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Spore germination and early gametophyte development of *Platycerium wandae* (Polypodiaceae) from Papua, Indonesia

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Abstract. *Praptosuwiryo TNg. 2017. Spore germination and early gametophyte development of* Platycerium wandae *(Polypodiaceae) from Papua, Indonesia. Biodiversitas 18: 175-182.* Studies on gametophyte development in ferns are very important, as the data generated is often useful for supporting taxa delimitation in ferns and fern-allies. This data is also very important for understanding the ecology, reproductive biology, evolution and distribution of ferns. The study reported here aimed to develop a deeper understand of morphogenesis in the fern *Platycerium wandae* Racib. by investigating the process of spore germination and early gametophyte development of the species on natural media. Fresh spores of *P. wandae* were germinated in a mixed media consisting of minced roots of *Cyathea contaminans* and charcoaled rice hulls (1: 1) under green house condition. Spores of *P. wandae* are monolete, ellipsoid, non-chlorophyllous, dark brown, and lacking perine. Spores germinated between 7 to 14 days after sowing (DAS). Spore germination of *P. wandae* is of the *Vittaria*-type and the prothallial development is of the *Aspidium*-type, characterized by early development of unicelullar trichomes (30-40 DAS). Only unisexual gametophytes were observed at 60-80 DAS. New data is provided concerning the morphogenesis of *P. wandae* from Papua, Indonesia, on natural media. The findings are relevant to reproductive biology, would contribute to establish an efficient *ex situ* propagation strategies for the conservation of epiphyitic ferns and to facilitate further *in situ* studies of gametophyte ecology.

Keywords: Gametophyte development, *Platycerium wandae*, spore germination, staghorn fern

INTRODUCTION

Gametophyte morphogenesis, including type of spore germination, early gametophyte development, and mature gametophyte trichomes and gametangia, has proven useful in characterizing fern taxa (Nayar and Kaur 1971; Prada et al. 1996; Huang et al. 2001; Pangua et al. 2003; Chen et al. 2008; Muñiz-Díaz de León et al 2008; Praptosuwiryo 2010; Puspitasari et al. 2015). These characters provide evidence of variations in the pattern of development, which are important criteria in fern taxonomy (Pryer et al. 1995). For example, *Lophosoria quadripinnata* (Gmel.) C. Chr. var. *quadripinnata* and *L. quadripinnata* var. *contracta* (Hieron.) R. & A Tryon. differ in prothallus shape and the number of antheridial cells (Mendoza et al. 1997). Gametophytes of *Asplenium obovatum* ssp. *obovatum* var. *protobillotii* and var. *deltoideum*, ssp. *numidicum*, and of *A. macedonicum* show significant differences in hair density (Herrero et al. 2002). *Asplenium scolopendrium* var. *americanum* L. and *A. scolopendrium* var. *scolopendrium* differed significantly in gametophyte ontogeny, morphology, and propensity for sexual and asexual reproduction. *Asplenium scolopendrium* var. *americanum* produced copious gametophytic outgrowths that were capable of developing into functional, independent thalii while *A. scolopendrium* var. *scolopendrium* does not (Testo and Watkins 2011). Comparative morphology of the fern gametophyte can also be an important tool in understanding different phyletic groups (Stokey 1951; Pryer et al. 1995). Therefore, further studies to understand the development of fern gametophytes are recommended to clarify taxonomic uncertainties.

Studies on gametophyte morphogenesis are also useful for in situ studies on the ecology of fern gametophytes (see Watkins et al 2007). These formed the basis for field identification of gametophytes, especially to the level of genus, and laid the groundwork for future gametophyte-based ecological studies (Farrar et al. 2008). Therefore integrative studies combining gametophyte development, morphology, and breeding system to understand the ecology and distribution of species would be promoted (Farrar 1967; Dassler and Farrar 1997, 2001; Chiou et al 1998; Chiou and Farrar 2002).

The staghorn fern genus, *Platycerium* Desv., is one of the most commonly grown ornamental ferns (Hoshizaki and Moran 2001; Darnaedi and Praptosuwiryo 2003). It is a very distinct genus of the family Polypodiaceae and differs from other ferns in the presence of stellate hairs (shared with *Pyrrosia* Mirbel) on the leaf lamina, the frond dimorphism, the differentiation of the leaves into litter collectors (mantle leaves or base fronds) and 'dichotomously forked trophosporophylls with coenosoroid to acrosoroid patches of sporangia' (Hennipman and Roos 1998; Hoshizaki and Moran 2001). *Platycerium* is one of the few pantropical epiphytic fern genera, with 18 species. Six species are found in Afro-Madagascar, 8-11 in subtropical to tropical Asia, Malesia, and Australia, and a single species in tropical South America (Kreier and

Schneider 2006). These plants grow predominantly as epiphytes or sometimes on rocks in subtropical to tropical lowland forests (Kreier and Schneider 2006; Hennipman and Roos 1998). They are among the most frequent vascular plant epiphytes in those forests and generally grow in relatively open conditions (Benzing 1990; Kreier and Schneider 2006; Hennipman and Roos 1998). These plants frequently have been used in cytological, morphological, developmental, physiological, and phylogenetic studies (see Hoshizaki 1970, 1972; Nagmani and Raghavan 1983; Kwa et al. 1995, Camloh et al. 1996, 1999; Teng and Teng 1997; Ambrožič-Dolinšek et al. 2002; Kreier and Schneider 2006; Espinosa-Matias et al. 2007; Janssen et al. 2007; Rut et al. 2008; Aspiras 2010) because they have great economic value and a special place among ferns (Camloh and Ambrožič-Dolinšek 2011). Staghorn ferns are becoming threatened in the wild because they are often collected by plant collectors for their majestic size and form, and are traded locally (Madulid 1985). Moreover, these ferns have spores that are difficult to germinate under natural conditions, further endangering their survival (Amoroso 1990, 1992; Amoroso and Amoroso 1998, 2003). Therefore, studies are required to find out differences in the developmental patterns of the gametophytes and sporophytes of this genus, and especially to inform the practice of *ex situ* conservation in this fern.

Indonesia has four species of *Platycerium*, namely *P. bifurcatum* (Cav.) C. Chr., *P. coronarium* (Konig ex Muller) Desv., *P. ridleyi* H. Christ, and *P. wandae* Racib. (Hennipman and Roos 1998). *Platycerium wandae* Racib. is differentiated from others species of *Platycerium* by the following characters: (1) Leave foliage is asymmetric with unequal lobes; (2) Soral patches are situated in sinuses near the base of the leave; the ultimate lobes are sterile; (3) Lateral soral patches have very short, simple, lateral sterile lobes (Hennipman and Roos 1998) (Figure 1.). *Platycerium wandae* are heliophilous through to semi-shade-tolerant epiphytes, growing solitary, high, in dry lower montane rain forest and lowland swamp forest, also in lowland areas in rubber and coconut plantations, and on waysides trees at altitudes from sea level to 1000 m (Joncheere de 1968; Hennipman and Roos 1998). This species is distributed in the eastern end of Malesia, *viz*., Moluccas, Aru Islands and New Guinea (Joncheere de 1968; Hennipman and Roos 1998).

Studies on the gametophytes of *Platycerium* were initiated by Bauke (1878) and Stokey and Atkinson (1954). Recently, studies on the spore germination and gametophyte development of *Platycerium* were reported on *P. andinum* (Espinosa-Matias et al. 2007; Rios et al. 2015), *P. bifurcatum* (Camloh 1993, 1999; García et al. 2013), *P. coronarium* (Awan and Rao 1981; Aspiras 2010), *P. grande* (Amoroso and Amoroso 2003; Aspiras 2010), *P. holttumii* (Manitayakul et al. 2006), *P. wandae* (Espinosa-Matias et al. 2007), and *P. wallichii* (Wang et al. 2011). Research relating to gametophyte morphology of *Platycerium* in Indonesia has not yet been reported in the literature. In addition, all of the works mentioned above were carried out by growing spores under in vitro condition on agar medium with nutrient solution. Natural media may be useful for studying normal spore germination and gametophyte development of the staghorn ferns. Mixed natural media composed of the minced roots of *Cyathea contaminans* together with charcoaled rice hulls has proven to be useful in studying spore germination and gametophyte development of *Asplenium nidus* (Praptosuwiryo 2010) and *Cibotium barometz* (Praptosuwiryo et al. 2015).

The aim of this study was to describe spore germination and early gametophyte morphological development of *Platycerium wandae* Racib. by germinating the spore on natural media −a mixed media of minced roots of *Cyathea contaminans* together with charcoaled rice hulls (1:1). This simple technique enabled the life cycle of *Platycerium* to be observed under more natural conditions than in in vitro methods. The purpose of the study was to provide information about gametophyte morphogenesis of *P. wandae* of Indonesia. Studying spore germination and gametophyte development among species of *Platycerium* should provide evidence on variation in their morphological patterns, and for alternative strategies for the *ex situ* conservation of epiphytic ornamental ferns. Studies on spore germination and gametophyte development of epiphtytic fern by using natural media would also be useful for *in situ* ecological gametophyte research which is critical to ultimately understanding sporophyte distributions.

MATERIALS AND METHODS

Spore collection, media preparation and spore germination

Fresh spores were collected directly from the personal living fern collection of Prof. Dr. Eko Baroto Walujo (Herbarium Bogoriense, Botany Division, Biology Research Center, Indonesian Institute of Sciences). This living specimen, collection number EBW s.n., was originally collected from Mount Meja, Manokwari, Papua (Irian Jaya), Indonesia.

Natural media was prepared consisting of minced roots of *Cyathea contaminans* together with charcoaled rice hulls (1: 1), mixed with water. A layer of mixed media 4-5 cm high was poured into a plastic box (35 x 25 x 11 cm^3). Hot water (about 100˚C) was poured over the media to sterilize it. The plastic box containing the mixed media was covered with transparent plastic and let stand for 24 hours for cooling down. Fresh spores shed from an unsterilized foliage frond were directly taped over the surface of media, and the plastic box was then covered with transparent plastic. The culture box was kept in a glass house at a temperature 25-32.5ºC with 68-85% relative humidity.

Observations of spore germination and gametophyte development

Observations were carried out every 5-6 days and terminated 80 days after sowing (DAS). Spore germination was defined as the emergence of a rhizoid through its spore coat (Camloh 1993). Early gametophytes were classified according to the gametophyte development stages described

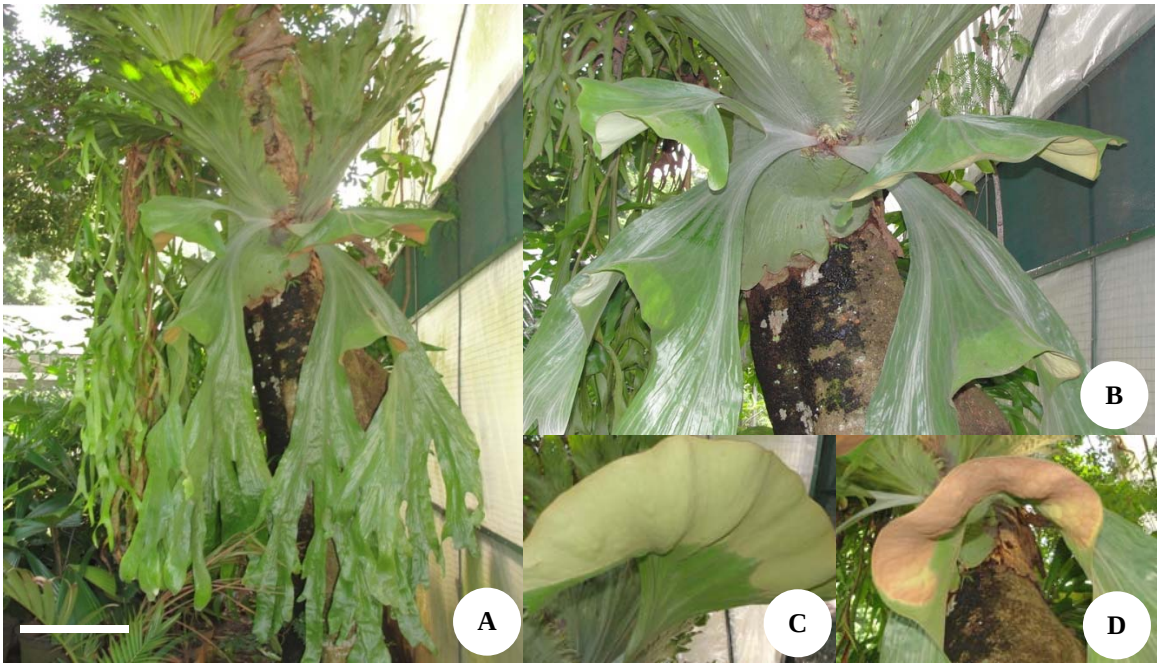

Figure 1. Habit and gross morphology of *Platycerium wandae*. A. *Platycerium wandae* of Papua cultivated in Bogor Botanic Gardens; B. Fertile patch showing its adaxial surface; C-D. Fertile patch showing its abaxial surface; C. Young fertile patch; D. Mature fertile patch. Scale bar = 30 cm for A.

by Rechenmacher et al. (2010): viz. gametophyte with chlorocyte and rhizoid (chlorocyte and rhizoid stage); filamentous gametophyte (filamentous stage); and laminar gametophyte (laminar stage). The laminar stage was divided into three sub-stages: *viz*. spatulate stage, lopsided stage, and young heart shape stage. In the young laminar stage, reproductive organs (antheridium and/or archegonium) have not yet formed, or if they have formed the organs are still immature.

The assemblage of prothalli were separated and observed under Nikon binocular microscope (Nikon SMZ-10A). In order to document spore germination and gametophyte development, photographs were taken using the Olympus microscope connected to a computer monitor camera (Olympus CX 31) fitted with objective lenses 4X-40x.

RESULTS AND DISCUSSION

Spore germination

For sporulating plants, germination is defined as the set of mechanisms occurring in the dormant spore that culminates in the growth of the embryo or cell to form a sporeling able to establish in the substrate (Gabriel y Galán et al. 2015). Germination can easily be detected by observing signs of a body emergence, such as a filament/prothallial cell or rhizoid or both simultaneously (Nester and Coolbaugh 1986; Bradbeer 1988; Pérez-García et al. 2010). Spores of *Platycerium* species usually germinate between 11-19 DAS under in vitro conditions (Table 1.). According to Manitayakul et al. (2006), spores of *Platycerium holttumii* Jonch. & Hennipm. require 14 days from sowing to emergence of the rhizoid. Spores of *P. grande* (Fee) C. Presl. germinated 19 DAS under in vitro condition in the study of Amoroso and Amoroso (2003). *Platycerium coronarium* (Koenig.) Desv. and *P. grande* required 11.50 and 16.75 days, respectively, to reach this gametophyte developmental stage (Aspiras 2010).

In this study, spores of *P. wandae* germinated between 7-14 days after sowing (DAS). The spores were bean-shaped, monolete, bilaterally symmetrical, ellipsoid, non-chloropyllous, dark brown, lacking perine, with smooth to scabrid, that fit with previous descriptions (Hoshizaki 1972; Tryon and Lugardon 1991; Pérez-García et al. 2010). In general, the first indication of germination was the changing of coloration from dark brown spores to greenish brown spores. The first cell produced on germination of the spores was rhizoid (Figure 2.A.) A rupture at the lower end of the spore caused by spore swelling led to the emergence of the rhizoid. It is generally considered that the criterion for spore germination is the emergence of the chlorocyte or the rhizoid (Chuter et al. 2008; Aspiras 2010). The rhizoid cell usually does not contain chloroplasts. It is produced on an upper surface of the basal cell or sometimes at one corner of the basal cell. In this study, the basal cell underwent a second division on the opposite direction to the rhizoid resulting in the emergence of chlorocyte (Figure 2.B.). Nayar and Kaur (1971) gave a detailed account of the patterns of spore germination and classified them on the basis of the plane cell division (in relation to the polarity of the spores) and the direction of growth of the primary

rhizoid and the prothallus. The elongation of the germ papilla (chlorocyte) and its continuous division gave rise to a protonema consisting of a uniseriate germ filament with 3-8 protonemal cells (Figure 2.C-F.). The protonemal cells usually arise from one of the three corners directed to the former side-walls. The chloroplast content increased in line with the growth and development of the prothallus.

The germination pattern of *P. wandae* was of the *Vittaria*-type. In accordance with the description of Nayar and Kaur (1971), the spore germination resulted in a uniseriate, elongated, germ filament composed of barrel-shaped chlorophyllous cells and bearing one or more rhizoids at the basal end. The *Vittaria*-type of germination pattern was also reported by Espinosa-Matias et al. (2007), Perez-García (2010) on *P. andinum* and *P. wandae*, and by Wang et al. (2011) on *P. wallichii*. In this study, germinative uniseriate filaments of *P. wandae* of Papua consisted of 3-8 protonemal cells. They were longer than those reported by Espinosa-Matias et al. (2007) from New Guinea (collection number: LD Gomez 26250) which had only uniseriate germ filaments with 1-5 cells long before bearing spatulate stages. The possible factors that influence this differences are the photoperiods of light and medium nutrients (Racusen 2002).

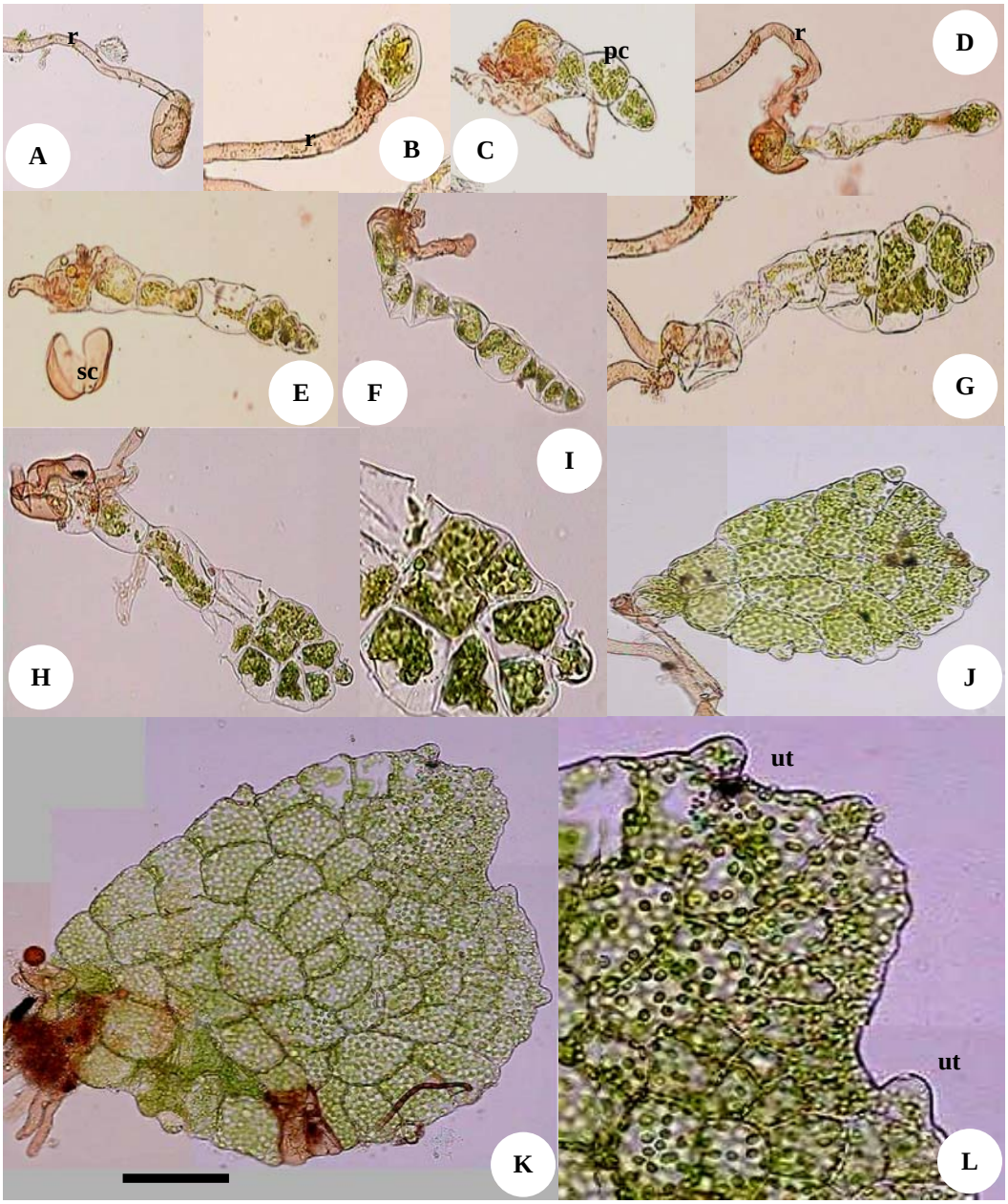

Figure 2. A. Spore germination and early gametophyte development of *Platycerium wandae* (EBW s.n., Papua). A-B. Germination at about one week. A. Spore swelling led to the emergence of the rhizoid; B. Chlorocyte-rhizoid phase. C-F. Filamentous phase, 3-8-celled filament, 7-14 DAS. G-I. Young phase of gametophyte (spatulate plate) (27 DAS). J-L. Young gametophyte with unicellular trichome (lopsided prothallus), one month (34 DAS). Rhizoid (r), unicellular trichome (ut), spore coat (sc), prothallial cell (pc). Scale bar = 30 µm for A-G.

Prothallial development

Spatulate prothallial plate formation. The young prothallus plate is initiated in the terminal cell of the filament by perpendicular divisions (Fig. 2). A small spatula consisted of 14-16 cells (Fig 2.G-H.). The spatulate phase occurred at about 14-30 DAS. A broad spatulate prothallial plate is formed by division of the anterior cells, including the terminal cell.

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 commonly to taxa of higher order under normal conditions of growth. Prothallial development of *P. wandae* is of the *Aspidium*-type, a pluricellular meristem being established directly from the anterior cells of broad spatulate young prothalli. The *Aspidium*-type prothallial development in *Platycerium* was also reported by Espinosa-Matias (2007) and Pérez-García et al. (2010), *viz*. in *P. andinum* Baker of Peru and *P. wandae* of New Guinea.

Lopsided prothallus formation. The continuous division of the anterior and terminal cells of the prothallial plate and the repeated longitudinal and transverse divisions of the daughter cells led to the formation of a lopsided prothallus (Fig. 2.J-L.). This gametophyte development stage occured at about 30-40 DAS.

Cordate prothallus formation. Further growth of the lopsided prothallus resulted in the formation of asymmetrical cordate prothallus. The young heart shape (cordate) prothallus of *P. wandae* of Papua was formed in two months (Figure 3). The length of time needed for reaching the heart-shaped stage of *P. wandae* in natural media was similar to the length of time needed by *P. coronarium* under in vitro conditions on Knop's agar medium (Awan and Rao 1981). The number of days for cordate prothallus formation among species of *Platycerium* may be significantly different. The gametophytes of *P. coronarium* required 27.50 days for reaching the cordate stage (Aspiras 2010), while *P. grande* required 21-33.75 days (Amoroso and Amoroso 2003; Aspiras 2010) in in vitro condition.

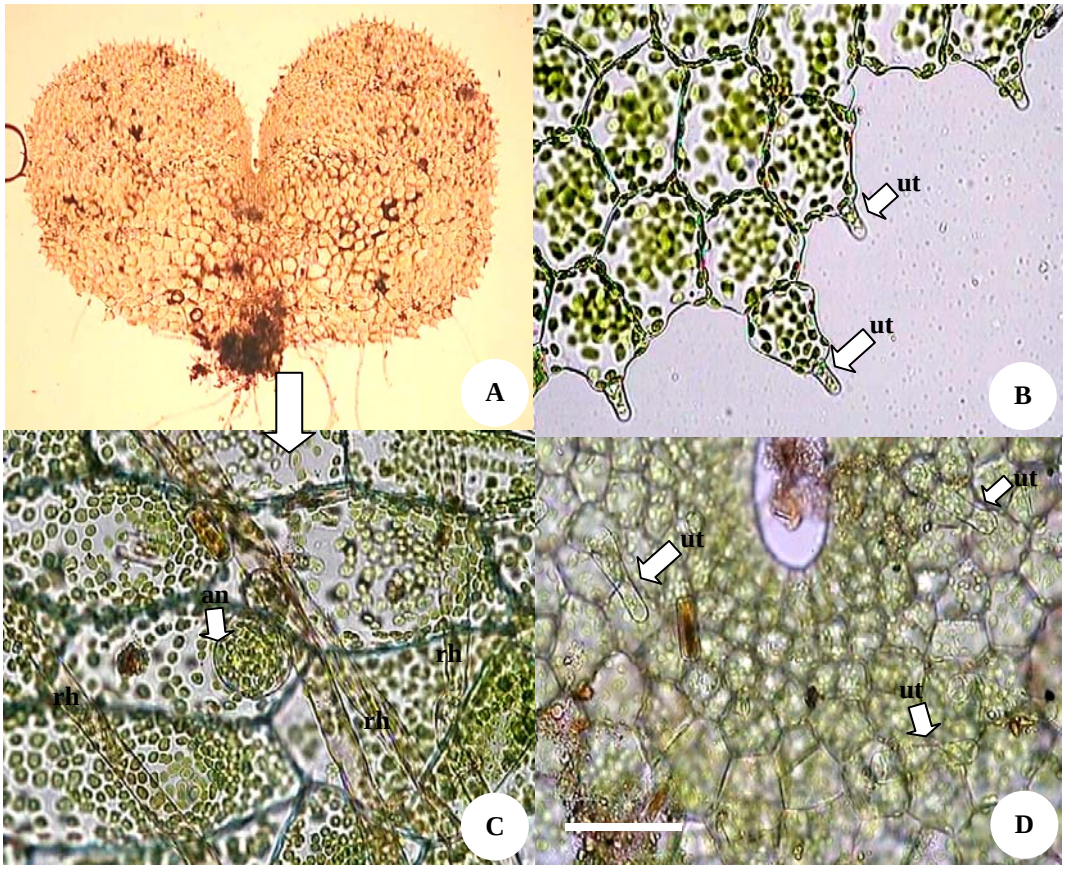

Figure 3. Unisexual young gametophyte and trichome morphology of *Platycerium wandae* of Papua. A. young cordiform-reniform gametophyte of *Platycerium wandae* (male), 61 DAS. B. Marginal part of gametophytes with unicellular trichome (ut); C. Basal part of gametophyte showing young antheridium (an) among rhizoids (rh). D. Meristematic zone on apical part of gametophyte with unicellular trichomes. Scale bar = 64 µm for A; 6 µm for B and D.

Table 1. Gametophyte development and morphological characters compared among species of *Platycerium*

Characters	*P. wandae* (Present study)	*P. wandae* (Pérez-García et al. 2010)	*P. andinum* (Pérez-García et al. 2010)	*P. coronarium* (Aspiras 2010)	*P. grande* (Aspiras 2010)	*P. holttumii* Manitayakul et al. (2006)	*P. wallichii* (Wang et al. 2011) [Abstract]
The medium employed	Natural substrate (minced roots of *Cyathea contaminans* with charcoaled rice hulls (1: 1)	10% agar in Thomson's media	10% agar in Thomson's media	Knudson C culture medium + 2% glucose	Knudson C culture medium + 2% glucose	Miller and Miller (MM) (1961)	Knop's agar media
The germination (DAS)	7-14	6-15	6-8	6	12	14	Not mentioned
Type of spore germination	*Vittaria*-type	*Vittaria*-type	*Vittaria*-type	*Gleichenia*-type	*Vittaria*-type	-	*Vittaria-type*
Prothallial development	*Aspidium*-type	*Aspidium*-type	*Aspidium*-type	*Drynaria*-type	*Drynaria*-type	-	*Drynaria*-type
Filamentous phase	3-8 cells	1-5 cells	1-5 cells	2-8 cells or more	2-8 cells or more	-	Not mentioned
Shape of laminar phase	Asymmetrical cordate-shape	Asymmetrical cordate-shape Asymmetrical cordate-spatulate Asymmetrical cordate-kidney shape	Asymmetrical cordate-shape Asymmetrical cordate-spatulate Asymmetrical cordate-kidney shape	Symmetrical cordate-shape	Symmetrical cordate-shape	Symmetrical cordate-shape	Symmetrical cordate-shape
Mature gametophyte	Asymmetrical cordate-shape Male	Cordiform-spatulate Male and female (unisexual)	Cordiforme-reniforme Bisexual	Symmetrical cordate-shape	Symmetrical cordate-shape	Symmetrical cordate-shape Male and female	
Wing cell shape	Isodiametric polygonal with almost straight side wall	Isodiametric polygonal with almost straight side wall	Isodiametric polygonal with almost straight side wall	Isodiametric polygonal with almost straight side wall	Isodiametric polygonal with almost straight side wall	Isodiametric polygonal with almost straight side wall	Not mentioned
Type of secretory trichome (prothallial hairs)	Unicellular	Bicellular; Multicellular	Bicellular; Multicellular	Not mentioned	Not mentioned	Not mentioned	Not mentioned
Distribution of secretory trichome	Margin, wing and meristematic zona	Margin, wing, chusion, and meristematic zona	Margin, wing, chusion, and meristematic zona	Not mentioned	Not mentioned	Not mentioned	Not mentioned

Reproductive organ formation. The male sex organ (antheridium) of *P. wandae* was noticed at 61 days after spore sowing, but it was still immature (Figure 3). The female sex organ (archegonium) of *P. wandae* had not been formed by 80 after sowing. Thus, there were only unisexual gametophytes, *viz.* male gametophytes. Pérez-García et al. (2010) reported that gametophytes of *P. wandae* of New Guinea were also unisexual, bearing both male and female gametophytes. Antheridia and archegonia of *P. holttumii* could be observed at 98 and 112 after sowing spores, respectively, under in vitro conditions (Mitayakul et al. 2006). Each species of *Platycerium* may have a different rate of growth and gametophyte development to reach maturity. Under in vitro conditions, the development of gametophyte reproductive organs in *Platycerium* may be relatively slower. Under in vitro conditions on Knop's agar medium, mature gametophytes of *P. coronarium* develop in 2 months, and 85 per cent of them were unisexual (both male and female) and 15 per cent bisexual (Awan and Rao 1981).

Gametophyte morphology of P. wandae and its related species. Table 1. Provides a gametophyte morphological comparison among species of *Platycerium*. The gametophyte of *P. wandae* of Papua has an asymmetrical heart-shape or cordate-shape on both laminar phase and mature stage. In this phase of development, the gametophytes develop unicellular secretory structures (unicellular trichomes), an important characteristic of *Aspidium*-type sensu Nayar and Kaur (1971). In the pattern of development of gametophyte of Aspidium-type (Nayar and Khaur 1971), early hair formation take place in the young prothalli (Figure 2.G-I). The trichomes are distributed on the marginal, wing and near meristematic zone of the ventral surface (Figure 3.A., B, and D).

Most species of *Platycerium* have a similar laminar shape; asymmetrical heart-shaped or cordate-shaped (Aspiras 2010; Espinosa-Matías and Pérez-García 2007; Pérez-García et al. 2010); except for *P. wallichi*, which has a symmetrical cordate-shape (Wang et al. 2011). However, the gametophyte of each species shows distinctive features (Table 1.). For example, young heart shape gametophytes with secretory trichomes were found in *P. andinum* (Pérez-García et al. 2010) and *P. wandae* (present study), but the morphological characters of the secretory trichomes of the two species are different. *Platycerium andinum* has bicellular and multicellular trichomes, while *P. wandae* has unicelullar trichomes.

In conclusions, spore germination of *P. wandae* is of the *Vittaria*-type and the prothallial development is of the *Aspidium*-type. *Platycerium wandae* of Papua has a unisexual asymmetrical heart-shape gametophyte with unicellular trichomes distributed on the marginal, wing and meristematic zones. New data on morphogenesis of *P. wandae* from Papua on natural media is provided. The findings also provide data relevant to reproductive biology and conservation of *Platycerium*. The straightforward method of germinating spores in a relatively simple natural substrate, such as minced roots of *Cyathea contaminans* with charcoaled rice hulls (1: 1), can be used for studying gametophyte development and propagating *Platycerium,* which may increase the availability of plants for ornamental purposes. This method also can be applied for *ex situ* conservation of endangered epiphytic ferns. Gametophyte plays an important role in the dispersal and reproductive biology of ferns, therefore the description of the gametophyte development also would be useful on further *in situ* studies on gametophyte ecology.

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