

Growth, development and morphology of gametophytes of golden chicken fern (*Cibotium barometz* (L.) J. Sm.) in natural media

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Abstract. *Praptosuwiryo TNg, Pribadi DO, Rugayah. 2015. Growth, development and morphology of gametophytes of golden chicken fern (Cibotium barometz (L.) J. Sm.) in natural media. Biodiversitas 16: 303-310.* The golden chicken fern, *Cibotium barometz* (L.) J. Sm., is an important export commodity for both traditional and modern medicine. To understand the reproductive biology of this species, spore germination, gametophyte development, morphological variation, and sex expression were studied by sowing spores on sterilized natural media consisting of the minced roots of *Cyathea contaminans* and charcoaled rice husks (1: 1) mix. Spores of *C. barometz* are trilete, tri-radially symmetrical, non chlorophyllous, and golden-yellow with a perine. Six stages of gametophyte development (rhizoid stage, rhizoid/protochorm stage, filament stage, spatulate stage, young heart stage, mature heart stage) were observed between 24-45 days after sowing. Spore germination of *C. barometz* is *Vittaria*-type. Prothallial development of *C. barometz* is *Drynaria*-type. Five morphological types of adult gametophyte were recorded: (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). The presence of morphological variations is presumed to be related to the population density, which significantly affects the sexual expression of gametophytes. The variation of sex expression in *C. barometz* also indicates that this species may has a mixed mating systems that resulted in genetic diversity within population and among populations.

Keywords: *Cibotium barometz*, gametophytes, golden chicken fern, spore germination, prothallial development

INTRODUCTION

Cibotium is a genus of 12 species (Hassler and Swale 2002) or 11 species of tropical tree fern (Zhang and Nishida 2013), which is subject to much confusion and revision. Two species of them occur in Indonesia, namely *Cibotium barometz* (L.) J. Sm and *C. arachnoideum* (C. CHR.) Holttum (Holttum 1963). The Golden Chicken Fern, *C. barometz*, is easily recognized because of the gold yellowish-brown, smooth and shining hairs covering its rhizome and basal stipe. The rhizome is usually prostrate or erect and rarely more than 1 m high.

Cibotium barometz differs from *C. arachnoideum* by a number of diagnostic characters as follows: *C. barometz* has sori 2-6 or more pairs on each pinnule-lobe of larger fronds, largest pinnules 20-35 mm wide, pinnules on the two sides of a pinna not greatly different in length, hairs on lower surface of costae and costules flaccid and never spreading. In contrast *C. arachnoideum* always has two pairs of sori on each pinnule-lobe of larger fronds, largest pinnules 15-26 mm wide, pinnules on basisopic side of lower pinnae much shorter than those on acroscopic side, hairs on lower surface of costae and costules rigid and spreading (Holttum 1963; Praptosuwiryo et al. 2011).

Economically, *C. barometz* is an important export commodity for both traditional and modern medicine in

China, Japan and France (Zamora and Co 1986; Praptosuwiryo 2003). The golden hairs on its rhizome and stipes have been used as a styptic for treating bleeding wounds (Dan and Nhu 1989). An extract from the rhizome ('gouji') is also used as an anti-rheumatic, to stimulate the liver and kidney, to strengthen the spine, as a remedy for prostatic disease, and to treat various other illnesses, including flatulence (Praptosuwiryo 2003; Yao 1996; Ou 1992). Although this species has many uses, it has not yet been cultivated for commercial trade. Therefore this species has been included in Appendix II of the Convention on International Trade in Endangered Species (CITES) since 1976. In order to utilize it, the NDF (*Non Detriment Findings*) system must be applied to determine the annual quotas. Biological aspects, such as reproductive biology, are some of the most information needed for the NDF.

Reproductive biology determines plant adaptation and species evolution (Farrar et al. 2008). Many traits are used to infer the reproductive biology of ferns, such as the mating system; the number of spores in each sporangium; sporogenesis; spore size; the lifespan of the gametophyte generation; gametophyte morphology and development; and reproductive systems (Manton 1950; Masuyama 1979, 1986; Walker 1979; Haufler et al. 1985; Lin et al. 1990; Kawakami et al. 1996; Huang et al. 2006, 2009).

Studies on the reproductive biology of *C. barometz* have been reported by some researchers from China. Chen et al. (2007) studied gametophyte development and its diversity in *C. barometz* from China by culturing spores in Knop's solution and solid medium. Li et al. (2010) reported *in vitro* culture and plant regeneration of *C. barometz* using Murashige & Skoog (MS) media. Deng et al. (2007) observed the gametophyte development of *C. barometz* from China by culturing the spores both in inorganic medium and in soil from the original habitat.

This paper reports the reproductive biology of *Cibotium* from Sumatra, Indonesia. The aims of this study were: (i) to verify the reproductive characteristics of *C. barometz* of Sumatra, including spore germination, gametophyte development and morphology; (ii) to study morphological variation and sex expression, and (iii) to show that a sterilized natural media consisting of the minced roots of *Cyathea contaminans* and charcoaled rice husks (1: 1) mix can be used as an alternative to study the gametophyte development and morphology of tree fern.

MATERIALS AND METHODS

Spore collection. Spores of *Cibotium barometz* were collected from Riau, Sumatra in June 2011. Spore-bearing pinnae of mature sporophylls were cleaned in running water to avoid spore contamination from other species. Spore-bearing pinnulae were dried and folded in a newspaper and then placed in an envelope (22 x 11 cm²). The specimens were kept at room temperature in a dry place. A few days later (7-10 days) most of spores had been released from their sporangia and were lying in the envelope. The spores were separated off from the sporangia by tilting the envelope paper and removing them from the envelope to a piece of glassine weighting paper which was folded into a pocket. The spore collections were kept at room temperature until the sowing day (not more than two month).

The spores (10 mg) were sown on a sterilized, mixed natural media of *Cyathea contaminans* roots and charcoaled rice husks (1: 1) mix in a transparent plastic box (7 cm height, 11 cm diam.) with the media layer 2.5-3 cm high. Preparation of the media was as follows: the mix media was boiled in water for 2.5-3 hours for sterilization. The sterilized media was kept in a sealed transparent plastic box (7 cm height, 11 cm diam.) for 24 hours at room temperature before it was used to sow spores. The plastic boxes were kept in a glasshouse at 25-32.5°C with 68-85% relative humidity. Watering was done once every week to maintain the humidity of the media.

Observations. Periodically, spore germination, gametophyte growth and differentiation and sex expression were observed under an Olympus trinocular fitted with Nikon Camera SMZ 10A 1.5X. Every 4-5 days 100-300 selected prothallus or gametophytes were observed. First observations using microscope were carried out 24 days after sowing. Photographs were taken using the microscope combined with a computer monitored camera (Olympus CX 31).

RESULTS AND DISCUSSION

Spore germination

Spores of *C. barometz* are perinate, trilete, tri-radially symmetric, non chlorophyllous and golden yellow in colour (Figure 1.A.). After 23 days from sowing, five stages of the gametophyte development were found (Figure 1-2.), viz. rhizoid stage, rhizoid/protochorm stage, filament stage, spatulate stage and young heart stage. The sixth stage of gametophyte development, mature heart stage, was first appeared 45 days after sowing. First germination occurred two weeks after sowing. Deng et al. (2007) also reported that spores of *C. barometz* of China germinated about 1-2 weeks after being sowed.

The first cell produced on germination of the spores was rhizoid (Figure 1.B.). This is called the rhizoid stage. The rhizoid cell usually does not contain chloroplasts. It is produced on the upper surface of the basal cell or sometimes at one corner of the basal cell. The second stage of gametophyte development is the rhizoid/protochorm stage in which the spore bears both rhizoid and the first cell of a filament.

The filamentous phase of *C. barometz* occurred within about 10-15 after sowing and is indicated by formation of a 3-12-celled filament. Every cell of the filament contains chloroplasts. As stated by Nayar and Kaur (1971) spore germination results in a uniseriate, elongated, germ filament composed of barrel-shaped chlorophyllous cells and bearing one or more rhizoids at the basal end.

Spore germination in *C. barometz* of Sumatra is of the *Vittaria*-type, producing a slender, uniseriate germ filament four to twelve cells long. The *Vittaria*-type is apparently common in *Cibotium*. Chen et al. (2007) reported *Vittaria*-type and *Cyathea*-type spore germination of this golden chicken fern from China. The spore germination of *C. barometz* reported by Deng et al. (2007) also belonged to the *Vittaria*-type, the first division giving rise to the rhizoid initial is perpendicular to the polar axis of the spore and the second division yielding the protonema initial that is perpendicular to the first (Nayar and Kaur 1968) (Figure 1.B-E).

Prothallial development

The young prothallus plate of *C. barometz* was initiated in the terminal cell of the filament in about 14-30 days by perpendicular divisions. It had two phases, namely a spatulate phase which had 10-21 cells and an early heart shape stage which had 13-42 cells (Figure 2). The meristematic cells of the spatulate stage and early heart shape stage are occurred in margin of the distal gametophytes. After repeated divisions these became mature thallus (Figure 3.A, 4.C-H.). Typically, the gametophyte of *C. barometz* is a heart-shaped monolayer of cells with an apical cell as the meristem. The spores took about 30-40 days after sowing to differentiate into a cordate thallus. Usually, a prothallus plate did not form reproductive organs until 40 days after sowing. The period of prothallial development of *C. barometz* of Sumatra is not different from that observed by Deng et al. (2007) for *C. barometz* of China, as its prothallial plates formed in 25 days after being sowed.



Figure 1. A. Spores of *Cibotium barometz* (SEM photograph). B. Germinated spore, about 5-7 days after showing. C-E. Filamentous phase, 3-10-celled filament, about 10-15 after showing. Bar = 30 μ m for A, 60 μ m for B and C, 100 μ m for D, 40 μ m for E

Cibotium barometz is a homosporous fern species, producing only one type of spores with both male and female reproductive organs in the resulting gametophyte. Nayar and Kaur (1971) showed that the prothallus of homosporous ferns follows a definite pattern of development leading ultimately to the characteristic adult form. This pattern is constant for each species and common to taxa of higher order under normal conditions of growth. Nayar and Kaur (1971) recognized seven different patterns of prothallial development among homosporous ferns, viz. *Adiantum*-type, *Aspidium*-type, *Ceratopteris*-type, *Drynaria*-type, *Kaulinia*-type, *Marattia*-type, and *Osmunda*-type. These types differ in the sequence of cell divisions; in the stages of development and the region at

which a meristem is established; and in the resultant form of the adult thallus. Prothallial development of *C. barometz* is of the *Drynaria*-type (Nayar and Khaur (1971): The spores germinate into a germ filament composed of barrel-shaped chlorophyllous cells with one or more rhizoids at the base cell; One of the cells at the top margin of the prothallus (the anterior marginal cells) then divides obliquely when it has 5-10, or more cells across its width; This results in an obconical meristematic cells; Division by this type of cell is parallel to each other and perpendicular to the rest of the cells, forming rows. This results in the formation of a notch at the anterior edge of the prothallus, giving it a roughly heart-shaped appearance.

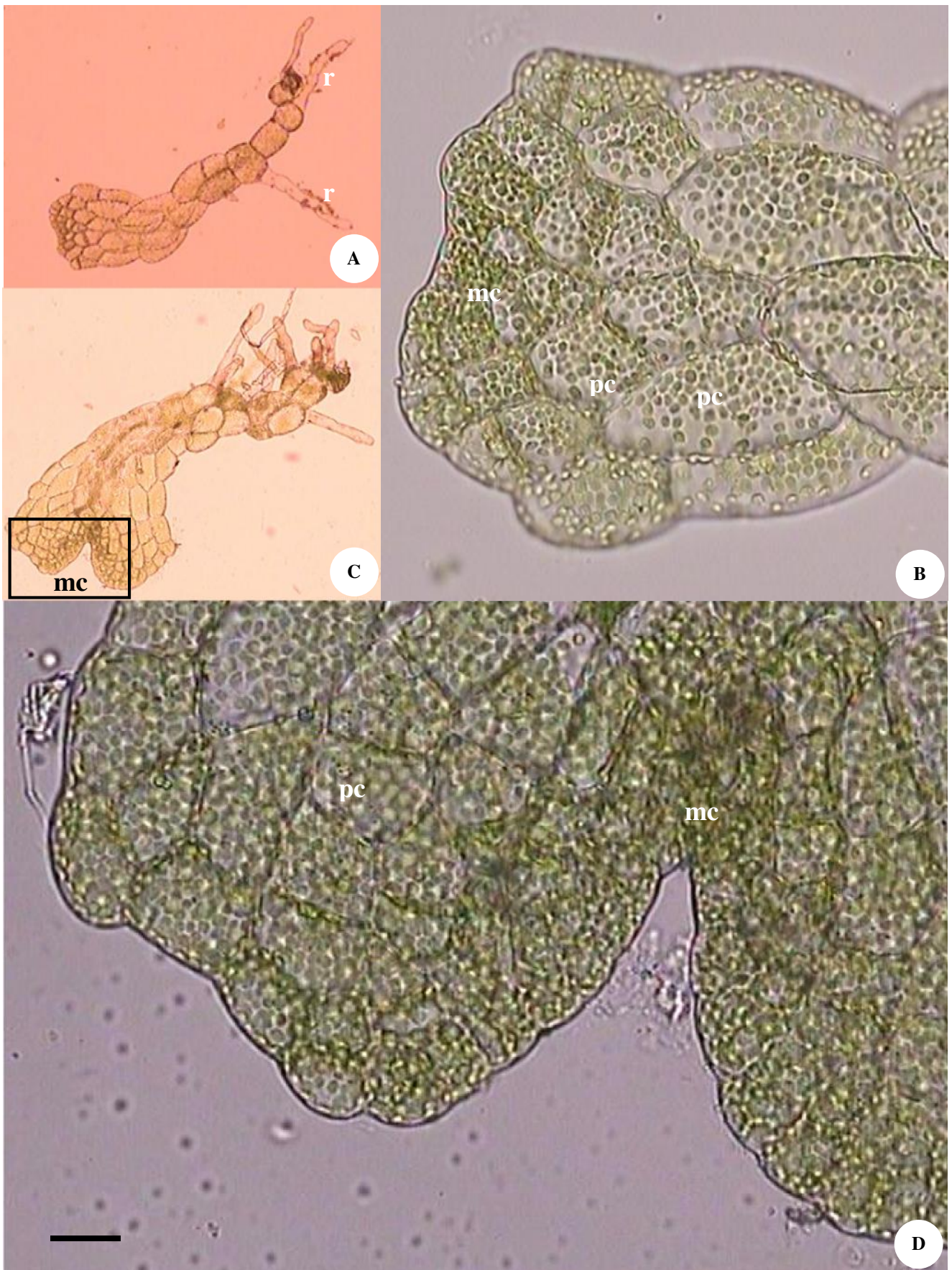


Figure 2. A-B. Young plate phase (spatulate plate), 27 days; B. Enlargement of A. C-D. Young gametophyte in elongated heart shape, after one month (38 days); D. Enlargement of C. Rhizoid (r), meristematic cells (mc), prothallial cell (pc). Bar = 120 μ m for B and D

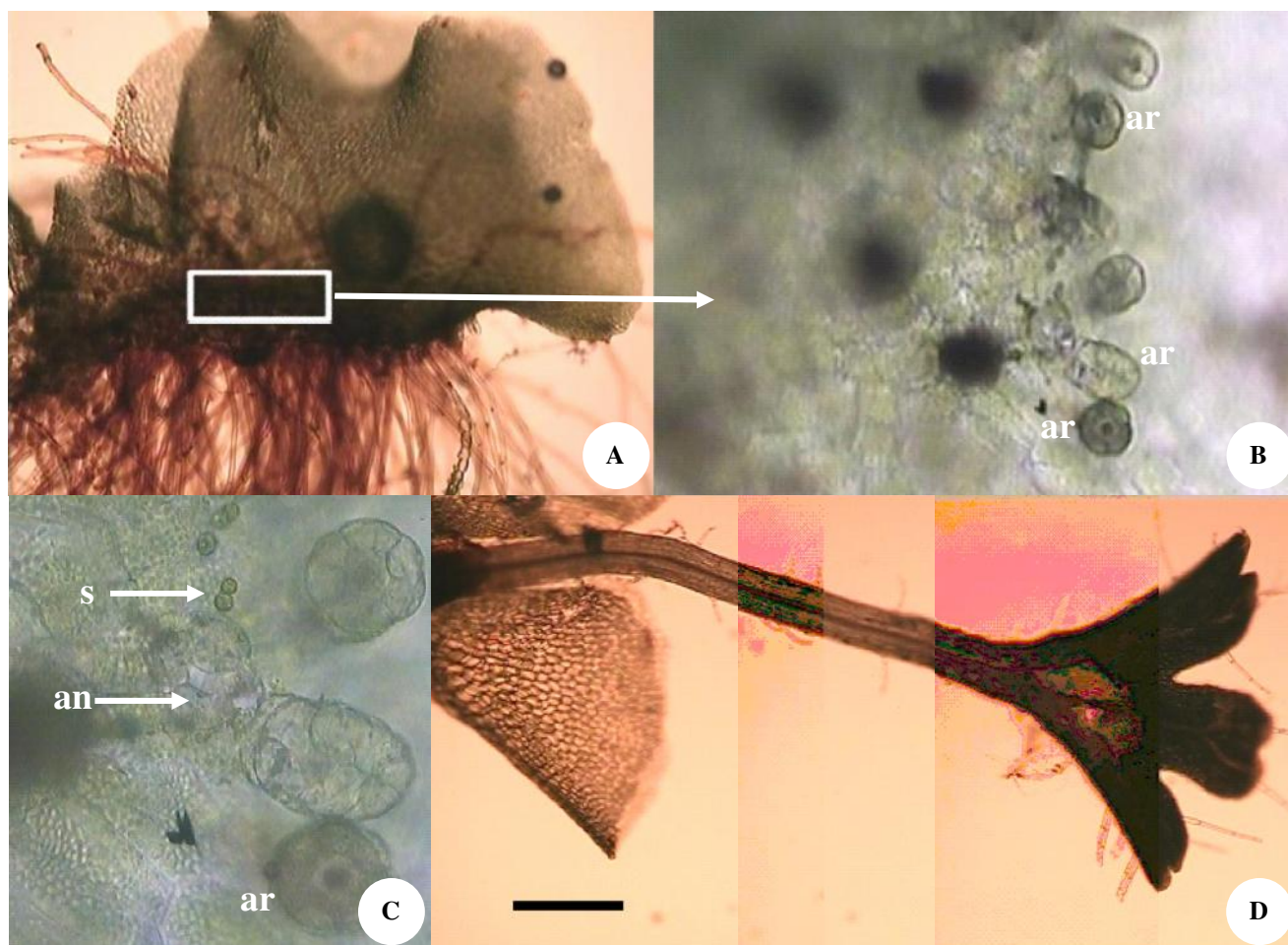


Figure 3. A. Normal heart-shape, adult gametophyte, bisexual (175 days after sowing); B. Archegonium (ar); C. Antheridium (an) releasing spermatozoid (s); D. Young sporophyte growing from gametophyte (178 days after sowing). Bar = 0.7 mm for A and D, 100 μ m for C

Morphological diversity of mature gametophytes and sex expression of *Cibotium barometz*

Mature gametophytes first appeared at around 45 days after sowing. They were male gametophytes. Mature gametophytes were indicated by the formation of reproductive organs, *viz.* male organ or antheridium and female organ or archegonium (Figure 3). Both unisexual and bisexual prothalli produced antheridia earlier than archegonia. The antheridia walls were composed of 5 cells. Mature archegonia appeared within 150-165 days after sowing.

Within two to six months after sowing spores we found the gametophyte populations in the culture consisting of male, female and hermaphroditic gametophytes. Moreover, five morphological types of adult gametophyte in *C. barometz* were observed as shown in Figure 4 and Table 1. These morphological types are clearly correlated with the gametophytes' sexual expression. Male gametophytes were of three shapes, namely scoop shaped, fan shaped and long heart shaped. On the other hand, female and bisexual gametophytes had only one shape. They were normal heart shaped.

As reported by Chen et al. (2007), normally the mature gametophytes of *C. barometz* are found to be symmetrically cordate. The presence of morphological variations of the gametophyte in the Sumatran fern of this study is presumed to be related to population density, which significantly affects gametophytes' sexual expression. As reported by Huang et al. (2004), the gametophyte size of *Osmunda cinnamomea* (Osmundaceae) is negatively related to the population density. A similar case is observed in *Woodwardia radicans* (De Soto et al. 2008). Under low density, the gametophytes of *W. radicans* mature sexually at a relatively large size and turn into females and subsequently into bisexuals. Under crowded conditions, the gametophytes of this species mature sexually at a smaller size and turn into males. How gametophyte population densities effect the shape of gametophyte? Huang et al. (2004) stated that gametophyte population density is one of the factors affecting sexual expression and growth in gametophyte communities. In overcrowded populations resources are limited by competition, and gametophytes are often asexual or male, and narrow (usually spatulate

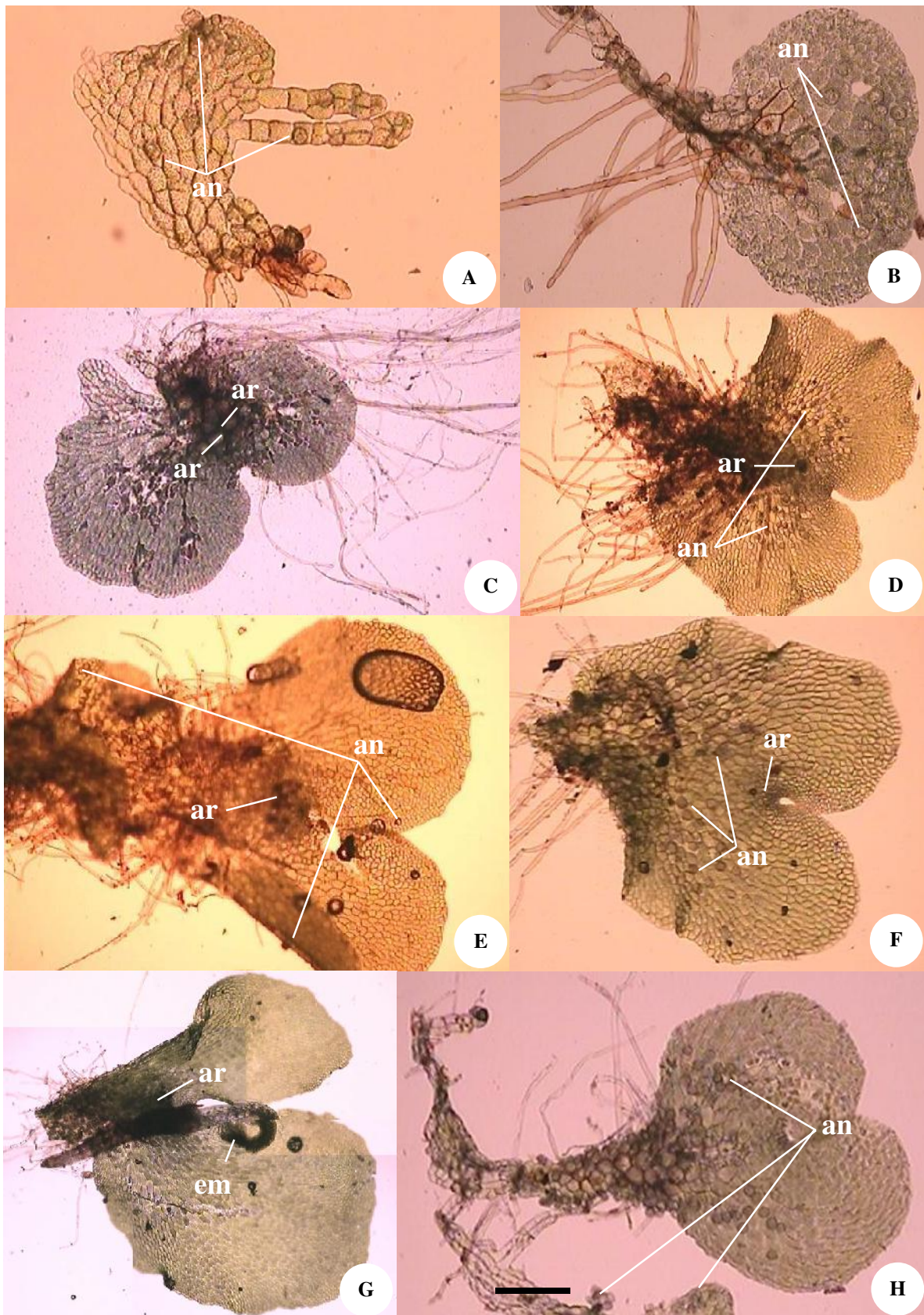


Figure 4. Morphological diversity of adult gametophytes of *C. barometz*. A. Irregular shape (male, type A); B. Fan shape (male, type B); C. Short heart shape or butterfly shape (Female, type C); D. Normal heart shape (bisexual, type D); E. Normal heart shape (bisexual, type D); F. Normal heart shape (bisexual, type D); G. Normal heart shape (bisexual) showing embryo; H. Elongated heart shape (male). Bar = 0.6 mm for all. an = antheridium. ar: archegonium. em: embryo

Tabel 1. Description of mature gametophyte types in *C. barometz*

Type	Description	Average size (mm)	Sex expression
A	Irregular scoop shape with filamentous branches: antheridia dispersed everywhere (Figure 4.A).	2 x 1.5	Male
B	Fan shape: antheridia dispersed one third from margin of wings area (Figure 4.B).	2 x 2	Male
C	Short heart shape or butterfly shape: archegonia were formed at thick cushion, on the anterior part of the midrib (Figure 4.C).	2.5 x 1.5	Female
D	Normal heart shape: Archegonia were produced on the anterior part of the midrib as it approaches the meristem; antheridia were produced on the wings and midrib of the ventral side (Figure 4.D. and 4.E-G).	4 x 4	Female, bisexual
E	Long heart shape: antheridia dispersed irregularly on the ventral side of wings (Figure 4.H).	5 x 3	Male

shape). Female and hermaphroditic gametophytes, on the other hand, often occur in sparse populations (Klekowski and Lloyd 1968; Cousens and Horner 1970; Lloyd and Gregg 1975; Cousens 1979). The effects of high gametophytes population density were summarized by Smith and Rogan (1970). Crowded conditions may alter the normal sequence of development of fern gametophytes. Increasing population density leads to an increase in gametophyte length and delay in the transition to two dimensional growths.

The variation of sex expression in *C. barometz* also indicates that this species may has a mixed mating systems (self-fertilization, intergametophytic selfing and intergametophytic crossing). However, some species of ferns do show mixed mating systems (Soltis and Soltis 1987, 1988), and by now several studies have indicated that mating systems on some species may vary greatly even between different genotypes within species (Peck et al. 1990; Suter et al. 2000; Wubs et al. 2010). The existence of mixed mating systems in this species will result in genetic diversity within population and among populations. As reported by You and Deng (2012), *C. barometz* in China showed 41.06% of genetic diversity among populations and 58.94% of genetic diversity within populations. Rugayah et al. (2009) also confirms the existence of genetic variation in *C. barometz* of Sumatra three morphological variants of *C. barometz* were reported from West Sumatra.

Most studies on gametophyte development and reproductive biology of tree ferns, including *C. barometz*, are carried out *in vitro*, using agar media in petri dishes (see Khare and Srivastava 2009; Khare et al. 2005; Chen et al. 2007; Li et al. 2010). Our study, using sterilized natural media, shows that all stages from spore germination to development of gametophyte, and from rhizoid stage to mature prothallial stage, are not significantly different from those obtained in studies using agar media in petridishes *in vitro*. The results of this study gives proof that a sterilized natural media consisting of minced roots of *Cyathea contaminans* with charcoaled rice husks (1: 1) mix can be used as an alternative medium for studying the development and morphology of gametophytes of a tree fern. This information is very important for *ex situ* conservation of ferns

In conclusion, six stages of gametophyte development (rhizoid stage, rhizoid/protochorm stage, filament stage, spatulate stage, young heart stage, mature heart stage) were

observed 24-45 days after sowing. The first cell produced after spore germination was rhizoid. Spore germination of *C. barometz* is *Vittaria*-type, producing a slender, uniseriate germ filament, four to twelve cells long. Prothallial development of *C. barometz* is *Drynaria*-type. The young prothallus plate was initiated in the terminal cell of the filament, approximately 14-30 days after sowing, by perpendicular division. Antheridia and archegonia were first observed at 45 and 150 days after sowing, respectively. Five morphological types of adult gametophyte were recorded: (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). The presences of morphological variations on the gametophyte are presumed to be related to the population density, which significantly affects gametophytes' sexual expression. *Cibotium barometz* may has a mixed mating systems (self-fertilization, intergametophytic selfing and intergametophytic crossing) that resulted in genetic diversity among population and within population. A sterilized natural media consisting of the minced roots of *Cyathea contaminans* with charcoaled rice husks (1: 1) mix can be used as an alternative medium for studying the development and morphology of gametophytes of the tree fern. The present study contributes to the understanding of the full life-cycle of *C. barometz*, providing information for cultivation, management and conservation of the species.

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