

ONTOGENETIC ORIGINS OF FLORAL BILATERAL SYMMETRY IN MORINGACEAE (BRASSICALES)¹

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Floral morphology of the 13 species of *Moringa* ranges from actinomorphic flowers with little hypanthium to highly zygomorphic flowers with well-developed hypanthia. Scanning electron and light microscopy were used to identify ontogenetic differences among two actinomorphic and eight zygomorphic species. All species show traces of zygomorphy between petal organogenesis and anther differentiation. At late organogenesis, zygomorphy is manifest by one petal being larger than the others, slight unidirectional maturation of the anthers, and in many species, some staminodes may be missing. At organ differentiation and beyond, the actinomorphic species show a trend toward increasing actinomorphy, whereas the zygomorphic features of early ontogeny are progressively accentuated throughout the ontogeny of the zygomorphic species. Because of the early traces of zygomorphy throughout the family, ontogeny in *Moringa* does not resemble that known from the sister taxon Caricaceae, which has flowers that are actinomorphic throughout ontogeny. Great intraspecific variation was found in floral plan in the actinomorphic-flowered species in contrast to the zygomorphic species. Each of the main clades in the family is distinguished by at least one feature of floral ontogeny. In general, ontogenetic differences that are congruent with deeper phylogenetic splits tend to occur earlier in ontogeny than those congruent with more recent divergences.

Key words: Brassicales; development; evolution; floral symmetry; homology; *Moringa*; ontogeny; phylogeny.

Bilateral floral symmetry (zygomorphy) has repeatedly arisen from ancestors with radially symmetrical (actinomorphic) flowers (Endress, 1999) and is a character of major systematic significance. Likewise, floral symmetry is one of the primary factors determining the spectrum of pollinators that a flower attracts (Dafni and Kevan, 1996). As a result of the importance of this character, the evolutionary processes leading to symmetry differences have received attention from a variety of perspectives, from molecular genetics (Theissen, 2000; Shepard and Purugganan, 2002) to comparative morphological studies of ontogeny (e.g., Tucker, 1984, 1997, 2000). The latter approach is particularly valuable when a phylogenetic hypothesis for the group in question is available, because the phylogeny can be used to identify the polarity of evolutionary transformations within a clade. For example, Hufford (1995) evaluated his studies of comparative ontogeny in the snapdragon relative *Besseyia* in the context of a phylogeny estimated from nonfloral data and arrived at the counterintuitive conclusion that evolutionary inversions and deletions of early

and intermediate ontogenetic stages are as common as modifications to the terminal phases of ontogeny.

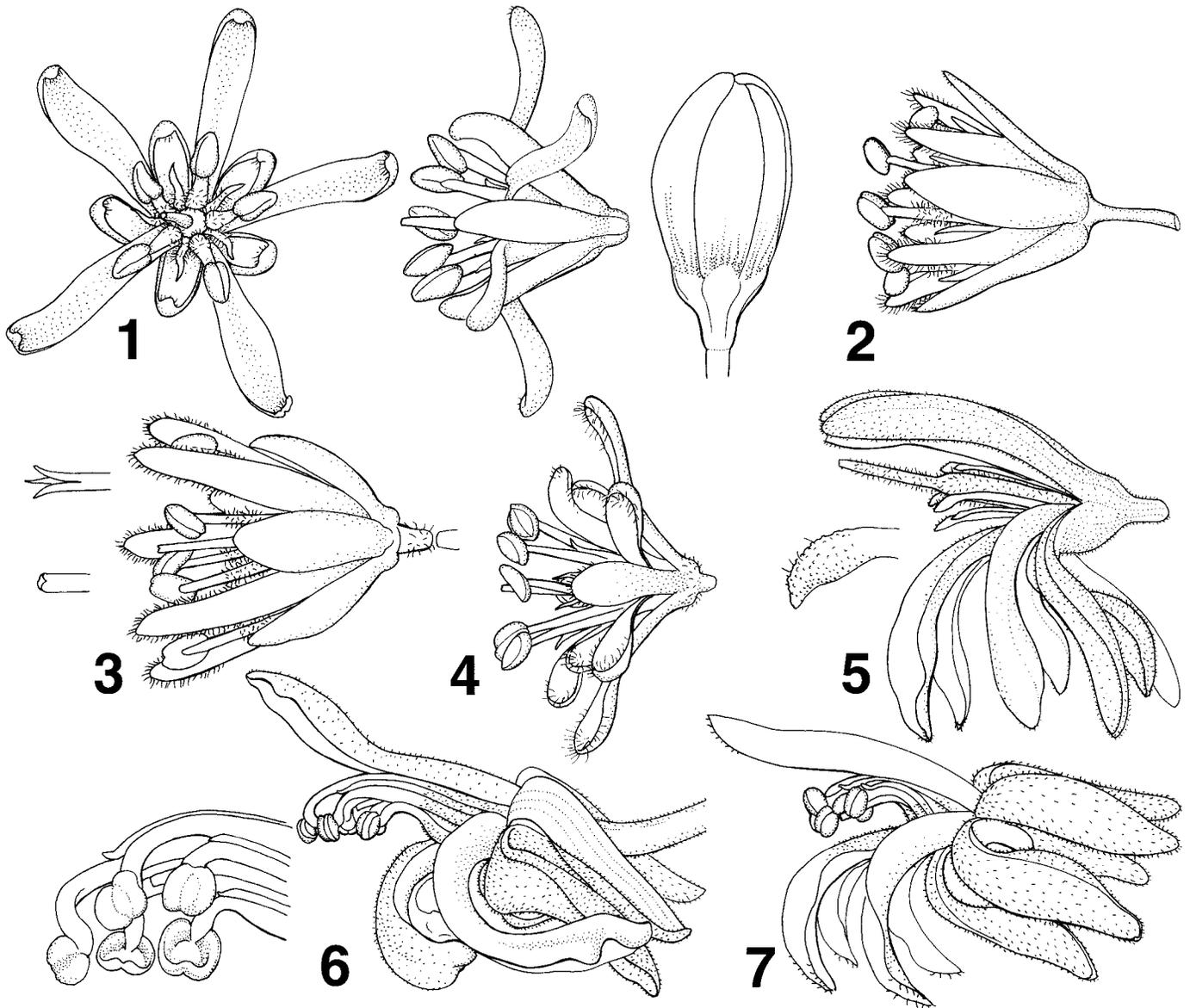
The 13 species of *Moringa* span the range from small, actinomorphic, cream-colored flowers with little hypanthium, to highly zygomorphic, intensely colored flowers with pronounced hypanthia (Figs. 1–13). Recent field work in dry tropical Asia, Africa, and Madagascar has made it possible to include ten of the 13 species in a comparative study of floral ontogeny with the following aims: (1) document floral organogenesis, anatomy, and ontogeny in *Moringa*, the only genus in Moringaceae; (2) test the expectation that actinomorphic flowers in *Moringa* are entirely so in all stages, as in the sister group Caricaceae (Ronse Decraene and Smets, 1999), and that zygomorphy becomes manifest in the later stages of ontogeny; (3) examine the congruence of ontogenetic characters with a previous phylogenetic reconstruction (Fig. 14); and (4) examine the correlation of the timing of ontogenetic divergences between species groups with degree of phylogenetic relationship. Because perturbations to early ontogeny may drastically affect subsequent events, numerous workers (e.g., Takhtajan, 1972; Tucker, 1984, 1997) have suggested that early stages should be more evolutionarily conserved while later stages are more evolutionarily labile. Therefore, divergences in early ontogeny should characterize deep phylogenetic splits, and divergences at later stages should characterize more recent evolutionary branching.

Moringa flowers (Figs. 1–13) have five sepals, five petals, five antesealous staminodes, and five antepetalous stamens with monotheical, bisporangiate anthers. The ovary is borne atop a gynophore of varying length, and the style is hollow. The four actinomorphic-flowered species, all massive pachycaul trees, form a paraphyletic assemblage at the base of the *Moringa* phylogeny (Fig. 14; Olson, 2002). Flowers in this group (the “actinomorphic grade”) are cream-colored with unreflexed sepals and petals that may be reflexed at anthesis (*M. drouhardii*, Fig. 1; *M. ovalifolia*, Fig. 4) or may project forward (*M. hildebrandtii*, Fig. 2; *M. stenopetala*, Fig. 3). The nine zygomorphic-flowered species form a monophyletic

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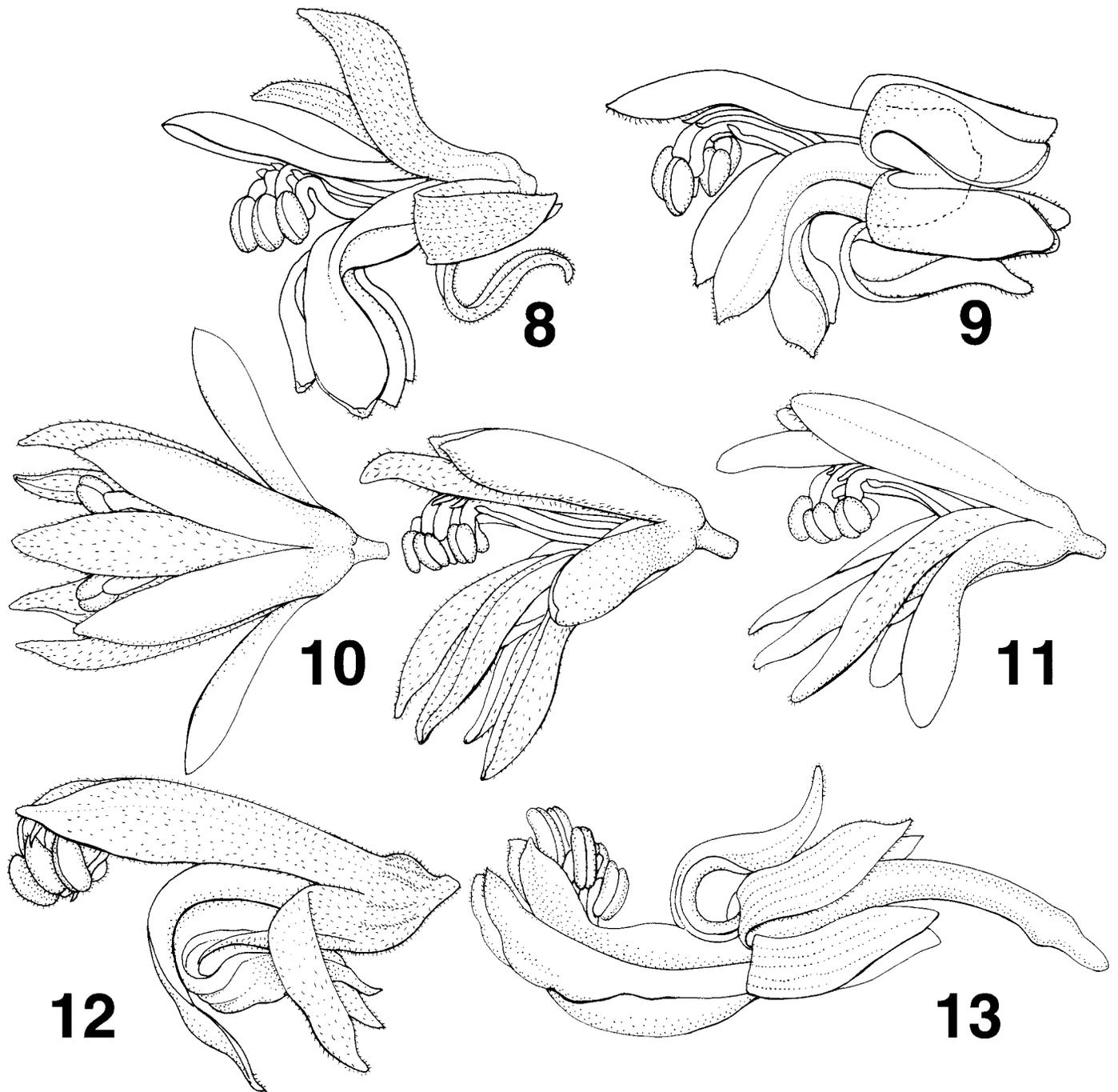
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Figs. 1–7. Flowers of all species in the actinomorphic grade and slender tree clades of *Moringa*. Side views unless specified. 1–4. Members of the actinomorphic grade showing actinomorphic or nearly actinomorphic arrangement of parts. 1. *Moringa drouhardii* Jum., apical and side views, showing reflexed petals, and bud showing irregular shape at base. 2. *Moringa hildebrandtii* Engl., showing slightly unequal filaments. 3. *Moringa stenopetala* (Baker f.) Cufod., with details showing shallow and deep lobing of style apex. 4. *Moringa ovalifolia* Dinter & A. Berger. 5–7. Members of the slender tree subclade of the zygomorphic clade. 5. *Moringa concanensis* Nimmo, flower with nonadhering filaments and reduced anthers, with detail of reduced anther. 6. *Moringa oleifera* Lam. with detail of three-tiered anther presentation. 7. *Moringa peregrina* (Forssk.) Fiori.

group (the “zygomorphic clade” in Fig. 14). Zygomorphy is apparent in the flowers of all of these species through differential flexion of petals, sepals, the androecium, and the gynoecium. The three principally Asian species (Figs. 5–7) have mostly cream-colored flowers and form a clade of slender trees (“S” in Fig. 14). The slender trees are the sister group to a clade of tuberous trees and shrubs that have diversified in the Horn of Africa (“T” in Fig. 14). This tuberous clade is subdivided into two subclades, the *rivae* clade (“ri” in Fig. 14), consisting of four species with yellowish flowers often blotched with maroon (Figs. 8–11). The remaining two species (Figs. 12, 13) have the only red flowers in the genus and are designated the red-flowered clade (“rf” in Fig. 14).

Like many taxa of the dry tropics, most species of *Moringa* have been little studied. The flowers of *M. ovalifolia* and *M. ruspoliana* are illustrated here for the first time (Figs. 4, 12), and, of those that have been illustrated previously, *M. arborea*, *M. borziana*, *M. pygmaea*, and *M. rivae* are illustrated for the first time in their correct orientations (Figs. 8–11). Previous studies of floral anatomy (e.g., Dutt, 1978; Dutt, Narayana, and Parvathi, 1978; Ronse Decraene, De Laet, and Smets, 1998) have examined only *M. oleifera* and *M. concanensis*, both members of the slender tree clade from the Indian subcontinent. Studies of wood anatomy, which also focused on the slender tree clade (e.g., Metcalfe and Chalk, 1950; Dutt, Rao, and Rai, 1978), greatly under-represented the diversity of



Figs. 8–13. Flowers of all species of the tuberous subclade of the zygomorphic clade in *Moringa*. Side views unless specified. 8–11. Members of the *rivae* subclade. **8.** *Moringa arborea* Verdc.; sepals may be entirely reflexed. **9.** *Moringa borziana* Mattei, showing nearly actinomorphic calyx. **10.** *Moringa rivae* Chiov. subsp. *rivae*; lateral views of flower from above and one side as it is oriented at anthesis. **11.** *Moringa pygmaea* Verdc. from type specimen. 12–13. Members of the red-flowered subclade. **12.** *Moringa ruspoliana* Engl. **13.** *Moringa longituba* Engl.

anatomical modes in the family (Olson and Carlquist, 2001; Olson, 2002). This bias toward the slender tree clade is due to the economic importance of *M. oleifera* as a food and oil plant and because these species are widely cultivated or grow in readily accessible localities. To present a fuller picture of floral diversity in the family, the present account includes the slender trees but emphasizes the species of the actinomorphic grade and the tuberous clade in the figures.

MATERIALS AND METHODS

Inflorescences of ten species were collected from the field or from cultivated specimens (see <http://ajbsupp.botany.org/v90/> for voucher information; species studied are noted with an asterisk in Fig. 14) and preserved in FAA (formalin-acetic acid-alcohol) or locally available alcohol. All specimens were transferred to 70% aqueous ethanol for storage. For scanning electron microscope (SEM) observation, samples were moved to 95% aqueous ethanol at

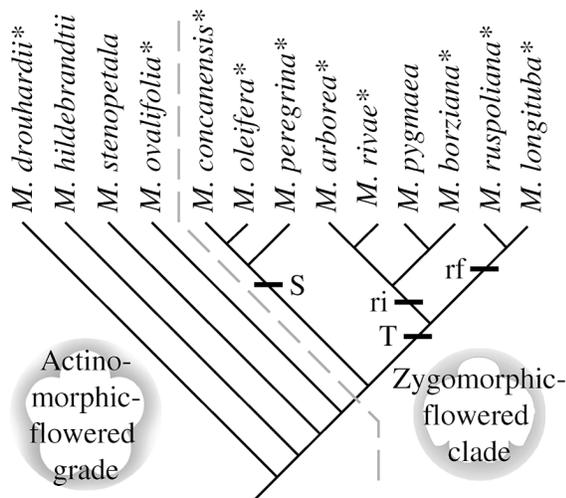


Fig. 14. Phylogenetic distribution of floral actinomorphy and zygomorphy in *Moringa* based on phylogeny of Olson (2002). The basal four species that make up the actinomorphic grade have radially symmetrical or nearly radially symmetrical flowers. The radially symmetrical symbol labelling this grade is repeated in the figures that follow to denote a member of this group. The rest of the family comprises the zygomorphic clade. The bilaterally symmetrical symbol shown labeling this clade is also repeated in the figures that follow. This clade is divided into two subclasses: the slender trees (S) and the tuberous shrubs and trees of northeast Africa (T). The tuberous clade is further subdivided into the *riva** clade (ri) and the red-flowered clade (rf). Species included in the microscopy component of the present study are indicated with an asterisk.

least 24 h before dissection to make the tissue more brittle, facilitating the removal of sepals and petals. After dissection, the material was passed through three changes of absolute ethanol, critical-point dried, mounted on stubs, sputter coated in a Polaron E-5000 (Polaron, Warrington, Pennsylvania, USA) and observed in an Hitachi S-450 SEM (Hitachi Scientific Instruments, Pleasanton, California, USA) at 20 kV. For light microscope (LM) observation, samples were dehydrated in an ethanol-tertiary butyl alcohol series, embedded in paraffin, sectioned on a rotary microtome at 13 μ m, and stained with a safranin-fast green combination. Hand sections stained with cresyl violet acetate were also employed to confirm observations made with SEM and LM, particularly for the later stages of ontogeny. The ontogenetic stages studied with each method are summarized for each species at <http://ajbsupp.botany.org/v90/>. Herbarium material of all 13 *Moringa* species was consulted from the following herbaria: BM, EA, FT, K, MO, ON, P, PAL, RHT, TAN, TEF, UPS, WIND. The phylogenetic hypothesis used here is the preferred topology presented in Olson (2002) and is based on variation in three DNA loci and morphological data. The morphological data set included seven floral characters whose main role was the placement of the unsequenced *M. pygmaea*.

RESULTS

In many cases it is possible to generalize regarding ontogenetic patterns in more than one species and avoid cumbersome descriptions of each species individually. Thus in many cases I refer to development in the “tuberous clade” to summarize observations in those species when I have found homogeneity among samples. The samples and stages on which these generalizations are based can be found at <http://ajbsupp.botany.org/v89/>.

Inflorescence structure—All of the species of *Moringa* bear axillary panicles. The simplest inflorescence is found in *M. ruspoliana*, which has one flower borne at the apex of the inflorescence (the “terminal” flower), with up to five addi-

tional flowers (“primary” flowers) borne on pedicels branching from the main inflorescence axis (diagram in Fig. 17; a very young inflorescence is shown in SEM images in Figs. 15–16). The pedicel of a primary flower is subtended by a bract on the main inflorescence axis (particularly conspicuous are those labeled “6c” and “7c” in Fig. 15). Each pedicel also bears two bracteoles (e.g., those labeled “2b” in Fig. 16). The terminal flower of the inflorescence may rarely be subtended by a bract (e.g., the flower labeled “1a” and its bract “1c” in Fig. 16; this bract is shown in gray in Fig. 17). Although they are indicated as such in the schematic Figs. 17–22 to distinguish them from the bracteoles, in all species the bracts are not always larger than the bracteoles at anthesis.

The rest of the species of the tuberous clade have more complicated inflorescences, with flowers initiated in the axils of the bracteoles (“secondary” flowers; Fig. 18). The internodes between the bracteoles may be very short, especially in *M. longituba*, *M. borziana*, and *M. pygmaea*, giving the inflorescences an almost dichasial appearance (Fig. 19). In *M. arborea* and *M. riva**, the pedicels may bear three rather than two bracteoles. In these two species, it is common to observe flowers (“tertiary” and “quaternary” flowers) in the axils of the bracteoles of the pedicels of the secondary flowers (Fig. 21).

Except for *M. ruspoliana* and the small tuberous shrub species *M. longituba*, *M. borziana*, and *M. pygmaea*, inflorescences in *Moringa* are often characterized by three or more repetitions of inflorescence units similar to those in Fig. 18 along a main axis (represented by the dashed line in Fig. 21). The main axis of such an inflorescence has a terminal flower, as do the branches (labeled as “t1” in Figs. 21 and 22). Often glandular bracts subtend the branches along the main inflorescence axis (shown in gray in Fig. 21). The inflorescences of the three species of slender tree and the actinomorphic-flowered species correspond to this type. The inflorescences of the slender trees are characterized by abundant initiation of flowers in the axils of the bracteoles of secondary and tertiary flowers (Fig. 21), and pedicels that elongate more markedly than those in the rest of the family. The inflorescence subunits close to the terminal flowers may be reduced with respect to those in the rest of the inflorescence. In the actinomorphic-flowered species, some of the internodes in the inflorescence may be very short, a characteristic most conspicuous in *M. ovalifolia*. The very short internodes in each congested group of ten flowers gives them a nearly fascicular appearance (Fig. 22 shows such clusters with slightly elongated internodes for clarity).

Variation in floral plan—Little variation in floral plan was observed in the flowers of the zygomorphic clade. The most common sepal arrangement in *Moringa* is found in two equally common enantiomorphs (Figs. 23, 24). Both consist of two outer sepals that are initiated first and second in ontogeny and usually remain larger than the others, two internal sepals, and another that is overlain only by the first sepal. Which enantiomorph a given flower displays does not appear to be correlated with terminal or lateral position in an inflorescence.

In the zygomorphic clade, the petal between sepals 3 and 5 is larger than the others and occupies a dorsal position at anthesis (Figs. 5–13). It is referred to as the “posterior petal” by Ronse Decraene, De Laet, and Smets (1998), but on the “anterior side” of the flower according to Dutt (1978). To avoid confusion, and because this petal appears analogous in function to the banner petal (vexillum) of many papilionoid

legumes, it is here referred to as the “banner,” and is indicated by a “b” in Figs. 23–86. The other four petals are lateral with respect to the banner and form two pairs. The lateral petals closest to the banner (the petals between the sepal pairs 1/3 and 2/5) are mirror images of one another and are here referred to as the “middle petal pair.” The two lateral petals farthest from the banner likewise are mirror images of one another and are referred to as the “lower petal pair.” Rare variants in the zygomorphic clade include sepal 5 being completely external, and the petal between sepals 2 and 4 being external (Fig. 25). These variants occur independently of one another.

In all members of the zygomorphic clade examined, the anthers point toward the banner in bud (Fig. 23). The variation that was observed mostly consisted of between-species differences in anther inclination with most species having the long axes of the anthers more or less parallel with the long axis of the banner in bud. In other species, such as *M. borziana*, the distalmost anthers (farthest at left in Fig. 9) are held strongly obliquely in bud.

Sepal arrangements in the actinomorphic species resemble those in the zygomorphic clade. However, the actinomorphic species show much more intraspecific variation in petal and anther arrangement. In the actinomorphic species, as in the zygomorphic species, one petal is usually external and larger throughout the early stages of ontogeny. But rather than the consistent location of this petal between sepals 3 and 5 as in the zygomorphic-flowered species, it was observed equally frequently between the sepal pairs 3/5, 1/3, or 2/4 (Figs. 27, 28). Because this petal resembles the banner of the zygomorphic species in its larger size and external position but is of inconsistent location, such a petal is referred to as a “banner-like” petal. Just as commonly, petal aestivation was observed to be entirely imbricate (Fig. 26). The petal arrangement shown in Fig. 28 was the one most commonly observed in *M. ovalifolia*, in which one external petal overlaps two petals, one of which is entirely internal and the other that is overlain only by the external petal. No consistent pattern was observed as to the handedness of these two petals (the internal petal could be on either side of the external petal). Likewise, no consistent orientation of this petal arrangement with respect to the sepals was observed, with the external petal having been observed between sepal pairs 1/3 (slightly more common than the other positions), 1/4, 2/5, and 3/5.

Anther orientation shows even more variation in these species, from the arrangement typical of the zygomorphic species (Fig. 23) to all having the same oblique orientation perpendicular to the floral axis (Fig. 26). The orientation most commonly observed consisted of four anthers facing the same side of their respective petals and one anther pointing to the other side (Fig. 27).

Floral ontogeny: stages—Floral ontogeny is summarized below for the ten species examined. The account of the continuous process of floral ontogeny is divided into the following phases for convenience: (1) organogenesis, consisting of the appearance of primordia; (2) differentiation, in which the primordia become distinguished from one another in shape and continue to enlarge; (3) enlargement, in which differentiated floral parts enlarge and change positions; (4) anthesis, which mostly involves flexion of the sepals, petals, and stamens, and elongation of the gynoecium. These four categories correspond, respectively, to Tucker’s (1997) early-, mid-, late-stage, and anthesis categories. In Figs. 29–86, radially symmetrical

symbols surround the figure number in images of actinomorphic-flowered species, and bilaterally symmetrical symbols surround those of zygomorphic species.

Floral ontogeny: organogenesis—In all species examined, sepal initiation begins well in advance of the initiation of the other floral parts. The floral primordium is tangentially elongate with the more distant poles coincident with the sites of initiation of the first sepals (Fig. 29 shows *M. arborea*), which rapidly begin to converge over the floral apex (Fig. 30 shows *M. longituba*; Fig. 16, flower 2a, is *M. ruspoliana*). As the sepals converge, their faces delimit a slightly raised pentagonal field at the angles of which the petal primordia will form. This stage is shown in *M. longituba* (Figs. 30, 31), *M. ruspoliana* (Figs. 32, 33), and *M. peregrina* (Fig. 34).

As the sepals converge over the floral apex, petal primordia appear simultaneously on the apices of the pentagonal field, in alternate positions with respect to the sepals (Figs. 33–37). The stamen primordia, which are antepetalous, form immediately after the appearance of petal primordia. Similar patterns of petal and stamen initiation were observed in all samples studied, including members of the tuberous clade (e.g., *M. longituba* in Fig. 35), the slender tree clade (e.g., *M. peregrina* in Fig. 36), and the actinomorphic grade (e.g., *M. drouhardii* Fig. 37).

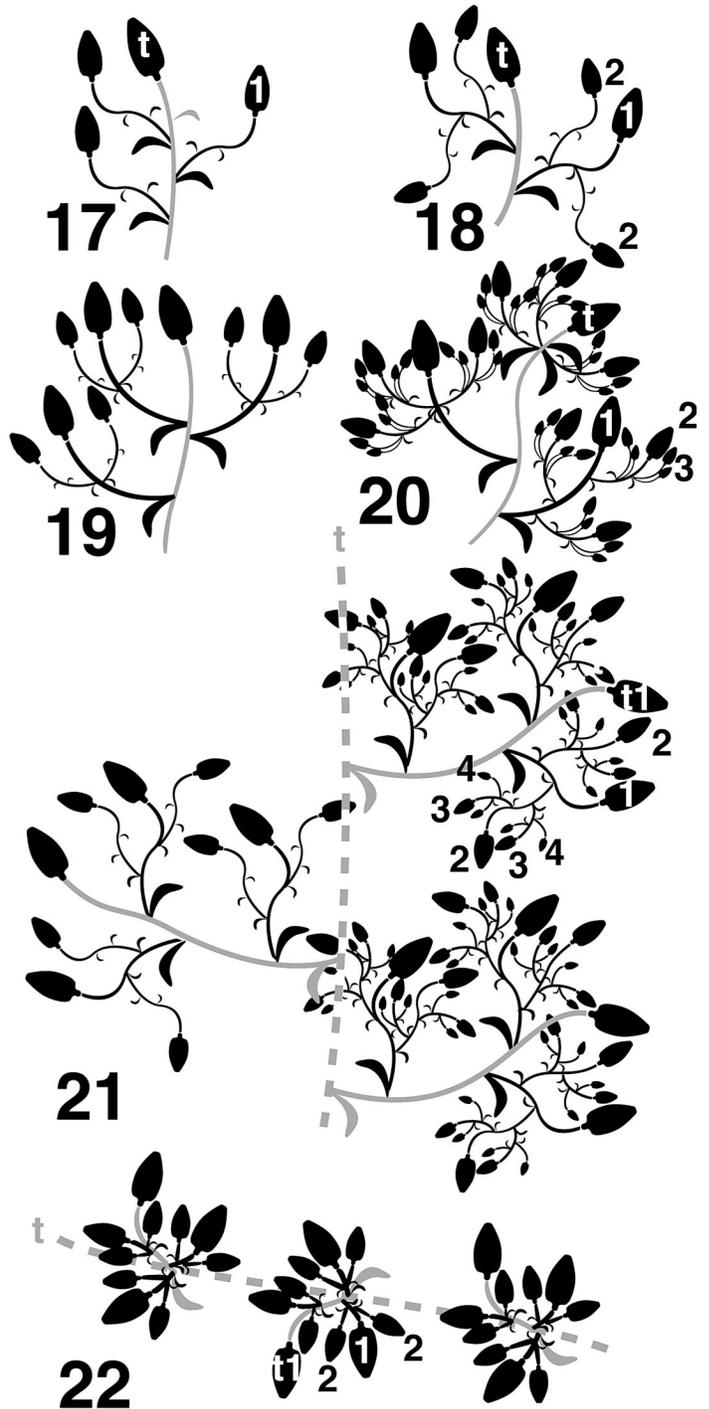
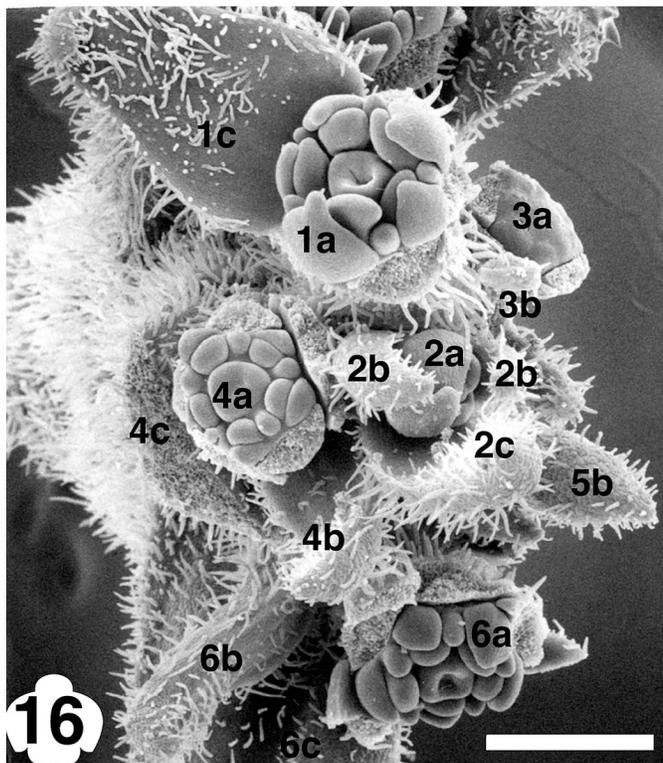
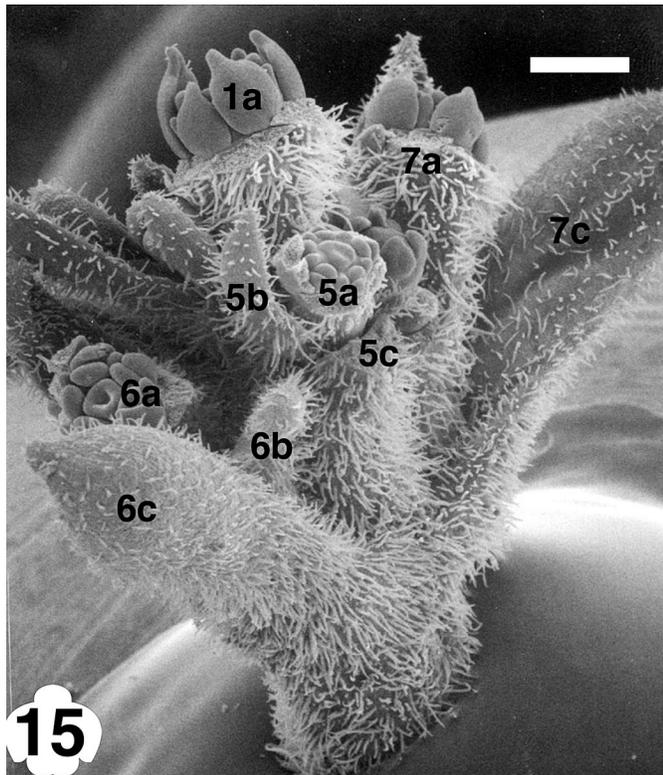
Despite extensive sampling of the early stages of floral ontogeny, no samples were encountered in which only one or two or even only three staminodes appear. When one staminode can be seen, at least three others can be detected. Therefore, it appears that the staminodes, like the petals and stamens, emerge for all practical purposes simultaneously. In the slender trees, the staminode opposite sepal 4, which is the lowermost staminode at anthesis, is often the smallest or fails to form entirely (e.g., Figs. 36, 39, but present in Fig. 44; “lost” in the sense of Tucker, 2000). The lack of a staminode in the actinomorphic clade is also common, but is not consistent in its location with respect to the sepals and petals (Figs. 37, 38, 56, 58). No flowers from members of the tuberous clade were observed to lack staminodes.

In the members of the actinomorphic grade and the tuberous clade, the gynoecium appears as a disc or annulus in the center of the flower concurrently with the initiation of petals and stamens (Figs. 35, 37, 41). In the slender tree *M. peregrina*, the gynoecium appears well after the initiation of the petals and stamens (Figs. 36, 39, 44).

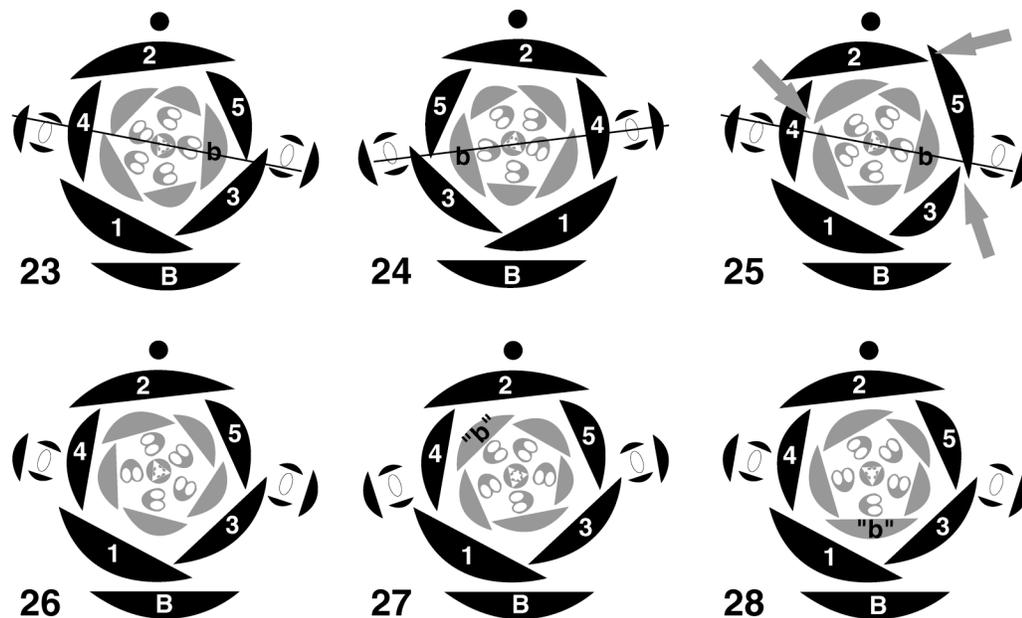
The floral buds of all species are zygomorphic at the end of organogenesis. The banner or banner-like petal and its associated stamen and flanking staminodes are larger than other similar groups in the flower, with the banner or banner-like petal extending farther radially from the axis of the flower than do the other petals (e.g., Figs. 35, 37, 40). This group of primordia is also distinctive in being bilaterally symmetrical, whereas the lateral petals are mirror images of one another (e.g., Figs. 36, 38, 40).

Floral ontogeny: early differentiation—During early differentiation, the sepals are already covering the more internal parts and are showing their pubescence patterns, the petal primordia continue to change shape, and the petals and anthers are involved in torsion apparently associated with the packing of the massive anthers in the confined space of the bud and the ultimate orientation of the anthers at anthesis.

The petals flatten ventrally, with the broad face appressed



Figs. 15–22. Inflorescence structures in *Moringa*. 15–16. *Moringa ruspoliana*. Entire panicle with some floral buds partially dissected, SEM micrographs. Structures marked “a” are flowers, “b” are bracteoles, and “c” are bracts. The numbers indicate the members subtended by the same bract: 1a is the top flower in the panicle, and 1c is its bract, while 2a is the next flower down in the inflorescence, 2b is its bracteoles, and 2c is its bract, etc. In some cases, bracts or bracteoles have been removed. Bars = 500 μ m. 15. Oblique view of panicle. 16. Apical view of the same panicle. 17–22. Schematic representations of inflorescences in *Moringa*. The main inflorescence axis is shown in gray; in black are groups of flowers consisting of one bract, a pedicel with two to three bracteoles, one flower borne at the end of the pedicel (the primary flower, indicated by a 1), flowers borne in the axils of the bracteoles (2 = secondary flowers), flowers borne in the axils of the bracteoles of the secondary flowers (3 = tertiary flowers), flowers borne in the axils of the bracteoles of the tertiary flowers (4



Figs. 23–28. Floral diagrams in *Moringa*. Black arc “B” = subtending bract; black lateral arcs are bracteoles subtending secondary flowers (white ovals with black outlines). Small black circle = inflorescence axis. Black numbered arcs = sepals with order of initiation; gray arcs = petals. **23, 24.** The most common enantiomorphs in *Moringa*. In the zygomorphic clade, the petal between sepals 3 and 5 is usually larger than the others and is borne at the top of the flower at anthesis (the banner = b). The other four petals are lateral with respect to the banner and are referred to as the upper petal pair (between sepals 2/5 and 1/3) and the lower petal pair (between sepals 1/4 and 2/4). **25.** Rare variations in the zygomorphic clade, with differences from Fig. 23 indicated by arrows. **26.** An arrangement with imbricate petals and identically oriented anthers that is common in the actinomorphic grade. **27, 28.** Common petal arrangement variants found in the actinomorphic grade. External petals are marked “b.”

to the opposite stamen primordium. If present, the banner is usually conspicuously larger than the rest of the petals (Figs. 38–49) and often maintains its abaxial face perpendicular to the axis of symmetry of the flower (e.g., Figs. 39, 40). *Moringa arborea* in SEM images (Figs. 41–43) and *M. borziana* and *M. rivae* in LM sections are characterized by massive primordia and young organs and very compact packing in the buds. Nevertheless, bilateral symmetry is apparent in the greater radial extent of the banner and associated anther (Fig. 41).

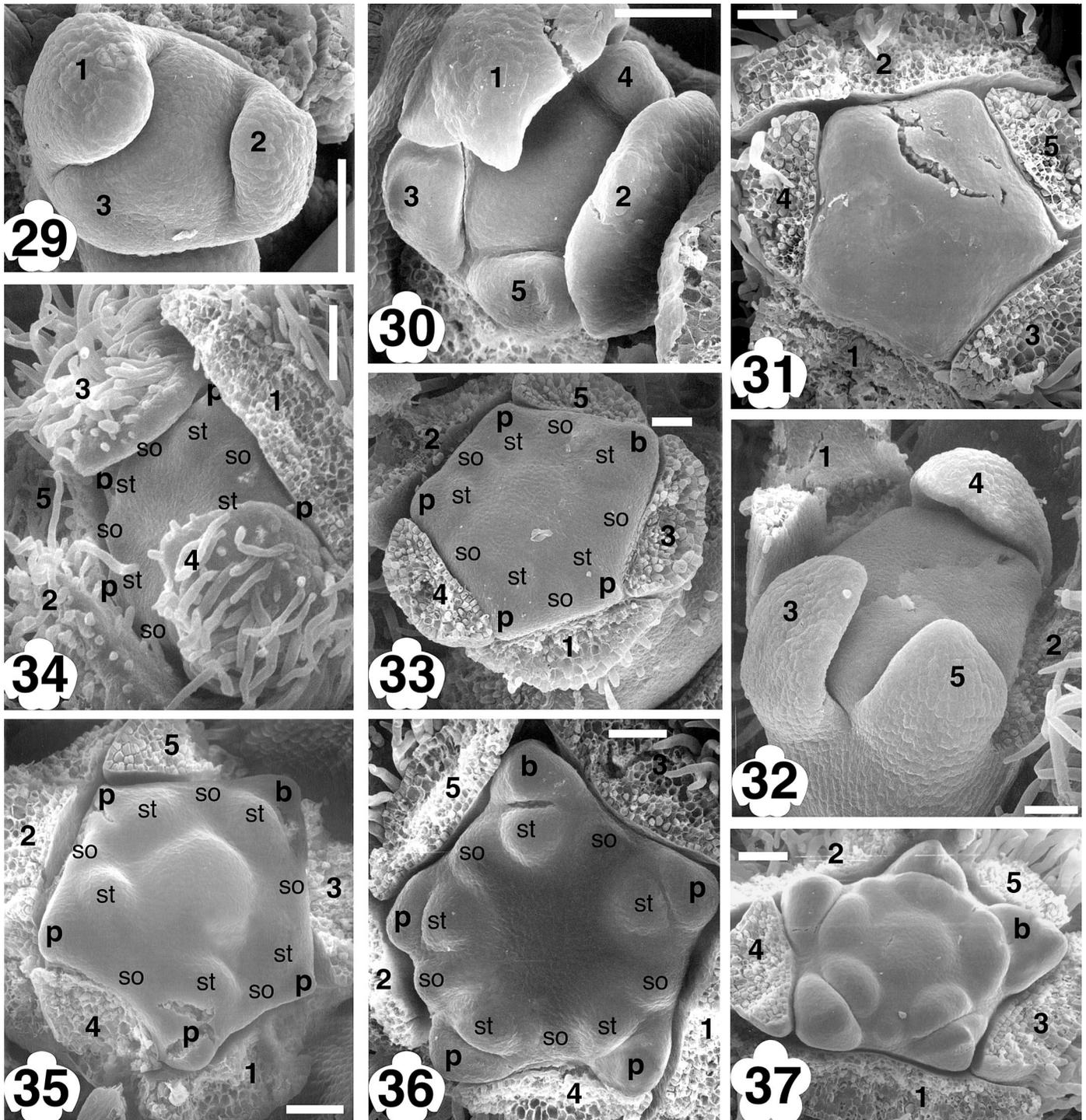
In the zygomorphic-flowered species, the features of bilateral symmetry established in the early stages of ontogeny, such as a larger banner and lost staminode opposite sepal 4, are maintained through the early stages of differentiation. Furthering the aspect of zygomorphy in these species, the petals and stamens undergo characteristic patterns of torsion or packing (“twisting” of Ronse Decraene, De Laet, and Smets, 1998) symmetrically on either side of the axis of symmetry. This first becomes manifest with the asymmetrical growth of the lateral petals. The lateral petals closest to the banner incline away from it concurrently with the orientation of their associated anthers to face the banner (Figs. 45–47, 49). In the process, the bulk of an anther is shifted away from the bulk

of its opposite petal, thereby maximally occupying the space available in the bud. A similar packing of forms takes place among the members of the lower petal pair. In this case, the petals move toward the banner while the anthers shift the majority of their bulk toward sepal 4, the bottom of the flower as viewed at anthesis (particularly conspicuous in Fig. 47, but also visible in Figs. 40, 45, 46). Similarly, the banner and the anther opposite to it may shift in opposite directions (Figs. 43, 47) or may show only minimal shifting (e.g., the anther opposite the banner in Fig. 45 points to the right of the figure, but the petal shows little torsion).

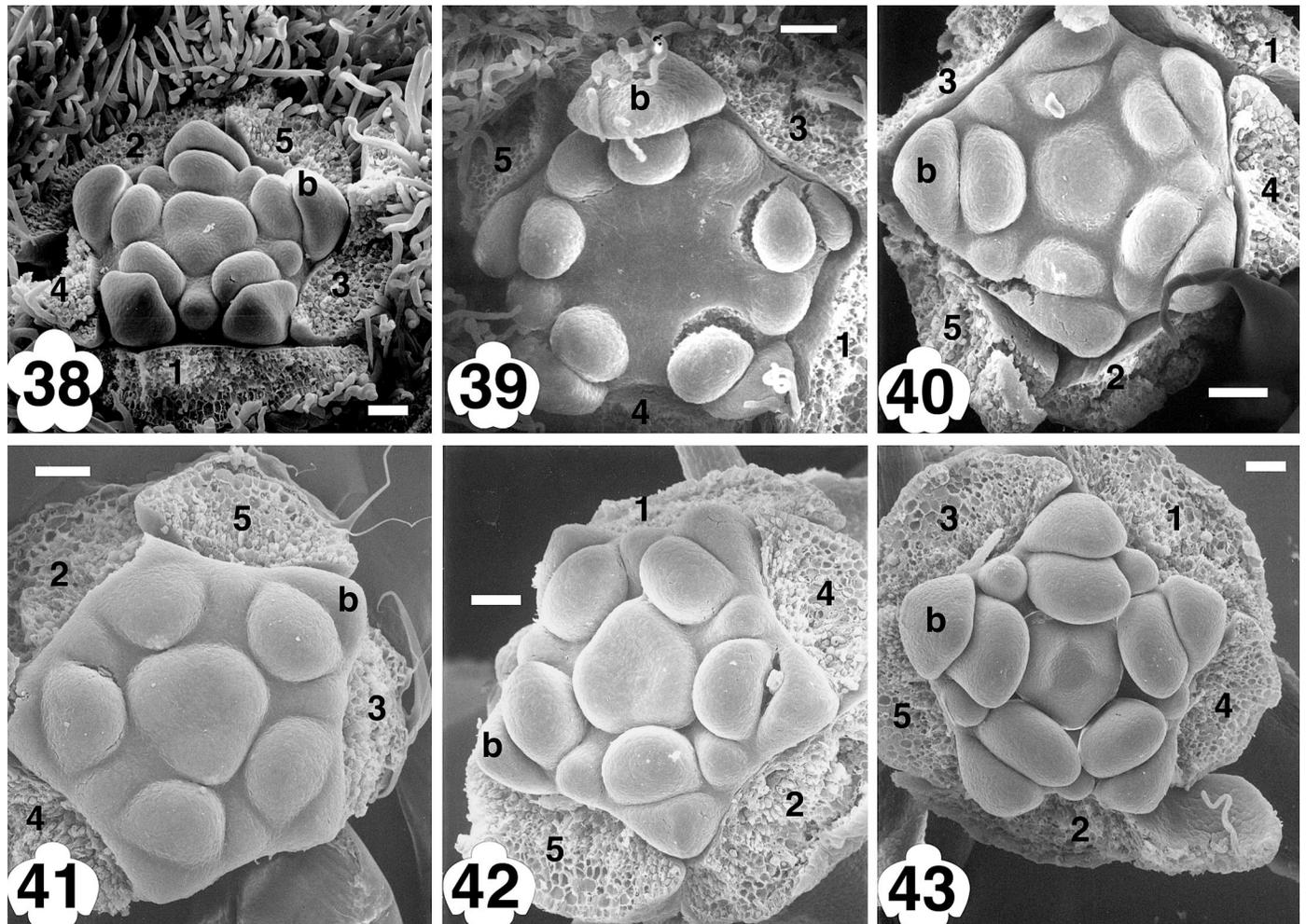
The actinomorphic-flowered species show a less consistent pattern. The packing of anthers and petals in *M. drouhardii* and *M. ovalifolia* was observed at times to follow a pattern similar to that seen in the bilaterally symmetrical species (e.g., *M. drouhardii* in Fig. 38). Just as commonly, flowers were found that deviated from this pattern (e.g., *M. drouhardii* in Fig. 48, in which the following members are shifted in opposite directions from the arrangement in the zygomorphic-flowered species: the petal between sepals 1 and 3 and its opposite anther, the anther opposite the petal between sepals

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= quaternary flowers); t = the terminal flower of the inflorescence; t1 = the terminal flower of of an inflorescence branch; bracts are shown as being larger than bracteoles, though this is often not the case. **17.** Basic inflorescence structure consists of an apical flower that rarely is subtended by a bract (shown in gray). Bracts on the main axis subtend pedicels that bear usually two bracteoles and a flower. **18.** Subsidiary flowers are often borne in the axils of the bracts (not shown) or bracteoles (as shown here). The pedicels of these flowers bear two bracteoles. **19.** Dichasium-like inflorescence common in some members of the tuberous clade. **20.** Similar inflorescence in *M. arborea* and *M. rivae* with multiple tertiary flowers in the axils of bracteoles of secondary flowers. **21.** Compound inflorescence composed of repetitions of a module like that in Fig. 18. along a main axis shown as a gray dashed line. Bracts at the base of inflorescence branches are shown in gray. Tertiary and quaternary flowers are shown on the right side of the inflorescence. For clarity, only primary and secondary flowers are shown on the left side. **22.** Inflorescence structure in *M. ovalifolia*.



Figs. 29–37. Organogenesis in *Moringa* flowers, SEM micrographs. Labels on sepals and petals correspond to Fig. 23 and as follows: p = non-banner petal primordia; st = stamen primordia; so = staminode primordia. Bars = 50 μm . 29. *Moringa arborea* bud at sepal initiation. 30. *Moringa longituba* floral bud prior to petal initiation showing sepals beginning to cover the floral apex shortly after sepal initiation. 31. *Moringa longituba* bud with sepals removed. 32. *Moringa ruspoliana* bud with sepals 1 and 2 removed to show that petals have not been initiated. 33. *Moringa ruspoliana* bud with sepals removed to show that petals and stamens form at angles of the pentagonal field only after sepals completely cover bud. 34. *Moringa peregrina* bud with sepal 1 removed and petal, stamen, and staminode primordia emerging. 35. *Moringa longituba* bud showing simultaneous emergence of petal, stamen (in the antepetalous positions), and ovary primordia. 36. *Moringa peregrina* bud showing bilateral symmetry in the form of elongation in the direction of the banner and suppression of the staminode opposite sepal 4. 37. *Moringa drouhardii* bud with sepals removed showing bilateral symmetry in the form of a banner that is larger than the other petals and a lost staminode opposite sepal 4.



Figs. 38–43. Organogenesis in *Moringa* flowers, SEM micrographs. Labels on sepals and petals correspond to Fig. 23 unless noted. Bars = 50 μm . **38.** *Moringa drouhardii* bud. Bilateral symmetry is manifest in the suppression of the staminode opposite sepal 4. **39.** *Moringa peregrina* bud showing banner overtopping its corresponding stamen and the lack of a staminode opposite sepal 4. **40.** *Moringa longituba* bud showing bilateral symmetry in the much larger banner and adjacent stamen primordia. **41–43.** *Moringa arborea* flowers showing massive primordia of the rivae clade.

2 and 4; the banner is also shifted to its left. Note the presence of all five staminodes).

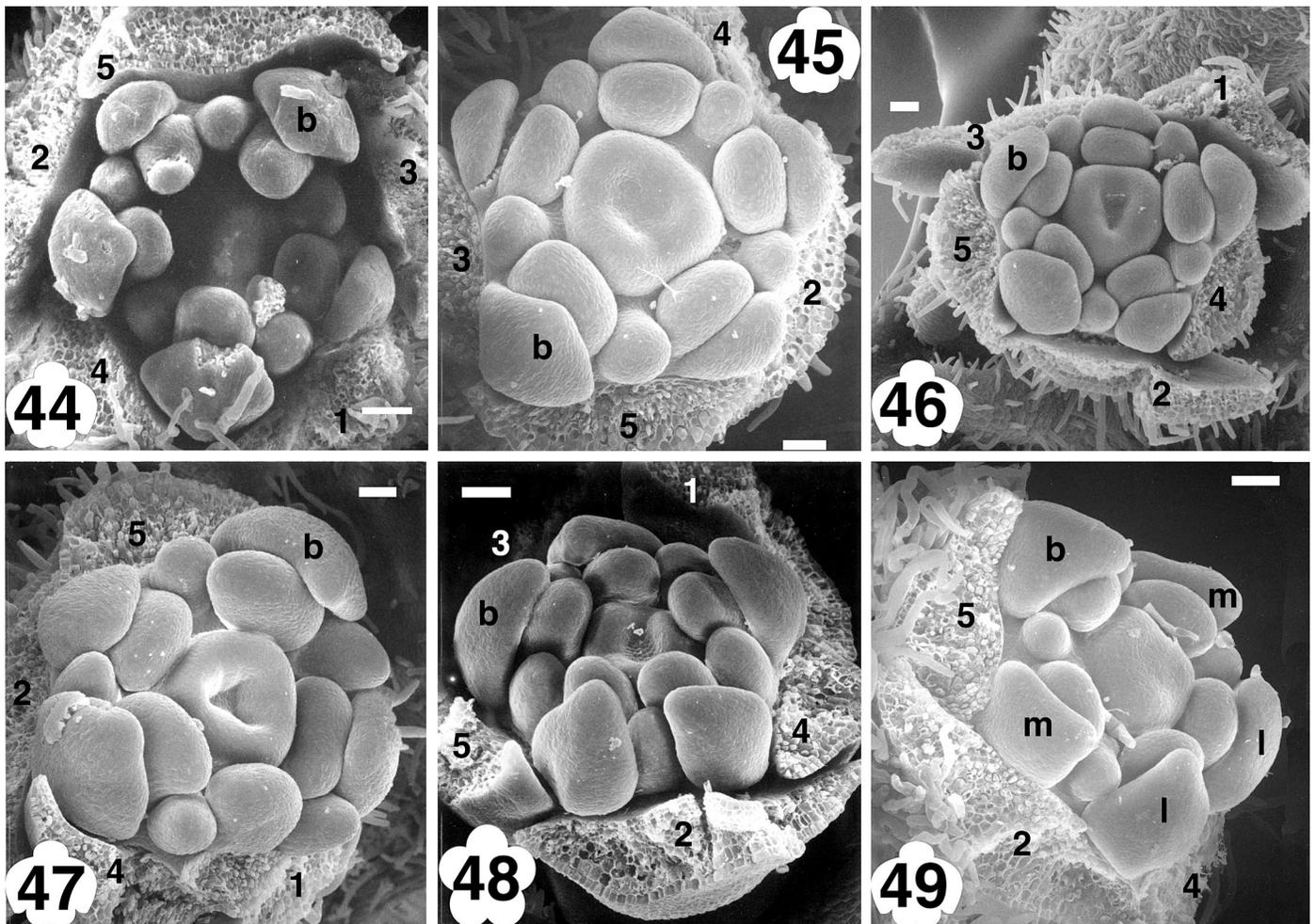
Floral ontogeny: late differentiation—This stage is defined as the period in which the petals begin to converge and establish aestivation, the stamen primordia differentiate into anthers and filaments, and the gynoecium becomes differentiated into an ovary and a short style.

In all species, the petals remain thickest at the midrib and acquire broad margins as they overtop the stamens. The largest petals may bear apiculi or small trichomes (Figs. 50–55). The gynoecium continues to elongate apically, being shaped both by the triangular form of the three carpels that compose it and also by the pressure of the surrounding organs (Figs. 50, 55). As the petal margins converge on each other, the torsion associated with packing that was previously apparent is now present as slight inclinations of the petals along their long axes (Fig. 54). It is at this point, when the buds are nearly 1 mm in diameter, that the aestivation of the petals begins to be established.

The variation illustrated in Figs. 23–28 becomes apparent at this stage. The banner is consistently external in all the

species of the zygomorphic clade (cf. Figs. 55 and 23). In contrast, the middle stages of ontogeny of the actinomorphic *M. drouhardii* and *M. ovalifolia* show greater variation in floral plan. For example, the petal between sepals 3 and 5 (the banner in the zygomorphic species) often is established as internal to another petal on at least one side, as in Fig. 51. In this image, the petal between sepals 1 and 3 is the largest one, and it overlaps the petal between sepals 3 and 5. Figures 56–58 illustrate a similar variability with respect to anther orientation. The typical pattern of orientation is seen in the anthers opposite the lateral petals in Figs. 56 and 57. The stamen to the left of the banner in Fig. 57 faces to the right of the image and is inclined to the left. The opposite pattern is shown in Fig. 58. The location of any staminode lacking in the actinomorphic-flowered species is not necessarily consistent with the expected position opposite sepal 4 as in the rest of the family or even on the opposite side of the flower from a banner-like petal if present (e.g., the staminode missing from the flower in Figs. 67 and 81 is opposite sepal 2 rather than sepal 4).

Anther differentiation begins as or shortly after the petals converge. This process involves a ballooning growth of the apical half or more of the stamen primordium (Figs. 56, 57,



Figs. 44–49. Late organogenesis and beginning of differentiation in *Moringa* flowers, SEM micrographs. Labels on sepals and petals correspond to Fig. 23 unless noted. Bars = 50 μm . **44.** *Moringa peregrina* flower polar view with petals beginning to overtop the stamens. This flower has five staminodes. Sepal arrangement corresponds to Fig. 24. **45–47.** *Moringa ruspoliana* buds showing packing of petals and anthers (especially conspicuous in the banner of Figs. 45 and 47 and the petal between sepals 1 and 4 in Fig. 46) and, in Fig. 47, the ovary cavity deepening in Fig. 47. **48.** *Moringa drouhardii* bud showing scant zygomorphy and irregular packing. **49.** *Moringa oleifera* bud showing bilateral symmetry in the symmetrical movements of the lower (l) and middle (m) petal pairs. In addition, the staminode opposite sepal 4 is lost.

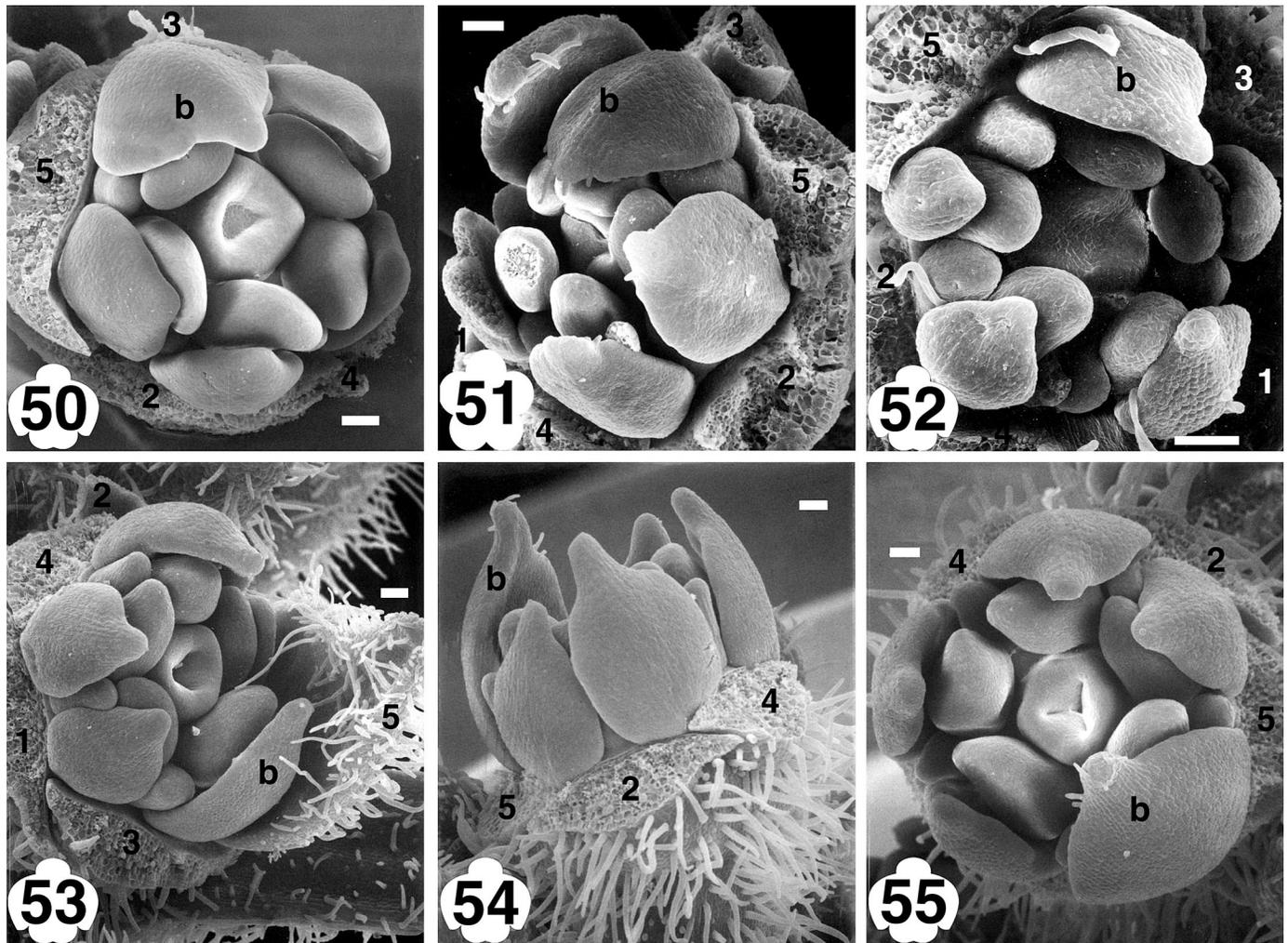
63, 74). The anthers initially appear basifixed, but the lower half of the anther grows downward, partially enveloping the filament (Figs. 77–80). In the slender trees and the actinomorphic-flowered species, the anthers at early differentiation have shallow longitudinal furrows marking the zone of dehiscence (Figs. 57, 58, 60). At similar and even later stages of development, members of the tuberous clade show only a very shallow invagination along this suture (Fig. 62). *Moringa peregrina* has anthers that are apiculate in intermediate stages of ontogeny (Figs. 60, 61).

In the zygomorphic species, the three anthers nearest the banner usually remain larger than the rest throughout anther differentiation and enlargement (Figs. 58, 61, 62, 64, 72, 73), giving the appearance of unidirectional differentiation and enlargement. Just as the staminode opposite sepal 4 may fail to develop in the actinomorphic-flowered species and the slender trees, the two anthers nearest to this sepal may remain smaller than the others in the mature flower in these species (*M. peregrina* in Fig. 61; but note the tiny anthers of the stamens opposite the petals of the middle petal pair in Fig. 64). Just as

they were never observed to lack staminodes, no members of the tuberous clade were observed with partially developed anthers at maturity. Anthers in the actinomorphic species are usually of equal sizes by this stage (Figs. 65, 82).

At organ differentiation, bilateral symmetry in the zygomorphic clade is enhanced by the rotation of the anthers and the continued asymmetric growth of the petals. The members of the tuberous clade establish the mature anther orientation depicted in Fig. 23 earlier in development than the other species. For example, in a flower of *M. longituba* that is scarcely 0.5 mm in diameter, all but one anther has already assumed the conformation seen at anthesis, and the remaining anther has nearly rotated to its final position (Fig. 59). Likewise, Fig. 62 shows the just-differentiating anthers of *M. arborea* in their mature orientation. In contrast, anthers in the slender trees are still rotating to their final orientation at mid-differentiation (Figs. 60, 61).

After elongating apically into a tubular structure (Figs. 60, 61), the gynoecium in all species differentiates into a squat ovary with a short, tubular style (Figs. 62–64, 74, 75). Ovule



Figs. 50–55. Organ development in *Moringa* flowers, SEM micrographs. Labels on sepals and petals correspond to Fig. 23 unless noted. Bars = 50 μm . **50.** *Moringa longituba*. Bud showing deepening ovary cavity and banner markedly larger than other petals. **51.** *Moringa drouhardii* bud with petals overtopping stamens. Sepal configuration corresponds to Fig. 24. The petal marked “b” corresponds in sepal position to the banner in the zygomorphic clade. This image illustrates a variant that is common in actinomorphic species, in which the petal between sepals 1 and 3 is the largest and is external. **52.** *Moringa peregrina* flower showing gynoecium emergence well after the organs of outer whorls have formed. **53.** *Moringa ruspoliana* flower with the last sepal to develop left intact, with the inflorescence axis visible below the flower. **54–55.** *Moringa ruspoliana* lateral and polar views of the same flower showing bilateral symmetry.

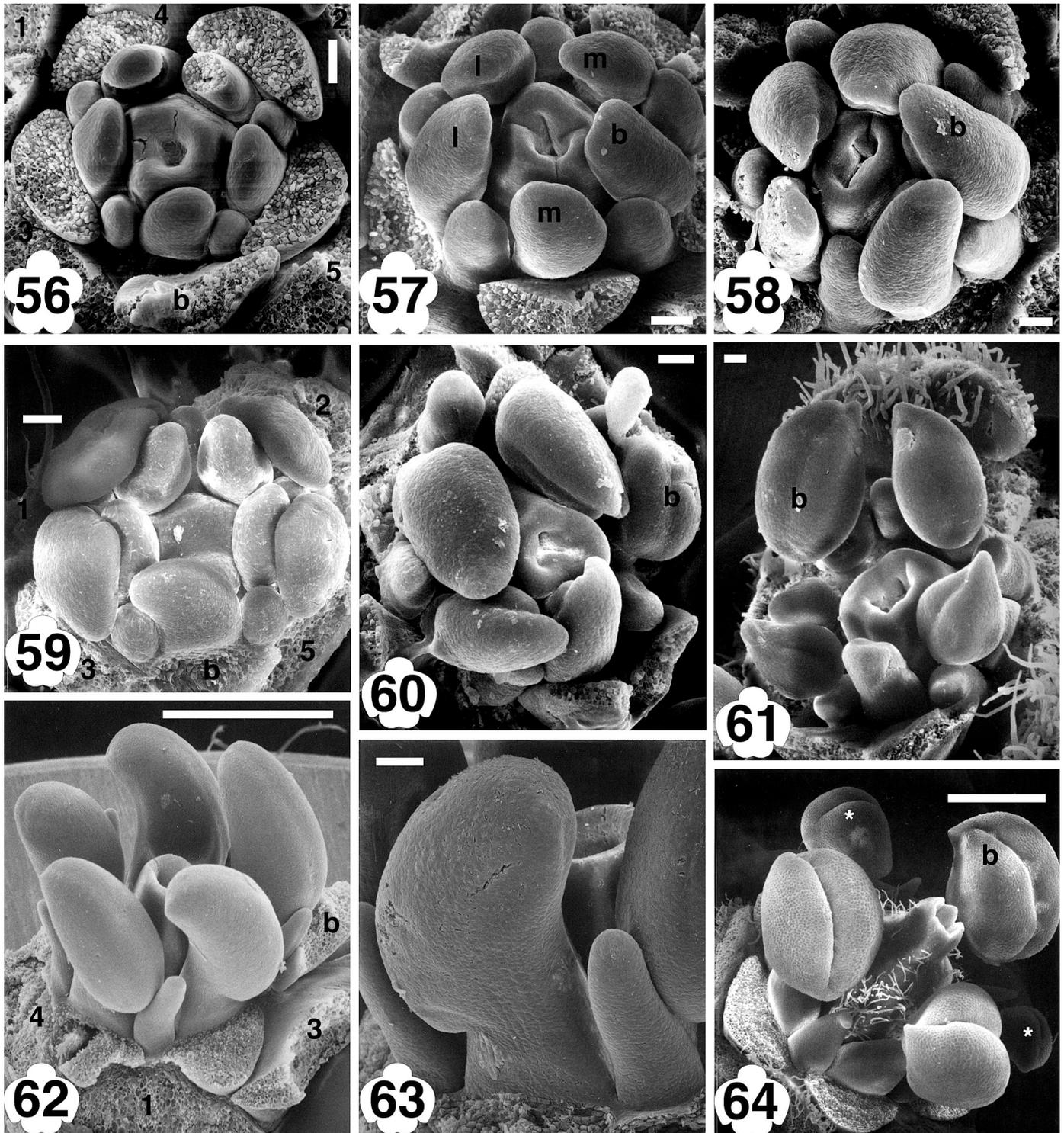
initiation coincides with or closely follows this differentiation (Figs. 68, 73, 74, 76). Concurrently, differentiation of the sporangia and pollen formation occurs (Figs. 72, 73). Aberrant androecial development was observed in the collection Olson 700 of *M. concanensis*, in which there were no flowers with functional anthers. The distal parts of the stamen primordia differentiate into apiculate bodies with a papillate texture distinct from the smooth surface of the filament (depicted in Fig. 5) but scarcely enlarge and remain without sporangia. The gynoecium of these flowers is of normal size.

Floral ontogeny: enlargement—This stage is characterized by enlargement of parts before the flower opens. The stamens are differentiated into anthers and filaments. Ovule and pollen formation may still be ongoing during this stage. It is at the early part of this stage that characteristics such as pubescence begin to become widespread on the central flower parts.

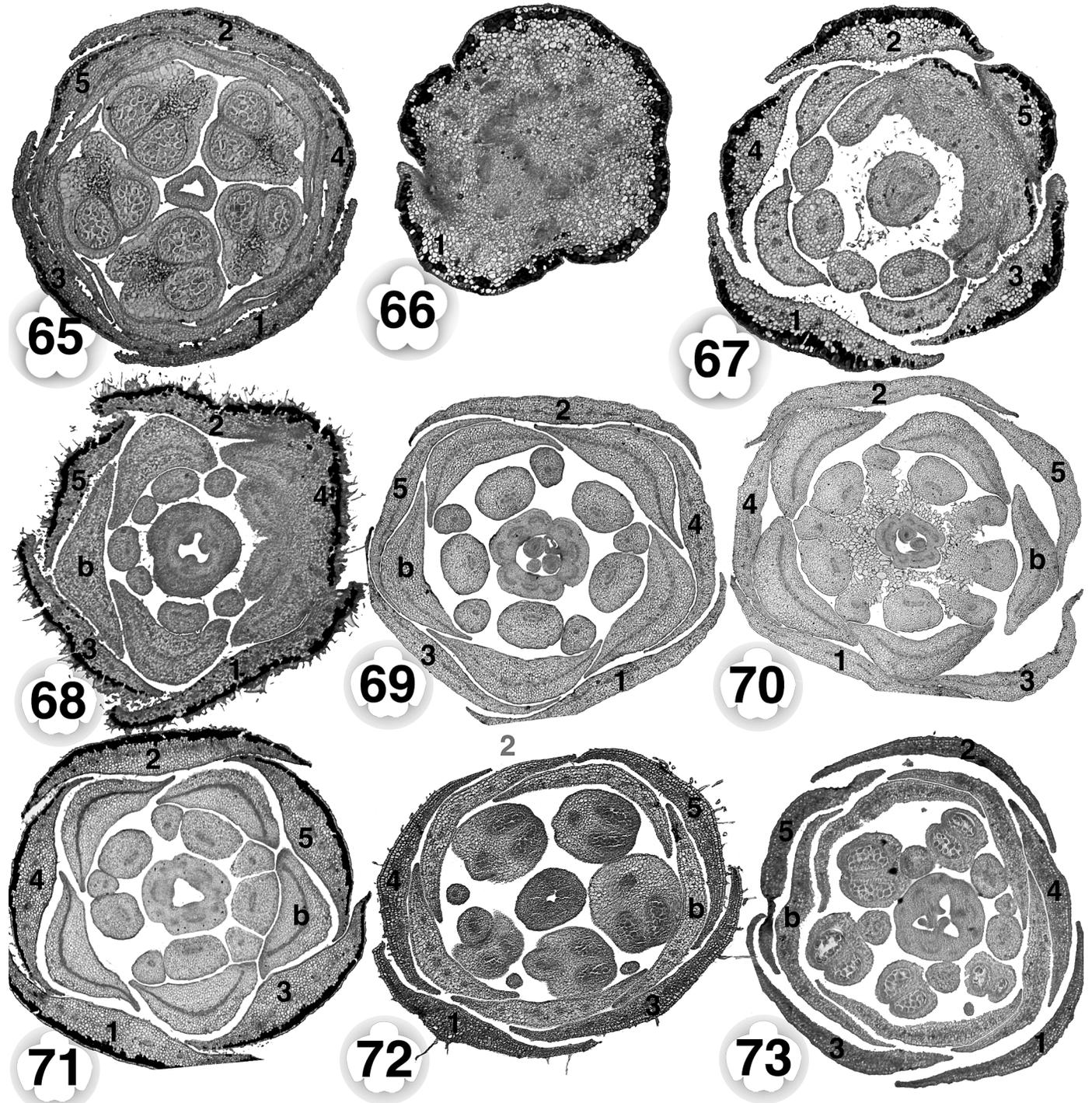
If they have not already done so, flowers in the members of the actinomorphic grade usually lose clear traces of zygo-

morphy during the enlargement phase. If a banner-like petal was present in the preceding stages, it is equalled in size by the other petals during enlargement. *Moringa drouhardii* and *M. ovalifolia* commonly show imbricate petal aestivation with petals of equal sizes and anthers that may all have the same oblique orientation (Fig. 65) or sets of four anthers facing the same side of their opposite petals and another that faces the opposite side (Fig. 82). The filaments of *M. hildebrandtii* may fail to elongate equally in this phase.

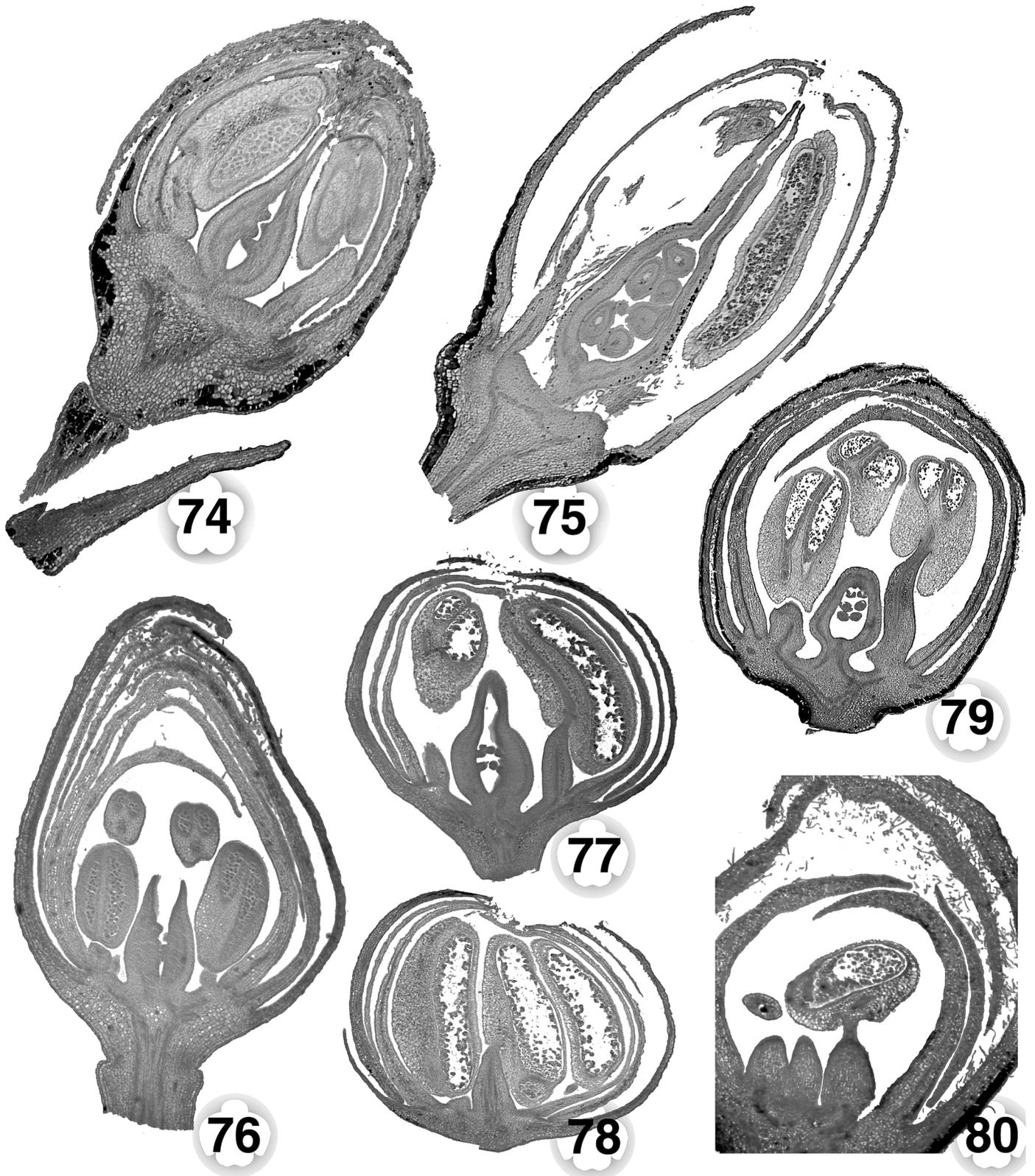
In all species, this stage is characterized by the continued appearance and growth of pubescence (e.g., Figs. 67–70). Members of the *rivae* clade still bear very thick, compact petals and androecial members (Figs. 69–71). This phase in all species is characterized by the enlargement and continued torsion of the stamens. As in the previous stage, enlargement in the zygomorphic clade appears to be slightly unidirectional, with the anther opposite the banner remaining larger than the others (Figs. 72, 73). In all species, just-differentiated anthers rapidly become massive structures tightly filling the available



Figs. 56–64. Organ development in *Moringa* flowers, SEM micrographs. Labels on sepals and petals correspond to Fig. 23 unless noted; b = banner petal or its opposite stamen. Petals and sepals are removed in all figures. Bars = 50 μm in Figs. 56–61, 63; = 500 μm in Figs. 62, 64. Figs. 56–58. *Moringa drouhardii*, showing incipient anther differentiation. **56.** Anther and gynoecium primordia enlarging. Bilateral symmetry is manifest as a lost staminode opposite sepal 4. **57.** Bud with the most common anther arrangement in the family, in which the middle petal pair (m) and the lower petal pair (l) are symmetrically arranged and the anther opposite the banner does not form a symmetrical pair with any other anther. **58.** Appearance of the anther dehiscence seam and anther packing pattern that differs from that depicted in Fig. 57. **59.** *Moringa longituba* showing rotation of the anthers very early in ontogeny. **60.** *Moringa peregrina* anthers rotating to face the banner simultaneously with differentiation into anthers and filaments. **61.** *Moringa peregrina* showing characteristic apiculi on young anthers that are differentiating from the filaments. **62.** *Moringa arborea* oblique view of young flower showing anthers that have already assumed mature orientation. **63.** *Moringa arborea* close view of differentiating anther and filament. **64.** *Moringa peregrina* flower with anthers differentiated from filaments and dorsifixed condition established. Gynoecium has differentiated into an ovary with a short style. Asterisks mark the very small anthers opposite the upper petal pair.



Figs. 65–73. Organ development in transsections of young *Moringa* buds, LM micrographs. Labels on sepals and petals correspond to Fig. 23, unless noted otherwise. Bud diameters are widest dimension. 65–67. *Moringa drouhardii*. 65. Bud, 1.5 mm in diameter, sectioned at mid-anther shortly after anther differentiation, showing sepal arrangement corresponding to Fig. 24 and petal and anther arrangement as depicted in Fig. 26. 66. Receptacle of young flower showing the extent of sepal 1. Diameter is 1 mm. 67. The same flower sectioned at the hypanthium apex to show the obliquity of the hypanthium. Diameter is 2 mm. 68. *Moringa peregrina* showing the most common configuration in *Moringa* at the hypanthium apex, with the banner conspicuous and separate from the lowermost edge of the hypanthium. Sepal configuration corresponds to Fig. 24. Diameter is 3 mm. 69. *Moringa rivae* bud, 3 mm in diameter, sectioned at mid-ovary and mid-filament, showing robust filaments and staminodes and developing ovules. Sepal arrangement corresponds to Fig. 24; the right margin of the banner is internal. 70. *Moringa borziana* bud, 2.5 mm in diameter, sectioned toward the bases of the filaments, showing the abundant trichomes that cover the empty space formed by the hypanthium. The filaments and staminodes opposite sepal 4 are adherent to one another. 71. *Moringa arborea* sectioned at mid-filament. The filaments and staminodes opposite sepal 4 are adherent. Diameter is 3 mm. 72. *Moringa ruspoliana* bud, 2 mm in diameter, at anther differentiation. Anther orientation is established before completion of microsporogenesis. 73. *Moringa longituba* bud, 2.2 mm in diameter, showing that the anthers toward the banner are larger than the others at the early enlargement stage. Sepal arrangement corresponds to Fig. 24.



Figs. 74–80. Organ development in long sections of young *Moringa* buds, LM micrographs. Labels on sepals correspond to Fig. 23, unless noted otherwise. Bud diameters are widest dimension. **74, 75.** *Moringa drouhardii*. **74.** Young flower at ovule initiation. The androecium is differentiated into anthers on very short filaments and the receptacle is massive relative to the rest of the flower. The pedicel and stalk between pedicel and bracteole are very short. Diameter is 1.2 mm. **75.** Bud, 2.5 mm in diameter, showing elongation of the receptacle. Ovule development and microsporogenesis are advanced as the bud elongates markedly. **76–78.** *Moringa longituba*, showing development of anthers and hypanthium. **76.** Anther differentiation and ovule initiation within the gaping ovary

space within the bud (Figs. 74–80). As anthesis approaches, the epidermal texture of the anthers become papillate and the anthers elongate markedly, as do the filaments to a lesser degree. (Fig. 75 shows a shortly preanthetic bud of *M. drouhardii*, and Fig. 79 shows an intermediate stage in *M. ruspoliana*.)

The enlargement of the gynoecium is accompanied by a marked constriction at the apex of the ovary and the elongation of the hollow style (Figs. 74, 75). After the appearance of the ovules as peg-like ingrowths of the inner wall of the ovary, the integuments of the ovules become differentiated. In the actinomorphic-flowered species, the ovary and style remain centered along the polar axis of the flower. In most of the zygomorphic-flowered species, the ovary is displaced toward the banner (Figs. 83, 84, 86, 87–90). The exception is *M. longituba*, in which the ovary is displaced toward sepal 4 (Fig. 85; cf. Figs. 1–13 and Figs. 91–94). The style may culminate in three stigmatic lobes (e.g., *M. stenopetala* in Fig. 3), but these are usually absent or very small.

The filaments and staminodes remain free of one another throughout the ontogeny of the actinomorphic species. As anthesis approaches in the zygomorphic species, the margins of the filaments and staminodes come into contact with one another and adhere via a thin layer of an adhesive that stains slightly in safranin. In *M. oleifera* and *M. peregrina*, the filaments and staminodes gather side-to-side in a crown centered between the banner and the gynoecium (Figs. 6, 7, 83). The filaments and staminodes adjacent to the banner remain straight, but those adjacent to the lateral petals, especially the lower ones, bend markedly at the base in the direction of the banner, achieving proximity to the other filaments and staminodes. The staminode opposite sepal 4, if present, flexes toward the gynoecium but does not adhere to its adjacent filaments. Filaments and staminodes in the available pickled material of *M. concanensis* (Olson, 700), with only reduced anthers, were not observed to adhere to one another, but herbarium specimens at BM, K, and RHT have fertile anthers and the same pattern of anther-filament adhesion described in the other slender trees.

Two differing patterns of filament-staminode adhesion were observed in the two subclades of the tuberous clade. In the members of the *rivae* subclade, as in the flowers of the slender trees, the filaments and staminodes form a crown opposite the banner (incipient stages of adhesion can be seen in a young bud of *M. arborea* in Fig. 71 and in a nearly mature bud of *M. borziana* in Fig. 84) but with filaments often directly adhering to one another and staminodes adhering to their outer surfaces (Fig. 84). In the red-flowered subclade, the stamens and filaments adhere in a continuous ring that always includes the staminode opposite sepal 4 (Figs. 85, 86).

One of the most conspicuous features of late ontogeny is hypanthium formation. After the anthers differentiate and begin to enlarge and the gynoecium begins to differentiate, growth of the rim on which the petals, sepals, filaments, and staminodes are inserted begins. Likewise, growth ensues at the base of the ovary. In this way, a hypanthial cup and a short

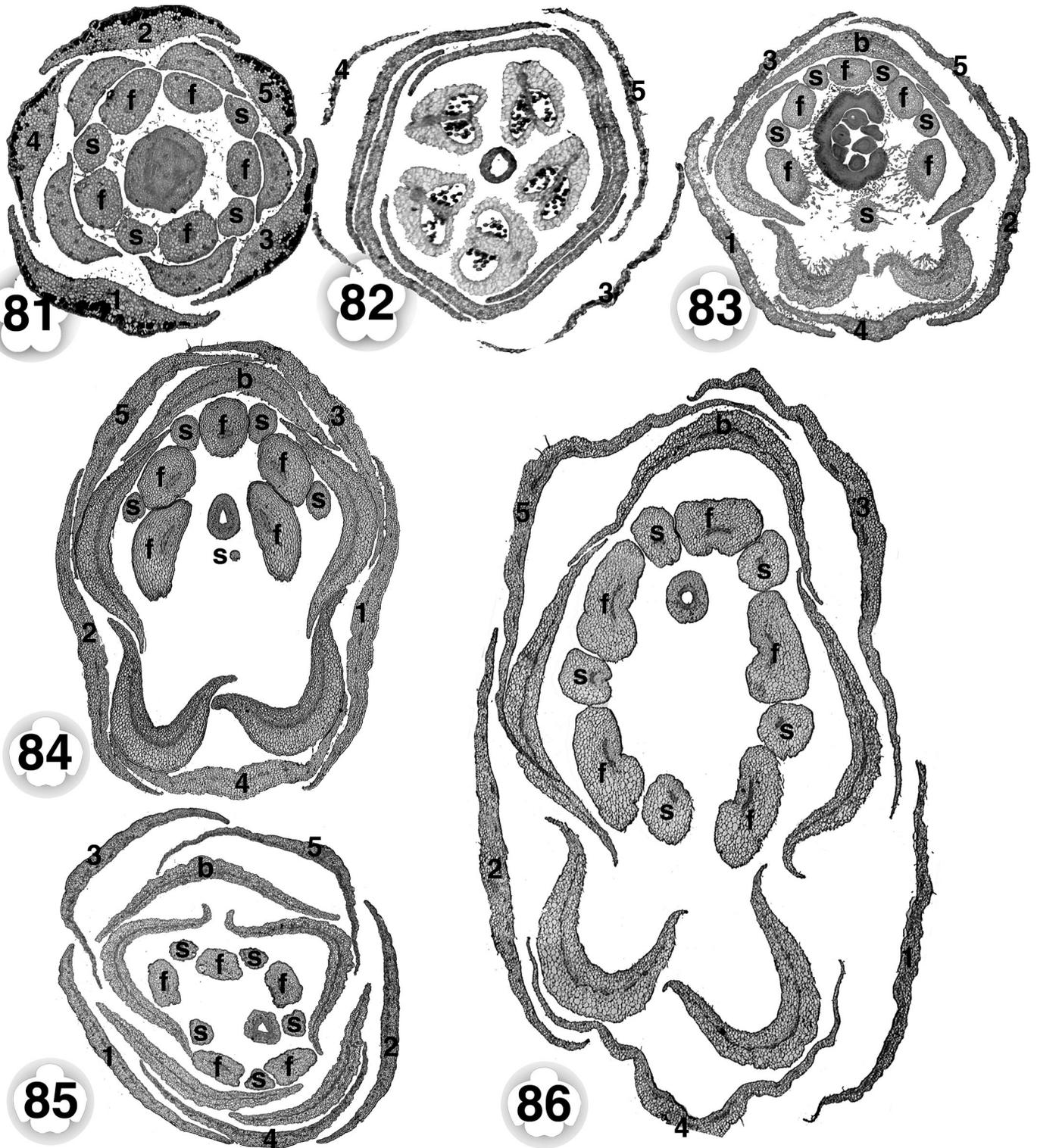
gynophore are formed. The species with the least tendency to form hypanthia, if they can be considered as such, are the actinomorphic-flowered species. In *M. drouhardii*, the gynophore elongates as the margins of the receptacle grow slightly apically (Figs. 74, 75). In the zygomorphic-flowered species, a similar pattern occurs initially. Slight deepening of the hypanthial cup accompanies anther differentiation and the beginning of anther enlargement. For example, in *M. longituba*, the species with the longest hypanthium, little hypanthium is visible at ovule initiation (Fig. 76). The same is true in the slender tree species *M. peregrina* (Fig. 80). As the anthers enlarge and the ovules develop, a slight extension of the gynophore and deepening of the hypanthial cup occurs (Figs. 77, 79, 87, 91). *Moringa arborea* illustrates an ontogenetic pattern common in the zygomorphic clade, in which the hypanthium deepens to 1–2 mm, with the gynophore elongating sufficiently to maintain the base of the ovary even with the hypanthium apex (Figs. 87–90). Unique in the family, *M. longituba* progresses well beyond this stage, with the hypanthium reaching up to 2 cm in length. In this species, hypanthial growth outpaces the elongation of the gynophore, and the ovary is drawn into the hypanthium and completely enclosed by it at maturity (Figs. 91–94).

In the zygomorphic species, the apex of the hypanthium reaches its farthest distal extent between the petals alternate with sepal 4, whereas the banner at this level remains free (Fig. 68 shows the hypanthium apex in *M. peregrina* in transection). Thus, the aperture of the hypanthial cup is tilted slightly upward at anthesis (e.g., Figs. 5, 12, 90, 94). The inclination of the hypanthium is less consistent in the actinomorphic-flowered species. Slight inclination in the insertion of the sepals can be seen in a bud of *M. drouhardii* in Fig. 1; Fig. 66 shows that sepal 1 is the first sepal to be separate from the receptacle as one sections up from the pedicel in *M. drouhardii*; Fig. 67 shows the inclination of the hypanthium from the area of sepal 5 at its distal extent to the area of the petal between sepals 1 and 4. The arrangement of the flower shown in Fig. 67 seems analogous to that in the zygomorphic flowered species because the greatest distal extent of the hypanthium is on the opposite side of the flower from a banner-like petal that is larger than the others. However, not only is this not the usual position of the banner with respect to the sepals, but flowers of *M. drouhardii* and *M. ovalifolia* were observed with the distalmost edge of the hypanthium anywhere from between the sepal pairs 3/5, 2/5, 2/4, or 1/4.

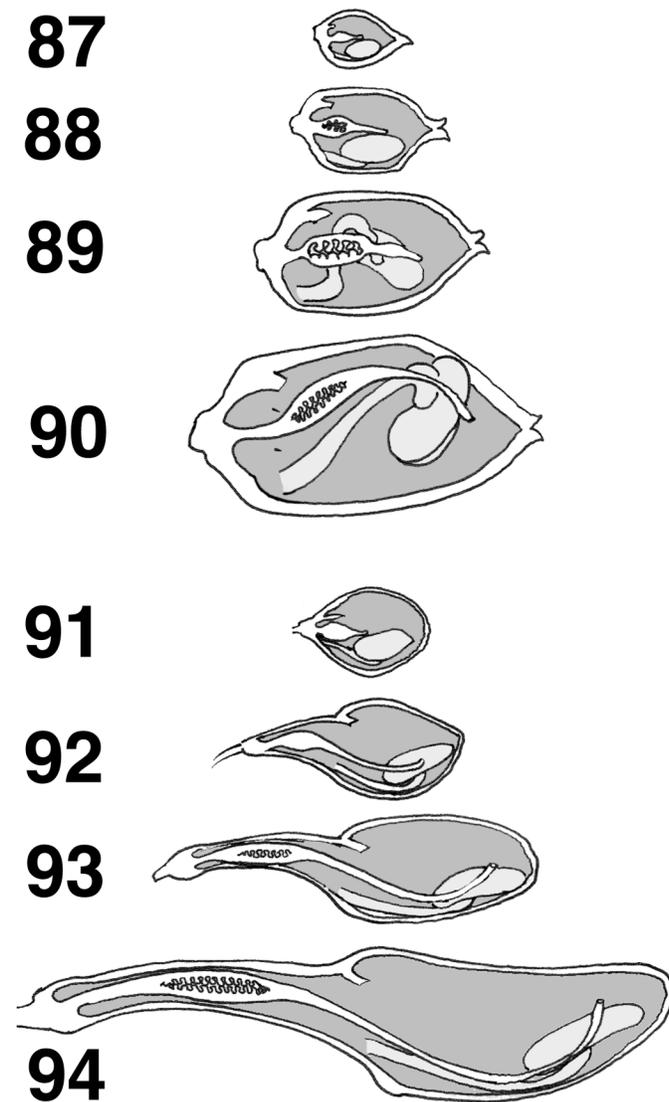
Floral ontogeny: anthesis—As the flowers open, each species is characterized by a unique degree of petal and sepal deflection, anther orientation, and style behavior. The illustrations of mature flowers in Figs. 1–13 may be used to represent the span of these differences, with a few generalizations noted here.

As anthesis approaches in the zygomorphic-flowered species, the petals that show the most flexion perpendicular to their longitudinal axes at anthesis begin to fold inward along

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cavity take place long before marked growth of the hypanthium. Diameter is 1.5 mm. **77.** Medial longitudinal section of a bud 2.1 mm in diameter with slightly advanced ovule development and marked enlargement of the anthers and only a very slight deepening of the hypanthium. **78.** Tangential section of the same flower to show the anthers nearly filling the entire space within the bud. **79.** *Moringa arborea* showing the elongation of filaments occurring before zonal growth of the hypanthium. Diameter is 2.2 mm. **80.** *Moringa peregrina* tangential section of a 2.1-mm bud showing differentiation of anthers before deepening of hypanthium.



Figs. 81–86. Organ positions in transsections of *Moringa* buds, LM micrographs. Labels on sepals and petals correspond to Fig. 23. The banner is shown at the top in the images of the bilaterally symmetrical-flowered species in Figs. 83–86. **81.** Transsection of *M. drouhardii* at mid-filament of the same 2-mm flower in Fig. 67 to show irregular symmetry. The petal between sepals 1 and 4 is markedly larger than the others, and the staminode opposite sepal 2 is lost. **82.** *Moringa ovalifolia*, showing the most common anther orientation in the actinomorphic species, with four anthers facing the same direction and one facing the opposite direction. Diameter is 3 mm. **83.** *Moringa peregrina* bud, at 4 mm, showing the crown of adherent filaments and stamens that forms opposite the banner and does not include the staminode opposite sepal 4. The petals of the lower lateral pair are markedly folded. The lateral opening of the gynoecium is atypical. **84.** *Moringa borziana*, showing arrangement of androecium and petal folding. Diameter is 3 mm. **85.** *Moringa longituba*. The crown of usually adherent filaments and stamens in this species encircles the gynoecium and includes the staminode opposite sepal 4. The most highly reflexed petals in this species are those in the upper lateral pair. Diameter is 4 mm. **86.** *Moringa ruspoliana*. As in *M. longituba*, the crown of filaments and stamens encircles the gynoecium, but the lower lateral petal pair in this species is reflexed and it is these petals that are most folded. Diameter is 4 mm.



Figs. 87–94. Drawings of longisections to show hypanthium development in *Moringa arborea* and *M. longituba*. The only androecial member shown is the left lower lateral stamen. All figures are 3× life size. 87–90. *Moringa arborea*. As the hypanthium deepens, the gynophore elongates, maintaining the ovary base at the hypanthium apex. The gynoeceum and lower lateral stamens are reflexed toward the banner, at top. 91–94. *Moringa longituba*. A gynophore forms, but hypanthial growth is so extensive that the ovary becomes completely enclosed by the hypanthium. The gynoeceum and androecium are deflected toward the bottom of the flower, a unique condition in the family.

their longitudinal axes. In all species but *M. longituba*, the members of the lower petal pair most exemplify this phenomenon as do, to a lesser extent, the members of the upper petal pair (Figs. 83, 84, 86). At anthesis in the zygomorphic clade, with the exception of *M. longituba*, the banner remains projecting forward, while the rest of the petals are downswept in a variety of arrangements, from slightly (e.g., *M. rivae* and *M. pygmaea* in Figs. 10, 11) to strongly reflexed (e.g., *M. oleifera* and *M. arborea* in Figs. 6, 8). *Moringa longituba* shows an opposite pattern, with the banner being borne upright or swept back and the lower petal pair projecting forward (Fig. 13). Likewise, it is the upper three petals in *M. longituba* that show

the greatest extent of folding along their longitudinal axes (Fig. 85).

There is a greater tendency for sepals than petals to display actinomorphy at anthesis. The actinomorphic-flowered species have radially symmetrical calyces at anthesis (Figs. 1–4). *Moringa arborea* and *M. borziana* also have calyces that are radially symmetrical or nearly so (Figs. 8, 9), though sometimes both sepals of a sepal pair are not deflected equally (not shown). The calyces of *M. rivae* and *M. pygmaea* are zygomorphic mainly due to slight differences in flexion among the sepals (Figs. 10, 11). The members of the slender tree clade, in addition to *M. longituba* and *M. ruspoliana*, have backswept sepals that mirror the patterns of flexion seen in the petals (Figs. 5–7, 12–13).

In the actinomorphic species, there may be a very slight tendency for the members of one petal pair to have a greater angle between them than that found between the other petals (Fig. 1). However, this is impossible to detect in pressed material and the pickled material available was distorted by compression of the flowers against one another during fixation, so this subtle difference in petal angle may be artefactual. In contrast, petal flexion is very marked in the members of the zygomorphic clade.

At anthesis, any anthers not in their final positions rotate to their mature orientations. In the actinomorphic-flowered species, the anthers are more or less obliquely introrse at anthesis (Fig. 1). In the zygomorphic-flowered species, the anthers are borne in three tiers: the anther opposite the banner forms the distalmost element in all species but *M. longituba*. The anthers opposite the lower petal pair are in a rank together closest to the base of the flower, with the anthers opposite the upper petal pair being intermediate (these tiers can be seen in the illustrations of mature flowers in Figs. 5–12 and are highlighted in *M. oleifera* in Fig. 6). In *M. longituba* the pattern is reversed, with the anther opposite the banner borne closest to the base of the flower, with those of the lower petal pair being the distalmost elements (Fig. 13; cf. differences in flexion between lower lateral stamens in Figs. 90 and 94). Observations of *M. borziana*, *M. concanensis*, *M. longituba*, and *M. oleifera* in cultivation indicate that the anthers dehisce over the first 2 d after anthesis, after which the style elongates markedly and projects well above the anthers.

DISCUSSION

The ontogenetic origin of bilateral symmetry—It might be suspected that radial symmetry in the basal four species of *Moringa* is a symplesiomorphy with the sister family Caricaceae. Floral development in *Carica papaya* has been examined in detail by Ronse Decraene and Smets (1999) and was found to be actinomorphic at all stages. In contrast, all species of *Moringa*, including the actinomorphic ones, are zygomorphic in early ontogeny (cf. *Bersama*, Ronse Decraene et al., 2001). This difference, along with many other floral differences between the families (see Ronse Decraene and Smets, 1999; Olson, 2002), may tentatively be thought of as autapomorphic for Moringaceae. However, the finding that a floral symmetry gene is expressed asymmetrically in the early stages of *Arabidopsis* flowers (Cubas, Coen, and Martínez Z., 2001), which are actinomorphic throughout ontogeny, suggests that other examples of early zygomorphy may be undetected in Brassicales.

That the flowers of all *Moringa* species are indistinguishable

at early organogenesis is represented at the top of the gray branching diagram of ontogenetic similarity between species in Fig. 95 (ontogenetic differences between the actinomorphic and zygomorphic species are given in greater detail in Table 1). At this point all species show traces of zygomorphy. The first divergence in Fig. 95 represents the point in ontogeny when a species group can first be distinguished from the rest of the family, with the *rivae* clade being conspicuous because of its massive primordia (Fig. 41). The next branch in Fig. 95 indicates when the rest of the zygomorphic species become distinguished from the actinomorphic species, most notably by a banner that is always located between sepals 3 and 5 that remains the largest petal throughout ontogeny, consistent patterns of packing of stamens and petals, and a missing staminode, if any, that is always opposite sepal 4. In Fig. 95, the *rivae* clade becomes "reunited" with the rest of the species of the tuberous clade to indicate that these species become indistinguishable from one another at mid-differentiation. Similarities shared by these species include early assumption of mature anther position and the consistent full development of all staminodes. In all species, the position of a flower in an inflorescence appeared to have little effect on floral symmetry.

That the early ontogeny of the actinomorphic species is much more variable than that of the zygomorphic species can be visualized with a Venn-type diagram (Fig. 96). The black box encloses symbols representing the stages of ontogeny in the actinomorphic species. The phases of late organogenesis through enlargement are characterized by great intraspecific variation in these species, with slight zygomorphy giving way to numerous slightly differing floral arrangements. This variation is represented by the numerous symbols at intermediate stages in Fig. 96. The distinct bilateral symmetry of the young flower does not show a consistent relative orientation of parts, e.g., a large, external, banner-like petal may occur between a variety of sepal pairs (Figs. 51 and 81). Lost staminodes, if any, are not necessarily found on the opposite side of the flower from a banner-like petal (cf. Figs. 38 and 56). Some of these variants correspond to the typical zygomorphic floral plan in Fig. 23 (represented in Fig. 96 by the symbol bisected by a plane of symmetry). All variants observed at early and middle ontogenetic phases converge at anthesis on radial symmetry. The differences between the resulting mature flowers, such as a missing staminode or differing petal aestivation, are almost certainly indistinguishable by pollinators (e.g., abundant trichomes mean that presence or absence of the dashed staminode in the mature flower in Fig. 96 has little effect on floral display).

The early floral ontogeny of the zygomorphic species of *Moringa* consists of a subset of elements found in the ontogeny of the actinomorphic-flowered species. The gray box in Fig. 96 encloses stages in the ontogeny of the zygomorphic species. One symbol per ontogenetic stage is shown to imply the low intraspecific ontogenetic variation in this clade. For example, in contrast to the array of floral plans found within an actinomorphic species at differentiation, only bilaterally symmetrical young buds with predictable axes of floral symmetry are found in the zygomorphic clade (as in Figs. 23, 24). Other features that are consistent in most individuals sampled within and across the species of this clade include inclination of the hypanthium (cf. Figs. 68 and 67), patterns of petal and sepal sizes at maturity, and anther orientation. Rather than traces of zygomorphy becoming diminished as ontogeny progresses, these features become more pronounced, as other charac-

teristics, such as stamen and gynoecium displacement, accentuate zygomorphy even further.

Development in zygomorphic flowers can thus be thought of as a stabilization and accentuation of characteristics present in the highly variable ontogeny of the actinomorphic-flowered species (serving as a possible example of the "recruitment" of incipient zygomorphy suggested by Cubas, Coen, and Martínez Z., 2001). The high variability observed in the actinomorphic-flowered species is consistent with the hypothesis that differences in aestivation, floral orientation, and presence or absence of staminodes have little effect on pollination in these species and are therefore subject to little selective pressure. These features, however, appear to be under strong selection in zygomorphic-flowered species based on the low degree of variability observed in these same characteristics in the zygomorphic clade.

Phylogenetic distribution of ontogenetic patterns—Numerous congruences are noted when the distribution of ontogenetic differences between species groups is superimposed on the phylogenetic hypothesis favored here (Fig. 97). Selected congruences are reviewed here, as are the smaller number of incongruences. Differences in ontogeny between species groups are summarized in Table 1.

Mature floral morphology and floral ontogeny are remarkably constant throughout the actinomorphic-flowered species and do not suggest that this group forms a paraphyletic assemblage (note the numerous similarities among these species indicated in Fig. 97). Characters that distinguish the zygomorphic clade are noted in Fig. 97. Particularly noticeable among these are the banner that persists throughout ontogeny, adhesion of the filaments and staminodes, displacement of the gynoecium, hypanthium formation, and three-tiered anther presentation.

Subclades within the zygomorphic clade each have distinguishing ontogenetic features (Fig. 97). In the slender trees, it is the position opposite sepal 4 that remains uninitiated, whereas in the actinomorphic-flowered species the staminode may be missing from a variety of locations. The pattern of stamen and filament adhesion observed in *M. peregrina* (Fig. 83) and *M. oleifera* (Fig. 6) is peculiar to the slender trees. The tuberous clade is distinguished, among other features, by always producing all five staminodes and anthers that differentiate nearly simultaneously, as opposed to the staggered or unidirectional differentiation shown by the rest of the family. Within the tuberous clade, the *rivae* clade is distinguished by buds with massive primordia (Figs. 42–43; see also later stages in Figs. 69–71). This clade also displays a distinctive pattern of filament and staminode adhesion (Fig. 84). The two red-flowered species share the third distinctive pattern of filament and staminode adhesion, with a continuous ring of alternating filaments and staminodes (Figs. 85, 86). Similarities that may be symplesiomorphies were noted between the slender trees and the actinomorphic-flowered species, most notably the tendency to lack a staminode and the early appearance of sutures on the anthers.

Moringa peregrina showed species-specific features earlier in ontogeny than any other species. In *M. peregrina*, initiation of the gynoecium lags distinctly behind that of the other floral organs (Figs. 39, 44, 52). This staggered emergence of the ovary well after the stamen primordial was not observed in any other species. *Moringa peregrina* also has anthers with apiculi that distinguish this species at anther differentiation

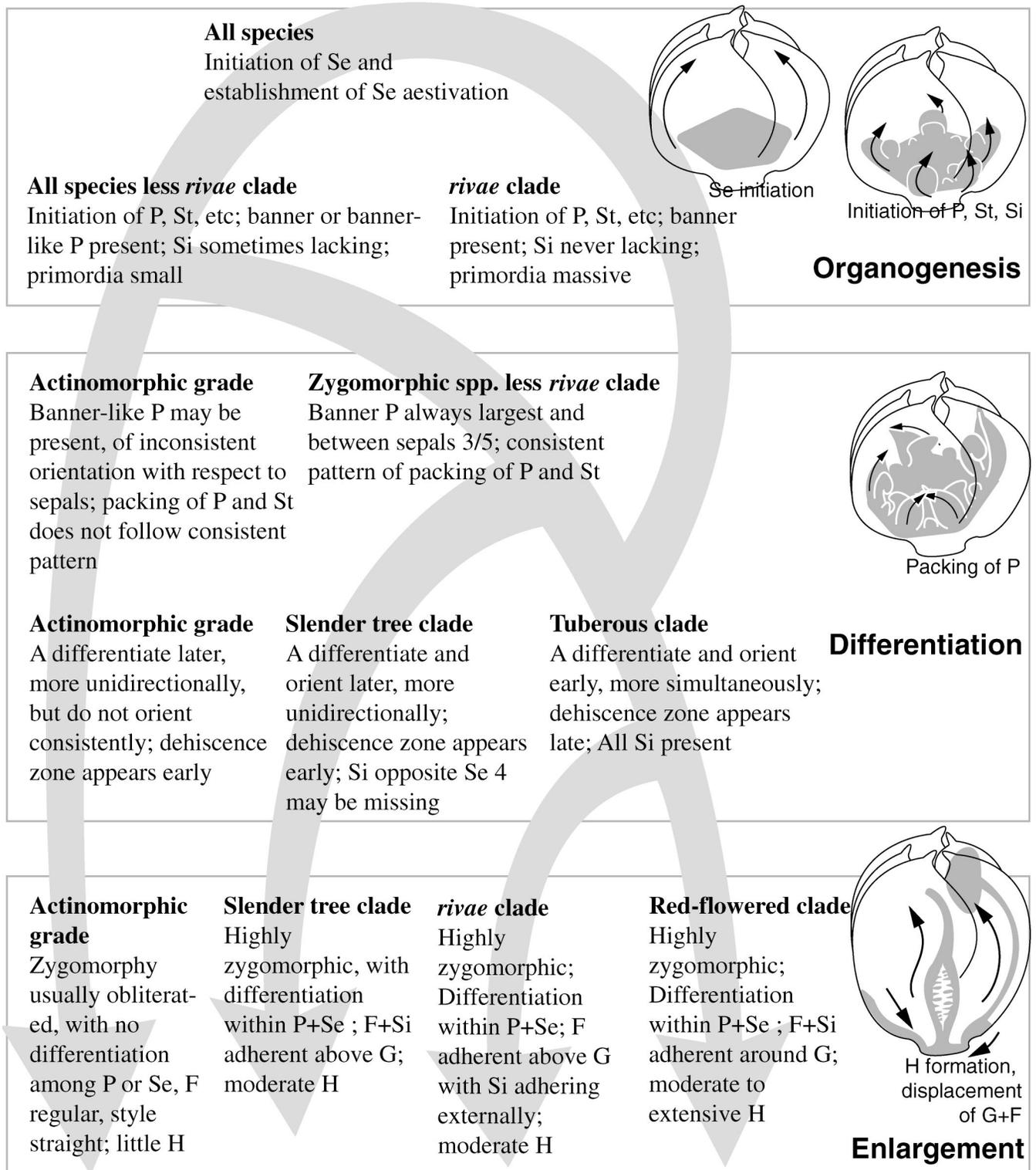


Fig. 95. Summary of ontogenetic programs in *Moringa*. The branching gray arrows indicate the potential to differentiate species groups based on a sample of a given ontogenetic stage (ontogenetic stages are labeled at right). For example, a sample of the *rivae* clade can be distinguished from the rest of the family at later organogenesis; a sample of the red-flowered clade can be distinguished from the rest of the family during the enlargement stage, etc. Abbreviations are as follows: A = anthers, F = filaments, G = gynoecium, H = hypanthium, P = petals, Se = sepals; Si = stamenes; St = stamens.

TABLE 1. Events associated with ontogenetic stages in phylogeny-based species groups of *Moringa*.

Stage and events	Actinomorphic grade	Zygomorphic clade I: the slender tree clade	Zygomorphic clade II: the tuberous clade
Organogenesis			
Emergence of sepals and establishment of sepal aestivation	Sepal aestivation corresponds to Fig. 23 or its enatiomorph in Fig. 24		
Emergence of petal, stamen, staminode, and carpel primordia	Emergence of carpel primordia very closely following anther emergence	Emergence of carpel primordia very closely following anther emergence in <i>M. oleifera</i> ; carpel emergence lags in <i>M. peregrina</i> ; <i>M. concanensis</i> not examined	Emergence of carpel primordia very closely following anther emergence
Establishment of bilateral symmetry	Banner-like petal of variable location present; lateral petals may form symmetrical pairs; one staminode of variable location often not initiated	Banner and surrounding organs larger than other petals and more or less regular; lateral petals form symmetrical pairs across an axis of symmetry bisecting banner and sepal four; staminode opposite sepal four often not initiated	Banner and surrounding organs larger than other petals and more or less regular; lateral petals form symmetrical pairs across an axis of symmetry bisecting banner and sepal four; staminode opposite sepal always initiated
Differentiation of organ form			
Organ size and shape differences associated with mature floral symmetry	Slight differentiation into banner-like petal and lateral pairs may be detected, but banner-like petal often no longer identifiable	Banner and opposite anther remain largest; staminode opposite sepal four, if present, often reduced	Banner and opposite anther remain largest; staminode not reduced
Flexion of petals and stamens associated with packing	The bulk of the petals and their antepetalous stamen primordia often shift in opposite directions, but with no consistent pattern	The bulk of the lower stamen pair is displaced toward sepal four as the lower petal pair twists away from sepal four; the middle stamen pair shifts toward the banner as the middle petal pair shifts away from the banner	
Establishment of petal aestivation	Corresponding to Fig. 23, 24, 26, 27, or 28	Almost always corresponding to Fig. 23 or 24	
Differentiation of stamen primordia into anthers and filaments	Order may be slightly unidirectional; early appearance of dehiscence suture		Differentiation more simultaneous; differentiation of dehiscence suture later
Rotation of anthers	No clear pattern	Rotate well after differentiation to face banner petal	Anthers face banner petal at differentiation
Pigmentation of sepals	Very slight	Very slight	May be very extensive
Enlargement			
Pigmentation of petals	Very slight	Very slight	May be very extensive
Displacement of style	None	Toward banner petal	Toward banner except in <i>M. longituba</i> , in which displacement is toward lower petal pair
Displacement and adhesion of filaments	None	Toward banner; filaments and staminodes alternate in a sheath above gynoecium	Toward banner except in <i>M. longituba</i> , in which displacement is toward lower petal pair; filaments adhere above ovary, often with staminodes adhering to the outside of this sheath in <i>rivae</i> clade; in <i>M. longituba</i> and <i>M. ruspoliana</i> , alternating filaments and staminodes surrounded ovary
Further changes in anther orientation	Slight	Continued rotation toward banner petal	Most anthers at this point are directed toward banner petal; anther opposite banner petal may still be in the process of rotating
Greatest hypanthial growth	Slight	Zonal growth at anther, petal, and sepal base deepens hypanthium, forming a shallow cup in all species but <i>M. longituba</i> , in which a long tube is formed	
Greatest elongation of gynophore	Slight	Zonal growth at base of ovary maintains base of ovary more or less even with hypanthium apex, except in <i>M. longituba</i> , in which ovary is drawn into hypanthium	
Anthesis			
Flexion of sepals and petals	No banner	Banner always present	
Anther orientation	Irregularly introrse	Facing banner petal in three tiers; origin of distal and proximal tiers in <i>M. longituba</i> reversed with respect to other spp.	
Elongation and flexion of style	Elongates slightly if at all	1–3 d after anther dehiscence, style elongates and curves beyond anthers	

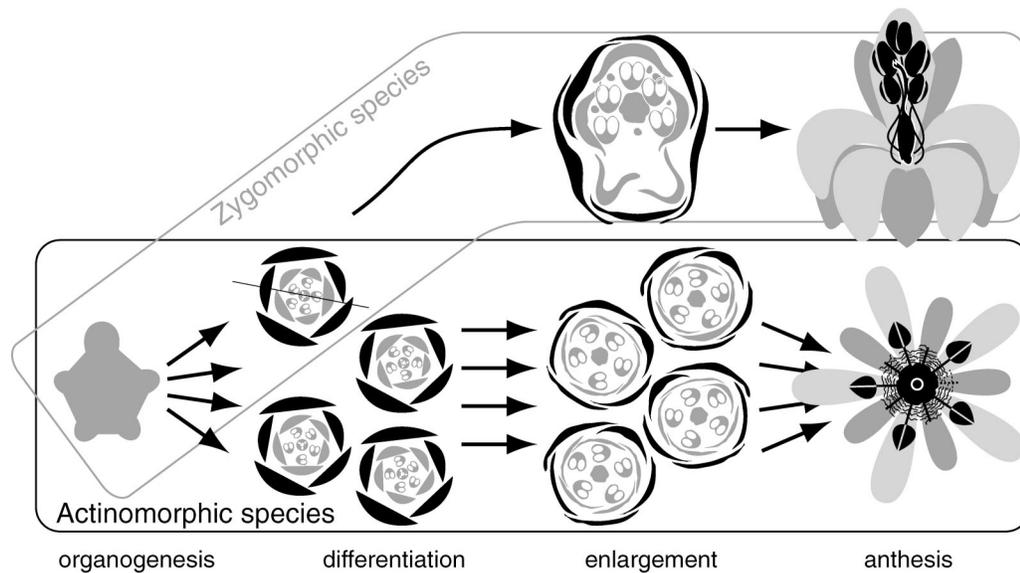


Fig. 96. Venn-type diagram showing that the floral arrangements found in the early ontogeny of the zygomorphic species are a subset of those found in the ontogeny of the actinomorphic species. Stages found in the actinomorphic species are boxed in black; those found in the zygomorphic species are boxed in gray. Many symbols are shown in the black box in the intermediate stages of ontogeny to contrast the large variety of possible arrangements in this group with the low variation seen in the zygomorphic species.

(Figs. 61, 64). Both the emergence of the gynoecium and the anther apiculi in *M. peregrina* seem likely to be additional examples of the many autapomorphies of *M. peregrina* (e.g., bluish leaves with deciduous leaflets).

Timing of ontogenetic divergence as correlate of phylogenetic branching—If ever-earlier ontogenetic divergences characterize ever-deeper phylogenetic splits, then the branching diagram in Fig. 95 should approximate the major nodes of the phylogeny in Fig. 14. This would be the case in *Moringa* were it not for one main incongruence, which is the early divergence of the *rivae* clade at organogenesis. These species are distinguished at this early stage by having larger primordia than the rest of the species, a quantitative difference that does not represent a major departure in ontogenetic pattern such as differences in anther rotation patterns or staminode loss. Divergences deep in the tree, such as that between the actinomorphic and zygomorphic species, occur very early in ontogeny. Those more distal in the tree, such as the split between the *rivae* and red-flowered clades, are manifested late in ontogeny. The phylogenetic distribution of ontogenies in *Moringa* thus appears largely consistent with the expectation that ontogenetic differences characterizing deeper phylogenetic splits occur at earlier stages.

Ontogeny and male-sterile flowers in *Moringa concanensis*—The aberrant androecial development that was observed in *M. concanensis* (Fig. 5) suggests that flowers that are functionally female are produced at least occasionally in this species. Developmentally, this breeding system variant is produced via suppression (in the sense of Tucker, 2000), through which organs are initiated but do not develop fully. The adhesion of filaments and staminodes was not observed in the available pickled material of *M. concanensis* (Olson 700) and was not noted by Dutt (1978) or Matthew and Britto (1983), though filaments bearing fertile an-

thers on numerous herbarium specimens examined do show the normal pattern seen in the slender trees. Male-sterile flowers are not known from any other species in the genus, but indicate the possibility of gynomonoeicy at least in *M. concanensis*. Little is known regarding breeding systems in *Moringa*, although *M. oleifera* is known to outcross and occasionally self (G. Muluvi, Kenya Forestry Research Institute, personal communication).

Conclusion—The study of floral ontogeny in *Moringa* substantially alters interpretation of the polarity of evolutionary transformations. When only mature flowers are examined, the actinomorphic flowers of the basal four *Moringa* species would likely be interpreted as expressing the same kind of actinomorphy as the sister group Caricaceae. Ontogenetic study reveals that the zygomorphic to actinomorphic transition in *Moringa* is dissimilar to the consistent actinomorphy observed in *Carica*. Symmetry differences in *Moringa* flowers are not the result of modifications to later ontogeny in the zygomorphic clade only. Symmetry differences can instead be viewed as the accentuation of zygomorphy present at organogenesis in the zygomorphic species and obliteration of these traces in the actinomorphic species. With some exceptions, ontogenetic events that characterize deep phylogenetic splits occur earlier in ontogeny than those that are associated with shallower nodes. In addition to suggesting ontogenetic mechanisms of evolutionary change, ontogenetic studies in *Moringa* provide numerous characters that are congruent with and therefore support a previous phylogenetic hypothesis. Interspecific functional differences in *Moringa* flowers remain unknown, but very low intraspecific variation in ontogeny in the zygomorphic clade suggests that characteristics such as presence and position of staminodes and aestivation are under strong selection in zygomorphic, but not actinomorphic, species.

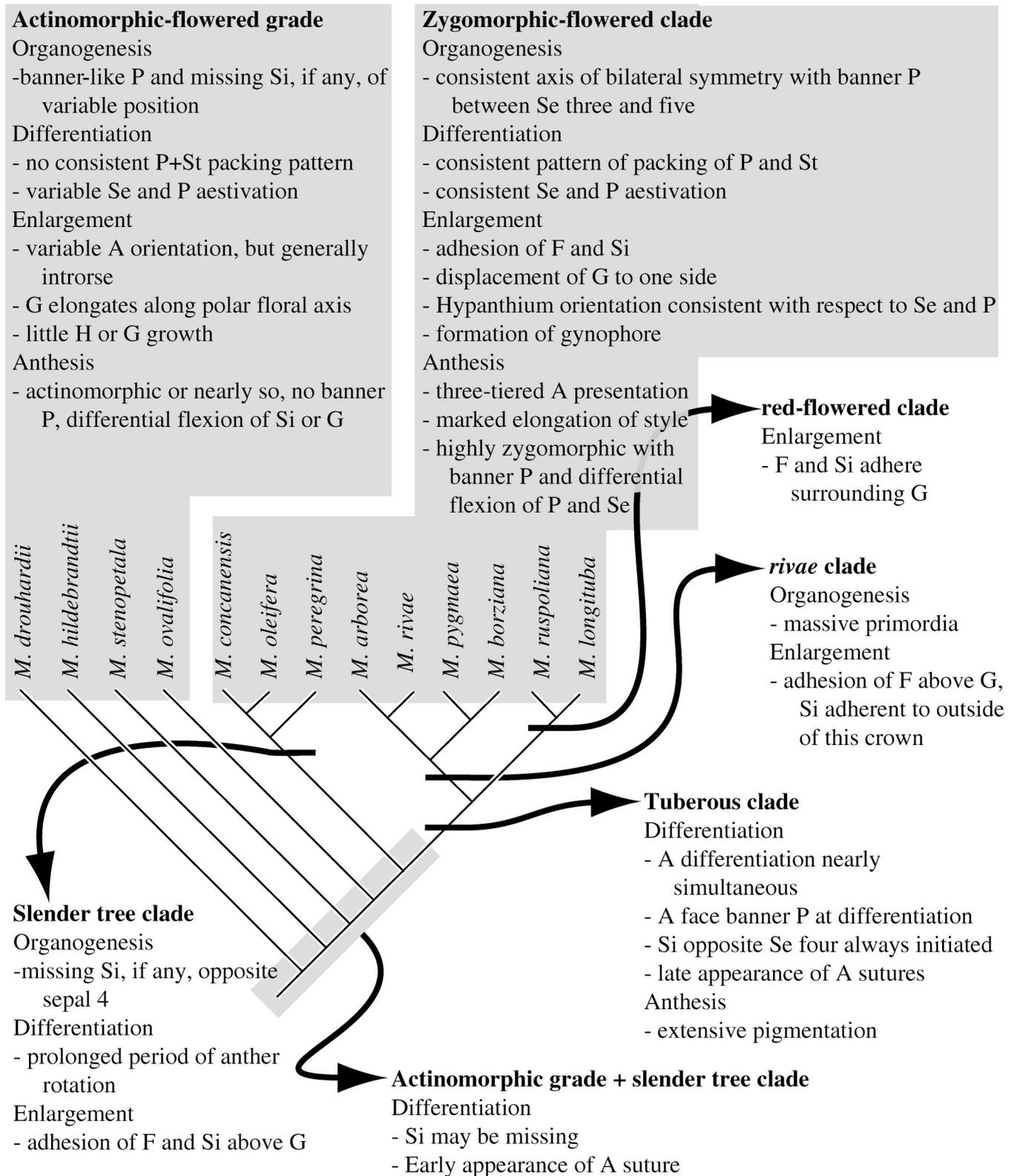


Fig. 97. Phylogenetic distribution of ontogenetic differences between species groups of *Moringa*. Major ontogenetic differences distinguishing the actinomorphic and zygomorphic species are shown in the shaded boxes at top. Differences diagnosing smaller groups are listed by arrows originating at the nodes of interest. Abbreviations are as in Fig. 95.

LITERATURE CITED

- CUBAS, P., E. COEN, AND J. M. MARTÍNEZ Z. 2001. Ancient asymmetries in the evolution of flowers. *Current Biology* 11: 1050–1052.
- DAFNI, A., AND P. G. KEVAN. 1996. Floral symmetry and nectar guides, ontogenetic constraints from floral development, colour pattern, rules and functional significance. *Botanical Journal of the Linnean Society* 120: 371–377.
- DUTT, B. S. M. 1978. Anther in *Moringa concanensis* Nimmo. *Current Science* 47: 589.
- DUTT, B. S. M., L. L. NARAYANA, AND A. PARVATHI. 1978. Floral anatomy of *Moringa concanensis* Nimmo. *Indian Journal of Botany* 1: 35–39.
- DUTT, B. S. M., P. S. P. RAO, AND B. H. RAI. 1978. A study of the secondary xylem of *Moringa concanensis* Nimmo with discussion on the relationships and the systematic status of Moringaceae. *Journal of the Indian Academy of Wood Science* 9: 111–119.
- ENDRESS, P. K. 1999. Symmetry in flowers: diversity and evolution. *International Journal of Plant Sciences* 160: S3–S23.
- HUFFORD, L. 1995. Patterns of ontogenetic evolution in perianth diversification of *Besseyia* (Scrophulariaceae). *American Journal of Botany* 82: 655–680.
- MATTHEW, K. M., AND S. J. BRITTO. 1983. Moringaceae. In K. M. Matthew [ed.], *Flora of the Tamilnadu Carnatic*, vol. 3, pt. 1, 313–314. Rapinat Herbarium, St. Joseph's College, Tiruchirapalli, India.
- METCALFE, C. R., AND L. CHALK. 1950. *Anatomy of the dicotyledons*. Clarendon Press, Oxford, UK.
- OLSON, M. E. 2002. Combining data from DNA sequences and morphology for a phylogeny of Moringaceae (Brassicales). *Systematic Botany* 27: 55–73.
- OLSON, M. E., AND S. CARLQUIST. 2001. Stem and root anatomical correlations with life form diversity, ecology, and systematics in *Moringa* (Moringaceae). *Botanical Journal of the Linnean Society* 135: 315–348.
- RONSE DECRAENE, L. P., J. DE LAET, AND E. F. SMETS. 1998. Floral development and anatomy of *Moringa oleifera* (Moringaceae): what is the evidence for a Capparalean or Sapindalean affinity? *Annals of Botany* 82: 273–284.
- RONSE DECRAENE, L. P., H. P. LINDER, T. DLAMINI, AND E. F. SMETS. 2001. Evolution and development of floral diversity of Melianthaceae, an enigmatic southern African family. *International Journal of Plant Sciences* 162: 59–82.
- RONSE DECRAENE, L. P., AND E. F. SMETS. 1999. The floral development and anatomy of *Carica papaya* (Caricaceae). *Canadian Journal of Botany* 77: 582–598.
- SHEPARD, K. A., AND M. D. PURUGGANAN. 2002. The genetics of plant morphological evolution. *Current Opinion in Plant Biology* 5: 49–55.
- TAKHTAJAN, A. 1972. Patterns of ontogenetic alterations in the evolution of higher plants. *Phytomorphology* 22: 164–171.
- THEISSEN, G. 2000. Evolutionary developmental genetics of floral symmetry: the revealing power of Linnaeus' monstrous flower. *Bioessays* 22: 209–213.
- TUCKER, S. C. 1984. Origin of symmetry in flowers. In R. A. White and W. C. Dickison [eds.], *Contemporary problems in plant anatomy*, 351–396. Academic Press, New York, New York, USA.
- TUCKER, S. C. 1997. Floral evolution, development, and convergence: the hierarchical-significance hypothesis. *International Journal of Plant Sciences* 158: S143–S161.
- TUCKER, S. C. 2000. Floral development in Tribe Detarieae (Leguminosae: Caesalpinioideae): *Amherstia*, *Brownea*, and *Tamarindus*. *American Journal of Botany* 87: 1385–1407.