



From pollination to embryo: A closer look into the anatomical reproductive development in *Epidendrum* (Orchidaceae; Epidendroideae)

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ABSTRACT

Analyses of reproductive development in Orchidaceae have significantly contributed to our understanding of ontogenetic diversity and reproductive strategies in the family. In this study, we describe the development of the female gametophyte and embryo in two species from one of the largest orchid genera *Epidendrum*: *Epidendrum fulgens* and *E. puniceoluteum*. To describe megasporogenesis, megagametogenesis and embryo development, we performed manual self-pollinations in first-day flowers of cultivated plants. Flowers and fruits at different stages were collected and the usual methodology for performing anatomical studies and scanning microscopy following established protocols. In both species, placenta formation and ovule development are triggered by pollination. The ovules are anatropous, bitegmic, and tenuinucellate, exhibiting monosporic development. The megaspore mother cell undergoes meiosis, forming a triad of megaspores and a female gametophyte with seven cells and eight nuclei. Fertilization occurs between 30 and 35 days after pollination and is evidenced by the presence of the zygote and the primary endosperm nucleus, which soon degenerates. The embryo is minute and by the time of seed dispersal, it shows protoderm differentiation, smaller cells in the apical region and larger cells in the basal region.

1. Introduction

Orchidaceae Juss., one of the largest families of angiosperms, exhibits a wide diversity of forms and colors and is characterized by structurally complex flowers. Orchid flowers are relatively uniform in terms of whorl number and arrangement, comprising three sepals and three petals, one of which is differentiated into a labellum (Dressler, 1981). The reproductive organs are fused into a single structure, the column or gynostemium, which typically contains one or, more rarely, two or three anthers and a stigmatic region formed by the fusion of the three stigmatic lobes (Dressler, 1981). The column exhibits significant complexity due to interspecific differences in its size, morphology, along with variations in anther arrangement, number, and structure, as well as polliniferous traits and other features. Investigating the evolutionary development of the orchid column is of considerable interest, and substantial morphological and molecular insights into its ontogeny have been documented (Pabón-Mora and González, 2024).

The flowers of this family also exhibit a unique reproductive feature

among angiosperms: at anthesis, the ovary contains underdeveloped ovules that develop only after pollination (Mayer et al., 2021). During the pollen tubes growth, auxin and ethylene are produced, promoting placenta proliferation and ovule development (Zhang and O'Neill, 1993; Novak et al., 2014). As a result, the period between pollination, fertilization, and seed development varies widely among orchids. Therefore, pollination is recognized as a two-step process: first stimulating ovule development and second promoting fertilization (Hildebrand, 1863).

Another remarkable feature of Orchidaceae is the formation of a large number of seeds per fruit. The seeds are minute, exhibit considerable structural diversity, and are characterized by a thin seed coat, highly reduced and undifferentiated embryos, and, in most cases, the absence of endosperm (Arditti, 1992; Barthlott et al., 2014). Kodahl et al. (2015) suggest that the absence of endosperm may be associated with the occupation of disturbed environments with adverse conditions, scenarios in which small seeds exhibit high adaptability. Moreover, significant changes in the nutritional strategies of young plants, particularly their symbiotic associations with mycorrhizal fungi, have

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effectively obviated the need for endosperm formation in Orchidaceae (Benzing, 1981). However, the double fertilization and the formation of a rudimentary endosperm are observed in some species of the family (see Yam et al., 2002; Yeung, 2017).

Given the particularities of the reproductive process in Orchidaceae and the large number of species, knowledge about the stages of reproductive development is still poorly explored. Studies on the development of the female gametophyte and embryo in the family reveal considerable diversity. Variations have been reported in the number of nuclei within the embryo sac (Swamy, 1949a; Law and Yeung, 1989; Mayer et al., 2011; Kodahl et al., 2015; Li et al., 2016; Duarte et al., 2019; Kolomeitseva et al., 2021; 2022) as well as in the number of ovule integuments (Swamy, 1949a; Abe, 1976; Arekal and Karanth, 1981; Krawczyk et al., 2016; Li et al., 2016; Alves et al., 2019; Ryabchenko et al., 2024). Differences in embryo size and suspensor type have also been documented (Swamy, 1949b; Yam et al., 2002; Mayer et al., 2011; Yeung, 2022; Kolomeitseva et al., 2023; 2024). These variations reflect the complexity of the family's reproductive processes and structures and underscore the importance of embryological studies for understanding the evolution and systematics of orchids.

Among the subfamilies of Orchidaceae, Epidendroideae stands out as the largest and most diverse (Chase et al., 2015; Freudenstein, 2025). Despite its morphological diversity, the anatomical characterization of the reproductive stages remains poorly documented for many genera within this subfamily. In *Epidendrum* L. (subtribe Laeliinae), which comprises ~1.500 species with wide geographic distribution and marked morphological variation (Karremans, 2021), embryological information remains available only for a few representatives. To date female gametophyte development has been studied in only nine species: *Epidendrum variegatum* Sw., *E. verrucosum* Sw., *E. globosum* Jacq., *E. cochleatum* L. (Sharp, 1912); *E. prismatocarpum* Rchb. f. (Swamy, 1948); *E. scutella* Lindl. (Cocucci and Jensen, 1969a), *E. ibaguense* H.B.K (Yeung and Law, 1989); *E. denticulatum* Barb. Rodr. and *E. orchidiflorum* Salzm. (Alves et al., 2025). Embryo development, has been described for eight species: *E. variegatum*., *E. verrucosum*, *E. globosum*, *E. cochleatum* (Sharp, 1912); *E. prismatocarpum* (Swamy, 1948); *E. ibaguense* (Yeung, 2022), *E. denticulatum* and *E. orchidiflorum* (Alves et al., 2025). In this study, we investigated post-pollination events in two species endemics to Brazil: *Epidendrum fulgens* Brongn and *E. puniceoluteum* F. Pinheiro & F. Barros. Our aim was to characterize female gametophyte and embryo development contributing to the understanding of embryological patterns in *Epidendrum*. Characterizing these stages provides valuable data for comparative analyses among species and clades, offering potentially informative characters for systematics and a enhancing understanding of evolutionary processes within the family.

2. Material and methods

2.1. Species analyzed and study area

Epidendrum fulgens (Fig. 1a) and *E. puniceoluteum* (Fig. 1b) occur in restinga vegetation, a type of vegetation covering sandy coastal sediments deposited during the Quaternary period, regardless of their physiognomy (Rabelo et al., 2024), in southeastern and southern Brazil. All species were collected and cultivated in the orchid collection from the Plant Sciences Department at University of Campinas, São Paulo State, Brazil. Voucher specimens were deposited at the Herbarium of the Universidade Estadual de Campinas (UEC), Campinas, São Paulo, Brazil, under the following record numbers: UEC 212842, UEC 212846.

2.2. Experimental pollination and optical microscopy

To analyze the female gametophyte and embryo development in the species studied, manual cross pollinations were carried out. Flower buds were bagged to prevent contact with floral visitors. Eleven pollinations were carried out on five individuals for each species. Two flowers per individual were used, and manual pollinations were carried out in February and March of 2022 and 2023. Eleven fruits were collected 10, 15, 20, 25, 30, 35, 40, 45, 50, 60 days after pollination (DAP) as well as at seed dispersal, in order to maximize the likelihood of capturing all developmental stages of the female gametophyte and embryo. The samples were fixed in 10 % neutral buffered formalin (Lillie, 1965), dehydrated in an increasing ethyl series and infiltrated in hydroxyethylmethacrylate (Gerrits and Smid, 1983). The samples were sectioned at a thickness of 3 µm using a Leica RM2245 rotary microtome (Leica Biosystems, Germany), stained with 0.05 % toluidine blue in phosphate buffer (pH 4.5) following Sakai (1973). The slides were analyzed under an Olympus BX-50 optical microscope and photographed with an Olympus DP-73 digital camera.

2.3. Scanning electron microscopy

Botanical material was fixed in 10 % neutral buffered formalin solution (Lillie, 1965), dehydrated in a graded ethanol series and critical point dried under carbon dioxide (CO₂) in a Balzers model CPD 030 Critical Point Dryer. Subsequently, the botanical material was mounted on metal supports and coated with colloidal gold using Emitech K550X Sputter Coater. Observations and electron micrograph recordings were carried out using a ZEISS EVO 40 scanning electron microscope at 15 kV.

3. Results

Both species exhibit similar patterns of ovule and seed development

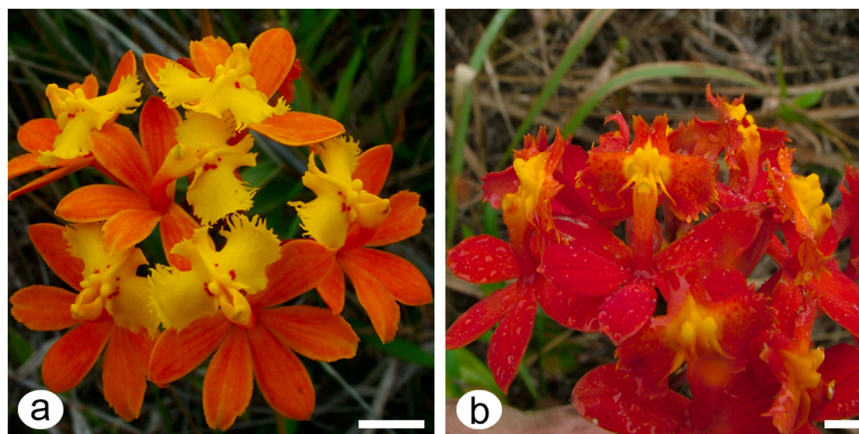


Fig. 1. *Epidendrum* flowers. (a) *Epidendrum fulgens*. (b) *Epidendrum puniceoluteum*. Scale bars= 1 cm.

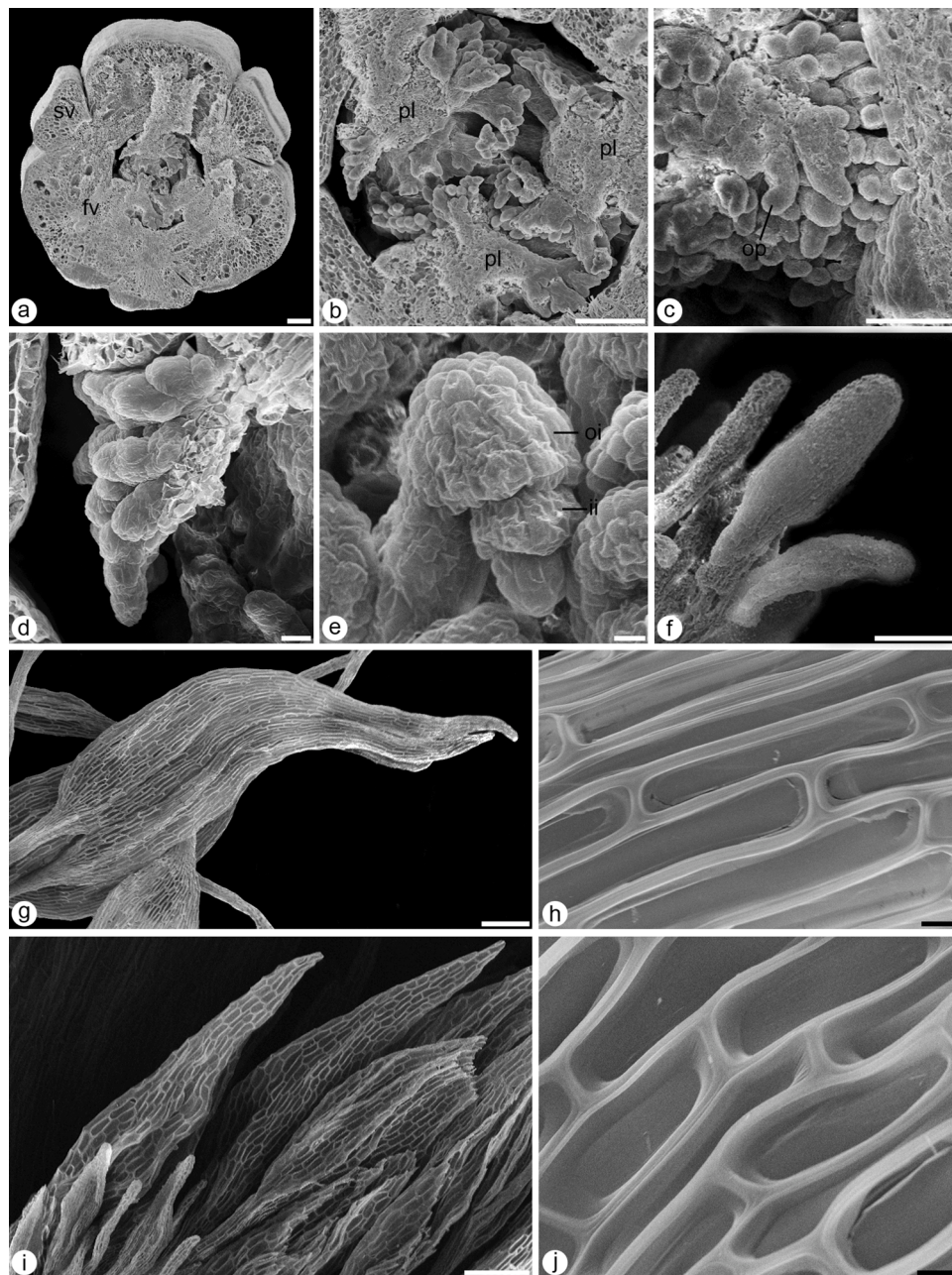


Fig. 2. Ovule and seed development in *Epidendrum*. a, b, e = *E. puniceoluteum*, c, d, f = *E. fulgens*. (a) Overview of the ovary showing sterile valve and fertile valve. (b) Detail of three placental regions. (c) Detail of ovule primordium. (d) Ovule primordium. Arrow indicates integument initiation. (e) Bitemic ovule. (f) Outer integument fully developed. (g, h). *Epidendrum puniceoluteum* seeds. (i, j) *Epidendrum fulgens* seeds. ii= inner integument; fv= fertile valve; oi= outer integument; op= ovule primordium; pl= placental region; sv= sterile valve. Scale bars: a, b, i = 200 μ m; c, f, g = 100 μ m; d = 20 μ m; e, h = 10 μ m.

(Fig. 2, Fig. 3, Fig. 4). A summary of the main developmental stages is provided in Table 1.

3.1. Female gametophyte development

Epidendrum fulgens and *E. puniceoluteum* have flowers at anthesis with an inferior ovary which then presents three carpels divided into six valves, three of which are sterile and three fertile, presenting the placental region, which at this stage lacks developed ovules and instead displays only small projections (Figs. 3a–b, 4a).

After pollination, placental proliferation occurs as a result of intense mitotic activity. Ovule differentiation within the same fruit is asynchronous and begins approximately 10 to 15 DAP (Figs. 2a–c, 3c, 4b). In the early stages of ovule development, a subepidermal cell differentiates

into an archesporial cell (Figs. 3d). Pollen tubes reach the placental region around 15 DAP in *E. fulgens* and 20 DAP in *E. puniceoluteum* (Fig. 3d), remaining near the developing ovules. At this same stage of development, cell divisions occur in the dermal layer, leading to the formation of the integuments (Figs. 2d–e, 3d, 4c), and to the ovule curvature, which characterizes it as anatropous (Figs. 3e, 4c). The inner integument develops more rapidly than the outer integument, and both consist of two cell layers (Figs. 3e, 4d). The nucellar epidermis consists of a single cell layer, characterizing the ovule as tenuinucellate (Figs. 3e, 4d).

The archesporial cell does not divide and directly gives rise to the megaspore mother cell, which has an evident nucleus (Figs. 3e, 4d). In both *E. puniceoluteum* and *E. fulgens*, the first phase of meiotic division in the megaspore mother cell produces a dyad of megaspores around 25

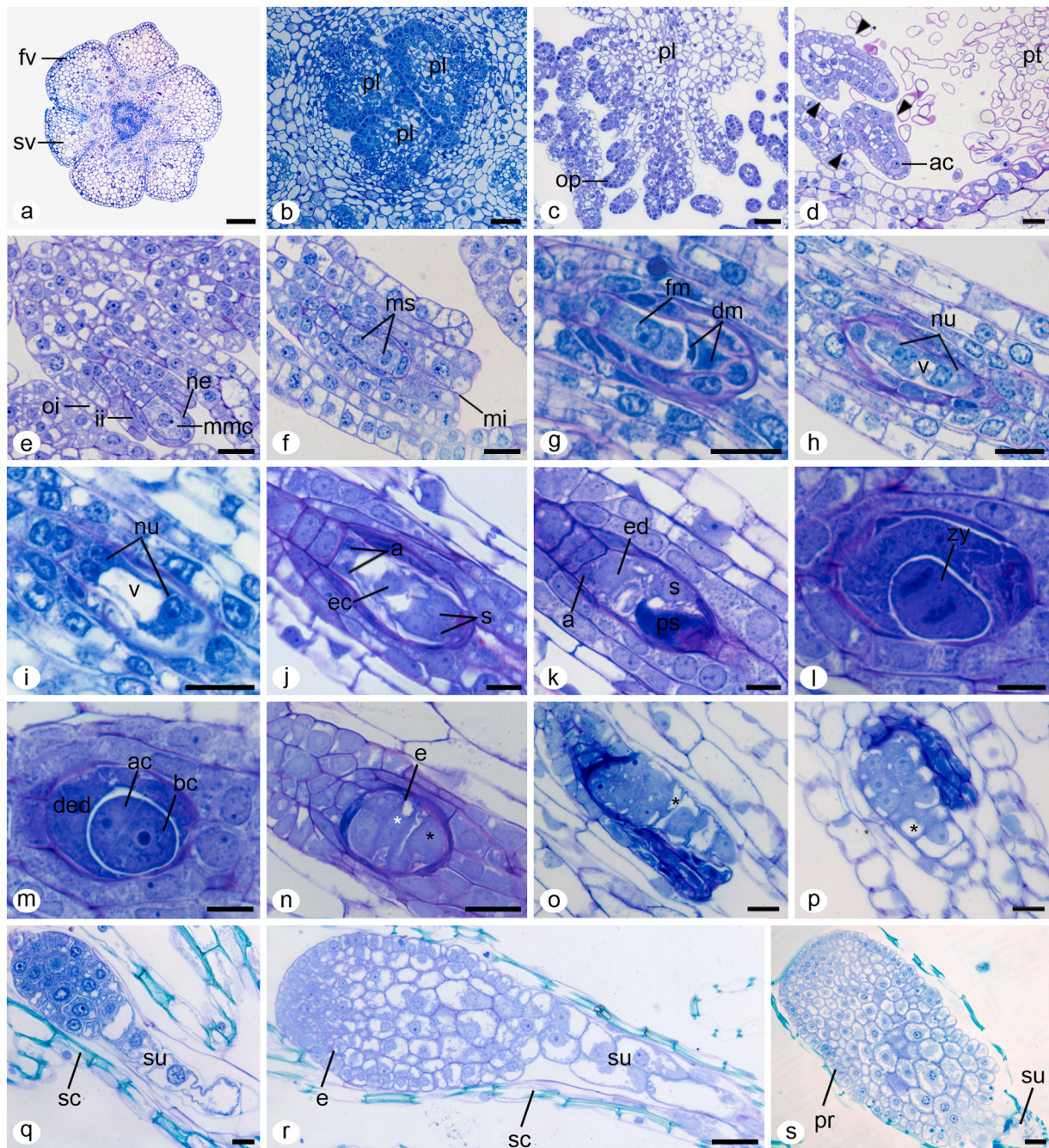
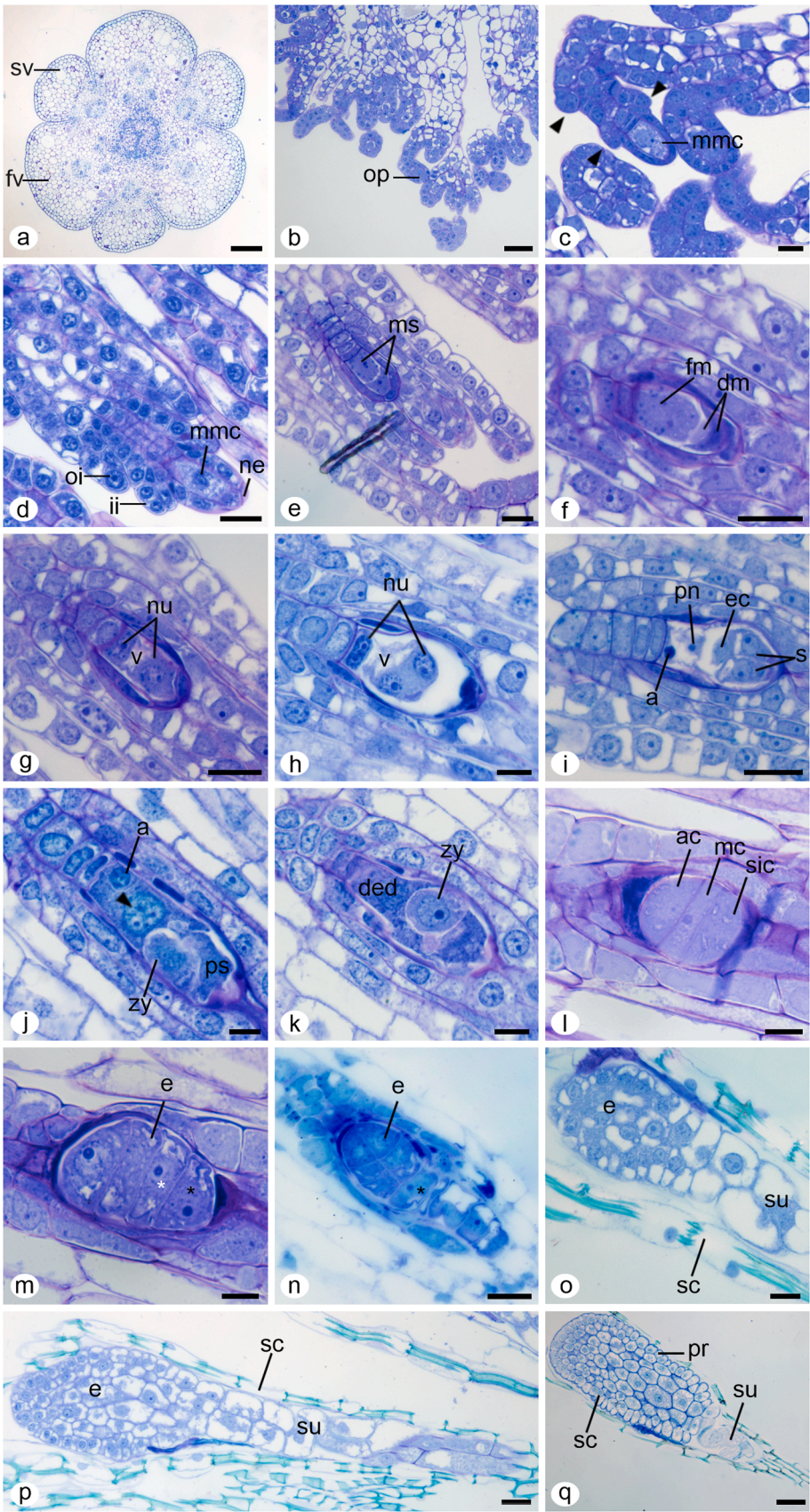


Fig. 3. Ovule and embryo development of *Epidendrum fulgens*. (a) Ovary presenting fertile and sterile valves. (b) Detail of the placental region of the ovary showing only small projections. (c) Placental regions with ovules in the early stages of development. (d) Differentiation of the initial archesporial cell and formation of integuments indicated by arrowheads. Observe numerous pollen tube near the placenta. (e) Megaspore mother cell. (f) Megaspores dyad. (g) Functional chalazal megaspore expanding and degenerating megaspores. (h) Binucleated female gametophyte. (i) Female gametophyte with three visible nuclei. (j) Female gametophyte with synergids, egg cell and two antipodes. (k) Penetrated synergid. (l) Zygote in the first division. (m) Bicellular embryo, showing apical and basal cell, and degenerating endosperm. (n) Proembryo with four cells. Black asterisk indicate suspensor initial cell and white asterisk indicate the middle cell. (o) Proembryo with six cells. Asterisk indicates the beginning of the suspensor. (p) Proembryo with eight cells. Asterisk indicates the beginning of the suspensor. (q) Embryo and suspensor. (r) Embryo with elongated suspensor. (s) Embryo at time of seed dispersal. a= antipodes; ac= archesporial cell; ded= degenerating endosperm; dm= degenerating megaspores; e= embryo; ec= egg cell; ed= endosperm; fm= functional megaspores; fv= fertile; ne= nucelar epidermis; nu= nucleus; op= ovule primordium; pl= placental region; pn= polar nuclei; pr= protoderm; ps= penetrated synergid; pt= pollen tube; s= synergids; sc= seed coat; sv= sterile valve; su= suspensor; v= vacuole. Scale bars: a = 200 µm; b, c, r, s = 50 µm; d, e, f, g, h, i, o, p, q = 20 µm; j, k, l, m, n = 10 µm.

DAP (Figs. 3f, 4e). At this stage, the outer integument elongates, delimiting the micropyle (Figs. 3f, 4e). Only the chalazal megaspore undergoes the second phase of meiosis, leading to the formation of a megaspore triad. The chalazal megaspore becomes functional and the other megaspores degenerate, characterizing the ovule development as monoporic (Figs. 3g, 4f).

The functional megaspore increases in size and undergoes the first mitotic division, giving rise to two nuclei that are separated by the formation of a large central vacuole (Figs. 3h, 4g). The two nuclei undergo the second mitotic division, resulting in four-nucleate female gametophyte (Figs. 3i, 4h). The third and final mitotic division leads to the formation of a female gametophyte with eight nuclei. Subsequently,



(caption on next page)

Fig. 4. Ovule and embryo development of *Epidendrum puniceoluteum*. (a) Ovary presenting fertile and sterile valves. (b) Placental regions with ovules in the early stages of development. (c) Differentiation of the megaspore mother cell and formation of integuments indicated by arrowheads. (d) Megaspore mother cell. (e) Megaspores dyad. (f) Functional chalazal megaspore expanding and degenerating megaspores. (g) Binucleated female gametophyte. (h) Tetranucleated female gametophyte. (i) Female gametophyte with synergids, egg cell, a polar nuclei and antipode. (j) Penetrated synergid, zygote and fusion of the polar nuclei and the male gamete indicated by the arrow. (k). Zygote and degenerating endosperm. (l) Proembryo with three cells. (m) Proembryo with four cells. Black asterisk indicate suspensor initial cell and white asterisk indicate the middle cell. (n) Proembryo with six cells. Asterisk indicates the beginning of the suspensor. (o, p) Embryo and suspensor. (q) Embryo at time of seed dispersal. a= antipodes; ded= degenerating endosperm; dm= degenerating megaspores; e= embryo; ec= egg cell; fm= functional megaspores; fv= fertile valve; mmc= megaspore mother cell; mc= middle cell; ms= megaspores; ne= nucelar epidermis; nu= nucleus; op= ovule primordium; pl= placental region; pn= polar nuclei; pr= protoderm; ps= penetrated synergid; s= synergids; sc= seed coat; sic= suspensor initial cell; sv= sterile valve; su= suspensor. Scale bars: a = 200 μ m; b, p, n, o = 50 μ m; c, d, e, f, g, h, i = 20 μ m; j, k, l, m = 10 μ m.

Table 1
Time after pollination, developmental phases of ovules and seeds in *Epidendrum*.

Days after pollination	Developmental phases	
	<i>Epidendrum fulgens</i>	<i>Epidendrum puniceoluteum</i>
10	Ovule primordia	Ovule primordia
15	Arquesporial cell	Arquesporial cell
20	Megaspore mother cell	Megaspore mother cell
	Integument development	Integument development
25	Megasporogenesis and megagametogenesis	Megasporogenesis and megagametogenesis
30	Megasporogenesis and megagametogenesis	Megasporogenesis and megagametogenesis
	Mature female gametophyte	Mature female gametophyte
	Fertilization	
35	First zygote division	Fertilization
	Embryo development	First zygote division
	Inner integument compression	Inner integument compression
40	Embryo and suspensor development	Embryo development
	Inner integument and endosperm degeneration	Endosperm degeneration
	Seed coat development and lignification	
45	Embryo development	Embryo and suspensor development
		Inner integument and endosperm degeneration
		Seed coat development and lignification
50	Embryo and enlogated suspensor	Embryo and enlogated suspensor
60	Embryo and enlogated suspensor	Embryo and enlogated suspensor
80		Seed dispersal
92	Seed dispersal	

the organization and cellularization of the female gametophyte begin. During this process, one nucleus from the micropolar pole and one nucleus from the chalazal pole migrate toward the center of the female gametophyte to form the polar nuclei. Three nuclei from the micropolar pole organize into a triangular arrangement to form the two synergids and the egg cell, while the three remaining nuclei from the chalazal pole form the three antipodes (Figs. 3j, 4i). Thus, at the end of its development, the female gametophyte is organized into seven cells containing eighth nuclei.

3.2. Embryo and seed development

Fertilization occurs 30–35 DAP in both species and is evidenced by the presence of penetrated synergids and zygote formation (Figs. 3k, 4j–k). The polar nuclei fuse with the male gamete nucleus (Fig. 4j), forming the primary endosperm nucleus, which soon degenerates (Figs. 3l–m, 4k). The first zygotic division is transverse and asymmetrical, giving rise to an apical cell and a basal cell (Figs. 3l–m). The basal cell undergoes a subsequent transverse division, producing the suspensor initial cell and the middle cell (Fig. 4l). In both species, the apical cell may divide either transversely (Fig. 4m) or longitudinally (Fig. 3n), and in both cases this division results in the formation of a four-celled proembryo (Figs 3n,

4m). The derivatives of the apical cell continue to divide, forming the embryo with six cells (Figs. 3o, 4n), eight cells (Fig. 3n) and the embryo proper. In the micropylar region the middle cell and the suspensor initial cell undergo repeated transverse and subsequent longitudinal divisions to form the suspensor (Figs. 3o–r, 4n–p). The suspensor cells then elongate and grow toward the micropylar region (Figs. 3o–r, 4n–p). Shortly after fertilization, the cells of the inner integument exhibit a denser cytoplasm (Figs. 3k–n, 4j–m). As embryo development progresses, the inner integument becomes compressed (Figs. 3o–p, 4n), while the outer integument contributes to the formation of the seed coat (Figs. 2g–j). During this process, the cells of the outer layer of the outer integument undergo lignification in its tangential and radial cell walls, as suggested by their positive reaction to toluidine blue. (Figs. 3q–r, 4o–p). By the time of seed dispersal, the embryo displays protoderm differentiation and consists of smaller cells in the apical region (chalazal pole), larger cells in the basal region (micropylar pole), and remnants of the suspensor (Figs. 3s, 4q).

4. Discussion

Orchid embryology is crucial for understanding the family’s evolutionary success, as their unique reproductive strategies such as pollination-dependent ovule development (Mayer et al., 2021), and specialized embryo-suspensor systems (Yeung, 2022; Lee and Yeung, 2023) may reflect adaptations to resource limitation and low reproductive success (Tremblay et al., 2005). Consistent with this pattern, the analyzed species in this study exhibit ovary at anthesis contain underdeveloped ovules, a condition widely reported in orchids (Mayer et al., 2021), and regarded as a strategy for resource allocation, ensuring that ovule development occurs only when seed formation is guaranteed. The ovary is differentiated into three carpels and six valves, three fertile and three sterile, as previously described for other orchid species (Rasmussen and Johansen, 2006). The fertile valve consists of the margins of two adjacent carpels along with the base of the opposite petal, while the sterile valve is formed by the margin of a single carpel and the base of the opposite sepal (Rasmussen and Johansen, 2006).

In ovules primordium, the subepidermal cell differentiates directly into an archesporial cell, corroborating previous reports for species of the genus *Epidendrum* (Sharp, 1912; Swamy, 1948; Yeung and Law, 1989; Alves et al., 2025) as well as for other genera within Orchidaceae, such as *Cymbidium* Sw., *Dendrobium* Sw., *Habenaria* Willd., *Oncidium* Sw., *Acianthera* Scheidw., *Pogoniopsis* Rchb. f., *Liparis* Rich., *Malaxis* Sol. ex Sw. (Swamy, 1949a; Yeung, 1996; Sood, 1986, 1992; Mayer et al., 2011; Duarte et al., 2019; Alves et al., 2021; Kolomeitseva et al., 2021; Ryabchenko et al., 2024). Although differentiation of the megaspore mother cell occurs at the same time, the development of the female gametophyte is asynchronous within the same fruit, corroborating what has been described for several orchid species (Swamy, 1949a; Mayer et al., 2011; Duarte et al., 2019; Alves et al., 2021; Kolomeitseva et al., 2021; Ryabchenko et al., 2024; Alves et al., 2025).

The stages of megasporogenesis proceed regularly in both species analyzed, with no detectable alterations during meiosis. The megaspore mother cell undergoes meiosis, giving rise to a megaspore triad, which results from the suppression of cytokinesis in the micropylar cell of the dyad. The formation of megaspore triads is similar to that described for

E. variegatum, *E. verrucosum*, *E. globosum*, *E. cochleatum*, *E. prismatocarpum*, *E. ibaguense*, *E. denticulatum*, and *E. orchidiflorum* (Sharp, 1912; Swamy, 1948; Yeung and Law, 1989; Alves et al., 2025), as well as for other species of the family (Swamy, 1949a; Sood and Mohana Rao, 1986; Sood, 1986, 1992; Yeung and Law, 1997; Duarte et al., 2019; Kolomeitseva et al., 2021; Alves et al., 2025). During megagametogenesis, the chalazal megaspore undergoes three synchronous mitotic cycles. These findings corroborate reports for *E. variegatum*, *E. denticulatum*, and *E. orchidiflorum* (Sharp, 1912; Swamy, 1948; Alves et al., 2025), but differ from *E. ibaguense*, which shows asynchrony during mitotic division, with one of the nuclei destined to form the antipodes dividing at a later stage (Yeung and Law, 1989). Following these mitotic cycles, the nuclei organize and cellularization occurs, resulting in a female gametophyte with seven cells and eight nuclei, which represents a modified *Polygonum* type. At the end of development, the ovules in both species are anatropous, bitegmic, and tenuinucellate, conforming to the pattern widely observed in Orchidaceae (Johri et al., 1992).

Fertilization was evidenced by the presence of a penetrated synergid, a zygote, and a primary endosperm nucleus, which soon degenerates. These results corroborate those described for *E. variegatum*, *E. verrucosum*, *E. globosum*, *E. cochleatum*, *E. prismatocarpum*, *E. denticulatum*, and *E. orchidiflorum* (Sharp, 1912; Swamy, 1948; Alves et al., 2025), but differ from observations in *E. scutella* and *E. ibaguense*, in which fusion of the sperm nucleus with the polar nuclei does not occur and no endosperm is produced (Cocucci and Jensen, 1969b; Yeung, 2022). Considerable variations in the degree of endosperm development are widely reported in Orchidaceae (Yam et al., 2002). In addition to the early endosperm degeneration observed in the species analyzed here, and to those in which endosperm formation is completely absent, a few taxa are reported to produce a nuclear-type endosperm containing up to 16 nuclei, as in *Cyrtosia septentrionalis* (Rchb. f.) Garay (= *Galeola septentrionalis* Rchb. f.) (Chugai, 1971).

The mechanisms responsible for the recurrent absence of endosperm in the family remain poorly understood. This phenomenon, however, has been associated with developmental anomalies affecting either ovules or male gametes. In many orchids, suppression of mitotic divisions gives rise to female gametophytes with reduced cell numbers, a condition referred to as the “striking phenomenon” (Yeung and Law, 1997). Competition for nutrients among large numbers of ovules may also impair central cell development, while inhibitory effects exerted by micropylar nuclei can prevent chalazal divisions (Haig, 1990). Regarding the male gametophyte, studies have shown that the chromatin density of the sperm nucleus is lower in the central cell than in the pollen tube, as observed in *E. scutella* (Cocucci and Jensen, 1969b) and *Phaius tankervilleae* (Banks ex L’Hér.) Blume (Ye et al., 2002), suggesting that male gametophyte may contribute to endosperm failure.

Before the first division, the zygote elongates, a characteristic consistently observed in *Epidendrum* species studied to date (Yeung, 2022; Alves et al., 2025). It then undergoes two transverse divisions, giving rise to a three-celled proembryo. At this stage, the apical cell may divide either transversely or longitudinally, corroborating Swamy’s (1949b) description for Orchidaceae, in which only the first two or three cell generations of the zygote follow a consistent pattern, whereas subsequent divisions occur without a defined sequence. The proembryo then undergoes successive transverse and longitudinal mitotic divisions, producing an embryo that shows protoderm differentiation and a cell mass with smaller cells at the apical pole and larger cells at the basal pole. These features are consistent with observations in other *Epidendrum* species (Yeung, 2022; Alves et al., 2025) and in other Orchidaceae, such as *Phalaenopsis amabilis* var. *formosa* (Lee et al., 2008), *Oncidium flexuosum* (Mayer et al., 2011), and *Gastrodia nantoensis* T.C.Hsu & C.M. Kuo ex T.P.Lin (Lee and Yeung, 2023). This differs from the pattern found in most Orchidaceae examined so far, in which embryos form a homogeneous mass of undifferentiated cells (Arditti, 1992). Structural differentiation between the apical and basal poles, reflected in differences in cell size and organization, has been associated with increased

germination capacity (Lee et al., 2008).

During early embryogenesis, the basal cell derived from the zygote divides to form the suspensor. In the species analyzed here, suspensor cells subsequently elongate and extend toward the micropylar region, acquiring a haustorial-like appearance. This elongation is largely attributed to the predominance of primary cell walls. As noted by Yeung (2022), the absence of a cuticle and phenolic compounds such as lignin in *Epidendrum* suspensor walls facilitates apoplastic water flow, thereby enhancing cell expansion. This elongation pattern appears to be widespread within the genus, as also reported in *E. prismatocarpum*, *E. ibaguense*, *E. denticulatum*, and *E. orchidiflorum* (Swamy, 1948; Yeung, 2022; Alves et al., 2025). The elongated suspensor is generally interpreted as an adaptation for nutrient translocation, compensating for the absence of vascular continuity between the placenta and developing seeds (Yeung, 2022). Within Orchidaceae, however, suspensor morphology is highly diverse (Swamy, 1949b; Clements, 1999; Yam et al., 2002). In addition to elongated or haustorial types, as observed here, multicellular suspenders that completely occupy the embryo cavity, as well as unicellular suspenders, have also been reported (Yam et al., 2002; Yeung, 2022; Lee and Yeung, 2023). This diversity has attracted considerable attention and underscores the taxonomic relevance of embryological characters within the family.

Seed coat development occurs concurrently with embryo formation. Shortly after fertilization, the inner integument begins to exhibit dense cytoplasm, a characteristic also observed in other *Epidendrum* species (Lee and Yeung, 2023; Alves et al., 2025) and in other orchid genera, such as *Calypso bulbosa* (L.) Oakes (Yeung and Law, 1992), *O. flexuosum* (Mayer et al., 2011), *A. johannensis* (Duarte et al., 2019), *L. parviflora* (Kolomeitseva et al., 2019), and *D. nobile* (Kolomeitseva et al., 2021). As the embryo develops, the inner integument becomes compressed, and the seed coat is formed solely from the outer tegument, as observed in most orchids studied to date (Sood and Mohana Rao, 1986; Sood, 1992; Mayer et al., 2011; Alves et al., 2019; Lee and Yeung, 2023). The only exception to this pattern appears to be in species of the genus *Vanilla* Mill., in which the seed coat is derived from both the outer and inner integuments, and the seeds are dark-colored (Swamy, 1947; Nishimura and Yukawa, 2010; Alves et al., 2019; Lee and Yeung, 2023). In the species analyzed here, lignification of the radial cell walls of the outer integument occurs during embryo development, a process commonly reported in Orchidaceae and associated with seed protection during dispersal (Lee and Yeung, 2023).

5. Conclusions

The present study demonstrates that *Epidendrum* species exhibit embryological features consistent with the general patterns observed in Orchidaceae. The pollination-dependent for ovule development support previous reports of resource allocation strategies in orchids with typically low reproductive success (Tremblay et al., 2005). The developmental sequence from archesporial cell differentiation, through megasporogenesis leading to triad formation and subsequent modified *Polygonum*-type gametophyte development, and endosperm degeneration appears largely conserved across studied taxa. We also observed embryos with smaller cells in the apical region and larger cells in the basal region. This cellular differentiation contrasts with the pattern reported for most Orchidaceae species, which typically exhibit embryos composed of a homogeneous mass of undifferentiated cells (Arditti, 1992). These findings contribute to our understanding of orchid reproductive biology and may facilitate future comparative studies examining evolutionary relationships within the family.

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CRedit authorship contribution statement

Mariana Ferreira Alves: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Fábio Pinheiro:** Writing – review & editing, Supervision, Conceptualization. **Karen Lúcia Gama De Toni:** Writing – review & editing, Supervision, Conceptualization. **José Fernando Andrade Baumgratz:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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Data availability

Data will be made available on request.

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