

DEVELOPMENT AND MORPHOLOGY OF FLOWERS AND INFLORESCENCES IN *BALANOPHORA PAPUANA* AND *B. ELONGATA* (BALANOPHORACEAE)¹

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Extreme modification and reduction in floral morphology presents an obstacle to determining the evolutionary relationships and homologies of the holoparasites in Balanophoraceae. Developing flowers and inflorescences of two dioecious species, *Balanophora papuana* and *B. elongata*, were compared to each other and to the monoecious *B. fungosa*. Intermingled with flowers in the male inflorescences are bracts (*B. elongata*) or bract parts (*B. papuana*). In the latter, early cessation of bract tip growth results in two half-bracts, which become displaced during inflorescence elongation, thus disproving the view that these bract-like structures are axial in nature. Male flower primordia emerge in positions axillary to the dividing bracts, and both arise in a spiral sequence. This pattern is modified in *B. papuana* by the formation of pseudowhorls of four. In both species, the staminate flowers consist of a generally four-merous perianth and a synandrium of congenitally fused stamens. Male flower and bract ontogeny (but not pollen sacs) conform to patterns seen in other angiosperms. More problematic are the carpellate flowers whose primordia arise in irregular order between club-shaped, radially symmetrical organs called claviform bodies. The interpretation that these bodies are homologous to the peltate bracts of Helosideae appears plausible, but cannot explain their nonspiral initiation and radial symmetry.

Key words: *Balanophora*; Balanophoraceae; floral development; floral morphology; parasitic plant; Santalales.

Comparative morphology of flowers has long occupied a central position in attempts to understand evolutionary relationships among flowering plants (Weberling, 1989; Endress, 1994). Further insights into such relationships can be gained when traditional approaches are complemented by ultrastructural, developmental, and genetic data interpreted in a phylogenetic context (Erbar, 2007; Wanntorp and De Craene, 2007). Reductions in vegetative and floral structures are well documented in parasitic flowering plants, but such reductions have reached an extreme in the genus *Balanophora* (Balanophoraceae), whose bottle-shaped female flowers are minute. With regard to their flower development, we address the basic question “do these highly derived parasites follow developmental patterns typical of most angiosperms?” Floral developmental data will inform work addressing the phylogeny of the family, a topic that has been controversial for over two centuries. Recent molecular phylogenetic analyses (Nickrent et al., 2005) yielded the result that the family Balanophoraceae is allied with the order Santalales, a relationship previously proposed by some workers (Engler, 1894; Fagerlind, 1945a; Cronquist, 1981; Thorne, 1992; Takhtajan, 1997) but questioned by Kuijt (1969) because of evolutionary convergence in ovule morphology. Assuming the Balanophoraceae–Santalales relationship is real, what can floral developmental features tell us with regard to the evolutionary pathway that connects these two groups?

All members of Balanophoraceae, a tropical and subtropical family of 16–17 genera of root holoparasites, attach to host roots by means of a structure called the “tuber” (for a discussion of its homology, see Holzapfel, 2001). The aboveground portion of the plant consists of a thickened, fleshy inflorescence, usually with scale leaves (bracts), but sometimes without (*Lathrophytum*, *Chlamydoxylum*). The inflorescences arise endogenously from within the “tuber” (Shivamurthy et al., 1981a, b) and bear either male or female flowers, or both. Many Balanophoraceae are dioecious; in monoecious species, the distribution of the sexes within the inflorescence is variable.

Floral morphology is highly variable among the genera of the family (for a survey see Kuijt, 1969; much general information can be also found in the paper on *Dactyloctenium* by Holzapfel, 2001). The flowers are always unisexual. A rudimentary perianth is found in the flowers of both sexes in *Dactyloctenium*, *Hachettea*, and *Mystropteron*, whereas in all other genera only the staminate flowers possess a perianth. Because the perianth consists of a single whorl, it may also be referred to as a perigone. The number of stamens usually equals the number of perianth lobes, and the stamens exhibit an antepetalous position. In some genera such as *Mystropteron*, both the filaments and the anthers are free. In others, the anthers appear fused, while the filaments remain free. In *Balanophora*, *Langsdorffia*, *Thonningia*, and *Rhopalocnemis*, congenital fusion of the filaments and anthers occurs, thus forming a compact synandrium. In these genera, the entire gynoecium of carpellate flowers is extremely reduced (for a review and discussion of the female flower of Balanophoraceae, see Holzapfel, 2001). Based on the number of styles, carpel number appears to range from one to three. Typical ovules (i.e., integumented megasporangia) are not formed, but rather a nucellar complex (“mamelon”) is present in the central part of the ovary containing the megaspores and subsequently the embryo sac (Holzapfel, 2001). The evolutionary process of reduction

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culminates in the genus *Balanophora*. Here the female flowers are represented only by a small multicellular body, of which a hypodermal cell becomes the archesporial cell, while from the outer cell layer a pointed extension grows out (Goebel, 1932). The single-seeded fruit is scarcely larger than the ovary from which it develops ("seed" used here as the functional unit). The embryo is usually very small, few-celled, and lacks cotyledons. *Balanophora* is often referred to as a dust seed plant; the seeds are among the smallest of all flowering plants (Harms, 1935).

In contrast to the attention paid to the anatomy and morphology of the "tuber," comparatively less information exists on the structure and development of inflorescences and flowers in Balanophoraceae. Hansen published floristic treatments for the neotropics (Hansen, 1980) and paleotropics (Hansen 1972, 1976, 1982, 1984, 1986), as well as papers devoted to morphological problems (Hansen and Engell, 1978). In the latter paper, light microscopy and SEM were used to illustrate various mature features; however, no developmental studies were conducted. Generally, most developmental studies in the family have focused upon embryology (e.g., Lotsy, 1901; Fagerlind, 1945b; Teriokhin and Yakovlev, 1967; Holzapfel, 2001).

In the present paper, we examine the early ontogeny of inflorescences and flowers of two dioecious species of *Balanophora*, *B. papuana* Schltr., and *B. elongata* Blume, using SEM. In addition, we compare the carpellate flower morphology of these species to *B. fungosa*, which is monoecious. Both *Balanophora papuana* (Fig. 1A, B) and *B. elongata* (Fig. 1C, D) are strictly dioecious.

MATERIALS AND METHODS

Material of three *Balanophora* species was collected in peninsular Malaysia, Java, and Australia. Specimens were deposited with WU (Herbarium, Institute of Botany, Wien, Austria), MB (Herbarium, Botanik, Philipps-Universität, Marburg, Federal Republic of Germany), or SIU (Herbarium, Plant Biology, Southern Illinois University, Carbondale, Illinois, USA). Plants and plant parts at various developmental stages were fixed in the field using denatured ethanol. A few samples were fixed with FPA (37% formaldehyde, propionic acid, ethanol, and water in a 1:1:9:9 ratio). Species and collection details are as follows: (1) *Balanophora papuana* Schltr., Pahang/Perak border, Gunung Irau, A. Weber 870623-2/16, 23 June 1987 (WU). (2) *Balanophora elongata* Blume var. *elongata*, West Malaysia, Perak, Bukit Larut (Maxwell's Hill), coll. S. Vogel and A. Weber s.n., 22 August 1979 (WU). (3) *Balanophora elongata* Blume var. *elongata*, Indonesia, West Java, Gunung Gedé, H. C. Weber, 25 September 1987 (MB). (4) *Balanophora fungosa* Forster & G. Forster, Australia, Queensland, Mossman Gorge, D. Nickrent 2825, 19 August 1991 (SIU).

Other specimens of *Balanophora papuana* and *B. elongata* provided by the Nationaal Herbarium Nederland, Universiteit Leiden Branch (L) (formerly Rijksherbarium Leiden) were examined for comparative purposes, as was the specimen of *B. fungosa* (subgenus *Balanophora*, section *Balanophora*).

The samples were cleaned in 70% ethanol using a stereomicroscope and specially sharpened steel needles. Dehydration was accomplished using FDA (formaldehyde dimethyl acetal) according to Gerstberger and Leins (1978). Critical point drying was with standard techniques, using CO₂ as the carrier gas. The samples were coated with gold in a Balzers (BAL-TEC AG, Balzers, Liechtenstein) sputter coater and viewed and photographed in a JEOL (Tokyo, Japan) T 300 scanning electron microscope at 10 kV.

RESULTS

***Balanophora papuana*: Male inflorescences**—These are about 2.5–5 cm long, spadix-like, and bear numerous four- (rarely

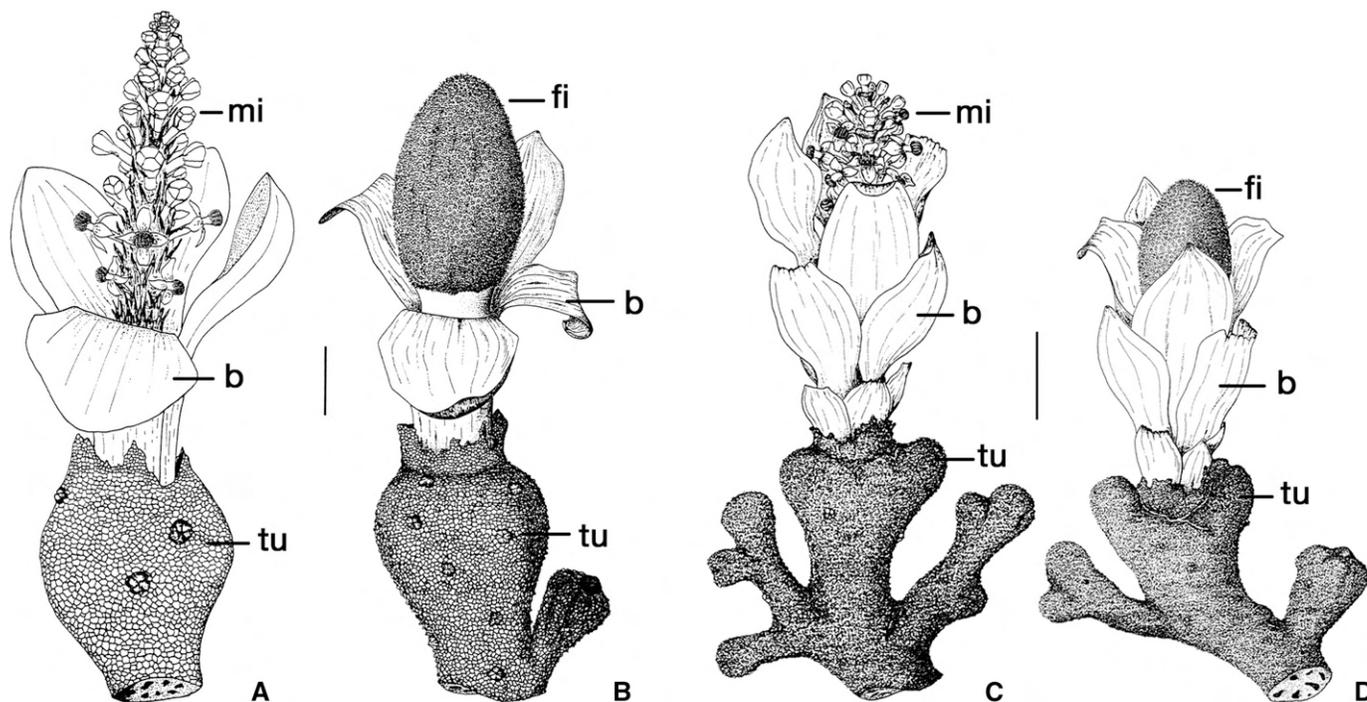


Fig. 1. Drawings of *Balanophora* species. *B. papuana*: (A) Male plant and (B) female plant. Note "tuber" side branch (in B) with inflorescence that has not yet broken through the "tuber" cortex; from coll. A. Weber 870623-2/16 (WU). *Balanophora elongata*: (C) male plant and (D) female plant. Note "tuber" branching; from coll. S. Vogel & A. Weber s.n., 22 Aug. 1979 (WU). Bar = 1 cm. For all drawings, the "tuber" has been severed above the point of the host connection. The inflorescences, including the basal bracts, appear above ground, and the "tuber" is mostly subterranean. All drawings by R. Eberwein. b, bract; fi, female inflorescence; mi, male inflorescence; tu, tuber.

five-)merous, bisymmetrical to slightly zygomorphic, pedicellate, staminate flowers. These flowers are positioned roughly in whorls of four. In the four-merous flowers, the tepals occur in orthogonal positions, with two lateral and two median (upper and lower) tepals. The anthers are fused into a complex synandrium, the individual pollen sacs of which open by longitudinal slits in the still closed flower. Forman (quoted in Hansen, 1972) observed that the staminate flowers of *Balanophora papuana* open on being touched.

The flower primordia arise from the apical meristem of the spadix in a faintly ascending spiral. The apical meristematic dome is broad and low (Fig. 2A, B; in contrast to *Balanophora elongata*) and produces a relatively large number of flower primordia in close succession. This results in a close packing of floral organs and bracts on the young spadix. Though the initiation of bract and flower primordia is principally spiral, there is, apparently due to the close packing and secondary shifts, some tendency toward an arrangement into (pseudo-)whorls. Orthostichies are recognizable, but these are not precise and are often

distorted (Fig. 2A, B: A', B', two of the orthostichies marked by thick arrows). The number of orthostichies is eight. If eight regular orthostichies (and eight clockwise and counterclockwise parastichies with the same angle of ascendance) were present, the result would be regular whorls of four, alternating with each other. Distortions ("breaks") in the orthostichies not only result from the primarily spiral initiation, but also secondarily by asynchronous growth of the primordia.

Scattered between the flower primordia, and more numerous than them, are structures that are roughly quadrangular in surface view. These result from the subtending floral bracts that split into two halves. Bract primordia begin as low mounds and initially expand isodiametrically (Figs. 3A, 4A). Soon growth becomes restricted to the margins, and the primordia expand along two lateral flanks (Figs. 3B, 4B). Later, the two flanks are further separated from each other as they are pushed apart by the developing floral primordium, eventually becoming located in the gaps between floral primordia (Figs. 3C, 4C–E). Upon inflorescence axis elongation, the bract halves shift in an

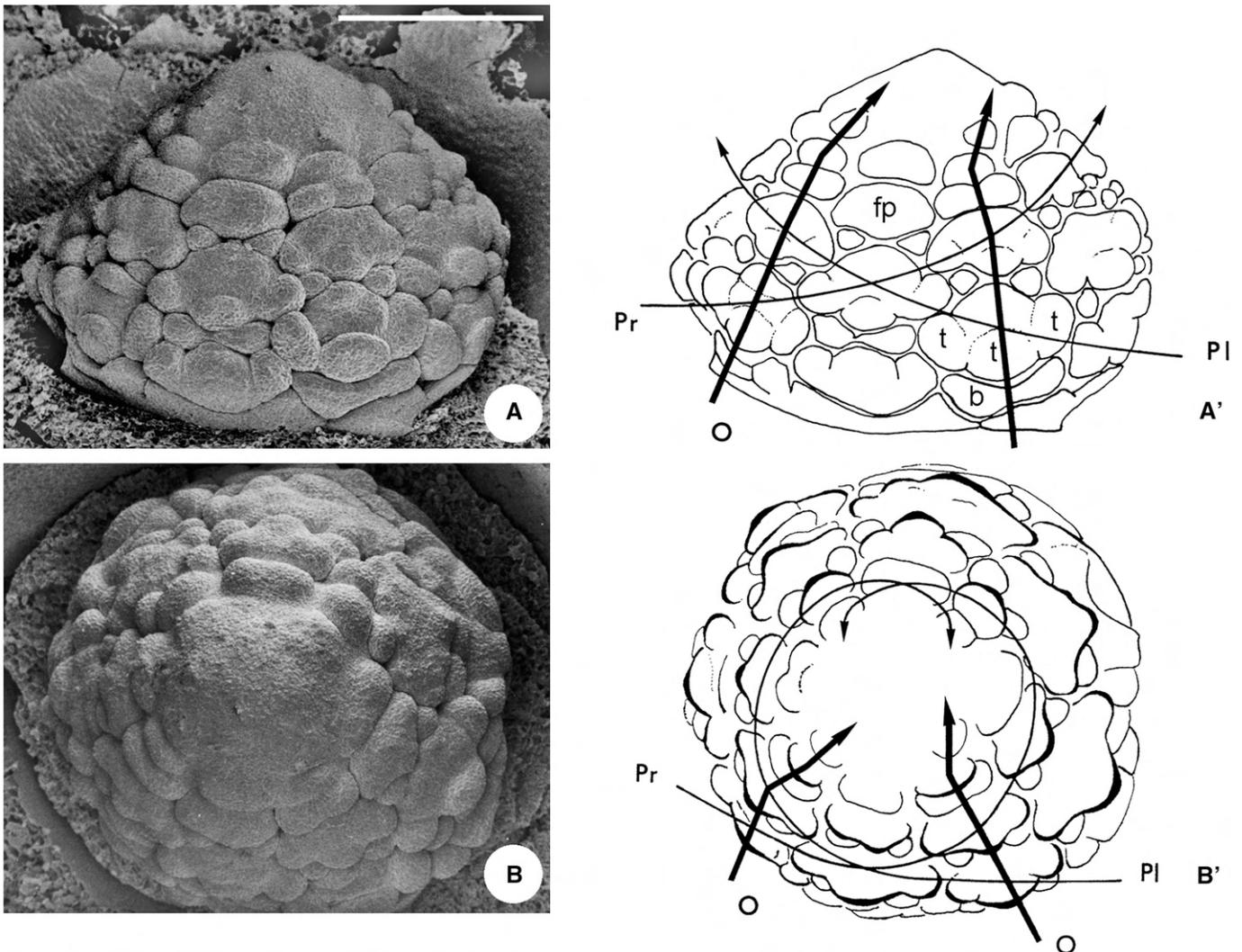


Fig. 2. SEMs of *Balanophora papuana*, male inflorescence development; (A) side and (B) top view of young inflorescence with bract and flower primordia; bar = 500 μ m. Figures A' and B', explanatory diagram of Figs. 2A and B. b, bract; fp, floral primordium; Pl, parastichy running left; Pr, parastichy running right; O, orthostichy; t, tepal.

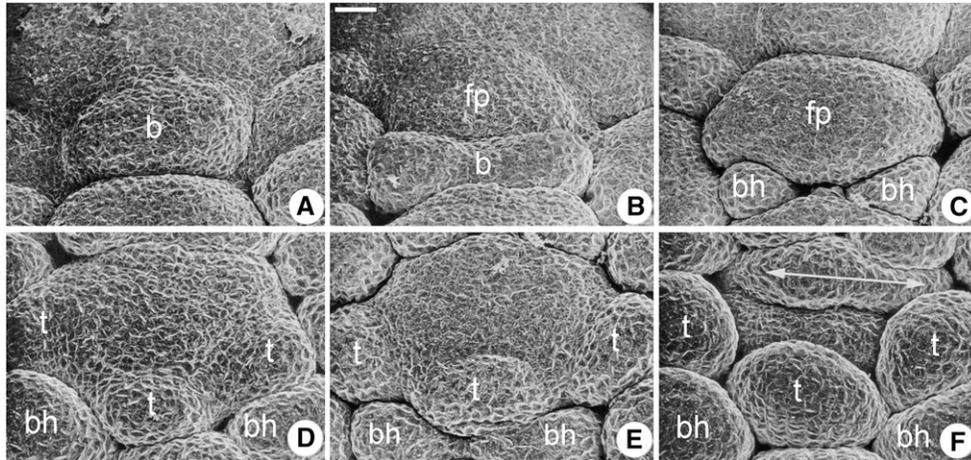


Fig. 3. SEMs of *Balanophora papuana*; development of subtending bracts and staminate flowers. (A) Emergence of bract primordium; (B) emergence of male flower primordium, early stage splitting of bract primordium; (C) bract halves after splitting; (D) emergence of three tepal primordia; (E) expansion of the three tepal primordia, the fourth (adaxial) is still lacking; (F) emergence and (frequent) lateral extension of the fourth (adaxial) tepal primordium (double arrow). Bar = 50 μ m. b, bract primordium; bh, bract halves; fp, male flower primordium; t, tepal primordium;

acropetal direction. Therefore, it is difficult or impossible to relate the bract halves to their respective axillary flowers, especially in the lower part of the inflorescence. In some inflorescences, between the base of the scale leaves and the lowermost flowers, a ring of sterile bracts can be observed that may likewise split into halves (Fig. 2A, 2A'). The extent of bract cleavage decreases continuously acropetally such that the uppermost bracts are only shallowly notched.

Soon after the emergence of the meristematic ridge of the bract primordium (Fig. 3A), an acropetal swelling appears (Fig. 3B), which is the staminate flower primordium. It initially has an elliptical outline (Fig. 3B) but later widens distinctly (Fig. 3C). At about this time, lateral and lower tepal primordia are initiated (Fig. 3C, C'); the upper tepal primordium appears later as a broad meristematic bulge (Fig. 3E, F). This dispropor-

tionately broad meristem may cleave in the middle, thus giving rise to two upper tepals instead of one. This results in flowers with a five-merous perianth.

The apical meristem of the floral primordium is completely used up by the formation of the synandrium. Even in the early stages, a subdivision of the primordium upper surface into low ridges can be observed (Fig. 5A, B). These ridges develop into the pollen sacs. The number, form, and arrangement of the pollen sacs are variable. They may end at the synandrium apex (Fig. 5C, D), they may traverse the synandrium apex in acropetal/basipetal or distal/proximal directions (Fig. 5E, F), or they (usually the lateral ones) may be U-shaped. Equating the synandrium bulges with the structure of conventional anthers is difficult by SEM; anatomical studies will be necessary. Likewise, the form and position of the synandrium cannot easily be

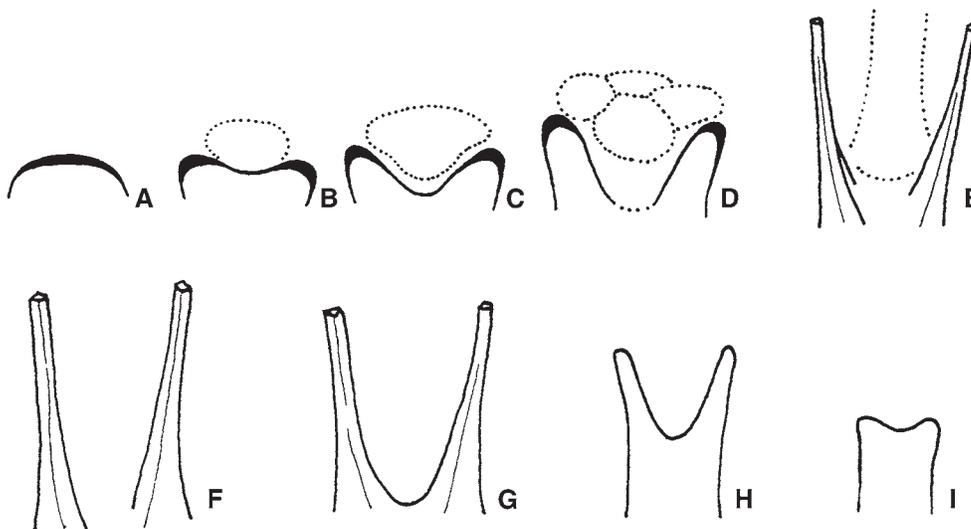


Fig. 4. *Balanophora papuana*, bract development and shape of adult bracts. (A-E) Developmental sequence of a bract and its axillary male flower (dashed lines); the bract is cleaved during the course of its development. (E) The completely separated bract halves and the pedicel of the axillary male flower (dashed lines). (F-I) Bract forms observed along the inflorescence axis (size and proportions do not reflect the natural condition). (F) The bract is split into separate halves. (G-I) Continuously decreasing amounts of emargination of the bract, proceeding upward on the inflorescence.

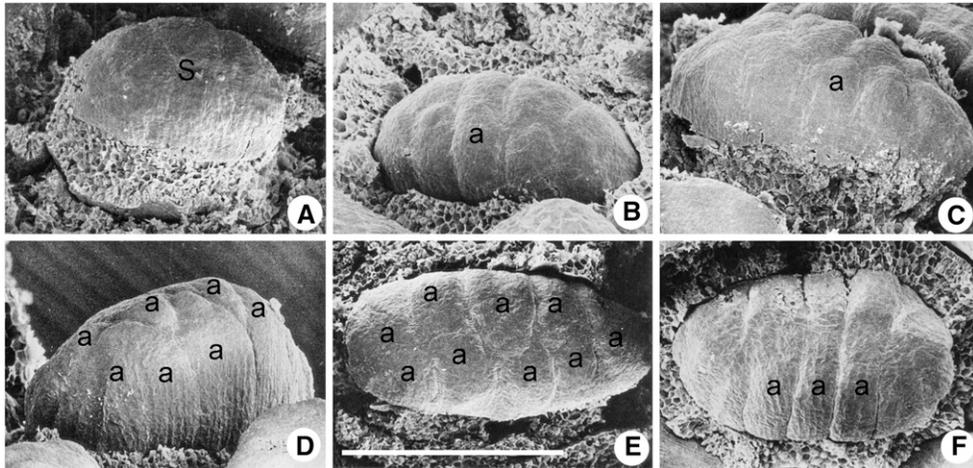


Fig. 5. SEMs of *Balanophora papuana*. (A–F) Primordium of the synandrium shown in various early developmental stages, all with tepals removed. (A) Very young synandrium with anther differentiation just beginning. (B) A somewhat later developmental stage. (C–E) Later stages with anthers interdigitated. (F) Later stage with anthers not interdigitated. Bar = 500 μ m. a, anther; S, synandrium.

related to tepal position because of secondary displacement of either or both floral parts.

***Balanophora papuana*: Female inflorescences**—These are 1.5–3 cm long with axes reaching 1–2.5(–3) cm in thickness. The inflorescence surface is studded with a large number of small, claviform (club-shaped) bodies, each ca. 1 mm in length. The carpellate flowers are located in large numbers between these structures. Carpellate flowers are without a perianth and are reduced to a style, an extremely simplified ovary, and a pedicel. The stylar end protrudes very slightly from the surface formed by the claviform bodies.

As in the male inflorescences, female inflorescence initiation is endogenous, and it eventually emerges from within the “tuber.” A whorl of four scale leaves is formed at the inflorescence base (Fig. 1B). These scale leaves cover the young inflorescence apex, which grows to considerable size before the claviform bodies are formed on its surface. There is no recognizable order or sequence of claviform bodies emergence.

Between the primordia of the claviform bodies, small protruberances become apparent early (Fig. 6A, A') that are the primordia of the carpellate flowers (6B, B', C, C'). A carpellate flower consists only of a pedicel and a gynoecium, which itself is differentiated into a slender ovoid ovary and a filiform style. Pedicels differ in length or may be lacking entirely (Fig. 6D, D'). Flowers are packed very tightly, and, by different lengths of the pedicels and by mutual pressure, arrange themselves into several (usually three to four) tiers. In this *Balanophora* species, flower primordia emerge directly from the axis of the inflorescence and do not appear to fuse with the axis of the claviform body. Although the flowers are tightly packed together, careful dissection reveals that the pedicels are free all the way to the inflorescence main axis.

***Balanophora elongata*: Male inflorescences**—These are between 3 cm and 5 cm long, i.e., roughly the same as in *B. papuana*, but usually with fewer flowers than *B. papuana* (Fig. 1C). Flower primordia emerge from the dome-shaped apical meristem (Fig. 7A) in a spiral sequence. The formation of (pseudo-) whorls can be observed only occasionally. The four- (rarely five-) merous, zygomorphic, pedicellate flowers are subtended

by undivided, spatulate bracts. The anthers are fused into a synandrium.

Bract primordia emerge in the form of low, transverse ridges. In contrast to *B. papuana*, they remain meristematic throughout their margins (Fig. 7B, D–F). They do not split into halves, but grow undivided to full length. Figure 7B shows a sterile subtending bract from the apical area of an inflorescence. Fully developed bracts are narrow-oblong and truncated at the tip. No ring of sterile bracts is found at the inflorescence base (Fig. 7A).

Staminate flower primordia arise as meristematic protuberances above the bract primordia (Fig. 7C). As in *B. papuana*, the lateral and the lower tepals differentiate first, and the upper tepal (usually the smallest of the four) is the last one to emerge (Figs. 7E–G, 8A, B). At a later stage, papillae grow out from the margins of the tepals (Fig. 7G). Papillae of the adjacent margins come into contact and interlock (Fig. 8A–F). The closure of the young floral bud is so tight that at some stages perianth lobes appear fused and the individual tepals cannot be recognized (Fig. 8D). The pollen sacs are already open in the closed flower (not shown). Given the close relationship of *B. elongata* to *B. papuana*, it is possible that the flowers in *B. elongata* also open upon touch and that the interlocking of the tepals may play a role in this function (Fig. 8E, F).

The developing synandrium primordium (Fig. 9A) widens (Fig. 9B), and the pollen sacs differentiate in an irregular sequence and in various arrangements (Fig. 9B–F). No positional relationship with the tepals has been found. The synandrium structure shown in Fig. 9F is one of many possibilities. Although this synandrium appears to be subdivided into individual stamens, in other samples such subdivision cannot be seen.

***Balanophora elongata*: Female inflorescences**—These are between 3 and 4 cm long with axes ranging from 1.8 to 3 cm in diameter. The spadix surface is covered by numerous claviform bodies, each ca. 1 mm long. Carpellate flowers, up to 1.3 mm in length, are located between the claviform bodies. The extremely reduced, perianthless flowers consist only of a pedicel, a simple ovary, and a threadlike style.

The inflorescence primordium is ovoid and, at the stage shown (Fig. 10A), its surface is still completely undifferentiated.

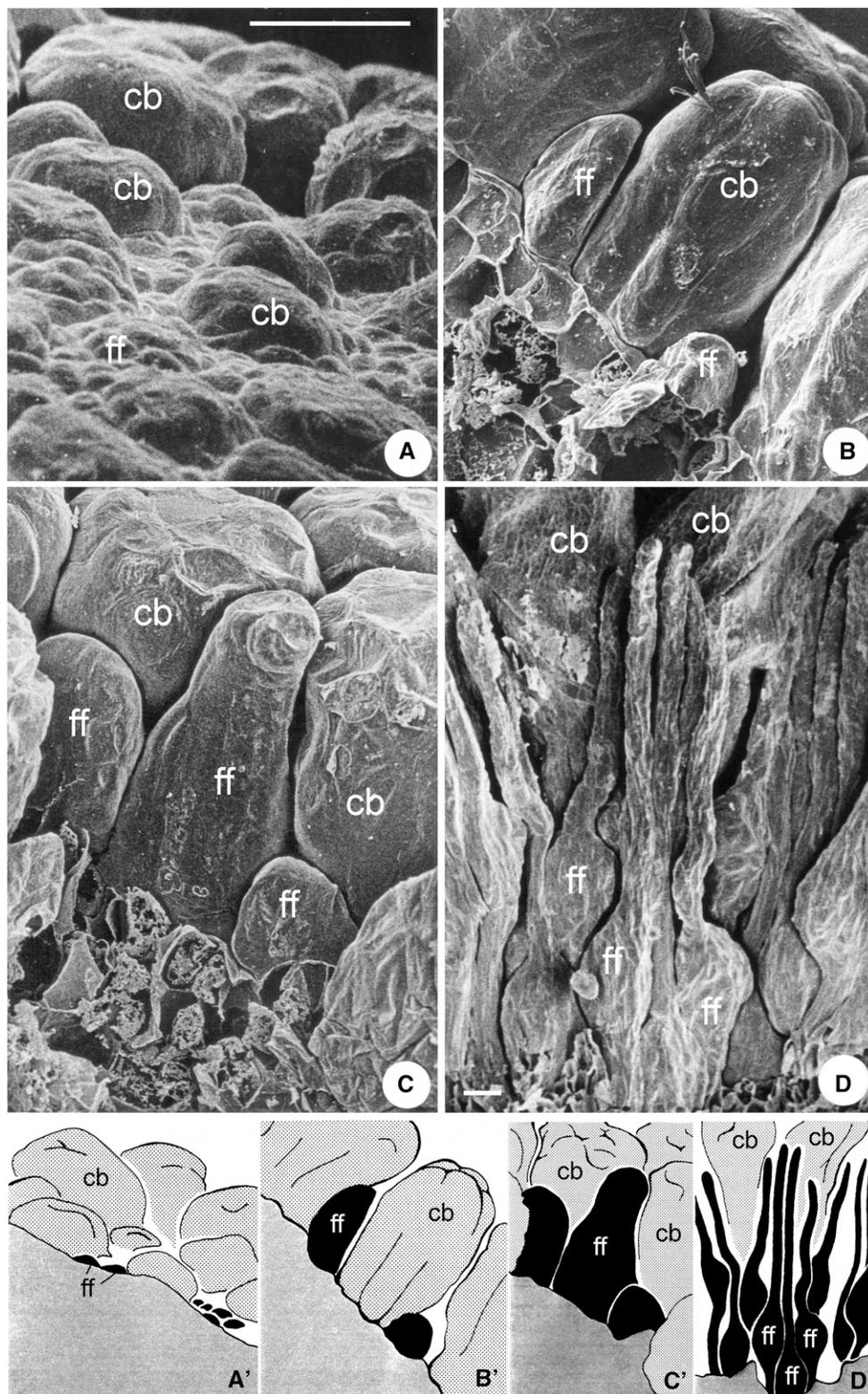


Fig. 6. SEMs of *Balanophora papuana*, development of claviform bodies and carpellate flowers in the female inflorescence. (A) Surface of spadix with emerging claviform body (cb) and female flower (ff) primordia (the latter colored black in the explanatory diagram a'). (B, C) Claviform bodies and flower primordia (in various developmental stages). (D) Young female flowers arranged in several tiers. Bar = 500 μ m. Figures A' to D', explanatory diagram of Figs. 6A–D.

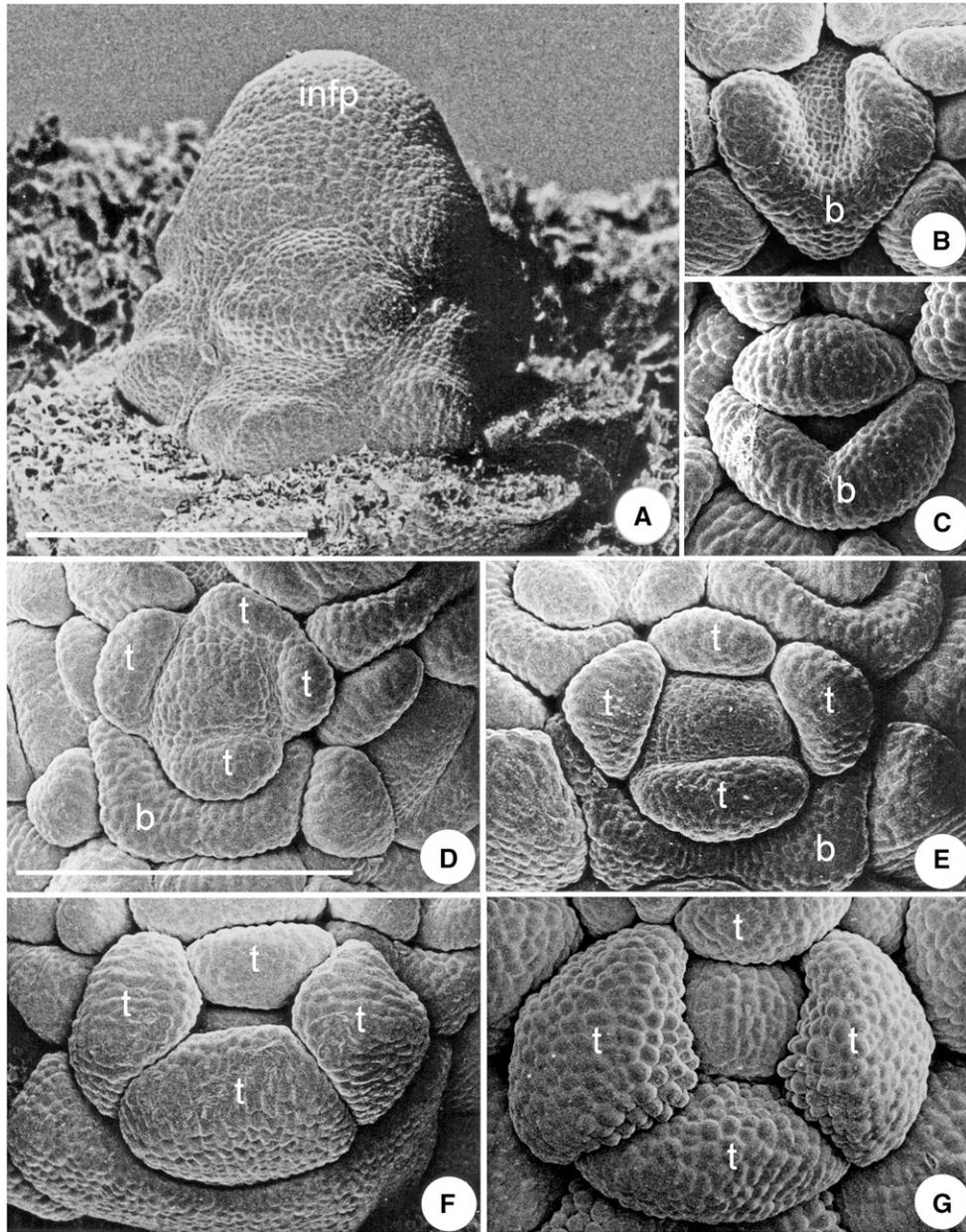


Fig. 7. SEMs of *Balanophora elongata*, development of the male inflorescence. (A) Inflorescence primordium (infp) with a large dome-shaped apical meristem. (B) Sterile (U-shaped) bract (b) from the apical region of a young inflorescence. (C) Bract (somewhat V-shaped) with floral primordium above. (D–G) Detachment of four tepal primordia (t) in prospective male flowers, each subtended by a bract; see Results, *Balanophora elongata*: male inflorescences for explanations). Bar = 500 μ m.

The first stage of differentiation is the almost simultaneous emergence of the claviform bodies (Fig. 10B). Only at the inflorescence apex and occasionally at the base is there some delay in the formation of the claviform bodies. These structures emerge as hemispherical knobs (Fig. 10C). The surface of the claviform bodies is made up of equal, tightly packed polygonal cells. The bodies closely resemble multiseriate glandular hairs and may have a secretory function (see Discussion). Carpellate flower primordia arise somewhat later, without clear order and without a recognizable association with the claviform bodies. When prospective carpellate flowers can be recognized (Fig. 10D), the claviform bodies have already attained a clavi-

form shape (Fig. 10D). The carpellate flowers then elongate rapidly (Fig. 10E) and finally slightly overtop the claviform bodies with their styles and stigmas (not shown).

***Balanophora fungosa*: Female inflorescences**—The individuals of this species examined were monoecious, thus according to Hansen (1972), this collection should be classified as *B. fungosa* subsp. *fungosa* (dioecious populations are *B. fungosa* subsp. *indica*). SEM photographs of the mature female inflorescence show the presence of female flowers (ca. 1.0 mm in length) densely packed around the claviform bodies (Fig. 11). The stigmas of most flowers are at or just below the surface formed by

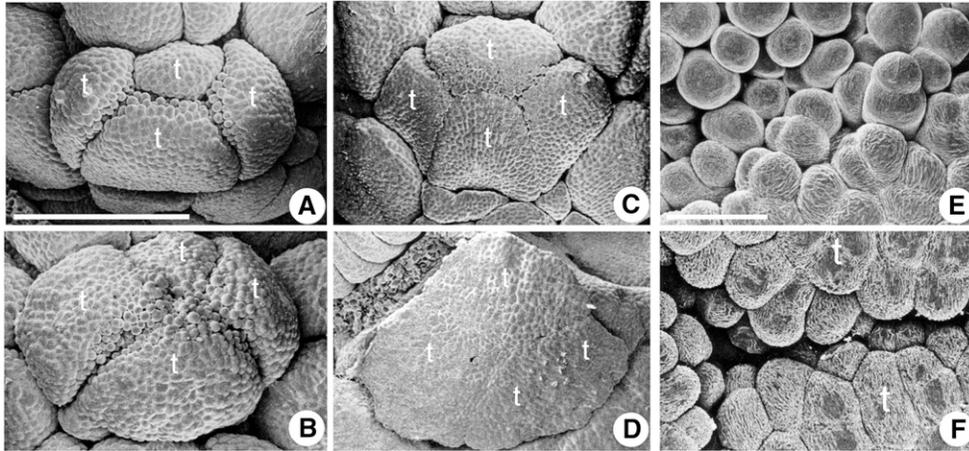


Fig. 8. SEMs of *Balanophora elongata*; perianth development in the male flower. (A–D) Various developmental stages showing tepals (t) interlocking by papillar outgrowths from their edges. Bar = 500 μm . (E, F) Closer views of tepal edges showing epidermal papillae. Bar = 50 μm .

the claviform body tips. Although the pedicels of many of the female flowers arise directly from the main inflorescence axis, others arise from the narrow basal stalk of the claviform body. Careful dissection shows that these pedicels are indeed fused to the claviform body, not simply attached to it by secretions.

DISCUSSION

In spite of the much reduced and seemingly peculiar nature of Balanophoraceae flowers and inflorescences, male flower ontogeny generally follows patterns seen in other angiosperms (including Santalales). However, the female flowers are more enigmatic. As evolutionary reductions and modifications become more extreme, it is increasingly difficult to relate observed patterns to those seen in other flowering plants. However, having a phylogenetic framework based on independent molecular characters is extremely helpful. For example, the evolutionary directionality (polarity) of perianth loss can be addressed given the preliminary molecular phylogeny of the family (Nickrent 1997). That tree shows that *Dactylanthus*, *Hachettea*, and

Mystropetalon (subfamily Mystropetaloidae of Harms 1935) are sister to the remaining genera. Because these genera have a perianth in female as well as in male flowers, its absence in the other genera represents at least one evolutionary loss in the family. Our results with *Balanophora* are compared with floral development and inflorescence architecture among various genera of Balanophoraceae.

Architecture of the male inflorescence—The helical developmental sequence of the flowers in the male inflorescence of *Balanophora elongata* and *B. globosa* was already noted by Fagerlind (1945a). This is confirmed here for *B. elongata*, and principally also for *B. papuana*. However, in the latter species, there is some tendency toward flowers arranged in whorls of four, which is indicated by the presence of eight (usually somewhat irregular) orthostichies. In plants with a whorl-like arrangement of organs that originate in a spiral manner, the pattern is described as false or pseudowhorls (Rutishauser, 1981).

Helical patterns usually involve organs not strictly defined in number, whereas whorls (including the decussate arrangement)

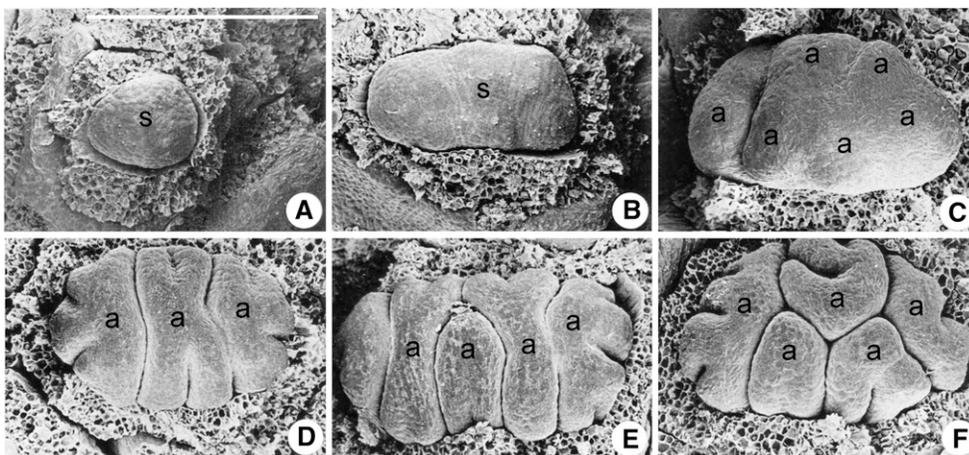


Fig. 9. SEMs of *Balanophora elongata*, development of the synandrium. (A–F) Primordia of the androecial synandrium (S) in various developmental stages and patterns of anther (a) differentiation; bar = 500 μm .

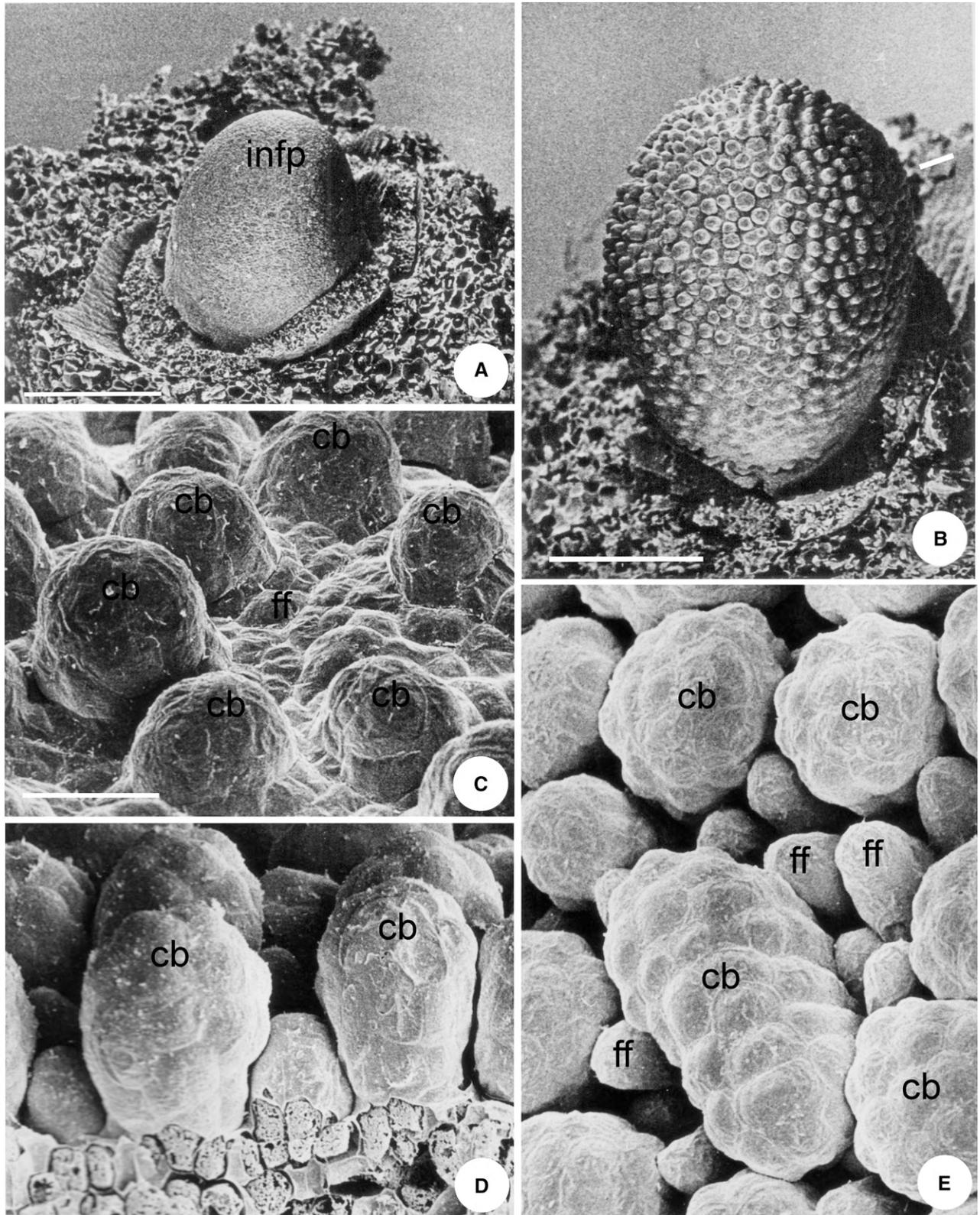


Fig. 10. SEMs of *Balanophora elongata*, development of the female inflorescence. (A) Inflorescence primordium (infp). (B) Primordium with young claviform bodies. Bar = 500 μ m. (C) Closer view of inflorescence primordium showing young claviform bodies (cb) and young developing female flower (ff); (D) Two claviform bodies with a female flower primordium between. (E) Top view of an older inflorescence primordium. Note young female flowers between the claviform bodies. Bar = 50 μ m.

have a more or less defined number of organs. The basal scale leaves are the organs that precede the subtending flower bracts, and their number (four) corresponds to the number of pseudowhorls of flowers and their subtending bracts. The tendency to form whorls, as in *B. papuana*, may explain the presence of four decussately arranged scale leaves at the base of the male inflorescence in that species. Although diagnostic of the species, this feature is based on an ontogenetically secondary event, i.e., the bract primordia emerge in a spiral manner, but due to effects of subsequent growth, later appear in a pseudowhorl. This ontogeny also explains the occasional occurrence of five scale leaves in a whorl, which was observed by Hansen (1976). Thus, the helical (*B. elongata*) and decussate (*B. papuana*) scale leaf arrangement patterns appear to be influenced by similar secondary ontogenetic processes that can convert the former into the latter pattern. This developmental similarity is not unexpected given that these two species are classified in the same section (*Dibalaniella*) and may be phylogenetically close.

The present developmental analysis shows that the initiation of bracts and the axillary origin and position of the male flowers follows a conventional pattern. No difficulties arise in the understanding of the male inflorescences and flowers. The only point of major interest is the synandrium.

Synandria—When synandria are observed, inevitably questions arise, such as of which parts they are composed, how these parts are arranged, and how fusion is achieved. Synandria, as found in *Balanophora*, occur in several groups of angiosperms (Endress and Stumpf, 1990). In both *Balanophora* species investigated, the apical meristem of the flower primordium is completely used up with the formation of the synandrium. Rudiments of a gynoecium or a central depression (i.e., a residual floral apex) do not occur. The differentiation into pollen sacs can be observed even during the initiation of the synandrial primordium. However, differentiation is irregular, and different from flower to flower. Therefore, it is not possible to associate the pollen sacs with individual stamens. In the two species investigated here, even the number of stamens forming the synandrium cannot be assessed. In *Balanophora involuocrata*, however, the synandrium contains only three pollen sacs, and in this case they occupy an antetepalous position (Hooker, 1856). Endress and Stumpf (1990) concluded that the *Balanophora* synandrium originally represented the fusion product of stamens that probably had two pollen sacs each. These authors further suggest that higher pollen sac numbers resulted from longitudinal septation, not by derivation from polysporangiate stamens.

The female inflorescence and its claviform bodies—In contrast to the male inflorescences, the female inflorescences have been interpreted differently by various authors. In the literature, the claviform bodies have been referred to as spadiceles (Eichler, 1873), spadiceles (Baillon, 1877), spadiceles (Fawcett, 1886), and the English translation of these terms, spadicles (Hansen, 1972, 1976; Hansen and Engell, 1978). These terms literally mean small spadix, which carries morphological implications. Specifically, the term implies that the claviform bodies are lateral axes derived from the primary inflorescence axis, and indeed this concept was accepted by many authors (Griffith, 1846; Weddell, 1850; Eichler, 1868, 1873; Baillon, 1877; Fawcett, 1886; Harms, 1935; Fagerlind, 1945a). Some authors abstain from an interpretation; for example, Engler (1894) used the

German term *Kölbchen* without further explanation. Backer and Bakhuizen van den Brink (1965) used the term *spadicle* explicitly as a technical term only, without interpretation.

The claviform bodies have also been interpreted as bracts. This concept probably first derives from Hooker (1856, 1859, 1886) and was revived and substantiated by the comparative studies of Hansen and Engell (1978). In support of their view, Hansen and Engell (1978) discuss *Balanophora wilderi* (the only monoecious species with male flowers above females on the inflorescence axis), where transitional organs between bracts subtending male flowers and claviform bodies are said to occur (Hansen, 1972). In the neotropical monoecious genera *Lathrophytum*, *Lophophytum*, and *Ombrophytum*, male and female flowers are borne on first order branches (brachyblasts), and these are covered by peltate scales interpreted as subtending bracts. But unlike *Balanophora*, these genera do not bear flowers on the main inflorescence axis. In genera of Helosidae, the first order branches are interpreted as being extremely reduced, even to the point of forming slight depressions in the main inflorescence axis. In much the same way, Hansen and Engell (1978) conclude that the spadicles in *Balanophora* are bracts (phyllaries), each subtending a depressed inflorescence branch.

The insertion points of the carpellate flowers on or near the claviform bodies may assist in assessing their homology. As stated by Hansen and Engell (1978), members of *Balanophora* subgenus *Balanophora* do not have carpellate flowers emerging from the claviform bodies. Our study suggests that this may also be the case with *Balanophora* subgenus *Balanophora* section *Dibalaniella* (*B. papuana* and *B. elongata*). Conversely, the female flowers in *B. fungosa* subsp. *indica* “ascend the basal parts of the bracts” (Hansen and Engell, 1978), as was illustrated by Fawcett (1886) for this species (as two synonyms, *B. gigantea* and *B. ramosa*). The current study shows such is also the case with *B. fungosa* subsp. *fungosa* (Fig. 11). Fagerlind (1945a), who also studied *B. elongata*, reported that when the spadicle develops, the flower rows at the base shift to the stalk-shaped basal part of the spadicle.

Flowering branches may exhibit metatopy, i.e., displacement and congenital fusion of organs such as stems and axillary buds (Weberling, 1989). Metatopic phenomena include concaulescence (acropetal displacement of an axillary bud to a position on the stem), recaulescence (displacement of the bud by stretching the common basal region of the leaf and bud), and syndesmy (where inflorescences are not free but incorporated into the stem axis). Although an ontogenetic study would be required to determine the exact form of metatopic displacement, the presence of multiple female flowers on all sides of a single claviform body suggests syndesmy. If the claviform body is interpreted not as a bract but as a first order branch, then metatopic displacement of flowers from the main inflorescence axis to this branch would be required, i.e., concaulescence. Evidence against this interpretation is the observation that flowers occur in spiral or whorled patterns on the claviform bodies (e.g., Fagerlind, 1945a). Such patterns would require adaxial, abaxial, and lateral displacements, shifts not known in the context of concaulescence.

Because of their surface structure and their general resemblance to multiseriate glandular hairs, the claviform bodies may be secretory structures. The secretion may be a sugary liquid (nectar) associated with pollination (Kawakita and Kato, 2002; Govindapa and Shivamurthy, 1975). Alternately, the watery liquid reported secreted from the dense layer of claviform bodies

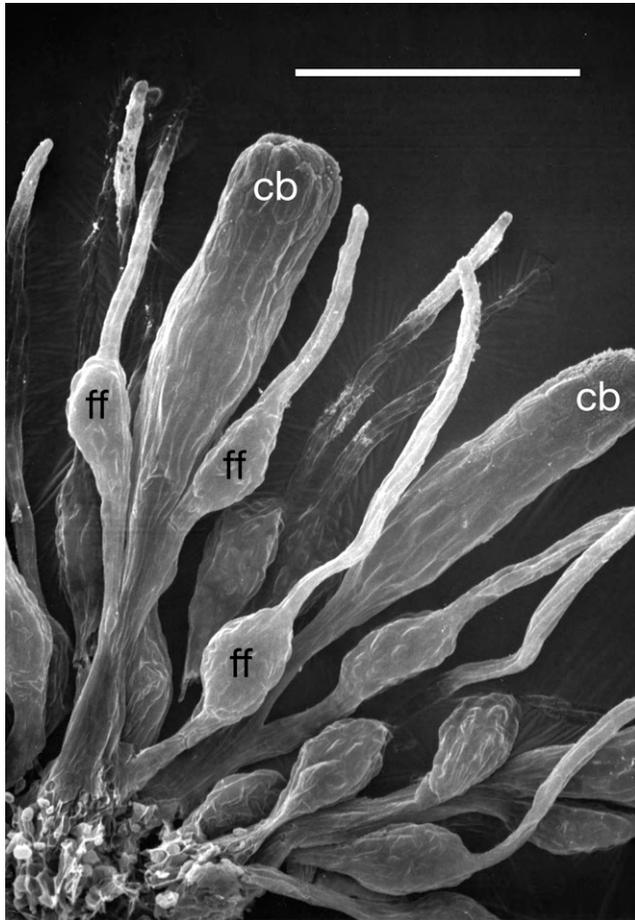


Fig. 11. SEM of *Balanophora fungosa*; portion of a female inflorescence showing the female flowers (ff) densely packed around and fused to the claviform bodies (cb). Bar = 500 μ m.

surrounding the naked and tiny carpellate flowers may protect them from desiccation.

Remaining questions—One of the difficulties of Fagerlind's (1945a) interpretation is the relationship between the male and the female inflorescences; the former are clearly simple spikes, whereas the latter would have to be regarded as double spikes, with the flowers representing lateral structures of the second order. An inconsistency arises with this interpretation. In the male inflorescence, the lateral structures (i.e., flowers) emerge in the axils of bracts and thus conform to the conventional pattern found in many (but not all) angiosperms. In the female inflorescence, the lateral structures (claviform bodies) do not have subtending bracts. To resolve the discrepancy, Fagerlind interpreted the male inflorescences as double spadices, where the secondary axis has widened and sunken into the primary inflorescence axis. It is only through such extreme hypotheses that congruence is reached between the male and female inflorescences. This male-inflorescence-as-double-spadices interpretation is, however, in clear conflict with the observable developmental facts. As shown by our SEMs, the subtending structures of the male flowers are bracts, as is evidenced by the organ number, by the laterally expanded form of the primordium, and by the spatial association with the prospective staminate flower. The split into two halves and subsequent bract

displacement observed in *B. papuana* is a peculiarity of this (and other?) species, and also cannot be seen as an indication of an axial nature. The bract split is clearly a developmentally secondary event in an initially undivided bract primordium.

The following issues about the claviform bodies remain unexplained and call for further investigation. These structures do not emerge in an acropetal sequence from the inflorescence apex like bract primordia in the male inflorescences. They arise almost simultaneously on the whole surface of the inflorescence primordium. The shape of the claviform body primordium is hemispherical, and no lateral extension can be observed. Thus, the primordia do not look like those typical of leaves or bracts, and more specifically, they are not similar to the bract primordia of the male flowers. Their distribution is dense and irregular. No orthostiches or parastichies can be observed that would indicate a spiral or other orderly sequence of emergence. The radial symmetry of the claviform bodies is retained throughout development. Marginal expansion is observed only occasionally (e.g., one of the bodies in Fig. 10E), but the claviform bodies never achieve a leaf- or bract-like form. Eberwein (2003, 2005) has shown that not all the bracts of *Rhopalocnemis* (Helosideae) have conventional leaf morphology. In *Rhopalocnemis*, the base of the inflorescence axis is densely covered by small flat bracts that pass into stalked-peltate structures in the fertile zone (with both male and female flowers embedded in a dense layer of hairs underneath the peltae). The pelta is hexagonal and has a distinct, wart-like or slightly recurved appendage in the center. Peltation of *Rhopalocnemis* bracts is not reached in the conventional way, by fusion of marginal blastozones (Hagemann, 1970), but by the formation of a protuberance both on the upper and the lower bract surface. The occurrence of unconventional bracts in *Rhopalocnemis* opens the possibility that the claviform bodies of *Balanophora* could be interpreted as unorthodox bracts. Eberwein (2005) proposed a number of possible ontogenies of peltate bracts that await confirmation by targeted studies of Helosideae, Lophophyteae, and Balanophoreae. Nonetheless, it seems clear that the bracts of Balanophoraceae possess a morphogenetic potential not known so far from other angiosperms.

Conclusions—Our analysis of *Balanophora papuana* and *B. elongata* shows that the male inflorescences follow a rather conventional pattern of development. Primordia of bracts emerge from the inflorescence apical meristem in a helical sequence. This pattern is retained in *B. elongata* during further development and maturation, but is modified in *B. papuana* by the formation of alternating pseudowhorls of four bracts, resulting in eight orthostichies. However, breaks (discontinuities) in the whorled pattern often occur, so that the architecture of the male inflorescences is not absolutely regular. Primordia of staminate flowers arise in close association with the bracts in axillary positions. Male flowers contain only two floral whorls: a four-(rarely five-)merous perianth, and stamens congenitally fused to a synandrium. The differentiation of pollen sacs does not follow strict rules; neither number, form nor position follows predictable patterns. It is thus impossible to recognize from a surface view the number of stamens making up the synandrium.

Female inflorescences are more difficult to understand; this difficulty is intimately tied to the interpretation of the claviform bodies, between and upon which are found the extremely reduced carpellate flowers. We prefer the interpretation of Hansen and Engell (1978) who considered claviform bodies homologous to the peltate scales (bracts) found in genera of Helosideae and Lophophyteae, but problems with this concept

still exist. In the two species we investigated, the claviform bodies arise almost simultaneously all over the developing inflorescence, with some delay in the apical and basal regions. An acropetal succession and/or helical pattern of emergence or position is not observed. The shape of the primordium is hemispherical, with radial symmetry and lack of a marginal blastozone. Neither by the succession of emergence nor by their primordium or adult form is their interpretation as conventional bracts supported. The same holds true for the (less probable) view that the claviform bodies represent lateral axes, sometimes with flowers borne on them. The claviform bodies do not emerge in the axils of bracts and are extra-axillary structures. Nonetheless, the developmental patterns seen in "typical" bracts (helical-acropetal initiation and arrangement, presence of a marginal blastozone, etc.) have not been observed in the claviform bodies of these two *Balanophora* species. Developmental and phylogenetic data from other Balanophoraceae are needed to present a definitive interpretation.

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