

GAMETOPHYTES OF THE BIRD NEST FERN *Asplenium nidus* L. (ASPLENIACEAE) FROM WEST KALIMANTAN

Gametofit paku sarang burung *Asplenium nidus* L. (Aspleniaceae)
dari Kalimantan Barat

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Abstrak

Gametofit *Asplenium nidus* L. dari Kalimantan Barat diamati dengan mengecambahkan spora pada media campuran akar paku pohon dan arang sekam (1:1). Penelitian ini bertujuan untuk mendapatkan bukti taksonomi baru untuk mendukung keberadaan jenis kompleks dalam *A. nidus*. Spora coklat tua simetris bilateral yang berkecambah menghasilkan benang berklorofil yang terdiri atas 4-6 sel. Rizoid berkecambah pada sisi polar, sedangkan benang berklorofil pada sisi ekuator. Pembentukan piringan berawal pada sel sub terminal dari benang yang berkecambah pada hari ke-12 dengan pembelahan sel secara mencong. Rizoid terbentuk lagi pada sel-sel pangkal atau semi pangkal pada piringan. Gametofit muda berbentuk jantung panjang dan anteridium muda mulai timbul pada umur 60 hari. Gametofit dewasa menjantung dan menghasilkan anteridium dewasa mulai umur 75 hari. Gametofit hermaprodit dan betina tidak ditemukan. Sporofit muda mulai muncul pada umur 90 hari. Hasil penelitian menunjukkan bahwa ciri-ciri morfologi gametofit menambah bukti taksonomi dalam mendukung keberadaan jenis kompleks atau jenis tersembunyi dalam *A. nidus*.

Kata kunci: *Asplenium nidus*, perkembangan gametofit, paku sarang burung, Borneo.

INTRODUCTION

Gametophyte observation on Malesian ferns are limited. There are only some species reported by several authors. Atkinson (1970) has studied gametophyte of *Pleocnemia conjugata* and *Arcypteris irregularis* from New Guinea and Malay Peninsula respectively. Gametophyte of *Diplazium simplicivenium* of Malaya was reported by Atkinson (1967).

Studies on gametophytic morphology may useful in recognizing the relationship among closely related

species of ferns and therefore these characters can be used in verifying the taxonomic status of species. Gametophyte characteristics in *Pleocnemia* and *Arcypteris* indicated close relationships to each other than to other members of Holttum's Tectarioideae, and supported separation of *Pleocnemia* and *Arcypteris* from *Tectaria* (Atkinson, 1970). On the study of gametophyte of some Jamaican thelypteroid ferns, Atkinson and Stokey (1973) showed that gametophytic characters supported the separation of *Gonioperis* from *Menischium*. The removal of *Dryopteris stellato-pilosum* Brause from genus *Dryopteris*, which then placed in

genus *Diplazium* as *Diplazium stellato-pilosum* (Brause) Holttum, is strongly supported by the morphology of gametophyte (Atkinson, 1967). A taxonomy of the gametophytes based on antheridium morphology corresponds very closely to the classification of subgenus *Equisetum* into three sections based on the coning behavior of the sporophytes (Duckett, 1973).

Asplenium is one of the large fern genera that consists of more than 700 species. The genus distributes all over the world, some species even extending into arctic and arid areas. It is a cosmopolitan genus, and one of the very few that represented about equally in all tropical areas (c. 30% in the neotropics, c. 22% in Africa, c. 33% in Asia, c. 10% in the Pacific (including Australia, etc.), c. 5% in Europe *s.l.*) (Kramer and Green, 1990).

Asplenium nidus L. is one of the most common and well known epiphytic fern species with large simple leaves, which occurs in the Old World tropics. The species is a member of section *Thamnopteris* (Holttum, 1974; Nakaike, 1975). It can be differentiated from the close related species, *A. antiquum* Makino and *A. australasicum* (J. Smith) Hooker, by its flat undersurface of rachis and petiole, narrow lanceolate scale, and short and dense sorus.

Study on gametophyte development of *A. nidus* has been initiated by Momose (1968) using spores collected from Trang, Thailand. However, *A. nidus* is a complex species. Molecular study on *A. nidus* complex from Gunung Halimun National Park using *rbcl* gene sequences has been reported by Muarakami *et al.* (1999a, b) and Yatabe *et al.* (2001). They suggested that *A. nidus* consists of several different reproductively isolated cryptic species. This paper presents the first observation on the gametophyte of *A. nidus* from West Kalimantan. The aim of the study is to obtain new taxonomical evidence in supporting the existence of the complex species of *A. nidus*.

MATERIALS AND METHODS

Fresh mature spores of *A. nidus* were collected from Dusun Ketiat, Cipta Karya Country, Sungai Betung Subdistrict, Bengkayang District, West Kalimantan on February 2009. The voucher specimen (No. Coll.: NI s.n.) was deposited in the Herbarium of Bogor Botanic Garden (BOHB). The fresh spores were germinated on

the mixture of tree fern roots and husk charcoal (1:1), in crowded spore cultures in the plastic box covered by transparent plastic on daylight in the glass house. Active charcoal is an effective agent on the growth and morphogenesis of ferns gametophyte. Therefore it does not influence 2-dimensional growth (Kato, 1973). The temperature, humidity, and illumination in the glass house were 26.1-29.8°C, 73-93%, and 1140-7380 lux, respectively.

Spores were sowed on March 5, 2009. First observation was carried out on 12 days after sowing and observation was carried out every 5-6 days. The crowded masses of prothallia were separated under binocular microscope of Nikon SMZ-10A. Preparative slides were prepared using water or 10% glycerin. Photographs were taken using microscope (Olympus CX 31). The data described here were based on examination of 5-10 slides containing 1-10 or more protonema or gametophytes per slide per observation.

RESULTS

Spore Germination. Most of spores were germinated in the sporangium (Fig.1A). The bilaterally symmetrical dark brown spore developed into 4-6-celled chlorophyllous filaments (Fig. 1B-C.). The filaments with 4-5 cells were the most common. The terminal cell was deltoid. Spores which germinated within the sporangium produce a longer filament. Rhizoid was germinated at the polar position, while the filament is at the equatorial position. The rhizoid was colorless or pale brown (Fig.1 B-C).

Gametophyte Development. The plate was initiated in the sub terminal cell (below the deltoid terminal cell) of the germinating filament on 12 days old by oblique division (Fig. 1D-E). The very young plate (Fig. 1 F-G) is sword-shaped; meristematic cells are located below or beside of the deltoid cell. The meristematic cells subsequently divided into isodiametric polygonal cells (common cells) and deltoid terminal cells to develop an irregular spatulate plate with free side of the marginal cells in sharply acute (Fig. 1 H-J) after 29 days. The free side acute marginal cells will be decreased and disappeared when the plates increased in age and size.

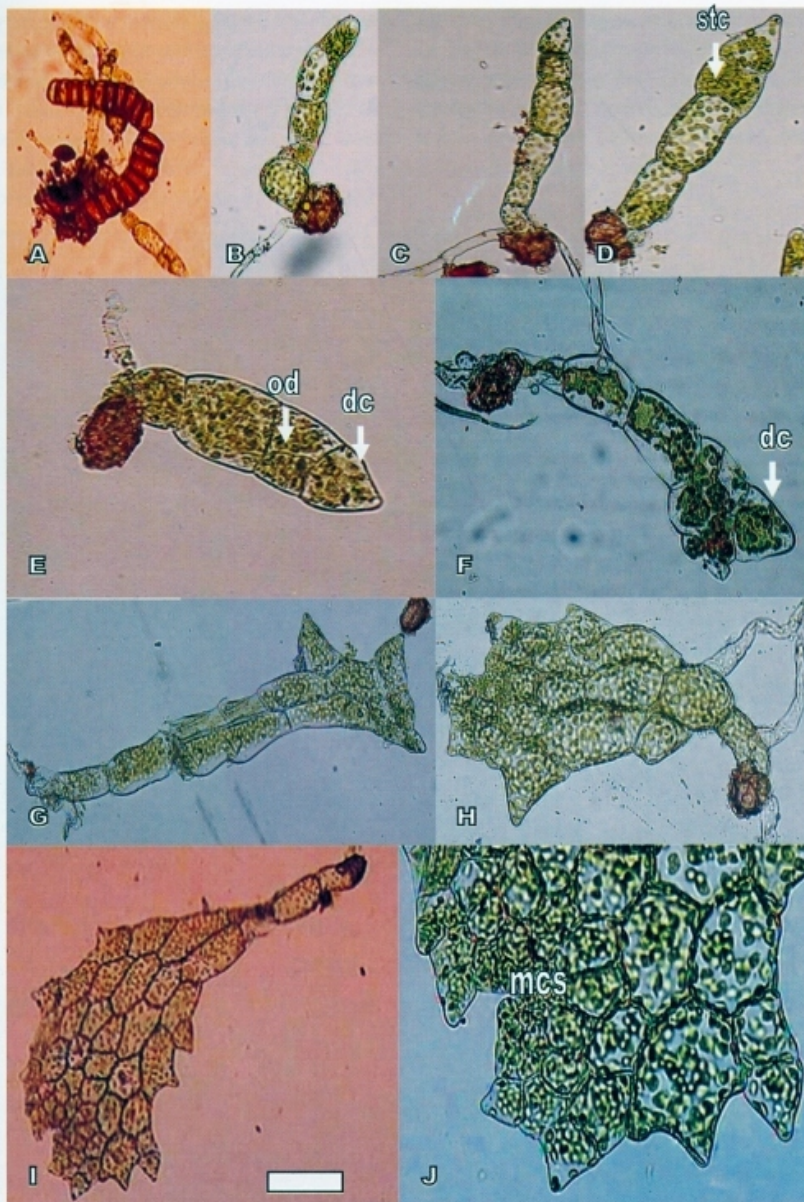


Figure 1. Spore germination and early gametophyte development of *Asplenium nidus*. (A) Spores germinate in the sporangium; (B-C) The bilaterally symmetrical dark brown spore gives rise to a 5-6-celled chlorophyllous filament. (D-E) The subterminal cells of filament divide into two cells by oblique division (od). (F-G) Terminal and subterminal cells of the filaments divide simultaneously forming irregular young spatulate gametophyte (25 days old). (H-J) Young spatulate-hearts shaped gametophyte (29 days). dc = deltid cell; stc = subterminal cell; msc = meristematic cells. Bar = 960 μ m for (A), 64 μ m for (B and C), 70 μ m for (D to F), 96 μ m for (G and H), 135 μ m for (I), 60 μ m for (J).

Sexual expression of *A. nidus* begins with the appearance of antheridium. Prothalli developed antheridia after 60 to 75 days, while archegonia were not formed (Fig. 2-3). These male gametophytes were long heart shape when young and cordate

when mature. Antheridium emerged in the one third of lower midrib to middle midrib between the rhizoid and meristem. The full description of the mature gametophyte is presented as follow.

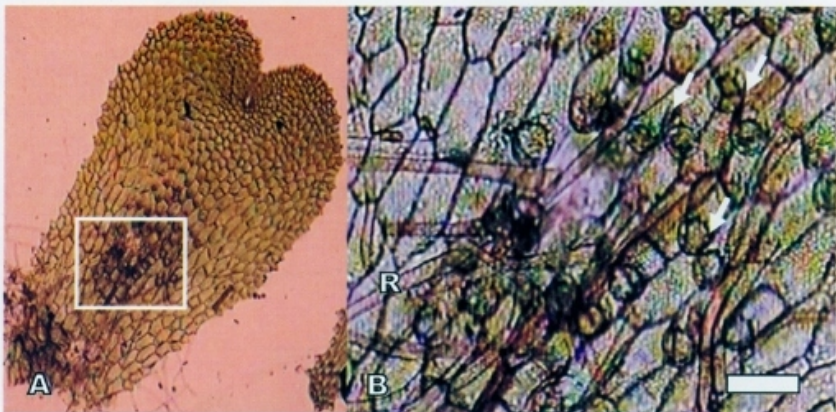


Figure 2. (A) A long heart shaped of young gametophyte. (B) The figure is magnification of A, marked by the white rectangle. Pale brown rhizoid (R), young antheridium (arrow), 63 days old. Bar = 480 μ m for (A), 180 μ m for (B).

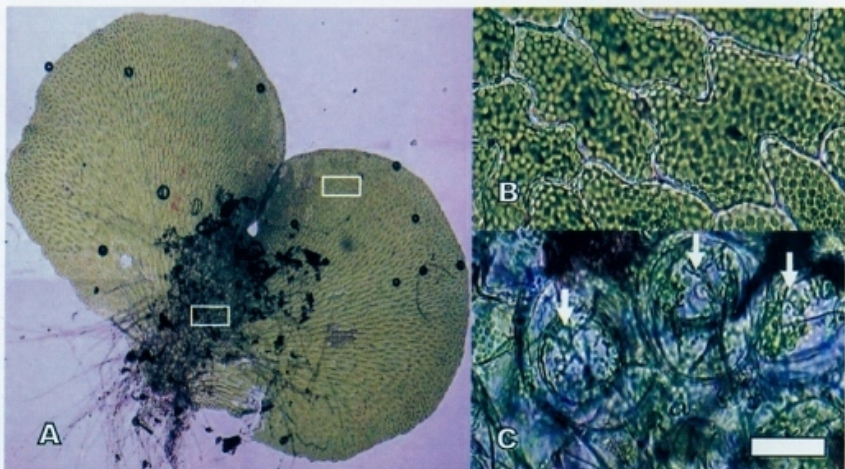


Figure 3. (A) Cordate shaped of mature male gametophyte with a dense rhizoid (68 days old). (B) Magnification of upper white rectangle of A; Isodiametric cells with almost straight side wall and curved/waved side wall composing the mature gametophyte wing; (C) Magnification of lower white rectangle of A; Mature antheridia with spirally coiled antherozoids (arrow), top view. Bar = 0.8 mm for (A), 48 μ m for (B and C).

The description of mature gametophyte. Mature gametophyte bilateral, broad heart-shaped, almost flat; apex deeply rounded and acutely cordate, inner sides of lobes approaching together, sometimes overlapped, closed or opened above the sinus; lower part of gametophyte rounded, steeply narrowing toward base. Wings equal in size, broadwise or upper sidewise expanded, margin almost entire; wing cells isodiametric polygonal with almost straight side wall or curved side wall; marginal cells smoothly arranged, isodiametric or elongate polygonal with

flat or concave free side. Midrib cushioned from the middle. Rhizoid superficial, on the lower part of gametophyte spreading aside to wings and nesting above the cushion, light brown in color. Antheridia are on all over the chusion; neck rather large and thick, archegonia are absent.

Sporophyte Formation. Very young tiny sporophytes (sporeling) were emerged after 90 days. They were formed at the middle midrib of the gametophytes among rhizoids and antheridia (Fig. 4).

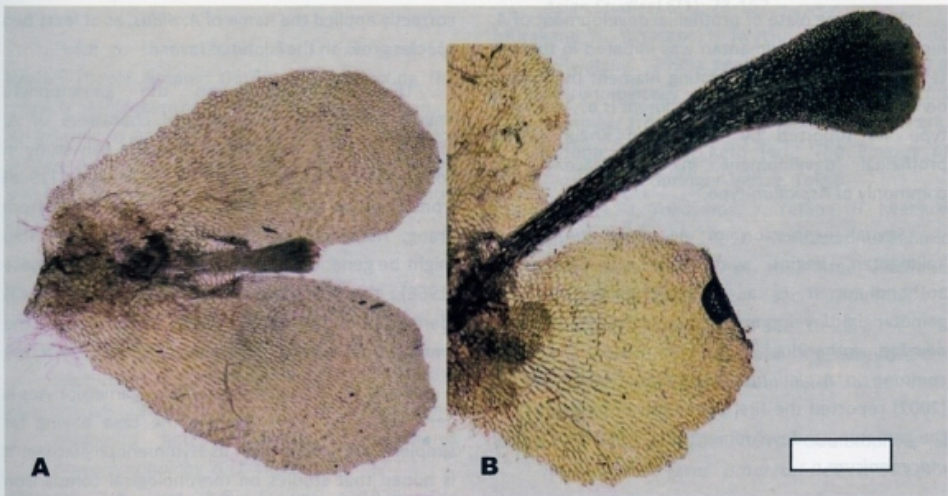


Figure 4. Young sporophyte of *A. nidus*. (A) An old gametophyte with young sporeling, 90 days old. (B) 96 days old of young sporeling. Bar = 1 mm for all.

DISCUSSION

Spore germination of *A. nidus* is *Vittaria* type, producing a proximal rhizoid and a uniseriat germ filament lateral to it. As reported by Nayar and Kaur (1971), most of species of Aspleniaceae have spore germination of *Vittaria* type. The bilaterally symmetrical dark brown spore gives rise to a 4-6

celled chlorophyllous filament. The filament with 4-5 cells was the most common. It is different to *A. nidus* of those described by Momose (1968) of Thailand in which the filaments were only consisting of 2-4 cells. The gametophyte development and morphological characters differences of *A. nidus* between the material from Trang, Thailand (Momose, 1968) and of the present study are presented on Table 1.

Table 1. The gametophyte development and morphological characters differences of *A. nidus* between plant material from Trang, Thailand (Momose, 1968) and the present study.

Characters	<i>A. nidus</i> of Trang, Thailand (Momose, 1968)	<i>A. nidus</i> of West Kalimantan (Present study)
Filament/protonema structure	2-4 cells	4-6 cells
Wing cells shape of mature gametophyte	Isodiametric polygonal with almost straight side wall	Isodiametric polygonal with almost straight side wall and curved or waved side wall
Sex type/Sexual expression	Bisexual/Hermaphrodite	Monosexual/Male

The young plate of prothallial development of *A. nidus* from West Kalimantan was initiated in the sub terminal cell of the germinating filament by oblique divisions. This prothallial development is of *Aspidium*-type. As reported by Nayar and Khaur (1971) prothallial development in *Asplenaceae* are commonly of *Aspidium*-type.

Sexual expression of *A. nidus* from West Kalimantan begins with the appearance of antheridium. It is a common pattern in the gametangial development. Four weeks age prothalli develop antheridia. This phenomenon might be common in *Asplenium*. In contrast, Herrero *et al.* (2002) reported the first formation of archegonia in the gametangial development in *A. foreziense* and *A. macedonicum*.

The mature gametophyte of *A. nidus* of Thailand give rise bisexual gametangial or hermaphrodite (Momose, 1968). On the other hand, *A. nidus* from West Kalimantan showed only unisexual gametophytes, namely male gametophyte, until the formation of young sporophyte with one leaf. Young sporophyte was formed on the midrib among the antheridium and rhizoids.

Johns (1996) stated that *A. nidus* is a species complex which consists of several closely related taxa. *Asplenium nidus* was originally collected from Java in 1750. The type specimen of this species is infertile and also lacks the base of the frond. Thus, the scales of the stipe which can also be diagnostic feature of the species were not existed. Johns (1996) recommended that field studies are required before

correctly applied the name of *A. nidus*, as at least two species grow on the island of Java.

Three differences on the gametophyte development and morphological characters of *A. nidus* from West Kalimantan shown in this study in comparison with those reported by Momose (1968) from Thailand give an indication that *A. nidus* from Trang, Thailand, and those from West Kalimantan might be genetically different. As noted by Momose (1968), the gametophyte of Trang, Thailand, is identical with that of the plants in the Bonins, the Ryukyus and Yukushima of Japan.

Raine *et al.* (1996) showed that gametophytes is very useful in systematic for the taxa having far simpler sporophytes such as *Hymenophyllaceae*. It is hoped that studies on morphological comparison on the gametophytes would be useful in revealing complex species or cryptic species of the simple frond species *A. nidus*. Therefore, further morphological comparison studies on the gametophyte of *A. nidus* complex from its distribution range are needed.

Conclusions

Spore germination of *Asplenium nidus* of West Kalimantan is *Vittaria*-type. Rhizoid is germinated at the polar position, while the filament is at the equatorial position. Four to six-celled formed the chlorophyllous filaments. Prothallial development is of *Aspidium*-type. The young plate is initiated in the sub terminal cell of the germinating filament in about

two weeks (12 days) by divisions oblique. Mature gametophyte is cordate and bear mature antheridium after 75 days. Hermaphrodite and female gametophyte were not found. Young sporophyte was appeared after 90 days old of germination and formed at the midrib gametophyte among rhizoids and antheridia. This study gives an indication that studies on morphological comparison on the gametophytes is useful in revealing species complex of *A. nidus* and the existence of cryptic species in this species.

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