ANNALS OF
BOTANY
Founded 1887

Floral development and anatomy of Salvadoraceae

Louis Ronse De Craene[1,*] and Livia Wanntorp[2]

[1]*Royal Botanic Garden Edinburgh, 20A Inverleith Row, Edinburgh EH3 5LR, UK and* [2]*Department of Phanerogamic Botany, Swedish Museum of Natural History, Svante Arrhenius väg 7, PO Box 50007, SE-104 05 Stockholm, Sweden*

• *Background and Aims* This study is an investigation into the floral development and anatomy of two genera of the small family Salvadoraceae, which belongs to the Brassicales in a clade with *Batis* and *Koeberlinia*. Salvadoraceae remains little known, despite its wide distribution in arid areas of the globe. Floral morphological data are scarce, and information on floral anatomy is limited to a single study, although morphological and ana-tomical characters are now used increasingly as a counterpart of molecular data. There remain a number of con-troversial morphological questions, such as the fusion of the petals, the number of carpels and the nature of the nectaries.
• *Methods* Floral anatomy and ontogeny were studied in two species of *Salvadora* and one species of *Dobera*. Only for *S. persica* could a full floral developmental sequence be done.
• *Key Results* The floral development demonstrates that the ovary of Salvadoraceae is basically bicarpellate and pseudomonomerous with a single locule and parietal placenta. The ovary of *Dobera* resembles *Azima tetracantha* in the presence of a false apical septum. Evidence for a staminodial nature of the nectaries is not decisive. In *Salvadora* petals and stamens are lifted by a short hypanthium.
• *Conclusions* Salvadoraceae share several morphological and developmental synapomorphies with *Batis* (Bataceae) and possibly *Koeberlinia* (Koeberliniaceae), supporting their close relationship as indicated by molecular phylogeny.

Key words: *Batis*, Brassicales, *Dobera*, *Emblingia*, floral development, floral anatomy, *Koeberlinia*, phylogeny, *Salvadora*, Salvadoraceae, SEM.

INTRODUCTION

The small family Salvadoraceae consists of three genera, *Azima* Lam. with three or four species, *Dobera* Juss. with two species, and *Salvadora* Juss. with three to five species (Kubitzki, 2003). Salvadoraceae are shrubs or small trees well adapted to arid environments and widespread in the Old World.

In pre-molecular classifications the family has always been considered an outsider, either as 'incerta sedis' (Thorne, 1992), or dumped in or close to Celastrales (Sleumer, 1960; Baillon, 1868; Cronquist, 1981; Takhtajan, 1997) or Oleales (e.g. Melchior, 1964; Goldberg, 1986). Dahlgren (1975) placed Salvadoraceae in an expanded order Capparales and he later segregated the family in the separate order Salvadorales (Dahlgren, 1989). The presence of mustard oils in Salvadoraceae was recognized early as an indicator of affi-nity with Capparales (Brassicales; e.g. Dahlgren, 1975, 1989), and it was only through the molecular support of studies based on several gene regions that an association with all mustard oil-producing families was finally confirmed (see Rodman *et al.*, 1993, 1994, 1996, 1998). In those molecular studies Salvadoraceae has been consistently associated with Bataceae as its sister family with strong support, and Koeberliniaceae is sister to those two families in a clade next to the core Brassicales. This association has also been supported by a recent combined morphological and molecular

analysis of the Brassicales (Ronse De Craene and Haston, 2006).

The family Salvadoraceae remains little known, despite its wide distribution in arid areas of the globe. Floral morphologi-cal data are scarce, often copied in different textbooks, and information on floral anatomy is limited to the study of Kshetrapal (1970). Flowers are described as tetramerous (rarely pentamerous) with connate sepals, distinct or shortly connate petals, alternipetalous, free or connate stamens, and a uni- or bilocular ovary with one or two basal ovules. Stamens usually alternate with globular glandular structures. *Azima* differs from other Salvadoraceae by the (occasional) presence of prophylls, generally unisexual flowers (dioecious), free petals and a bilocular ovary with 1(2) ovules. *Dobera* is hermaphroditic or polygamous-dioecious and has one fertile locule, a stamen tube and free petals, while *Salvadora* is reported to have basally fused petals with stamens adnate to the tube and a single locule with a single ovule. *Dobera* and *Salvadora* share presence of antepetalous glands, which are absent in *Azima* (e.g. Thonner, 1915; Sleumer, 1960; Kubitzki, 2003).

However, despite its wide distribution on the globe, the flower morphology of Salvadoraceae remains little known and some of the information available from the literature, such as carpel number, fusion of the corolla and nature of the nectaries, is doubtful and needs to be checked. The number of carpels is uncertain, whether there are two or a single carpel as described in the literature. Engler (1921) reported and illustrated two locules for *Dobera*, of which

* For correspondence. E-mail l.ronsedecraene@rbge.ac.uk

one is sterile, while only a single locule was reported by Kubitzki (2003). *Dobera* would be intermediate between *Azima* with two fertile ovules and *Salvadora* with only a single locule. Baillon (1868) doubted that the corolla of *Salvadora* is really fused; he believed that the corolla lobes are held pressed together by the flattened filaments, contrary to other descriptions of the family. Although antepetalous glands are obvious structures, they were ignored by Baillon (1868) and Engler (1921). The glands are generally described as teeth, disc-glands or an intrastaminal disc, but have sometimes been interpreted as staminodial (e.g. Thonner, 1915; Ozenda, 1958; Watson and Dallwitz, 1992) or as fused stipules of the filaments (e.g. Glück, 1919 in Sleumer, 1960).

Ronse De Craene (2005) investigated the floral development of *Batis maritima* (Bataceae) and suggested that several characters are synapomorphic for Bataceae and Salvadoraceae. No information is currently available on the floral development of Salvadoraceae. However, floral developmental evidence is increasingly used to assess other morphological and molecular evidence for discussing affinities of plants (e.g. Ronse De Craene and Haston, 2006; Wanntorp and Ronse De Craene, 2009). The aim of the present study is to contribute additional support for the postulated relationship of Salvadoraceae with Bataceae and Koeberliniaceae through flower developmental and morphological investigations.

MATERIALS AND METHODS

Pickled floral material of *Salvadora* and *Dobera* was obtained from the alcohol collections in Kew (K) and Wageningen (WAG; Table 1). Further material of *Salvadora persica* was collected by T. Miller in Yemen. Buds and mature flowers were dissected and prepared using a Wild MZ8 stereomicroscope (Leica, Wetzlar, Germany), dehydrated in an ethanol–acetone series, and critical point dried with a K850 Critical Point dryer (Emitech Ltd, Ashford, Kent, UK). The dried material was later coated with platinum using an Emitech K575X sputter coater (Emitech Ltd, Ashford, Kent, UK) and examined with a Supra 55VP scanning electron microscope (LEO Electron Microscopy Ltd, Cambridge, UK). Reference material (in ethanol) is kept at RBGE.

For light microscopy, flower buds were embedded in Kulzer's Technovit (2-hydroxyethyl methacrylate), as described in Igersheim and Cichocki (1996) and sectioned with a Leitz Minot 1212 rotary microtome fitted with metal blade. The sections (about 8 μm thick) were stained with ruthenium red and toluidine blue and enclosed in DMX.

TABLE 1. *Origin of material studied*

Species studied	Origin of the material
Dobera glabra (Forsk.) R.Br.	Herbarium Vadense (WAG), pickled collection – J. Reitsma 323
Salvadora angustifolia Turrell.	Royal Botanic Gardens, Kew pickled collection 32247 – McWhirter 136, Madagascar
Salvadora persica L.	(1) Royal Botanic Gardens, Kew Pickled collection 36279 – Ash 715, Ethiopia; (2) Ronse De Craene 948 Lo – Miller (RBGE), s.n., Yemen

RESULTS

Salvadora persica (Figs 1–5 and 8)

Flowers are grouped in racemose inflorescences or panicles with shorter lateral branches. The flowers are initiated acropetally on a flattened apical meristem (Fig. 1A–D). The first organs to emerge are the bract primordia, which are initiated basipetally in a spiral sequence (Fig. 1B–D). They rapidly increase in size and become triangular in shape; two hemispherical stipule primordia arise laterally of each bract (Fig. 1C–F, arrows). The bract has already attained a considerable size when a small hemispherical flower primordium arises between bract and inflorescence axis (Fig. 1A, C, E, F). As the flower increases in size, it is squeezed between bracts and stipules. The resulting flower bud becomes rectangular in shape (Figs 1A, E and 2A). As the bract and emerging flower primordium take up more space, the stipules are pushed upwards and are nested on each side of the flower (Fig. 1E and F). Stipules grow rapidly up to about 20–30 μm and develop as triangular to pear-shaped colleter-tipped structures (Figs 1E, F and 2B, D) before they shrivel and fade away in later stages of development. The flower primordium increases radially in size and becomes bilaterally symmetrical while two sepal primordia initiate transversally in a rapid sequence (Fig. 1E, F); they attain half the size of the flower bud before a median abaxial sepal primordium is initiated against the bract. Space for the median adaxial sepal is limited and the primordium is squeezed between bracts of younger flowers (Figs 1F and 2A). As a result the sepal primordium is triangular and slightly displaced right or left of the symmetry line. The lateral sepal lobes start to arch over the flower bud when four petal primordia are initiated almost simultaneously in the four corners (Fig. 2A, B). In pre-anthetic buds, sepal aestivation reflects their initiation sequence with the lateral sepal lobes covering the two inner.

The two abaxial petal primordia grow slightly faster than the adaxial primordia and all petals attain a considerable size when the androecium is initiated (Figs 2B, D, E and 3A, B). The residue of the floral bud has taken a cross-shape and four stamens are initiated simultaneously or in a rapid sequence: it was not possible to see stages where the two median stamens arise before the lateral stamens, but they appear to be split off the central meristem before the lateral stamens (Fig. 2B–E). The calyx develops into a basal tube with four lobes (Fig. 3C–E). Sepal growth lags behind those of the petals which push the four sepal lobes open; the upper margin of the sepals is fimbriate as is the margin of the bract (Fig. 3C, E). At maturity bract and sepals are present as small lobes. Petals grow rapidly and develop a broader base; they remain unequal in size for some time before arching over the flower bud in a contorted aestivation (Fig. 3C, E). Because of the compression of the flower between the bract and inflorescence axis, flowers appear disymmetric (Fig. 3A, C, D), or the orientation of the organs is slightly displaced with petals appearing in a median position (Fig. 3B). Stamen primordia develop into broad anthers; the dorsal pollen sacs are connected by a broad plate, while the ventral pollen sacs remain distinct (Fig. 3D, I). When the stamens start differentiating anther tissue, the square central floral residue develops into the gynoecium (Fig. 3B, D, F). One of the adaxial corners opposite a petal develops as a protuberance that differentiates as an ovule
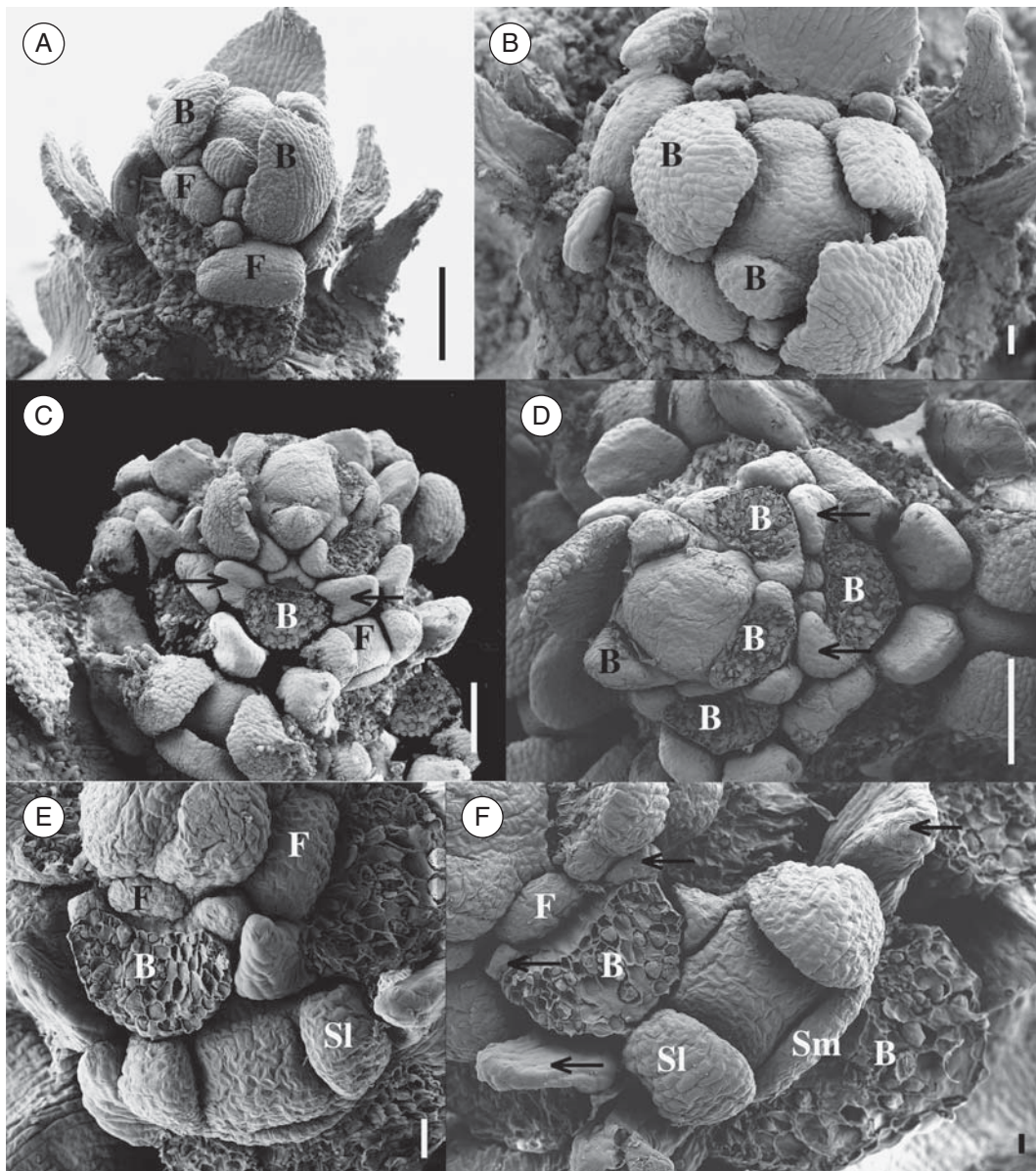
FIG. 1. *Salvadora persica*. Early inflorescence and floral development. (A, B) Lateral and top view of early bract and flower inception. (C) Lateral view of young inflorescence – some bracts removed. (D) Apical view of young inflorescence, some bracts removed. Note the large stipules. (E) Partial view of inflorescence. Initiation of lateral and median sepals. (F) Slightly older stage with initiation of adaxial sepal. Scale bars: (A, C, D) = 100 μm; (B, E) = 20 μm; (F) = 10 μm. Abbreviations: B, bract; F, flower; Sl, lateral sepal; Sm, median sepal. Arrows in (C), (D) and (F) refer to stipules.

primordium (Fig. 3F, G, arrow, H), while the surrounding tissue expands around the central depression. At the same time the sides of the carpel in line with the ovule primordium grow faster than the lateral walls at the time filaments and petal bases become peripherally connected by common zonal growth (Fig. 3H, I); the resulting gynoecium is an obliquely oriented two-lobed tube with an apical slit (Figs 3I and 4A). This tube grows into a saccate organ while the apical part differentiates as a stigmatic depression (Fig. 4B, J). The ovule occupies a basal position and curves into an anatropous structure (Figs 4D–G and 5A). The limit of ovule and stylar area is formed by the presence of a flattened plug-like obturator (Figs 4F, G, arrow, and 5A; cf. Kshetrapal, 1970). Two integuments are initiated; the outer integument develops as a

continuous rim around the fragmented inner integument (Fig. 4G). A filament starts growing only when anthers are well differentiated (Fig. 4A, B, H–J). Filaments are connected at the base in a short tube (Figs 3I and 4A). Alternating with the stamens, four hemispherical primordia are initiated on the connecting tissue; they develop as nectaries in preanthetic buds (Fig. 4B, I). No vascular connection was found at the base of the nectaries (Fig. 5A, B).

Preanthetic buds have the introrse stamens curved around the flask-shaped ovary. Petal bases and filament bases are lifted by common basal growth, resulting in a short tube (Figs 4I and 5A, B). The upper region of the ovary develops as a flat circular stigma covered with papillae and surrounding a central opening filled with exudates (Fig. 5C); the stylar
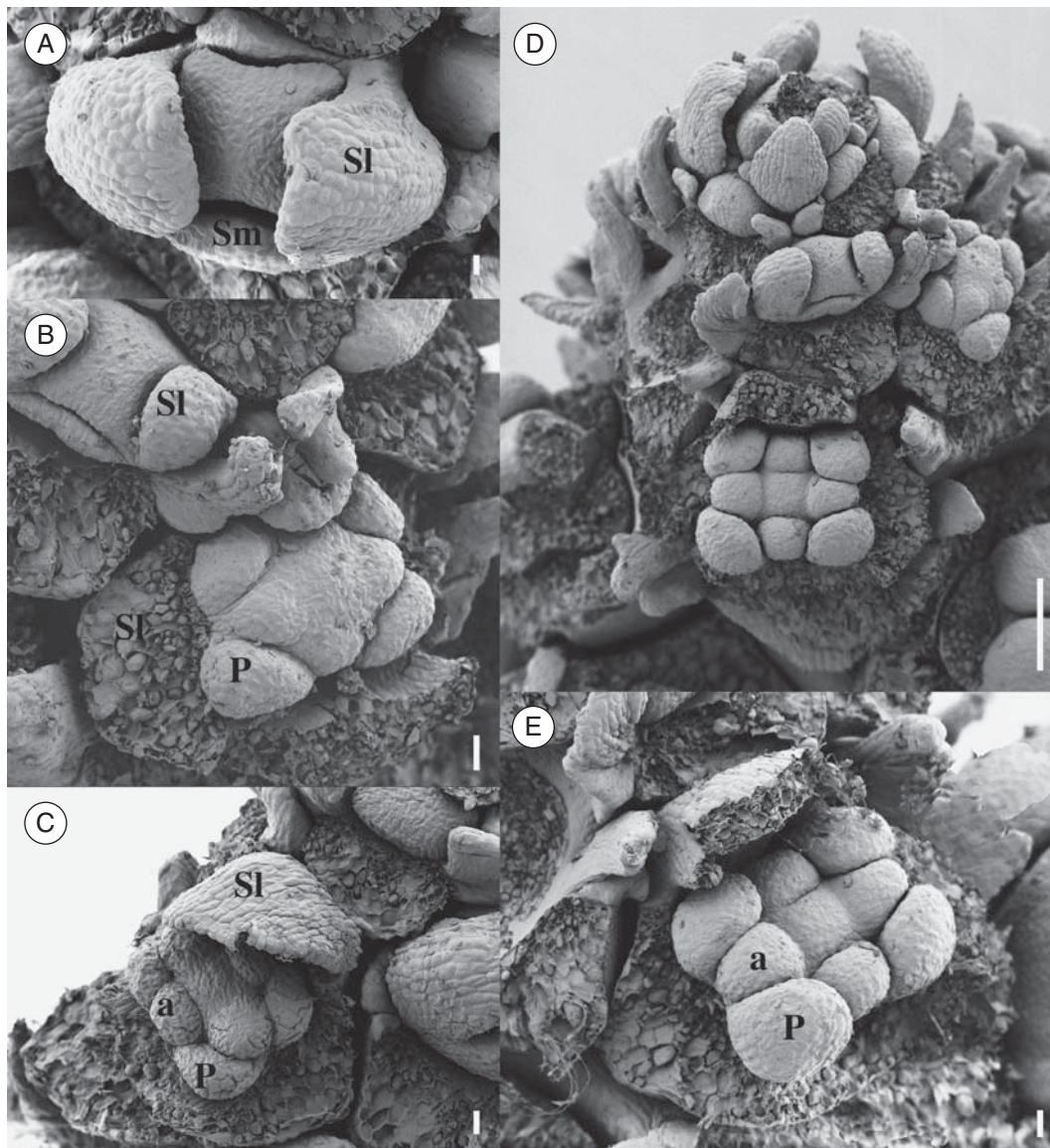
FIG. 2. *Salvadora persica*. Petal and stamen initiation. (A) Early initiation of petal primordia. (B) Older stage showing differentiation of the androecium. (C) Successive initiation of stamens. (D) Overview of young inflorescence with older central flower below. (E) Detail of older flower in (D). Scale bars: (A) = 10 μm; (B, C, E) = 20 μm; (D) = 100 μm. Abbreviations: a, stamen primordium; Sl, lateral sepal; Sm, median sepal; P, petal.

slopes are covered with stomata (Fig. 5D). A short gynophore is present in *Salvadora* (Fig. 5A).

At anthesis, petal lobes are rolled backwards and the erect anthers are exposed by extension of the filaments (Fig. 4J). The calyx appears as a circular rim below the petal lobes (Fig. 4H). Anthers dehisce introrsely by lateral slits and are bent outwards, avoiding contact with the stigmatic plug. By unequal elongation of internodes flowers lower on the inflorescence tend to be arranged decussately in alternating pairs. Pedicels are very short and flowers are inserted at right angles to the inflorescence. Younger flowers at the top of the inflorescence abort, leaving a few basal flowers to reach maturity. Petals and stamens are persistent in fruit.

Some inflorescences were distorted by cabbage-like outgrowths and proliferations (Fig. 5E, F). While the flower retained a tetramerous appearance the inner organs were in higher numbers and had a phyllomatic shape, occasionally bearing distorted pollen-bearing appendages (Fig. 5F).

Salvadora angustifolia *(Fig. 6A–F)*

Only mature and preanthetic stages were available. Flowers resemble *S. persica* closely in morphology, including the presence of a short gynophore (Fig. 6A). Differences are the extent of development of the nectaries (larger and running along the slopes of the longer hypanthium: Fig. 6B, E, F), aestivation of the petals (imbricate descending; Fig. 6C) and the non-reflexed petal lobes (Fig. 6D, E). Nectaries are covered with stomata (Fig. 6F). The presence of a short tube connecting stamens and petals is more clearly visible in this species than in *S. persica* (Fig. 6D, E). Petals of *S. angustifolia* and *S. persica* have only a single vein.
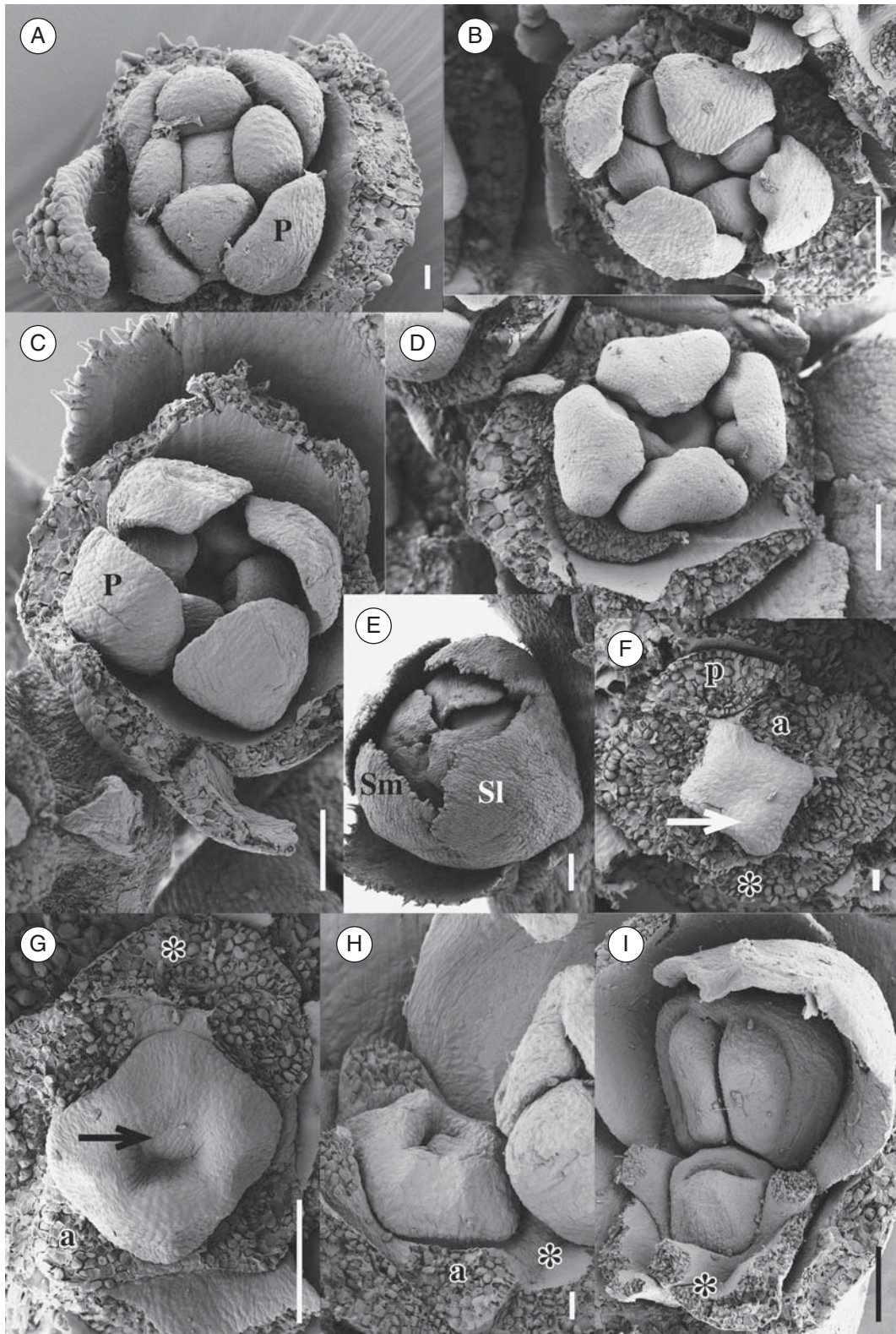
FIG. 3. Development of stamens and gynoecium. (A) Development of young anthers. (B) Differentiation of central gynoecium. (C) Petals start to cover the stamens. (D) Detail of developing anthers and gynoecium. (E) Closed flower bud showing perianth aestivation. (F) Detail of early gynoecium initiation, with initiation of adaxial protuberance. (G) Detail of gynoecium differentiation and early placenta formation (arrow). (H) Lateral view of similar stage. (I) Carpel closure and anther differentiation, three anthers removed. Scale bars: (A, F, H) = 20 μm; (B–E, G, I) = 100 μm. Abbreviations: a, stamen primordium; Sl, lateral sepal; Sm, median sepal; P, petal. In (F–I), the asterisk indicates the adaxial side of the flower.
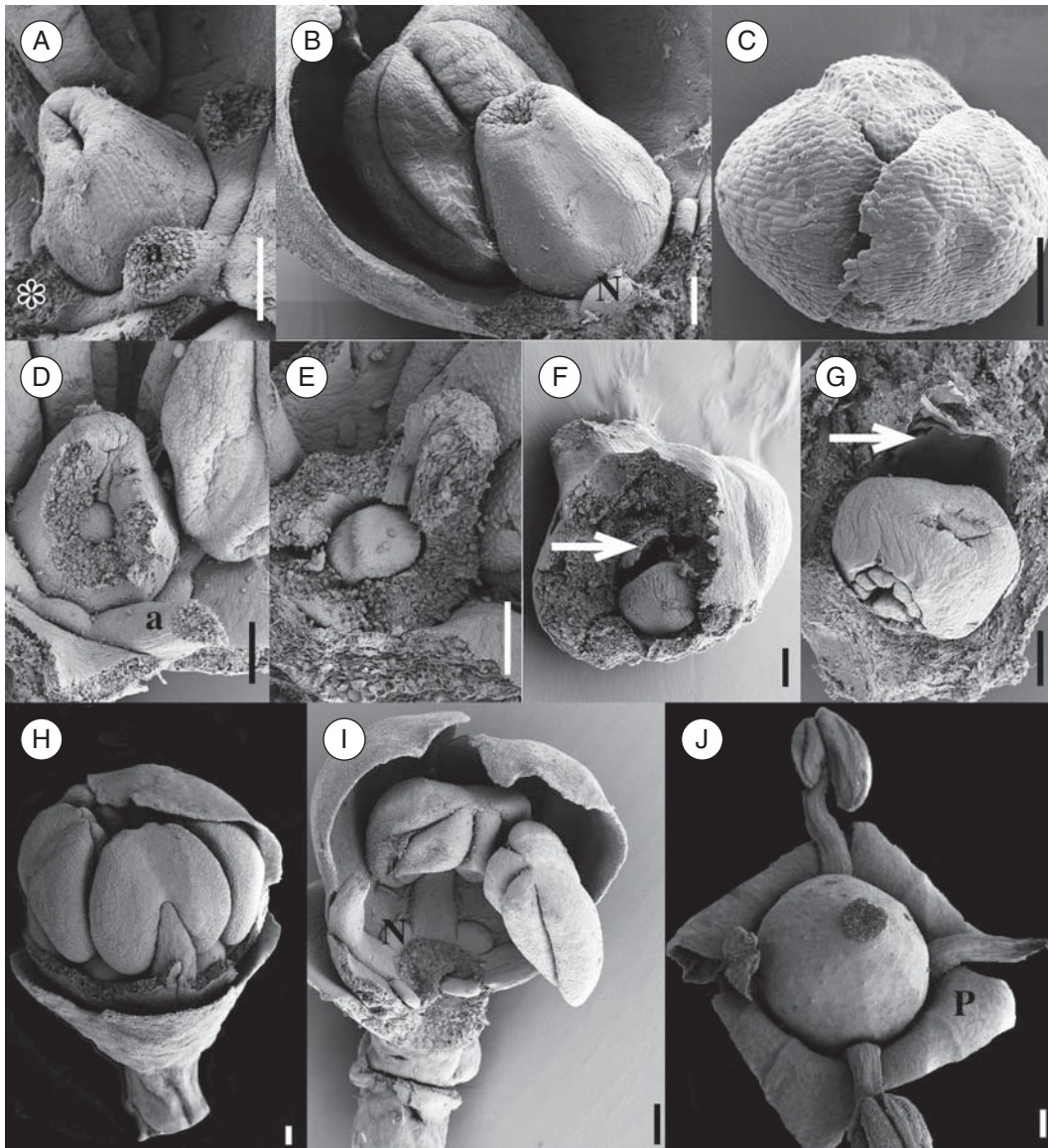
FIG. 4. *Salvadora persica*. Gynoecium and late stages of development. (A) Development of gynoecial dome with apical slit. (B) Differentiation of stigmatic tissue – note the early development of nectaries. (C) Apical view of petals showing the aestivation. (D) Early ovule initiation. (E) Early stage in the anatropous curving of the ovule. (F) Ovary from below showing apical plug (arrow) and curving ovule. (G) Differentiation of two integuments; arrow points to obturator. (H) Lateral view of young preanthetic bud, three petals removed. (I) Similar flower, dissected. (J) Flower at anthesis – note the reflexed petals. Scale bars: (A–H) = 100 μm; (I) = 200 μm. Abbreviations: a, stamen; N, nectar gland; P, petal. In (A) the asterisk indicates the adaxial side of the flower.

Dobera glabra *(Figs 6G–I and 7)*

Only preanthetic and mature flowers were available for study. Flowers are either tetramerous (Figs 6I and 7C, D, G) or trimerous (Figs 6G and 7B, E, F, H, I). Mature flowers resemble *S. persica* in the reflexed petals (Fig. 6I). Main differences are the strongly developed filament tube and nectaries lying outside the tube (Figs 6G and 7B, C), free three-veined petals with imbricate aestivation, as well as a bicarpellate ovary with two ovules (Fig. 6H). Floral anatomical sections (Fig. 7) show that the ovary is pseudomonomerous with two parallel ovules arising at the base of the single locule (Fig. 7A–E). In the upper part of the ovary, a septum divides the ovary in half and is crossed by pollen-transmitting tissue linked to the stigmatic area (Fig. 7A, E–H). Ovules are antitropous (Fig. 7A) with the micropyle formed by the inner integument. Petals surround the nectaries and remain adherent to the stamen tube but are not fused (Fig. 7B–E). Aestivation of petals is contorted (Fig. 7F, H). Stamens are introrse with a flat plate-like connective area (Figs 6G and 7I).

DISCUSSION

Floral developmental and anatomical data demonstrate important differences between the two genera studied. These include merism, extent of fusion of the stamens and petals and number of carpels. The gynoecium was described as
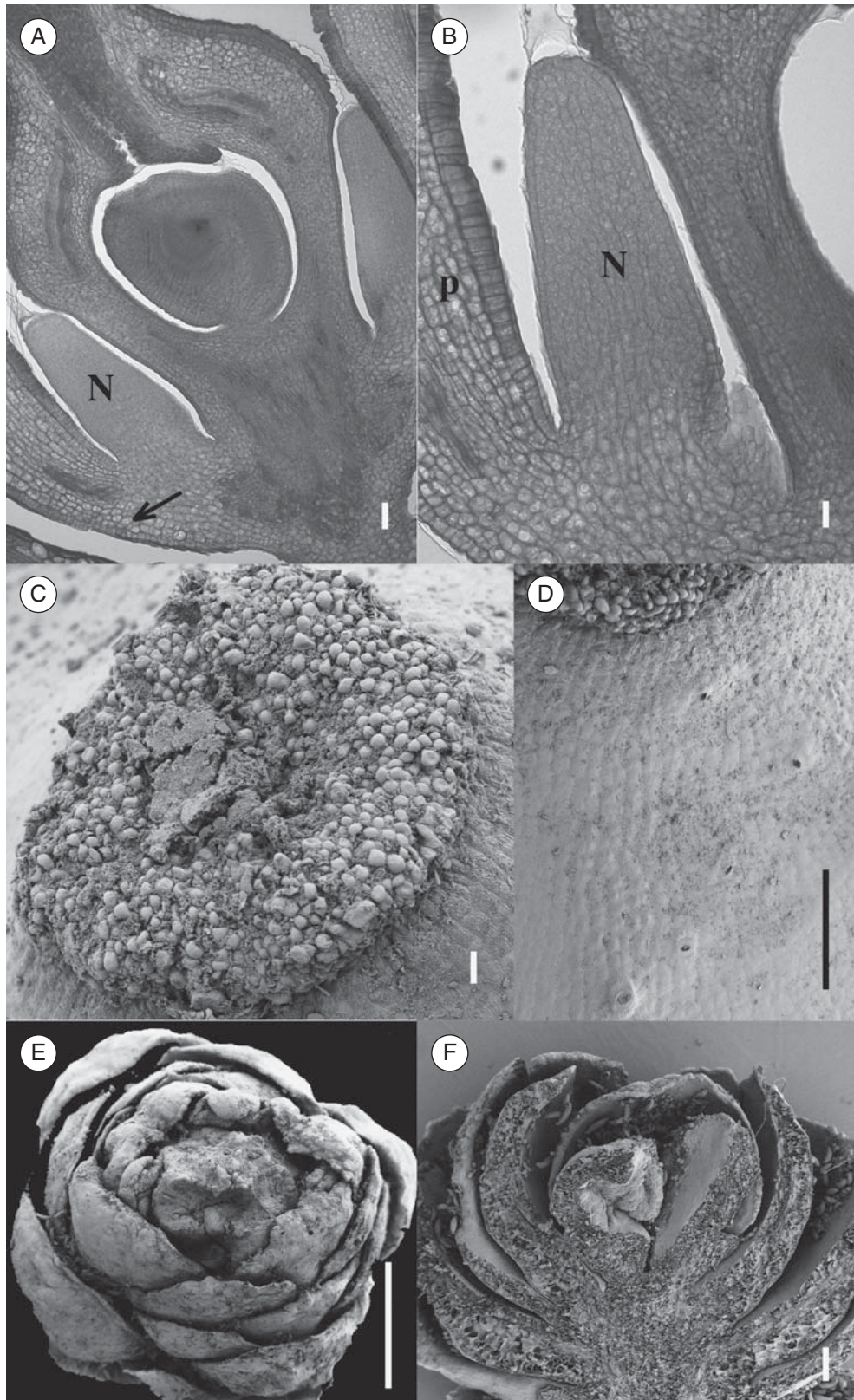
FIG. 5. *Salvadora persica*. (A) LS of preanthetic flower – note the apical plug at the top of the ovary and short hypanthium (arrow). (B) Detail of nectary and hypanthium. (C) Detail of stigma with apical secretion. (D) Detail of the carpel wall – note the presence of stomata. (E) Double flower mutant of *Salvadora* – note the arrangement of several sepal whorls. (F) LS through mutant flower – note the half-developed central anther. Scale bars: (A, C) = 20 μm; (B) = 10 μm; (D) = 100 μm; (E) = 1 mm; (F) = 200 μm. Abbreviations: N, nectar gland; P, petal.
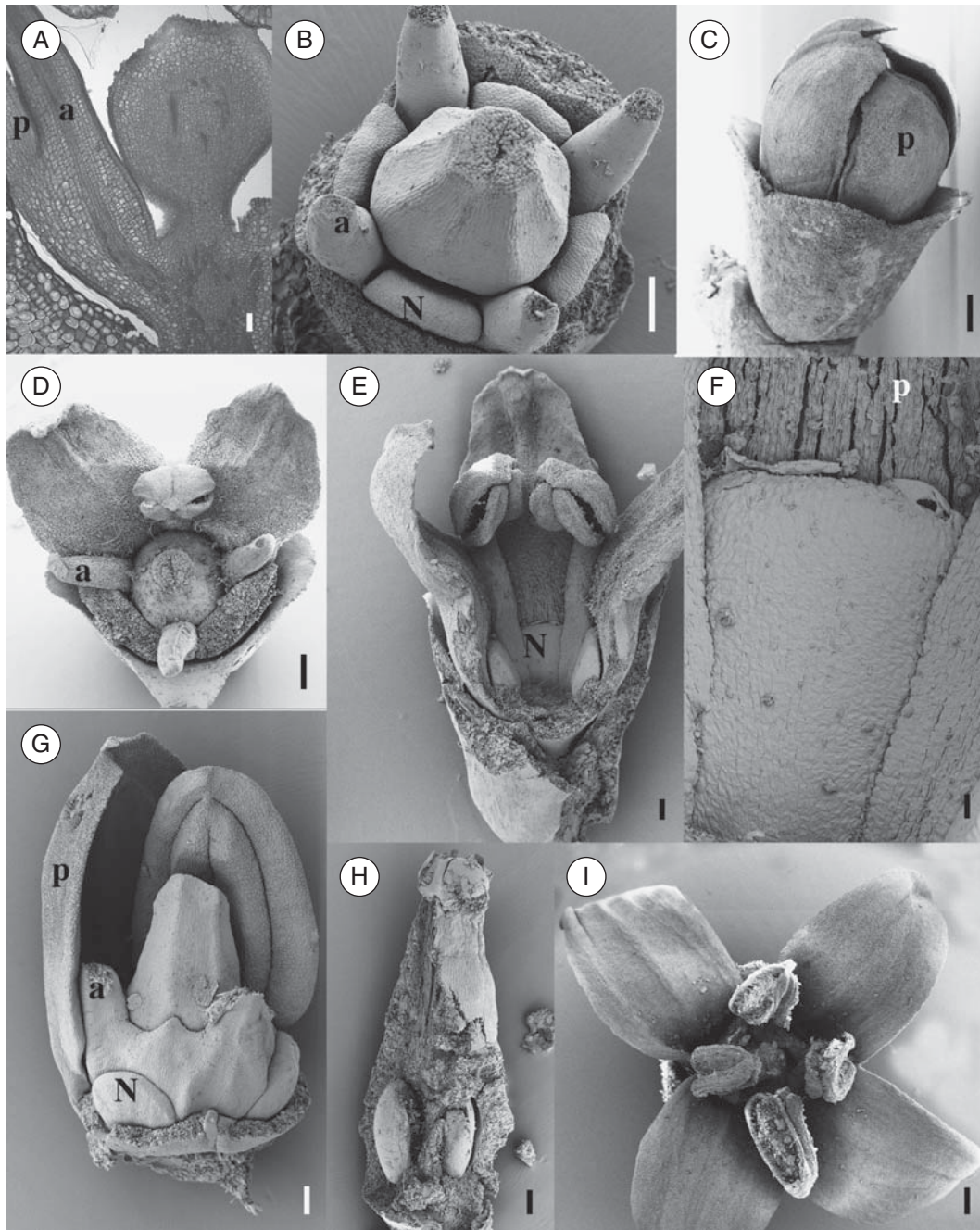
FIG. 6. *Salvadora angustifolia* (A–F) and *Dobera glabra* (G–I). (A) LS of young flower showing hypanthium with base of sepals and petals. (B) View of filaments and gynoecium, anthers removed. (C) Lateral view of flower bud preanthesis. (D) Apical view of flower at anthesis, two petals removed. (E) Section through flower, gynoecium removed. (F) Detail of nectary. (G) Lateral view of dissected flower with extra-staminal nectary and stamen tube. (H) LS through young ovary showing the two ovules. (I) Apical view of flower at anthesis. Scale bars: (A, C, E, G, H) = 100 μm; (B) = 10 μm; (D, I) = 200 μm; (F) = 20 μm. Abbreviations: a, stamen; N, nectar gland; p, petal.

bicarpellate–unilocular in *Salvadora* and *Dobera*, and bilocular in *Azima* (e.g. Kshetrapal, 1970; Kubitzki, 2003). The placentation was described as basal with a single ovule in both *Salvadora* and *Dobera*. However, illustrations of *Dobera glabra* by Engler (1921) show a bi-ovulate, pseudomonomerous gynoecium, as one of the carpels is reported to be sterile. Here the observations on *Dobera glabra* (Fig. 6H and 7A–E) confirm the presence of a single placenta with two basal

ovules. The floral sections of *Dobera* closely resemble those of *Azima tetracantha* presented by Kshetrapal (1970). Floral anatomical sections of *Dobera* and *Azima* demonstrate that the two ovules are situated on one basal-parietal placenta, while the other one is absent; at higher levels a partition develops from the placenta, separating the two ovules and creating the impression of a bilocular ovary (Fig. 7D and E; Kshetrapal, 1970). The partition resembles a false septum,
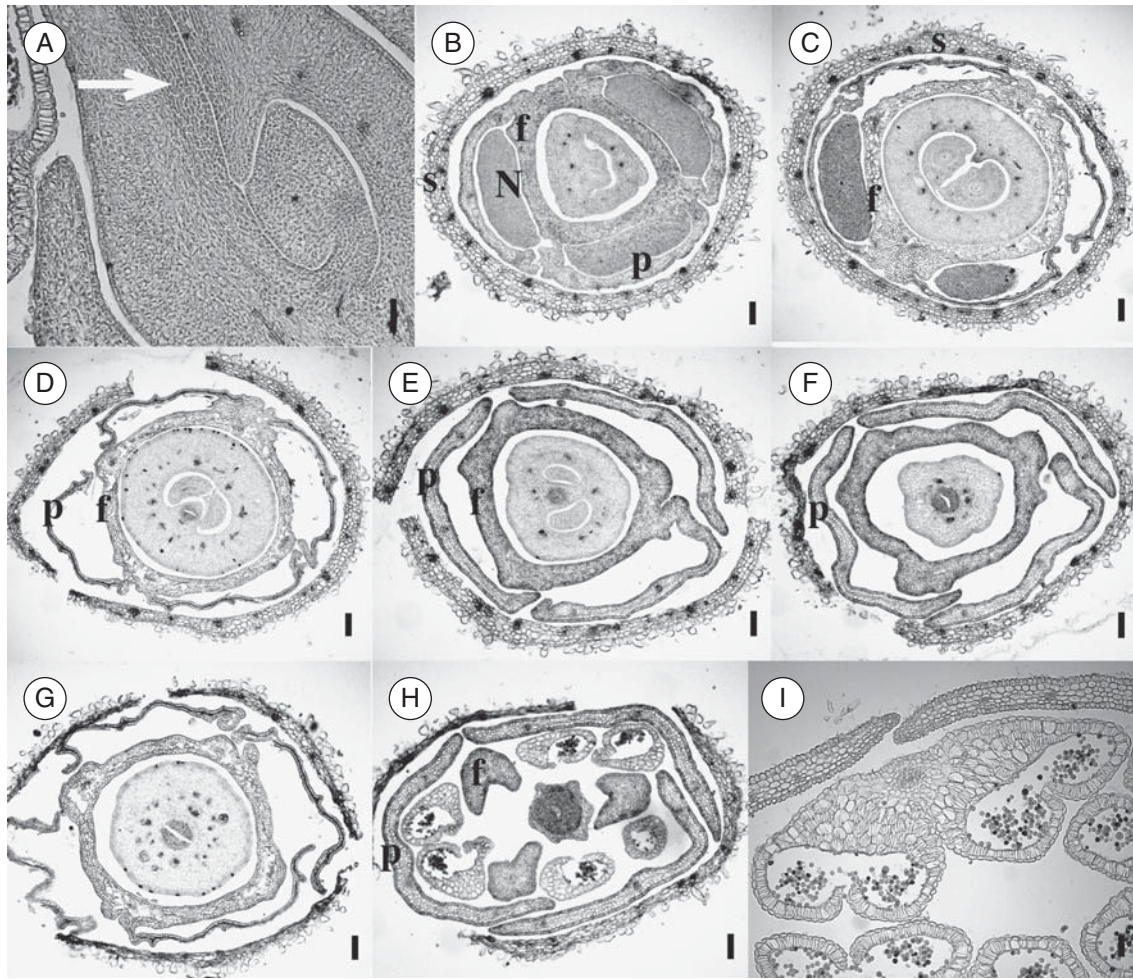
Fɪɢ. 7. LS and TS of *Dobera glabra*. (A) LS of young ovary showing one of the ovules. The arrow points to the pollen-transmitting tract. (B) TS through basal part of ovary with base of placenta – note trimerous flower with three nectaries. (C) Section at the level of the two ovules. (D) Section in the middle of the ovary – note the formation of tube with excretion. (E) Top of ovary showing partitioning of the locule. (F) Section through base of the stigma, with central slit. (G) Similar view – note the stamen tube. (H) Section at the level of the stigma – note three filaments and basal part of the anthers. (I) Section through anther. Scale bars: (A–H) = 100 μm; (I) = 50 μm. Abbreviations: f, filament or stamen tube; N, nectar gland; p, petal; S, calyx tube.

which is also reported for *Batis* (Ronse De Craene, 2005). This observation contradicts the reported presence of a bicarpellate, bilocular ovary with fertile ovules in *Azima*. Depending on the angle of sectioning, one gets the impression of a bilocular ovary in *Azima* (cf. fig. 76 g in Kubitzki, 2003). There is little indication of a pseudomonomerous ovary in *Salvadora*. However, early stages of development differ from truly unicarpellate gynoecia in the development of a nearly symmetrical structure with two main growing points and a single locule (Fig. 3G, H). The ovule is initially marginal but shifts in a basal position at maturity (Figs 3F–H, 4F, G and 8). This indicates that the gynoecium of Salvadoraceae was probably originally bicarpellate and unilocular with parietal placentation followed by the complete loss of one placenta in *Salvadora* and *Dobera*. While the stigma of *Azima* and *Dobera* is bilobed (Kshetrapal, 1970; Fig. 6H), a single apical stigma is formed in *Salvadora* (Fig. 5C). An apical obturator as found in *Salvadora* is absent in *Azima* and *Dobera*, where the apical septum is crossed by a two-lobed secretory zone connecting the stigmatic area (Fig. 7D–G). The ovaries of *Azima* and

Dobera have a similar elongated fruit, while *Salvadora* has globular fruits. Baillon (1868) mentioned the presence of four ovules and two false septa in the basal part of the ovary of *Azima* (*Actegeton*) *sarmentosa*. This would represent a direct link with *Batis* that has a similar ovary. Unfortunately no material was available to evaluate the existence of two fertile carpels in this species and to check this assumption of a false septum.

Glands are either interstaminal (in *Salvadora*) or extrastaminal (in *Dobera*). A nectary is apparently absent in *Azima* (Kubitzki, 2003). Although the presence of glands is evident in the flower (Figs 4I, 5A, B, 6B, E, G and 8), nectaries were not mentioned by Baillon (1868), Engler (1921) or Kshetrapal (1970). Other authors interpreted interstaminal glands as staminodes (e.g. Thonner, 1915; Ozenda, 1958; Watson and Dallwitz, 1992). Glück (1919, in Sleumer, 1960) interpreted the glands as fused stipules of the filaments. Cronquist (1981) and Takhtajan (1997) described the glands as a disc without discussing homologies. The late development of glands and absence of vascular connection do not support
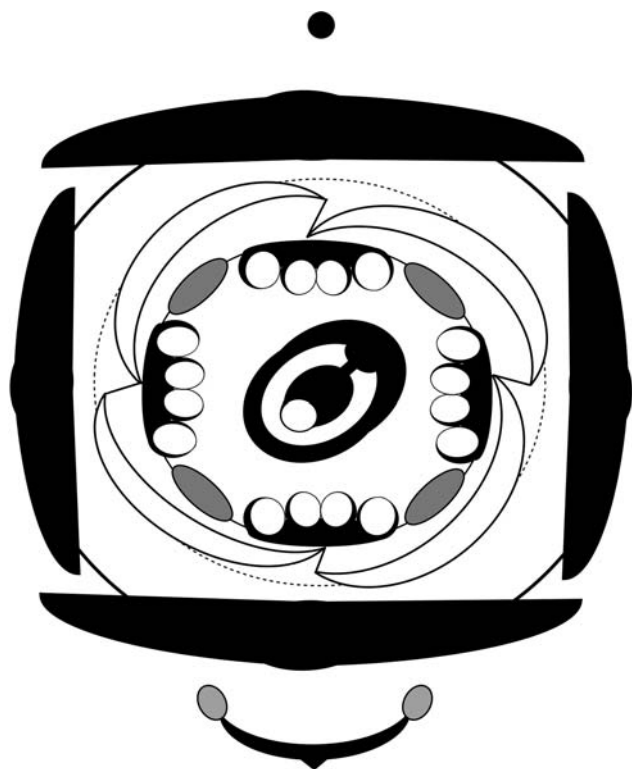
Fɪɢ. 8. Floral diagram of *Salvadora persica*. Grey ovals indicate nectaries and colleter-tipped stipules. Black arcs connected by a circle indicate fused sepals, white arcs indicate petals. The broken line indicates the hypanthial tube connecting the stamen tube and petals.

a staminal origin, although it is not excluded that a second whorl of stamens was present in ancestors of Salvadoraceae. Stamens and petals of *Salvadora* are connected through a short hypanthium arising through common basal growth (Figs 4H, 5A, 6A, E and 8). A short stamen tube is present in *Salvadora*, while it is well developed in *Dobera*. In *Dobera* the stamen tube becomes connivent with the petals without fusing (Fig. 7B–F).

The floral development of *Salvadora* reveals striking similarities with *Batis*, thus supporting a close affinity between the two families Salvadoraceae and Bataceae. Morphological characters shared by *Salvadora* and *Batis* (Ronse De Craene, 2005; Ronse De Craene and Haston, 2006) that might represent synapomorphies in these two genera are: bracts with colleter-tipped stipules, the almost horizontal orientation of mature flowers relative to the inflorescence, a similar unidirectional floral developmental sequence (although it runs from the adaxial to the abaxial side in *Batis*, contrary to *Salvadora*), a similar stamen morphology, an imbricate descending petal aestivation (not in *S. persica*), and the same tetramerous floral organization. Both genera are disymmetric, at least in early stages of development in *Salvadora*. Wind-pollination in *Batis* is associated with a highly divergent morphology at maturity. However, some Salvadoraceae (*Azima*) are constantly dioecious, although staminodes or carpellodes are present. Other differences of *Batis* are the strictly decussate arrangement of the flowers in the inflorescence (although this pattern is visible in mature *Salvadora*), saccate calyx

development and organization, and shape of petals. Salvadoraceae differ from *Batis* in the pseudomonomerous flowers with the reduction of one placenta. The ovary of *Salvadora* is arranged obliquely (Figs 3G and H and 8), contrary to *Batis* with transversal carpels, which is also the common arrangement in core Brassicales. However, *Dobera* has its carpels arranged transversally. An oblique orientation of carpels was observed in *Koeberlinia* (Koeberliniaceae) and some Capparaceae (Mehta and Moseley, 1981; Ronse De Craene and Smets, 1997). Wood anatomy also supports a sister relationship between Salvadoraceae and Bataceae: they share bands of mostly non-subdivided axial parenchyma cells, wide multiseriate, heterocellular rays, and a storied wood (Carlquist, 2002). There is a strong similarity in the presence of stipules that are initially large and are associated with leaves as well as bracts. Rudimentary stipules that are associated with bracts in the inflorescence are relatively common in core Brassicales (see Ronse De Craene, 2002, 2005) and are often secretive at a young stage covered with colleters to humidify the young buds.

Koeberlinia has been associated with *Batis* and *Salvadora* in a single clade on the basis of molecular evidence with moderate to weak support (Rodman *et al.*, 1996, 1998). The floral morphology of *Koeberlinia* was discussed by Mehta and Moseley (1981), although nothing is known of the floral development of the genus and the general floral morphology remains largely understudied. Ronse De Craene and Haston (2006) mentioned several synapomorphies for the *Koeberlinia–Batis–Salvadora* clade, such as pollen, mainly tetramerous flowers, opposite leaves, reduced colleter-like stipules, a comparable flower diagram, a micropyle formed by the inner integument, and a straight embryo, besides a number of wood anatomical similarities enumerated by Carlquist (2002). *Koeberlinia* is diplostemonous with eight stamens and it is logical to accept the loss of antepetalous stamens. The presence of tetramerous, disymmetric flowers with diagonal imbricate petals, an extrastaminal nectary (in *Dobera*), bicarpellate ovary with reduced ventral traces and basally inserted ovules are characters shared by the core Brassicales and the *Koeberlinia–Batis–Salvadora* clade that may or may not represent synapomorphies. Ronse De Craene and Haston (2006) associated the Australian monotypic *Emblingia* with the same clade, although molecular analyses show a stronger support with the core Brassicales (Hall *et al.*, 2004). *Emblingia* is unusual for the clade in having highly zygomorphic and pentamerous flowers, although it shares the reduced stipules, sub-opposite leaves, fused sepals, bicarpellate ovary with axile-basal ovule, and absence of a style. The genus shares other typical characters of the core Brassicales not found in the *Batis* clade, such as an extrastaminal disc (although comparable to the extrastaminal glands of *Dobera*), curved embryo, and androgynophore. However, a short gynophore is present in *Salvadora* (Figs 5A and 6A) and *Koeberlinia*, which is a core Brassicales character. Interestingly, curious teratological flowers of *Salvadora* with a proliferation of leafy organs were found (Fig. 5E and F). These resemble triple mutants of *Arabidopsis* (Brassicaceae), and may have a similar genetic origin.

In this investigation, it has been shown how flower developmental and morphological data support a close relationship

between *Salvadora* and *Batis* (Bataceae). Future studies will, however, need to concentrate on the floral development of *Koeberlinia* and *Emblingia* to understand patterns of floral evolution in the *Koeberlinia–Batis–Salvadora* clade relative to the core Brassicales.

ACKNOWLEDGEMENTS

LITERATURE CITED

Baillon H. 1868. Recherches sur l'organisation et les affinités des Salvadorées. *Adansonia* **9**: 277–290.

Carlquist S. 2002. Wood and bark anatomy of Salvadoraceae: ecology, relationships, histology of interxylary phloem. *Journal of the Torrey Botanical Society* **129**: 10–20.

Cronquist A. 1981. *An integrated system of classification of flowering plants.* New York, NY: Columbia University Press.

Dahlgren R. 1975. A system of classification of the angiosperms to be used to demonstrate the distribution of characters. *Botaniska Notiser* **128**: 119–147.

Dahlgren G. 1989. The last Dahlgrenogram. System of classification of the dicotyledons. In: Tan K. ed. *The Davis and Hedge Festschrift. Plant taxonomy, phytogeography and related subjects.* Edinburgh: Edinburgh University Press, 249–261.

Engler A. 1921. Salvadoraceae. In: Engler A, Drude O. eds. *Vegetation der Erde IX. Band 3,2.* Leipzig: Engelmann, 247–250.

Goldberg A. 1986. *Classification, evolution and phylogeny of the families of Dicotyledons.* Washington, DC: Smithsonian Institution Press.

Hall JC, Iltis HH, Sytsma KJ. 2004. Molecular phylogenetics of Core Brassicales, placement of orphan genera *Emblingia, Forchhammeria, Tirania*, and character evolution. *Systematic Botany* **29**: 654–669.

Igersheim A, Cichocki O. 1996. A simple method for microtome sectioning of prehistoric charcoal specimens, embedded in 2-hydroxymethyl methacrylate (HEMA). *Review of Palaeobotany and Palynology* **92**: 389–399.

Kubitzki K. 2003. Salvadoraceae. In: Kubitzki K, Bayer C. eds. *The families and genera of vascular plants.* Berlin: Springer, 342–344.

Kshetrapal S. 1970. A contribution to the vascular anatomy of the flower of certain species of the Salvadoraceae. *Journal of the Indian Botanical Society* **49**: 92–99.

Mehta IJ, Moseley MF. 1981. The floral anatomy of *Koeberlinia* Zucc.: systematic implications. *American Journal of Botany* **68**: 482–497.

Melchior H. 1964. *Engler's Syllabus der Pflanzenfamilien.* Berlin: Gebr. Borntraeger.

Ozenda P. 1958. *Flore du Sahara Septentirional et Central.* Paris: Centre National de la Recherche Scientifique.

Rodman J, Price RA, Karol KG, Conti E, Sytsma KJ, Palmer JD. 1993. Nucleotide sequences of the *rbc*L gene indicate monophyly of mustard oil plants. *Annals of the Missouri Botanical Garden* **80**: 686–699.

Rodman J, Karol KG, Price RA, Conti E, Sytsma KJ. 1994. Nucleotide sequences of *rbc*L confirm the Capparalean affinity of the Australian endemic Gyrostemonaceae. *Australian Systematic Botany* **7**: 57–69.

Rodman J, Karol KG, Price RA, Sytsma KJ. 1996. Molecules, morphology, and Dahlgren's expanded order Capparales. *Systematic Botany* **21**: 289–307.

Rodman J, Soltis PS, Soltis DE, Sytsma KJ, Karol KG. 1998. Parallel evolution of glucosinolate biosynthesis inferred from congruent nuclear and plastid gene phylogenies. *American Journal of Botany* **85**: 997–1006.

Ronse De Craene LP. 2002. Floral development and anatomy of *Pentadiplandra* (Pentadiplandraceae): a key genus in the identification of floral morphological trends in the core Brassicales. *Canadian Journal of Botany* **80**: 443–459.

Ronse De Craene LP. 2005. Floral developmental evidence for the systematic position of *Batis* (Bataceae). *American Journal of Botany* **92**: 752–760.

Ronse De Craene LP, Haston E. 2006. The systematic relationships of glucosinolate-producing plants and related families: a cladistic investigation based on morphological and molecular characters. *Botanical Journal of the Linnean Society* **151**: 453–494.

Ronse De Craene LP, Smets E. 1997. A floral ontogenetic study of some species of *Capparis* and *Boscia*, with special emphasis on the androecium. *Botanische Jahrbücher für Systematik* **119**: 231–255.

Sleumer H. 1960. Salvadoraceae. In: Engler A, Prantl K. eds. *Die Natürlichen Pflanzenfamilien* 20b. Berlin, Duncker and Humblot, 232–239.

Takhtajan A. 1997. *Diversity and classification of flowering plants.* New York, NY: Columbia University Press.

Thonner F. 1915. *The flowering plants of Africa.* London: Dulau & Co.

Thorne RF. 1992. An updated phylogenetic classification of the flowering plants. *Aliso* **13**: 365–389.

Wanntorp L, Ronse De Craene LP. 2009. Perianth evolution in the sandalwood order Santalales. *American Journal of Botany* **96**: 1361–1371.

Watson L, Dallwitz MJ. 1992 onwards. *The families of flowering plants: descriptions, illustrations, identification, and information retrieval.* http://delta-intkey.com, accessed 9 March 2006.