Pl. Syst. Evol. 143, 151—161 (1983)

Plant Systematics and Evolution

Ultrastructural, Micromorphological and Phytochemical Evidence for a "Central Position" of *Macarthuria* (*Molluginaceae*) within the *Caryophyllales*

By

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(Received October 18, 1982)

Key Words: Angiosperms, *Caryophyllales*, *Molluginaceae*, *Macarthuria*.— Sieve-element plastids, pollen, ultrastructure of exine, seed-coat surface, anthocyanins.

Abstract: Subtype PIII sieve-element plastids, anthocyanins, spinulose, perforate-tectate pollen grains and the specific seed-coat sculpturing found in two *Macarthuria* species (*M. australis*, *M. neocambrica*) consolidate their placement within *Molluginaceae*. The unique form of the sieve-element plastids, i.e. with cubic crystals and starch grains (PIIIc"fs), finds its closest counterpart in *Limeum*. The multiple intertwinement of different genera of the *Molluginaceae* with many other centrospermous families led to a consideration of their more central position within *Caryophyllales*.

The family *Molluginaceae* is an integral part but probably the least investigated of the otherwise morphologically, ultrastructurally and chemically well defined order *Caryophyllales* (see MABRY & BEHNKE 1976). Separated from the *Aizoaceae* s.l. already earlier, HUTCHINSON (1926) established the *Molluginaceae* as a distinct family. Recent classifications of the *Caryophyllales* which place the *Molluginaceae* close to the *Caryophyllaceae* (DAHLGREN 1980, CRONQUIST 1981) or even treat the two families as the suborder *Caryophyllineae* (THORNE 1976, TAKHTAJAN 1980) are supported by the detection of anthocyanins in both taxa (BECK & al. 1962, MABRY & al. 1976) as well as HOFMANN's (1973) morphological monograph of the *Molluginaceae*. All available data also demonstrate that the *Molluginaceae* are heterogeneous in many respects. The presence of betalains in *Gisekia* clearly can be resolved by its exclusion from the *Molluginaceae* (MABRY & al. 1976) and an incorporation into the *Phytolaccaceae* (TAKHTAJAN 1980). However, the coexistence of anthocyanin-synthesizing genera together with (apparently) nonpigmented genera (e.g. *Limeum*) and of sieve-element plastids with a globoid crystal (form PIIIcf, in the tribe *Mollugineae*, BEHNKE & al. 1983) and those containing cuboid crystals (form PIIIc″f, *Limeum*) awaits an explanation. HOFMANN (1973) had stressed that the closest relationships of the *Molluginaceae* after the *Aizoaceae* were with the *Phytolaccaceae*; EHRENDORFER (1976) presented arguments in favour of a "central position" of the *Molluginaceae—Aizoaceae—Phytolaccaceae* in the order *Caryophyllales*; DAHLGREN (1980) puts *Limeum* back into the *Phytolaccaceae* (where it had been placed previously by MOQUIN-TANDON 1849). Undoubtedly, after HOFMANN's (1973) thorough morphological study there is an obvious need for a closer inspection of more genera of the *Molluginaceae* with modern methods, to which the present report is a beginning.

Materials and Methods

Living plant material was obtained by air mail from the following sources: *Macarthuria australis* HUEGEL ex ENDL. collected 1982-02-16 and 1982-03-25 at Herbarium Garden, Dept. of Agriculture, South Perth, Western Australia by K. F. Kenneally (No 8027); *Macarthuria neocambrica* F. v. MUELL. collected 1982-03-29 at Evans Head, N.S.W., Australia by P. Hind (No 3084). Voucher specimens are deposited in the Western Australian Herbarium and at NSW, respectively.

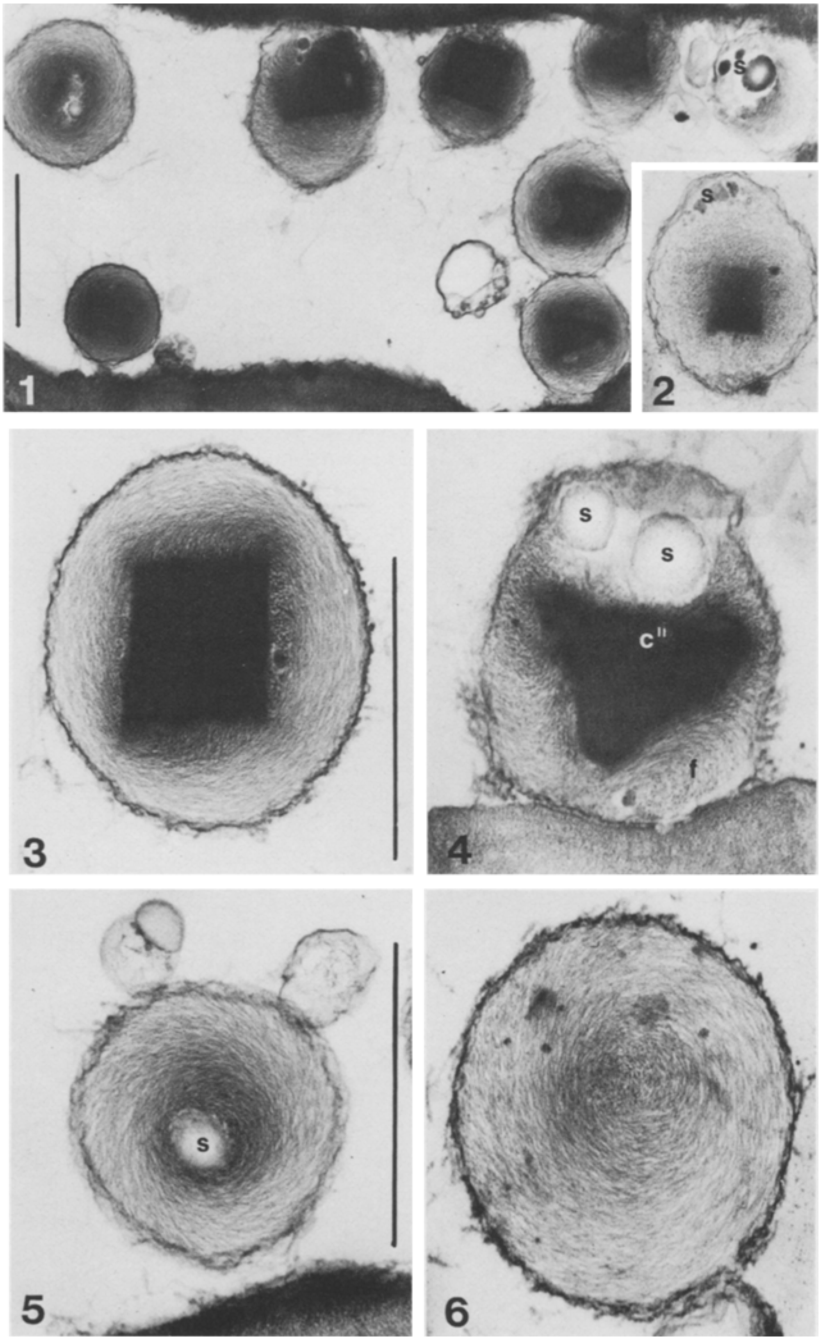
Transmission electron microscope investigations included: (1) for sieve-element plastids a fixation of longitudinal hand sections of stem pieces in a formaldehyde-glutaraldehyde solution followed by a post-fixation in 1% osmic acid; (2) for pollen exine a fixation of entire anthers in 1% osmic acid overnight. All samples were processed according to standard procedures including an embedment in epoxy resins (see BEHNKE 1982a, for details).

Scanning electron microscope investigations of acetolyzed pollen grains and fresh seeds were performed—after sputtering with gold—with a Cambridge Stereoscan 600 microscope.

For pigment analysis reddish pigmented tissue of both species were selected and subjected to standard procedures (MABRY & al. 1975). The test for C-glycosylflavonoids used standard flavonoid identification techniques (MABRY & al. 1970) including two-dimensional paper chromatography and acidic hydrolysis conditions.

Results

Sieve-Element Plastids. In *Macarthuria* as in all other members of the *Caryophyllales* the sieve-element plastids are of the subtype PIII. However, the specific form represented—PIIIc″fs—has not been recorded elsewhere. *M. australis* (Figs. 1, 3-6) and *M. neocambrica* (Fig. 2) both contain subtype PIII plastids which, in addition to the filaments



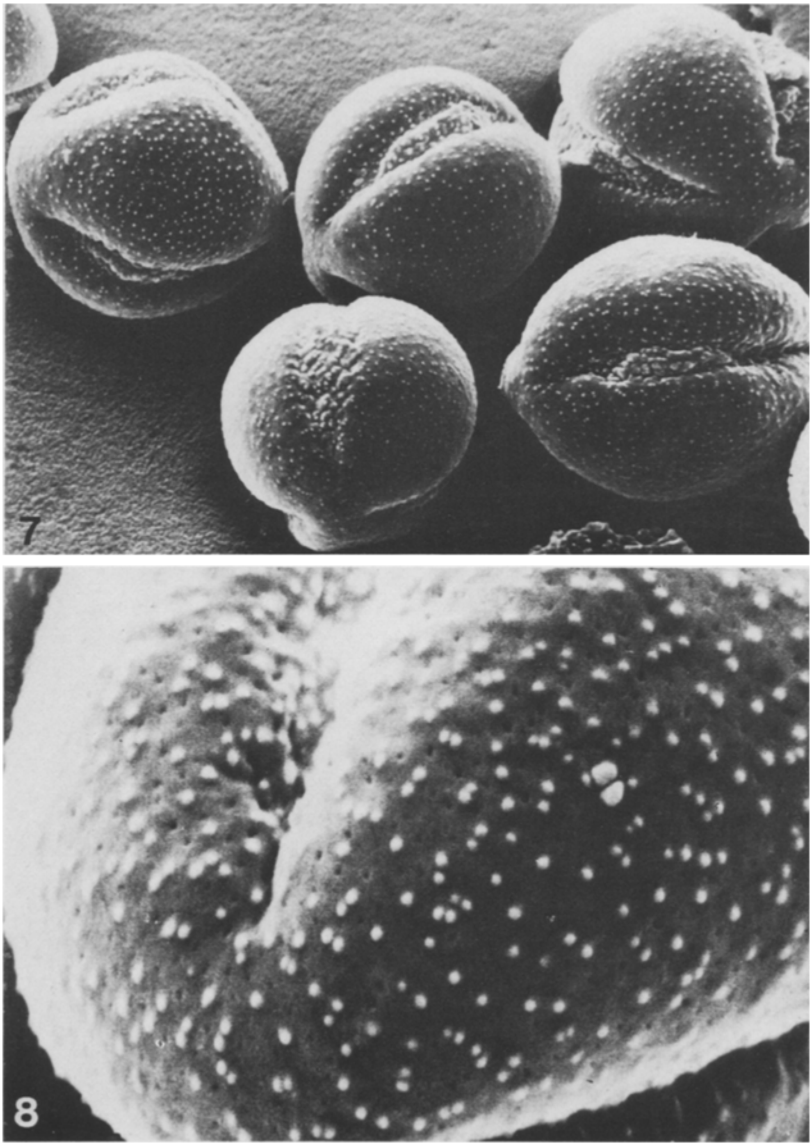
Figs. 1-6. Form-PIIIc″fs sieve-element plastids of *Macarthuria* with central crystal (c'') of cuboid system, peripheral filaments (f) and starch grains (s). Depending from the angle of sectioning smaller and larger portions of the plastids are depicted which exhibit either all kinds of inclusions (Fig. 4: c'', f, s) or only some of them (Figs. 3, 5, 6). Figs. 1, 3-6: *M. australis.* Fig. 2: *M. neocambrica.*—Figs. 1, 2: TEM, ×20,000; Figs. 3-6: TEM, ×40,000.—Scales = 1 μm

(Fig. 4, f), are characterized by a crystal of the cubic system (Fig. 4, c″) and a few starch grains (Fig. 4, s). The crystal is near a central position. The most frequent tri- and quadrangular outlines exhibited in thin sections (Figs. 1-4) suggest that it is a cube-like, a parallelepiped or similarly regular body. It is clearly distinguished from central crystals of form PIIIc′f which are polygonal in thin sections (cf. BEHNKE 1976a). The starch grains are always located at the periphery of the plastids (Figs. 1, 2, 4), partly ensheathed by the filaments (Figs. 1, 5). Some sections cut through these plastids show only protein filaments (Fig. 6). The four different views of form PIIIc″fs sieve-element plastids depicted in Figs. 3-6 impressively demonstrate the need for serial or sequential sectioning for the definite determination of a plastid type (cf. BEHNKE 1977).

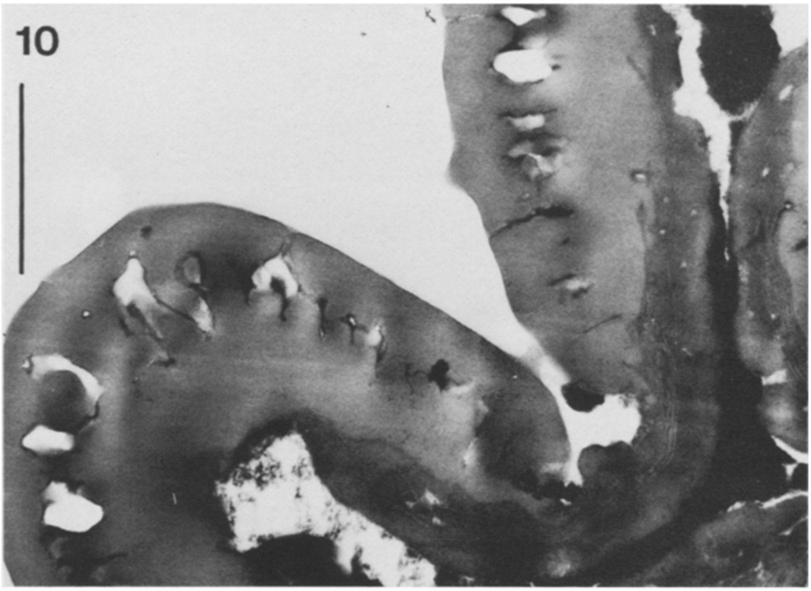
Pollen Exine. The scanning electron micrographs of the tricolpate pollen grains demonstrate a spinulose punctate exine sculpturing for both *M. neocambrica* (Figs. 7, 8) and *M. australis* (Fig. 9). Unlike the situation with many other centrospermous pollen exines there are no anuli formed surrounding the puncta ("tubuliferous tectum" of NOWICKE 1975 and "anulopunctate tectum" of LEUENBERGER 1976). Transmission electron micrographs of *M. australis* (Figs. 10, 11) exhibit an exine composed of a thick tectum supported by very broad and thick columellae which in turn sit on a heavy foot layer. A lamellated endexine is visible and especially prominent in apertural regions (Fig. 10). Small supratectal spinulae and narrow perforations (Fig. 11) correspond to the surface pattern described with the SEM.

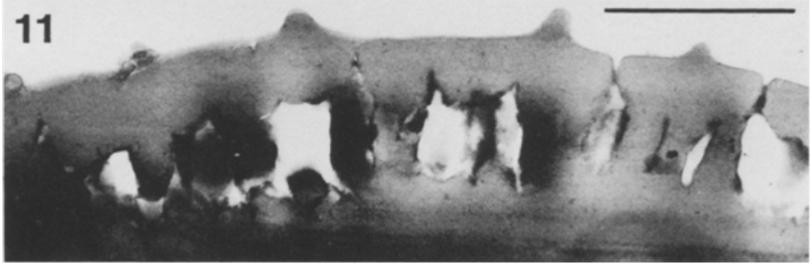
Pigments. Examination of the red pigments in the stems and flowers of *M. australis* and leaves and stems of *M. neocambrica* by standard electrophoretic procedures (MABRY & al. 1975) clearly established that these species produce anthocyanins and not betalains. The pigment from *M. australis* was further identified as a cyanidin 3,5-dimonoside (probably a 3,5-diglucoside based on color reactions and co-chromatography of both the aglycone and glucoside with authentic standards). When the aqueous methanol extract of leaves of *M. neocambrica* were examined by two-dimensional paper chromatography (MABRY & al. 1970) only flavonoids which appeared to be O-glycosides were detected. This was confirmed by treating the extract under acidic hydrolysis conditions; under these conditions no flavonoid glycosides remained and only flavonol aglycones could be detected. Thus, *M. neocambrica* does not contain C-glycosylflavonoids.

Seed Coat. The seeds of *Macarthuria neocambrica* are ovoid, with a length of about 1.7 mm. Attached to the seed is a small and dry aril

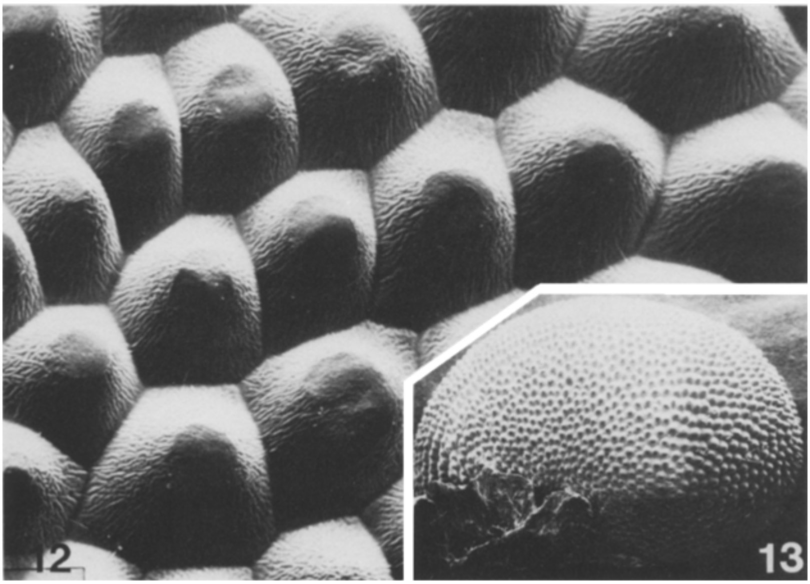


Figs. 7–8. Tricolpate pollen grains of *M. neocambrica* with spinulose and finely punctate exine sculpturing.—Fig. 7: SEM, × 1,750; Fig. 8: SEM, × 7,000





Figs. 9-11. *M. australis*.—Fig. 9: Spinulose and finely punctate pollen exine sculpturing. SEM, ×8,500.—Figs. 10-11. Sections through pollen wall exhibiting tectate perforate exine with thick tectum, broad columellae and thick foot-layer. Lamellated endexine (in 10) very conspicuous below apertures. Fig. 10: TEM, ×25,000; Fig. 11: TEM, ×30,000.—Scales = 1 μm



Figs. 12–13. Seed-coat sculpturing of *Macarthuria neocambrica*.—Fig. 12: Hexagonal testa cells aligned in regular longitudinal rows are covered by a pattern of central-field type cuticular foldings. SEM, ×500.—Fig. 13: Entire seed with dry aril. SEM, ×30

(Fig. 13). The seed coat is black. The testa cells are arranged in regular longitudinal rows along the dorsal juncture (Fig. 12). In the top view a single cell is isodiametrical, often hexagonal and cone-shaped. The surface is covered by a very prominent pattern of cuticular foldings, i.e. of the central-field type (Fig. 12). The specific arrangement of testa cells along the dorsal juncture, the presence of the dry aril plus the central field type of cuticular ornamentation are very characteristic for major groups of *Caryophyllales* (e.g. many *Cactaceae* and *Portulacaceae*) and are part of the "centrospermoid" seed syndrome (BARTHLOTT 1983).

Discussion

The ultrastructural and micromorphological data presented—subtype PIII sieve-element plastids, spinulose and perforate-tectate exine, and specific seed-coat surface—clearly integrate *Macarthuria* in the *Caryophyllales*. Moreover, the presence of anthocyanins and absence of betalains support the inclusion of *Macarthuria* in the *Molluginaceae*. Previously, RICHARDSON (1981) suggested that herbaceous members of the anthocyanin family *Molluginaceae* and the betalain family *Aizoaceae* could be distinguished on the basis of the presence of C-glycosylflavonoids in the former and their absence from members of the latter familiy. Our finding of only flavonoid O-glycosides and no C-glycosylflavonoids in the molluginaceous *M. neocambrica* provides an exception to this generalization.

A close inspection of the exine structure of *M. australis* reveals similarities—viz. proportion and subdivision of strata—likewise with the *Molluginaceae* (e.g. *Limeum*: NOWICKE & SKVARLA 1977, *Gisekia*: NOWICKE & SKVARLA 1979), but also with some *Aizoaceae* (*Mesembryanthemum*: SKVARLA & NOWICKE 1976, NOWICKE & SKVARLA 1977). The micromorphology of the seed-coat surface is identical to a pattern known from several *Cactaceae* (VOIT 1979) and very similar to some *Portulacaceae* (BEHNKE & BARTHLOTT 1982).

The unique form PIIIc″fs sieve-element plastids of *Macarthuria* have only one counterpart in the *Caryophyllales*, the form PIIIc″f plastids of *Limeum*. Although previously filed among PIIIc′f (i.e. with a polygonal crystal), additional serial sectioning proved that the central crystal fits into the cubic system—see also quandrangular crystal of the plastid of *Limeum* published earlier (Fig. 13 in BEHNKE 1976 b). Thus, the nearly identical forms of subtype PIII sieve-element plastids strongly support the tribal arrangement made by HOFMANN (1973) after a detailed morphological and anatomical study of the flowers whereby *Limeum* and *Macarthuria* are placed together into the tribe *Limeeae*.

HOFMANN (1973) previously mentioned multiple close relationships between the *Molluginaceae* and the *Caryophyllaceae*, *Portulacaceae*, and *Phytolaccaceae* which suggest a more central position among the *Caryophyllales* for the *Molluginaceae*. Since then additional results obtained by various methods corroborate this view, e.g. the detection of betalains in *Gisekia* (MABRY & al. 1976) now placed into *Phytolaccaceae*, the comparable exine stratifications in *Molluginaceae* and *Aizoaceae*, the identical seed coat patterns between *Macarthuria* and some *Portulacaceae* and *Cactaceae*. The form PIIIc″f(s) sieve-element plastids combine *Limeum* and *Macarthuria* with *Phytolaccaceae* and *Caryophyllaceae*: *Stegnospermataceae* and *Achatocarpaceae*—as subfamilies often included in *Phytolaccaceae* s.l.—contain polygonal crystals (c′) in their PIII-plastids. These latter types of crystals are found in all tested genera of the *Caryophyllaceae* (BEHNKE 1976 a, 1982 b) which are supposed to be closer to "cubic" crystals (c″) than to the globular crystals (c) of the great majority of the *Caryophyllales*. Starch in subtype PIII plastids is found in *Molluginaceae*, *Nyctaginaceae* (2 genera, each) and *Phytolaccaceae* (1 genus). All of this evidence would agree with a more basic and central position of the *Molluginaceae*—as e.g. proposed by EHRENDORFER 1976 (see also MABRY 1977)—than reflected in recent classifications of the angiosperms.

We are deeply indepted to Drs. K. F. KENNEALLY (Western Australian Herbarium, South Perth) and P. HIND (Royal Botanic Gardens, Sydney) who collected living material from *Macarthuria* and acknowledge the great help in this matter given to the senior author by Drs. JANICE JACOBS and DIANA BRIGGS (Sydney). Mrs. L. POP and Mrs. D. LAUPP (both Heidelberg) assisted with the processing of the material for electron microscopy. We thank Dr. J. B. HARBORNE (Reading, England) for co-chromatographing the cyanidin derivative and its aglycone with standard samples and DOUGLAS GAGE and SUSAN McCORMICK (both Austin) for technical assistance in the chemical analyses.

Supported by Deutsche Forschungsgemeinschaft (grants to HDB) and by the National Institutes of Health (Grant HDO-4488 to TJM) and the Robert A. WELCH Foundation (Grant F-130 to TJM).

This paper was part of a lecture on "Ultrastructurelle und phytochemische Untersuchungen zur Evolution der Centrospermen" presented by the two senior authors during a symposium on "Ultrastructural and Phytochemical Investigations of Plants" held at June 23, 1982, at Heidelberg and celebrating ten years of research cooperation between members of UT-Austin Department of Botany and Zellenlehre and Pharmazeutische Biologie der Universität Heidelberg.

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