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Embryological studies of *Magonia pubescens* (Dodonaeaeae, Sapindaceae): development of male and female gametophytes in both floral morphs and its phylogenetic implications

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Abstract. *Magonia pubescens* A.St.-Hil. (Dodonaeaeae, Sapindaceae) is a monoecious species exhibiting two floral morphs, namely staminate flowers, with gynoecium reduced to a pistillode, and morphologically hermaphrodite but functionally pistillate flowers. It presents the basic type of antheral wall development. Microsporogenesis is normal, forming tetrahedral and decussate tetrads. Anatomical differences in anthers between floral morphs become visible at the stage of callose wall degradation and release of tetrads. In staminate flowers, the endothecium develops fibrous thickening, and the two middle layers, the tapetum and the parenchymal septum that separates both locule, are degraded. At dehiscence, permanent calymmate tetrads are released. *Magonia* is the only genus of the family with this type of pollen unit. In pistillate flowers, the endothecium exhibits fibrous thickening only in three to five cells on the dorsal loculus, and only the inner middle layer collapses. The septum that separates both locules remains unaltered, the stomium is non-functional, mature anthers are indehiscent and show collapsed tetrads. In staminate flowers, the gynoecium is reduced to a tricarpellar pistillode, trilocular, with ovules that degenerate after megasporogenesis. In pistillate flowers, the gynoecium has a tricarpellary ovary, with six to eight ovules per carpel; they are campylotropous, bitegmic, mixed crassinucellate, and exhibit a well-developed obturator. The phylogenetic implications of these embryological characters are discussed in the context of the family.

Additional keywords: calymmate tetrads, developed obturator, embryology, monoecious, ovules campylotropous.

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Introduction

Sapindaceae *s.l.* is a moderately large family of trees, shrubs and lianas that comprises ~141 genera and ~1900 species (Acevedo-Rodríguez *et al*. 2011). It is widely distributed in tropical to subtropical regions, with some genera extending to temperate regions of Eurasia and America. The first complete taxonomic treatment of the family *s.s.* was performed by Radlkofer (1931–1934), who identified the subfamilies Sapindoideae (= Eusapindaceae) and Dodonaeoideae (= Dyssapindaceae). In this classification system, the monotypic genus *Magonia* A.St.-Hil. was included in the second subfamily as belonging to the tribe Harpullieae. A revision of Radlkofer's system of the family on the basis of macromorphology and pollen morphology was provided by Muller and Leenhouts (1976). These authors accepted the original system proposed with slight modifications and stressed that Sapindoideae represents the most derived group.

Using an evolutionary framework, on the basis of molecular phylogenetic analysis, Harrington *et al*. (2005) and Buerki *et al*. (2009) detected that the infrafamilial groupings were paraphyletic, with the exception of the Paullinieae tribe. However, *Magonia* samples have not been included in phylogenetic analyses conducted so far. Recently, on the basis of macromorphological characters, Acevedo-Rodríguez *et al*. (2011) placed this genus in the tribe Dodonaeaeae of the subfamily Dodonaeoideae. The current treatment follows the approach of Acevedo-Rodríguez *et al*. (2011) for the taxonomic classification.

Magonia pubescens A.St.-Hil. is a monoecious species occurring in the 'cerrado', that presents economics importance because of its tannins with potential larvicidal activity against *Aedes aegypti* (Diptera, Culicidae; Silva *et al*. 2004). It is distributed in eastern Bolivia, northern Paraguay and northern,

central-western and eastern Brazil (Joly *et al*. 1980; Ferrucci 1991). Flowering occurs from June to September and the fruits are ripe from May to August. This species is usually recognised by its conspicuous and colorful flowers with an extra-staminal annular unequal nectary, and the fruit, a woody capsule that is perhaps the most reliable character to identify the genus (Ferrucci 1991). Moreover, the tetrads as pollen units and the number of ovules per carpel (6–8) observed in this species are found to be remarkable and unique to the family.

Embryological information contributes to a more complete circumscription of the genera and is helpful to complement molecular phylogenetic data, so as to resolve infrafamily relationships. Embryological studies are currently limited to a small number of species in Sapindaceae. Banerji and Chaudhuri (1944) analysed the life cycle of *Litchi chinensis* Sonn. Appanah (1982) provided interesting data on pollination and detection of androdioecy in *Xerospermum intermedium* Radlk., a tree with a late self-compatibility. Ha *et al*. (1988) studied the reproductive patterns of four species of the Malaysia rain forest, namely of *Pometia pinnata* J.R.Forst. and G.Forst., *Allophylus cobbe* Bl. (monoecious species), *Xerospermum intermedium* (dioecious species or androdioecious), and *Nephelium lappaceum* L. (androdioecious species), focusing on the floral biology, fruit, seed and embryology. More recently, Cao *et al*. (2008) discussed the systematic position of *Handeliodendron bodinieri* (Lévl.) Rehder at the family level on the basis of embryological characters. Solís *et al*. (2010) analysed microsporogenesis and microgametogenesis in *Cardiospermum grandiflorum* Sw. and *Urvillea chacoensis* Hunz. (Paullinieae), and concluded that in pistillate flowers, male sterility would be associated with the persistence of tapetal cells. Vary *et al*. (2011) related flowers of *Tina striata* Radlk. to cross-pollination. Zhou and Liu (2012) studied the embryogenesis of *Xanthoceras* Bunge and its phylogenetic implications. Zini *et al*. (2012) analysed differences in the development of anther-walls and pollen grains in pistillate and staminate flowers of *Melicoccus lepidopetalus* Radlk. González *et al*. (2014) provided knowledge about the reproductive anatomy of *Allophylus edulis* (A.St.-Hil.) Niederl. in both floral morphs.

With the aim to comprehend the embryology of the monotypic genus *Magonia* and to contribute to the understanding of the systematic position of this genus within the family, we (1) analysed aspects of reproductive anatomy, such as anther sporogenesis, gametogenesis and pollen unit in both floral morphs, and (2) compared these results with the embryological information previously known about the family. The results of the present study will contribute to the morphological and functional characterisation of both floral types of *M. pubescens*, complementing the available embryological information about Sapindaceae.

Materials and methods

Buds of staminate and pistillate flowers at different development stages were fixed in formalin–acetic acid–alcohol (FAA) for anatomical and scanning electron microscopy (SEM) examination. The voucher specimens were deposited in the herbarium of the Botanical Institute of the North-east (CTES), Argentina.

Examined material

Magonia pubescens. BOLIVIA. Departament Santa Cruz, 18.VII.2013, *Ferrucci et al. 3124* (CTES); Idem, 20.VII.2013, *Ferrucci et al. 3134* (CTES).

Light microscopy

To prepare permanent slides, the fixed material was dehydrated in an ethanol series with a pre-impregnant rinsing of tertiary butyl alcohol (Gonzalez and Cristóbal 1997). Infiltration in paraffin was performed using the technique of Johansen (1940). The material was later embedded in Histoplast (Biopack, Buenos Aires, Argentina). Serial longitudinal and transverse sections (10–12 μm in thicknesses) were cut with a rotary microtome and stained with astra blue–safranin (Luque *et al*. 1996), before mounting with synthetic Canada Balsam (Biopur, Buenos Aires, Argentina).

Morphological and anatomical analyses were performed under a Leica MZ6 stereomicroscope and a Leica DM LB2 compound microscope (Leica, Wetzlar, Germany) respectively, both equipped with a digital camera.

Pollen study

Pollen samples from staminate and pistillate flowers were obtained from herbarium specimens of the Botanical Institute of the North-east, Corrientes (CTES). The material was acetolysed according to the technique of Erdtman (1960), mounted in glycerinated gelatin and deposited in the Palinoteca of the UNNE (PAL–CTES). The terminology used was basically that of Erdtman (1966); Punt *et al*. (2007) was also consulted. The polar axis (P) and equatorial axis (E) were measured in the equatorial view of 20 grains per sample.

Scanning electron microscopy (SEM)

Fixed material was dehydrated through an ethanol series of increasing ethanol concentrations. The material was then critical point-dried with solvent-substituted liquid carbon dioxide and coated with a thin layer of gold palladium. SEM micrographs were obtained with a JEOL 5800 LV scanning electron microscope (JEOL USA, Peabody, MA, USA) operating at 20 kV.

Results

Floral morphology

Flowers are greenish-purple, obliquely monosymmetric, zygomorphic, functionally unisexual and comparatively large, being 24–33 mm in diameter (Fig. 1A, B). Sepals five, moderately thick, welded at the base, narrow oblong lobes rounded at apex, abundant curly hairs on the abaxial face (Fig. 2A), significantly reduced in number on the adaxial face (Fig. 2B). Petals five, narrow oblanceolate or oblong, revolute margin in basal half; greenish, with a dense indument of curly and brief hairs on abaxial face (Fig. 2C), and purple and slightly pubescent on the adaxial face (Fig. 2D).

The annular nectary is unequal; anterior view simple, deeply folded, posterior one bicupular, laminar, with the outer edge higher than the inner one (Fig. 2E–G).

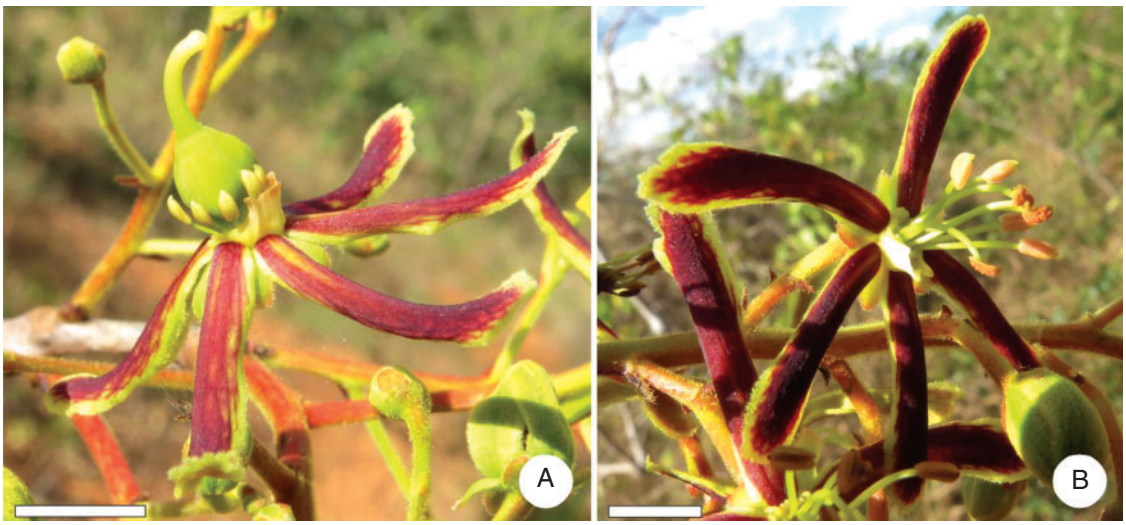

Fig. 1. Flower morphology of *Magonia pubescens*. A. Pistillate flower; B. Staminate flower. Scale bars: 0.5 cm. Photo credit: Juan Domingo Urdampilleta.

The androecium consists of eight exerted stamens, filament glabrous and well developed in staminate flowers (6–10 mm long; Fig. 2E) and much shorter in pistillate flowers (2–3.6 mm long; Fig. 2F, G). The anthers are oblong, glabrous, with longitudinal dehiscence in staminate flowers (Fig. 2H), and indehiscent in pistillate ones, characterised by a small apical connective-tissue expansion (Fig. 2I). The insertion of the anther filament is basifixed in both flower types (Fig. 2H, I).

The gynoecium is reduced to a short, tricarpelar and trilocular pistillode in the staminate flower; the ovary is pubescent, the style is short and the stigma is trifid, with small branches (Fig. 2J). The pistillate flower presents a tricarpelar and trilocular gynoecium; ovary ovoid and pubescent (Fig. 2F, G), with six to eight ovules per carpel; style filiform, curved and glabrous; and papillose stigma with three welded lobes (Fig. 2K).

Anther ontogeny

During ontogenetic development, both floral morphs exhibit anther primordium formed by a group of undifferentiated meristematic cells, bounded by a monostratified protodermis. As antheral development proceeds, the subepidermal layers of cells undergo mitotic divisions that result in the formation of four microsporangium lobes separated by connective tissue. In each microsporangium, homogeneous archesporial cells are differentiated below the epidermis; they are isodiametric, with dense cytoplasm and conspicuous nucleus. These cells divide periclinally to form two groups of cells: on the outer side are primary parietal cells, and on the inner side is a tissue containing sporogenous cells. The primary parietal cells, through periclinal and anticlinal divisions, give rise to external and internal secondary parietal layers, which again divide periclinally to form the endothecium, two middle layers and the tapetum delimiting the anther locule. The latter sporogenous group of cells, belonging to the primary sporogenous tissue, undergoes another periclinal division, leading to secondary sporogenous cells, which are then differentiated into microspore mother cells (Fig. 3A). As in most angiosperms, young anthers are bithecal and tetrasporangiate. The differentiated anther wall exhibits as follows: the monostratified epidermis, which presents rectangular to subrectangular cells, with dense cytoplasm, conspicuous nuclei, and thin walls, with a thick cuticle; the endothecium with tangentially narrow rectangular cells, with a large central nucleus; two middle layers of tangentially elongated cells; and the secretory tapetum, uniseriate with large cells, uninucleate or binucleate with large nuclei (Fig. 3B, C).

Development of microsporangia, microgametogenesis and microsporogenesis

Common stages to staminate and pistillate morphs

During microsporogenesis, sporogenous primary cells divide by mitosis, forming microspore mother cells (mmc), which are surrounded by a callose wall in Prophase I. The division of mmc is simultaneous, originating tetrahedral and decussate tetrads at the end of meiosis (Fig. 3D). Subsequently, callose degrades and young tetrads are released into the locule. The callosic wall around the pollen mother cell disappears, but tetrads are permanent because they do not have callose between the microspores (Fig. 3D). At this stage, the tapetum still remains attached to the antheral wall, although its cells have begun to downsize.

Staminate flowers

Once callose surrounding each tetrad dissolves, tetrads increase in size. On completion of the formation of pollen grains, they remain in permanent tetrads until they are released, at bicellular state (Fig. 3F, G).

Development of the anther wall continues. The epidermis presents globose cells that are gradually reduced; endothecium cells increase in size and the fibrous lignin thickening in the radial walls of a few cells located at the outermost part of the dorsal locules begins to differentiate. There are some binucleate endothecium cells. The two middle layers remain somewhat compressed and tapetum cells, which are significantly reduced

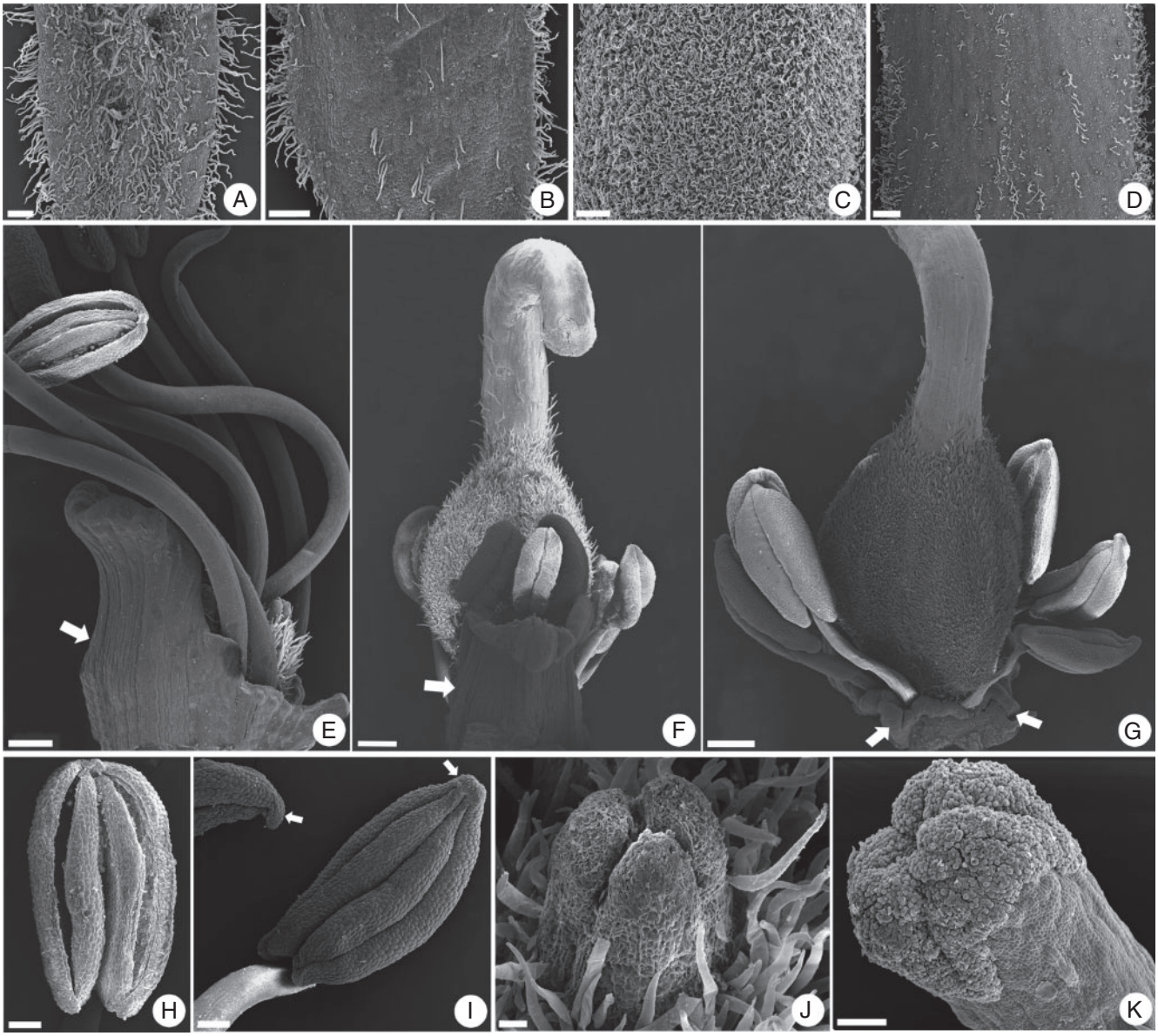

Fig. 2. Scanning electron micrographs of floral morphology of *Magonia pubescens*. A. Sepal, abaxial surface. B. Sepal, adaxial face. C. Petal, abaxial surface. D. Petal, adaxial face. E. Staminate flower; note posterior region of nectary (arrow). F. Pistillate flower; arrow indicates nectary posterior view. G. Pistillate flower; arrow indicates the anterior view of nectary. H. Dehiscent anther of staminate flower. I. Indehiscent anther of pistillate flower; note apical growth of connective tissue (arrows). J. Pistillode; note the trifid stigma. K. Detail of papillose stigma of pistillate flower; note the three connate lobes. Scale bar: 300 µm (A–D), 500 µm (E–G), 40 µm (J), 200 µm (K, H) and 150 µm (I).

in size, begin to disintegrate, showing dense and stained red cytoplasm (Fig. 3E).

When pollen tetrads are mature, and before release, anther walls have epidermal cells presenting compressed cytoplasm, which is thinned at stomium level. Endothecium thickening increases and extends onto the remaining endothecium cells; concomitantly, the two middle layers disintegrate and remnants of tapetal cells remain recognisable in the inner wall of the microsporangium (Fig. 3F).

At anthesis, the parenchymal septum that separates both pollen sacs of each theca also disappears (Fig. 3F). The released tetrads are tetrahedral and decussate (Fig. 4A, B). The mature anther wall shows dehydrated epidermal cells, with contracted cytoplasm, and endothecium with conspicuous fibrous thickening on internal radial and tangential walls in all its extension; the middle layers and the tapetum have disintegrated (Fig. 3G).

Anther dehiscence occurs longitudinally at stomium level (Fig. 3G).

Pistillate flowers

Young microspores remain joined forming tetrads, which are released into the locule. Tetrads gradually collapse, showing

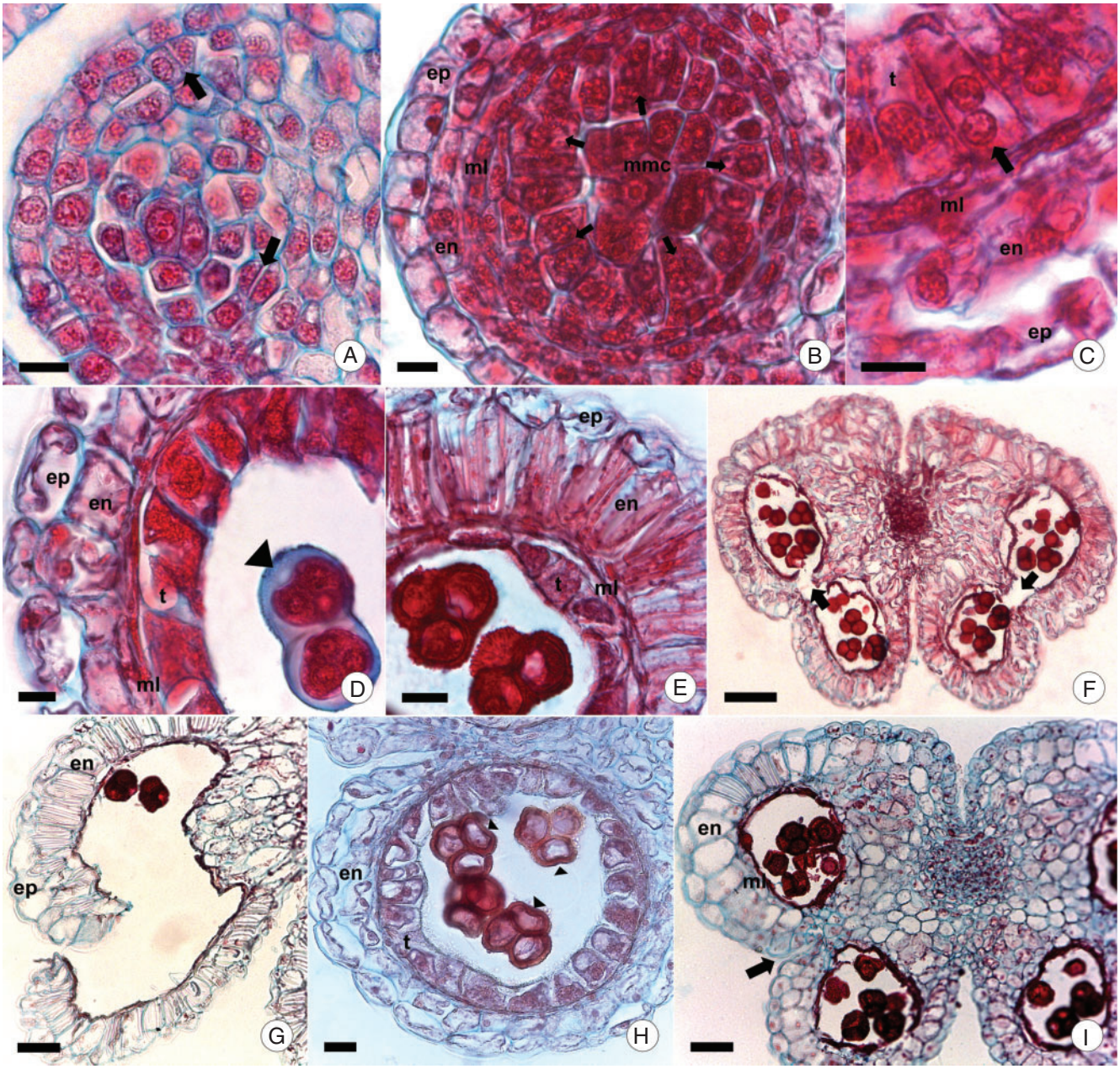

Fig. 3. Light micrographs of anther development in cross-section of staminate and pistillate flower of *Magonia pubescens*. A. Young microsporangium with primary sporogenous cells and differentiation of parietal layers, note dividing cells (arrows). B. Microspores mother cells (mmc) surrounded by a well-differentiated tapetum (arrows), two thin middle layers (ml), endothecium (en) and epidermis (ep). C. Detail of binucleate tapetal cells (arrow). D. Detail of tetrahedral tetrads surrounded by callose (arrow head). Staminate flower. E. Young bicellular tetrads free in the locule; note tapetal cells (t) slightly reduced in size, the middle layers (ml) partially compressed, and the endothecium (en) radially expanded with radial fibrous thickening. F. Anther, showing degradation of the septum that separates both microsporangium (arrows), at stomium level. G. Dehiscent anther releasing the tetrads. Pistillate flower. H. Detail of collapsed tetrads released into the locule; tapetal cells (t) still exhibit the same size. I. Mature anther, showing the endothecium with fibrous thickening in three to five cells on dorsal loculus, a non-functional stomium (arrow), collapsed pollen grains and remnants of tapetal cells and middle layers. Scale bars: 10 µm (A–D), 20 µm (E, H), 100 µm (F, I) and 60 µm (G).

contracted walls and cytoplasm, whereas tapetal cells still exhibit the same size (Fig. 3H).

When tetrads are totally collapsed, antheral wall shows epidermis with small and globose cells; endothecium with radially elongated cells develops fibrous thickening only in radial walls of three to five cells as is observed in cross section, located on the upper side of the dorsal loculus. This process does not extend to other endothecium cells; remnants of inner middle layer are present, whereas the outer middle layer remains unchanged, and tapetal cells disintegrate

(Fig. 3I). The parenchymal septum that separates both locules is preserved; however, epidermal cells become thinner at stomium level, resulting in a non-functional stomium, because these mature anthers are always indehiscent (Fig. 3I).

Pollen morphology

Magonia pubescens presents permanent calymmate tetrads as a dispersal unit. These are tetrahedral and decussate tetrads of ~50–58 µm in diameter (Fig. 4A, B). Pollen grains united in tetrads are equal in size, trizonocolporate, radio-symmetric, with a triangular outline and angulaperturate. The apertures are distributed according to Fisher's law; this is the most frequent type of arrangement, in which apertures are located in pairs forming six points in the tetrad (Fig. 4A).

Pollen grains of pistillate flowers present striate-rugulate exine, with nanogranules homogeneously distributed on the surface of striae and warts (Fig. 4E). In staminate flowers, the exine is striate-rugulate without nanogranules (Fig. 4D). The tectum with nanoperforations is shared in the pollen wall of both floral types.

Indehiscent anthers of pistillate flowers exhibit collapsed pollen units (Fig. 4C).

Megasporangium, megasporogenesis and female gametophyte

Ovule ontogeny

Ovule primordium initiates from the placenta with a small protrusion of meristematic cells that are in constant mitotic division in the central body. At later stages of development, the megaspore mother cell or megasporocyte differentiates in the central body (Fig. 5A).

The integuments originate from periclinal and anticlinal cell divisions from the dermal layer of the ovular primordium, with the inner integument forming first (Fig. 5A) and, then the outer one. The inner integument grows faster and embraces the nucellus, forming the endostome, whereas the outer integument is slightly shorter (Fig. 5A–H).

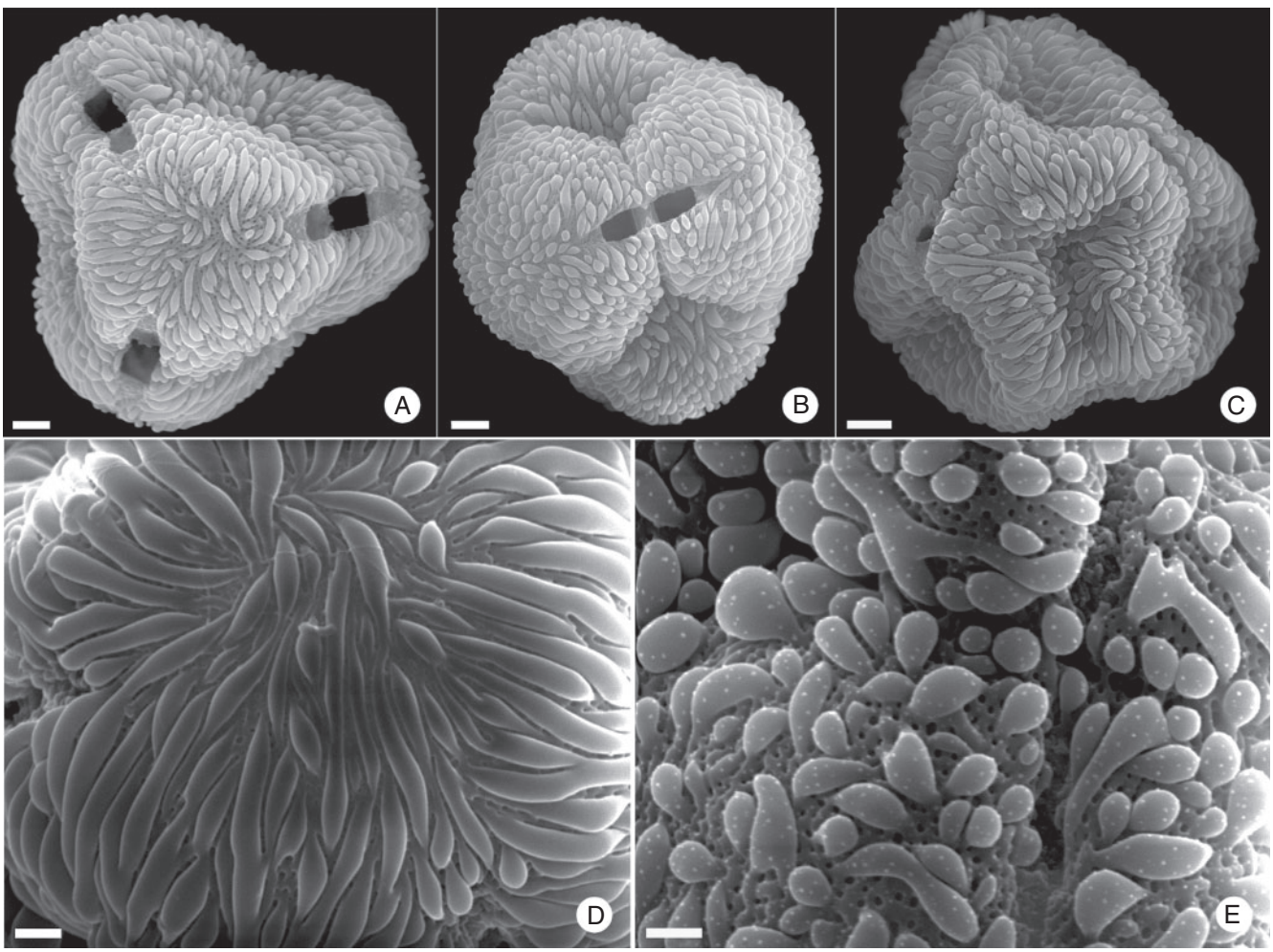

Fig. 4. Scanning electron micrographs of tetrads of *Magonia pubescens*. A, B, D. Staminate flower. A. Tetrahedral permanent calymmate tetrads; the apertures are distributed according to Fisher's law. B. Decussate permanent calymmate tetrads. D. Detail of striate-rugulate exine. C, E. Pistillate flower. C. Collapsed pollen unit. E. Detail of striate-rugulate exine, with nanogranules. Scale bars: 5 µm (A–C) and 2 µm (D, E).

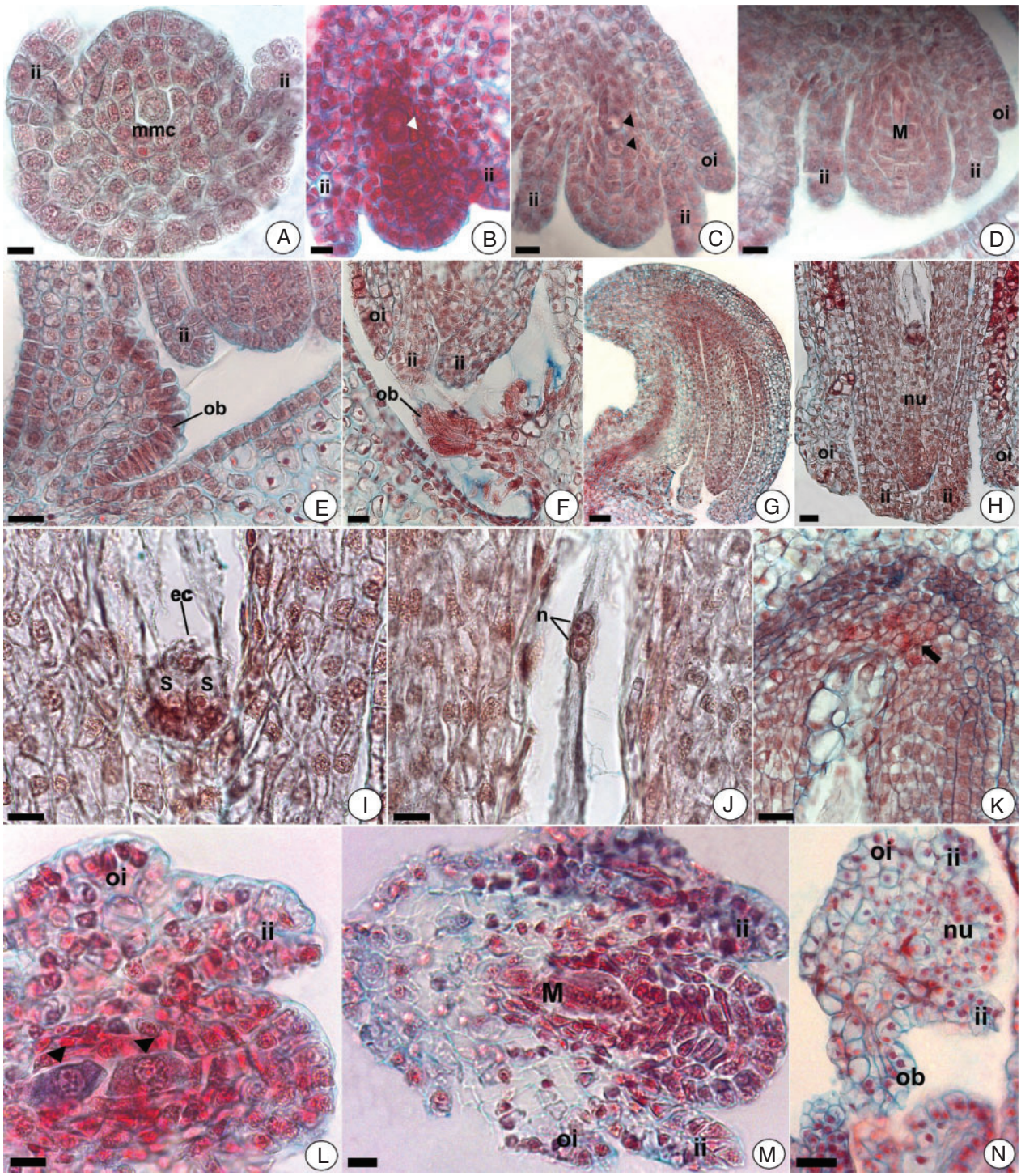

Fig. 5. Light micrographs of ovule development in longitudinal-section in both morphs of *Magonia pubescens*. A–K. Pistillate flower. A. Ovule primordium, showing the megaspore mother (mmc) and inner integuments (ii). B. Young ovule with the megaspore mother cell at the stage of first meiotic division (arrowhead). C. Ovule with two megaspores (arrowhead), inner (ii) and outer (oi) integument. D. Functional megaspore (M). E. Detail of obturator (ob) in young ovule; note trichomatic epidermis. F. Detail of obturator in mature ovule; note unicellular secretory trichomes. G. Campilotropous ovule; note the conspicuous parenchymal excrescence in the chalazal extreme. H. Micropylar detail of mature ovule; note the nucellus (nu), inner (ii) and outer (oi) integument. I–K. Detail of mature female gametophyte. I. Synergids cells (S) and egg cell (ec). J. Central cell with two nuclei (n) close to merge. K. Chalazal portion, showing the hypostase (arrow). L–N. Staminate flower. L. Young ovule, showing a dyad of megaspores (arrowheads). M. Young ovule at megaspore state; note degradation of chalazal region. N. Atrophied ovule, with reduction and degradation of nucellus cells (nu) and both integuments (ii, oi). Scale bars: 10 μm (A, B, I, J, L, M), 15 μm (C, D), 20 μm (E, F, H, K, N) and 50 μm (G).

Pistillate flowers

Ovules are described here as campilotropous, bitegmic and of crassinucellate mixed type, with axilar placentation.

Next to the micropyle, the obturator of funicular origin develops (Fig. 5E); it is formed by unicellular secretory trichomes, elongated radially, with dense cytoplasm and tannic components (Fig. 5F).

The integument begins to be distinguished during the formation of the functional megaspore. The inner integument grows faster than the outer one, embracing the nucellus and delimiting the micropyle. The inner integument is formed by four layers of homogeneous cells with dense cytoplasm and conspicuous nuclei, with the number of cells increasing towards the apical edge. Three or four layers of isodiametric cells (Fig. 5G, H) form the outer integument.

During the formation of the female gametophyte, the ovule elongates. In the mature ovule, there is a conspicuous parenchymal excrescence in the chalazal extreme (Fig. 5G).

At micropylar level, the nucellus shows small irregular cells with conspicuous nuclei and thin walls (Fig. 5H). There is no evidence of epistase, but there is hypostase. The latter is a differentiated tissue in the chalazal area that contacts with the megagametophyte; it presents irregular cells with tannic cytoplasmic component and large nuclei (Fig. 5K).

Megasporogenesis and megagametogenesis development is normal. One hypodermal archesporial cell divides to form several layers of subepidermic parietal cells outside and the megaspore mother cell or megasporocite inside. The megaspore mother cell differentiates at a depth of three or four cell layers of the nucellar tissue. It is characterised by its prominent size and irregular shape, differing from the remaining cells; the cytoplasm is dense with small vacuoles, a conspicuous central nuclei and thin wall (Fig. 5A). Then this cell undergoes one meiotic division, giving rise to a linear tetrad of megaspores; the upper three degenerate soon, with only the chalazal megaspore remaining viable (Fig. 5D). The latter increases in size and undergoes three mitotic divisions, resulting in a female gametophyte of Polygonum type, with eight nuclei being distributed in seven cells, including two synergids and the egg cell (small cells and vacuolated, with conspicuous nuclei), an elongated and binucleate central cell (occupying most of the total volume of the female gametophyte, with large central vacuole, and antipodes (three small and ephemeral cells; Fig. 5I–K).

Staminate flowers

The gynoecium is reduced to a small trilocular pistillode, with six to eight ovules per locule of axillar placentation. Each of these ovules begins its development normally, as in pistillate flowers; however, ontogeny stops and the ovules begin to degenerate before the second meiotic division occurs (Fig. 5L) or, in some cases, reach the megaspore state (Fig. 5M, N), followed by a reduction and degradation of nucellus cells and both integuments (Fig. 5N).

Ovules are innervated by vascular bundles arising from ventral bundles of each carpel. They begin development in an upright position, and then curve until becoming hemitropous (Fig. 5N). Before ovules fail to be functional, they are bitegmic and crassinucellate, with two integuments that fail to form a true micropyle; they exhibit a slightly developed obturator that degenerates.

Discussion

Magonia pubescens has the distinctive and dominant floral structure in the family, including functionally unisexual flowers, with staminate types showing a gynoecium reduced to a pistillode, and morphologically hermaphrodite flowers functioning as pistillate because of their indehiscent anthers (Acevedo-Rodríguez 1993, 2003). In a study conducted in *Handeliodendron bodinieri* (Lévl.) Rehder, Cao *et al*. (2008) defined this pattern of sexual dimorphism as ‘pseudo-bisexual flowers’, apparently bisexual but functionally unisexual. Flowers are bisexual at very early stages of development. This sexual dimorphism is the mechanism that would ensure cross-pollination (Ha *et al*. 1988), and is one of the two main pathways from co-sexuality to dioecism (Barrett 2002).

According to Bawa (1977), in *Cupania guatemalensis* Radlk., the possibility that the presence of anthers in pistillate flowers in most species implies an adaptive advantage cannot be ruled out, increasing the attractiveness of flowers but without providing pollen to pollinators. Thus, the visits that this kind of flowers may receive would be a fortuitous event or a deception. In ‘cryptic dioecious’ species, one or both of the functional unisexual morphs appear to have perfect flowers, making the dioecious condition difficult to detect. Regarding retention of non-functional sexual organs, Mayer and Charlesworth (1991) postulated that there has not been enough evolutionary time for their removal. These floral types probably represent a transitory state in the evolution from unisexuality to dioecy.

Anther ontogeny

According to the classification of Davis (1966), the species here studied presents in both floral morphs, anther-wall development corresponding to the basic type. This type was cited for species from other tribes, such as *Cardiospermum halicacabum* L. (Nair and Joseph 1960); *Pometia pinnata*, *Allophylus cobbe*, *Xerospermum intermedium* and *Nephelium lappaceum* (Ha *et al*. 1988), *Handeliodendron bodinieri* (Cao *et al*. 2008), *Cardiospermum grandiflorum*, *Urvillea chacoensis* (Solís *et al*. 2010), *Houssayanthus incanus* (Radlk.) Ferrucci, *Paullinia elegans* Cambess., *Serjania meridionalis* Cambess. (Solís 2011) and *Allophylus edulis* (González *et al*. 2014). However, this type of anther-wall development differs from what is documented for species such as *Lophostigma plumosum* Radlk., *Thinouia mucronata* Radlk., *Allophylus zeylanicus* L., *A. alnifolius* (Baker) Radlk. and *Lepidopetalum jackianum* Radlk., which exhibit a dicotyledonous type (Mathur and Gulati 1980, 1981, 1989; Solís 2011).

In *M. pubescens*, the secretory tapetum is uniseriate, with uninucleate or binucleate cells. The binucleate tapetum state is shared with other Sapindaceae species, such as *Filicium decipiens* (Wight and Arn.) Thwaites (Gulati and Mathur 1977), *Nephelium lappaceum, Pometia pinnata*, and *Xerospermum intermedium* (Ha *et al*. 1988), *Melicoccus lepidopetalus* (Zini *et al*. 2012), *Xanthoceras sorbifolium* (Zhou and Liu 2012) and *A. edulis* (González *et al*. 2014). However, the tapetum is a variable

character within the family. In species such as *H. incanus, L. plumosum, P. elegans, S. meridionalis, T. mucronata* (Solís 2011), *C. halicacabum* (Nair and Joseph 1960), *C. grandiflorum*, *U. chacoensis* (Solís *et al*. 2010) and *H. bodinieri* (Cao *et al*. 2008), uninucleate tapetal cells have been documented, whereas in *A. zeylanicus*, the tapetum can be trinucleate (Mathur and Gulati 1989), in *A. alnifolius* it varies from uninucleate to multinucleate (Mathur and Gulati 1980), and in *L. jackianum* it is multinucleate (Mathur and Gulati 1981).

In *M. pubescens*, both floral morphs have common anatomical features at early stages of anther development. At more advanced stages, pistillate flowers show anatomical differences from staminate flowers. Similar patterns have been described for *C. grandiflorum*, *U. chacoensis* (Solís *et al*. 2010), *H. incanus*, *L. plumosum*, *P. elegans*, *S. meridionalis* (Solís 2011), *Melicoccus lepidopetalus* (Zini *et al*. 2012) and *A. edulis* (González *et al*. 2014).

In mature anthers of staminate flowers of *M. pubescens*, endothecial cells elongate radially and develop fibrous thickenings in radial walls, a character that has been widely documented in the family (Mathur and Gulati 1980, 1981, 1989; Solís *et al*. 2010; Solís 2011; Zini *et al*. 2012; González *et al*. 2014). In pistillate flowers, only a few cells from the upper side of the dorsal locules of the endothecium increase radially in size, developing fibrous thickenings on radial walls. This feature diverges from that observed in *M. lepidopetalus* (Zini *et al*. 2012) and *A. edulis* (González *et al*. 2014), in which endothecium cells retain their original size, without developing fibrous thickenings in radial walls. However, a few species present the same fibrous thickenings in both floral morphs, such as *C. grandiflorum* and *U. chacoensis* (Solís *et al*. 2010) and *X. sorbifolium* (Zhou and Liu 2012), whereas in *X. sorbifolium* only anthers of staminate flowers are dehiscent.

At the tetrad stage, the middle-layer cells begin to be radially compressed, which is in agreement with findings reported by Mathur and Gulati (1981, 1989) and Solís *et al*. (2010). Subsequently, in staminate flowers, the inner middle-layer cells show signs of degradation; the cytoplasm and cellular wall collapse, followed by the cells of the outer middle layer that persist after the release of the tetrads in locule. In pistillate flowers, remnants of inner middle layer remain, and the outer middle layer remains complete until the end of the process; this phenomenon has still not been documented for other species of the family.

In staminate flowers of *M. pubescens*, tapetal cells disorganise before parenchymal septum degradation, whereas tetrads increase in size and complete wall development. However, in pistillate flowers, the tapetum maintains its structure, whereas tetrads start to collapse; nonetheless, by the end of ontogeny, the tapetum undergoes complete degradation. This character differs from observations reported for pistillate flowers of *C. grandiflorum, U. chacoensis, H. incanus, L. plumosum, P. elegans, S. meridionalis, T. mucronata, M. lepidopetalus* and *A. edulis*, in which the tapetal cells maintain their shape or disintegrate only partially (Solís *et al*. 2010; Solís 2011; Zini *et al*. 2012; González *et al*. 2014).

At anthesis, anthers of staminate flowers present unilocular thecas. Anther dehiscence is longitudinal. In pistillate flowers, anthers are always indehiscent; stomium cells do not undergo degradation. In most Sapindaceae species studied, the parenchymatous septum that separates the sporangia remains intact; nevertheless, in mature pistillate flowers of *T. mucronata* (Paullinieae–Thinouiinae), anthers are unilocular, because of the degradation of the septum (Solís 2011). In all cases, pollen units are not released outside; instead, they gradually collapse and contract the cytoplasm by the end of development (Solís *et al*. 2010; Solís 2011, Zini *et al*. 2012, González *et al*. 2014).

Pollen morphology

In angiosperms, the analysis of pollen morphology has great taxonomic value; palynology has provided a wealth of phylogenetically useful information. In Sapindaceae, Radlkofer based his taxonomic treatment on a wide range of evidence, including pollen morphology (Radlkofer 1895, 1934). Later, Muller and Leenhouts (1976) presented a general survey of pollen types in relation to taxonomy of the family. More recently, Ferrucci and Anzótegui (1993) postulated an evolutionary trend in the Paullinieae tribe on the basis of pollen morphology and other morphological characters, and Acevedo-Rodríguez *et al*. (2017) included pollen characters to define genera in their paper on the new concept of supertribe Paulliniodae.

In Sapindaceae, *Magonia pubescens* is the only species with permanent calymmate tetrads as pollen units. Our results are consistent with those documented by Muller and Leenhouts (1976), who also concluded that the tetrads of *Magonia* show rather inconspicuous apertures and are arranged according to Fisher's rule. Concerning sculpture patterns, Muller and Leenhouts (1976) mentioned a distinctive rugulate sculpture, whereas in a taxonomic revision of the genus, Joly *et al*. (1980) proposed that the distinction between *M. pubescens* and *M. glabrata* is not possible because of a continuous gradient that exists in a series of characters analysed, also including two exine patterns, namely rugulate and striate. In our study, we report a combination of both patterns and the tectum with nano-perforations. In addition, our results showed differences in pollen ornamentation between floral types. In contrast, Joly *et al*. (1980) based their pollen-analysis study on male flowers because previous studies showed that there were no differences between pollen grains of both flower morphs.

Regarding evolution of pollen units, monads represent the basic angiosperm pollen unit. Among the 50 angiosperm families with pollen in tetrads, 27 only rarely have tetrads, including Sapindaceae (Walker and Doyle 1975). In Sapindaceae, the presence of tetrads in *Magonia* could be related to the number of ovules per locule ensuring seed production, assuming the hypothesis that the most taxa of Sapindaceae present one or two ovules per locule, and all these species are linked to monads as pollen units (except *Magonia* and *Xanthoceras* species).

Gynoecium

Megasporangium development in functionally pistillate flowers is normal, resulting in a megagametophyte of Polygonum type, a conserved character in the family (Nair and Joseph 1960; Gulati and Mathur 1977; Mathur and Gulati 1980,

1981, 1989; Ha *et al*. 1988; Cao *et al*. 2008; Solís 2011; Zhou and Liu 2012; González *et al*. 2014). The presence of bitegmic and crassinucellate ovules with a well-developed obturator is a character shared with other species of Sapindaceae (Mathur and Gulati 1980, 1989; Ha *et al*. 1988; Solís 2011; González *et al*. 2014); within Sapindales, obturators also occur in Rutaceae (Weckerle and Rutishauser 2005). It is developed from micropilar side of the funicule, to guide pollen tube towards the micropyle; Shamrov (1998) classified Sapindaceae obturators as funicular. According to Weckerle and Rutishauser (2005), the epidermis of the obturator is secretory, in this case with glandular trichomes. Obturator development varies in the family, i.e. Paullinieae species (Weckerle and Rutishauser 2005); in *M. pubescens*, it is approximately a quarter to a fifth as long as the ovule. The absence of this structure in *Xanthoceras* is notable (Zhou and Liu 2012). The campilotropous ovule type is the most frequent in the family (Nair and Joseph 1960; Mathur and Gulati 1980; Ha *et al*. 1988; Cao *et al*. 2008; Solís 2011, Zhou and Liu 2012); however, anatropous and hemianatropous types were also recorded (Gulati and Mathur 1977; Mathur and Gulati 1981, 1989; Ha *et al*. 1988, Zhou and Liu 2012).

An interesting aspect in *M. pubescens* is the presence of hypostases, because the absence of this character has been cited as a constant for the family (Corner 1976). However, it was recorded in other taxa of the group, i.e. *A. alnifolius*, *A. zeylanicus*, *C. grandiflorum* and *C. halicacabum* (Nair and Joseph 1960; Mathur and Gulati 1980, 1989; Solís 2011, Zhou and Liu 2012). The absence of epistasis is shared only with *X. intermedium* (Ha *et al*. 1988), whereas this character is present in most of the species studied (Nair and Joseph 1960; Mathur and Gulati 1980, 1989; Solís 2011; González *et al*. 2014).

In functionally staminate flowers, drastic changes are evident during ontogeny, resulting in the interruption of ovule development. Although ovule degeneration was widely recorded in the family, the process is interrupted at different stages. The ovules are atrophied before forming tetrads of megaspores or at a functional megaspore state, i.e. in *C. grandiflorum*, *H. incanus*, *S. meridionalis*, *P. elegans* (Solís 2011), before differentiation of the archesporial cell in *A. edulis* (González *et al*. 2014) or, even before the integuments are differentiated in *Xanthoceras* (Zhou and Liu 2012). Cao *et al*. (2008) reported that ovules of *H. bondineri* degenerate at the state of two-cellular embryo sac.

Phylogenetic implications

The genus *Magonia* is recognised by two apomorphies, namely pollen tetrad units and the presence of six to eight ovules per locule. It was placed in the subfamily Dodonaeoideae, tribe Dodonaeaeae, which is rather widespread and comprises 14 genera (Acevedo-Rodríguez *et al*. 2011). However, it is noted that the high number of ovules per locule puts *Magonia* closer to *Xanthoceras* (5–8 ovules), a genus classified in the subfamily Xanthoceroideae (Acevedo-Rodríguez *et al*. 2011). On the basis of molecular sequence data, general morphology and biogeography, Buerki *et al*. (2010) confirmed that *Xanthoceras* is sister to all other members of the Sapindaceae *s.l.*; thus, they recognised the new family Xanthoceraceae. However, the most recent phylogenetic analysis by Buerki *et al*. (2011) keeps Xanthoceroideae as one of the four subfamilies within Sapindaceae *s.l.* Therefore, a molecular phylogeny that includes *Magonia* is necessary to determine whether it is maintained in its current taxonomic position or a new subfamily is determined, as proposed for *Xanthoceras*. Although the results presented here have shown that *Magonia* shares many embryological features with Sapindaceae *s.s.* (actually subfam. Dodonaeoideae + Sapindoideae), such as anther-wall structure, fibrous endothecium, secretory tapetum, simultaneous microsporogenesis, tetrahedral or isobilateral tetrads, two-celled mature pollen, tricarpellate pistil, campilotropous ovule, presence of obturator, bitegmic integument and crassinucellate ovule, these embryological characters suggest that *Magonia* is closely related to other members of Sapindaceae *s.s*.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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