Embryology of *Syngonanthus nitens* var. *nitens* (Eriocaulaceae)

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In *Syngonanthus nitens* (Bong.) Ruhl. var. *nitens* the microsporangium wall consists of an epidermis, an endothecium with fibrous thickenings, a middle layer and a layer of glandular tapetum with uninucleate cells. The spiraperturate pollen is three-celled when shed. Embryo sac development is of the Polygonum type. The three antipodal cells fuse to form a cyst. Endosperm development is *ab initio* nuclear. Both integuments contribute to the seed coat formation. It is concluded that the creation of the subfamily Syngonanthoideae is justified on morphological and embryological grounds.

Keywords: Endosperm, megasporogenesis, microsporogenesis, monocotyledons, seed coat, Syngonanthoideae, systematics.

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Introduction

The family Eriocaulaceae, called the Compositae of the monocotyledons (Rendle 1930), is represented by 14 genera and 1 200 species (Mabberley 1987). Based on the number of stamens and the presence or absence of glands on the perianth, the family is divided into two subfamilies, the Eriocauloideae and the Paepalanthoideae. The Eriocauloideae is represented by two genera: *Eriocaulon* L. (with *ca.* 400 species) and *Mesanthemum* Koern. (with 12 species). The remaining 12 genera are placed in the Paepalanthoideae (Koernicke 1863; Ruhland 1930; Hamann 1964; Thorne 1983; Kral 1989). Thorne (1992) considered two genera, *Philodice* C. Martius (with two species in Brazil) and *Syngonanthus* Ruhl. (with *ca.* 200 species in Tropical America, Africa and Madagascar) in a third subfamily, Syngonanthoideae. The present detailed study on *Syngonanthus nitens* (Bong.) Ruhl. var. *nitens* was to evaluate the status of Syngonanthoideae from an embryological viewpoint. Also, based on the available embryological literature, we wanted to determine whether the Eriocauloideae is more advanced (Lowe 1961; Tomlinson 1969) than the Paepalanthoideae (Ruhland 1930; Thanikaimoni 1965). Except for a brief note on the embryogeny (Ramaswamy & Arekal 1982a), this is the first detailed embryological report on the genus (Nagendran & Dinesh 1989; Johri *et al.* 1992).

Materials and Methods

Flowering and fruiting inflorescences of *S. nitens* var. *nitens* were collected in the province of Goyaz, Brazil and were passed on to us by Prof. Graziela M. Brosso, University of Brasilia, Brazil. The voucher specimen has been deposited in the herbarium of the University of Mysore, Manasa Gangothri, Mysore. Microtome sections were cut at 8 to 14 µm thickness and stained in Heidenhain's iron alum – haematoxylin, with erythrosin in clove oil as counter stain.

Results

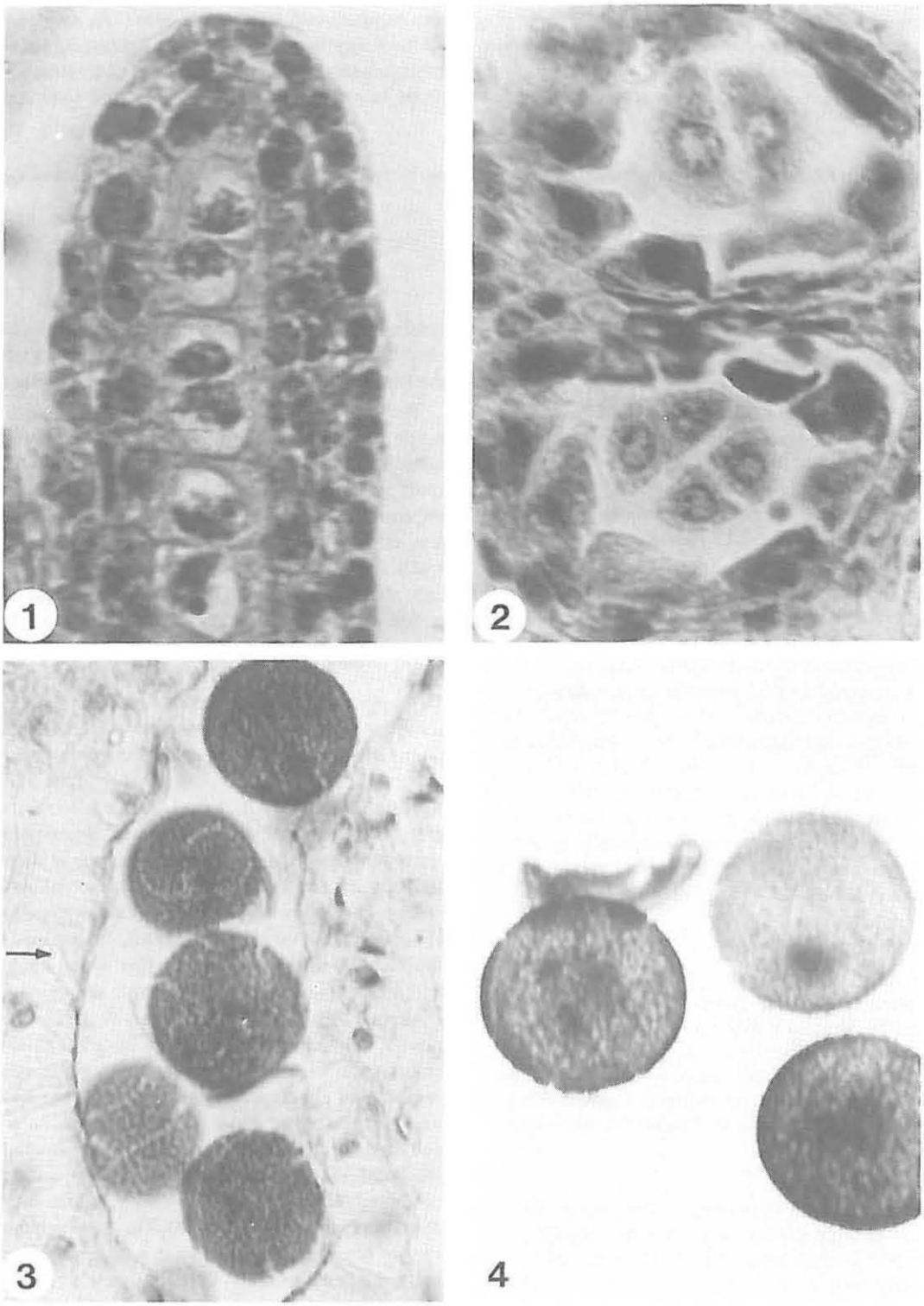
Microsporogenesis and male gametophyte. The young anther is four-lobed. A row of large, nucleated, hypodermal, densely cytoplasmic cells appear in each lobe. They divide periclinally, producing the primary parietal and sporogenous cells. The primary parietal cells, after periclinal and anticlinal divisions, contribute to the three layers of the anther wall (Figure 1). The innermost layer functions as the tapetum and its cells enlarge conspicuously with prominent nuclei and dense cytoplasm (Figure 2). During later stages of microsporangial development, the middle layer is crushed and the uninucleate tapetal cells break down and are absorbed. The endothecial cells develop characteristic band-like thickenings (Figure 3), except at the region of the dehiscence.

The cells of the sporogenous layer, which are arranged in a single row, differentiate into microsporocytes (Figure 1). After meiosis and successive type of quadripartition, the microsporocytes give rise to isobilateral microspore tetrads (Figure 2). Subsequently, the microspores separate, enlarge and assume a spherical shape. The spore nucleus eventually divides, resulting in a small generative cell and a large vegetative cell. The generative cell, after mitosis, produces two male gametes. At shedding, the pollen grains have a thin intine and thick spiraperturate exine bearing minute spinules, and are three-celled (Figures 3 & 4).

Megasporogenesis and female gametophyte. A hypodermal archesporial cell is organized very early in the ovular primordium. It enlarges and elongates, functioning as the megasporocyte. After meiosis I, it produces two dyad cells, which after meiosis II results in T-shaped or linear tetrads of megaspores (Figure 5). Of the tetrad, the three megaspores towards the micropyle usually degenerate and the one at the chalazal end is functional. Rarely, two megaspores of a tetrad may show signs of further development. The functional megaspore enlarges and elongates as it becomes vacuolated. Its nucleus undergoes two free nuclear divisions, forming first a two-nucleate (Figure 6) and then a four-nucleate embryo sac. By this time, the nucellar epidermal cells around the mid-part of the embryo sac are completely obliterated. Another free nuclear division renders the embryo sac eight-nucleate. The micropylar quartet of nuclei organize an egg apparatus consisting of two synergids, an egg and the micropylar polar nucleus (Figure 7). Three antipodal cells and the chalazal polar nucleus result from the chalazal quartet. Soon, the walls of the antipodal cells disorganize and their protoplasts fuse, resulting in a darkly stained antipodal cyst with hypertrophied nuclei (Figure 8). The three nuclei of the cyst may remain separate, or fuse to form a large polyploid nucleus. The antipodal cyst extends upwards, pushing the chalazal polar nucleus towards the micropylar polar nucleus. The polar nuclei fuse at the centre of the embryo sac, forming the secondary nucleus. The central cell enlarges and elongates. The antipodal cyst finally breaks down, leaving only the remnants at the extreme chalazal end.

The Polygonum type of organized embryo sac is more or less fusiform and is in direct contact with the inner layer of the inner integument, except at the chalazal and micropylar ends. The two beaked synergids and a pear-shaped egg constitute the egg apparatus. The remains of the antipodal cyst persist as a darkly stained body (Figure 12). A small nucellar cap over the micropylar end of the female gametophyte also persists (Figures 7, 8).

Endosperm. The primary endosperm nucleus is located at the extreme chalazal end of the embryo sac and above the remains of the antipodal cyst. Its first division is not followed by a wall. The resulting nuclei divide again, forming four nuclei which become embedded in dense cytoplasm. After further free nuclear divisions, the endosperm nuclei become distributed throughout the embryo sac in a thin peripheral layer of cytoplasm (Figure 9),

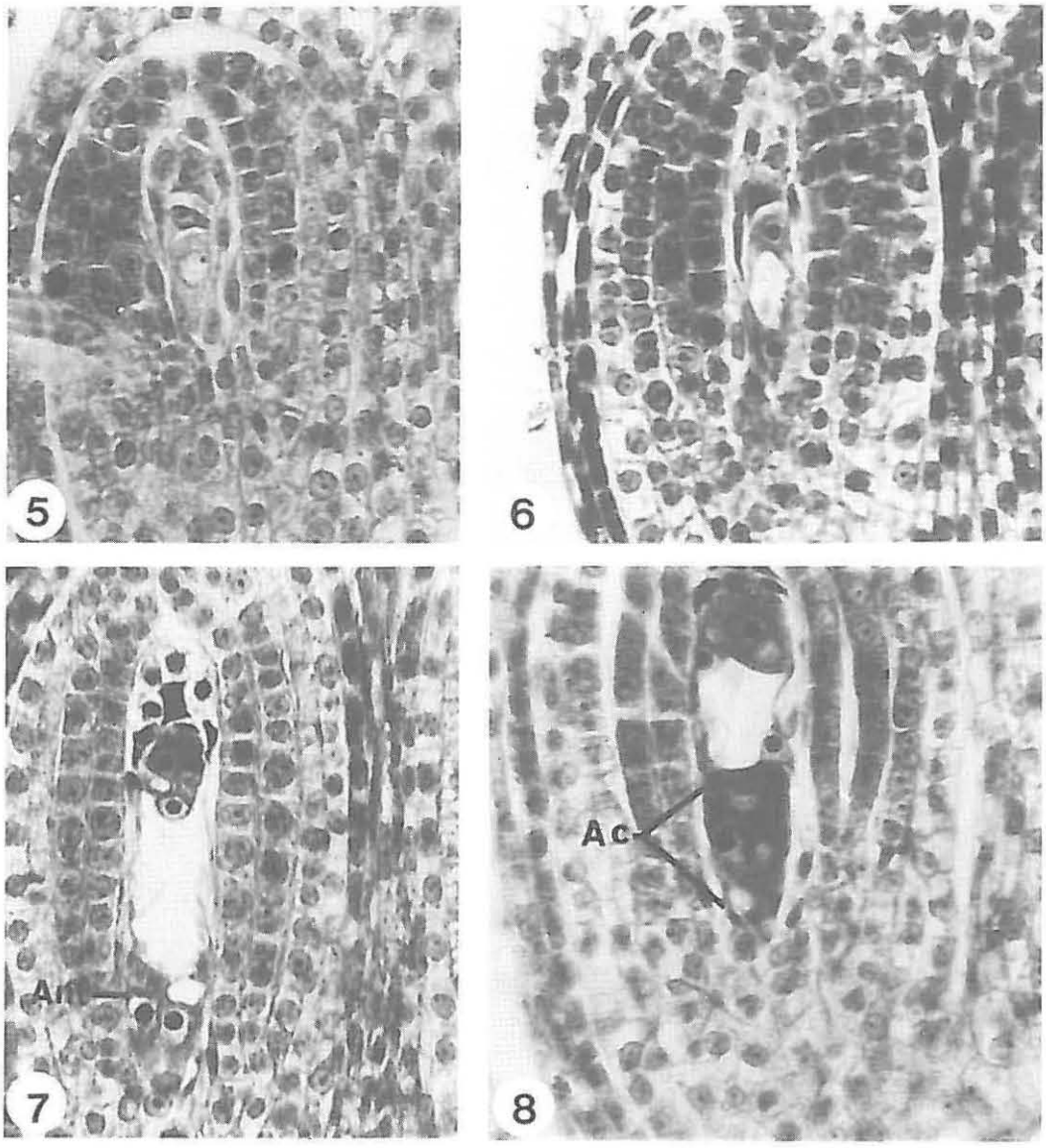


Figures 1–4 Microsporogenesis and male gametophyte in *Syngonanthus nitens* var. *nitens*. **1.** Longitudinal section of part of a young anther lobe showing a 4-layered anther wall enclosing a row of microspore mother cells; × 1 200. **2.** Transverse section of anther lobe showing a dyad and a microspore tetrad in microsporangia; × 1 200. **3.** Transverse section of old anther lobe showing fibrous thickenings in endothecium (arrow); note also spiraperturate pollen; × 800. **4.** Mature pollen grains with 3-celled condition; × 800.

although most of them are confined to the chalazal end. Wall formation begins at the chalazal end of the embryo sac and it gradually extends to the micropylar region. When the initial wall formation is complete, a layer of endosperm cells surrounds the large central vacuole in the embryo sac. Further growth of the endosperm is centripetal and is by cell division. Ultimately, the central vacuole becomes replaced by large, thin-walled endosperm cells (Figures 10, 11 & 12). In older stages of seed development, three regions could be distinguished in the endosperm tissue: the chalazal endosperm consisting of three to six narrow cells with little starch, the peripheral part with densely protoplasmic, elongated cells also with little starch, and the central mass of endosperm with large cells, engorged with starch and oil. Even in the ripe seed, this chalazal region of endosperm is clearly discernible (Figure 12).

Ovule, seed coat and fruit wall. The ovules are orthotropous, tenuinucellate and bitegmic. Each of the two integuments is usually two-layered (Figure 13), although the outer integument becomes three-layered at its base. After fertilization, marked changes occur in the cells of both layers of each integment. The cells of the inner layer of both integuments acquire heavy thickenings on their tangential walls (Figure 14). Tannin-like materials accumulate in cells of the inner layer of the inner integument. During the same period, the cells of the inner layer of the outer integument become vacuolate and lose their contents. The thickening gradually extends to the radial and outer tangential walls. When the central mass of endosperm increases in volume, the outer layers of the integuments become obliterated. As a result, the coat in the ripe seed includes tannin-filled cells of the inner layer of the inner integument and cells of the inner layer of the outer integument, having heavy thickenings on their tangential and radial walls (Figure 14).

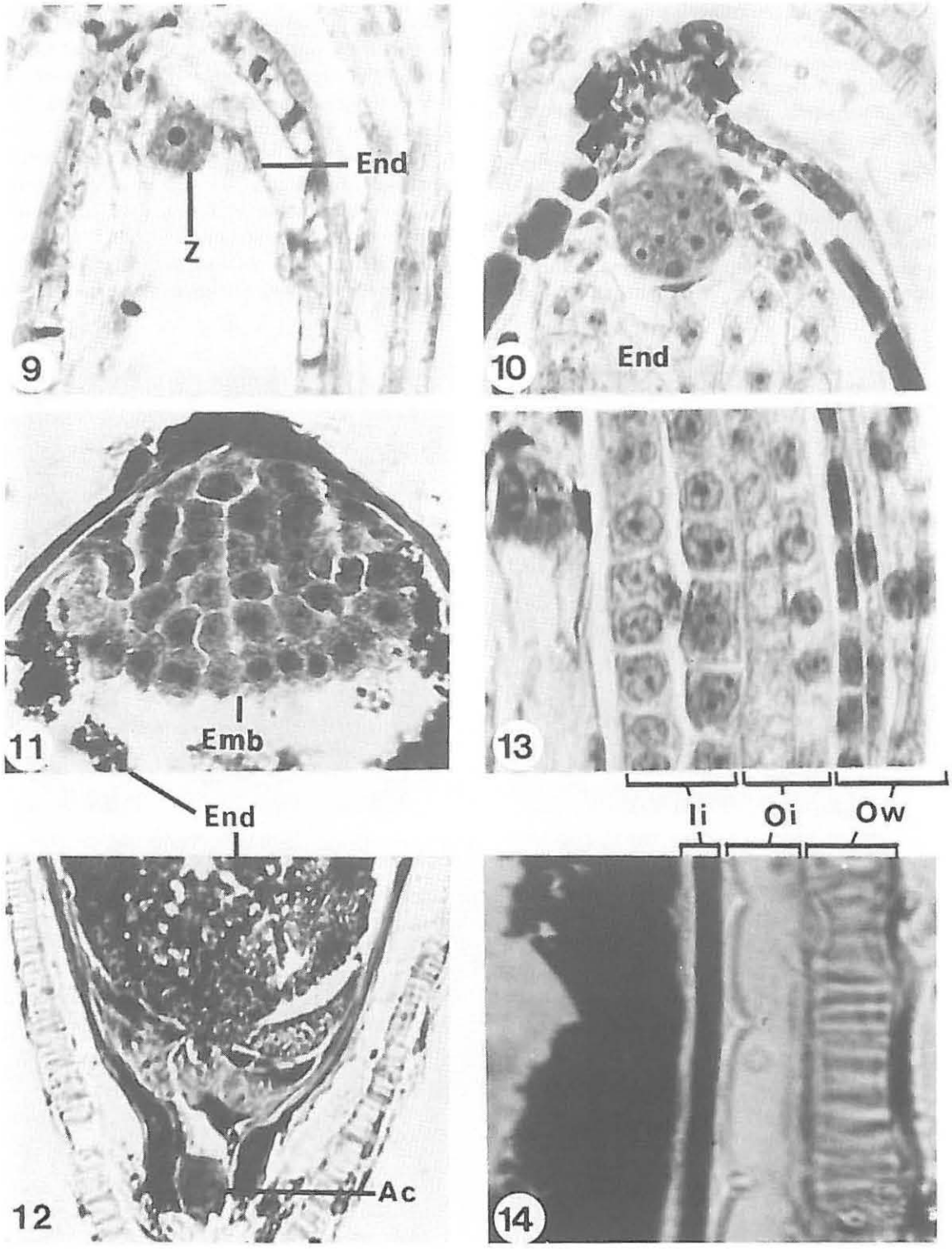
The young ovary wall is made up of four layers of vertically elongated cells (Figure 13). The two middle layers later get crushed and are absorbed. During post-fertilization stages, the cells of the inner layer become vertically stretched. As the fruit ripens, the inner layer of the pericarp develops band-like thickenings and the outer layer persists as a membrane (Figure 14).



Figures 5–8 Megasporogenesis and female gametophyte in *Syngonanthus nitens* var. *nitens*. **5.** Part of longitudinal section of ovule with a linear tetrad of megaspores in nucellus; × 800. **6.** A 2-nucleate embryo sac; × 800. **7.** Longitudinal section of ovule with organized embryo sac; × 800. **8.** Longitudinal section of embryo sac with antipodal cyst; × 800 (Ac, antipodal cyst; Ant, antipodal cells).

Discussion

Syngonanthas nitens var. *nitens* resembles other members of the Eriocaulaceae in having the following features: the general plan of development of the microsporangium, secretory type of anther tapetum, spiraperturate pollen, unisexual flowers with a single orthotropous, bitegmic, tenuinucellate ovule per carpel, micropyle organized by the inner integument, a prominent antipodal cyst in the female gametophyte, *ab initio* nuclear endosperm, embryo internally differentiated into cotyledon and epicotylary sectors but externally undifferentiated, and seed coat formed by



Figures 9–14 Endosperm, seed coat, and fruit wall in *Syngonanthus nitens* var. *nitens*. **9.** Longitudinal section of micropylar end of fertilized ovule showing zygote and endosperm nuclei in peripheral cytoplasm; × 700. **10.** Longitudinal section of micropylar end of young seed; × 700. **11.** Micropylar part of ripe seed with endosperm and embryo; × 2 000. **12.** Longitudinal section of chalazal part of ripe seed to show endosperm cells; × 700. **13.** Longitudinal section of integuments and ovary wall before fertilization; × 2 000. **14.** Longitudinal section of integuments and ovary wall at the ripe seed stage; × 2 000 (Ac, remains of antipodal cyst; Emb, embryo; End, endosperm; Ii, inner integument; Oi, outer integument; Ow, ovary wall; Z, zygote).

both inner and outer integuments (Arekal & Ramaswamy 1980; Begum 1968; Monteiro-Scanavacca & Mazzoni, 1978; Patel & Patel 1964; Ramaswamy & Arekal, 1981a, b, c, d, 1982a, b; Ramaswamy *et al.* 1981). During the development of the fruit wall, the middle layers get crushed and the cells of the inner layer develop band-like thickenings on the inner side of their radial walls.

The taxon differs from the investigated taxa of the Eriocauloideae (species of *Eriocaulon*) in having more than two layers of cells in the outer integument, a four-layered ovary wall, and early wall formation in the endosperm. While the epidermal cells of the mature anther are prominent in all the investigated species of Eriocauloideae, they are not so in *S. nitens*. Embryological studies have been carried out in only two genera of the Paepalanthoideae: *Leiothrix* Ruhl. and *Paepalanthus* Mart. (Monteiro-Scanavacca & Mazzoni, 1978; Ramaswamy & Arekal, 1981d, 1982a). *P. bifidus* (Schard.) Kunth differs from *S. nitens* in having the following features: (i) two-celled pollen at shedding stage, (ii) absence of histological zonation of the endosperm, and (iii) the absence of tannin deposition in the inner integument. Similarly, *P. bifidus* differs from *S. nitens* in having a multilayered ovary wall with conspicuous vascularization.

In addition to the above differences in embryological features, the two genera *Syngonanthus* and *Philodice* differ from other Paepalanthoideae in having inner perianth segments connate in the middle (Ruhland 1930). In fact, the name *Syngonanthus* is from the Greek *syngonos*, which means joined together, and *anthos*, meaning flower, from the connate petals of the carpellate flowers (Kral 1989). Further, the presence of bisexual flowers in the monotypic Venezuelan genus *Wurdackia* Mold. (Steutzel 1985) reveals the heterogeneity in the Paepalanthoideae; embryological studies on *Leiothrix*, *Paepalanthus* and *Syngonanthus* reveal that there is considerable diversity in the subfamily Paepalanthoideae *sensu lato*, thus supporting Thorne's (1992) decision to separate the Syngonanthoideae from the Paepalanthoideae.

The Eriocauloideae are more advanced than the Paepalanthoideae *sensu stricto* in having the following features: (i) herbaceous habit, (ii) absence of vascularization in the ovary wall, and (iii) absence of a nucellar cap at the micropylar part of the ovule. The Syngonanthoideae are evolutionarily between the Eriocauloideae and the Paepalanthoideae.

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