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ORIGINAL ARTICLE

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# Embryology of Gomortegaceae (Laurales): characteristics and character evolution

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**Abstract** The embryological characteristics of Gomortegaceae, which are poorly understood, were investigated on the basis of *Gomortega nitida*, the only species of the family, to understand better the evolution of this group within Laurales. Comparisons with other Laurales and Magnoliales (a sister group of Laurales) show that *Gomortega* has many embryological features in common with the other lauralean families. Notably, *Gomortega* shares a testa without or with at best only a poorly developed mesotesta as a synapomorphy with all other Laurales. The genus further shares anthers dehisced by valves as a synapomorphy with the other Laurales (except for Calycanthaceae and Monimiaceae), and a non-multiplicative testa and bisporangiate anther as synapomorphies with Atherospermataceae and Siparunaceae (although the non-multiplicative testa occurs as a homoplasy in Monimiaceae, and the bisporangiate anther in Monimiaceae pro parte, Lauraceae pro parte and Hernandiaceae, respectively). *Gomortega* shows simultaneous cytokinesis to form pollen grains, a one-celled ovule archesporium and non-specialized chalaza, all or part of which may be synapomorphies shared with Atherospermataceae. *Gomortega* appears to have no embryological autapomorphies, but further comparison with Atherospermataceae is required.

**Key words** Embryology · *Gomortega* · Gomortegaceae · Laurales

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## Introduction

Gomortegaceae, a monotypic lauralean family, which consists of *Gomortega nitida* Ruiz & Pavon (syn. *G. keule*) endemic to southern Chile (Kubitzki 1993), are poorly understood embryologically. A literature review of the embryological features of Laurales (comprising seven families including Gomortegaceae) has suggested that 16 embryological characters (e.g., mode of cytokinesis in the microspore mother cell, anther dehiscing mode, number of integuments) are variable within the order and likely to be useful in discussions of morphology-based relationships among and within the seven lauralean families (Kimoto and Tobe 2001). As regards Gomortegaceae, the data available are restricted to the following: anthers dehisced by valves; ovule orthotropous to hemianatropous, or almost orthotropous; ovule bitegmic and crassinucellate; both inner and outer integuments non-multiplicative and two to three cell-layers thick throughout development; micropyle formed by both integuments; young seeds pachychalazal; endosperm oily in mature seeds; the embryo straight; seed coat thin-walled (for review see Kimoto and Tobe 2001, p 260). Other than these features, little is known of the embryological characteristics of *Gomortega*.

The purpose of the present paper is to fill the gap in embryological information on *Gomortega*, and to clarify characteristic features of Gomortegaceae to discuss character evolution in Laurales.

## Materials and methods

Flower buds and fruits of *Gomortega nitida* were collected by one of the authors (M.R.) from plants growing at the arboretum, Universidad Austral de Chile, Valdivia, Chile (no vouchers), and fixed with FAA (5 parts stock formalin; 5 parts glacial acetic acid; 90 parts 50% ethanol). Flower buds and fruits in various developmental stages were dehydrated through a *t*-butyl alcohol series, embedded in para-

plast with a melting point of 57–58°C, and sectioned with a rotary microtome using standard methods. Sections cut to a thickness of 5–8 μm were stained with Heidenhain's hematoxylin, safranin O, and fastgreen FCF, and mounted with Entellan (Merck, Germany). To examine fine structure, a few flower buds and fruits were embedded in Technovit 7,100 (Kulzer, Wehrheim, Germany), after dehydration through an ethanol series (Kimoto and Tobe 2003). Serial resin sections cut to a thickness of 4–6 μm were stained with 0.5% toluidine blue for 10 s or with Heidenhain's hematoxylin. To examine vasculature and inserted positions of integuments (a testa and tegmen), a few ovules and young seeds were bleached with sodium hypochlorite (NaOCl) at room temperature for 12–24 h. After being washed several times with water, they were dehydrated through an ethanol series, cleared with Herr's 41/2 clearing fluid, and observed on Raji slides (Herr 1974; Kimoto and Tobe 2003). To count the generative cells in mature pollen, mature pollen grains were stained with 1% acetocarmine. The terminology of seed coat structure followed Corner (1976) and Schmid (1986).

## Results

### Anthers and microspores

Flowers are bisexual, each borne on a pedicel with two bracteoles. Inside of five or six perianth members, there are eight to ten stamens outside and two to four staminodes inside (Fig. 1, *1*, *2*). Anthers of the stamens are bisporangiate (Fig. 1, *2*, *5*). Although it is unclear how it is formed, the anther wall (of the valve) prior to maturity comprises five to six cell layers: an epidermis, an endothecium, two or three middle layers, and a tapetum (Fig. 1, *3*). The tapetum is glandular, and its cells become bi-nucleate (Fig. 1, *4*). During maturation, epidermal cells become flattened and the endothecium develops fibrous thickenings, while the middle layers degenerate (Fig. 1, *4*, *5*). Thus the mature anther wall (i.e., valve) consists of the flattened epidermis and the fibrous endothecium (Fig. 1, *6*, *7*).

Meiosis in the microspore mother cell is accompanied by simultaneous cytokinesis (Fig. 1, *8*). Resultant microspore tetrads are tetrahedral in shape (Fig. 1, *9*). Each anther opens via two valves (Fig. 1, *6*, *7*) as already reported by Kubitzki (1993) and Endress (1994). Pollen grains are two-celled at the time of shedding, although only the nucleus of the generative cell is stained by acetocarmine (Fig. 1, *10*).

### Ovule, nucellus and megagametophyte

Flowers usually have two or three uniovulate carpels, which are united to form an inferior compound ovary (Fig. 2, *11*). After arising from the axial placenta, an ovule primordium gradually curves downwards as it develops (Fig. 2, *12–14*). When it reaches maturity, the ovule becomes hemianatropous with half the raphe free and with a micropyle oriented downward (Fig. 2, *15*, *16*). The ovule has a short and thick funiculus (Fig. 2, *15*, *16*).

The ovule is crassinucellate. The archesporium is nearly always one-celled (Fig. 3, *17*) and very rarely two-celled. The archesporial cell divides into a primary sporogenous cell and a primary parietal cell. The primary parietal cell divides further to form a parietal tissue of two to three cell layers (Fig. 3, *18*). The primary sporogenous cell differentiates into a megaspore mother cell (Fig. 3, *18*). The megaspore mother cell undergoes meiosis (Fig. 3, *19*, *20*), forming a linear tetrad of megaspores (Fig. 3, *21*). The chalazal megaspore is functional, while the others degenerate (Fig. 3, *21*). The functional megaspore develops successively via two (Fig. 3, *22*) and four nuclei (Fig. 3, *23*) into an embryo sac with eight nuclei. A mature embryo sac has an egg cell, two synergids, two polar nuclei, and three antipodal cells (Fig. 3, *24*). Thus the mode of embryo sac development is of the Polygonum type. The mature embryo sac is elliptic to narrowly elliptic in shape and positioned near the apex of the nucellus. The antipodal cells degenerate soon after fertilization. No conspicuous starchy grains are observed in the embryo sac.

During megagametogenesis, a nucellar cap of two to four cell layers is formed by periclinal divisions of epidermal cells of the nucellus (Fig. 3, *21*; Fig. 4, *30*). Nucellar tissue remains around the mature embryo sac (Fig. 3, *25*) and even in young seeds (Fig. 4, *32*, *33*). An obturator was not found.

### Integuments

The ovule is bitegmic (Fig. 2, *14*; Fig. 3, *25*). Both integuments are initiated by periclinal divisions of epidermal cells of the ovule primordium at the archesporial cell stage (Fig. 4, *26*). Both the inner and the outer integuments are thin and not multiplicative (Fig. 4, *27*). However, the inner integument is somewhat thicker on the raphal side than antiraphal side in general (Fig. 4, *25*). Indeed, it initially consists of two to three cell layers, and, except for the apical part, it later develops to four cell layers due to anticlinal cell division on the raphal side (Fig. 3, *25*). The outer integument consists of two to three cell layers throughout the development (Fig. 4, *27*).

The micropyle is formed by the inner integument alone (Fig. 4, *28*), contrary to the description of Endress and Igersheim (1997). No endothelium is formed. The vascular bundle running through the raphe ends in the chalaza, and it is not ramified there (Fig. 4, *29*, *31*).

### Fertilization, endosperm and embryo

Fertilization is porogamous (Fig. 4, *28*). The mode of endosperm formation is of ab initio Cellular type (Fig. 4, *30*, *32*, *33*). Embryogenesis was not observed.

### Seed and seed coat

Fruits are drupes with a thick fruit wall (Fig. 4, *34*). At maturity they have a hard endocarp that comprises stony cells (Fig. 5, *35*). Seeds are not pachychalazal, contrary to

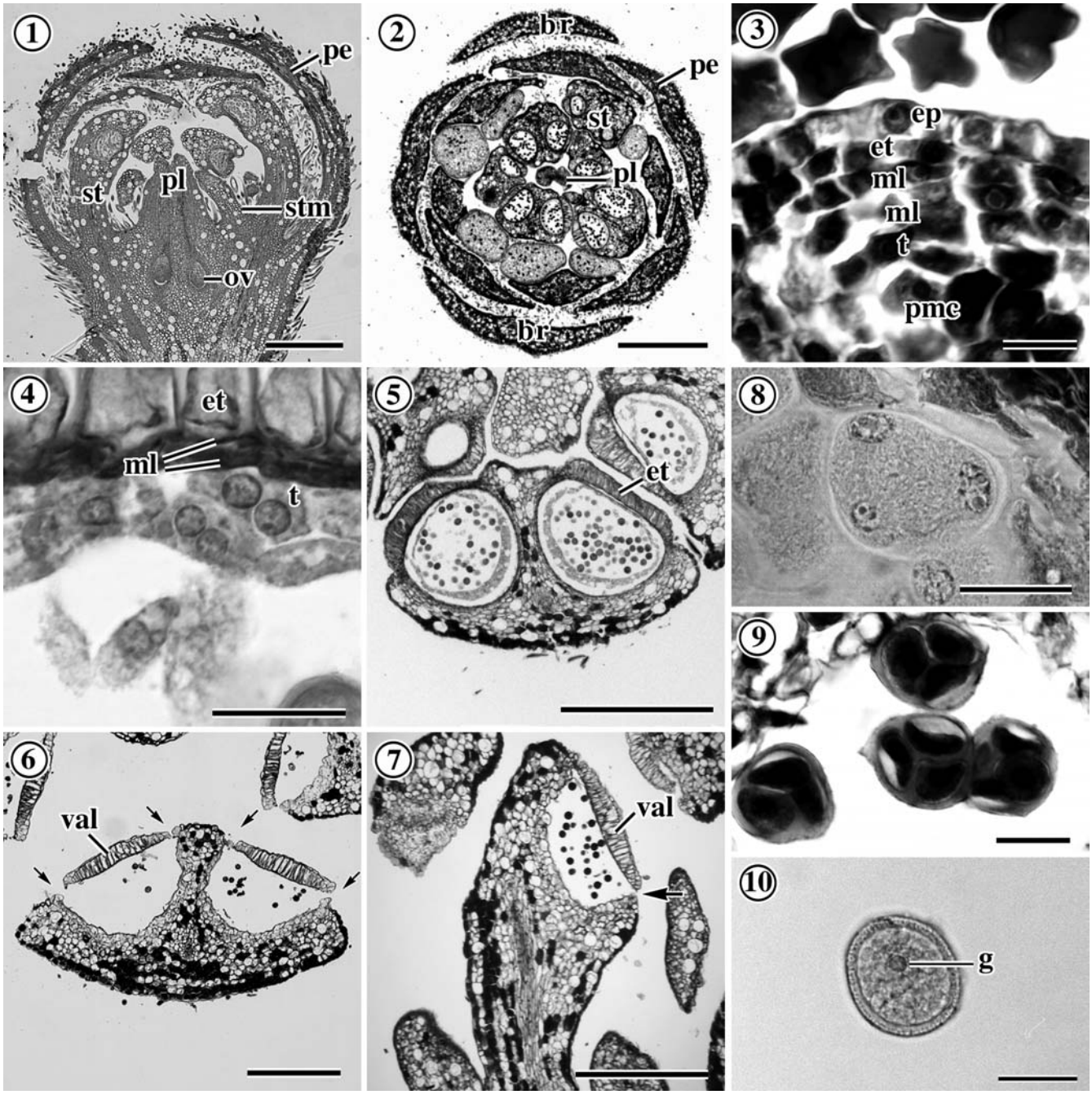



**Fig. 1.** Development of anthers and microspores in *Gomortega nitida*. *1* Longitudinal section (LS) of young flower bud. *2* Transverse section (TS) of older flower bud. *3* TS of young anther wall, showing anther wall formation. *4* TS of young anther wall, showing bi-nucleate tapetal cells. *5* TS of older anther wall. *6* TS of anther dehisced by two valves; *arrows* indicate openings formed by valves. *7* LS of mature anther wall; an *arrow* indicates an opening formed by valves. *8* Microsporogenesis showing that cytokinesis occurs after the second meiotic division. *9* Tetrads of pollen grains. *10* Mature pollen. *br* Bracteole, *ep* epidermis, *et* endothecium, *g* generative cell, *ml* middle layer, *ov* ovule, *pe* perianth member, *pl* pistil, *pmc* pollen mother cell, *st* stamen, *stm* staminode, *t* tapetum, *val* valve. *Scale bars* represent 1 mm in *2*; 500 μm in *1* and *5–7*; 50 μm in *3* and *4*; and 20 μm in *8–10*

the description of Endress and Igersheim (1997). Neither the size nor shape of the chalaza changes throughout development (Fig. 2, *15*; Fig. 4, *33*). The junction between the inner and outer integuments is always positioned lower than the junction between the nucellus and the inner integument (Fig. 4, *31*), thus showing no sign of perichalazy as in Siparunaceae (Kimoto and Tobe 2003). The hypostase is differentiated in the chalazal region below the embryo sac in mature ovules or young seeds (Fig. 4, *31*, *33*).

Usually two or three seeds mature in a fruit. Mature seeds are tangentially bilaterally flattened (Fig. 5, *36*), and are widely obovate when looked at from the adaxial or abaxial side (Fig. 5, *35*). They are 9–12 mm long and 5–7 mm wide, and have no appendages such as arils or wings. The

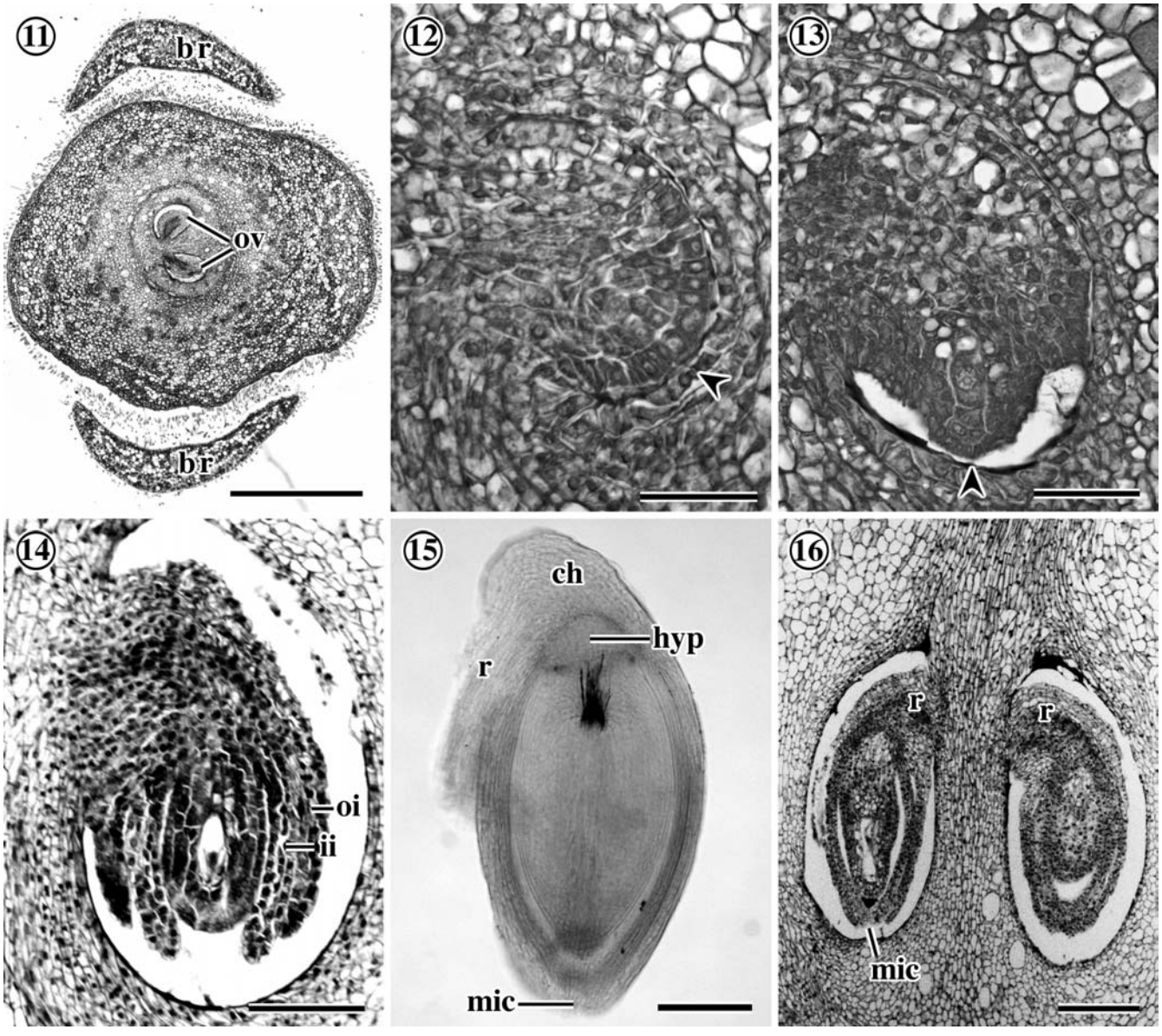



**Fig. 2.** Development of ovules in *Gomortega nitida*. *11* TS of flower bud with a bicarpellate pistil. *12–14* LS of young to old ovules, showing gradual change of the position of a nucellar apex (*arrowheads*). *15* Cleared ovule stained with safranin, showing a raphal vascular bundle terminated at chalaza. *16* LS of ovary. *br* Bracteole, *ch* chalaza, *hyp* hypostase, *ii* inner integument, *mic* micropyle, *oi* outer integument, *ov* ovule, *r* raphe. *Scale bars* represent 1 mm in *11*; 200 μm in *15–16*; 100 μm in *14*; and 50 μm in *12–13*

mature seeds are albuminous, containing a copious endosperm and no persistent nucellar tissue (i.e., perisperm) (Fig. 5, *36*). Cells of the endosperm contain oil droplets (Fig. 5, *37*). An embryo in the mature seed is straight and symmetrical.

The seed coat is thin. As the seed develops, cells of the tegmen (i.e., developed inner integument) are crushed and disappear, while the testa (i.e., developed outer integument) remains at a thickness of two or three cell layers (Fig. 5, *38*, *39*). Cells of the exotesta, as well as those of the mesotesta if present, develop reticulate thickenings and persist, while those of the endotesta collapse and remain as a darkly stained layer (Fig. 5, *39*). The seed coat is thus "exotestal."

## Discussion

### Summary of embryological characteristics of *Gomortega*

As presented in our previous paper (Kimoto and Tobe 2001, p 260, Table 3), much embryological data on *Gomortega* was missing. Not only does the present study provide most of this missing information, but it also revises the data on several important characteristics relevant to the integument and seed. The overall information is summarized below, and asterisks are used to indicate new or revised data.

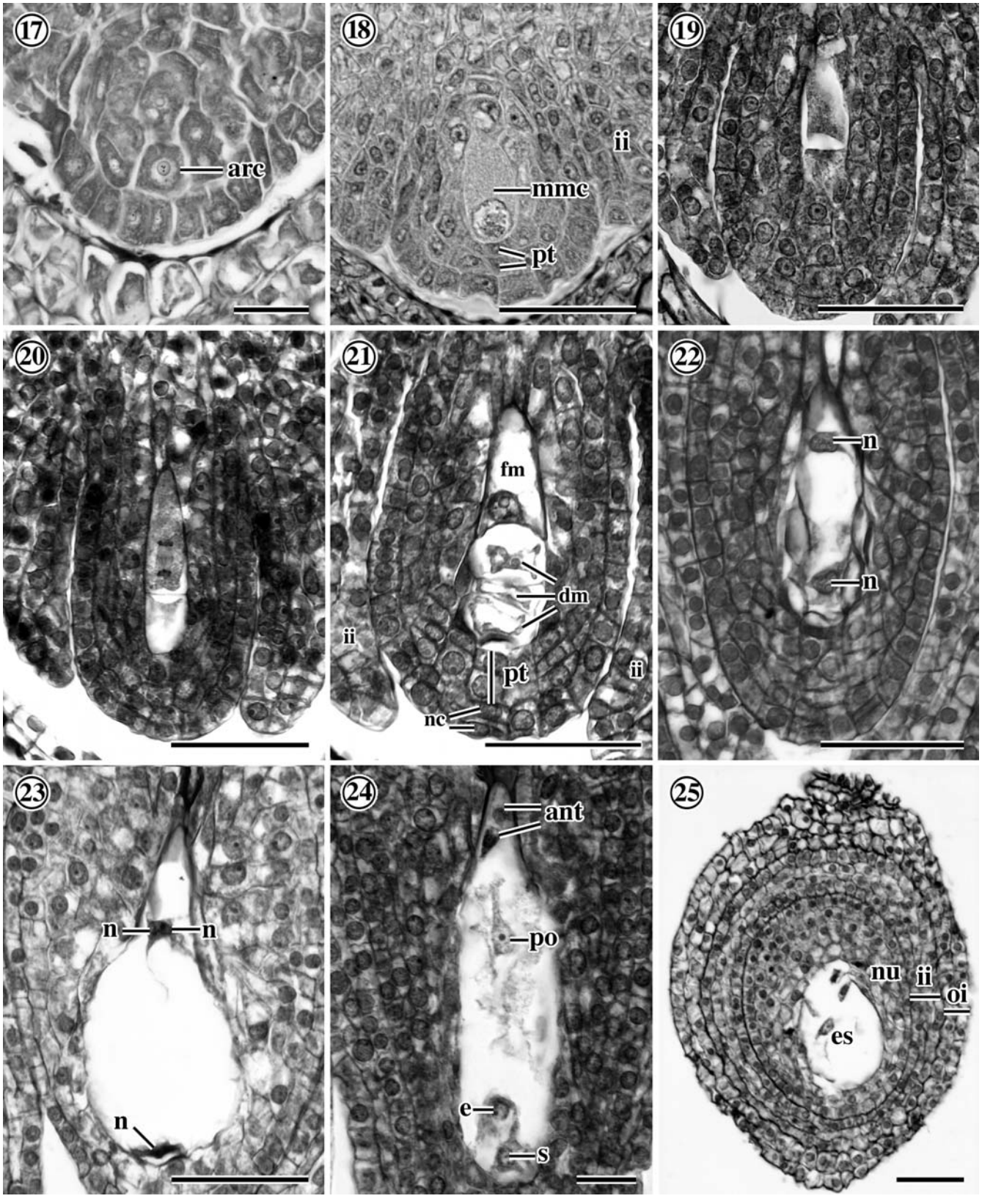
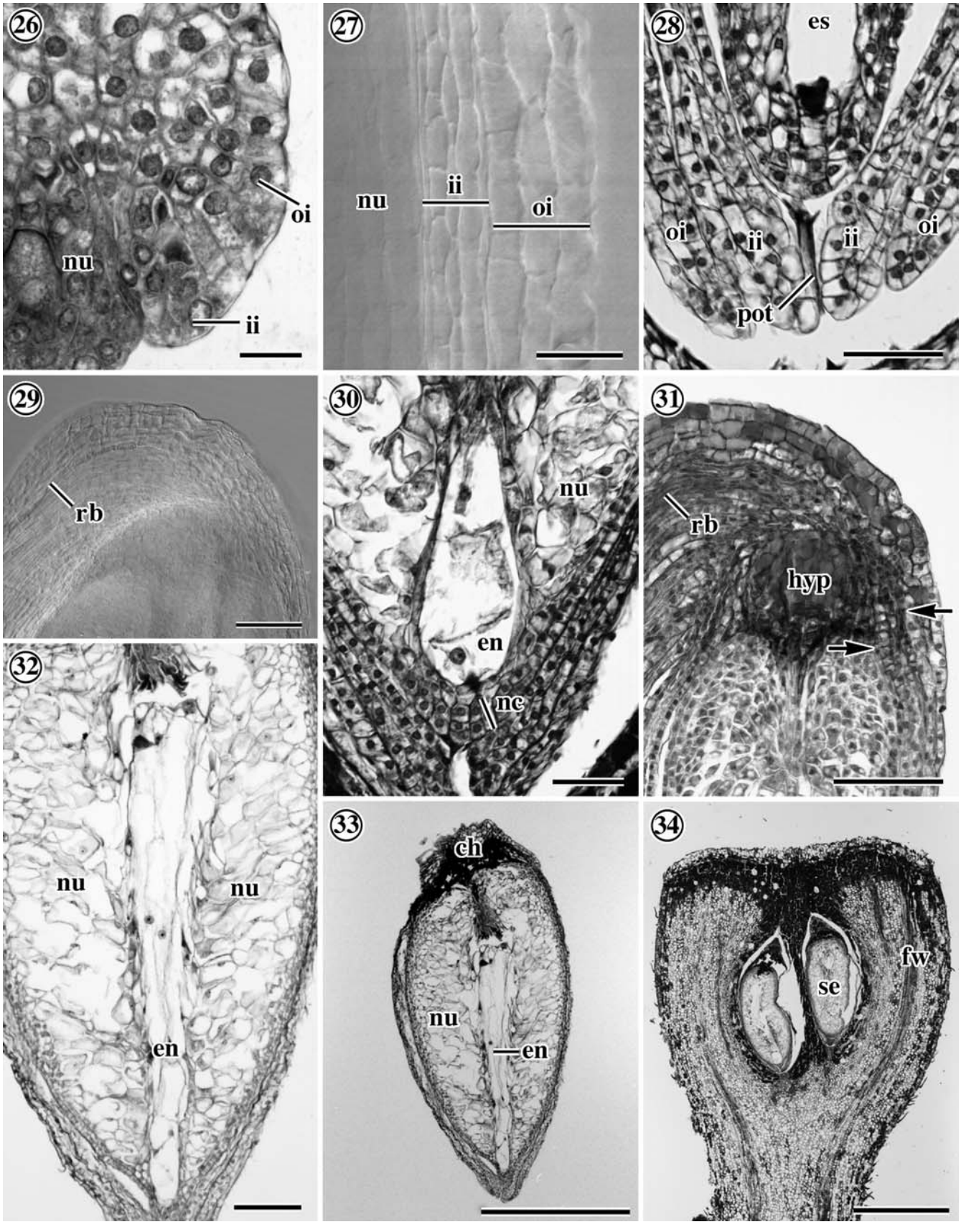



**Fig. 3.** Development of nucellus and megagametophyte in *Gomortega nitida*. *17* LS of young ovule with an archesporial cell. *18* LS of young ovule with a megaspore mother cell with parietal tissue consisting of two cell layers. *19–20* Dyad of megaspores. *21* Tetrad of megaspores; three megaspores on the micropylar side are degenerating and a nucellar cap of two to three cells is formed. *22* LS of immature ovule with a bi-nucleate embryo sac. *23* LS of immature embryo sac with four nuclei; one nucleus appears in the subsequent section. *24* LS of mature ovule with an organized embryo sac. *25* TS of mature ovule. *arc* Archesporial cell, *ant* antipodal cell, *dm* degenerated megaspore, *e* egg cell, *es* embryo sac, *fm* functional megaspore, *ii* inner integument, *mmc* megaspore mother cell, *n* nucleus, *nu* nucellus, *oi* outer integument, *po* secondary polar nucleus, *pt* parietal tissue, *s* synergid. *Scale bars* represent 50 µm in *18–25* and 20 µm in *17*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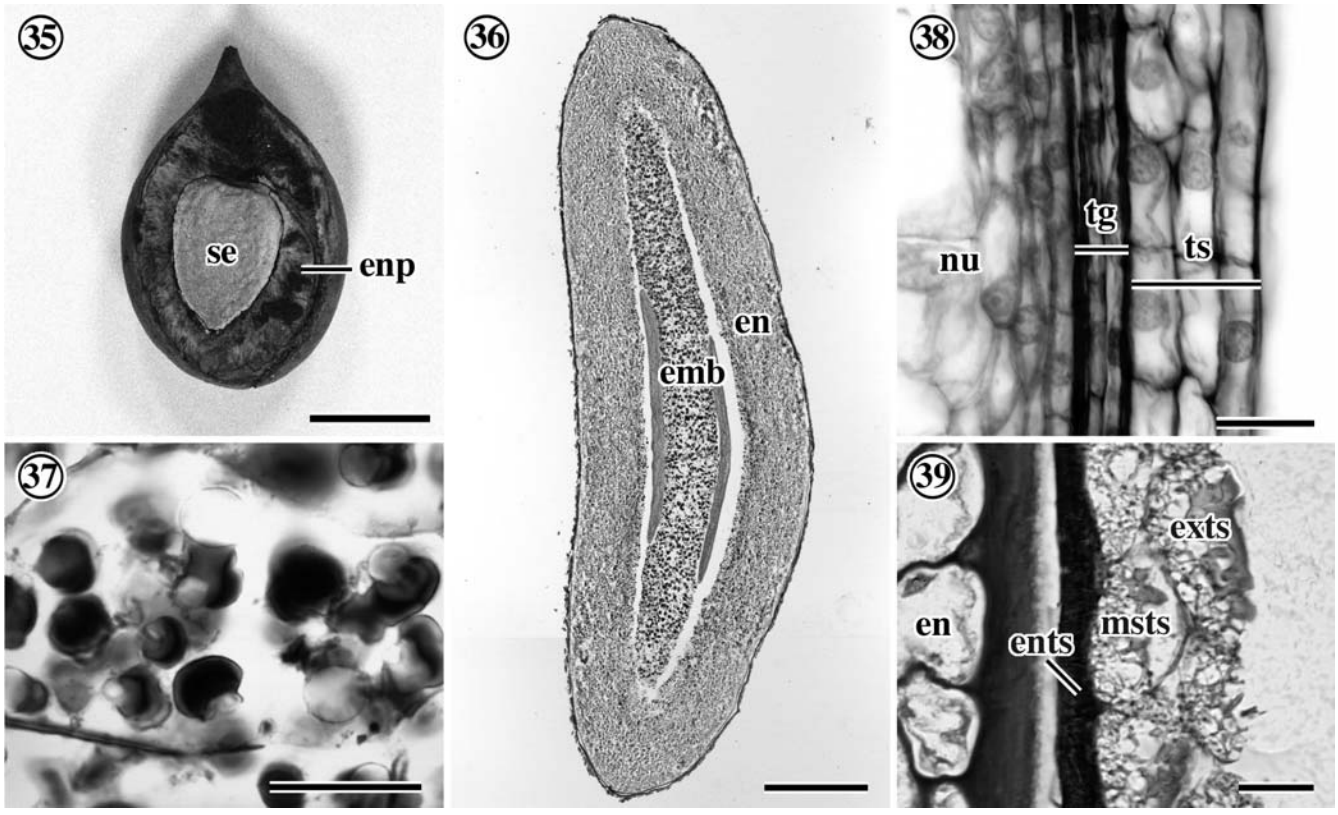


**Fig. 5.** Structure of mature seeds and seed coat development in *Gomortega nitida*. *35* Longitudinally sectioned mature fruit with fleshy part of the fruit wall removed, showing a seed enclosed by a hard endocarp. *36* TS of mature seed. *37* Mature endosperm containing oil droplets. *38* LS of young seed coat. *39* TS of mature seed coat. *enp* Endocarp, *emb* embryo, *en* endosperm, *ents* endotesta, *exts* exotesta, *msts* mesotesta, *nu* nucellus, *tg* tegmen, *ts* testa, *se* seed. *Scale bars* represent 10 mm in *35*; 1 mm in *36*; and 20 µm in *37–39*

– Anther bisporangiate (*); anther wall prior to maturation consists of five to six cell layers (*); anther epidermis flattened and persistent (*); mode of anther wall formation uncertain; endothecium fibrous (*); middle layers crushed (*); tapetum glandular (*), and its cells binucleate (*); cytokinesis in the microspore mother cell simultaneous (*); microspore tetrad tetrahedral in shape (*); mature pollen grains two-celled (*); anther dehisced by valves.
– Ovule hemianatropous (*) and crassinucellate; ovule archesporium one-celled (*); parietal tissue two to four cells thick (*); megaspore tetrad linear (*); embryo sac formation conforming to the *Polygonum* type (*); mature embryo sac nearly always single, and positioned near the apex of the nucellus (*); its shape elliptic to narrowly elliptic (*); antipodal cells ephemeral (*); starchy grains not conspicuous in mature embryo sacs (*); nucellar cap consisting of two to four cell layers formed (*); nucellar beak not formed (*); nucellar tissue remaining in mature ovules; obturator not found (*).
– Ovules bitegmic and not multiplicative; integuments two to three cells thick throughout the development. Integuments histogenetically of dermal origin; no endothelium formed (*); no vascular bundles present in the integuments (*); the micropyle formed by the inner integument alone (*).
– Fertilization porogamous (*); endosperm formation of ab initio Cellular type (*); embryogenesis uncertain.
– In young seeds, hypostase formed (*); the funicular or raphal vascular bundle not ramified at the chalaza (*). Seed coat not multiplicative (*); both testa and tegmen consist of two to three (or four) cell layers in young seeds (*); young seeds not pachychalazal (*).
– Mature seed exarillate (*) and containing abundant, oily endosperm; perisperm absent (*); embryo straight with two straight cotyledons; seed (endosperm) not ruminated (*). Type of seed coat "exotestal" (*); only cells of the exotesta (and, if present, of mesotesta) developing

**Fig. 4.** Development of integuments, endosperm, and seeds in *Gomortega nitida*. *26* LS of young ovule showing the initiation of both integuments. *27* Cleared mature ovule showing the thickness of both integuments on the lateral side of ovule. *28* LS of young seed just after fertilization. *29* Cleared young seed, showing a raphal vascular bundle. *30* LS of young seed, showing the beginning of endosperm formation. *31* LS of young seed just after fertilization, showing structure of the chalazal region; *arrows* indicate the junction between the two integuments and between the nucellus and the inner integument. *32* LS of young seed, showing cellular endosperm. *33* LS of young seed. *34* LS of young fruit. *ch* Chalaza, *en* (cellular) endosperm, *es* embryo sac, *fw* fruit wall, *hyp* hypostase, *ii* inner integument, *nu* nucellus, *oi* outer integument, *pot* pollen tube, *rb* raphal bundle, *se* seed. *Scale bars* represent 1 mm in *34*; 500 µm in *33*; 100 µm in *29*, *31* and *32*; 50 µm in *28* and *30*; and 20 µm in *26* and *27*

persistent reticulate thickenings (*); cells of tegmen crushed (*).

## Evolution of embryological characters within Laurales

In establishing the data missing from Table 3 of Kimoto and Tobe (2001), the present study confirms that *Gomortega* has many embryological characteristics in common with the other lauralean families.

Based on a comparison with Magnoliales, a sister group to Laurales, Kimoto and Tobe (2001) suggested a multi-celled ovule archesporium and a crushed mesotesta as synapomorphies of all Laurales. *Gomortega* has a one-celled, rather than multi-celled, ovule archesporium. In other Laurales, a one-celled archesporium is reported from *Laurelia* of Atherospermataceae (Sampson 1969a). Within Atherospermataceae, however, a multi-celled archesporium is also reported from genera such as *Daphnandra* and *Doryphora* (Foreman 1984). Therefore, it is unclear yet whether the one-celled archesporium is a homoplasy or a synapomorphy, and one must await a comprehensive study of Atherospermataceae. As regards the mesotesta, families of Magnoliales such as Degeneriaceae, Himantandraceae, Myristricaceae, and Magnoliaceae all have a thick, persistent mesotesta that may be oily as in Degeneriaceae and Magnoliaceae. *Gomortega* has no mesotesta or a mesotesta of only one cell layer in mature seeds. Therefore, *Gomortega* is consistent with other Laurales in having no mesotesta or, at most, a poorly developed mesotesta that is unlikely to be comparable to the thick mesotesta in Magnoliales.

Within Laurales, *Gomortega* shares anthers dehisced by valves as a synapomorphy with the other lauralean families except for Calycanthaceae and Monimiaceae. In addition, the family shares the non-multiplicative testa and the bisporangiate anther as synapomorphies with Atherospermataceae and Siparunaceae. As described in this paper, in *Gomortega* the mature seed coat is composed of a testa of two or three cell layers, and the cells of the exotesta (and, if present, the mesotesta) develop reticulate thickenings. However, the non-multiplicative testa is known as a homoplasy from Monimiaceae; likewise, the bisporangiate anther also occurs as a homoplasy in Monimiaceae pro parte, Lauraceae pro parte and Hernandiaceae, respectively (Endress 1994; for character evolution see Fig. 1 of Kimoto and Tobe 2001).

Molecular phylogenetic analyses show that *Gomortega* is sister to Atherospermataceae, and that a clade of *Gomortega* and Atherospermataceae is sister to Siparunaceae (Renner 1999). Based on data available for Atherospermataceae, which are still very poorly studied embryologically (Kimoto and Tobe 2001), no obvious embryological synapomorphies exist between *Gomortega* and Atherospermataceae. One characteristic that *Gomortega* may share with Atherospermataceae is the mode of cytokinesis in meiosis of pollen mother cells. Unlike Siparunaceae (and other Laurales) (with successive cytokinesis), *Gomortega* shows simultaneous cytokinesis, and Atherospermataceae "modified simultaneous" cytokinesis (for a review of characteristics in families other than *Gomortega* see Kimoto and Tobe 2001). According to Sampson (1969b), cytokinesis is "not strictly simultaneous" in *Laurelia novae-zelandiae* (Atherospermataceae), where the heterotypic cell plate is formed shortly before the homeotypic cell plate. We will have to see from ongoing studies of Atherospermataceae how critically the "modified simultaneous" cytokinesis (as in *Laurelia*) is different from the true simultaneous cytokinesis (as in *Gomortega*) and whether it is prevalent within Atherospermataceae or not.

Notably the non-specialized chalaza of *Gomortega* may be a synapomorphy shared with Atherospermataceae or an autapomorphy because other Laurales (including Siparunaceae) have perichalazal or pachychalazal seeds (though still uncertain in Calycanthaceae). If the non-specialized chalaza is a synapomorphy, then *Gomortega* may have no autapomorphies except for the aforementioned one-celled ovule archesporium. This must be confirmed by comparison with Atherospermataceae when the family is investigated.

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