terrestrial fern, *monolete* spore type, *oblate* shape, polar 28.9 µm, equatorial 44.9 µm, P/E 0.64 medium size spore, *rugulate* exine ornamentation (Figure 8).

Figure 8. *Nephrolepis radicans* (Burm.) Kuhn: Habitus (a), leaf (b), root (c), sori (d) and exine shape (e)

9. *Pyrrosia nummularifolia* (Sw.) Ching
Epiphyte fern, *monolete* spore type, *subospheroidal* shape, polar 68.7 µm, equatorial 47.8 µm, P/E 0.69 big size spore, *verrucate* exine ornamentation (Figure 9).
Figure 9. *Pyrrosia nummularifolia* (Sw.) Ching: Habitus (a), leaf (b), root (c), sori (d) and exine shape (e)

10. *Pyrrosia penangiana* (Hook.) Holtt. Epiphyte fern, *monolete* spore type, *oblate*, polar 67.9 µm, equatorial 52.5 µm, P/E 0.77 big size spore, *echinate* exine ornamentation(Figure 10).

Figure 10. *Pyrrosia penangiana* (Hook.) Holtt.: Habitus (a), leaf (b), root (c), sori (d) and exine shape (e)

4. Conclusion
Ten fern species were found with two type spores monolete and trilete. Spore types were mainly monolete (9 species) and only one trilete (*P.calomelanos*). Based on the spore size, there were big size and medium size. Big size spores were found in 4 types of ferns namely *P. Calomelanos, N. falcata, P. nummularifolia* and *P. penangiana*, while 6 other types had spores of medium size. The spore shape
mainly subspheroidal (4 species) and oblate (6 species). Exine ornamentation, there were rugulate, it found in 3 species, baculate two species and the others were faveolate, scabrate, gemmate, verrucate and echinate with polar/equatorial score (P/E) 0.64 – 0.97.

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