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Floral Ontogeny of the Afro-Madagascan Genus *Mitrasacmopsis* with Comments on the Development of Superior Ovaries in Rubiaceae

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• *Background and Aims* Members of Rubiaceae are generally characterized by an inferior ovary. However, *Mitrasacmopsis* is cited in the literature as having a semi-inferior to superior ovary. It has previously been hypothesized that the gynoecial development of Rubiaceae with semi-inferior to superior ovaries takes place in the same way as in *Gaertnera*, one of the most commonly cited rubiaceous genera with a superior ovary. To test this hypothesis, a floral ontogenetic study of *Mitrasacmopsis* was carried out with special attention paid to the gynoecial development.

• *Methods* Floral ontogeny and anatomy of *Mitrasacmopsis* were examined using scanning electron and light microscopy.

• *Key Results* At an early developmental stage, a concavity becomes visible in the centre of the floral apex simultaneously with the perianth initiation. A ring primordium surrounding this concavity expands vertically forming the corolla tube (early sympetaly). Stamen primordia develop inside the corolla. From the bicarpellate gynoecium only two carpel tips are visible because the ovary is formed by a gynoecial hypanthium. In the basal part of each carpel, a placenta primordium is initiated. Two septa divide the ovary into two locules. In each locule, the placenta becomes mushroom shaped and distinctly stalked. Eventually, the inferior ovary of *Mitrasacmopsis* develops into a beaked capsule. Only very late in the fruiting stage, the continuously expanding ovary is raised above the insertion point of the persistent calyx, changing the ovary position of *Mitrasacmopsis* from basically inferior to secondarily semi-inferior.

• *Conclusions Mitrasacmopsis* follows an epigynous pattern of floral development. However, the presence of a prominent beak in the fruiting stage gives the whole ovary a semi-inferior appearance. This kind of secondarily semi-inferior ovary is shown to be different from the secondarily superior ovary observed in *Gaertnera*.

Key words: *Mitrasacmopsis quadrivalvis*, *Gaertnera*, floral ontogeny, gynoecial development, epigyny, secondary semi-inferior, secondary superior, scanning electron and light microscopy.

INTRODUCTION

Mitrasacmopsis is a herbaceous genus of the Rubiaceae occurring in continental Africa and Madagascar. Its only species, *Mitrasacmopsis quadrivalvis*, is restricted to rocky areas in high altitude regions (up to 1500 m). Based on the presence of an apparently superior ovary, Jovet (1941) originally placed *Mitrasacmopsis* in Loganiaceae-Spigelieae. Bremekamp (1966) transferred the genus from Loganiaceae to Rubiaceae. Because of the occurrence of raphides, the absence of intraxylary phloem and the herbaceous habit of the genus, he placed *Mitrasacmopsis* in the tribe Hedyotideae (sub-family Rubioideae) close to *Oldenlandia* and its allies (*Hedyotis*, *Houstonia* and *Kohautia*). Based on molecular data, Bremer (1996) merged Hedyotideae with Spermacoceae.

Members of Rubiaceae are generally characterized by the presence of an inferior ovary. However, *Mitrasacmopsis* is cited in the literature as having a semi-inferior to superior ovary (Jovet, 1941; Bremekamp, 1966; Robbrecht, 1988; Endress, 2003). Within Rubiaceae, semi-inferior to superior ovaries are also reported in *Gaertnera*, *Pagamea*, *Coryphothamnus*, *Tresanthera*, *Canthiopsis*, *Mastixiodendron*, *Synaptantha*, *Lucya*, *Leptomischus*, *Pleiocraterium*, *Arcytophyllum*, *Oldenlandia* and *Astiella* (Robbrecht, 1988).

By investigating the gynoecial development of *Gaertnera*, Igersheim *et al.* (1994) demonstrated that during floral development the ovary position changes from inferior to secondarily superior. The ovary tissue undergoes a marked increase in size and extends in an upward direction by intercalary growth, thereby contributing to the superior appearance of the ovary. This was considered to be a secondary modification of the 'typical' inferior ovary characteristic for the vast majority of Rubiaceae.

Igersheim *et al.* (1994) hypothesized that the gynoecial development of other Rubiaceae with semi-inferior to superior ovaries takes place in the same way as in *Gaertnera*. To test this hypothesis, a floral ontogenetic study of *Mitrasacmopsis* was carried out.

In general, the ovary position is determined based on the point of attachment of the perianth with respect to the ovary in a mature flower or fruit. The description of the ovary of *Mitrasacmopsis* as semi-inferior or superior may be due to the presence of a prominent beak in the fruiting stage that, upon superficial observation, gives the whole ovary a semi-inferior to superior appearance. However, floral ontogenetic investigation can reveal whether this semi-inferior to

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superior ovary position is primary or secondary (Igersheim *et al.*, 1994; Kuzoff *et al.*, 2001; Soltis *et al.*, 2003). Flowers begin development with either a hypogynous or an epigynous ground plan and result in flowers with a superior or an inferior ovary, respectively. In a hypogynous flower, all floral organs are initiated on a convex floral apex. Epigynous flowers also begin floral organogenesis with a convex floral apex; however, during or just after perianth initiation, a concavity develops in the centre of the floral apex (Leins, 1972).

Here observations on the floral development and anatomy in the genus *Mitrasacmopsis* are presented. In particular, an attempt is made to determine whether the ovary of *Mitrasacmopsis* is initially inferior and shifts to a semi-inferior or superior position, or if it is semi-inferior or superior by initiation.

MATERIALS AND METHODS

Material of *M. quadrivalvis* Jovet (Dessein *et al.* 266, 422, 515, 583 and 1283, BR) collected in the field in Zambia and preserved in 70 % ethanol was studied by scanning electron microscopy (SEM) and light microscopy (LM).

For SEM studies, floral buds and fruits at different developmental stages were dissected in 70 % ethanol under a Wild M3 stereomicroscope (Wild Heerbrugg Ltd, Heerbrugg, Switzerland). The material was washed repeatedly in 70 % ethanol and dehydrated in a 1 : 1 mixture of ethanol : dimethoxymethan (DMM or formaldehyde-dimethylacetal) for 5 min and in pure DMM for 20 min. After critical-point drying (CPD 030, BAL-TEC AG, Balzers, Liechtenstein), the dried material was mounted on aluminium stubs using Leit-C and coated with gold (SPI Module Sputter Coater, Spi Supplies, West Chester, PA, USA) prior to observation with a JEOL JSM-6360 SEM (Jeol Ltd, Tokyo, Japan).

For LM, samples were dehydrated through a graded ethanol series and embedded in KULZER's Technovit 7100 (based on hydroxyethyl-methacrylate, HEMA) as detailed by Igersheim (1993). Sections, 2 mm thick, made with a rotation microtome (Microm HM 360, Walldorf, Germany), were stained with 0·1 % toluidine blue. Photographs were taken under a Leitz Dialux 20 microscope (Wetzlar, Germany) equipped with an Olympus DP50 camera (Hamburg, Germany).

RESULTS

Early floral development

In the terminal inflorescences, flowers are organized in dichasia, subtended by a series of fimbriae. The uppermost flower of each dichasium develops first, followed by the two lateral flowers (Fig. 1A).

Flower primordia are initially globular (Fig. 1A). Soon, four sepal primordia originate, of which two opposite primordia are more developed than the other two (Fig. 1B). At this stage, a central concavity becomes visible (Fig. 1B). Subsequently, the floral apex elongates and differentiates into a ring primordium surrounding the shallow depression in the centre of the floral apex (Fig. 1C). On this ring primordium, four thickened zones occur, alternating with the sepal primordia (Fig. 1C). The ring primordium expands vertically forming the corolla tube (Fig. 1D). The four bumps on the ring primordium grow out into four corolla lobes. The corolla lobes meet centrally and cover the floral apex in a valvate aestivation (Fig. 1D, E). Prior to anthesis, the abaxial cells of the epidermis of the calyx and corolla become globular (Fig. 1E) and subsequently some of them develop into trichomes (Fig. 3A).

Covered by and alternating with the corolla lobes, four stamen primordia are borne inside the corolla (Fig. 1E, F). The stamen primordia differentiate into anthers and filaments. The anthers are introrse, dithecal and tetrasporangiate (Fig. 1G).

Gynoecial development

During the differentiation of the stamens, two carpel tips become visible at the top of the ovary (Fig. 1E, F). They represent the upper part of the bicarpellate gynoecium. These tips extend and fuse to form a solitary style ending in a two-lobed stigma (Fig. 1G) with a papillose surface (Fig. 3A). At this early stage, the ovary roof is already elevated around the base of the style (Fig. 1G). Surrounding this elevation, a circular zone with paracytic stomata and trichomes is observed (Fig. 1H).

More-or-less simultaneously with the initiation of the carpel tips (Fig. 1E, F), two placenta primordia are formed in the basal part of the ovary (Fig. 2A, B). Two septa grow centripetally and divide the ovary into two locules (Fig. 2B). In each locule, the placenta primordium forms a short-stalked disc-shaped structure (Fig. 2C). At the periphery of the discs 10–12 ovule primordia are initiated, the upper part of the placental discs remaining sterile (Fig. 2C, D). While the developing ovules increase in size, filling all available space in the locules, the placentas become conspicuously stalked and consequently somewhat mushroom shaped (Figs 2E, F, and 4B). The proportion of the disc with respect to the stalk gradually changes in favour of the disc (Figs 2G, H, and 5B).

Development from mature flower to young fruit

During development of the gynoecium, all floral parts continue to grow. Elongation of the stamen-corolla tube elevates the corolla tube *sensu stricto*, the corolla lobes and anthers, inserted at the throat of the corolla, above the level of the calyx (Fig. 3A).

Meanwhile, the style also elongates until it reaches the same height as the anthers (Fig. 3A). Flowers of *Mitrasacmopsis* are isostylous with anthers and stigma never protruding above the corolla throat. The corolla lobes have a papillate inner surface (Fig. 3B). The anthers are attached dorsimedifixed. Below and above the insertion zone of the anthers, trichomes with cuticular striations occur (Fig. 3C).

Eventually all the floral parts wither, with the exception of the persistent calyx (Fig. 3D). At this point, the central elevation of the ovary roof (beak) is still rather limited.

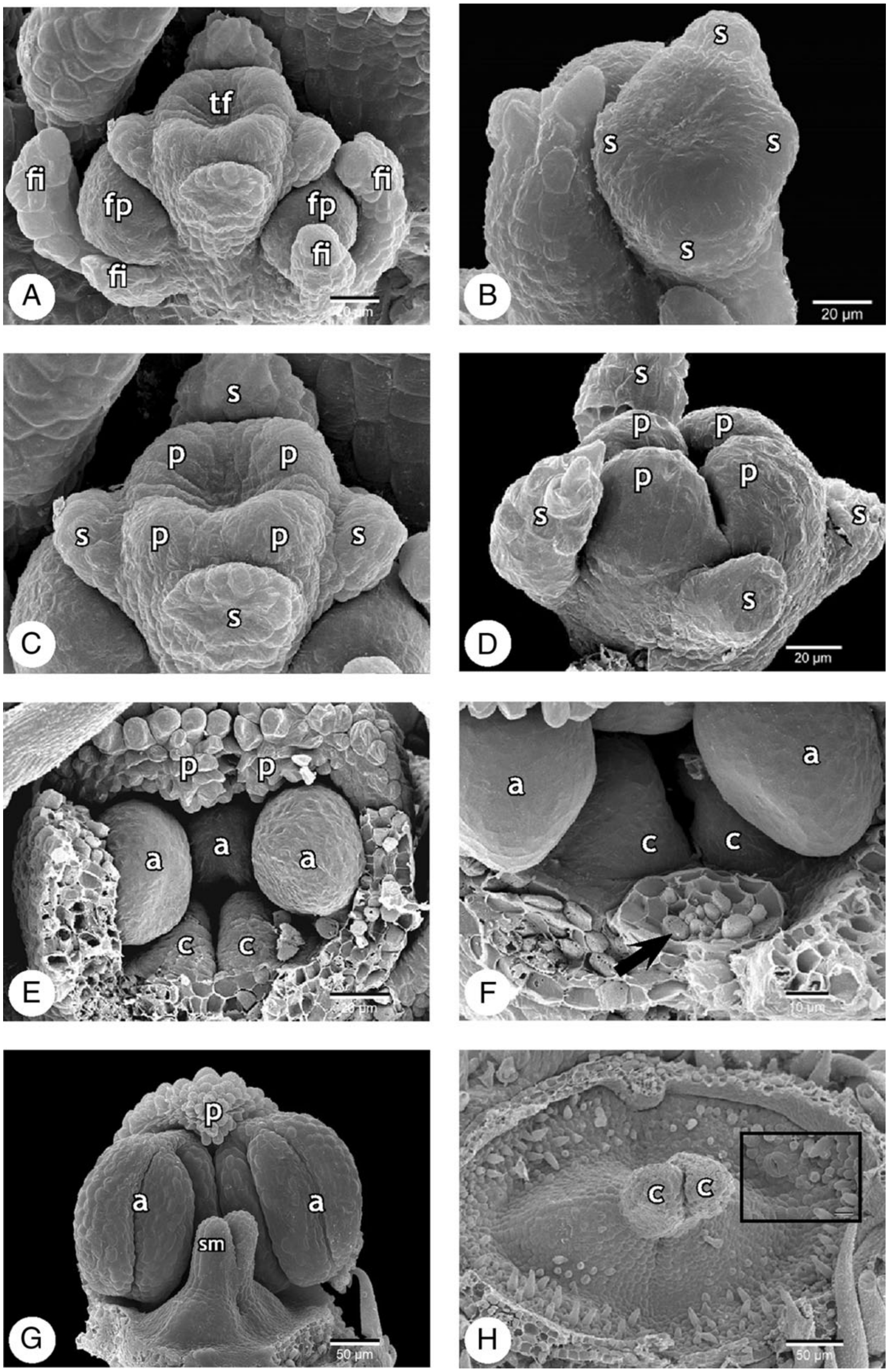

FIG. 1. Early floral development of *Mitrasacmopsis quadrivalvis*. (A) Dichasium with top flower and two younger lateral flower primordia. (B) Four sepal primordia develop on the floral apex and a central concavity becomes visible. (C) Initiation of corolla ring primordium surrounding a central depression. (D) Older stage with the corolla lobes covering the apex. Note the differentiation of a short corolla tube. (E) Flower bud with petals removed showing stamen primordia and two carpel tips in the centre. (F) Insertion point of stamen primordium. (G) Partly dissected flower bud showing anthers and style. (H) Top view of circular zone around the style with stomata and trichomes. Detail in frame. Abbreviations: a, stamen primordium/anther; c, carpel; fp, floral primordium; fi, fimbriae; p, petal (primordium); s, sepal (primordium); sm, stigma (primordium); tf, top flower.

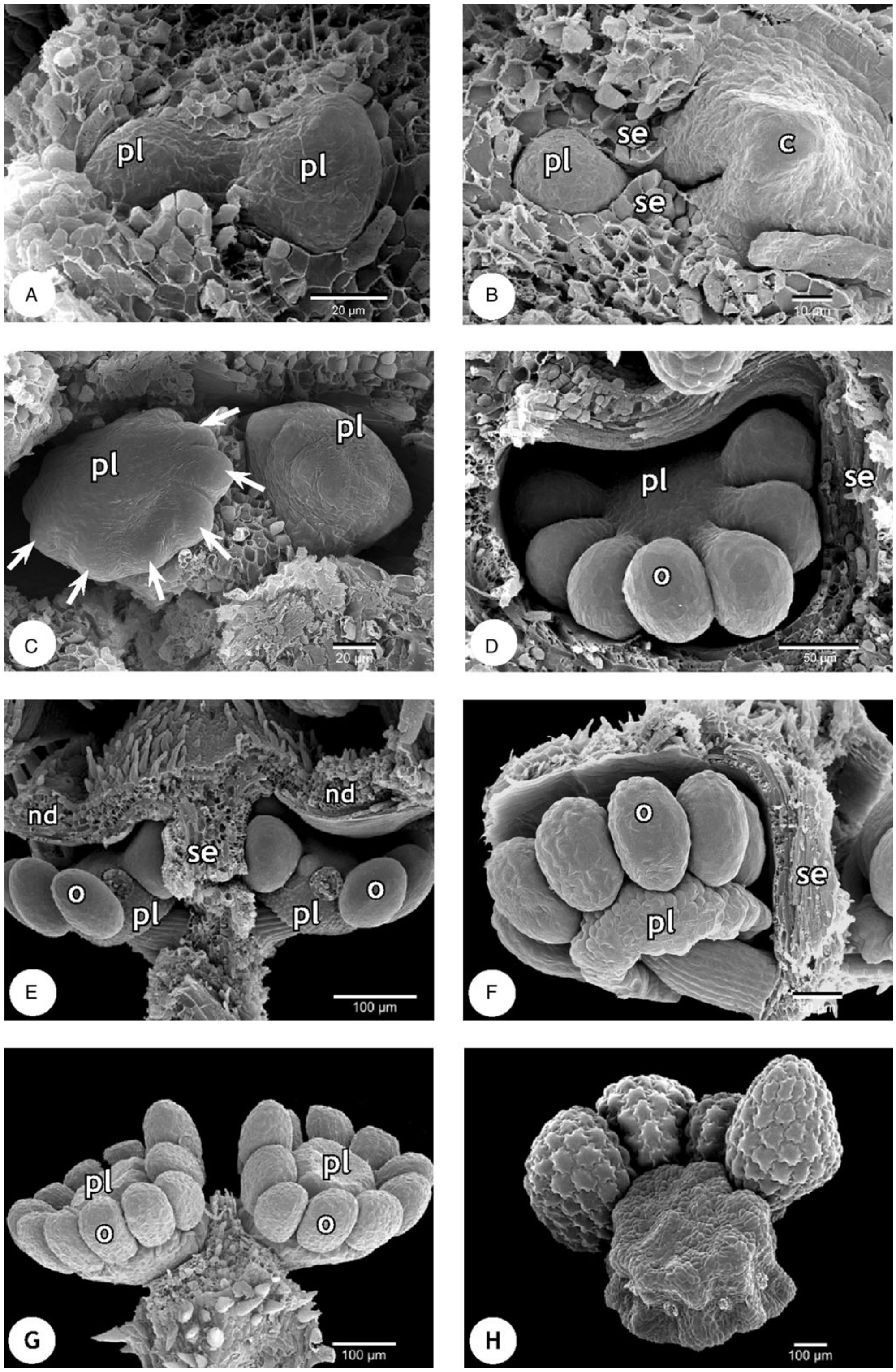

FIG. 2. Gynoecial development of *Mitrasacmopsis quadrivalvis*. (A) Two congenitally fused placenta primordia initiated at the base of the inferior ovary. (B) Detail of a young carpel before differentiation of placental tissue; the other carpel has been removed to show the placenta primordium. (C) Placenta primordia differentiate into disc-shaped structures bearing ovule primordia at the periphery (arrows). (D–F) Ovules increase in size, filling the whole locular space. The placenta becomes mushroom shaped and stalked. (G, H) The proportion of the placental disc with respect to the stalk gradually changes in favour of the disc. Abbreviations: c, carpel; nd, nectary disc; o, ovule; pl, placenta (primordium); se, septum.

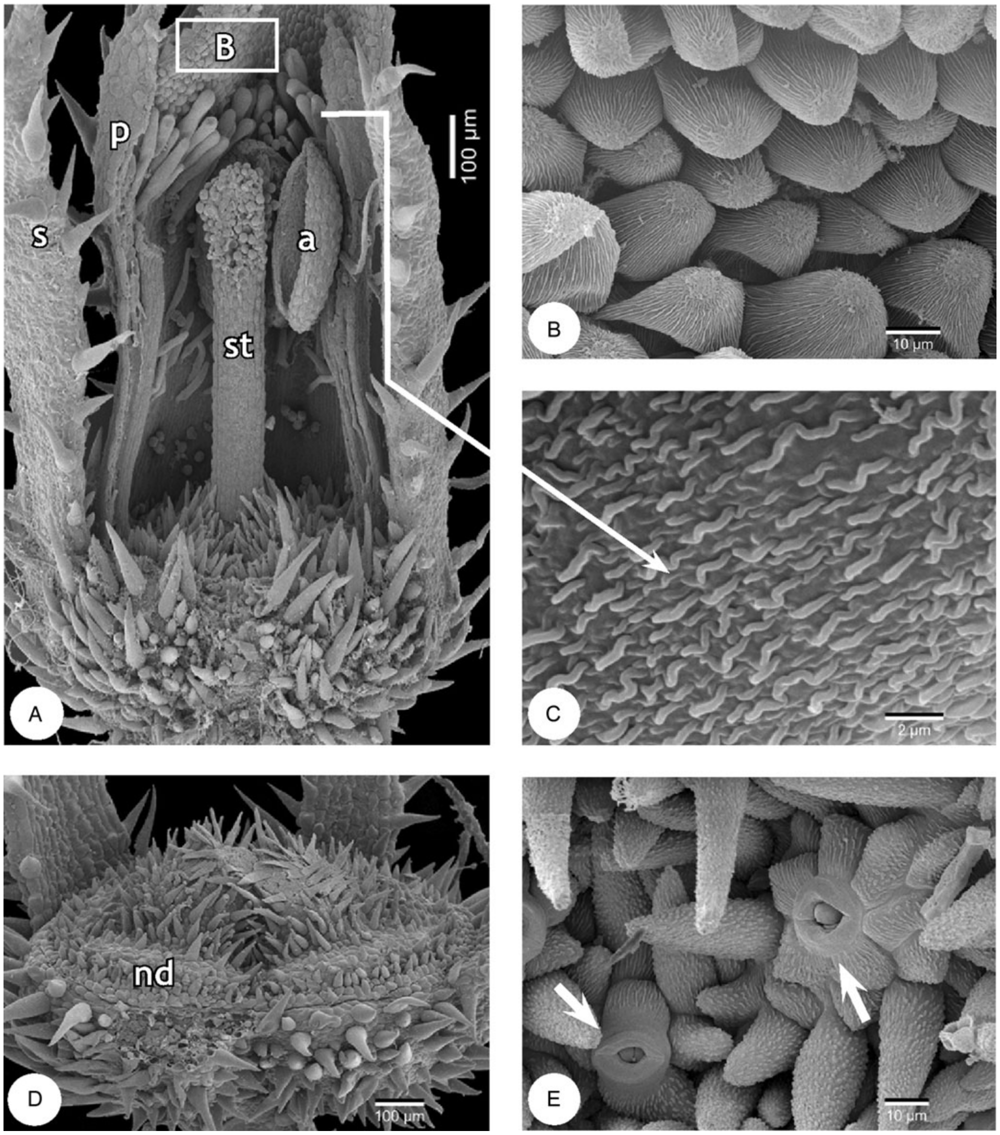

FIG. 3. Mature flower to fruit development of *Mitrasacmopsis quadrivalvis*. (A) Mature flower partly dissected showing style and anthers. (B) Papillate inner surface of corolla lobes. (C) Detail of the cuticular wax on the trichomes situated around the insertion point of the anthers. (D) Young fruiting stage with persistent calyx. All other floral parts have dropped off. (E) Detail of nectarostomata (arrows) and trichomes with cuticular wax. Abbreviations: a, anther; nd, nectary disc; p, petal; s, sepal; st, style.

The nectary disc around the elevation is now completely covered with long unicellular trichomes and nectarostomata that are surrounded by radially oriented cells. Both trichomes and stomata have cuticular striations on their surface (Fig. 3E).

Gynoecial anatomy

To understand the structure of the ovary and its development into a fruit with a distinct beak, longitudinal sections at bud (Fig. 4) and fruit stage (Fig. 5) were prepared.

Before anthesis, the ovary appears obtriangular in shape in longitudinal sections (Fig. 4B). At this stage, the carpel wall already consists of three distinct layers: exo-, meso- and endocarp (Fig. 4C). The exo- and mesocarp form a thick layer of parenchymatic cells. Chloroplasts and raphides are present in the mesocarp. The endocarp is thin but nevertheless recognizable as a few layers of sclerenchymatic cells. The elevation of the ovary at the base of the style, as observed with SEM (Figs 1G and 2E), results from a local thickening of the mesocarp (Fig. 4D). This is further intensified by a depression of the ovary roof below the nectary disc (Figs 4B and 2E).

During fruit development, the ovary shape changes conspicuously from widely obtriangular to widely triangular (Fig. 5A, B). Longitudinal sections show that the outer two carpel layers, exo- and mesocarp, do not undergo marked changes (Fig. 5C, D). On the other hand, the endocarp enlarges considerably, and forms a massive sclerenchymatic layer at maturity (Fig. 5C, D). The change in ovary shape is caused by both an upward growth of the ovary tissue as a whole and a thickened endocarp.

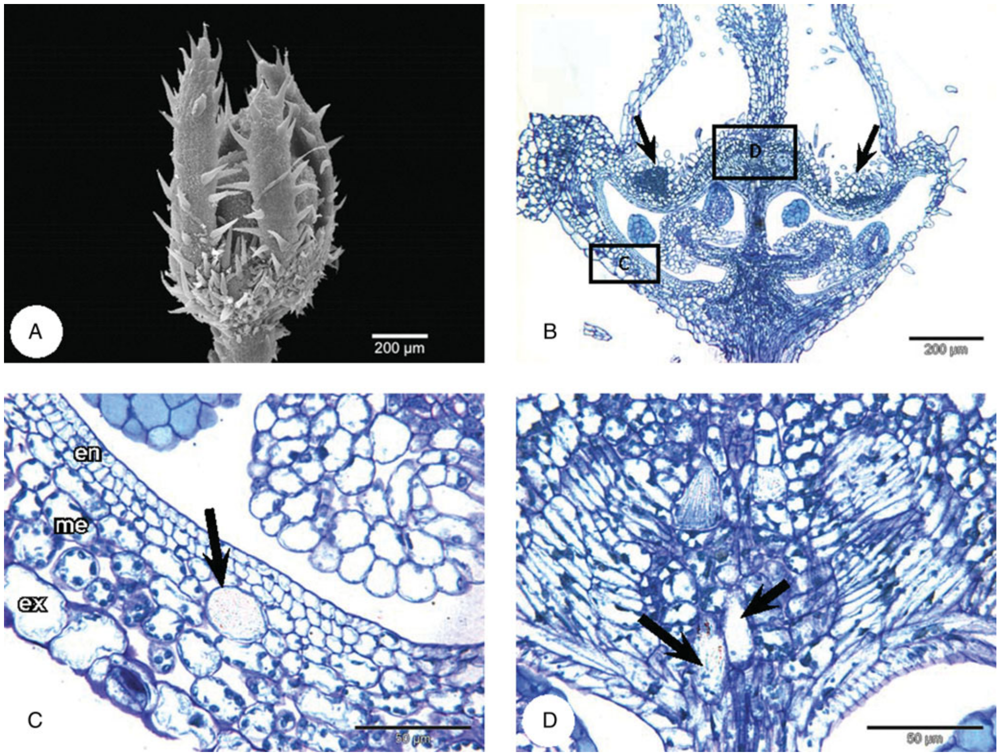

FIG. 4. Sections through flower bud of *Mitrasacmopsis quadrivalvis*. (A) SEM of flower bud. (B) Longitudinal LM section through flower bud. Note the presence of a nectary disc (arrows) around the central elevation. (C) Detail of the carpel wall. Note the presence of raphides (arrow). (D) Detail of the projection around the style (beak). Note the presence of chloroplasts and raphides (arrows). Abbreviations: en, endocarp; ex, exocarp; me, mesocarp.

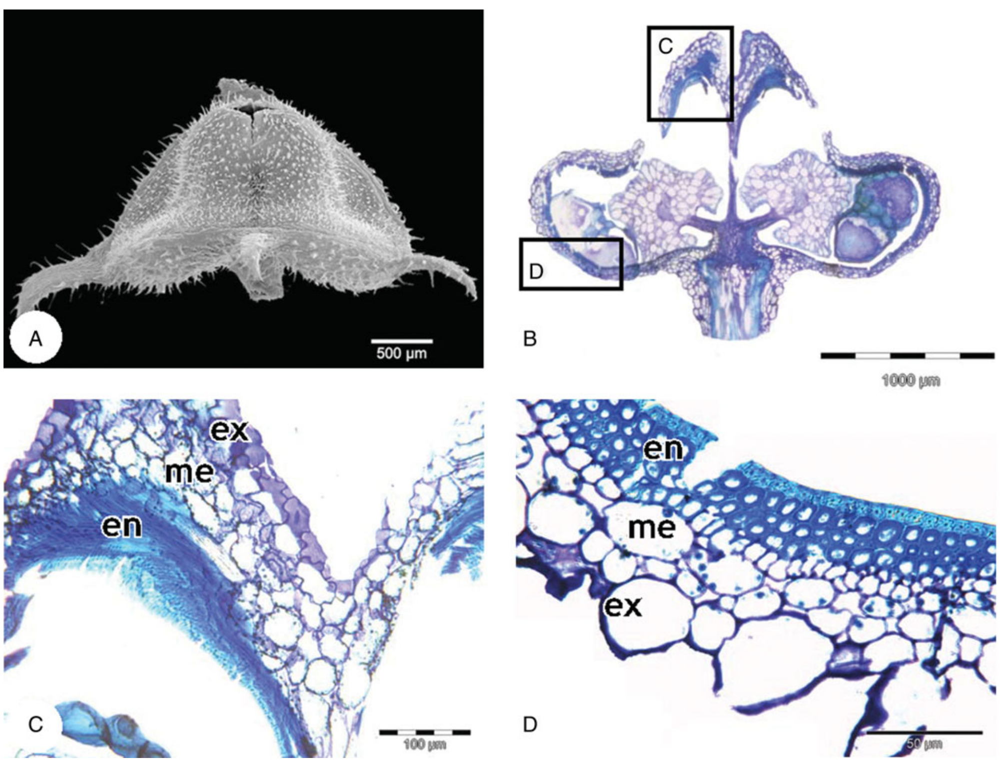

FIG. 5. Sections through mature fruit of *Mitrasacmopsis quadrivalvis*. (A) SEM of mature fruit. (B) Longitudinal LM section through fruit. (C) Detail of the beak. (D) Detail of the fruit wall. Abbreviations: en, endocarp; ex, exocarp; me, mesocarp.

DISCUSSION

Floral ontogeny

Floral ontogenetic investigations in Rubiaceae are scarce [e.g. *Coffea* (Marchand, 1864); *Coprosma* (Leinfellner, 1941); *Gaertnera* (Igersheim *et al.*, 1994; Erbar and Leins, 1996*b*); *Galium* (Fagerlind, 1937; Pötter and Klöpfer, 1987); *Galopina* (Ronse de Craene and Smets, 2000); *Paederia* (Svoma, 1991); *Rubia* (Payer, 1857); *Spermacoce* (Vaes *et al.*, 2006); *Theligonum* (Rutishauser *et al.*, 1998)]. However, they are essential to interpret certain morphological features of the flower, such as sympetaly, ovary position and, in the case of *Mitrasacmopsis*, the formation of the beak.

The present floral ontogenetic results of *Mitrasacmopsis* support several earlier observations within Rubiaceae. Initial unequal sepal development was previously demonstrated in *Galopina tomentosa* (Ronse de Craene and Smets, 2000). The corolla development of *Mitrasacmopsis* corresponds to early sympetaly *sensu* Erbar (1991), observed in some other Rubiaceae (Erbar and Leins, 1996*a*,*b*; Ronse de Craene and Smets, 2000; Vaes *et al.*, 2006).

Superior or inferior ovary?

Several authors described the ovary of *Mitrasacmopsis* as semi-inferior to superior (Jovet, 1941; Bremekamp, 1966; Robbrecht, 1988; Endress, 2003). However, the present results show that flowers of *Mitrasacmopsis* are initially epigynous with inferior ovaries. The organogenesis begins with a convex floral apex. As the perianth grows up, a concavity is formed in the centre of the floral apex. Stamen primordia originate inside the corolla. Stylar primordia are initiated below the stamen primordia (Kaplan, 1967; Leins, 1972).

However, this basic epigynous ground plan can still be modified. A diverse array of ovary positions, ranging from completely inferior to apparently superior ovaries (termed pseudo-superior) as well as the entire range of intermediate ovary positions (semi-inferior), can be produced through differential growth in the superior and inferior region of the ovary, as demonstrated by Kuzoff *et al.* (2001) and Soltis *et al.* (2003). The superior region (SR) of the ovary extends from the insertion point of the perianth (IP) to the apex of the ovary. The inferior region (IR) extends from the IP to the base of the ovary.

Until late in the development, the ovary of *Mitrasacmopsis* is inferior, situated entirely below the IP. However, during fruit development, the relative length of SR and IR changes dramatically, causing a shift in the ovary position. More growth in the SR results in a distinctly beaked capsule. By raising a large part of the ovary above the IP, this prominent beak gives the whole capsule a semi-inferior appearance.

On the basis of ontogenetic studies, Igersheim *et al.* (1994) concluded that the superior ovary of *Gaertnera* is achieved through a process that follows the principle of variable proportions (Troll, 1948). During development, the ovary of *Gaertnera* gets an almost horizontal insertion area and becomes superior primarily due to a strong vertical expansion of the SR just after anthesis. In *Mitrasacmopsis*, the situation differs because growth mainly takes place in the IR. Half of the ovary of *Mitrasacmopsis* remains situated well below the IP. The relative vertical distance between the ovary base and the IP increases, whereas in *Gaertnera* it remains more or less the same throughout organogenesis.

Other taxa with secondarily superior ovaries

Before it became general practice to map character states on phylogenetic trees, it was commonly believed that there was a unidirectional trend in evolution from superior to inferior ovary position; reversals were thought to be rare (Grant, 1950; Stebbins, 1974; Cronquist, 1988; Takhtajan, 1991). However, recent research has shown that a shift from inferior to semi-inferior or superior ovary position also occurs outside Rubiaceae and has been observed within Araliaceae (*Tetraplasandra*; Costello and Motley, 2004), Haemodoraceae, (*Wachendorfia*; Simpson, 1998) and Saxifragaceae (*Lithophragma*; Kuzoff *et al.*, 2001).

Mitrasacmopsis, as well as *Gaertnera*, *Tetraplasandra* and *Wachendorfia*, are all well nested within epigynous families. Floral development indicates that the semi-inferior to superior ovaries found in these genera are derived from an inferior ovary (Igersheim *et al.*, 1994; Costello and Motley, 2004; Simpson, 1998, respectively). Because the genera mentioned above belong to families that are generally epigynous, their flowers can be referred to as secondarily hypogynous with secondarily semi-inferior or superior ovaries to distinguish them from true superior ovaries in ancestrally hypogynous lineages.

The development of the beak

In a study of *Payera* (Rubiaceae), Buchner and Puff (1993) concluded that the development of the beaked fruits of the genus is intimately linked with the fate of the endocarp during fruit maturation. According to them, the growth of the beak is correlated with an increase in extent of the endocarp. Indeed, longitudinal sections of the ovary in *Mitrasacmopsis* show that during the development from ovary to fruit the endocarp undergoes a conspicuous increase in thickness, whereas the exo- and mesocarp do not change remarkably. However, the semi-inferior appearance of the fruit is essentially caused by an upward growth of the ovary tissue as a whole in the superior region, in combination with a thickened endocarp.

In her morphological study of the Rubioideae, Hayden (1968) stated that some genera of Spermacoceae *sensu stricto* have semi-inferior fruits. According to Robbrecht (1988), this statement is based on the strong expansion of the top of the nectary disc in the fruiting stage. However, semi-inferior ovaries within Spermacoceae *s.s.* have not been observed. Nevertheless, within Spermacoceae *sensu lato*, several other taxa, apart from *Mitrasacmopsis*, are characterized by the presence of a beak at fruit stage (*Conostomium* spp., *Hedythyrsus* spp., *Kohautia* spp. and *Oldenlandia* spp.). These beaks are not remnants of the nectary disc and probably originate in a way similar to in *Mitrasacmopsis*. However, the ovaries of these species do not undergo a remarkable reverse in shape in the fruiting stage as observed in *Mitrasacmopsis*. In general, the presence of a beak gives the ovary a semi-inferior appearance.

Decisions concerning the ovary position have been based primarily on the point of attachment of the perianth relative to the ovary in a mature flower or fruit. Although it is crucial to evaluate mature floral architecture, this study shows that early floral ontogeny helps to distinguish more accurately between ovary positions.

Placentation

Like most Rubiaceae (Robbrecht, 1988), *Mitrasacmopsis* is characterized by an axile placentation. Within Rubiaceae, the insertion of the axile placentation is very variable; it may be attached to the entire septum or only to a small part of it. The Hedyotideae and associated tribes are a good example of the extreme variability of axile multi-ovulate placentas. Robbrecht (1988) proposed a progression series in the group from peltate multiovulate placentas completely adnate to the septum to small-stalked globoid placentas with relatively few ovules. According to this progression series, placentation in *Mitrasacmopsis* is nearest to type iv (small $\pm$ spherical or elongated placenta inserted at the base of the locule with a reduced number of ovules). However, to understand better the unusual placentation found in *Mitrasacmopsis*, a more detailed study of the placentation forms within the whole Spermacoceae *sensu lato* tribe is needed.

CONCLUSIONS

The present findings show that flowers of *Mitrasacmopsis* are initially epigynous with inferior ovaries. Continued expansion of the upper part of the ovary in the fruiting stage results in a change in the ovary position of *Mitrasacmopsis* from basically inferior to secondarily semi-inferior.

Differential growth during gynoecial development also explains the transition to a secondarily superior ovary observed in *Gaertnera* (Igersheim *et al.*, 1994). However, the shift to a secondarily superior ovary position in *Gaertnera* is already completed at anthesis, whereas the inferior ovary of *Mitrasacmopsis* does not show a notable change in position until very late in the fruiting stage. Moreover, the ovary of *Gaertnera* becomes almost completely situated above the IP during development, whereas half of the ovary of *Mitrasacmopsis* stays well below the IP.

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