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Pleuropetalum Hook. f. is still an anomalous member of Amaranthaceae Juss. An embryological evidence

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Summary: The present investigation was aimed at revealing embryological characters of *Pleuropetalum*, a 'problem' member of Amaranthaceae, and elucidating affinities of this genus. Rather many embryological traits are shared by *Pleuropetalum* as well as by core Amaranthaceae. Most of these traits, however, are also inherent in the vast circle of centrosperms outside the family Amaranthaceae. *Pleuropetalum* contrasts with core Amaranthaceae but resembles some members of the closely related Portulacaceae and Cactaceae in exotesta structure. Contrary to stalactiform thickenings of the outer cell walls of the exotestal cells – which are typical of Amaranthaceae – such walls in *Pleuropetalum* are evenly thick, show a gradated staining and are penetrated by polysaccharide dendrites and numerous pore channels just like the ones in some Portulacaceae and Cactaceae. *Pleuropetalum* also differs from core members of Amaranthaceae in having aleuron grains and lipid globules in its embryo cells and thus is similar to representatives of Portulacaceae, even Didiereaceae, and Caryophyllaceae. *Pleuropetalum* is strikingly similar to Amaranthaceae in its multiseriate anther archesporium, whereas the anther archesporium of Portulacaceae is always uniseriate. Calcium oxalate crystals in pollen are a character shared by *Pleuropetalum* and some Phytolaccaceae. Such crystals have not been revealed in core Amaranthaceae so far. *Pleuropetalum* differs from Phytolaccaceae in a much thinner nucellar cap, invariably 2-layered integuments with an air chamber in between, and neither placental nor funicular obturators. Seemingly unique traits of *Pleuropetalum* are (i) an exostome which supersedes the endostome after legitimate fertilization, (ii) an exudate that precipitates to plug the micropyle of the unfertilized ovule, and (iii) aleuron grains and protein crystals as reserve material in perisperm cells. It's concluded from our new embryological data that *Pleuropetalum* is even more a 'problem member' of Amaranthaceae than it has been supposed before.

Keywords: *Pleuropetalum*, Amaranthaceae, embryology, anther, ovule, archesporium, microsporogenesis, megasporogenesis, embryo sac, embryo, seed coat

Embryological traits of *Pleuropetalum* have never been scrutinized although rather controversial affinities of the genus should have provoked comprehensive investigations of the taxon. *Pleuropetalum* was described by Joseph Hooker, Jr. as a member of Portulacaceae Juss. (vide Bentham & Hooker 1883). This classification was reproduced later in Bd. III/1b of Engler & Prantl's 'Die natürlichen Pflanzenfamilien' (Pax 1889). However, the genus was transferred to Amaranthaceae Juss. s. str. (excl. Chenopodiaceae Vent.) in the second edition of 'Die natürlichen Pflanzenfamilien' Bd. III/1a only 4 years later (Schinz 1893). Since then, *Pleuropetalum* has invariably stayed within traditional Amaranthaceae (Duke 1961; Willis 1973; Takhtajan 1987, 1997; Eliasson 1988; Kadereit et al. 2003; etc.). It was usually assigned to *Celosia* and its relatives because only these plants (within all members of the Amaranthaceae s.l. incl. Chenopodiaceae) have multi-ovulate unilocular ovaries.

However, *Pleuropetalum* does not fit well *Celosia* affinity because of a certain number of distinctive characters of its flower (Eliasson 1988; Ronse Decraene et al. 1999), leaf vasculature (Timonin 1987), and molecular traits (Müller & Borsch 2003; Kadereit et al. 2003). Therefore,

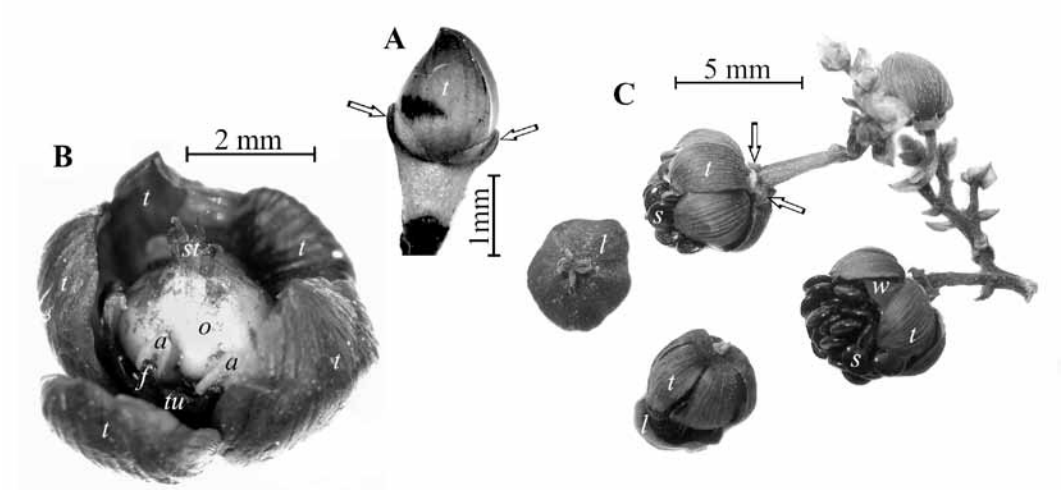

Figure 1. Flower bud (A), open flower (B), and ripe dehiscent pyxidia and shed lid (C). *a* – anther; *f* – filamentum; *l* – lid; *o* – ovary; *s* – seed; *st* – stigmata; *t* – tepal; *tu* – stamen tube; *w* – pyxidium wall; *arrows* show paired bracteoles.

Pleuropetalum was segregated from *Celosia* as a monotypical suprageneric taxon whose rank varied from subfamily to tribe (Takhtajan 1997; Kadereit et al. 2003).

Nobody dared to segregate the genus from the family Amaranthaceae, however, though it was denominated as an 'anomalous' or 'problem' representative of this family by Ronse Decraene et al. (1999) and Stevens (2008), respectively. Paradoxically enough, *Pleuropetalum* was compared with members of Chenopodiaceae (Volgin 1987; Eliasson 1988; Takhtajan 1966, 1987; Ronse Decraene et al. 1999; Kadereit et al. 2003), Achatocarpaceae (Takhtajan 1966, 1987), Phytolaccaceae (Takhtajan 1966, 1987; Eliasson 1988; Ronse Decraene et al. 1999), even with Polygonaceae (Ronse Decraene et al. 1999), but it has never been compared with members of Portulacaceae since 1889 (vide Pax 1889). Besides, when comparing *Pleuropetalum*, botanists always completely ignore its embryological characters.

Now we present embryological data concerning *Pleuropetalum darwinii* Hook. f. in the context of a broader comparison with centrosperms with special attention to core Amaranthaceae, Portulacaceae, and Phytolaccaceae.

Materials and methods

Developing flower buds, open flowers and very young fruits were collected from plants grown in the greenhouse of Tsitsin Main Botanical Garden of Russian Academy of Science, Moscow. The samples were routinely fixed with FAA fixative (formaldehyde – acetic acid – ethanol), washed, dehydrated by means of ethyl alcohol series, moved to pure xylene via increasing alcoholic solution of xylene, and embedded in paraffin wax. Embedded samples were cut with a rotary microtome MSE (London). 10 µm thick sections were mounted on microscope slides, dewaxed in xylene and rehydrated in xylene/ethyl alcohol and ethyl alcohol series. The sections thus prepared were mainly stained with principal Rawitz's haematoxylin and additional with both, light green and alcian blue. Some sections were processed with PAS reaction and Procion brilliant blue RS reaction for detecting polysaccharides and proteins, respectively (Barykina et al. 2004). Then, all sections were dehydrated via ethyl alcohol, ethyl alcohol / xylene, and xylene series and embedded

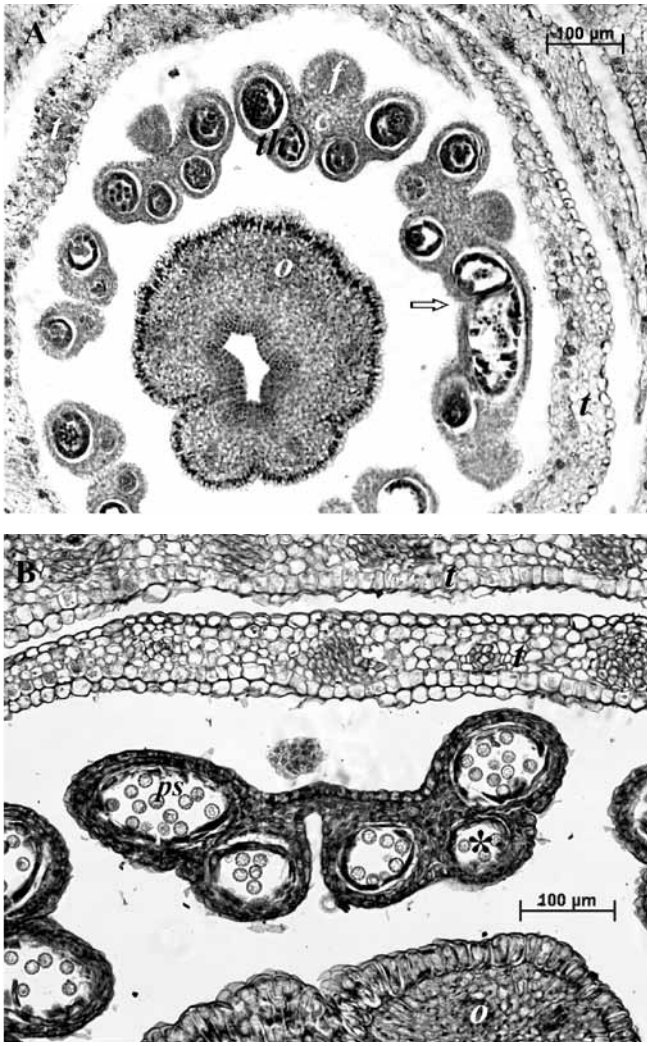

Figure 2. Cross-sectioned anthers. A – typical anthers and concrescent ones; B – anther with supernumerary pollen sac. *c* – connective; *f* – filament; *o* – ovary; *ps* – pollen sac; *t* – tepal; *th* – theca; *asterisk* – supernumerary pollen sac; *arrow* shows concrescent anthers.

in Canada balsam. The sections were examined and photographed using a light microscope 'Axioplan 2 imaging' equipped with digital camera 'AxioCam MRc'.

Results

The complete flower (Fig. 1 B) is 5–7 mm in diameter when opened; perianth of 5 separate, non-scarious light yellow tepals; 2 smaller yellowish scales below the perianth (Fig. 1 A, C), resembling paired sepals typical of Portulacaceae (they were proved to be bracteoles by Ronse Decraene et al. 1999); androecium of (6)8 stamens whose filament bases are evidently connated to a tube; anthers dorsifixed, tetrasporangiate; gynoecium mostly pentamerous, syncarpous sensu Leinfellner (1950); ovary superior; fruit pyxidium (Fig. 1), not a berry (in contrast to Ronse Decraene et al. 1999). Autogamous pollen grains are unable to grow on the stigma whereas allogamous ones have nearly 100% fertility.

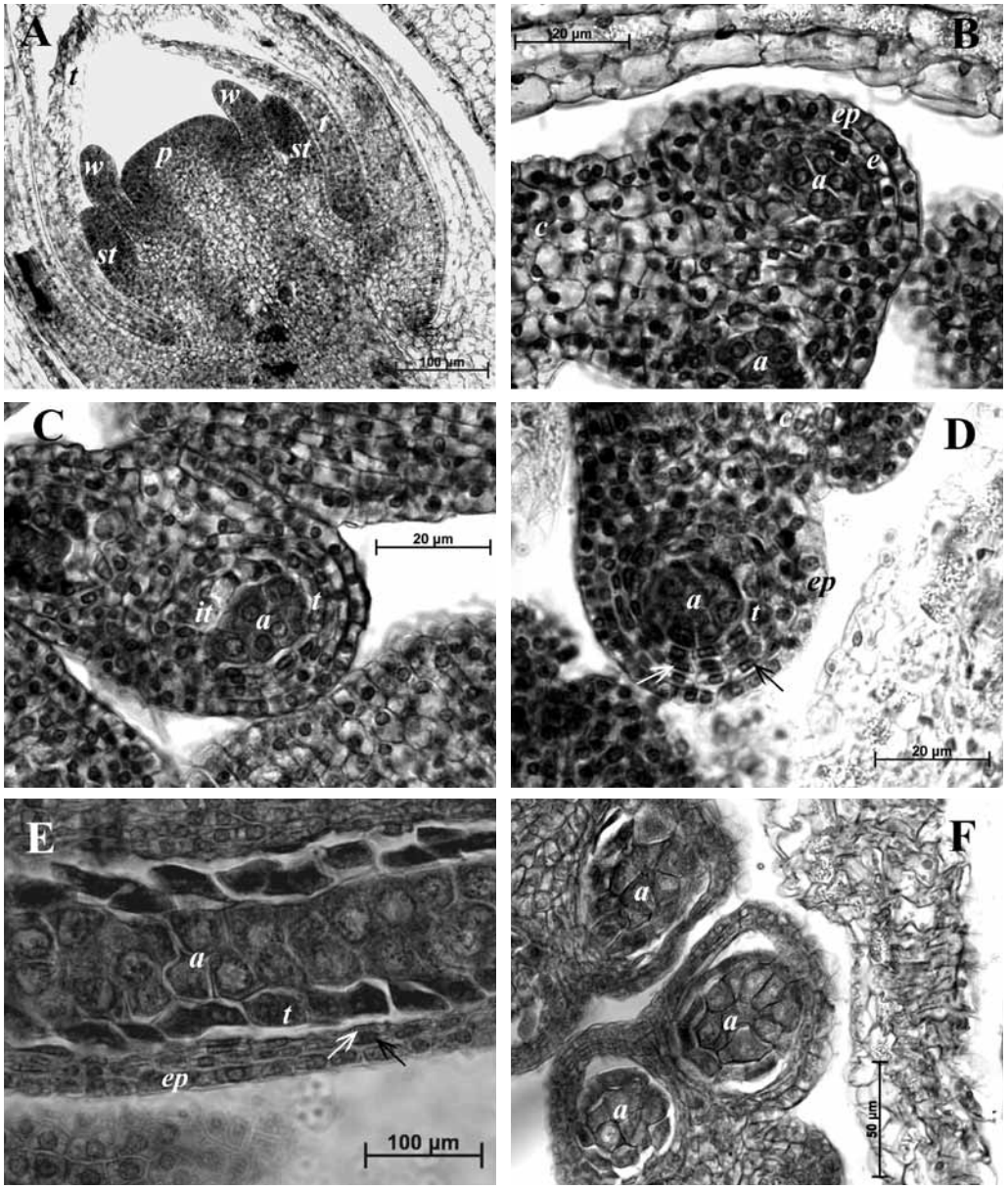

Figure 3. Anther development. A – anther primordia, longitudinal section; B–D – development of the anther wall, cross-sections; E – microsporocytes, longitudinal section; F – multiseriate archesporium, cross-section. *a* – secondary archesporium (sporogenous cells); *c* – connective; *e* – endothecium; *ep* – epidermis; *it* – internal part of the tapetum; *p* – developing placenta; *st* – developing stamen; *t* – tapetum; w – ovary wall; *white arrow* shows middle layer; *black arrow* shows endothecium.

Anther development

The anther starts as 2 laterally-adaxial oblong protrusions on the top of the stamen primordium, each typically developing into bilobed thecae (Fig. 2 A)[1]. The protrusion consists of an epidermis in progress and an inner meristem (Fig. 3 A). The subepidermal cell layer of the meristem changes

1) 3-lobed thecae (Fig. 2 B) and neighbouring stamens that are post-genitally concrescent by their thecae (Fig. 2 A) occur abnormally.

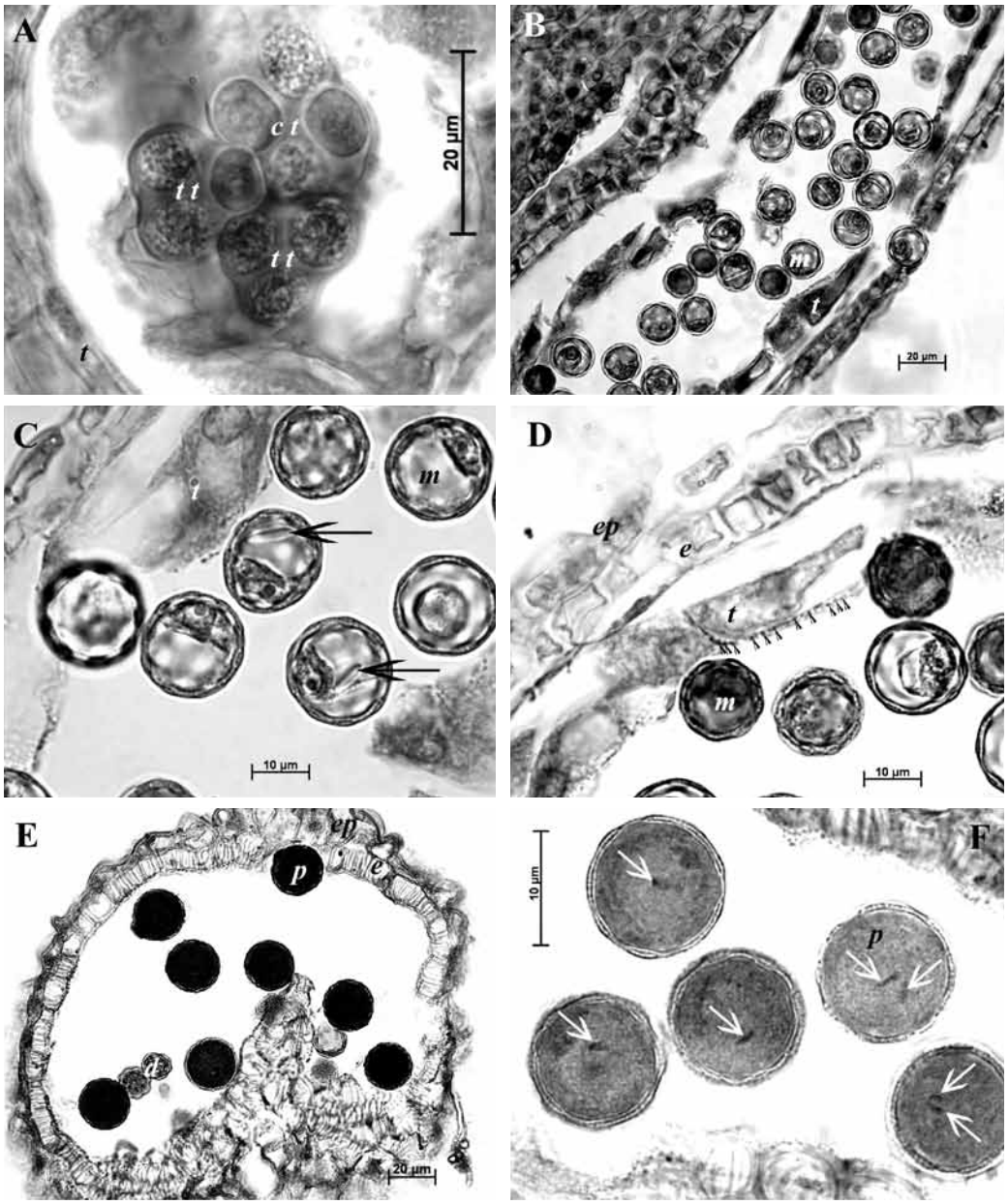

Figure 4. Pollen development. A – tetrads of microspores; B – microspores at late post-tetrad period; C – calcium oxalate crystals in microspores; D – orbicules (Ubish bodies) on tapetal cell walls; E – protein-filled pollen grains; F – 3-celled pollen grains. *ct* – cross tetrad; *d* – degrading microspore; *e* – endothecium; *ep* – epidermis; *m* – microspore; *p* – pollen grain; *t* – tapetum; *tt* – tetrahedral tetrad; *black arrows* show calcium oxalate crystals; *white arrows* show sperm cells; *arrow heads* show orbicules (Ubish bodies).

into a multicellular primary archesporium in each thecal lobe. The primary archesporium produces outwards a primary parietal layer and inwards a secondary archesporium (sporogenous cells) by periclinal divisions of its constituting cells. Periclinal cell-divisions of the primary parietal layer give rise to 2 secondary parietal layers, of which the outer one differentiates into the endothecium (Fig. 3 B), whereas periclinal cell divisions of the inner secondary parietal layer result in the outer middle layer and the inner cell layer that develops into the principal, external part of the tapetal

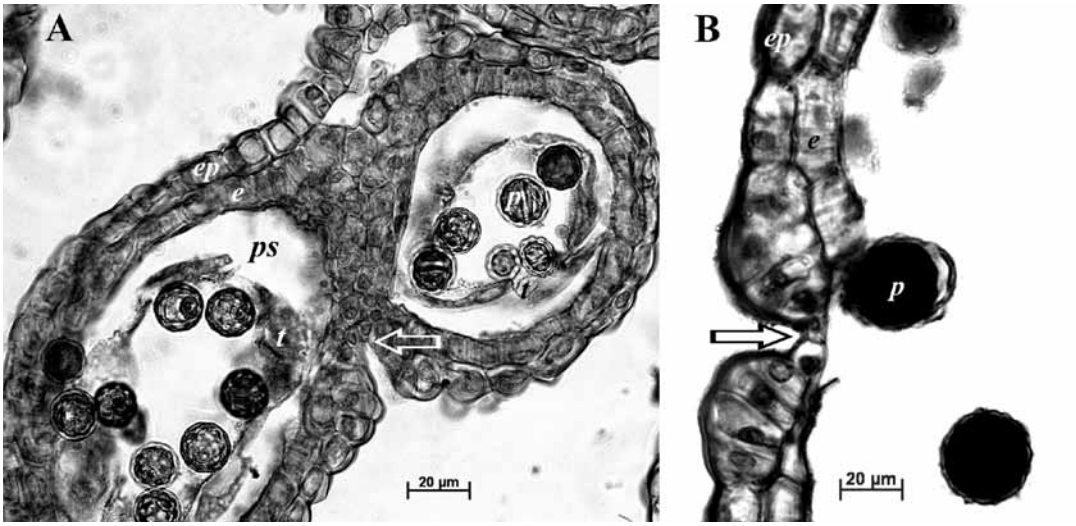

Figure 5. Anther wall. A – theca of premature anther, cross-section; B – detail of mature anther wall, cross-section. *e* – endothecium; *ep* – epidermis; *p* – pollen grain; *ps* – pollen sac; *t* – remnant of the tapetum; *arrow* shows dehiscence site of the wall.

layer (Fig. 3 C). The internal part of the latter precedes the external one and comes directly from the connective meristem adjoining the sporogenous cells. Cells of this part of the tapetal layer originally differ from their counterparts of the external part and from the cells of the connective meristem in their shapes and sizes, in a lighter stained cytoplasm, and in larger lobate nuclei (Fig. 3 C). The anther wall thus developed consists of epidermis, endothecium, middle layer, and tapetum (Fig. 3 D, E).

The anther wall later changes as follows: 2 dehiscence sites are formed in the epidermis of both thecae in the premature anther (Fig. 5). Each site is accompanied by an inner septum between both pollen sacs of the theca and consists of a narrow strip of small cells alias 'lock' which is flanked by enlarged epidermal cells on each side. Endothecial cells produce internal fibrous thickenings on their walls (Fig. 4 E) after the meiosis is accomplished in microsporocytes. Some neighbouring cells of the connective form similar thickenings, too (Fig. 4 E). Thus transformed, the cells cooperatively constitute an one cell thick fibrous layer which is located around every pollen sac and discontinues in septae (Fig. 5 A). The middle layer is rather ephemeral, because its cells become highly compressed in periclinal plane and contain a pycnotic nucleus at the very beginning of the microsporogenesis (Fig. 3 F). When the microsporogenesis is finished, the cells of the middle layer mostly undergo a complete autolysis (Fig. 4 B, D). Tapetal cells of different origin gradually resemble each other. At the beginning of the microsporogenesis the mostly 1-layered tapetum consists of uniform enlarged cells with double nucleus, an intensely stained and distinctly granular cytoplasm, and few small vacuoles (Fig. 3 E). The tapetum starts degrading by autolysis just after meiosis has taken place in the microsporocytes (Fig. 4 B). Nevertheless, its dissolving cellular structure remains quite recognizable up to the bi-cellular pollen stage (Fig. 5 A). They completely disappear afterwards (Figs 4 E–F; 5 B). Degrading of the tapetal cells coincides with accumulating orbicules (Ubish bodies) on their inner walls (Fig. 4 D) which change into a durable tapetal membrane. Its remnants are still detectable in the mature anther. The wall of the mature anther only consists of an epidermis and a fibrous layer (Fig. 5 B).

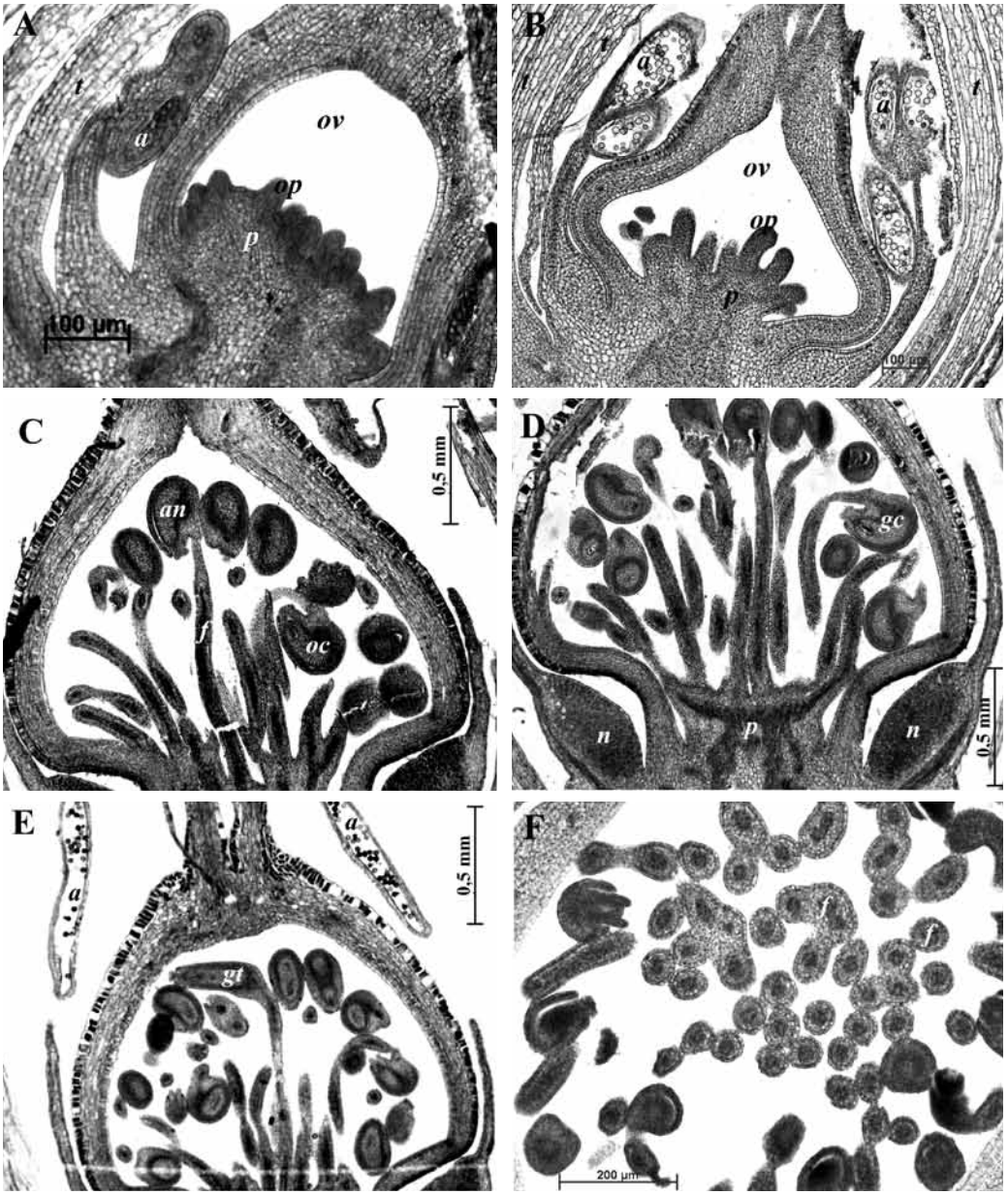

Figure 6. Ovule development. A – initiating ovules in longitudinally sectioned ovary; B – fissured placenta bearing developing ovules in longitudinally sectioned ovary; C–E – ovules of different types in longitudinally sectioned ovary; F – funiculi in cross-sectioned ovary. *a* – anther; *an* – anacampylotropous ovule; *f* – funiculus; *gc* – gemicampylotropous ovule; *gt* – anomalous 'gemitropous' ovule; *n* – nectary; *oc* – orthocircinotropous ovule; *op* – ovule primordium; *ov* – ovary; *p* – placenta; *t* – tepalum.

Pollen release is preceded by destruction of the septae (Fig. 5 B) which is starting when the bicellular pollen has arisen in pollen sacs. The mature anther introrsely opens by 2 longitudinal splits through the 'locks'.

Microspore and male gametophyte

The sporogenous cells that develop directly from the primary archesporium generate by mitosis up to 8 longitudinal files of microsporocytes per pollen sac (Fig. 3 F). All microsporocytes of

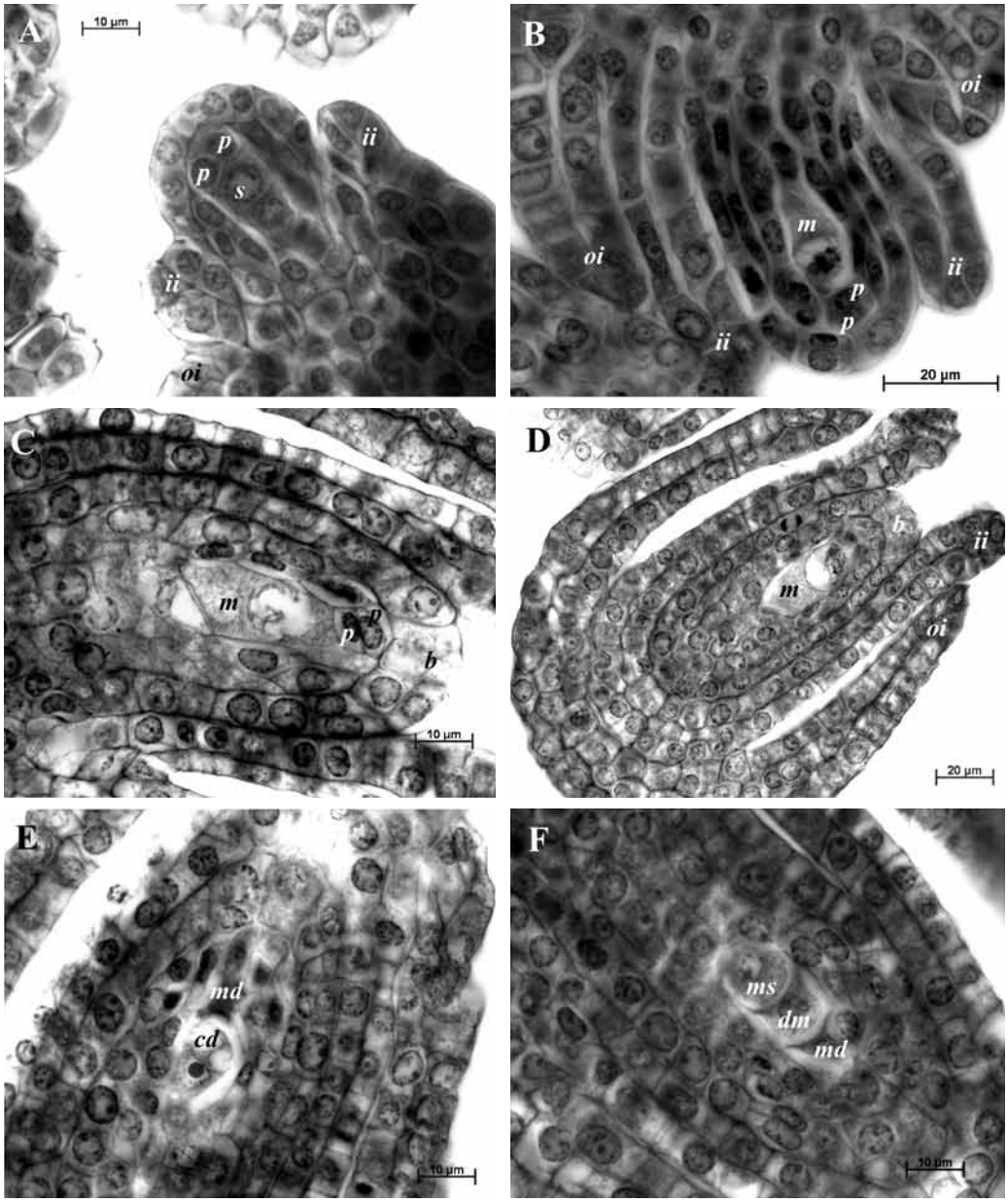

Figure 7. Megasporogenesis. A – sporogenous cell and 2 parietal cells; B–D – megaspore mother cells (megasporocytes) entering meiosis; E – dyad; F – triad. *b* – nucellar cap; *cd* – chalazal cell of dyad; *dm* – dead megaspore; *ii* – inner integument; *m* – megasporocyte; *md* – dead micropylar cell of dyad; *ms* – megaspore; *oi* – outer integument; *p* – parietal cell; *s* – sporogenous cell.

a pollen sac synchronously start meiosis to give rise to microspore tetrads. Microsporocytes of different pollen sacs start meiosis synchronously in some anthers and a bit asynchronously in others. Simultaneous microsporogenesis results in either tetrahedral or cross tetrads of microspores capsulated in callose (Fig. 4 A). They grow spherical due to their loose arrangement there. After releasing from the callose capsule, the microspores show a conspicuous panaperturate (panporate) exine, a distinctive intine, an intensely stained cytoplasm without a vacuole, and a central nucleus.

Later, the microspores grow larger and develop a bulky vacuole which dislocates the nucleus from its central position (Fig. 4 B). Many microspores contain 1 to 2 needle-shaped crystals of calcium oxalate (Fig. 4 C). Such crystals are quite visible even in bi-cellular pollen grains but they are always lacking in mature ones (Fig. 4 F). Most microspores unequally divide into vegetative and much smaller generative cells to give rise to bi-cellular male gametophytes. The latter accumulate starch and protein (Fig. 4 E). Still in the pollen sac the gametophytes become 3-cellular as their generative cells generate 2 small lenticular sperm cells (Fig. 4 F). The exine surface is uniformly granular throughout except for smooth patches around the pores which appear therefore annulate. There are microspores, however, which are remarkable for their distinctively larger size, thinner exine, and light-stained cytoplasm. They produce bi-cellular male gametophytes simultaneously just like their normal counterparts do. These larger male gametophytes are nevertheless unable to accomplish their development. The generative cell in such a gametophyte becomes pycnotic and only remnants of both, generative cell nucleus and vegetative cell nucleus, are present when the normal gametophytes store reserve starch and protein. Such aberrant pollen grains must completely disappear by anther dehiscence because all released pollen grains are morphologically uniform, alive, and fertile.

Ovule development

The ovary consists of an inhibited sterile synascidiate zone, a prominent fertile symplicate zone, and a very short central column. The column is terminated by a bulky, deeply fissured placenta (Fig. 6 B). More than 100 ovules develop centrifugally throughout the placenta (Fig. 6 A). The nucellus of the ovule precociously develops distalmost a primary archesporium of a single subepidermal cell that divides periclinally to give rise to an inner secondary archesporium cell (Fig. 7 A) and an outer parietal cell. The first division of the latter is either anticlinal (Fig. 7 A) or periclinal (Fig. 7 B, C). Both derivatives of the parietal cell contribute much later to the formation of a 2- to 4-layered parietal tissue (Figs 7 D; 8 A). Repeating periclinal cell divisions in the epidermis of micropylar part of the nucellus follow the origin of the secondary archesporium to generate the nucellar cap (Figs 7 C, F, 8 A; 9 F). These divisions are accompanied by intensive chalazal growing of the nucellus resulting in a crassinucellate ovule (Fig. 9). The inner and outer integuments successively start developing at the time when the secondary archesporium cell arises (Fig. 7 A). Both integuments, each 2-layered, completely girdle the developing nucellus (Fig. 7 D). The inner one highly exceeds its outer counterpart to form the endostome of the mature ovule (Fig. 9 A, C–D). There is a discernible air cavity between the bases of the two integuments (Fig. 9 A, C). An unexpectedly long funiculus lifts the ovule (Fig. 6 C–E).

The ovules thus completed are mostly connected in pairs or triads at the bases of their funiculi, whereas their vascular strands are separate there (Fig. 6 F). All the ovules have nearly equally long funiculi, but the curvatures of the latter are highly diverse. The more peripheral the ovule is, the more curved is its funiculus. The ovules vary from peripheral ortocircinotropous to central anacampylotropous (Fig. 6 C–D). Due to this diversity a host of ovules fits into the limited interior of the ovary. The micropyle is orientated from downward in anacampylotropous ovules to upward in ortocircinotropous ones. Nearly hemitropous ovules (Fig. 6 E) were noticed twice among the central ones. They both can be considered precociously inhibited following the example of Iljina's (1971) interpretation of similar ovules in Papaveraceae.

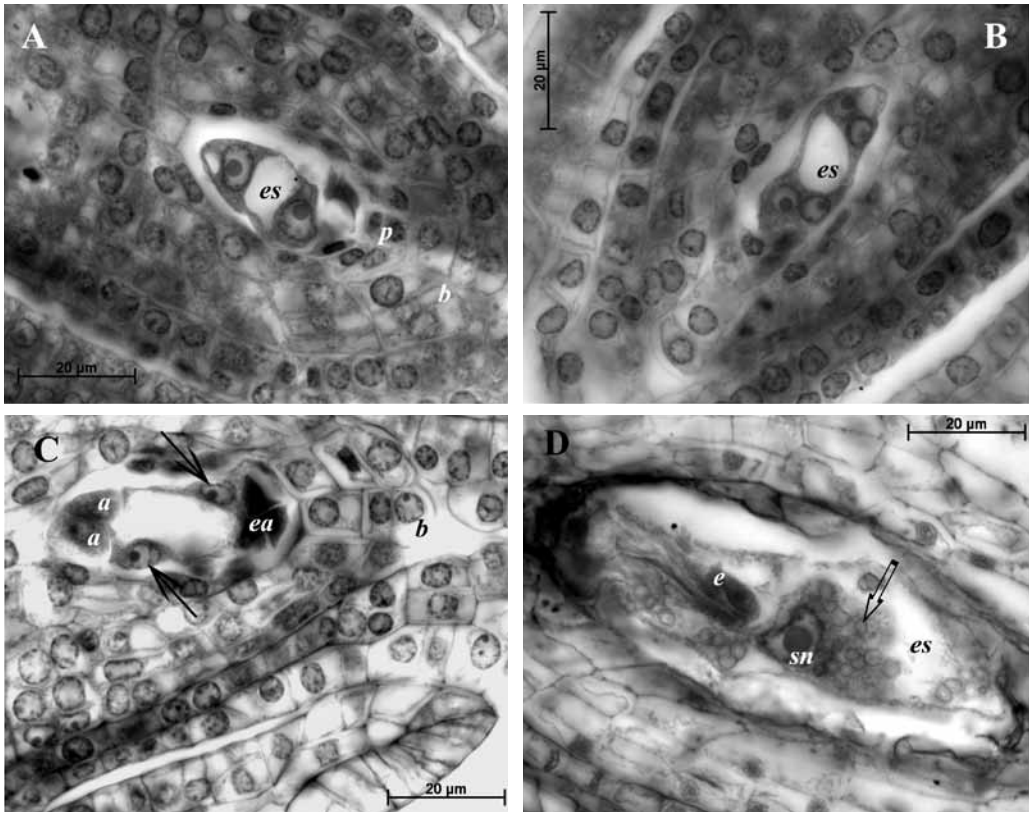

Figure 8. Embryo sac development. A – 2-nucleate embryo sac; B – 4-nucleate embryo sac; C – 7-celled 8-nucleate embryo sac; D – old unfertilized embryo sac. *a* – antipode; *b* – nucellar cap; *e* – egg; *ea* – egg apparatus; *es* – embryo sac; *p* – parietal layers; *sn* – secondary nucleus of embryo sac central cell; *black arrows* show polar nuclei; *double arrow* shows starch grains.

The inner integument cells, which enwrap the micropylar duct, grow larger. They enlarge their nuclei and thicken their inner cell walls during the embryo sac is ripening (Fig. 9 B, D). Accordingly, the cells of the nucellar cap and those of the parietal tissue grow larger and longer towards the embryo sac. The micropylar duct is filled with exudates (Fig. 9 C) which contain polysaccharides and proteins. The exudates plug the duct of the unfertilized ovule (Fig. 9 D–E).

Megaspore and embryo sac

The secondary archesporium cell elongates and develops into a megasporocyte (Fig. 7 B–D) proceeding meiotic divisions. The first division gives rise to paired cells (dyad) of which the micropylar one typically ceases without subsequent dividing (Fig. 7 E). The chalazal cell generates 2 megaspores by the second division. Then, a cell triad appears (Fig. 7 F). The micropylar megaspore dies while the chalazal one results in the embryo sac of Polygonum type (Fig. 8 A–C). The egg apparatus and the antipodes are originally very similar in cells sizes, shapes, and cytoplasm stain. The embryo sac thus arisen progresses as follows. The egg and both synergids develop each a large contrariwise located vacuole. The polar nuclei of the central cell approach each other and then unite (Fig. 8 D). All antipodes cease and disappear. The central cell accumulates numerous starch grains (Fig. 8 D). We have noticed an embryo sac that has no synergids in its egg apparatus but 2 eggs. Such embryo sac might sporadically reveal an atypical seed with 2 embryos.

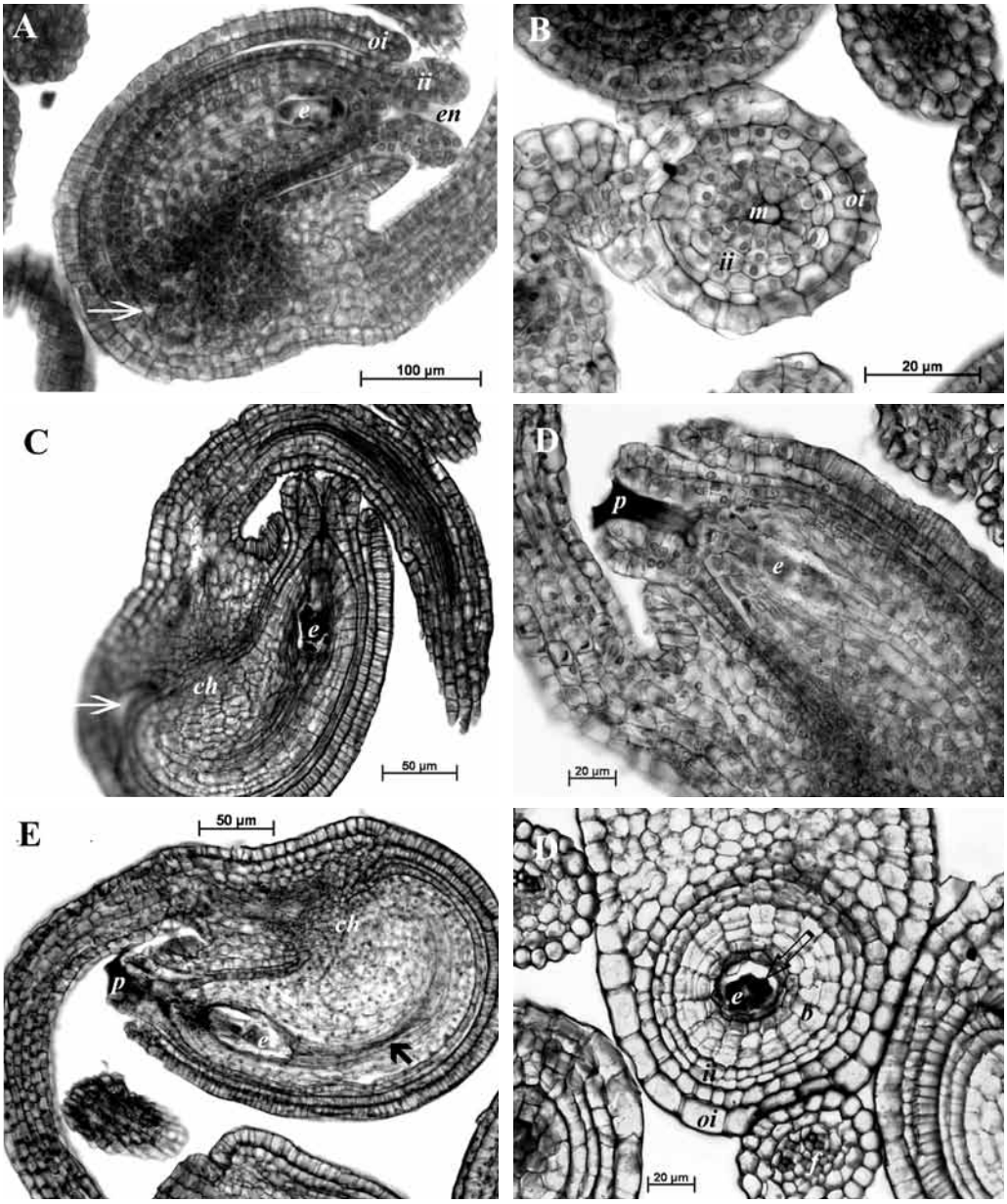

Figure 9. Mature ovules. A – unfertilized ovule; B – cross-sectioned micropyle duct; C – ovule with exudates in micropyle duct; D – plugged micropyle duct; E – strand of destroyed cells in nucellus; F – cross-sectioned nucellar cap. *b* – nucellar cap; *ch* – chalaza; *e* – embryo sac; *en* – endostome; *f* – funiculus; *ii* – inner integument; *m* – micropyle; *oi* – outer integument; *p* – exudates plug; *black arrow* shows strand of destroyed cells; *double arrow* shows starch grains in embryo sac; *white arrow* shows air cavity.

Seed development

A strand of destroyed nucellar cells appears between the embryo sac and the chalaza (Fig. 9 E). This is the first detectable step of the ovule to seed development. The strand precedes the fertilization. Seed development will progress only if legitimate fertilization takes place. Otherwise, the egg apparatus collapses and causes the dying of the whole ovule. Legitimate fertilization results in an elongated zygote and a nuclear endosperm (Fig. 10 A) and triggers a considerable growth of the

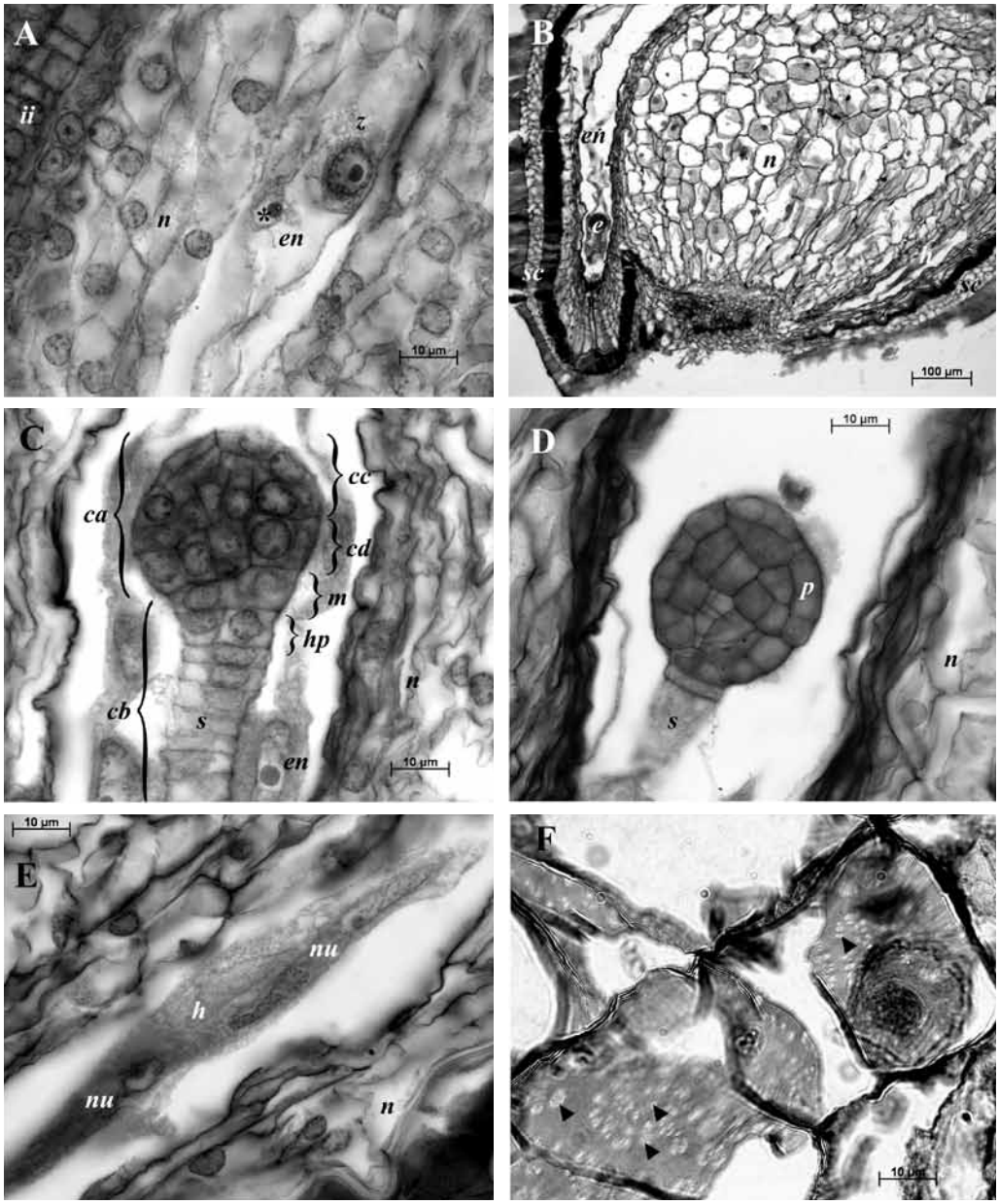

Figure 10. Developing seed. A – zygote; B – longitudinal section of seed; C, D – globular proembryos; E – endosperm haustorium; F – nucellus cells. *ca* – derivatives of proembryo's *a* cell; *cb* – derivatives of proembryo's *b* cell; *cc*, *cd* – derivatives of the *ca* cell; *e* – proembryo; *en* – endosperm; *h* – endospermal haustorium; *hp* – hypophysis; *ii* – inner integument; *m* – middle layer; *n* – nucellus; *nu* – haustorium nucleus; *p* – protodermis; *s* – suspensor; *sc* – seed coat; *z* – zygote; *asterisk* – endosperm nucleus; *arrowheads* show pores.

chalaza. The nuclear endosperm grows much longer towards the funiculus through the strand of collapsed nucellar cells around the center of the nucellus (Fig. 10 B). Therefore it becomes crescent. The growing chalazal part of the endosperm changes into a haustorium with enlarged, variably united nuclei (Fig. 10 E). Cytokineses extend through the endosperm from its micropylar end to the chalazal end at the time the cotyledons initiate.

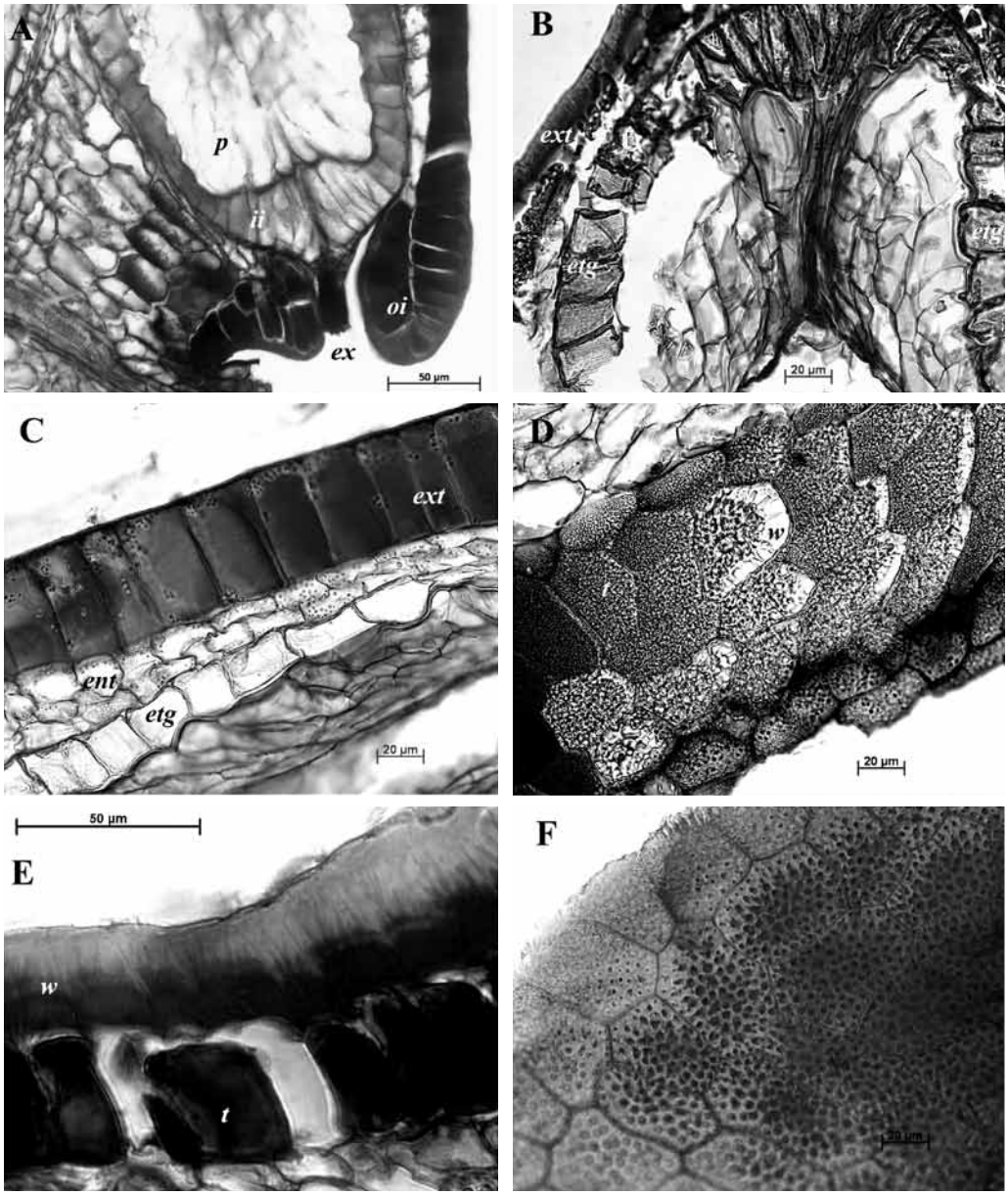

Figure 11. Developing seed coat. A – exostome; B – endotegmen; C – initiating testa and tegmen; D – periclinal section of initiating exotesta; E, F – underdeveloped exotesta with pore channels and polysaccharide dendrites in outer cell walls in anticlinal and periclinal sections, respectively. *ent* – endotesta; *etg* – endotegmen; *ex* – exostome; *ext* – exotesta; *ii* – inner integument; *oi* – outer integument; *p* – perisperm; *t* – tannins; *w* – cell wall.

The globular proembryo has a long uniseriate 10–12-celled suspensor (Fig. 10 B–C). The terminal cell of the suspensor contacts nucellar cells with its thickened outer cell wall. Protodermis differentiation proceeds from the *cc* and *cd* derivatives shown in Figure 10 D towards the base of the proembryo.

The developing embryo exhausts the endosperm except for two 1- to 2-layered remnants (alias endospermal caps) at the tops of the radicula and the cotyledons (Fig. 12 C). The remnants consist of cells abundant in aleuron grains. The embryo cells contain aleuron grains (Fig. 12 E),

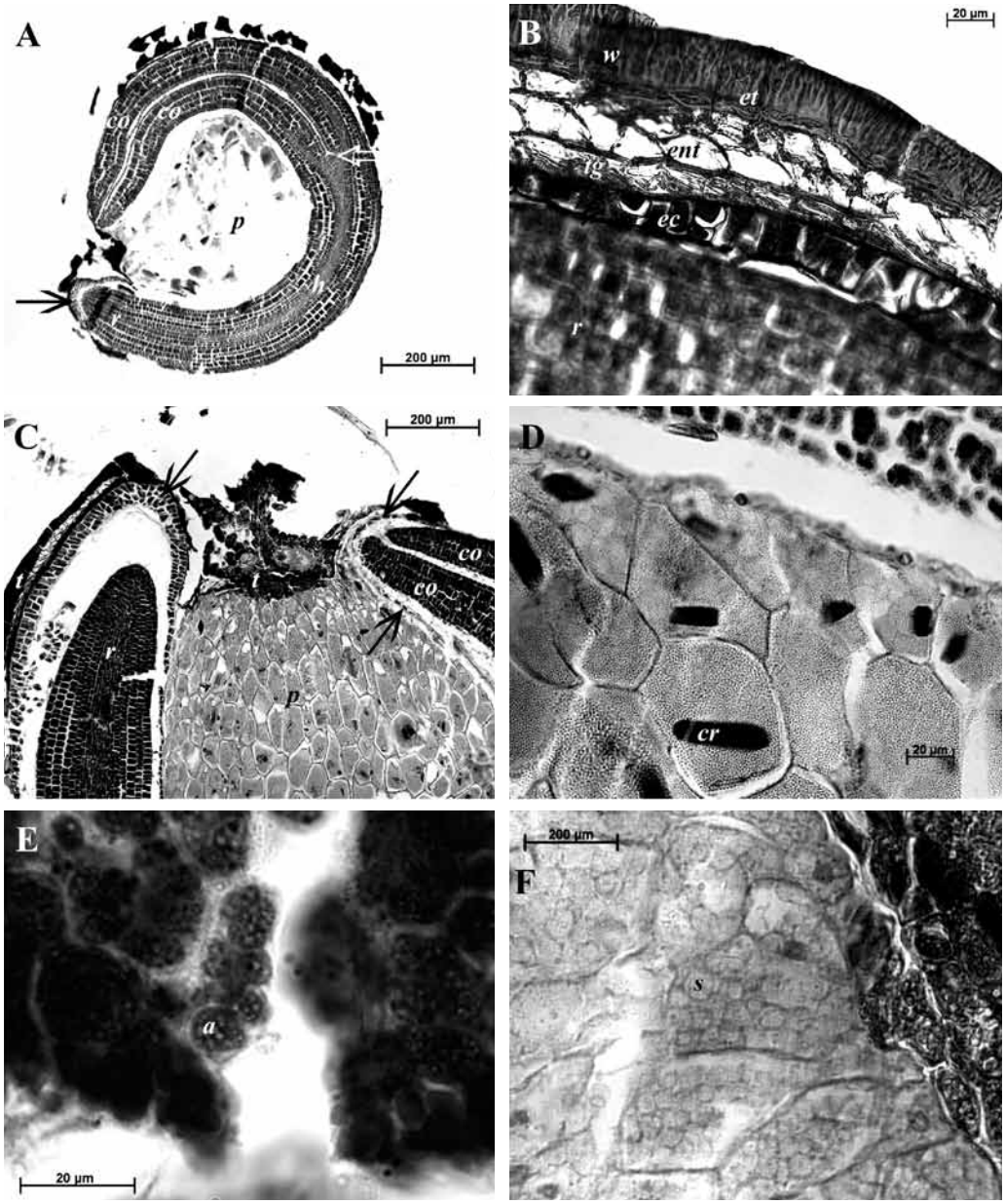

Figure 12. Mature seed. A – longitudinally sectioned seed; B – seed coat; C – endospermal caps; D – protein crystals in perisperm cells; E – aleuron grains in embryo cells; F – starch grains in perisperm cells. *a* – aleuron grains; *co* – cotyledon; *cr* – protein crystal; *ec* – endospermal cap; *ent* – endotesta; *et* – exotesta; *h* – hypocotyl; *p* – perisperm; *r* – radicle; *s* – starch grain; *t* – testa; *tg* – tegmen; *w* – outer cell wall; *black arrow* shows endospermal cap; *white double arrow* shows plumula.

some lipid globules, but no starch grains. A crescent embryo curves round the central part of the nucellus which gradually transforms into perisperm from the globular proembryo stage onwards as follows. Its cells develop numerously pored cell walls (Fig. 10 F) for transporting substances. They accumulate a great many globular compound starch grains consisting of numerous minute granules (Fig. 12 F), some aleuron grains of variable sizes, and a few prismatic to rhombohedral protein crystals (Fig. 12 D). Some perisperm cells by the micropyle and raphe accumulate tannins.

Both integuments start developing into the seed coat just after the legitimate fertilization. The endostome cells die off and collapse whereas the outer integument elongates to form exostome (Fig. 11 A). The outer cells of the inner integument completely collapse by the globular proembryo appearing, contrariwise their inner counterparts enlarge, especially by the micropyle (Fig. 11 A). Besides, they develop thin, anticlinally finely striped cell walls and lyse their protoplasts forming an endotegmen (Fig. 11 B) which later becomes compressed (Fig. 12 B). The outer integument develops into the testa. Its inner cell layer locally increases up to 2 or even 3 layers (Fig. 11 C); the cells retain thin cell walls and accumulate starch grains, but they undergo in time autolysis of their protoplasts and obliteration except of those located near by the radicula (Fig. 12 B). The outer cell layer develops exceedingly thick outer cell walls penetrated with pore channels and polysaccharide dendrites (Figs 11 D–E; 12 B). The polysaccharide content of the thickened wall highly increases inwards as evidenced by the PAS reaction. The cell protoplasts accumulate tannin globules which unite in time (Fig. 11 C, F). They also contain rather much polysaccharides and proteins for a time, but loose them during the seed coat development. In the ripening seed coat the thickened outer cell walls are evenly pervaded with tannin. The exotesta thus arises.

The flattened reniform ripe seed consists of a black shining exotestal-endotegmal seed coat, a large crescent peripheral embryo (comprising radicle, hypocotyl, 2 cotyledons and plumula in between), 2 endospermal caps, and a bulky central perisperm (Fig. 12 A).

Comparisons and conclusion

Though not scrutinized, the embryo of *Pleuropetalum* is believed here to correspond to the Chenopodiad type as evidenced by the cell pattern of the globular proembryo (Fig. 10 C). A rather long zygote generates an apical *ca* and a basal *cb* cell by transverse division. They transversely divide again to give rise to a linear 4-celled proembryo. Of these cells, *cc* and *cd*, both generated by the *ca* cell, produce two top tiers of the globular proembryo whereas the cell *cb* forms the middle tier of the proembryo (*m*), the hypophysis (*hp*), and a long uniseriate suspensor (*s*).

The centrosperms are monotonous in most of embryological traits and only a few peculiarities are inherent in their families (Veselova 1990). General characters of the centrosperms are: mostly centripetal development of the 4- to 5-layered anther wall, secretory tapetum, tapetal membrane with Ubish bodies, simultaneous microsporogenesis, 3-celled pollen, usually 2-layered integuments, successive megasporogenesis resulting in cell triad, ephemeral antipodes, exotestal-endotegmal seed coat, finely striped cell walls of the endotegmen, ephemeral endosperm, endospermal caps, bulky perisperm, crescent peripheral embryo with evident radicula, hypocotyl, paired cotyledons and plumula in between (Veselova 1990). The endostome of *P. darwinii* was considered as an indicator of close affinity between this taxon and Amaranthaceae by Ronse Decraene et al. (1999). However, the endostome is really typical of all centrosperms (Veselova 1990).

Pleuropetalum darwinii is similar to Amaranthaceae in multiseriate archesporium of each pollen sac and sharply contrasts to Portulacaceae in their uniseriate archesporium. The multiseriate archesporium is inherent in the vast majority of centrosperms (Veselova 1990) and in Phytolaccaceae (Kamelina 1983). Size heterogeneity of developing microspores and / or pollen is revealed in both, *P. darwinii* and *Myosoton aquaticum* (L.) Moench (Caryophyllaceae) (Veselova 1995). The panaperturate pollen, which is indeed a shared character of *P. darwinii*

and Amaranthaceae (Ronse Decraene et al. 1999), was discovered in a few Portulacaceae members, too (Kuprianova & Aleyoshina 1978), although the Portulacaceae mostly have 3- or multicolpate pollen. *P. darwinii* shares calcium oxalate crystals in the pollen with *Phytolacca americana* L. (Phytolaccaceae) (Kamelina 1983). Such crystals have been revealed so far, neither in Amaranthaceae s.l. nor in Portulacaceae.

Pleuropetalum darwinii resembles Amaranthaceae in circinotropous ovules, short cells of the nucellar cap during embryo sac development, evidently Chenopodiad type of the embryo development, and equal cotyledons. All characters mentioned are not unique to Amaranthaceae, unfortunately. Uniseriate suspensor, typical of *P. darwinii*, is also inherent in most but a few Amaranthaceae (Savina 1983). Exotesta cells of Amaranthaceae develop a peculiar thickening of outer cell wall that was named stalactiform due to its shape (Butnik & Zhapakova 1991). Such thickenings are always lacking in the exotesta cells of *P. darwinii*. Instead, the exotesta cells of *P. darwinii* – like those of some Pereskioideae (Cactaceae) (Vishenskaya 1991) – develop an exceedingly thick outer cell wall penetrated with polysaccharide dendrites. An inward increasing staining of the thickened outer cell wall of the exotesta cells is inherent in *P. darwinii* and some cacti (Vishenskaya 1991). *P. darwinii* is similar to some members of Opuntioideae (Cactaceae) (Vishenskaya 1991) and *Claytonia virginica* L. (Portulacaceae) (Plisko 1991) in numerous pore channels penetrating the outer cell wall of the exotesta cells.

Embryos of Amaranthaceae store starch. Contrary to them, the embryos of the *P. darwinii*, just like those of Caryophyllaceae (Gvinianidze & Fedotova 1991), Didiereaceae (Kravtsova 1991), and Portulacaceae (in prep.), contain aleuron grains and lipid globules instead of starch.

Few embryological traits of *P. darwinii* are likely to be unique. These are (1) exostome following the fertilization, (2) micropylar exudates that precipitate in the unfertilized ovule to plug the micropyle, and (3) aleuron grains and protein crystals stored by the perisperm cells.

As we have extracted from published data, Phytolaccaceae strikingly differ from *P. darwinii* as well as from most centrosperms in a much thicker (up to 17 cell layers) nucellar cap, a 5- to 6-layered outer integument, placental and funicular obturators, and integuments without air chamber in between. The only character shared by *P. darwinii* and Phytolaccaceae is the presence of calcium oxalate crystals in pollen. Therefore, the affinities between *P. darwinii* and Phytolaccaceae as established by Ronse Decraene et al. (1999) have been highly overestimated.

Some embryological similarities between *P. darwinii* and the closely interrelated families Portulacaceae and Cactaceae are evident, but *P. darwinii* seems to be more similar to Amaranthaceae. However, a series of conspicuous differences between *P. darwinii* and the core Amaranthaceae are really present. So, we have to ascertain that *Pleuropetalum* is even more anomalous and a problem member of Amaranthaceae than it was considered elsewhere due to its unique embryological traits and its unique combination of non-specific embryological characters.

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