J Indian bot Soc Vol 73 (1994) 95-99

SPORE GERMINATION AND GAMETOPHYTE DEVELOPMENT IN *PRONEPHRIUM ARTICULATUM* (HOULST. & MOORE) HOLTTUM

K.R. LEENA AND P.V. MADHUSOODANAN
Dept. of Botany, University of Calicut - 673 635, Kerala, India
(Accepted October 1993)

Spore germination, prothallial development and juvenile sporophyte morphology of *Pronephrium articulatum* (Houlst. & Moore) Holttum are described and compared with other thelypteroid species. Its bearing on phylogeny of thelypteroid ferns discussed.

Key words : *Pronephrium articulatum*, spore germination, prothallial development, juvenile sporophyte.

Gametophytes have been considered as a subject of investigation being a possible source of taxonomic characters since long in fern systematics (Stokey, 1951; Atkinson and Stokey, 1964). As a comparative morphological tool, gametophytes seem to be most useful to the systematists at the family level and in certain cases at the generic level. Loyal (1991) reported that the gametophytic features of the Thelypteridaceae can be regarded as genus specific. Among the earliest workers to examine gametophyte morphology of Thelypteridaceae were Schmelzeisen (1933) who studied prothallial development of *Thelypteris parasitica* and Momose (1938) studied *T. parasitica* and *T. acuminata*. Mehra and Loyal (1956) studied the effect of colchicine on the prothalli of *Goniopteris multilineata* (Wall.) Bedd. and *G. prolifera* Roxb. Nayar and Kaur (1971) reported that the spore germination in Thelypteridaceae is of Vittaria-type and the prothallial development of the Drynaria-type. Chandra (1972) described the spore germination and prothallial development in 5 thelypteroid species. Huckaby and Raghavan (1981) reported that imbibed spores of some species germinated in less than 72 hours of exposure to continuous light. The gametophyte development of 16 Jamaican thelypteroid ferns and 5 Old World species were explained by Atkinson (1973, 1975). Studies of Khare and Kaur (1979) show that the frequency of sporophyte production in *Cyclosorus parasiticus* (L.) Tardieu was maximum in composite and lower in isolate populations. Mehra and Sulklyan (1969) developed gametophytic callus of *Ampelopteris prolifera* in culture by treating germinating spores with 2,4-D. Apospory has been induced on cotyledonary, juvenile and adult type leaves, root and rhizome segments of *A. prolifera* (Mehra and Sulklyan, 1969) and in the root callus of *Cyclosorus dentatus* (Mehra and Palta, 1971). Formation of synthetic structures, *i.e.*, structures possessing characters of both sporophyte and gametophyte were reported in *Cyclosorus dentatus* by Mehra and Palta (1971).

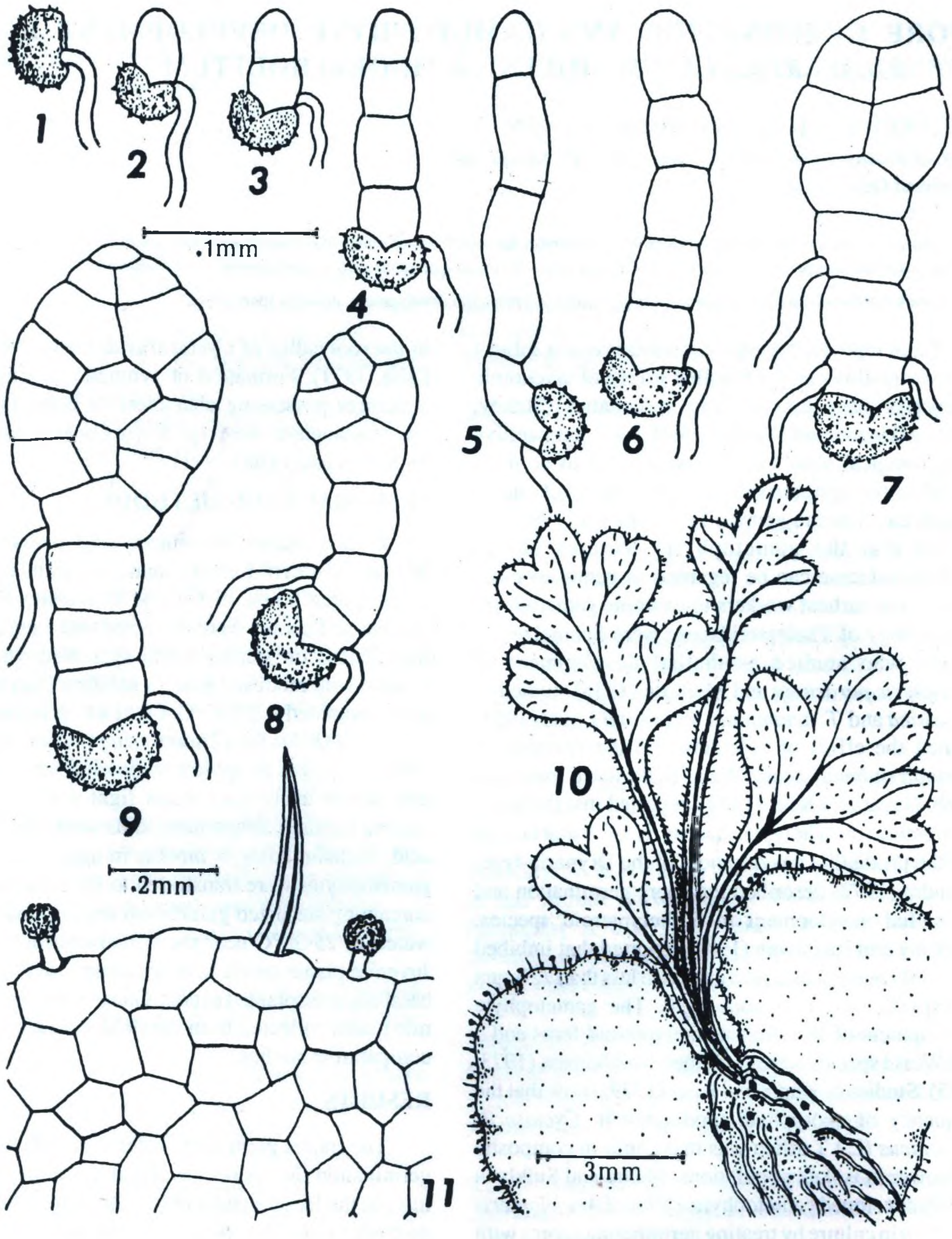
MATERIALS AND METHODS

P. articulatum specimens were collected from Wynad District of Kerala (South India) and cultivated in the green house of Calicut University Botanical Garden and spores were collected and gametophytes were raised on sterilised 8% agar medium supplemented with modified Knop's solution (Nayar, 1962) and maintained at 20-30°C. Light was given at the rate of 3000-4000 lux for 12 hours using fluorescent lamps. Different stages of spore germination were observed and drawn using Carl Zeiss light microscope and camera lucida. The spermatozoids were stained in 1% acid fuchsin. After 4 months in agar culture, some gametophytes were transferred to small earthern pots containing sterilized garden soil kept in troughs with water, at 25-35°C near the window/partial sunlight. Juvenile plants raised in agar cultures were also successfully transplanted to pots. Gametophytes and juvenile plants collected from the field were also used for comparative studies.

RESULTS

The spores germinate within 3 or 4 days. During germination the spores swell and the spore coat ruptures at the laesura and a uniseriate slender germ tube protrudes out. The spore germination is of Vittaria-type (Nayar and Kaur, 1971) *i.e.*, the first division of the spore is parallel to the equatorial axis which divides the spore into two unequal cells. Of these, the proximal

Received April 1993



Figures : 1-11. Gametophyte and sporophyte development in *Pronephrium articulatum* (Houlst. & Moore) Holttum. 1-9. different stages from spore germination to formation of prothallial plate, 10. cordate thalloid prothallus with juvenile sporophyte showing vasculature, 11. margin of prothallus with acicular and glandular hairs.

smaller one develops a rhizoid which comes out first. The first rhizoid generally emerges prior to the germ tube and is non-chlorophyllous and unicellular. The larger distal cell divides by a vertical wall into two equal cells of which one remains inactive. The other by a series of divisions parallel to the second division forms the prothallial filament. So the rhizoid and prothallial filament stand at right angles (Figs. 1-6).

The prothallial development is of Drynaria-type (Nayar and Kaur, 1971). The spore germination results in a slender uniseriate germ filament within 5-12 days. A broad spatulate prothallial plate is formed by repeated longitudinal and transverse divisions of its anterior cells and the expansion of the resultant daughter cells (Figs. 7-9). The prothallial plate often becomes 6-14 cells wide and broadly ovate but is devoid of any organised meristem. Establishment of meristematic cell is much delayed. Later an obconical meristematic cell is differentiated by two oblique divisions in one of the marginal cells at the anterior end of the prothallial plate. By this time the apical meristematic cell is replaced by a pluricellular meristem and a few cells thick midrib is developed. The thallus is dorsiventrally flattened, subcircular in outline, with a notched anterior end having well differentiated meristem located at the bottom of the notch and consisting of 4-8 cells thick median midrib and broad, one-cell thick, semicircular wings with wave margins, on either side. The sex organs and rhizoids are restricted to the midrib on ventral surface. Wing margins are turned up and raised above the substratum.

The young prothallial are naked and development of trichomes occurs only later after the prothallial plate becomes cordate thalloid. Marginal trichomes are unicellular acicular type developing from small lens-shaped initial cells formed towards the middle of the peripheral wall of the prothallial cells. These hairs are non-glandular, slender, elongated, needle-like devoid of contents when fully developed and with nearly hyaline, thickened walls. Papillate hairs are borne both marginally and superficially on the prothallus and are widely distributed. Papillate prothallial hairs are short cylindrical with a rounded anterior end, thin-walled and with vacuolated protoplasmic contents. They are secretory in function, secreting an extracellular, yellowish, waxy substance, which forms a cap crowning the hair (Fig. 11). Papillate hairs on the surface of the prothallus are generally larger in size compared to those borne on the margin of the same thallus.

Sex organs are produced within three months; antheridia develop first on either side of the midrib away from the notch; archegonia develop later, usually after the collapse of antheridia, near the notch region on the midrib itself. Sex organs are of the common leptosporangiate type. Antheridia are developed early often before the prothallial plates establish an apical meristematic cell. However, they are rather spares in the early stages of prothallial development. Some of the irregularly-shaped poorly developed gametophytes are entirely antheridial and produce clusters of antheridia all over. These remain one cell-thick throughout but may bear the characteristic type of hairs both on the margin and surface. Many of the proliferations formed on the young cordate prothallus develop into irregularly shaped antheridial lobes. On cordate mature prothalli, antheridia are generally restricted to the posterior half of the midrib on the lower surface and the area of the wings close to the midrib. Antheridium has a simple structure consisting of a subglobose to hemispherical mass of spermatozoids sheathed all round by a one-cell-thick wall. Each antheridium is small and produces 16-32 spermatozoids. Its wall consists of three cells, of which the one at the anterior end is circular, the cap cell (operculum); that at the posterior end is the basal cell by which the antheridium is attached to the prothallus, and the middle one (the ring cell) is annular, surrounding the central mass of spermatozoids.

The archegonia are usually restricted to the anterior half of the midrib. The archegonial neck is slender, elongated and composed of four longitudinal rows of cells, each row made up of 4 or 5 slightly elongated cells and is strongly curved away from the apex of the prothallus. The neck canal cell is binucleate and slightly swollen towards the anterior end at maturity. Ventral canal cell is ephemeral. Fertilization and development of sporophytes occur profusely in the culture.

Generally only one sporophyte is formed per prothallus. The first and often two or three succeeding juvenile leaves have a broadly obcuneate to reniform lamina (never spatulate) supplied by a solitary vein forked 2 or 3 times. The outer margin is lobed corresponding to the venation (Fig. 10) so that the lamina is of the flabellate type. A midrib is formed as a separate branch originating from near the base of one of the branches of the first dichotomy. Midrib may be found in the first juvenile leaf itself in some cases but usually only in the 3rd and 5th leaf. After the midrib becomes

established, the apex of the lamina becomes pronounced, and the lamina elongates markedly with the midrib bearing pinnate branches. The lamina becomes pinnatifid in successive leaves and finally pinnate. Union of veins as found in adult leaves occurs only after the leaf becomes distinctly pinnate and the pinnules develop marginal lobes corresponding to the main lateral veins. All young leaves bear capped papillate hairs of the type found on the prothallus. Hairs of the stipe are larger than those on the lamina. These hairs are stouter than those on the prothallus and are similar to hairs on the adult leaf. Towards the base of the stipe, elongated pluricellular hairs occur in the early juvenile leaves. In succeeding leaves these metamorphose to form paleae.

DISCUSSION

The thelypteroid ferns in South India and elsewhere show much diversity and morphological plasticity to a great extent. But in the prothallial development all show Vittaria-type of germination and Drynaria-type of prothallial development. Regarding its phylogeny there has been much difference of opinion among pteridologists. Holttum (1971) found many resemblance between *Cyathea* and *Thelypteris* and suggested a common origin. But, Pichi Sermolli (1977) is of the opinion that they are akin to Dryopteridaceae and Aspleniaceae. Nayar and Kaur (1974) suggested a close relationship of Thelypteridaceae with Aspleniaceae, both families probably being originated from the cyatheoid stock. Nayar (1980) also finds a close affinity with Grammitidaceae. In Thelypteridaceae and Aspleniaceae, the gametophytes are similar in development and morphology and at maturity possess characteristic papillate hairs but they differ in the shape of juvenile lamina. The first juvenile leaf of thelypteroid ferns are with flabellate margins and in this respect it differs from that of Asplenioid ferns where the first juvenile leaves are spatulate. In Grammitidaceae the gametophytes have a prolonged and extensive filamentous stage and elongated strap-shaped adult prothallus bearing branched pluricellular glandular hairs in addition to acicular hairs. The most common type of hair in the Grammitidaceae is branched, multicellular, club-shaped hair basically similar to the multicellular hairs of the Polypodiaceae but often more complex. The gametophytes of Grammitidaceae are comparatively most advanced being strap-shaped bearing profuse acicular hairs as well as branched club-shaped hairs. The prothalli of the Cyatheaceae, though of the cordate type, bear characteristic, superficial multicellular, 'bristle-like' hairs developing from special initial cells (Stokey, 1930), in contrast to the capped, unicellular, papillate hairs borne on the margins and surfaces of the prothalli of thelypteroid ferns. The acicular hairs found on mature prothalli of Thelypteridaceae are markedly different from those of the Cyatheaceae in structure and development. In Dryopteridaceae the prothallial development is of the Aspidium-type, the terminal segment of the germ filament produces a unicellular papillate hair crowning it and becomes sluggish, taking little part in development. The gametophyte morphology of the thelypteroid ferns including that of *P. articulatum* shows more resemblance towards the asplenioid ferns indicating a phylogenetic affinity but they differ in the shape of juvenile lamina.

We are grateful to Late Prof. R.E. Holttum of Royal Botanic Gardens, Kew, England for valuable suggestions and encouragements extended during the initial part of this study.

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