BIODIVERSITAS
Volume 18, Number 1, January 2017
Pages: 312-320

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d180141

Morphological variations and sex expression in gametophytes of *Cibotium barometz* under in vitro conditions

TITIEN NGATINEM PRAPTOSUWIRYO♥, YUPI ISNAINI
Center for Plant Conservation-Bogor Botanical Gardens, Indonesian Institute of Sciences. Jl. Ir. H. Juanda No. 13, Bogor 16122, West Java, Indonesia.
Tel.: +62-251-8322187, Fax.: +62-251-8322187, ♥email: tienpferns@yahoo.com

Manuscript received: 5 March 2016. Revision accepted: 17 January 2017.

Abstract. *Praptosuwiryo TNg, Isnaini Y. 2017. Morphological variations and sex expression in gametophytes of* Cibotium barometz *under in vitro conditions. Biodiversitas 18: 312-320.* Characteristics of gametophytes in ferns have been shown to be phylogenetically significant. Studies of fern gametophytes have become essential to fill the gaps in our knowledge of fern morphology, ecology, reproduction, evolution and distribution. The purposes of this study were: (i) to observe morphological variations and sex expression in gametophytes of *Cibotium barometz* (L.) J. Sm., and (ii) to understand how gametophyte densities affect sex expression in the species. Spores of five collection numbers of *C. barometz* from Sumatra, Indonesia, were sown on half-strength Murashige & Skoog (½MS) basal medium. Eleven weeks after germination of spores, prothalli were subcultured on ½MS medium along with sugar (30 g/L) and Naphthalene Acetic Acid (NAA, 0.5 mg/L). After eight months subculturing of prothalli (prothallus density: 100-150 individuals per cm^2), 100 gametophytes were observed for each collection number to determine their shapes and sex expression. Between 9- 12 months after subculturing, gametophytes growing at different population densities (between 100-500 individuals per cm^2) were sampled. One hundred prothalli were selected among the ten replicates for each collection number. The percentage of each identified gametophyte shape and their genders were recorded. Eight morphological types of adult gametophyte were recorded: (i) Branching filament (asexual), (ii) ribbon-like shape (male), (iii) spatulate shape (asexual, male, female), (iv) heart shape (male, female, bisexual), (v) gemmiferous heart shape (asexual, female, bisexual), (vi) long heart shape (male, bisexual); (vii) gemmiferous long heart shape (asexual, male, bisexual), and (viii) gemmiferous irregular shape (asexual, male, female, bisexual). We conclude that gametophyte morphology is simply "too plastic" to be used in suporting species delimitation in ferns if the prothalli is to be cultured in a high population density. There is a correlation between gametophyte size, shape and sex expression that is related to the population density. The presence of unisexual and bisexual gametophytes indicates that both intergametophytic and intragametophytic selfing occur in *C. barometz*.

Keywords: *Cibotium barometz*, Environmental Sex Determination theory, gametophyte, morphology, sex expression

INTRODUCTION

Ferns and other pteridophyte groups are seedless vascular plants which are unique among land plants in that they possess two morphologically independent generations; free-living and alternating phases. The more conspicuous form is the diploid generation consisting of vascularized sporophyte plants which meiotically produce spores. Derived from those spores is the haploid generation, consisting of a non-vascularized gametophyte that produces gametes by mitosis; it is much smaller in size and simpler in shape than the sporophyte.

Gametophyte morphology, including type of spore germination, early gametophyte development, and details of mature gametophytes, trichomes and gametangia, has been used to characterize fern taxa (Nayar and Kaur 1971; Prada et al. 1996; Huang et al. 2001; Pangua et al. 2003; Puspitasari et al. 2015). These characters provide evidence about variation pattern, which is one of the distinguishing criteria in fern taxonomy (Pryer et al. 1995). Data on fern gametophytes are also very important for understanding ecology (Dassler and Farrar 1997, 2001), evolution (Stokey 1951; Miller 1968; Nayar and Kaur 1971; Atkinson 1973; Windham and Haufler 1986), demography and distribution (Watkins et al. 2007; Flinn 2006). Therefore the data can be used in determining systematic and phylogenetic relationships in ferns and fern-allies (Pryer et al. 1995).

Gametophytes of homosporous ferns are generally cordate-thalloid with a midrib (cushion), but are sometimes noncordate, displaying various shapes such as tuberous, strap-like, ribbon-like, or filamentous, depending on the taxon (Bower 1923; Orth 1936; Nayar and Kaur 1971; Raghavan 1989; Imaichi 2013). Heart-shaped gametophytes can be found in Aspleniaceae (Herrero et al. 2002; Praptosuwiryo 2010), Cyatheaceae (Chen et al. 2008), Dryopteridaceae (Guo and Liu 2013), Lygodiaceae (Takahashi et al. 2015) and Pteridaceae (Puspitasari et al. 2015). Strap- and ribbon-shaped gametophytes are found in members of the Hymenophyllaceae, Vittariaceae and Polypodiaceae (Farrar et al. 2008; Takahashi et al. 2009). Strap-shaped gametophytes with shallowly notched apices and little branching are found in the family Elaphoglossaceae (Nayar and Kaur 1971; Chiou et al. 1998). Gametophyte form in the Polypodiaceae is more variable (Nayar and Kaur 1971; Chiou and Farrar 1997). Gametophytes in the Polypodiaceae are reported to range from cordate or strap-shaped and branched with apical notch, to ribbon-like (Nayar 1963).

Understanding sex expression in the gametophyte generation is critical, as this process ultimately determines

the outcome of crossing events (Pangua et al. 2011). Sex expression in the gametophyte generation influences the genetic structure of the sporophyte population. In some fern species, a pheromone, 'antheridiogen', that stimulates antheridia initiation is produced by maturing female gametophytes, thus promoting out-crossing (Pangua et al. 2003). Three modes of sexual reproduction are recognized in ferns and fern-allies (following Klekowski 1969): (i) Intragametophytic selfing, a zygote is formed from the same gametophyte; (ii) Intergametophytic selfing, a zygote is formed via the cross-fertilization between different gametophytes produced by a single sporophyte, and (iii) Intergametophytic crossing, a zygote is formed via the cross-fertilization between different gametophytes produced by different sporophytes (Soltis and Soltis 1987). A bigametophytic system, consisting in most cases of male and female prothalli, provides evidence for outcrossing of fern species (Pajarón et al. 1999).

All homosporous ferns have the capacity to be co-sexual, producing a single kind of spore that develops into potentially bisexual gametophytes (Nayar and Kaur 1971), producing both sperms and eggs and consequently produce strictly homozygous sporophytes (Haufler et al. 2016). However, in many species actual gender depends on the environment. This mechanism is known as Environmental Sex Determination (ESD), a mechanism by which sex is decided after conception, depending on the environment, rather than being genetically fixed (Bull 1981). Environmental Sex Determination is a form of phenotypic plasticity, by which individuals produce either female, male, or both sex organs depending largely upon environmental circumstances (Bull 1981; Leimar et al. 2004). Gametophytes of many species tend to become male under poor growing conditions, such as low nutrient availability (Korpelainen 1994), poor light level and quality (Guillon and Fievet 2003) or high density (Huang et al. 2004). Conversely, gametophytes normally become female under rich growing conditions. Recent study has shown that most homosporous ferns are capable of initiating sporophyte progeny in vitro via gametophytic selfing as well as via sporophytic selfing or sporophytic outcrossing (Sessa et al. 2016).

Study on the gametophyte morphology of *Cibotium* in Taiwan revealed that typical gametophytes of *Cibotium barometz* (L.) J. Sm. were heart-shaped, and naked; male, female, and hermaphroditic gametophytes of this species were simultaneously produced by 8-weeks cultures (Huang et al. 2003). Previous study on the gametophyte of *C. barometz* of Sumatra, Indonesia, by germinating spores on the natural media -the minced roots of *Cyathea contaminans* and charcoaled rice husks (1:1) mix- resulted five morphological types of adult gametophyte, *viz*.: (i) irregular spatulate shape (male), (ii) fan shape (male), (iii) elongated heart-shape (male), (iv) short heart or butterfly shape (female), and (v) normal heart shape (bisexual) (Praptosuwiryo et al. 2015). This work was carried out to clarify the findings by germinating spore and subculturing the gametophyte in vitro in a heavy population density. The purposes of this study were: (i) to observe morphological variations and sex expression in gametophytes of *Cibotium barometz* (L.) J. Sm., and (ii) to understand how gametophyte densities affect sex expression. This work was carried out to answer two questions: (i) Whether the gametophyte form of *C. barometz* is plastic in high population densities; (ii) Whether we can explain the sex expression of *Cibotium* gametophytes with the ESD (Environmental Sex Determination) theory.

MATERIALS AND METHODS

Studied species

Cibotium barometz (L.) J. Sm. is a tree fern belonging to the family Cibotiaceae (Smith et al. 2006). It is an evergreen fern distributed in the tropical and subtropical regions of Asia, including North East India, Myanmar, Thailand, Laos, South China, Malaysia, the Philippines, Indonesia, Japan and Viet Nam (Holttum 1963). *Cibotium barometz* can be easily recognized by the smooth, shiny, golden hairs covering its rhizome and basal stipes, and 2-6 or more pairs of cup-shaped sori on each pinnule-lobe (Holttum 1963; Rugayah et al. 2009; Praptosuwiryo et al. 2010, 2011). *Cibotium barometz* grows in warm and humid environments, in hilly or mountain forests, often in valleys, forest edges and open places in forests of elevations ranging from (50-) 200-600 (-1300-1600) m (Holttum 1963; Praptosuwiryo 2003; Zhang et al. 2008; Rugayah et al. 2009; Praptosuwiryo et al. 2011).

Spore collection and sterilization

Spore collection procedures follow those described by Praptosuwiryo et al. (2015). Fresh spores of four collection numbers of *C. barometz* were collected from plants growing at three botanical gardens in West Java, Indonesia, i.e.: Bogor Botanical Gardens, Cibodas Botanical Gardens and Ecopark of the Cibinong Science Center. One collection was collected directly from the field (Table 1).

The sterilization procedures followed the modified procedure of Isnaini (2013). Spores were sterilized with commercial Clorox at concentration 20%,10%, and then 5%, with 1-2 drops of tween-80 as a wetting agent in 20 mL of distilled water. The sterilized spores were rinsed 3 times in sterile distilled water for 5 minutes each time to remove all traces of sterilizing agent. All the sterilization work was carried out in a sterile environment in a Laminar flow cabinet.

Spore culture in vitro on a half-strength MS medium

Half-strength Murashige & Skoog (½MS) basal medium was used as a medium for germination of spores; 3% sugar was added as a carbon source. The pH of the media was adjusted to 5.7 ± 0.01 prior to sterilizing in an autoclave. Gelrite was used as a solidifying agent at a concentration of 0.2% w/v. Spores with sterile water were sown on the sterilized media in 5 cm Petri dishes. The Petri dishes were sealed with strip of plastic wrap film to inhibit drying and contamination. For germination, spores were incubated in Petri dishes at 20-25^{o}C at 2000 lux for 16 h photoperiod. There were at least ten replicates per collection number.

BIODIVERSITAS
Volume 18, Number 1, January 2017
Pages: 312-320
ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d180141

Tabel 1. Spore collection and living collection sites for the *Cibotium barometz* used in in vitro culture

Spore code	Location	Living collection site	Collection number	Deposited herbarium	Spore harvest
Cb	Medang Village, Air Hangat Subdistrict, Kerinci District, Jambi Province. 840-850 m asl.	Bogor Botanical Gardens	TNgP 2509	BOHB	2012
Cb-1	Soriak Hill, Lima Puluh Kota District, West Sumatra Province	Ecopark, Cibinong Science Center	TNgP 3353	BOHB	2013
Cb-2	Barisan Hill, Seberang Air Village, Lareh Sago Halaban Subdistrict, Lima Puluh Kota District, West Sumatra Province	Cibodas Botanical Gardens	TNgP 2780H	BOHB	2013
Cb-4	Rambut Tulang Hill, Tanjung Gadang Village, Lareh Sago Halaban Subdistrict, Lima Puluh Kota District, West Sumatra Province	Cibodas Botanical Gardens	TNgP 2844	BOHB	2013
Cb-5	Sikek Hill, Kampung Air Putih, Sari Lamak Village, Harau, Subdistrict Lima Puluh Kota District, West Sumatra Province	(not planted in the garden)	TNgP s.019	BOHB	2013

Note: TNgP = Titien Ng. Praptosuwiryo; BOHB (Herbarium of Bogor Botanical Gardens)

Prothalli sub-culturing

Eleven weeks after germination of spores, prothalli were obtained. The prothalli consisted of the spatulate stage and young heart-shaped stage. The prothalli were then sub-cultured on half-MS medium along with sugar (30 g/L) and Naphthalene Acetic acid (NAA, 0.5 mg/L), in the culture glass bottles (250 mL volume, 9.5 mm high, 6.5 mm diam., on 1.5 mm thick media), in which they multiplied successfully. There were at least ten replicates per collection number.

Observations of gametophyte morphology and sex expression

After 8 months subculturing of prothalli, 100 mature gametophytes were selected from among the ten replicates for each collection number. The prothallus density after 8 months subculturing was 100-150 individuals per cm^2. Each gametophyte was observed to determine its size, shape and sex expression (Table 2). The results were compared with the results for gametophytes grown in isolated conditions or at a low density (1-5 individuals per cm^2). Between 9-12 months after subculturing, gametophytes growing at different population densities (150-200, 200-250, 250-300, 300-350, 400-450 individuals per cm^2) were sampled. One hundred prothalli were selected from one culture glass bottle among the ten replicates for each collection number. The percentage out of the total number of gametophytes represented by each gametophyte shape and gender was recorded (Table 3). The gametophyte density was determined by counting all individual gametophytes in 1-3 cm^2 of purposively selected growth medium area.

Gametophyte shape and sex expression were observed under binocular Nikon SM2-10A microscope (objective lens 4.9x). An Olympus microscope U-TV0, 5XC-3 5H12344 (objective Lens 4x and 10x) and trinocular Optica microscope SZ-CTV (objective Lens 1.5x-2x) connected to a digital camera with computer monitor was also used to document the morphology and sex expression of gametophytes. Gametophyte shapes were labeled according to the categories for fern gametophyte morphology depicted in the figures of Nayar (1963) and Nayar and Kaur (1971).

RESULTS AND DISCUSSION

High population density affects gametophyte growth and morphological variations in *Cibotium barometz*

Eight types of gametophyte shape for *C. barometz* were found in the mass prothallus after subculturing, *viz*.: (i) branching filament (amorphous gametophytes), (ii) ribbon-like shape, (iii) spatulate shape, (iv) heart shape, (v) gemmiferous heart shape, (vi) long heart shape, (vii) gemmiferous long heart shape, and (viii) gemmiferous irregular shape (Figure 1-2; Table 2.). On the other hand, in isolated conditions or at a low density, gametophytes of *C. barometz*, both in the early development and adult stages, showed a relatively stable morphology; their form is heart-shaped (Figure 3). Huang et al. (2003) reported that *C. barometz* of Taiwan has heart-shaped gametophytes with male, female, and hermaphroditic sex expression. Table 2 also shows the average sizes of each form of gametophyte of *C. barometz*. Female gametophytes of *C. barometz* were much larger than the other types of gametophytes. Hermaphroditic gametophytes of *C. barometz* were larger than male gametophytes, which were larger than asexual gametophytes. These results are similar to the gametophyte observations on *Culcita macrocarpa* reported by Ghosh et al. (2012).

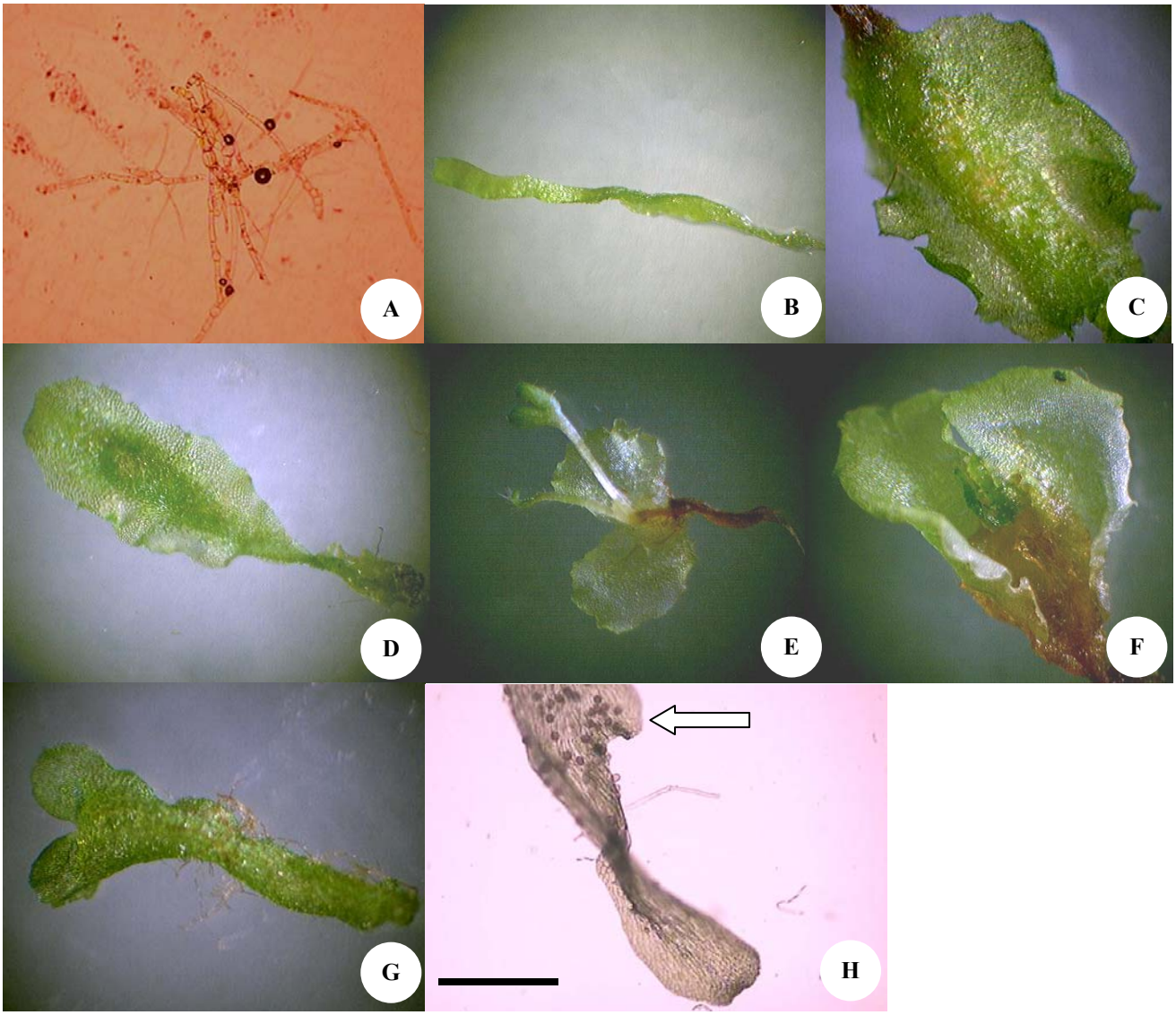

Figure 1. Gametophytes of *Cibotium barometz* at high population density, after 8 months subculturing. A. Branching filament (amorphous gametophytes, asexual); B. Ribbon-like shape (male); C-D. Spatulate shape (male); E-F. Heart shape (bisexual); G-H. Long heart shape (male), white arrow showing antheridia at abaxial surface. Scale bar = 1.25 mm for A; 1 mm for C-D; 2 mm for B and G; 3 mm for E-F; 0.75 mm for H

These results showed that the morphology of prothalli of *C. barometz*, whether they are in an early stage of development or a mature stage, will become plastic if they are cultured at a heavy population density. Some other research has also shown that gametophyte morphology is influenced by population density. Studies on *Osmunda cinnamomea* reported by Huang et al. (2004) revealed that population density of gametophytes affects gametophyte growth and sex expression of *Osmunda cinnamomea.* Gametophyte size of *O. cinnamomea* is negatively related to the population density, which significantly affects gametophytes' sex expression.

Gametophyte density affects sex expression on *Cibotium barometz*

Data on sex expression of *C. barometz* under in vitro conditions after subculturing is presented in Figure 4. Results of this study support previous research dealing with the sex expression of homospore fern species (see Carafa 1990; De Soto et al. 2008). The males gametophyte become dominant in the high population density (Figure 4). The percentage of male gametophytes out of the total ranged from 25 to 95% in a prothallus density ranging from 150 to 450 individuals per cm^2. Higher gametophytic densities probably promote maleness through the more rapid attainment of effective concentrations of antheridiogen. Ranker and Houston (2002) provided evidence that sex ratios in fern gametophytes are influenced by the population density of gametophytes. Ranker and Houston (2002) showed that field populations of gametophytes had a higher ratio of males to females than did laboratory cultures. Populations in the laboratory may not be representative of population sex ratios in the field. The study conducted by Ranker and Houston (2002) included

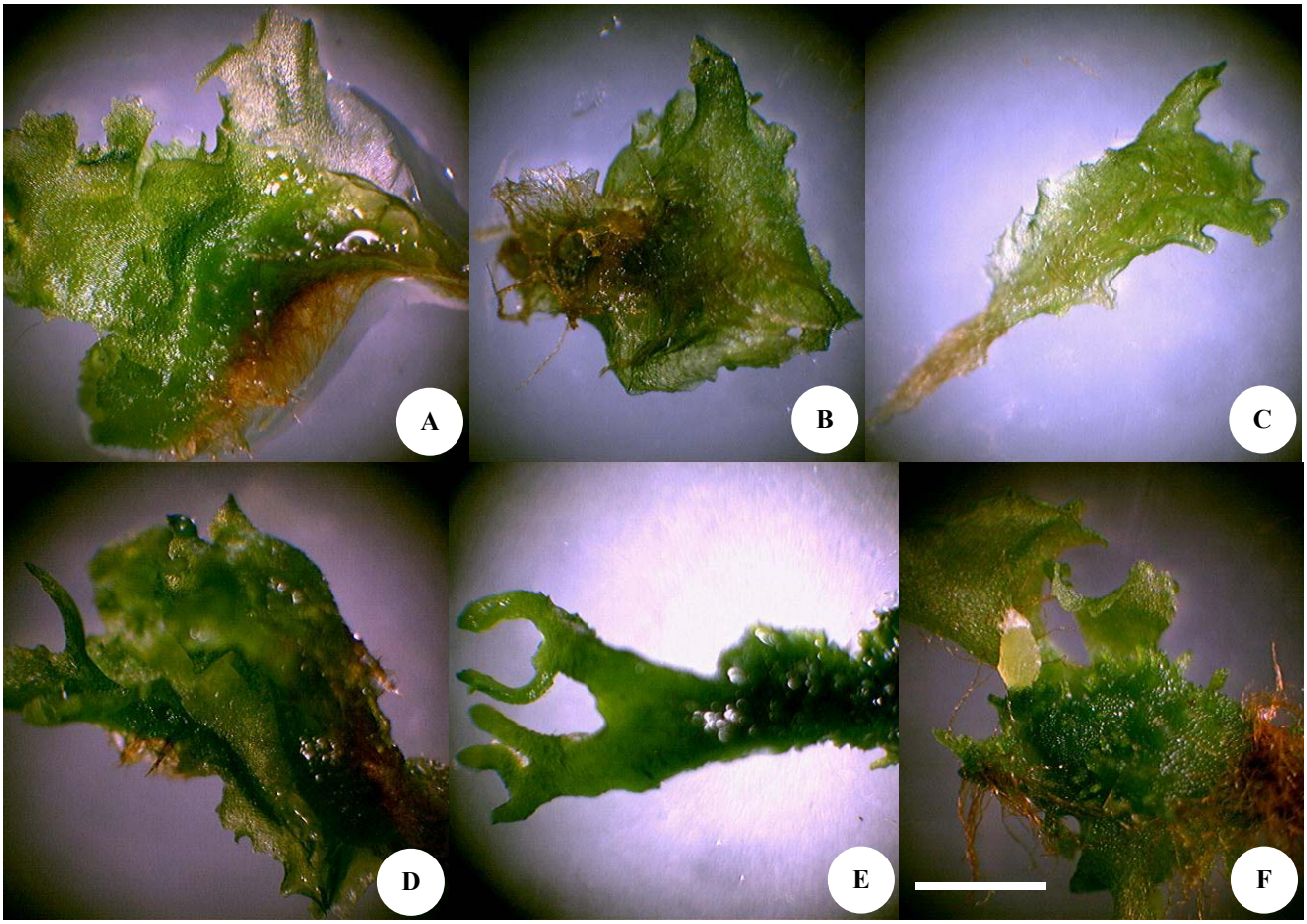

Figure 2. Gametophytes of *Cibotium barometz* at high population density, after 8 months subculturing**.** A-B. Gemmiferous heart shape (bisexual); C. Gemmiferous long heart shape (male); D. Gemmiferous long heart shape (bisexual); E-F. Gemmiferous irregular shape, E Asexual, F. Bisexual. Scale bar = 3.25 mm for A-B; 2.5 mm for C-D; 5 mm for E-F.

a direct comparison between laboratory and field gametophytes, using naturally occurring gametophyte populations and populations created under standard laboratory sowing and culture conditions. Keeping in mind the evidence they provided, Skelton (2007) developed hypotheses to explain the discrepancy between field and laboratory results and decided to test these experimentally in the laboratory to see if they could replicate the higher number of males in the population by altering some of the laboratory conditions to more accurately reflect conditions in nature.

Interactions between isolated gametophytes, and also among gametophytes in populations will result in different expression. Peck (1985) pointed out that interactions between gametophytes in isolated conditions are different from those in populations. Peck (1985) suggests that isolated gametophytes may express different: 1) germination potential, 2) developmental patterns, 3) sexual sequence and expression, and 4) reproductive capability than do gametophytes in populations.

Sex expression of gametophytes is affected by the pheromone 'antheridiogen'. Strain et al. (2001) showed how the pheromone antheridiogen secreted by the hermaphrodite gametophyte of the fern genus *Ceratopteris* induces male and represses female development in other young, sexually undertermined gametophytes. Strain et al. (2001) concluded that the presence of antheridiogen leads to the activation of the *FEM1* gene, which not only promotes the differentiation of male traits, but also represses female development by activating the *NOT1* gene. *NOT1* represses the *TRA* genes necessary for the development of female traits in the gametophyte.

Sex expression in *C. barometz* gametophytes can be explained within the ESD (Environmental Sex Determination) theoretical framework (Figure 4). In general, the total percentage of males gametophyte of Cb-5 with the density of 150-200 individuals per cm^2 was lower than Cb-2 with the density of 200-250 individuals per cm^2. The expression of gametophytes of *C. barometz* is clearly affected by gametophyte density. The results regarding sex expression agree with the observations of Masuyama (1974) and Chiou (1985). Masuyama (1974) and Chiou (1985) established that, under favorable growing conditions (light, temperature, and humidity), gametophytes grown in

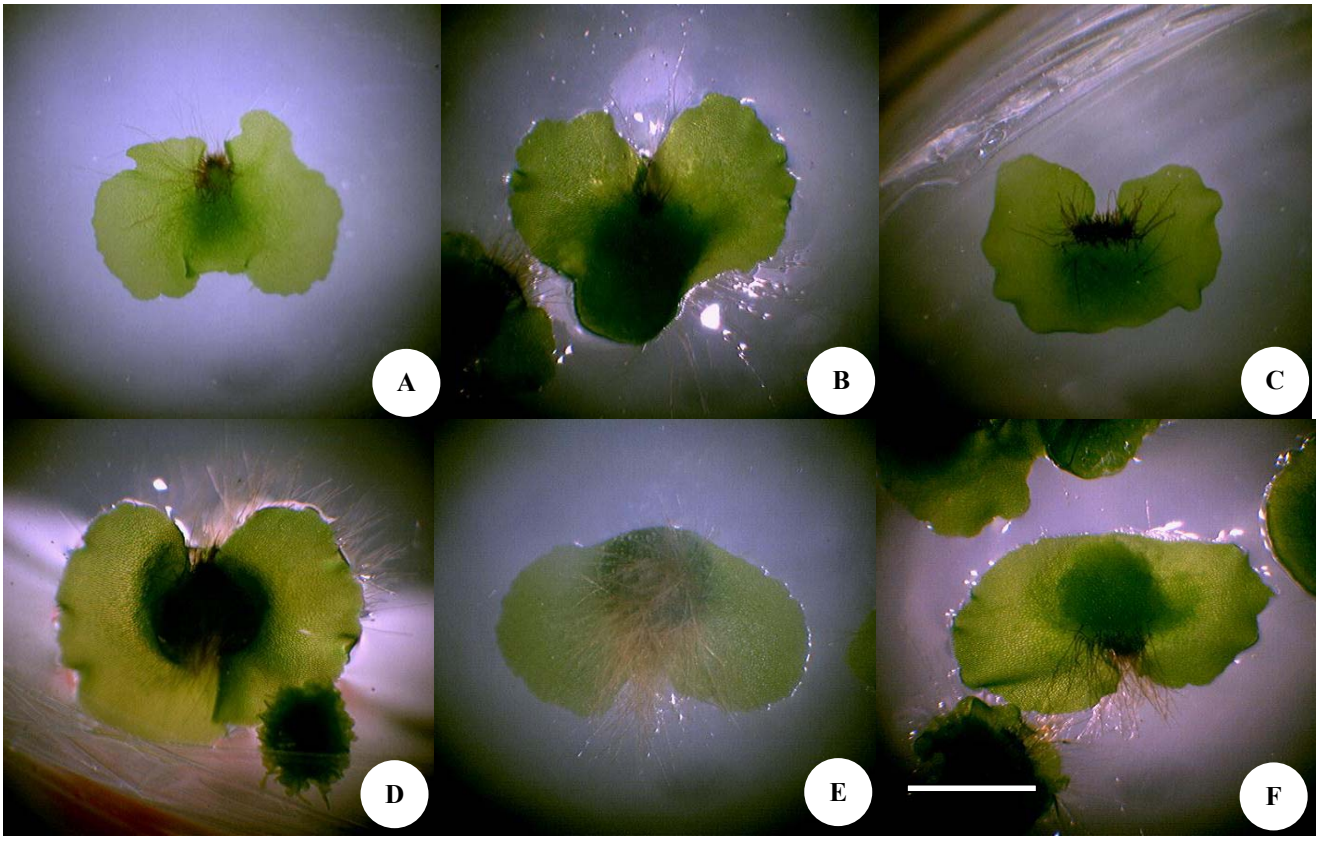

Figure 3. Gametophyte of *Cibotium barometz* (Spore Code: Cb-4) at low population density (1-4 individuals per cm^2), 17 weeks after sowing. A-B. Gametophytes in isolated condition (one individual per cm^2). C-E. Gametophytes in the density of 2 individuals per cm^2. F. Gametophytes in the density of 4 individuals per cm^2. Scale bar = 2.5 mm for all

Table 2. Morphological description of prothallus/gametophytes of *Cibotium barometz* under in vitro conditions after subculturing, at high population density

Morphology	Descriptions	Sex expression
Branching filament	Filamentous prothallus, uniseriate, much branching, only one dimension, sustaining filamentous architecture, ca. 0.25- 10.00 mm length.	Asexual
Ribbon-like shape	Ribbon-like prothallus with 2-4 cells wide, to 10.00 mm length and 0.50 mm wide	Male
Spatulate shape	Laminar prothallus with spoon form, the basal part consisting of 2 or more cells. The spatulate shape is usually composed of 12 or more cells, 1.00- 7.00 mm length and 0.01- 2 mm wide.	Asexual, male, female
Heart shape	Normal heart shape, mature prothallus 5.00- 7.00 mm length and 2.00- 5.00 mm wide (female); 4.00-13.00 mm length and 3.00-5.00 mm wide (bisexual).	Male, female, bisexual
Gemmiferous heart shape	Prothallus having heart shape, forming many lobes, on certain parts more than one cell thick, its size much larger than the normal heart shape, 13.00-14.00 mm length and 8.00-12.00 mm wide; female or bisexual bearing gemmae to produce new prothallus.	Asexual, female, bisexual
Long heart shape	Elongated normal heart shape, one cell thick, 5.00-18.00 mm length and 0.05-4.00 mm wide (male), 8.00-23.00 length and 1.50-3.00 mm wide (bisexual).	Male, bisexual
Gemmiferous long heart shape	Elongated heart shape, on certain parts having more than one cell thick, and bearing gemmae to form new prothallus; 10.00-12.00 mm length and 4.00-5.00 mm wide.	Asexual, male, bisexual
Gemmiferous irregular shape	Prothallus having irregular form, more than one cell thick, much branching and forming lobes, bearing gemmae to form new prothallus; 15.00-21.00 mm length and 1.00-3.00 mm wide (female); 7.00-26.00 mm length and 1.00-9.00 mm wide (bisexual)	Asexual, male, female, bisexual

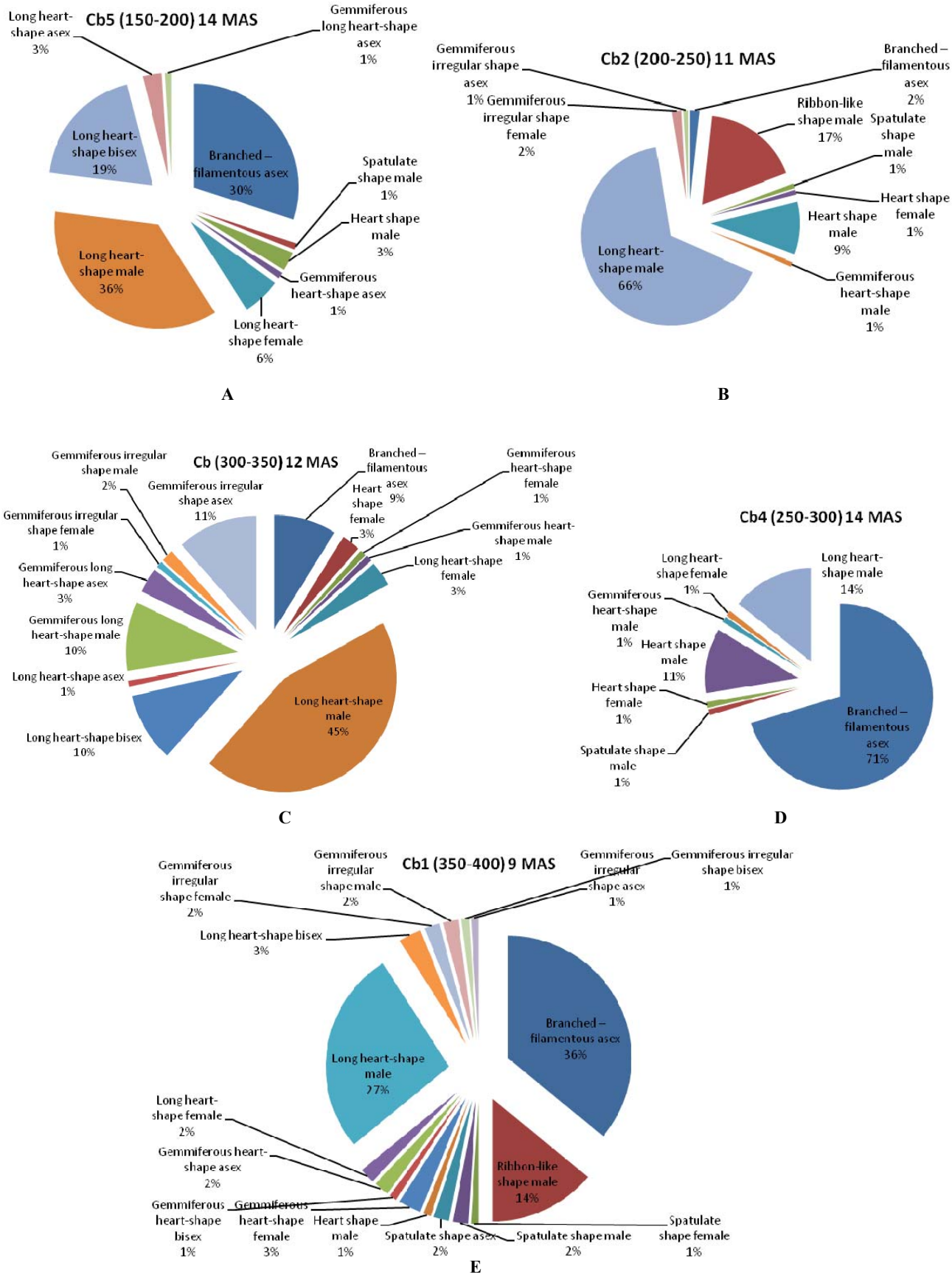

Figure 4. Percentage of morphological variations and sex expression in gametophytes of *Cibotium barometz* after subculturing. MAS = month after subculturing. Asex = asexual. Bisex = bisexual. Number in the round bracket is gametophyte density size per mm^2

high densities will produce mainly male gametangia, whereas low densities of gametophytes favor the establishment of female gametangia. The same occurrences were also shown in *Woodwardia.* The sex expression of gametophytes of *Woodwardia radicans* is affected by sowing density, by the presence of antheridiogen in the culture medium and by the nutritional conditions of gametophytes (Carafa 1990). De Soto et al. (2008) also showed that stress (limited nutrient supply, crowding) affects sex expression in *Woodwardia radicans* gametophytes. It was, in a way, compatible with ESD (Environmental Sex Determination). Under good growth conditions (low density or high nutrients), gametophytes matured sexually at a relatively large size and turned into females and subsequently into bisexuals; under harsh growth conditions, gametophytes matured sexually at a smaller size and turned into males (De Soto et al. 2008).

In conclusion, germinating spores and sub-culturing prothalli of *C. barometz* under in vitro conditions in a high population density has resulted in eight morphological types of adult gametophytes. Gametophyte morphology is simply "too plastic" to be used in supporting species delimitation in ferns if the prothalli is to be cultured in a high population density. Sexual expression in *C. barometz* gametophytes can be explained within the ESD (Environmental Sex Determination) theoretical framework. The sex expression of gametophytes of *C. barometz* is affected by population density, by the presence of antheridiogen in the culture medium and by the nutritional conditions of gametophytes. The presence of unisexual and bisexual gametophytes support the previous study that revealed that both intergametophytic and intragametophytic selfing occur in *C. barometz*.

ACKNOWLEDGEMENTS

This research was funded by a Grant-in-Aid for Scientific Research from The Ministry of Research and Technology, Government of Indonesia (GoI), 03/SU/SP/Insf-Ristek/III/11, under The *Program Insentif Peneliti dan Perekayasa LIPI TA 2011* and *Program Kompetitif/Program Riset Unggulan LIPI TA 2013-2015*. We are grateful to anonymous reviewer from Department of Botany, National Museum of Nature and Science (TNS), Amakubo, Tsukuba, Japan, for his insightful, suggestion, and constructive comments on the early version of this article. We thank Dr. Rugayah (Research Center for Biology, LIPI) for reading and giving some comments to improve the manuscript. We are indebted to Dr. Graham Eagleton (Australia) for providing valuable comments on an early draft and correcting the English of the manuscript.

REFERENCES

Atkinson LR. 1973. The gametophyte and the family relationships. In Jeremy AC et al. (eds). The phylogeny and classification of the ferns. Bot J Lin Soc 67 (suppl.1): 73-90.

Bower FO. 1923. The ferns (Filicales). Vol. 1. Anatomical examination of the criteria of comparison, reprint eds. (1963). Today & Tomorrow's Book Agency, New Delhi.

Bull JJ. 1981. Evolution of environmental sex determination from genotypic sex determination. Heredity 47 (2): 173-184.

Carafa AM. 1990. Gametophyte development of *Woodwardia radicans* (L.) Sm.: Effect of population density and antheridiogen on sex expression. Giornale Botanico Italiano 124 (5): 571-580.

Chen GJ, Cheng X, Liu BD, Jiao Y. 2008. Comparative studies on gametophyte morphology and development of seven species of Cyatheaceae. Amer Fern J 98 (2):83-95.

Chiou W, Farrar DR, TA Ranker. 1998. Gametophyte morphology and reproductive biology in *Elaphoglossum*. Can J Bot 76:1967-1977.

Chiou W, Farrar DR. 1997. Comparative gametophyte morphology of selected species of the family Polypodiaceae. Amer Fern J 87:77-86.

Chiou WL. 1985. The gametophytes of *Platycerium bifurcatum* (Cav.) C. Chr. Yushania 2: 19-23.

Dassler CL, Farrar DR. 1997. Significance of form in fern gametophytes: clonal, gemmiferous gametophytes of *Callistopteris baueriana* (Hymmenophyllaceae). Intl J Plant Sci 158: 622-639.

Dassler CL, Farrar DR. 2001. Significance of gametophyte form in long distance colonization by tropical, epiphytic ferns. Brittonia 53: 352-369.

Farrar DR, Dassler CL, Watkins JE Jr, Skelton C. 2008. Gametophyte ecology. In: Ranker TA, Haufler CH. (eds) Biology and Evolution of Ferns and Lycophytes. Cambridge University Press, Cambridge.

Flinn KM. 2006. Reproductive biology of three fern species may contribute to differential colonization success in post-agricultural forests. Amer J Bot 93 (9): 1289-1294.

Ghosh L, Jiménez A, Quintanilla LG. 2012. Effect of nutrients on environmental sex determination and size of gametophytes in *Culcita macrocarpa.* J Life Earth Sci 7: 109-113.

Guillon JM and Fievet D. 2003. Environmental sex determination in response to light and biased sex ratios in *Equisetum* gametophytes. J Ecol 91: 49-57.

Guo ZY, Liu HM. 2013. Gametophyte Morphology and Development of Three Species of *Cyrtogonellum* Ching (Dryopteridaceae). Amer Fern J 103 (3):153-165.

Haufler CH, Pryer KM, Schuettpelz E, Sessa EB, Rarrar DR, Moran R, Schneller JJ, Watkins Jr JE, Windham MD. 2016. Sex and the single gametophyte: Revising the homosporous vascular plant life cycle in light of contemporary research. BioScience 66: 928-937.

Herrero A, Prada C, Pajarón S. 2002. Gametophyte morphology and gametangial ontogeny of *Asplenium foreziense* and related taxa (Aspleniaceae: Pteridophyta). Bot J Linn Soc 139: 87-98.

Holttum RE 1963. Cyatheaceae. In Steenis CGGJ Van, Holttum RE (eds) Flora Malesiana. Martinus Nijhoff, The Hague, Netherlands.

Huang YM, Chiou WL, Lee PH. 2001. Morphology of the gametophyte and young sporophytes of Cyatheaceae Native to Taiwan. Taiwania 46 (3): 274-283.

Huang YM, Chou HM, Chiou WL. 2004. Density affects gametophyte growth and sexual expression of *Osmunda cinnamomea* (Osmundaceae: Pteridophyta). Ann Bot 94: 229-232.

Huang YM, Kuo CY, Chiou WL. 2003. Morphology of gametophytes and young sporophytes of *Cibotium* Kaulf. native to Taiwan. Taiwan J Forest Sci 18 (3): 163-170.

Imaichi R. 2013. A new classification of the gametophyte development of homosporous ferns, focusing on meristem behaviour. Fern Gazette 19: 141- 156.

Isnaini Y. 2013. In vitro germination of two species of pitcher plants (*Nepenthes ampullaria* and *N. rafflesiana*) from the island of Batam. Prosiding Seminar Nasional Perhorti, Bogor, 9 October 2013. [Indonesian].

Klekowski EJ Jr. 1979. The genetics and reproductive biology of ferns. In: AF Dyer (ed). The Experimental Biology of Ferns. Academic Press, London.

Leimar O, Van Dooren TJM, Hammerstein P. 2004. Adaptation and constraint in the evolution of environmental sex determination. J Theor Biol 227: 561-570.

Masuyama S.1974. The sequence of the gametangia formation in homosporous fern gametophytes. I Sci Rep TKD Sect B 16: 47-79.

Miller JH. 1968. Fern gametophytes as experimental material. Bot Rev 34: 361-440.

Nayar BK, Kaur S. 1971. Gametophytes of homosporous ferns. Bot Rev 37: 295-396.

Nayar BK. 1963. Contributions to the morphology of some species of *Microsorium*. Ann Bot (London) 27: 89-100.

Orth R. 1936. Morphologische und physiologische untersuchungen an farnprothallien. Planta 25: 104-150. [Germany]

Pajarón S, Pangua E, García-Álvarez L. 1999. Sexual expression and genetic diversity in populations of *Cryptogramma crispa* (Pteridaceae). Amer J Bot 86 (7): 964-73.

Pangua E, Pérez-Ruzafa I, Pajarón S. 2011. Gametophye features in apeculiar annual fern, *Anogramma leptophylla.* Ann Bot Fennici 48: 465-472.

Pangua E, Quintanilla LG, Sancho A, Pajaro´n S. 2003. A comparative study of the gametophytic generation in the *Polystichum aculeatum* group (Pteridophyta). Intl J Plant Sci 164 (2): 295-303.

Peck CJ. 1985. Reproductive biology of isolated fern gametophytes. Iowa State University. Retrospective Theses and Dissertations. Paper 8762.

Prada C, Pangua E, Herrero A, Pajarón S. 1996. A comparative study of the gametophytes af *Asplenium majoricum* Litard. (Aspleniaceae) and related taxa. Anales Jard Bot Madrid 54: 126-136.

Praptosuwiryo TNg, Pribadi DO, Puspitaningtyas DM, Hartini S. 2011. Inventorying of the tree fern genus *Cibotium* of Sumatra: Ecology, population size and distribution in North Sumatra. Biodiversitas 12 (4): 204- 211.

Praptosuwiryo TNg, Pribadi DO, Rugayah. 2015. Growth, development and morphology of gametophytes of golden chicken fern (*Cibotium barometz*) in natural media. Biodiversitas 16 (2): 303-310.

Praptosuwiryo TNg. 2003. *Cibotium barometz*. (L.) J. Smith. In: de Winter WP, Amoroso VB. (eds.) Plant Resources of South-East Asia 15 (2) Cryptogams: Ferns and Ferns Allies. PROSEA, Bogor.

Praptosuwiryo TNg. 2010. Gametophytes of the bird nest ferns *Asplenium nidus* (Aspleniaceae) from West Kalimantan. Buletin Kebun Raya 13 (1): 1-7.

Pryer KM., Smith AR, Skog JE. 1995. Phylogenetic relationships of extant ferns based on evidence from morphology and *rbcL* sequences. Amer Fern J. 85 (4): 205-282.

Puspitasasri DS, Chikmawati T, Praptosuwiryo TNg. 2015. Gametophyte morphology and development of six species of *Pteris* (Pteridaceae) from Java Island Indonesia. J Trop Life Sci 5 (2): 98-104.

Raghavan V. 1989. Developmental Biology of Fern Gametophytes. Cambridge University Press, Cambridge, UK.

Ranker TA, Houston HA. 2002. Is gametophyte sexuality in the laboratory a good predictor of sexuality in nature? Amer Fern J 92: 112-118.

Rugayah, Praptosuwiryo TNg, Puspitaningtyas DM. 2009. Morphological variation of *Cibotium barometz* from West Sumatra. Proceedings on the International Conference on Biological Science. Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia, 16-17 October 2009.

Sessa EB, Testo WL, Watkins JE Jr. 2016. On the widespread capacity for, and functional significance of, extreme inbreeding in ferns. New Phytologist 211: 1108-1119.

Skelton CL. 2007. Investigations into gametophyte morphology and population sex ratios through direct comparisons between laboratory-grown and field-grown fern gametophytes. Iowa State University. Ames, Iowa. Retrospective Theses and Dissertations. Paper 15062.

Smith AR, Pryer KM, Schuettpelz E, Korall P, Schneider H, Wolf PG. 2006. A classification for extant fern. Taxon 55: 705-731.

Soto De L, Quintanilla LG, Méndez M. 2008. Environmental sex determination in ferns: effects of nutrient availability and individual density in *Woodwardia radicans.* J Ecol 96: 1319-1327.

Stokey AG. 1951. The contribution by the gametophyte to classification of the homosporous ferns. Phytomorphology 1: 39-58.

Strain E, Hass B, Banks JA. 2001. Characterization of mutations that feminize gametophytes of the fern *Ceratopteris.* Genetics 159: 1271-1281.

Takahashi N, Kami C, Ota I, Morita N, Imaichi R. 2015. Developmental morphology of the typical cordate gametophyte of a homosporous leptosporangiate fern, *Lygodium japonicum* (Lygodiaceae), focusing on the initial cell behavior of two distinct meristems. Amer J Bot 102 (2): 197- 207.

Watkins JE Jr., Mack MK, Mulkey SS. 2007. Gametophyte ecology and demography of epiphytic and terrestrial tropical ferns. Amer J Bot 94 (4): 701-708.

Windham MD, Haufler CH. 1986. Biosystematic uses of fern gametophytes derived from herbarium specimens. Amer Fern J 76: 114-128.

Zhang XC, Jia JS, Zhang GM. 2008. Non-detriment finding for *Cibotium barometz* in China. NDF Workshop Case Studies. WG 2- Perennials. Case Study 1: *Cibotium barometz.* International Expert Workshop on CITES Non-Detriment Findings Cancun, Mexico, November 17th-22nd, 2008.