Floral evolution in the monocot family Nartheciaceae (Dioscoreales): evidence from anatomy and development in Metanarthecium luteo-viride Maxim.

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The placement of Nartheciaceae within Dioscoreales is an unexpected result of molecular phylogenetics. Nartheciaceae generally differs from the rest of Dioscoreales in having less specialized flowers. Studies of this family are important to elucidate the evolutionary history of the order. Using scanning electron microscopy and light microscopy, we describe the details of the flower structure, initiation, and development in Metanarthecium, which is unique amongst Nartheciaceae in possessing both an almost superior ovary and septal nectaries. This is the first member of Dioscoreales for which all stages of organogenesis have been studied. Within Nartheciaceae, the presence/absence of septal nectaries and the position of the ovary are labile. The presence of post-genital fusion in the gynoecium correlates with the presence of septal nectaries. Septal nectary morphology is complicated in Metanarthecium, which raises the question of whether its floral structure (including superior ovary) is plesiomorphic within Dioscoreales. The septal nectaries of Metanarthecium show homoplastic similarity with those of Allium (Asparagales). The presence/absence of a compitum is probably variable at the infraspecific level in Metanarthecium as a result of alternative possibilities of post-genital fusion between ventral carpel margins. © 2008 The Linnean Society of London, Botanical Journal of the Linnean Society, 2008, 158, 1–18.


INTRODUCTION

Nartheciaceae are a small family of the monocot order Dioscoreales (Angiosperm Phylogeny Group II, 2003) native to North and South America, Asia, and Europe. The family includes four (Zomlefer, 1997) to five (Takhtajan, 1997) genera, i.e. Narthecium, Aletris, Metanarthecium (often placed in Aletris; see also Tamura, 1998), Lophiola, and Nietneria. The largest genera are Aletris and Narthecium, both having disjunctive distributional areas in the Northern Hemisphere. All representatives of the family are perennial herbs with a sympodial rhizome and a basal rosette of linear to broad elliptical leaves. The flowers are arranged in long bracteate racemes. The flowers of Nartheciaceae (as well as many other monocots) are bisexual (rarely polygamous), trimerous, with six tepals in two whorls, six stamens in two whorls, and a gynoecium of three united carpels (for example, Takhtajan, 1997; Zomlefer, 1997). Having the same floral groundplan, members of Nartheciaceae differ considerably in the degree of tepal fusion, ovary position, and presence or absence of septal nectaries (Zomlefer, 1997; Tamura, 1998; Smets et al., 2000; Rudall, 2002). The presence of septal nectaries has been demonstrated in Aletris (Zomlefer, 1997; Rudall, 2002) and Metanarthecium (Utech, 1978), whereas they are absent in Narthecium (Sterling, 1979; Rudall, 2002; Remizowa, Sokoloff &
Rudall, 2006b), Lophiola (Simpson & Dickison, 1981), and Nieteria (Sterling, 1979).

As far as is known, septal nectary formation is associated with post-genital fusion between plicate parts of the carpels (for example, van Heel, 1988; Rudall, 2002; Remizowa et al., 2006b). In Narthecium ossifragum, carpels are congenitally united up to the stigma and there are no free carpel parts during gynoecium development (Remizowa et al., 2006b). Short, free stylodia are visible in some flowers of Narthecium balansae, but no traces of septal nectaries can be found (Remizowa et al., 2006b). The moniliform stamen hairs of Narthecium may contribute to pollinator attraction.

As Nartheciaceae is sister to all other Dioscoreales have been studied anatomically (Utech, 1978; Rudall, 2002), but certain features of the gynoecium structure are still unclear. For example, the presence of a synascidiate zone in the gynoecia of Aletris and Metanarthecium is questionable. Developmental data for Nartheciaceae and Dioscoreales are poor. Late flower development in Dioscoreaceae and Burmanniaceae has been studied by Caddick, Rudall & Wilkin (2000), and in N. ossifragum by Remizowa et al. (2006b). Data on the early development of septal nectaries in Dioscoreales seem to be lacking so far.

This paper investigates the floral anatomy and development in a member of Nartheciaceae that bears septal nectaries: Metanarthecium luteo-viride Maxim. These data are compared with the floral structure and development in Narthecium, a genus that lacks septal nectaries.

MATERIAL AND METHODS

Metanarthecium luteo-viride was selected for anatomical and developmental study as it is a species having septal nectaries and an almost superior ovary (character states that are traditionally considered to be primitive). Some aspects of floral morphology and development were studied in N. balansae. Material of Metanarthecium was collected by the authors in Japan (Honshu, Hiroshima Prefecture, Higashi-Hiroshima surroundings, November 2004 and May–July 2005). Plant material of N. balansae was collected in Russia (Caucasus, Teberda State Reserve, near Klukhor Pass, August 2005, V.G. Onipchenko).

For scanning electron microscopy (SEM) investigations, plant material was fixed in 70% ethanol; parts of young inflorescences and floral buds were dehydrated in acetone using standard methods, critically point dried, mounted onto SEM stubs using double-sided adhesive tape, coated with Pt–Pd using a sputter coater, and examined using a Hitachi SEM S-405A. In total, more than 100 digital images were saved.

For light microscopy observations, material of Metanarthecium was sectioned using the standard methods of paraffin embedding and serial sectioning (thickness, 12–14 μm). Sections were stained in safranin and water blue or Delafield’s haematoxylin and acid fuchsin, and mounted in Euparal. In total, 20 flowers at different stages were sectioned.

Voucher specimens of both species are deposited at the Herbarium of Moscow University (MW).

Although the majority of authors suggest the placement of the mono- or bispecific genus Metanarthecium into synonymy with Aletris (reviewed in Zomlefer, 1997; Tamura, 1998), we continue to use the name Metanarthecium luteo-viride because of tradition (for example, Ohwi, 1965; Czerepanov, 1995) and the equivocal nature of molecular phylogenetic data. Merckx et al. (2003), using evidence from atpB-rbcL spacer, trnL intron, trnL-trnF spacer, and morphology, showed that Metanarthecium is nested within Aletris. However, analyses of the matK gene (Fuse & Tamura, 2000) and matK plus rbcL (Tamura et al., 2004) suggest that Aletris is sister to the rest of Nartheciaceae.

RESULTS

Metanarthecium luteo-viride

Organography

Metanarthecium luteo-viride is a perennial herb with a short, stout, vertical, sympodial rhizome. The foliage leaves on vegetative shoots are in a basal rosette, spiral, bifacial, broad, and have a characteristic yellowish light-green colour (Figs 1–4). On reproductive shoots, leaves are reduced up the stem. Prophylls on lateral shoots are in an adaxial position (Fig. 3). They have the same size and shape as foliage leaves, but differ in the presence of two conspicuous keels on the abaxial side.

The flowers are arranged in long racemes (Fig. 5). Apart from a terminal raceme, nearly all inflorescence examined possessed two to four paraccladia. The flowers are subtended by long, linear bracts and have a small bracteole in the upper part of the pedicel.

The flowers are actinomorphic, trimerous, bisexual, with six basally connate tepals in two whorls, six
Connate stamens in two whorls, and three (sometimes two or four) entirely united carpels forming an elongate gynoecium. In one flower, we found a gynoecium with free stylodia, but this is exceptional for this species. Stamens are without connective appendages and moniliform hairs on their filaments.

Each carpel consists of a very short (syn)ascidiate zone and a long plicate zone (Figs 6–30). The carpels

Figures 1–5. *Metanarthecium luteo-viride* in its natural habitat. Figs 1, 2. Plant with young inflorescence. Fig. 3. Plant dissected to show prophyll (pr) of lateral shoot and young terminal inflorescence (arrowhead). Fig. 4. Plant with reproductive shoot of previous season (arrowhead). Fig. 5. Inflorescence at anthesis.
Figures 6–12. Metanarthecium luteo-viride, ovary, transverse serial sections. Fig. 6. Synascidiate zone. Figs 7, 8. Level of cross zones. Fig. 9. Same as in Figure 8, magnified. Figs 10–12. Plicate zone with post-genitally united carpels. ft, floral tube; white arrowheads, main body of septal nectary; black arrowheads, opening canal of septal nectary. Scale bars: Figs 6–8, 200 μm; Fig. 9, 50 μm; Figs 10–12, 100 μm.
Figures 13–18. *Metanarthecium luteo-viride*, upper part of transverse section series of gynoecium of Figures 6–12. Fig. 13. Ovary, upper part. Fig. 14. Style, lowermost part. Fig. 15. Style, middle part. Fig. 16. Style, uppermost part. Figs 17, 18. Stigma. Scale bars: Fig. 13, 100 μm; Figs 14, 17, 18, 50 μm; Figs 15, 16, 25 μm.
are united along their entire length. The ovary is semi-inferior. The inferior part of the ovary is represented by the sterile synascidiate zone (Figs 6, 7, 19, 20). The superior part of the ovary is plicate and trilocular (Figs 8–10, 13, 21–26). In each locule, numerous ovules are inserted on massive placentae in two rows. The ovules are anatropous, bitegmic, and syntropous (sensu Endress, 1994). Seeds lack chalazal

**Figures 19–30.** Metanarthecium luteo-viride, transverse serial sections, schematic drawings made from the same flower as in Figures 6–18. Fig. 19. Ovary, synascidiate zone. Fig. 20. Ovary, level of cross zones. Fig. 21. Ovary, level of orifices of septal nectaries. Figs 22, 23. Ovary, sections showing both main bodies and opening canals of septal nectaries. Figs 24–26. Ovary, plicate zone with post-genitally united carpels. Figs 27–30. Style. Dotted lines, post-genital fusion; grey areas, septal nectaries. Scale bars: Figs 19–21, 200 μm; Figs 22–26, 100 μm; Figs 27, 30, 50 μm; Figs 28, 29, 25 μm.
appendages. Further up, the plicate zone forms a long, narrow style which contains three separate stylar canals (Figs 14–18, 27–30). These canals extend up to the open at its top (Figs 17, 30). There is no post-genital closure of the stylar canals (Angiospermy type 1 sensu Endress & Iger-sheim, 2000).

In its upper part, the style is covered by three stigmatic parts (Fig. 18). Unicellular stigmatic papillae are located around each carpel mouth, which looks to the outside (towards the stamens and perianth). It is not clear whether papillae from the three areas interact to make an external compitum. An internal compitum is absent.

Septal nectaries are present between the locules in the plicate (superior) part of the ovary (Figs 8–12, 21–25). Nectaries have quite a complicated structure. Each nectary consists of two parts: (1) main body, and (2) opening canal. The main body is a sac, longitudinally elongated. In transverse sections, it is narrow and occupies more than one-half of the septum radius. Nectar-secreting cells of the sac wall are rectangular in outline (in cross-section) and have dense, darkly stained cytoplasm and large nuclei in comparison with the other cells of the septum. All epidermal cells of the main body of the nectary seem to be secretory because of their similar differentiation. The opening canal is slightly shorter than the main body of the nectary. It is located outside the secretory area towards the peripheral part of the septum (Figs 10, 11, 21–23). The cells restricting the canal cavity are square in cross-section and have light cytoplasm. They are not secretory. The orifices of the canals are situated at the level of the upper end of the floral tube (where the tepals become distinct). The opening canal and the main body of the nectary are continuous in the upper part of the nectary (Figs 12, 24, 25).

Raphide idioblasts are present in all floral organs.

Floral development
Inflorescences are initiated at the end of the vegetative season. In November, the perianth is already initiated in the floral buds. In the following spring, the floral buds continue their development; anthesis occurs in July and August. The inflorescences are terminal, but appear lateral, because of the leading position of a new lateral vegetative shoot (Figs 1–4).

Flowers are initiated in axes of spirally arranged bracts (Figs 31, 32). Floral primordia are hemispherical or slightly compressed in a transverse direction (because of pressure from the large subtending bract). The floral primordium expands laterally and thickens to become ellipsoidal, and then the bracteole appears (Fig. 33). The bracteole can be initiated either at the right or left side of the floral meristem, but always in (almost) the transverse position. The outer tepals arise in a spiral sequence (Figs 34–36). The first tepal is initiated strictly opposite the bracteole. The second tepal primordium appears between the first tepal and the bracteole, close to the bracteole. The third tepal initiates between the bracteole and the first tepal on the abaxial side of the flower. At the stage of outer tepal initiation, the bracteole grows rapidly and bends over the young flower to protect the developing parts. The sequence of initiation of the inner tepals is also spiral and the reverse of the outer tepals (Fig. 37). The stamen whorls are initiated rapidly and stamen primordia within the same whorl appear almost simultaneously (Fig. 38). The young bracteole and the outer tepals have multicellular hairs at their tips, which hold these organs together and aid in floral bud protection (Figs 36, 39).

Three free, dome-shaped, carpel primordia arise on the slightly concave receptacle. There is some space left between the carpel primordia. In some cases, a continuous ring uniting the peripheral edges of the carpel primordia is present on the floral apex. It is not clear whether it is a product of congenital fusion of the carpels or the upper edge of the concave receptacle. Soon after initiation, the carpels become horseshoe-shaped (Fig. 40). The horseshoe-shaped parts elongate and fuse post-genitally to form the style and superior part of the ovary (Figs 41–50). Massive placenta with numerous ovules appear relatively early when the carpels are quite small and free (Figs 46, 47). Post-genital closure of the ventral slits occurs slightly later than post-genital fusion between the carpels. Post-genital fusion begins from the base of the gynoecium and proceeds from the centre towards the periphery (Figs 54–58, 61–64). Stigmatic papillae appear around each carpel mouth. The synascidiate zone begins its development late, after the style and ovary, with ovules already completely differentiated (Figs 50–53, 59, 60). It is difficult to detect and illustrate the process of its growth.

At the latest stages of gynoecium formation, incomplete post-genital fusion between the carpels results in the formation of septal nectaries (Figs 65–73). Septal nectary development begins with the formation of the main body (Figs 65–67). The peripheral parts of the synascidiate zone grow intensively to form a ‘bowl’ divided into three parts by oblique septae. At this stage, the plicate parts of the carpels are almost free and their bases are situated inside the ‘bowl’ formed by the concave synascidiate zone. The upper edge of the synascidiate zone forms the outer (peripheral) wall of the basal part of the main body of the septal nectary. The inner part of the septal nectary is closed by post-genital fusion between adjacent carpels, but, at the earliest developmental stages of the septal nectary, a single triradiate cavity may be
present here. With the post-genital fusion between the plicate zones of neighbouring carpels in the upper parts of the gynoecium, the main bodies of the nectaries are formed and opening canals appear (Figs 68–73). The inner wall of the opening canals is formed by the peripheral part of the synascidiate zone, and its outer wall is a result of post-genital fusion between adjacent carpels (Fig. 73). Thus, the radial region between the main body and opening canal of the septal nectary is the congenitally fused upper edge of the synascidiate zone.

**NARTHECIIUM BALANSAE**

**Organography**

*Narthecium balansae* is a perennial herb with a slender, creeping, sympodial rhizome. The foliage leaves on vegetative shoots are mostly in a basal

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**Figures 31–39.** *Metanarthecium luteo-viride*, flower development, young stages. Fig. 31. Initiation of floral subtending bracts. Fig. 32. Floral primordium in axil of its bract (removed). Fig. 33. Bracteole initiation. Figs 34–36. Initiation of outer tepals. Fig. 37. Initiation of inner tepals. Fig. 38. Stamen initiation. Fig. 39. Young floral bud. br, bract; brl, bracteole; fp, floral primordium; ot, outer tepal; it, inner tepal; os, outer stamen; is, inner stamen; numbers indicate sequence of initiation. Scale bars: Figs 31, 32, 80 μm; Figs 33, 34, 40 μm; Figs 35, 36, 38, 60 μm; Figs 37, 39, 100 μm.
rosette, distichous, ensiform, and unifacial (laterally flattened). Reproductive shoots possess both basal, ensiform, distichous leaves and small, bifacial, plicate, spirally arranged scale leaves. Prophylls on lateral shoots are in the adaxial position and well distinguishable from the foliage leaves. They are two-keeled, small, and scaly.

The flowers are arranged in long, simple, bracteate racemes. Each flower has a small linear bracteole in the upper third of the pedicel.

The flowers are actinomorphic, trimerous, bisexual, with six basally connate tepals in two whors, six stamens in two whors, and three (sometimes four) almost united carpels forming a cone-shaped gynoecium. The stamens have short, triangular connective appendages. Each stamen filament is woolly from numerous moniliform hairs.

Each carpel consists of a (syn)ascidiate and a relatively long (sym)plicate zone. In mature gynoecia, the plicate zone is slightly longer than the ascidiate zone.

Figures 40–50. Metanarthecium luteo-viride, gynoecium development. Fig. 40. Carpel initiation. Figs 41–44. Carpel elongation, plicate zone development. Fig. 45. Single carpel dissected to show ventral side. Fig. 46. Longitudinal section of carpel to show developing placenta (arrowhead). Fig. 47. Placenta development (ov, future ovules). Figs 48, 49. Different stages of carpel fusion (arrowhead in Fig. 49, future orifice of septal nectary). Fig. 50. Mature gynoecium (arrowhead, orifice of septal nectary). Scale bars: Fig. 40, 80 μm; Figs 41–46, 48, 49, 100 μm; Fig. 47 (left), 100 μm; Fig. 47 (right), 30 μm; Fig. 50, 300 μm.
Figures 51–58. *Metanarthecium luteo-viride*, gynoecium development, transverse serial sections of young flower with partly fused carpels. Figs 51, 52. Synascidiate zone. Fig. 53. Level of cross zones. Fig. 54. Level at which carpels are congenitally united at the periphery and partially post-genitally united in the centre. Figs 55–58. Plicate zone. Arrowheads, future septal nectaries. Scale bars: Figs 51–53, 100 μm; Figs 54–58, 50 μm.
The carpels are united almost along their entire length (see also Remizowa et al., 2006b). Only the uppermost parts of the carpels are free and form three very short stylodia. The stylodia are hollow and have a stylodial canal inside. In the stylodia, the ventral slit remains unfused, even in the mature gynoecium. Each stylodium possesses a capitate stigma with secretory unicellular papillae (see also Remizowa et al., 2006b).

The symplicate zone forms a long, hollow style, which contains a stylar canal. In its upper part, the stylar canal divides into three parts extending into the stylodial canals, which open at the top of the stigmas. There is no post-genital closure of the stylodial canals (Angiospermy type 1 sensu Endress & Igersheim, 2000).

The ovary is trilocular in the synascidiate region and unilocular in the symplicate region. It is slightly semi-inferior as a result of the union of the floral tube and the base of the gynoecium. Septal nectaries are absent.

Numerous small ovules are inserted on broad placentae in the synascidiate zone and on narrow placentae in the lowermost part of the symplicate zone. The ovules are anatropous and bitegmic, antitropous (sensu Endress, 1994) in the synascidiate region and

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Figures 65–70. *Metanarthecium luteo-viride*, septal nectary development, transverse serial sections of young flowers with partly fused carpels. Figs 65–67. Early stage. Fig. 65. Ovary, level of cross zones. Fig. 66. Lower part of ovary. Fig. 67. Upper part of ovary with partly fused carpels. Figs 68–70. Later stage. Fig. 68. Ovary, synascidiate zone. Fig. 69. Ovary, level of cross-zones. Fig. 70. Detail of septum. White arrowheads, main body of septal nectary; black arrowheads, opening canal of septal nectary. Scale bars: Figs 65–67, 70, 25 μm; Figs 68, 69, 50 μm.
syntropous in the symplicate region. The ovules have a conspicuous long outgrowth on the chalazal side. In mature fruits, long, narrow seed appendages are situated within the stylar canal. The whole symplicate zone may act as a compitum.

In some flowers of *N. balansae*, the carpels are entirely fused right up to the stigma (Remizowa et al., 2006b). In such flowers, there are no stylodia, and the stigma is situated at the gynoecium tip and extends around the open stylar canal. In this case, it is triangular or rounded from the top (Remizowa et al., 2006b).

**Gynoecium development**

The inflorescences are initiated in the previous summer. In autumn, all the organs of a flower are well differentiated. In the summer of anthesis, the peduncle and pedicels elongate, and the flowers reach their final size. The carpels are initiated as three (very rarely four) free, single, hemispherical primordia on a slightly concave receptacle. Soon after initiation, the carpels become horseshoe-shaped (Fig. 74). The horseshoe-shaped parts elongate to give rise to free stylodia. The symplicate zone then appears as a continuous rim below the young stylodia (Figs 75–77). The carpel ring subsequently elongates to form a long, narrow style. There are three longitudinal ribs along the symplicate zone, corresponding to the placentae. These ribs alternate with the free parts and represent congenitally fused carpel margins. The synascidiate zone appears spherical. During the development of the synascidiate zone, the
carpel walls and septae grow simultaneously. The triradiate internal cavity of the gynoecium remains open at the top of the gynoecium during almost all developmental stages. Numerous small ovules appear on the placentae after the formation of all zones of the gynoecium.

In flowers without stylodia, the gynoecium starts its development from a continuous folded ring around the central depression of the floral meristem. Carpel tips are visible on the primordial ring as small, almost inconspicuous bulges at the edges of the ring.

**DISCUSSION**

**FLORAL DIVERSITY AND EVOLUTION IN NARTHECIACEAE**

Similarities between *Metanarthecium* and *Narthecium* include inflorescence and perianth morphology and development. In both genera, the flowers are situated in the axils of well-developed linear bracts and have a bracteole in a transverse position (see also Ambrose, 1980; Tamura, 1998). It has been suggested that the presence of a bracteole is a strong regulatory factor for a spiral tepal initiation (for example, Greller & Matzke, 1970; Remizowa et al., 2006a). We have observed a spiral sequence of tepal initiation in *Metanarthecium*, as predicted previously for members of Nartheciaceae (Remizowa et al., 2006a). In *Metanarthecium*, as well as in *Narthecium* and other genera of the family (Zomlefer, 1997), tepals and stamens form a short floral tube.

Although the gynoecia of *Narthecium* and *Metanarthecium* are superficially similar, there is a conspicuous structural and developmental gap between them. Two zones are present in the gynoecium of *N. balansae* (see also Remizowa et al., 2006b): (1) trilocu-

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**Figures 74–77. Narthecium balansae**, early stages of gynoecium development. Fig. 74. Young carpels. Figs 75–77. Growth of symplicate and plicate zones. Scale bars: Fig. 74, 30 µm; Figs 75, 76, 50 µm; Fig. 77, 100 µm.
lar syncasidiate, and (2) unilocular symplicate. Usually, free stylodia are also present, but this is a variable character in this species. Typically, representatives of the family Nartheciaceae do not have stylodia (Takhtajan, 1997). In *Metanarthecium*, the entire gynoecium is trilocular and mainly plicate (with post-genitally united carpels); only the lowermost sterile part is syncasidiate. It is noteworthy that the lowermost (inferior) part of the ovary is sterile in both genera. In *Metanarthecium*, the syncasidiate zone appears very late in gynoecium development when the placenta and ovules are already differentiated. As all ovules are initiated almost simultaneously in this plant, this may explain why the syncasidiate zone is sterile. In *Narthecium*, the presence of a short, sterile, basal region within the wholly syncasidiate ovary can be explained by a spatial constraint for ovules and seed development. Antitropous ovules and seeds should have enough space for growth towards the gynoecium base. Thus, their funiculus cannot be inserted at the very base of the ovary.

An internal compitum is present in *Narthecium* and absent in our *Metanarthecium* material. Septal nectaries are well differentiated in *Metanarthecium* and absent in *Narthecium*. The presence or absence of nectaries is correlated with androecium structure and the type of gynoecium development and carpel fusion within the genera examined. In *Metanarthecium*, the flowers offer nectar to pollinators, and therefore there is no need to produce something in addition to pollen and nectary. In *Narthecium*, the flowers attract pollinators by pollen and, possibly, by moniliform hairs on stamen filaments. It has been demonstrated that similar moniliform hairs have evolved independently in several unrelated angiosperm lineages with pollen flowers (Endress, 1994). It should be noted that both moniliform hairs and septal nectaries are absent from *Nietneria* and *Lophiola* (Nartheciaceae). Their developmental morphology and anthecology merit future investigations.

With regard to gynoecium development, the presence of septal nectaries in monocots is always associated with post-genital carpel fusion and dominance of the plicate zone in gynoecium structure (Baum, 1948; Hartl & Severin, 1981; van Heel, 1988; Endress, 1995; Kocyan & Endress, 2001; Rudall, 2002; Stauffer, Rutishauser & Endress, 2002; Stauffer & Endress 2003; Remizowa et al., 2006b). *Metanarthecium* is not an exception in this respect. The growth activity in its initially free carpels consists mainly of elongation of the plicate zone, which is followed by post-genital carpel fusion and septal nectary formation. In evolutionary lineages without septal nectaries (particularly in *Narthecium*), the mode of carpel fusion, with very rare exceptions (Remizowa et al., 2007), is congenital (for example, van Heel, 1988; Endress, 1995; Rudall, 2002; Remizowa et al., 2006b). In that case, both symplicate and syncasidiate zones of the gynoecium are usually present, but their relative size and fertility can vary. In *Narthecium*, syncasidiate and symplicate zones are of equal length, the syncasidiate zone is fertile, and the symplicate zone is mainly sterile (see also Remizowa et al., 2006b).

Representatives of the family Nartheciaceae, which is sister to the remainder of Dioscoreales, reveal a high variability in gynoecium structure and development from quite simple to somewhat complex. The ovary position varies between almost superior in *Narthecium* and *Metanarthecium* to more or less inferior in *Aletris* and *Lophiola* (Zomlefer, 1997; Tamura, 1998; Rudall, 2002). The relative size of the gynoecial zones and ovule position are also highly plastic in the Nartheciaceae. A similar pattern has been described in some other early divergent lineages. For example, 'lower' Asparagales are quite variable in gynoecium structure and the distribution of septal nectaries (Kocyan & Endress, 2001; Rudall, 2002).

The presence of septal nectaries is a primitive condition in monocots (for example, Dahlgren, Clifford & Yeo, 1985); however, septal nectaries are a key innovation of monocotyledons and an exclusive monocot feature (Daumann, 1970; Schmid, 1985; van Heel, 1988; Endress, 1995; Smets et al., 2000; Igersheim, Buzgo & Endress, 2001; Rudall, 2002). Septal nectaries were lost several times during monocot evolution (for example, in some Iridaceae and in some Alliaceae: Rudall et al., 2002; Rudall, Manning & Goldblatt, 2003). However, the type of carpel fusion is also important for reconstructing the phylogeny and character evolution. In monocotyledons, post-genital carpel fusion in the plicate zone is associated with septal nectary formation. In all monocot gynoecia without septal nectaries (except *Isidrogalvia*, Tofieldiaceae: Remizowa et al., 2007), the carpels are congenitally united. The absence of septal nectaries in gynoecia with congenital carpel fusion is probably always a derived condition in the monocotyledons, although this view partially contradicts the character optimizations in Doyle & Endress (2000). Another important feature is the presence of a compitum. Compitum formation is a strong factor of reproductive success (Endress, 1982; Armbruster, Debevec & Willson, 2002). In many early divergent monocots, an internal compitum is absent. An internal compitum arises in two different ways: (1) by post-genital carpel fusion of plicate carpel zones without post-genital closure of ventral slits, and (2) by development of a symplicate zone (i.e. by congenital fusion between the plicate zones). Both of these types of compitum
formation occur within Nartheceaeae. Thus, Nartheceaeae are diverse in ovary position, the presence or absence of nectaries, and the presence or absence and morphogenesis of an internal compitum. There is a tendency to a secondary loss of nectaries, congenital carpel fusion, and compitum formation in Nartheceaeae. This tendency is also present in other families of Dioscoreales. Within Dioscoreales, Thismiaceae lack septal nectaries completely and possess entirely congenital carpel fusion (Caddick et al., 2000). In other groups of the order, some genera have septal nectaries and other genera lack them (Caddick et al., 2000).

**INFRASPECIFIC VARIATION IN THE GYNOECIUM STRUCTURE OF METANARTHECIUM**

The floral anatomy of *M. luteo-viride* has been described previously by Utech (1978). He studied mainly vasculature, but details of gynoecium morphology were also observed. The gynoecium was sterile in the epigynous zone in Utech's material, as well as in our plants. However, it is not clear whether the sterile zone in the gynoecium studied by Utech corresponds to the synascidiate zone because vestigial placentae have been indicated there. The upper third of the gynoecium was represented by a hollow style and was therefore unilocular. The stylar canal was not post-genitally closed at the top and stigmatic papillae were situated around the gynoecium mouth (i.e. Angiospermy type 1 sensu Endress & Igersheim, 2000). Special cubical cells were found on the inner surface of the style in the area of the ventral sutures (Utech, 1978). The same cells were also observed on the funiculi (oburator) and over the top of the capitate stigma. The distribution of this cell type corresponds to the pollen tube transmitting tract. Thus, an internal compitum can be present within the unilocular part of the gynoecium.

We found many differences between the gynoecia examined by Utech (1978) and our material. The inferior part of the ovary is undoubtedly synascidiate and sterile, the locules are rounded in cross-section, and no traces of placentae are visible in our material. The style contains three separate stylar canals which open at the gynoecium top in our material. An internal compitum is lacking. Special cubical cells at the internal gynoecial surfaces are absent. Each stigmatic region is located around an individual carpel mouth in our material. The main differences concern the septal nectary structure. In Utech's material, each nectary had two opening areas: peripheral and central. Peripheral orifices were found at the level of the floral tube at the base of the nectary (as in our material), but the special non-secretory opening canal running from the top of the nectary towards its base was not found by Utech (1978). According to Utech (1978), a central ‘intragland connection’ was located at the middle part of the ovary. In our material, we found only peripheral openings via special non-secretory canals. In general, the gynoecium of *Metanarthecium*, as described by Utech, should be functionally similar to that of *Narthecium*. Both genera have a lowermost sterile part of the gynoecium, a trilocular ovary, a hollow style with stylar canal, and a capitate stigma; the main difference is that *Narthecium* completely lacks septal nectaries. Differences must also occur in the details of development.

All Japanese material of *Metanarthecium* is usually assigned to a single species: *M. luteo-viride* (for example, Ohwi, 1965). The species is variable, and var. *nutans* Masam. from Kyushu is recognized. Both our material and that studied by Utech was collected from Honshu, and the morphology of our plants does not fit var. *nutans*. It seems that *M. luteo-viride* is more variable than previously thought. It is especially interesting that such important features as the presence/absence of a compitum and septal nectary morphology are variable in this genus. Several flowers have been sectioned in both studies of *Metanarthecium*. Utech studied mature flowers and illustrated mature ovules; therefore, differences between his and our material cannot be ascribed to different developmental stages.

The differences between our material and that studied by Utech reflect different distributional patterns of post-genitally fused regions in the gynoecium. In many taxa with septal nectaries (including *Metanarthecium*), post-genital fusion commences relatively late (and proceeds quickly), often after the formation of the ovules. Regions designated for post-genital fusion are the narrow strips along the carpel edges (‘sticky margins’). Two different post-genital fusion processes (and developmental programmes) can be recognized in a growing gynoecium, namely post-genital fusion between carpels and post-genital closure of each carpel in the ventral region. In different taxa with syncarpous gynoecia, fusion between carpels and carpel closure occur simultaneously or in a sequence, and often at different levels. In species with non-simultaneous processes of carpel closure and fusion between carpels, even a slight shift in these programmes (change in timing or loss of one programme at a certain level) may result in superficially very different gynoecium architecture. In certain cases, the variation in patterns of post-genital fusion appears to occur at the infraspecific level.

**HOMOPLASTIC SIMILARITY BETWEEN NECTARIES OF ALLIUM AND METANARTHECIUM**

It is noteworthy that, in *Metanarthecium*, which possesses a combination of seemingly primitive character
states, internal septal nectaries have a surprisingly complicated structure. The nectary has a main nectariferous body and an opening non-secretory canal with the orifice at the base of the superior part of the ovary. This nectary type has not been described in other representatives of Dioscoreales. A similar septal nectary structure has been documented in Allium (Asparagales) (for example, Hartl & Severin, 1981; van Heel, 1988). However, the entire gynoecium of Allium is very different from that of Metanarthecium.

The similarity of the septal nectaries in Metanarthecium and Allium represents an interesting example of an obvious evolutionary parallelism. It is possible that this specialized septal nectary type has an adaptive significance as nectar release is more elaborated here [see schematic drawing of nectar deposition in Allium in Hartl & Severin (1981)]. Thus, the similarity between Metanarthecium and Allium could be explained as a similar adaptation in pollination biology that evolved in parallel in different lineages. However, adaptations may not be the same in Metanarthecium and Allium. In contrast to Metanarthecium, Allium has a gynobasic style, a feature that may have a functional correlation with the lateral location of nectary openings (see Rudall et al., 2002). Another (possibly complementary) explanation may involve a hypothesis that epigny was an ancestral character state in Dioscoreales. The vast majority of extant members of the order have epignous flowers. If the almost completely superior ovary position in Metanarthecium is a secondary condition, the unusual nectary structure could be explained by a complicated evolutionary history of the gynoecium in this group. Rudall (2002) discussed the possibility of secondary hypogyny in at least some higher Asparagales, including Allium. A similar evolutionary history of the ovary position might explain the similar nectary morphology in Metanarthecium and Allium. However, putative secondary hypogynous members of Haemodoraceae (Commelinaceae) and Bromeliaceae (Poales) have a different type of septal nectary (Simpson, 1998; Sajo, Rudall & Prychid, 2004). In addition, septal nectary morphology is quite diverse amongst different members of Alliaceae (see Rudall et al., 2002).

Future detailed phylogenetic and developmental studies of Aletris, a large genus of Nartheciaceae with a variable ovary position, should contribute significantly to the understanding of the primitive floral structure in the order Dioscoreales.

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