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Gametophyte Morphology and Development of Six Species of *Pteris* (Pteridaceae) from Java Island Indonesia

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ABSTRACT

The morphology of sporophyte, the type of reproduction, and cytology of *Pteris* had been reported, while the gametophyte morphology of *Pteris* in Java island has not been studied yet. The objective of this study was to describe the gametophyte morphology and development of *P. biaurita, P. ensiformis, P. exelsa, P. longipinnula, P. tripartita*, and *P. vittata* in Java island. Spores were obtained from fertile leaves of *Pteris* plants originated from several locations in Java island. The number of spores per sporangium was counted from fresh fertile leaves with mature sporangia. As much as 0.002 g spores was sown in a transparent box with sterile medium contain of vermiculite, sphagnum moss, and perlite with ratio 2:2:1. The gametophyte development of each species was observed under a microscope every 7 days. The spores of *P. ensiformis* were germinated faster, ten days after sowing, while the spores of *P. longipinnula* were germinated slower, 18 days after sowing. The pattern of spore germination is *Vittaria*-type. The development of gametophyte is *Ceratopteris*-type in common, but in a few cases is the *Adiantum*-type. The gametophyte development of observed *Pteris* species is varied in six characters including the number of filament cell, germinated time, the formation time of notch and gametangia, margin shape, and development type.

Keywords: *development, gametophyte, Java, morphology, Pteris*

INTRODUCTION

Pteris L. is a large fern genus belong to the family Pteridaceae, that are distributed in tropical and subtropical regions [1]. It is estimated about 250 *Pteris* species in the world [1,2] and 19 species of them distributed in Java island, Indonesia [3]. The species of *Pteris* grow in warmer temperature areas, but they also found in cold temperatures. They grow either terrestrially or lithophytically on rocks in several habitats, shaded canopy, and open areas, forests, coastal, and xeric niches [1,4]. Some species also survive in soil contaminated arsenic or other metals [5].

The *Pteris* species are easily distinguished from their sori characters. Their sori are linear and located in marginal of leaves, but are usually not reaching the apices of segments. A false indusium protected each sorus [1,6].

Several studies on gametophyte morphology of *Pteris* have been done. The gametophyte development of several *Pteris* species including, *P. vittata, P. finotii, P. fauriei, P. exelsa, P. wallichiana, P. ensiformis, P. cretica, P. multifida, P. deflexa, P. denticulata, P. tristicula, P. faurirei, P. incompleta, P. berteroana, P. chilensis,* and *P. tripartita* were reported having some unique characters [7-13]. The similarity of these species was on spore germination, the development gametophyte, and gametangia type. Among these species are differed in some gametophytic characters such as the number cell of the filament, germinated time, the formation time of notch, the formation time of gametangia and type of gametophyte development.

Pteris is taxonomically very interesting taxon since it is a species complex [14]. The morphology of the sporophyte, the type of reproduction and cytology of some *Pteris* species in Java island such as *P. biaurita,*

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Table 1. Collection site of observed *Pteris*

No	Species	Number Collection	Location
1	*P. biaurita*	DSP 050	Krikilan, Sangiran, Sragen, Center Java
2	*P. ensiformis*	DSP 035	Agatis Street, IPB Dramaga, Bogor, West Java
3	*P. exelsa*	DSP 045	Cibodas Botanical Garden, Cianjur, West Java
4	*P.longipinnula*	DSP 037	Situgedhe, Dramaga, Bogor, West Java
5	*P. tripartita*	DSP 021	Tanglebun, Ciomas, Bogor, West Java
6	*P. vittata*	DSP 051	Mampang prapatan XV, Mampang, South Jakarta

P.ensiformis, P. multifida, P. vittata, and *P. tripartita*, were reported by several authors [15-19], while the gametophyte morphology of *Pteris* has not been studied before. The information on gametophyte phase is important to study the evolution, phylogeny and reproductive biology of ferns [20,21]. Therefore, it is necessary to examine the development and morphology of fern gametophyte, especially on the genus *Pteris*.

The purpose of this study is to describe the gametophyte morphology and development of six species of Javanese *Pteris*, *P. biaurita, P. ensiformis, P.exelsa, P. longipinnula, P. tripartita*, and *P. vittata*.

MATERIALS AND METHODS

Spores were obtained from fertile leaves of *Pteris* plants originated from several locations in Java island (Table 1). Fertile leaves were put in paper envelopes under the dry condition to release spores from sporangia. After 6-7 days, the contents of the envelopes were sifted to eliminate sporangial fragments and other debris [22].

The number of spores per sporangium was counted from fresh fertile leaves with mature sporangia, and five to 10 sporangia were observed for it [23]. Sporangium with 64 normal spores were treated as sexual reproduction type while the sporangium with 32 spores treated as apogamous one [14].

As much as 0.002 g spores was sown in a transparent box with sterile medium contain of vermiculite, moss and perlite with ratio 4:4:2. Medium were covered with paper filter [24]. Before planting, the medium was soaked in hot water for a day. All cultures were maintained at the temperature ranged from 27-30°C. The gametophyte development of each species was observed under a microscope Olympus Stemi 1000 and Nikon Eclipse E100 every 7 days. The photograph was documented using Optilab Advance.

Data of cell growth was analysis with One-way Analysis of Variance (ANOVA) followed by Duncan test on the α<0.05.

Table 2. Spore size and reproduction type of six *Pteris* species

Species	Equatorial diameter (μm)	Polar length (μm)	The number of spores per sporangium	Reproduction type
P. biaurita	43.0 – 46.2	36.0 – 45.7	32	Apogamous
P. ensiformis	31.3 – 36.8	29.8 – 34.5	64	Sexual
P. exelsa	21.4 – 50.9	17.9 – 46.7	64	Apogamous
P.longipinnula	50.8 – 57.6	44.5 – 50.5	32	Apogamous
P. tripartita	50.2 – 51.6	40.7 – 48.7	64	Sexual
P. vittata	43.7 – 54.3	41.7 – 51.9	32	Apogamous

RESULTS AND DISCUSSION

Reproduction type and spore morphology of Pteris

Among six *Pteris* species that have been observed, two species (*P. ensiformis* and *P. tripartita*) have a sexual reproduction type and four others (*P. biaurita, P. exelsa, P. longipinnula*, and *P. vittata*) have apogamous reproduction type (Table 2).

Pteris exelsa has 64 spores per sporangium but are classified into apogamous type because it has various spore shape and size (Figure 1). The number of 64 spores per sporangium with various shapes and sizes have also been found in *P. vittata* [19]. Apogamous type is affected by the abnormal sporogenesis and sporangium that undergoes the abnormal process, thus, it produces variation in the number of spores, the size and shape of spores per sporangium [25].

Most *P. biaurita* have apogamous reproduction type than sexual types. Previous research reported that among 90 of individuals *P. biaurita* observed, found 78 individuals have an apogamous type, and 12 individuals have mixture type, but the sexual type was never found [15]. Sexual reproduction type in *P. biaurita* are relatively few in Bogor and never found in Taiwan [26,27]. The regulation the reproduction type of fern is influenced by environmental factors such as light, altitude, and temperature of the area [28]. The ethylene gas, succinic acid, naphthalene acetic acid (NAA), gibberellic acid, high phosphorus concentrations, particular wavelengths of light, and drought can induce apogamous type [29].

Sexual type on *Pteris* especially *P. vittata* and *P. ensiformis* were most often found in Java. Among 14 individuals of *P. ensiformis,* 13 individuals were the sexual type, but one individual has apogamous type [18].

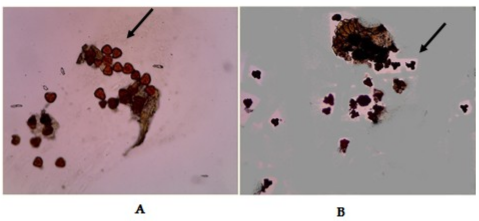

Figure 1. Spores of two fern reproduction types. A. Normal spores of sexual type in *P. tripartita* , B. Abnormal spores of Apogamous type in *P. exelsa*

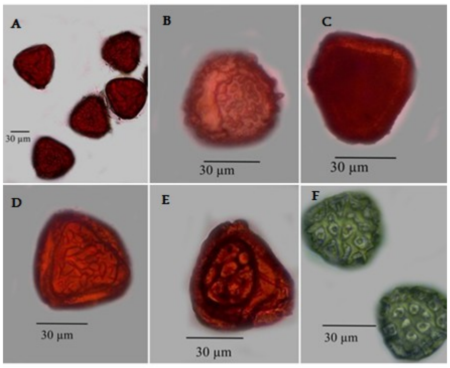

Figure 2. *Pteris* spores. A). *P. biaurita;* B). *P. ensiformis;* C). *P.exelsa;* D). *P. longipinnula;* E). *P. tripartita;* F). *P. vittata*

A previous study reported among 37 individuals of *P. vittata* in Java, 35 individuals have a sexual type, and 2 individuals were apogamous [19].

The spores of all species are trilete (Figure 2), except in *P. exelsa* have various shapes from triangles, rectangles, and ellipses. *Pteris* spore is triangular to rectangular [1]. Spore size, length polar and the equatorial diameter varies among species (Table 2). *Pteris longipinnula* has the biggest spore sizes of the different types. The most size variation spores found in *P. exelsa*. Spore color is also varied, *P. biaurita, P. longipinnula*, and *P. tripartita* have brown spore, *P. ensiformis* has dark brown spore, *P. exelsa* has blackish brown spore, and *P. vittata* has whitish-brown spore.

Gametophyte Morphology and Development of Pteris
Spore germination

The observed *Pteris* spores were germinated in 10 to 18 days after sowing (DAS) (Figure 3). Comparing to other observed *Pteris* spesies, the spores of *P. ensiformis* were germinated faster, 10 DAS, while the spores of *P. longipinnula* were germinated slower, 18 DAS. Spore germination type of all spesies is *Vittaria*

Table 3. Gametophyte development of observed *Pteris* and a previous study [10]

Species	The number of filament cell		The gametophyte development	
	Observation Result	Zhang *et al.* (2008) [10]	Observation Result	Zhang *et al.* (2008) [10]
P. biaurita	5-9	-	*Ceratopteris*	-
P. ensiformis	2-4	2-25	Modification of *Adiantum* and *Ceratopteris*	*Ceratopteris* -
P. exelsa	5-9	2-19	*Ceratopteris*	*Ceratopteris*
P. longipinnula	5-32	-	*Ceratopteris*	-
P. tripartita	3-5	-	*Adiantum*	-
P. vittata	7-9	2-22	*Ceratopteris*	*Ceratopteris*

Table 4. Average of cell number *Pteris* species for 10 week

Species	Week								
	2	3	4	5	6	7	8	9	10
P. biaurita	0.00^{a}	2.07^{a}	10.60^{a}	20.50^{b}	24.33^{a}	53.30^{cd}	62.30^{b}	70.63^{a}	175.03^{b}
P. ensiformis	1.30^{b}	3.10^{a}	11.10^{a}	17.30^{ab}	29.40^{bc}	48.57^{c}	50.17^{a}	72.06^{a}	79.23^{a}
P. exelsa	1.20^{b}	6.03^{b}	11.00^{ab}	17.27^{ab}	20.07^{a}	27.87^{a}	44.97^{a}	58.27^{a}	100.93^{a}
P. longipinnula	1.30^{b}	8.57^{c}	15.80^{cd}	27.67^{c}	32.27^{cd}	35.97^{c}	61.97^{b}	65.50^{a}	117.03^{a}
P. tripartita	1.27^{b}	2.70^{a}	11.00^{a}	15.50^{a}	27.20^{bc}	33.90^{ab}	80.03^{c}	155.57^{c}	161.27^{b}
P. vittata	1.33^{b}	6.10^{b}	17.60^{c}	34.87^{d}	32.27^{d}	58.33^{d}	86.83^{c}	125.30^{b}	232.27^{c}

***different alphabet on each average cell number indicates significant differences based on Duncan test.**

type, with the first cell, emerged from the spores is perpendicular to the first rhizoid [20].

The filamentous stage of each *Pteris* species is varied in the cell number. The filament of *P. ensiformis* has the shortest filament formed of 2-4 cells while *P. longipinnula* had the longest range of filamentous sizes (5-30 cell). *Pteris tripartita* had short filamentous size (2-5 cells), but the filamentous stage of other observed *Pteris* are consisted of 5-9 cells long (Table 3). The filamentous size of Javanese *Pteris*, *P. ensiformis, P. vittata*, and *P. exelsa*, are differed to those described by Zhang et al. (2008) [10], which have wider size variation (Table 3). The differences of this result may be due to Zhang et al. (2008) [10] grew spores in the mixture of black soil and sand.

The growth rate of *Pteris* gametophyte is varied among observed species in every week (Figure 4) and were significantly different among species based on ANOVA and Duncan test (Table 4). The growth rate of *Pteris* gametophyte began to differ among species on the second week, and then the gametophyte growth was continuously differed by the gametophyte age. *Pteris biaurita* did not grow until the second week. *Pteris vittata* showed the fastest growth rate during the fourth until the eighth week. On the eight-week, the growth of *P. ensiformis* gametophyte showed the slowest rate while that of *P. tripartita* showed the fastest. On the ninth week, the growth of *P. longipinnula* gametophyte showed the slowest rate while that of *P. tripartita* showed the fastest. On the tenth week, *P. vittata* grew the fastest while *P. ensiformis* grew the slowest.

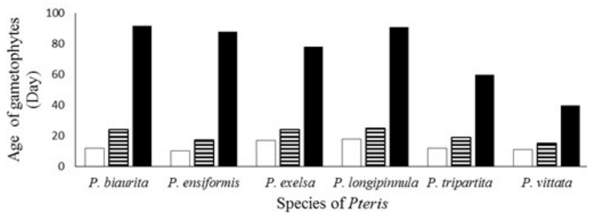

Figure 3. Comparison of gametophyte development time among *Pteris* spesies. Germinated time ( ), formation time of laminar ( ), formation time of gametangia ( )

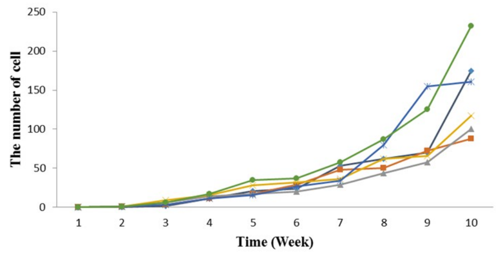

Figure 4. The cell growth of gametophyte *Pteris*, *P. biaurita* ( ), *P. ensiformis* ( ), *P.exelsa* ( ), *P. longipinnula* ( ), *P. tripartita* ( ), *P. vittata* ( )

Laminar phase

The type of gametophyte development varies within the genus *Pteris*, type *Ceratopteris*, *Adiantum*, and modifications of the two kinds (Figure 5). However, previous studies had reported that the gametophyte development in *Pteris* genus has *Ceratopteris*-type [20].

The gametophyte development of all *Pteris* species is the Cerato*pteris*-type, except *P. ensiformis* and *P. tripartita*. This pattern is similar to that of *P. deflexa, P. tristicula, P. cretica, P. berteroana, P. fauriei, P. incompleta, P. multifida, P. vittata*, and *P. wallichiana* [7-12]. In this type, the first cell division is in apical and sometime in subapical, following by repeated division (Figure 5I). Since the meristem is in lateral position, the cell differentiation in the laminar stage is asymmetric. By the time, the activity of meristematic cells gives rise to form a cordate stage after 15-25 days.

P. ensiformis has two types of gametophyte development, the *Adiantum*-type in the early stage, and the *Ceratopteris*-type in the next phase of its prothallia development. During the early phase of this type, the oblique wall division occurs in a terminal cell of the filament and was followed by a second oblique wall division at the right angles. A wedge-shaped then was formed in an apical region (Figure 5G). By the time, the activity of meristem cell in lateral position develops a young asymmetric gametophyte. Previous studies reported that the type of development gametophyte of *P. ensiformis* follow *Ceratopteris*-type [7,10].

The gametophyte development of *P. tripartita* follows the *Adiantum*-type as described by Nayar and Kaur (1971) [20]. In this type, the first division in the terminal cell is parallel to the long axis of the filament. The second division is oblique, then was followed by all cell divisions in the meristematic cells (Figure 5H). An expanded one-cell-thick of the obovate prothallia plate is formed by repeated transverse and longitudinal divisions. The location of the meristematic cell turned into the notch is in the thallus apex. Among these six species, the apical notches of *P. tripartita* were formed earlier than that of the others. Rhizoids were usually formed on the ventral surface of the prothallus, but they were sometimes found on the dorsal surface of the cushion or the wing margins. Results of this study differ from Ravi et al. (2014) [13] who reported that gametophyte development of *P. tripartita* is *Ceratopteris*-type.

Mature Gametophytes and Sexual Expression

The shapes of the mature cordate prothalli are varied among all *Pteris* species (Figure 6 D-I). The mature gametophytes of *P. biaurita, P. longipinnula, P. vittata*,

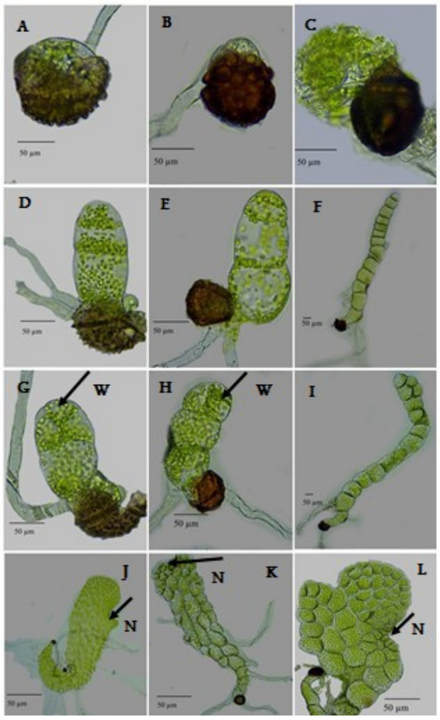

Figure 5. Germination, filamentous and laminar stages. A-C. Germination. A. *P. ensiformis*; B. *P. tripartita*; C. *P.-longipinnula.* D-F. Filamentous. D-E. Filamentous of *P. ensiformis* and *P. tripartita* formed of 3 cell; F. Filamenthous of *P.longipinnula* formed of 13 cell. G. Laminar with wedge-shaped meristematic cell of *P. ensiformis*. H. Laminar with wedge-shaped meristematic cell of *P. tripartita*. I. Laminar with apical cells twice devide; J. Young laminar plate with notch in lateral of *P. ensiformis*. K. Young laminar plate with notch in apex of *P. tripartita*. L. Young laminar with a meristematiz zone. W= wedge-shaped meristematic cell, N= Notch pointed by an arrow.

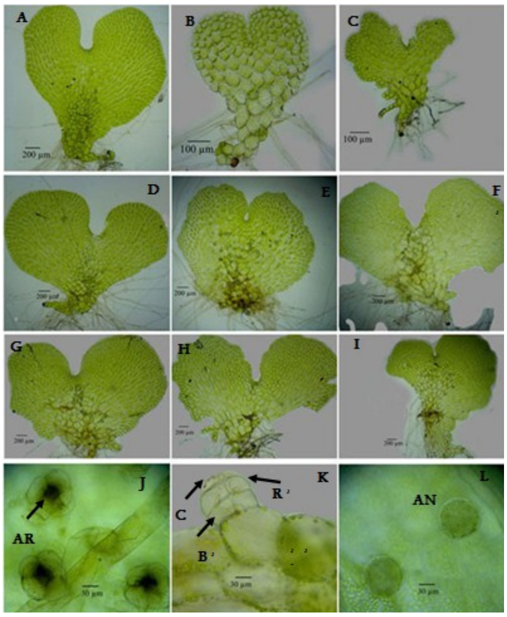

Figure 6. Development stages of the gametophyte and sex organs of *Pteris*. A-C Young gametophyte A. *P. ensiformis*; B. *P. tripartita*; C. *P.longipinnula*. D-I Mature Gametophyte, D. Asymetric cordate of *P. ensiformis*; E. Symetric cordate of *P. tripartita*; F. Asymetric cordate of *P.longipinnula*; G. Asymetric cordate of *P. biaurita*; H-I Asymetric cordate of *P. exelsa* and *P. vittata*; J. Top view of the archegonia *P. tripartita*; K. Side view of antheridium *P. exelsa* with a cap cell (C), a ring cell (R), and a basal cell (B); L. Top view of Antheridia. AR= Archegonium, AN= Antheridium. B, C, R, AR are pointed by an arrow.

and *P. exelsa* are asymetric cordiform, whereas *P. tripartita* and *P. ensiformis* are nearly symetric cordiform.

Formation time of sex organ is varied among species. *Pteris* gametophytes began to produce antheridia 40–92 DAS. *Pteris vittata* produced sex organ (antheridia) the earliest, 40 DAS, while *P. longipinnula* is the last producing sex organ, on 92 DAS (Figure 3). The hermaphrodite gametophyte produced antheridia earlier than archegonia. The archegonia were produced on the ventral surface or notches of the prothalli, 74–102 DAS. Typically, antheridia were produced on the ventral surface of the wings, distributed over the dorsal surface, along the wing margins, or both sides of the gametophytes. Antheridia consist of a cap cell, a ring cell, and a basal cell (Figure 6K).

Sex organs of *P. exelsa, P. longipinnula, P. vittata*, and *P. biaurita* give rise to monosexual/male gametophyte. *P. ensiformis* spores produced 17% of hermaphrodite gametophyte and 83% of the male gametophyte

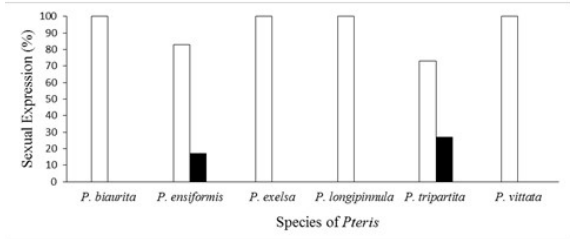

Figure 7. Appearance percentage of sexual expression, male gametophyte (□) and hermaphrodite gametophyte (■).

while *P. tripartita* produced 27% of hermaprodite and 73% of the male gametophyte (Figure 7).

Apogamous types of *P. exelsa, P. biaurita, P. longipinnula*, and *P. vittata* produced only the functional antheridium. The same evident also found in *P. cretica, P. pellucidifolia*, and *P. wulaensis* [30]. However, *P. wulaensis* produced only archegonium, and the embryo appeared from the gametophyte cells indicating that it has apogamous reproduction type. Laird and Sheffield (1986) [31] reported that apogamous *P. cretica* is hermaprodite, but the archegonia had lost function because of the failure of the neck canal to open.

Results of this study differ from the previous study on *P. exelsa, P. ensiformis,* and *P. vittata* that produces 100% gametophyte hermaphrodites [10]. The differences of this result may be due to physical factors such as nutrition, density gametophyte, the influence of light, and the interaction between the gametophyte [32, 33].

CONCLUSIONS

Spore germination of *Pteris* genus is the *Vittaria* type. *Pteris* spores were germinated in 10 to 18 DAS. The spores of *P. ensiformis* were germinated faster, 10 DAS while the spores of *P. longipinnula* were germinated slower, 18 DAS. The number of cells in each week of gametophyte growth were significantly different among species based on ANOVA and Duncan test. The gametophytes among *Pteris* species are differed in the number of filament cell, germinated time, the formation time of notch and gametangia, margin shape, and the development type. Gametophyte morphological characters can be used as a genus characteristic.

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