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Biology Department, Faculty of Mathematics and Natural Sciences, University of Jember

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Integrated Biological Sciences for Human Welfare

PROCEEDINGS

The Panorama Hotel and Resort Jember
East Java, Indonesia
August 7 - 8, 2017

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THE 2nd INTERNATIONAL CONFERENCE ON LIFE SCIENCES AND BIOTECHNOLOGY (ICOLIB): INTEGRATED BIOLOGICAL SCIENCES FOR HUMAN WELFARE

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CHARACTERIZATION OF TERRESTRIAL SPORES FERN PLANTS FROM WILDLIFE HIGHLAND "YANG" THE ARGOPURO MOUNTAIN

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Abstract

This study was conducted to determine the spore characteristics of terrestrial fern plants. It covered in highland “Yang" wildlife moss forest, Argopuro Mountains. The specimens of terrestrial fern collections were carried out by a structured random method along 2 km mossforest climbing route that was divided into 20 points. In each pointwas 100 meters away then made a rectangular plot with a length 15 meters and 6 meters wide. In each plot measurements of abiotic data such as temperature, humidity, light intensity, geospatial location and site altitude. The spores obtained were prepared by using acetolysis, then it observed under a microscope with magnification 400 times. Spore parameters were type, shape, size, and exinornamentation. Twelve kinds of terrestrial fern plants with 2 types of spores were trilete and monolete. The trilete spore was found in a single fern plant of *Pteristripita*. While monolete type were found in *Aspleniums colopendrium, Asplenium excisum, Asplenium normale, Blechnum nudum, Lastreopsis rufescens, Lastreopsis munita, Lastreopsiss mithiana, Lastreop sisgrayi, Sticherus lobatus, Diplazium pallidum and Athyrium mearnsianum*. The spores form observed were suboblasts (1 species), prolate (3 types), peroblics (2 types), oblate (1 type) and subspheroidal (5 types) with 5 exine ornamentation forms were psilate, verrucate, scabrate, echinate and regulate. This study that characteristics spore of fern plants in moss forest, Argopuro Mountains for better understanding the key characters from morphological features of spore fern plant.

Keywords: characterization of spore, terrestrial fern plants, Argopuro Mountains.

1. Introduction

Pteridophyteis one of flora diversity in Indonesia. It has a vessel system (kormophyhte). The spore as generative propagation tools instead of seed. Fern plants hadmetagenesis phase between gametophyte phase and sporophyte phase. Spore is an early development the gametophyte phases of fern plants [1]

Fern plants produce 2 kinds of spores, homospores or heterospores. In heterosporaproduce two types of spores, namely macrospores and microspore, whereas in the homospores produce only one type of spore. The spores of fern plants are composed of a thick outer part called an exine, and a thin inner part called an intin. According to Kapp [2] spore fern plants is divided into 2 types namely monolete and trilete. The difference between each type of spores is based on the presence or absence of a thin structure that resembles the aperture of tetrad spore scars.

Fern plants are pioneer plants in every type of forest area and it plays an important role for compiling forest ecosystems. Fern plants mostly live in a high humidity levels such in highland forests. This relates to the adaptation of epiphytic and terrestrial fern plants requiring the presence of water to maintain its viability and as a medium for sperm transfer during fertilization (Loveless, 1999).

One of highland in East Java is the Argopuro Mountains. It has a peak with an altitude of 3088 meters above sea level. One of the conservation areas is a moss forest it located in the altitude range of 1000-2000 meters above sea level which it located in the "Highlands Yang" Wildlife. Argopuro Mountains is located in 4 districts in East Java: Probolinggo, Jember, Bondowoso, and

Situbondo (Bksdajatim, 2012). Based on preliminary survey on 12thFebruary 2015 in themoss forest area are found many moss plants and fern plants, because both of plants require habitat has a high humidity, enough sunlight, and water availability.

Fern plant exploration and research, especially research on spores in the Argopuro Mountains region has never been done. In addition, the spores of each fern plant division have variations of shape and size [3] and the characteristics of each spore can be basis for distinguishing each species of fern plant. Therefore this study conducted to determine the characteristics of spores terrestrial fern plants in the Highland "Yang" Lichen Forest Argopuro Mountains are very important.

2. Materials and Methods

The tools used in this research are herbarium press tool, GPS (Global Positioning System), knife, microcentrifuge tube, centrifugator, falcon tube, vial tube, beaker glass (50 mL, 100 mL, and 500 mL), OptiLab, plastic cup, measuring cup (10 mL, 100 mL, and 500 mL), object glass, cover glass, waterbath, binocular microscope, stereo microscope, pipette, luxmeter, hygrometer, thermometer, and altimeter.

The materials used in this study were mature spores from terrestrial fern plants obtained in the Argopuro Mountains, aquadest, 70% alcohol, fern polish, 45% glacial acetic acid, glycerin, 45% glacial acetic acid mixture and concentrated sulfuric acid 9: 1 ratio, and safranin.

Research procedure

The Sampling was conducted by a randomly structured method around2km along moss forest tracking. The location were 20 points with each point was 100 meters. From each point, 15 meter straight line is drawn in the forest and then 6 meter transverse line is formed therefore a rectangular plot of 15 meters long and 6 meters wide is formed (Fig 1).

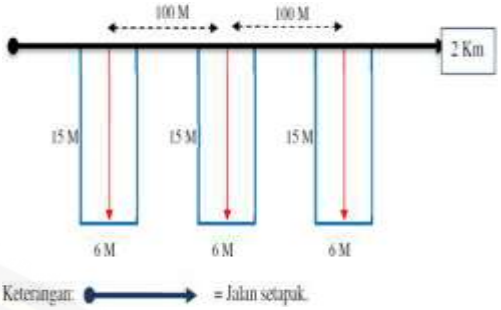

Figure1. Plot design

Sample collections of terrestrial fern plants obtained in each plot were put into large plastic bags for making herbariums. In each plot were mounted abiotic data such as temperature, humidity, light intensity, geospatial location, and height of research place. Each sample of the fern plants was labeled, morphologically characteristic, including the type, shape, color, and position of sorus to the leaf. It purposedfor identifying the species.

The Samples that mature spores (sori) were collected directly from terrestrial fern plants by scraping the sorus with a toothpick and then collected in a small plastic. The mature Soruswere characterized by a blackish brown sorus color and broke. The Identification of fern plants based on the morphological characteristics include shape and color of stem, branching stems, shape and color of leaves, shape of leaf bone, leaf margin, size and sorus location, the shape of the indigenous, the shape of the scales, and the paraphysis [4]. The morphological characteristics result compared with literatures [5], [6], (Winter and Amoroso, 2003a), and collected it to be Jemberiense Herbarium collections.

Preparation of Spore preservation

Preparation of spore preservation based on [7]. Spore preserved without incision (whole mount). The steps of spore preservation were acetolysis, cleaning, staining and mounting (attachment).

a. Acetolysis

The spores put into microcentrifuge tube were lyzed using 1 mL 24 hour glacial acetic acid then centrifuged at 2000 rpm for 30 minutes. The pellet added with a mixture of glacial acetic acid and 1 Ml sulfuric acid with a ratio of 9: 1 while warming it in the

waterbath for 10 minutes. Sample centrifuged at 2000 rpm for 30 minutes then removed the supernatan.

b. Cleaning

The pellet was added by aquadestthen centrifuged at 3000 rpm for 10 minutes three times. It purposed to clean pollen free with chemical substances such as the fixative substances in the spores to be prepared [7].

c. Staining

This step aims to clarify the spore wall ornament shape and facilitate the measurement of spores. This step was used safranin which has strong chloride and alkaline.Safranindissolved with aquadest for about 90 minutes. Spore stained with safranin for 1- 2 minutes. It washed with aquadest twice to remove the remaining dye. This repetition is necessary until the spore is good quality [7].

d. Mounting

Mounting was done by addition of glycerin which has been heated and stirred evenly. Glycerin dripped on the top of glass and trimmed it then spores dripped on top of the object glass and then closed with a cover glass and the edges smeared with fern polish. During the mounting process is kept in order not toformed air bubbles. The last preparations were dried and labeled.

Observation and Determination of Spores

Spore preservation observed under a microscope used 400 times magnification. Spore parameters were spores, size, exine ornamentation, and the presence or absence of thin structures resembling the aperture of tetrad scar on the spores.

This observation determined by comparing the observed features with several literatures such as [2] and Jones (1979).

3. Results and Discussion

1. *A. scolopendrium* Lour.

The spore was monolete, suboblaced, had a polar side length of 36.8 µm, Equatorial side 60.6 µm, and a ratio of P / E 0.83 µm, Exine ornamentation psilate shaped was 4.9 µm. The ornamentation element had a smooth surface that bulging on apical (Figure 2).

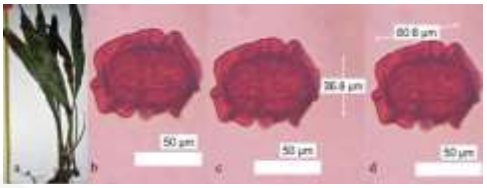

Figure 2. A. *Scolopendrium* Lour. A. Habitus; B. Spore forming; C. Polar side; D. Equatorial side

2. *A. excisum* C.Presl

Monolete spore type, peroblated, had a polar side length of 22.5 µm, Equatorial side 47.1 µm, and had ratio of P / E 0.47 µm, exine ornamentationechinate-shaped was 2.5 µm (Figure 3).

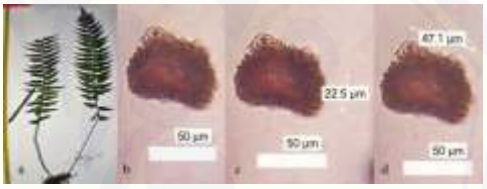

Figure 3. A. *Excisum* C. Presl a. Habitus; B. Spore form; C. Polar side; D. Equatorial side

3. *A. normale* D.Don

Monolete spore type, oblate-shaped, had a polar side length 25.7 µm, Equatorial side 44.80 µm, and had ratio of P / E 0.57 µm, exine ornamentation verrucate-shaped was 4.5 µm. The ornamentation element was Isodiametric shaped (Figure 4).

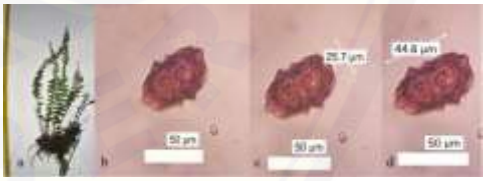

Figure 4 A. *Normale* D.Don a. Habitus; B. Spore form; C. Polar side; D. Equatorial side

4. *B. nudum* (Labill.) Wakef.

Monolete spore, subspheroidal, had a polar side length of 45.8 µm, Equatorial side 35.3 µm, and had ratio of P / E 1.29 µm, the exine ornamentation rugulate-shaped had 7.9 µm and Isodiametric (Fig. 5).

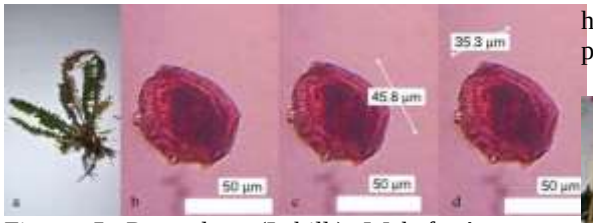

Figure 5 *B. nudum* (Labill.) Wakef. A. Habitus; B. Spore form; C. Polar side; D. Equatorial side.

5. *L. rufescens*(Blume) Ching

Monolete spores, prolate-shaped, had 60.5 µm polar side lengths, Equatorial side 33.6 µm, and had ratio of P / E 1.8 µm, Oxine ornamentation verrucate form measuring 2.9 µm. Ornamentation element is isodiametric shape (Figure 6).

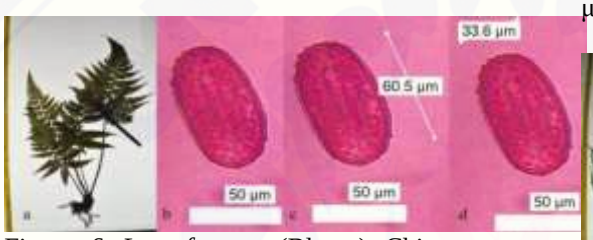

Figure 6 *L. rufescens* (Blume) Ching a. Habitus; B. Spore form; C. Side Polar; D. Equatorial side

6. *L. munita*(Mett.) Tindale

Monolete spore type, prolate-shaped, had 55.7 µm polar side, Equatorial side size 42.0 µm, and had ratio of P / E 1.32 µm, the exine ornamentation psilate shaped 5.1 µm. Ornamentation element is isodiametric shape (Figure 7).

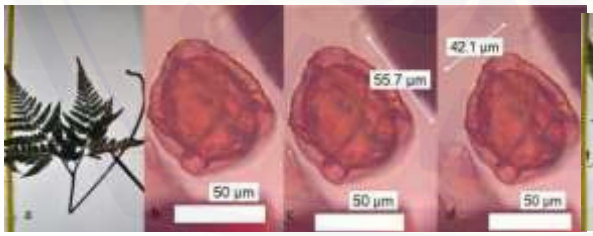

Figure 7 *L. munita* (Mett.) Tindale a. Habitus; B. Spore form; C. Polar side; D. Equatorial side

7. *L. smithiana* Tindale

Monolete spore type, subspheroidal shaped, had a polar side length of 55.6 µm, 38.6 µm equatorial side, and had ratio of P / E 1.44 µm, Oxine ornamentation psilate shaped 8.9 µm. The ornamentation element had smooth surfaces and inflated on apical parts (Figure 8).

Figure 8 *L. Smithiana* Tindale a. Habitus; B. Spore form; C. Polar side; D. Equatorial side

8. *L. grayi* D.L.Jones

Monolete spore type, subspheroidal, had a polarized side length of 28.6 µm, Equatorial side 52.6 µm, and had ratio of P / E 0.53 µm, Oxine ornamentation 2.7 µmrugulate shaped (Figure. 9).

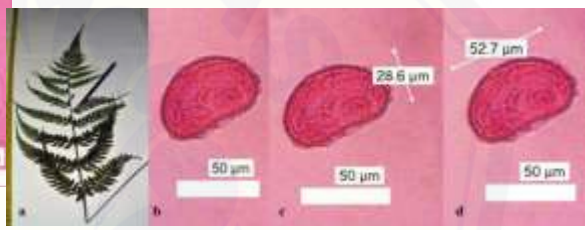

Figure 9 *L. Grayi* D.L.Jones a. Habitus; B. Spore form; C. Polar side; D. Equatorial side

9. *S. lobatus* N.A.Wakef.

Monolete spore type, peroblated, had a polar side length of 17.5 µm, equatorial side 39.3 µm, and had ratio of P / E 0.44 µm, the exine ornamentation psilate-shaped length 0.9 µm. Ornamentation element is isodiametric shape (Figure 10).

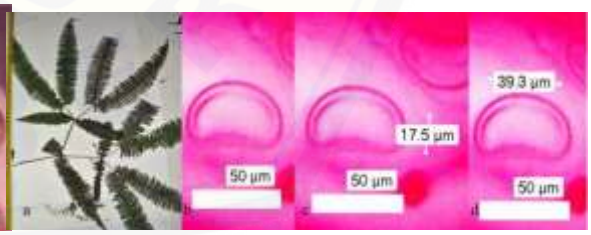

Figure 10 S. *lobatus*N.A.Wakef. A. Habitus; B. Spore form; C. Polar side; D. Equatorial side

10. *D. pallidum* (Blume) T.Moore

Monolete spore type, prolate form, had a polar side length 60.9 µm, Equatorial side 36.9 µm, and hadratio of P / E 0.61 µm, exine ornamentation Psilate-shaped 5.4 µm. The ornamentation element had a surface as

smooth as the wings and inflatable on the apical part (Figure 11).

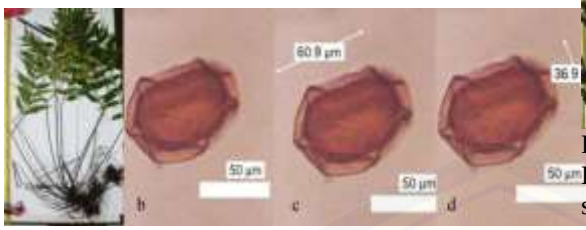

Figure 11 *D. Pallidum* (Blume) T.Moore A. Habitus; B. Spore form; C. Polar side; D. Equatorial side

11. *A. mearnsianum*(Copel.) Aldrew.

Monolete spore type, subspheroidal, had a polar side length 52.9 µm, 42.1 µm equatorial side, and had ratio of P / E ratio of 1.25 µm, the exine ornamentation is a verrucate 5.4 µm. The Ornamentation element isodiametric shape (Figure. 12).

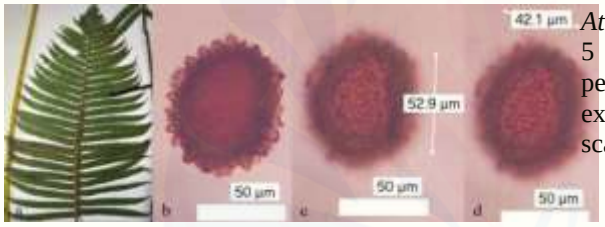

Figure 12 A. mearnsianum (Copel.) Aldrew. A. Habitus; B. Spore form; C. Polar side ; D. Equatorial side

12. *P. tripartita* Swartz.

Trilete spore type, subspheroidal, had a polar side length is 74.1 µm, the equatorial side is 78.5 µm, and had ratio of P / E 0.94 µm, exine ornamentation psilate-shaped is 12.8 µm. The ornamentation element had smooth surface and bulge on the apical (Figure. 13).

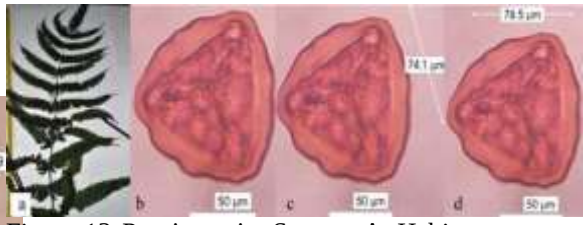

Figure 13 *P. tripaartita* Swartz. A. Habitus; B. Spore form; C. Polar side; D. Equatorial side

4. CONCLUSION

In moss forest Argopuro mountain found 12 types of terrestrial fern plants with 2 types of spores such as trilete and monolete. Trilete spores were found in *Pteristripartita*. Monolete spores were found in 11 species such as *Aspleniumscolopendrium*, *Aspleniumexcisum*, *Aspleniumnormale*, *Blechnumnudum*, *Lastreopsisrufescens*, *Lastreopsismunita*, *Lastreopsissmithiana*, *Lastreopsisgrayi*, *Sticheruslobatus*, *Diplaziumpallidum* and *Athyriummearnsianum*. The spore form were 5 kind namely suboblasts, prolles, peroblasts, oblasts and subspheroidal. The exineornamentation werepsilate, verrucate, scabarate, echinate and regulate.

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